The Veterinary Journal 268 (2021) 105604

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Serum hyaluronic acid, a marker for improved liver perfusion after gradual surgical attenuation of extrahepatic portosystemic shunt closure in dogs

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ARTICLE INFO

Keywords:
Canine
ELISA
Liver perfusion
Vascular anomaly

ABSTRACT

Current liver function tests used in dogs do not consistently normalise after successful surgical attenuation of portosystemic shunts (PSS). Serum hyaluronic acid (sHA) concentrations in dogs with PSS are reported to be higher at diagnosis than in healthy dogs. The objective of this study was to assess sHA as a marker of liver perfusion by measuring sHA concentrations in dogs before and after gradual surgical attenuation of extrahepatic (EH)PSS and by determining whether sHA concentrations could differentiate closed EHPSS from persistent shunting. Specificity of sHA was assessed by comparing sHA concentrations in dogs with EHPSS to those in dogs with other liver diseases.

Twenty dogs with EHPSS had sHA concentrations measured at diagnosis, 1, 3, and 6 months postoperatively. In addition, sHA concentrations were determined in 10 dogs with other liver diseases. At EHPSS diagnosis, median sHA concentration was 335.6 ng/mL (43.0–790.7 ng/mL). All dogs had a significant decrease in sHA concentrations from 1 month postoperatively onwards ($P < 0.05$), regardless of surgical outcome. At all postoperative follow-up visits, there was a significant difference between the median sHA concentration in dogs with closed EHPSS vs. those with persistent shunting ($P < 0.05$). Median sHA concentration in dogs with other liver diseases was 89.8 ng/mL (22.9–160.0 ng/mL), which was significantly lower than dogs with EHPSS at diagnosis ($P < 0.001$). In conclusion, sHA is a promising non-invasive biomarker that can help to determine liver perfusion after surgical attenuation of EHPSS. In addition, sHA could potentially be used to differentiate dogs with EHPSS from dogs with other liver diseases.

Introduction

A portosystemic shunt (PSS) is an aberrant vessel that forms a communication between the portal vein and the venous systemic circulation, causing blood originating from the gastrointestinal tract to bypass the liver parenchyma (Suter, 1975). Surgical attenuation of the aberrant vessel is the treatment of choice (Greenhalgh et al., 2014). Although several surgical techniques have been described, the evidence base comparing the ability of different techniques to achieve complete PSS closure without development of multiple acquired (MA) PSS remains weak (Tivers et al., 2017; Serrano et al., 2019).

Hyaluronic acid (HA) is a linear heteropolysaccharide that is secreted by fibroblasts and consists of repeating disaccharide units containing *N*-acetyl-D-glucosamine and D-glucuronic acid (Fraser et al., 1997). It is typically found in connective tissue and influences tissue viscosity, osmosis, shock absorption, and wound healing. Hyaluronic acid is locally metabolised and, when it reaches the blood via the lymphatic system, it is rapidly catabolised, mainly by hepatic sinusoidal endothelial cells (Fraser et al., 1997; Kogan et al., 2007). Consequently, increased serum (s)HA concentrations in dogs with PSS can be an indication of decreased clearance secondary to decreased liver perfusion. In two studies, sHA concentrations were higher in dogs with PSS compared to those in healthy dogs (Seki et al., 2010; Ceplecha et al., 2018). One of those

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http://dx.doi.org/10.1016/j.tvjl.2020.105604
1090-0233/

studies also reported that sHA concentrations decreased significantly 2 weeks after surgical attenuation of the extrahepatic (EH) PSS (Seki et al., 2010). A third study failed to identify differences in sHA concentrations between dogs with and without PSS (Lidbury et al., 2016), but only four dogs with congenital PSS were included in this study.

The aims of this study were: (1) to assess sHA concentration as a measure of liver perfusion by evaluating sHA in dogs before and after gradual surgical attenuation of EHPSS and by determining whether the test could differentiate between closed EHPSS and persistent shunting (patent EHPSS or MAPSS); and (2) to assess the specificity of sHA for diagnosing shunting, by comparing sHA concentrations in dogs with EHPSS to those in dogs with other liver diseases.

Materials and methods

Remainders of serum specimens obtained in the course of a previous prospective study (Devriendt et al., 2020a) were used. The study was approved by the local ethical and deontological committee (Approval number, EC 2014/179, 29 January, 2015; Approval number, DC 2015N03, 2 April, 2015).

Dogs with extrahepatic portosystemic shunts

All dogs were diagnosed with EHPSS based on routine serum biochemical analysis followed by medical imaging. After at least 4 weeks of medical stabilisation using a combination of liver-support diet, lactulose (0.5 mL/kg three times daily, adjusted to clinical effect) and metronidazole (7.5–10 mg/kg twice daily), all dogs underwent surgical attenuation using gradual attenuation devices, which were placed as close as possible to the insertion of the EHPSS into the systemic circulation. If sufficient space could be created after careful dissection of the EHPSS, an ameroid constrictor was placed. In cases where an ameroid constrictor was judged to be too bulky or if there was a risk of kinking, then thin film banding was applied. Whenever thin film banding was applied, the diameter of the EHPSS was slightly reduced. Follow-up visits were performed 1-, 3-, and 6-months postoperatively. Blood analysis (including haematology, biochemistry, ammonia and serum bile acid concentrations) was performed during all follow-up visits. At the 3-month follow-up visit, transsplenic portal scintigraphy (TSPS) was performed to determine the status of EHPSS closure. Shunt fractions <4.3% were considered in the reference range (Cole et al., 2005). Computed tomography angiography (CTA) scans to better evaluate the vascular anatomy were recommended when TSPS was equivocal or was unable to differentiate between patent shunting and MAPSS. If TSPS indicated that the EHPSS was closed, a commercially-available balanced diet was recommended. If there was a patent EHPSS, a second surgery was recommended to attempt to fully ligate the EHPSS. After the second surgery, further follow-up visits were as for the first surgery. Owners of dogs with MAPSS and dogs with patent EHPSS that did not undergo a second surgery were advised to continue a liver-support diet. Medical therapy was at the discretion of the attending clinician.

Dogs with other liver diseases

Dogs with increased pre- and/or postprandial bile acids and signalment characteristics typical for EHPSS (i.e. young, small breed dogs), or those with clear signs of chronic hepatopathy (i.e. persistent increase of serum liver enzyme activities and/or serum bilirubin), were eligible for inclusion. In all dogs, complete blood count and serum biochemistry analysis, including fasting ammonia and pre- and postprandial bile acids, were performed. PSS was excluded in all dogs by abdominal ultrasonography, TSPS, and/or CTA. Maltese dogs were considered as a separate group as it has been reported that they can have moderately increased postprandial bile acids without having other evidence of liver disease (Tisdall et al., 1995). Hence, liver biopsy was not performed in Maltese dogs.

Sample collection and storage

In dogs with EHPSS, blood samples were taken as part of the routine follow-up at diagnosis and at 1-, 3- and 6-months postoperatively. Blood samples after both the first and second surgeries were included. Blood samples were collected once in dogs with other liver diseases. A request was made for dogs to be fasted for at least 12 h prior to blood collection. Excess serum was stored at −80 °C until analysis.

Analysis

Serum HA was measured in all samples using a commercially-available ELISA kit (Hyaluronan Quantikine, R and D systems), according to manufacturer's instructions. Optical density was determined using a microplate reader (Multiskan GO, Thermo Fisher Scientific) set to 450 nm with wavelength correction set at 550 nm.

Although the kit used in the study had previously been evaluated for use in dogs (Ceplecha et al., 2018), three kits with the same batch number were used to perform a limited in-house validation; all samples were subsequently analysed in one batch. The limited in-house validation comprised determination of intra- and inter-assay precision via the coefficients of variation (CV; Appendix A: Supplementary material Tables S1 and S2). Convenience samples were used for in-house validation. The correlation coefficient of the standard curve (r) was determined and the recovery was calculated as the ratio of observed vs. expected sHA concentrations (Appendix A: Supplementary material Table S3). To determine the effect of haemolysis, plasma from one dog was collected in two separate EDTA tubes. One tube was shaken to induce visible haemolysis; sHA concentration was determined for both samples (Appendix A: Supplementary material Table S4). Preliminary tests were performed on a limited number of serum samples to determine the optimal dilution factor. Subsequently, based upon anticipated sHA concentrations, samples were divided in two groups: 'diseased' samples (e.g. samples from dogs with EHPSS collected at the time of diagnosis, postoperative samples of dogs with persistent shunting, and samples from dogs with other liver diseases) which were diluted 1 in 20; and 'healthy' samples (all postoperative samples from dogs with closed EHPSS as determined by TSPS 3 months postoperatively) which were diluted 1 in 4. The diluent (Calibrator Diluent RD5-18) was provided in the kit. The final analysis was performed on 1 day and all analyses were performed by the same person.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 26 (IBM). Data were tested for normality using Shapiro–Wilk testing. Kruskal–Wallis tests were performed to evaluate outcomes between dogs with closed vs. persistent shunting at different times. Mann–Whitney U tests were performed to compare dogs with EHPSS vs. dogs with other liver diseases at the time of diagnosis. Friedman two-way analyses with Bonferroni correction were performed to compare outcomes over time for dogs with closed EHPSS and persistent shunting. For dogs that underwent a second surgery, only data from the time of diagnosis, and 1- and 3-months postoperatively were available for analysis following the first surgery. Data from time-points after the second surgery were analysed according to the outcome of that surgery i.e. whether the EHPSS had been successfully closed or whether persistent shunting remained. Results were considered significant if $P < 0.05$.

Receiver operating characteristics (ROC) curves to assess the diagnostic accuracy of sHA to determine shunt closure status were plotted using values from EHPSS dogs at the time of diagnosis, and 3 and 6 months postoperatively. To assess the diagnostic accuracy of sHA to differentiate dogs with EHPSS from those with other liver diseases at diagnosis, ROC curves were plotted using values from dogs in each group. The area under these ROC curves (AUROC) was calculated with 90% confidence interval (CI). Subsequently, the optimal cut-off value using the Index of Union method (Unal, 2017) and the cut-off value resulting in 100% sensitivity to diagnose dogs with persistent shunting, were determined. Values above the cut-off were considered to indicate persistent shunting, and values below the cut-off were considered to indicate EHPSS closure. Concurrent sensitivity and specificity, with 95% CI, were calculated using 2 × 2 contingency tables. Sensitivity and specificity, with 95% CI, for the optimal cut-off value to differentiate dogs with closed EHPSS from those with persistent shunting, and to differentiate dogs with EHPSS at diagnosis from those with other liver diseases, were calculated using 2 × 2 contingency tables.

Results

Study population

Twenty dogs with EHPSS (median age, 7.5 months; age range, 2–74 months; median bodyweight, 3.8 kg; bodyweight range, 1.5–8.4 kg) were enrolled. Breeds included were as follows: Maltese (n = 4); Dachshund (n = 3); Chihuahua, Bichon Frisé, and Cross breed (n = 2 each); and Shih Tzu, Miniature pinscher, Standard Schnauzer, Border collie, Havanese, Yorkshire terrier and West Highland white terrier (n = 1 each). Twelve dogs were male (n = 4 neutered prior to diagnosis), and eight were female (n = 2 neutered prior to diagnosis). Surgical attenuation was performed using thin film banding in five dogs and an ameroid constrictor in the remaining 15 dogs. As TSPS was not performed at the time of diagnosis, preoperative shunt fractions were not available for analysis. Postoperatively, median shunt fraction in dogs with closed EHPSS was 2.5% (range, 1.0–4.2%) and the median shunt fraction in dogs with persistent shunting was 86.5% (range, 80.6–96.0%). In one dog, the

TSPS at 3 months was of poor quality, rendering the scan result inconclusive. Because the dog did not show any clinical signs, the owners declined further imaging and, therefore, the surgical outcome remained uncertain. For this reason, in this dog, only sHA concentrations at the time of diagnosis was included in the statistical analysis. Of the remaining 19 dogs, five (26%) had persistent shunting after the first surgery; three of the five had MAPSS (one diagnosed using CTA, one with TSPS, and one with both TSPS and CTA); and the other two had patent EHPSS (determined by TSPS). Both dogs with patent EHPSS underwent successful second surgeries, during which the EHPSS was completely ligated. Complete EHPSS closure was achieved in 84% of dogs. Frozen serum was available from all follow-up visits, with the exception of one 3-month postoperative sample in a dog with closed EHPSS, and one 6-month sample from a dog with MAPSS.

Ten dogs with other liver diseases were included (median age, 43.5 months; age range, 6.0–77.0 months; median bodyweight, 5.1 kg; bodyweight range, 2.2–27.0 kg). Liver histopathology from biopsies was available from seven dogs. Four dogs of different breeds (Chihuahua, Dachshund, Griffon terrier and Tibetan terrier) were diagnosed with portal vein hypoplasia and three dogs of different breeds (Chesky Fousek, Groenendael and Large Münsterländer) with chronic hepatitis. The remaining three were Maltese; one had increased paired bile acid concentrations, and the other two had normal preprandial bile acids and moderately increased postprandial bile acid concentrations.

Serum hyaluronic acid concentrations

At diagnosis, median sHA concentration in all dogs with EHPSS was 335.6 ng/mL (range, 43.0–790.7 ng/mL). The median sHA concentrations at diagnosis did not differ between dogs in which surgery resulted in closed EHPSS and those with persistent postoperative shunting (P = 0.229; Table 1).

In dogs with closed EHPSS and those with persistent shunting, sHA concentrations were significantly reduced by 1 month postoperatively (P = 0.001 and P = 0.011, respectively) and remained lower than at diagnosis (Fig. 1). However, there was a significant difference in median sHA concentrations between dogs with closed EHPSS vs. those with persistent shunting at all follow-up visits (Table 1). Median sHA concentrations in dogs with other liver diseases was 89.8 ng/mL (range, 22.9–160.0 ng/mL), which was significantly lower than sHA concentrations in dogs with EHPSS at diagnosis (P < 0.001; Table 2; Figs. 2 and 3).

The AUROC of dogs with EHPSS was 0.97 (90% CI, 0.94–1.00), suggesting that sHA concentration is very good test to differentiate dogs with closed EHPSS vs. those with persistent shunting postoperatively. Two cut-off values were retained: one (55.9 ng/mL) had an optimal combination of sensitivity (96.9%; 95% CI, 82.0–99.8%) and specificity (81.8%; 95% CI, 66.8–91.3%) for diagnosis of persistent shunting. The other cut-off value (42.5 ng/mL) had the highest possible specificity (61.4%; 95% CI, 45.5–75.3%) with 100.0% sensitivity for diagnosis of persistent shunting (95% CI, 86.7–100.0%; Fig. 1). For the cut-off of 55.9 ng/mL to differentiate dogs with EHPSS-pre-surgery from dogs with other liver disease, sensitivity to detect dogs with EHPSS was very high (95.0%; 95% CI, 73.1–99.7%), but specificity to rule out other liver diseases was low (40.0%; 95% CI, 13.7–72.6%; Fig. 2).

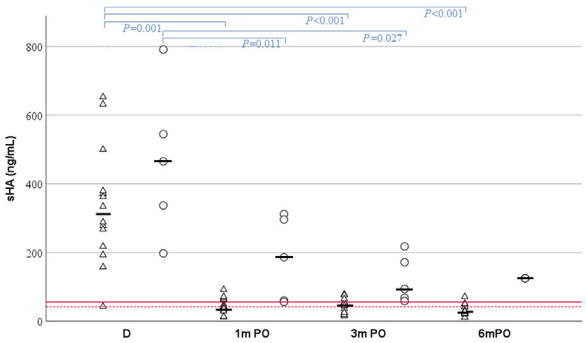

Fig. 1. Serum hyaluronic acid (sHA) concentrations at different time points (diagnosis, 1-, 3-, and 6-months postoperatively, respectively) in dogs with closed extrahepatic portosystemic shunts (EHPSS; triangles) or with persistent shunting (circles). In both groups, sHA decreased significantly by 1 mPO (P < 0.001) and remained low (P < 0.05). Median values are represented with horizontal lines. The continuous red line represents the optimal cut-off value to differentiate dogs with closed EHPSS from those with persistent shunting; the dotted red line represents the cut-off value resulting in 100% sensitivity for the diagnosis of persistent shunting. D, diagnosis; PO, postoperatively; 1 m, 1 month; 3 m, 3 months; 6 m, 6 months.

Table 2
Median (range) serum hyaluronic acid concentrations (sHA) in dogs with an extrahepatic portosystemic shunt (EHPSS) and dogs with other liver diseases at the time of diagnosis.

Dataset	n	sHA (ng/mL)	P
EHPSS	20	335.6 (43.0–790.7)	<0.001[a]
Other liver diseases	10	89.8 (22.9–160.0)	
Chronic hepatitis	3	114.8 (64.7–138.0)	
Portal vein hypoplasia	4	39.5 (23.1–138.5)	
Maltese	3	119.6 (22.9–160.0)	
Healthy dogs[b]	41	37.2 (14.0–123.2)	

[a] P for comparison between EHPSS and other liver diseases.
[b] Data from a previous study of healthy dogs have been added for comparison (Ceplecha et al., 2018); ELISA kits made by the same manufacturer were used for both studies.

Discussion

This study suggests that sHA has great potential as a non-invasive biomarker to determine EHPSS closure after surgical

Table 1
Median (range) serum hyaluronic acid concentrations (sHA) at different time point in dogs with extrahepatic portosystemic shunts (EHPSS).

Surgical outcome	Diagnosis		1 month PO		3 months PO		6 months PO	
	sHA (ng/mL)	n	sHA (ng/mL)	n	sHA (ng/mL)	n	sHA (ng/mL)	n
Closed[a]	311.29 (43.04–652.78)	14	36.76 (13.51–92.24)	16	44.98 (19.14–78.66)	15	28.91 (11.45–71.30)	16
Persistent[b]	465.22 (197.90–790.66)	5	186.28 (56.44–312.04)	5	93.78 (58.94–217.78)	5	130.43 (125.16–135.70)	2
P	0.229		0.008		0.005		0.025	

PO, Postoperatively.
[a] sHA concentrations obtained after the second surgery of dogs that underwent two surgeries and that resulted in a closed EHPSS are included.
[b] All sHA concentrations obtained before the second surgery of dogs with persistent surgery after the first surgery are included.

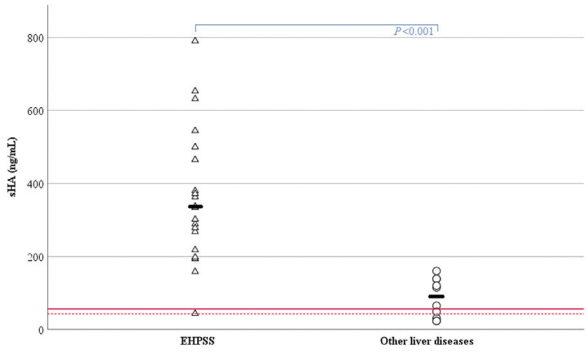

Fig. 2. Serum hyaluronic acid (sHA) concentrations at diagnosis in dogs with extrahepatic portosystemic shunts (EHPSS; triangles) and dogs with other liver diseases (circles). Median values are represented with horizontal lines. The continuous red line represents the optimal cut-off value to differentiate dogs with closed EHPSS and those with persistent shunting; the dotted red line represents the cut-off value resulting in 100% sensitivity for the diagnosis of persistent shunting.

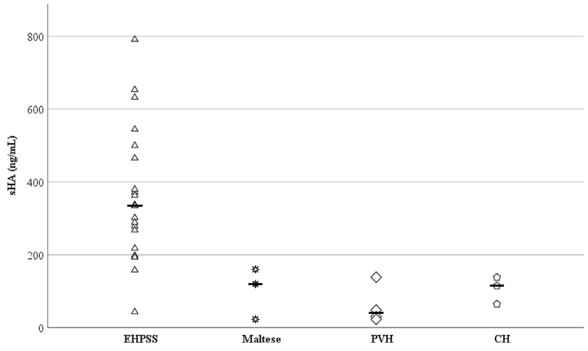

Fig. 3. Serum hyaluronic acid (sHA) concentrations at diagnosis in dogs with extrahepatic portosystemic shunts (EHPSS) at diagnosis (triangles). Maltese dogs with increase serum bile acids and absence of PSS, stars; dogs with portal vein hypoplasia (PVH) diamonds; dogs with chronic hepatitis (CH), pentagons. Median values are represented with horizontal lines.

attenuation. We confirmed that sHA concentration decreased in the first month after surgery in all dogs treated for EHPSS, as previously described (Seki et al., 2010). Nevertheless, even at the first follow-up visit, sHA concentrations were significantly lower in dogs that achieved complete EHPSS closure compared to those with persistent shunting, suggesting increased sHA clearance. Furthermore, since sHA concentrations were much higher in dogs with untreated EHPSS than in those with other liver diseases, sHA could potentially differentiate EHPSS from other hepatic conditions. Nevertheless, more research is needed to consolidate these findings in a larger group of dogs with a variety of liver diseases.

It is interesting that there was significant reduction in sHA in the first month after surgery, especially as the surgery aimed for gradual EHPSS occlusion. Ameroid constrictors can occlude intra-abdominal vessels in 10 days to 5 weeks (Vogt et al., 1996; Besancon et al., 2004). No comparable studies have been performed to determine the characteristics of intra-abdominal vessel occlusion using thin film banding; however, results from extra-abdominal models suggest that thin film banding results in slower occlusion of vessels (Youmans and Hunt, 1999). Regardless of the time needed for complete occlusion, some acute reduction in EHPSS flow can be expected after ameroid constrictor placement due to partial compression of the blood vessel, and after thin film banding due to a partial reduction in blood vessel diameter and the change in blood vessel profile to a teardrop shape. In contrast to commonly used liver function tests that measure the functional capacity of hepatocytes (van Straten et al., 2015; Bristow et al., 2017; Devriendt et al., 2020b), sHA concentrations primarily reflect liver perfusion, as HA is cleared from the blood by the hepatic sinusoidal endothelial cells (Fraser et al., 1997; Kogan et al., 2007). After gradual attenuation of EHPSS, the direction and velocity of the blood flow in the portal vein should gradually increase the blood flow towards the liver (Szatmári et al., 2004b) and, consequently, increase liver perfusion. Our results suggest that even in dogs with persistent shunting, dynamic changes in hepatic blood flow are associated with significant improvement in liver perfusion.

Despite significantly improved liver perfusion in dogs with persistent shunting, as demonstrated by decreased sHA concentrations, there was significantly better liver perfusion in dogs with closed EHPSS than in those with persistent shunting. Until now, it is not known how much persistent shunting can be tolerated on a long-term basis in dogs after surgical attenuation of EHPSS in dogs. Furthermore, it is important to realize that the direction of blood flow through a patent shunt in some patients will remain hepatofugal whereas it might become hepatopetal in others (Szatmári et al., 2004b). Determination of sHA concentrations might aid in clinical decision making when considering the potential benefits of a second surgery in dogs with patent EHPSS.

In this cohort of dogs with EHPSS, the optimal cut-off value resulted in a sensitivity of 96.9% and specificity of 81.8% for persistent postoperative shunting. However, when using this cut-off, additional tests and/or medical imaging are needed to definitively confirm shunt closure. When a cut-off value with 100% sensitivity was chosen to diagnose persistent shunting, specificity declined to 61.4%. However, using the lower cut-off value, only dogs with sHA concentrations higher than this value would need additional tests and/or advanced imaging to evaluate shunt closure. Using the optimal cut-off value, the resulting sensitivity of sHA concentrations is better than reported for commonly used liver function tests after surgical attenuation (Devriendt et al., 2020b). The sensitivities of postsurgical postprandial serum bile acids, fasting ammonia and ammonia tolerance tests are 85%, 19–44%, and 89%, respectively (van Straten et al., 2015; Vallarino et al., 2019) and their specificities are 74%, 100%, and 85%, respectively (van Straten et al., 2015; Vallarino et al., 2019). Thus, the specificity of fasting ammonia and ammonia tolerance tests is superior to sHA concentration. This is unsurprising, since hyperammonaemia in the absence of PSS is rare (Szatmári et al., 2004a), and our study demonstrated that sHA clearance improved even in dogs with persistent shunting.

Reference values have been reported for sHA in healthy dogs; in two studies, similar values were reported, ranging from 14 to 123 ng/mL (Seki et al., 2010; Ceplecha et al., 2018); but in a third study, the range of sHA concentrations was wider (84–1464 ng/mL; Lidbury et al., 2016). In our study, only one dog with EHPSS had a low sHA concentration at the time of diagnosis (43 ng/mL), and in all dogs with closed EHPSS, sHA concentrations were within the reference values for healthy dogs, as reported by Seki et al. (2010) and Ceplecha et al. (2018). In our study, two dogs with persistent shunting also had low sHA concentrations postoperatively, indicating a substantially improved sHA clearance, and only three dogs with other liver diseases had sHA concentrations >123 ng/mL (highest concentration,160 ng/mL). Based on the optimal cut-off value to distinguish dogs with closed EHPSS from those with persistent shunting (55.9 ng/mL), the specificity of sHA to discriminate dogs with EHPSS from those with other liver diseases was only 40%. The inclusion of healthy control dogs could change sensitivity and specificity results. However, as only a small number of dogs were included in our study, our cut-off values should be interpreted with caution. Larger studies, including more diseased and healthy dogs, are needed to consolidate our results.

We used a commercially available sHA ELISA that demonstrated satisfactory in-house validation parameters, and required only a small volume of serum (<100 μL). Individual samples cannot be measured with this kit, but conversion to either a point-of-care or individual auto-analyser immune-assay would overcome this disadvantage. In human medicine, automatic XP analysers have been used to determine sHA concentrations (Puigvehi et al., 2016). These automated immunoassays have a detection range from 1.6 to 1000 ng/mL, which would be sufficient for the values reported in this study. Nevertheless, automated immunoassays are not widely available at the time of writing.

To obtain accurate results, fasted samples should be analysed, as it is known in humans that sHA concentrations increase after eating (Fraser and Gibson, 2005). Although dog owners were asked to fast their dog at each follow-up visit, two dogs (both with closed EHPSS) were not fasted at the 6-month follow-up visit. Nevertheless, the sHA concentrations obtained from the non-fasted samples were well within the range of concentrations obtained from other dogs with closed EHPSS at their 6-month follow-up visits.

As HA is found in all connective tissues, but mainly in skin, bones and cartilage, a number of factors could influence sHA concentrations. For example, decreased lymphatic drainage and cardiac conditions can decrease sHA concentrations (Fraser et al., 1997). Local HA metabolism increases during inflammation, which could increase sHA concentrations (Fraser et al., 1997; Kogan et al., 2007). Additionally, either increased synthesis by hepatic stellate cells, and fibroblasts in case of hepatic fibrosis, or dysfunction of sinusoidal endothelial cells, can increase sHA concentration (Gudowska et al., 2016). Decreased kidney function could also increase sHA concentration. In healthy humans, although the kidneys extract approximately 10% of sHA, only 1–2% is excreted in the urine (Fraser et al., 1997). In dogs with congenital PSS, perisinusoidal hepatic stellate cells proliferate and transform into myofibroblast-like cells, causing mild to moderate reversible portal fibrosis (Baade et al., 2006). During the transformation into myofibroblast-like cells, various extracellular matrix components are released, including HA. As the half-life of HA in blood is only 2−5 min (Rostami and Parsian, 2013), the influence of this transformation is likely to be minor compared to decreased sHA clearance caused by the substantial amount of blood bypassing the liver. Finally, in humans, sHA concentrations increase with age (Engström-Laurent et al., 1985). All dogs in the current study were young to middle-aged; however, statistical comparisons by age were not possible due to low sample size.

This study had some limitations. The number of dogs included was relatively low. Of 20 dogs with EHPSS included, only five dogs had persistent shunting, three of which had MAPSS. The number of dogs with various other liver diseases was also relatively low and different liver diseases were represented. Although all non-Maltese dogs underwent diagnostics to exclude PSS and liver biopsies were performed to confirming the presence of hepatic disease, Maltese dogs had only medical imaging to exclude PSS and liver biopsies were not performed. Although clinically healthy Maltese dogs can have moderately increased postprandial bile acids (Tisdall et al., 1995), portal vein hypoplasia cannot be excluded in these dogs. Another potential limitation was that some of the serum samples were haemolysed. Nevertheless, during our internal validation, the presence of haemolysis did not interfere with sHA analysis. Finally, as all samples were analysed as a batch and the follow-up period was 6 months, the oldest samples were stored for 38 months. Although there were no indications that sample age affected the results obtained, there are no published studies investigating the stability of sHA at −80 °C.

Conclusions

In dogs with EHPSS, sHA is a promising non-invasive biomarker of liver perfusion that could help to determine the integrity of EHPSS closure after surgical attenuation. Significant differences in sHA concentrations are present as early as 1 month postoperatively, despite the placement of gradual attenuation devices. At diagnosis, sHA concentration might be helpful in correctly differentiating dogs with other liver diseases from those with EHPSS.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper.

Acknowledgements

Preliminary results were presented at the ECVIM-ca congress 2019 in Milan, Italy.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tvjl.2020.105604.

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