SURGICAL ATTENUATION OF CONGENITAL PORTOSYSTEMIC SHUNTS IN DOGS

TECHNIQUES, COMPLICATIONS AND PROGNOSIS

Anne Kummeling

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SURGICAL ATTENUATION OF CONGENITAL PORTOSYSTEMIC SHUNTS IN DOGS

TECHNIQUES, COMPLICATIONS AND PROGNOSIS

Chirurgische vernauwing van congenitale portosystemische shunts
bij de hond
technieken, complicaties en prognose

(met een samenvatting in het Nederlands)

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Chapter 1

Aims and Scope of the thesis

Surgical treatment of congenital portosystemic shunts (CPSSs) in dogs is ultimately aimed to restore portal blood flow into the liver and to normalize liver function. Until now, the general, worldwide, discussion has been focused on comparison of different surgical techniques, all designed to produce a maximum degree of attenuation of the shunt and optimal treatment outcome. However, several papers conclude that individual surgical treatment response is variable and unpredictable with all currently used techniques.[1,2] Reliable preoperative predictors of postoperative prognosis are difficult to identify.

The general aim of this thesis is to identify factors associated with outcome after surgical attenuation of congenital portosystemic shunts in dogs and, consequently, to gain insight in underlying mechanisms of postoperative recovery in this disease.

The following questions were addressed in this thesis:

- Is the surgical technique that is used to achieve CPSS attenuation critical for prognosis?
- Is individual outcome after CPSS attenuation related to the degree of attenuation during surgery?
- Is individual outcome after CPSS attenuation related to the diameter of the portal vein (portal development) before surgery?
- Can we identify factors in the blood coagulation cascade that predict or cause severe short-term complications after surgery?
- Is hepatic growth after surgical CPSS attenuation relevant for individual outcome after surgical CPSS attenuation?
- Is the preoperative expression of genes that are involved in hepatic and vascular growth and regeneration associated with individual long-term outcome after surgical CPSS attenuation?
- Can we identify prognostic markers that may clarify mechanisms or biochemical pathways that are involved in recovery or failure after surgical CPSS attenuation?

References

1. Sereda CW, Adin CA. Methods of gradual vascular occlusion and their applications in treatment of congenital portosystemic shunts in dogs: a review. Vet Surg 2005;34:83-91.
2. Berent AC, Tobias KM. Portosystemic vascular anomalies. Vet Clin Small Anim 2009;39:513-541.

Chapter 2

General Introduction

Introduction

Congenital portosystemic shunts (CPSSs) are vascular anomalies that divert portal blood directly into the systemic circulation, bypassing the liver parenchyma. The absence of a normal hepatic portal circulation has two important consequences:
(1) impaired hepatic development and function, and (2) direct systemic effects of toxins, nutrients, hormones, and other factors that originate from the splanchnic area.

CPSSs have been described in the dog for sixty years.[1] These congenital anomalies are also found in cats and occasionally in humans.[2-12] In a few other mammalian species incidental cases have been reported.[13,14] CPSSs commonly occur as single vessels that may have an intrahepatic (20-33%) or extrahepatic localization (66-80%).[2,15-18] Extrahepatic CPSSs are mainly seen in small-sized dog breeds, whereas intrahepatic CPSSs often occur in larger dog breeds.[2,6,16,18,19] Extrahepatic shunts commonly arise from the splenic vein, the left and right gastric vein, the gastroduodenal vein or the main trunk of the extrahepatic portal vein and drain into the caudal caval vein (76%) or the (hemi)azygos vein (24%).[2,3,18,20-22] Intrahepatic shunts are divided in left, central or right divisional shunts, depending on their localization, and drain into the hepatic caval vein or a hepatic vein.[5,23-25] CPSSs are expected to have a hereditary origin in predisposed breeds, but the mode of inheritance is often not known.[26-31]

Left divisional shunts are reported to be the most common intrahepatic shunts and result from a failure of the ductus venosus to close after birth (persistent ductus venosus).[32] Functional closure of the ductus venosus normally occurs within three days after birth.[5,24] Right-sided intrahepatic shunts that are presently categorised as central divisional shunts, were reported as right ductus venosus.[4,32,33] Since there is no clear evidence for such an embryological structure, this malformation could also be the result of persistence of the right omphalomesenteric vein, between the right umbilical vein and the cranial anastomosis of the vitelline veins.[24,32] Central and right divisional shunts may also represent malformations of hepatic sinusoids, possibly developing in the absence of a normal ductus venosus, in order to provide a venous bypass in the embryo.[32]

Extrahepatic CPSSs are caused by developmental errors that result in anomalous functional communications (inappropriate anastomoses) between the foetal cardinal (prehepatic caval vein or azygos vein) and vitelline systems (portal vein), with or without hypoplasia or aplasia of the portal trunk distal to the CPSS.[5,24,34] The presence of non-functional extrahepatic portosystemic venous anastomoses is normal. These anastomoses often become functionally significant to compensate obstruction of portal flow when portal hypertension develops (acquired portosystemic shunts).[24,35] Occasionally two or multiple CPSSs are described, with intrahepatic or extrahepatic localization.[20,36,37]

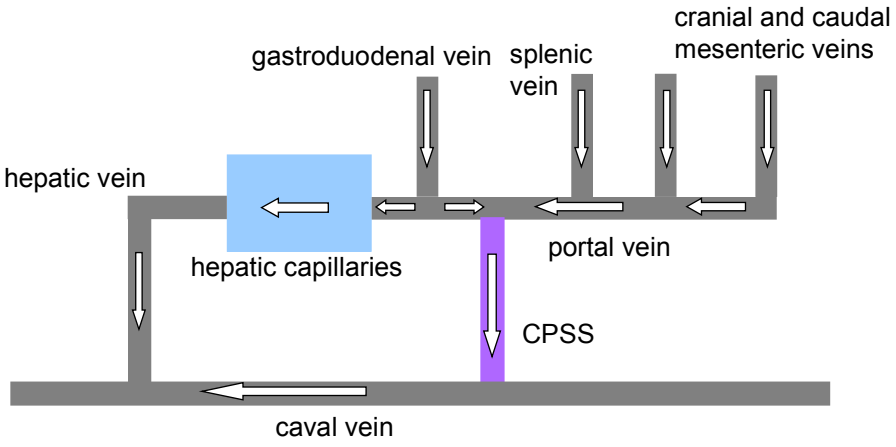

Figure 1. Schematic diagram of an extrahepatic congenital portosystemic shunt (CPSS)

The portal vein collects all venous blood from the stomach, intestines, spleen and pancreas and normally contributes up to approximately 80% of the total hepatic blood flow.[23] While the portal vein supplies the larger amount of blood to the liver, the liver is not capable of directly controlling this flow. The only control of blood flow into the liver is via the hepatic artery. Probably the arterial flow is not controlled by hepatic oxygen demands, but changes inversely in response to altered portal blood flow, explaining the secondary arteriolar proliferation in dogs with a CPSS. However, increased arterial flow cannot compensate for reduced portal blood supply.[5,32,35,38]

CPSSs are usually vessels with large diameters compared to the width of the portal vein cranial to the shunt in extrahepatic CPSSs, or the portal branches that supply the other liver lobes in intrahepatic CPSSs, providing little resistance to the large fraction of shunted blood. Intrahepatic CPSSs are usually larger diameter vessels than extrahepatic CPPSs.[39] In some dogs the CPSS appears to be a mere continuation of the portal vein that directly drains into the systemic circulation, without any portal branches to the liver tissue itself (aplasia of the distal portal vein or intrahepatic portal vasculature).[34] Small liver size and histological changes such as hepatocellular atrophy and degeneration, portal vein hypoplasia and hepatic steatosis are consistent features of

portal vein anomalies and may further increase the resistance for portal circulation into the liver.[3,5,18,32,38,40,41] The effect of the relatively small resistance of portal blood to flow through the shunt is further compounded by the absence of valves in the portal system.[3,35] All these factors are responsible for the high shunted fraction of portal blood that is usually found in dogs with a functional portosystemic shunt.[41]

The absence of a normal hepatic portal circulation results in hepatic insufficiency and a variety of clinical signs.[4,5,40,42] The majority of signs are related to the elevated plasma ammonia concentration, resulting in central nervous system signs (hepatic encephalopathy), precipitation of ammonium urate uroliths and gastrointestinal signs (vomiting and diarrhoea). Other clinical signs include polydipsia, retarded growth or leanness.[2-5,15,16,23,40] Usually signs appear at a young age, although dogs with portoazygos shunts are commonly older when clinical signs develop.[4,5,16] It has been suggested that portoazygos shunts may cause less severe signs because of intermittent compression of the shunt by the diaphragm or stomach.[15] On the other hand, dogs with an intrahepatic CPSS may develop signs at an earlier age, perhaps because of larger shunt diameter and therefore larger shunt fractions.[39]

Measurement of fasting plasma ammonia concentration and, if necessary, a complementary ammonia tolerance test, are the first choice methods to diagnose portosystemic shunting.[5,43,44] In addition, per rectal or trans-splenic portal scintigraphy provide a good estimation of the shunted fraction of portal flow past the liver (shunt index).[5,45-47] To obtain accurate information with respect to the anatomy of the liver, the shunt and the portal vasculature, ultrasonography and computed tomography are commonly used as reliable, non or minimally invasive techniques.[48-53]

Surgical treatment: techniques, complications and outcome

The first reported treatment of CPSS dogs consisted of a low-protein diet combined with antibiotics.[40] Later, surgical closure of the shunt was advised as therapy of choice, because conservative therapy often resulted in progressive hepatic insufficiency, uncontrollable seizures and shorter survival periods and with surgery a successful long-term outcome can be obtained.[4,20,23,54-56] The traditional technique for CPSS attenuation is ligation, usually with a single silk ligature.[2,20,21,23,32,39,54,57-63] Other surgical techniques were introduced, including perivascular ameroid constrictors, cellophane banding, intravascular thrombogenic coils and hydraulic occluders.[65-68]

CPSS ligation

Treatment of dogs with a CPSS preferably consists of complete surgical closure of the shunt (complete ligation). However, due to hypoplasia or aplasia of the portal vasculature outside and/or inside the liver, complete closure of the CPSS often results in severe portal hypertension. If not relieved by loosening the ligature around the shunt, portal hypertension may result in acute shock and death. Complete closure is not possible in approximately 50 to 86% of cases, with higher incidence in intrahepatic shunts compared with extrahepatic shunts.[2,16-18,21,22,54,62,64,69-71] When complete closure cannot be achieved, the CPSS is attenuated to the maximum degree of attenuation that is tolerated without portal hypertension (partial closure).[2,20,22,32,63] The ligature around the shunt is tied over a gauged rod of known diameter, after which the rod is carefully withdrawn. The shunt is then considered to be narrowed to the diameter of the rod.[2] In a small number of dogs, the CPSS cannot be attenuated at all, due to portal venous atresia or aplasia.[34,70,71]

To prevent portal hypertension after shunt attenuation, measurement of portal venous pressure rise can be used to determine the highest acceptable degree of shunt closure (maximum values accepted by different authors, varied from 9 to 25 cm H_2O or 8 to18 mm Hg pressure).[20,21,32,57,62] Baseline portal pressures were not significantly different between dogs with complete or partial ligation, but the intraoperative rise in portal pressure was greater in dogs with partially ligated CPSSs.[21,69] A reason for this association could be an underdeveloped intrahepatic portal system and thus higher pressure rises in partially closed CPSSs.[21] Reported effects of intraoperative portal pressure changes on long-term outcome or survival are controversial.[18,21,69] Although measurement of portal pressure during surgery is widely used, variations in technique make the interpretation of pressure changes often unreliable.[19,32,39,61,69] Other variables are reported to be more reliable tools to evaluate effects of shunt closure during surgery on the portal system, including examination of the intestines and pancreas for venous stasis and congestion, heart rate, end-expiratory CO_2%, mean arterial and central venous blood pressures.[2,21,58,61]

Closure of intrahepatic shunts often is technically more challenging than closure of extrahepatic shunts and several methods for intrahepatic shunt attenuation were developed, depending on type and localization of the shunt. In most dogs with a left divisional shunt, the shunt is exposed between the left lateral lobe and the papillary process of the caudate liver lobe, before entering the left hepatic vein. Typical central divisional shunts often take a straight course from the portal vein, which may locally be dilated, into the right medial lobe to connect with the hepatic vena cava or the central hepatic vein. Sometimes these shunts are no more than portosystemic foramina. Right divisional shunts usually pass as a broad loop through the right lateral lobe before entering the vena cava.[25,32] Extravascular techniques include ligation of the shunt after it has been dissected at a point where the shunt is not completely surrounded by liver

tissue (most left divisional shunts), blind ligation of the shunt using ultrasonographic guidance, or attenuation of the portal branch that supplies the shunt, by a directly or indirectly placed ligature.[32,39,57,72] Examples of intravascular techniques are intraluminal shunt closure via a thoracic caval venotomy or via a portal venotomy.[32,57,73,74]

Complications and outcome after CPSS ligation

Peri-operative or early postoperative mortality after ligation of intra and extrahepatic CPSSs varies from 2.1 up to 29%.[2,19,20,32,61,69,70] Besides portal hypertension, intraoperative complications include tearing of and bleeding from the shunt or one of the larger vessels during dissection and hepatic congestion.[18,32,34,39] Numerous postoperative complications are associated with surgical attenuation of a CPSS and intensive observation of the dog in the immediate postoperative period is extremely important.[62,75] Potential fatal complications shortly after surgery include portal hypertension, abdominal haemorrhage and postligation neurological dysfunction.[20,39,61,62,69,70]

Portal hypertension is not always evident during surgery but can develop several hours after CPSS attenuation.[20,39,61,62,69] Due to surgical manipulation of the CPSS and the surrounding tissues, postoperative vasospasm and swelling may temporarily increase vascular narrowing and result in mild to severe portal hypertension.[59] Severe portal hypertension can also be caused by thrombus formation resulting in acute and complete CPSS and portal vein obstruction.[61,62,76] Clinically, portal hypertension is characterised as a painful abdomen, haemorrhagic diarrhoea, cardiovascular collapse and death.[62] Severe signs of portal hypertension require immediate re-operation and removal of the ligature.[70]

Ascites is often a consequence of mild to moderate portal hypertension and may be increased by a low oncotic pressure, secondary to hypoproteinemia. Mild to severe ascites usually resolves in a few weeks with or without additional treatment.[21,39,61,62,70]

Postoperative abdominal haemorrhage is reported in several reports as a serious complication and is possibly related to hepatic dysfunction.[18,20,61,73] Dogs with a CPSS have lower plasma concentrations of fibrinogen and increased coagulation times compared to normal dogs, but prolonged coagulation times alone were not associated with a bleeding tendency during surgery.[77]

Postoperative generalized seizures and dysfunction of the central nervous system (synonyms: postligation seizure syndrome, postligation neurological dysfunction) are serious, often fatal complications that are regularly reported after CPSS ligation (5-15%).[39,61,69,78-80] Signs are usually progressive and in some cases refractory to treatment.[61,69,79-81] Older dogs might be more susceptible to neurological dysfunction than younger dogs.[18,23,79,80] Although postligation seizures occur more often in small breed dogs, they have been reported after attenuation of extrahepatic as well as

intrahepatic CPSSs.[23,39,61,80,82-84] Neurological signs or seizures usually occur within 12 hours to 3 days after surgery.[61,79,80,82,84] We have observed neurological abnormalities in a single case after general anaesthesia for diagnostic imaging that was not subjected to surgery (personal observation).

The cause of postligation neurological dysfunction is not determined. Suggested causes are hypoglycaemia, hypoxia, pre-existing brain disease, continued hepatic encephalopathy with cerebral edema and/or necrosis, decreased plasma calcium and potassium concentrations.[70,79,83] A plausible explanation might be a rapid decrease of abnormal metabolites in the central nervous system after CPSS attenuation, such as endogenous benzodiazepines, which have anticonvulsant effects.[79,85] Treatment with prophylactic anticonvulsants, early detection and aggressive treatment may avoid progression to refractory seizures and permanent brain injury.[80]

Long-term mortality after ligation of a CPSS is reported to vary from 7 to 16%.[19,32,69] Excellent to good results after long-term follow-up of dogs with ligated CPSSs are obtained in 67 to 85% of cases.[22,41,70] Overall, indications or evidence of recurrent or persistent portosystemic shunting are noted in approximately 22 up to 29% of dogs following surgical CPSS ligation.[41,64,69,70] In contrast to complete ligation, partial ligation is much more often reported to be associated with persistence or recurrence of clinical signs (up to 41%).[16,58,69,70] Causes of recurrent problems with portosystemic shunting commonly are patency of the original shunt (26% of partially attenuated dogs) and evidence of multiple or acquired shunts (3-7% of partially attenuated dogs).[69,70] However, there is no need to increase postsurgical risks associated with portal hypertension by ligating CPSSs completely, in order to attain a favourable outcome in most dogs with CPSSs.[41] The population of dogs with partially attenuated CPSSs can be divided into three groups:

1) In the majority of dogs with a partially ligated CPSS, complete functional closure of the shunt is observed within one to three months. These dogs make a complete clinical and metabolic recovery and a second procedure to further attenuate the shunt is not needed for successful long-term clinical outcome.[32,41,59,64,70]

2) A smaller fraction of dogs that have their CPSS partially ligated, do not recover completely. Clinical signs and attenuation-induced portal hypertension often disappear, but metabolic evidence of persistent portosystemic shunting remains present. When visualised with ultrasonography, the original shunt is often still patent. So, dogs with evidence of persistent shunting can be clinically completely normal, but it is not known if liver function remains sufficient over time.[22,58,59] Previous reports documented complications or recurrence of signs related to hepatic dysfunction, more than 12 months after a good initial clinical response after surgery.[58,70] A second attempt to

ligate the shunt completely can be useful in these dogs to prevent clinical recurrence and is recommended in all dogs after partial shunt closure in some reports.[16,32,62,69]

3) Lastly, in a few dogs partial ligation does not induce any improvement. Although high portal pressures are usually not noted during surgery,[69] persistent portal hypertension may lead to the development of acquired CPSSs after 1-3 months. Surgical attempts to further ligate the shunt are not useful in these dogs.[32,64,78]

Changes in shunt fractions after partial ligation depend on vascular resistance to blood flow through the shunt and the liver.[59,86] Van Vechten (1994) assumed an increased hepatic vascular resistance at the time of surgery compared with the situation in normal dogs and a decrease of hepatic resistance over time in dogs with normalization of their shunt fractions postoperatively.[59] Besides decreasing hepatic resistance, progressive shunt occlusion after partial attenuation was also thought to be necessary for reduction of shunting.[41,59] Progressive occlusion was attributed to scar formation after an inflammatory response to the silk suture material or formation and organization of a thrombus.[41,59] Unfortunately, implanted silk sutures are slowly broken down in a few years, causing recurrent portosystemic shunting through the originally closed CPSS in some cases.[64,86] Other suture materials may therefore be more suitable because they guarantee a more permanent ligation of a CPSS, such as polypropylene.[23,64] Although nonabsorbable, this material is inert and the least thrombogenic suture of all currently available materials.[87] If progressive attenuation of a CPSS depends mainly on fibrosis around the ligature, the fibrotic response and the ultimate degree of closure achieved with polypropylene, may be less than with silk ligatures. However, no significant reduction of vessel diameters was found 6 weeks after placement of silk ligatures around femoral veins.[86] Furthermore, no significant differences in occlusion were seen between CPSSs attenuated with different gauges of silk suture.[70] This suggests that progressive decrease of flow through a ligated CPSS may not be a result of a progressive decrease of shunt diameter by fibrosis. Probably a combination of factors, including reduction in portal vasculature resistance, is responsible for progressive functional shunt occlusion after partial attenuation.[59,70]

Failure of long-term recovery due to recurrent portosystemic shunting is also reported in dogs after complete shunt closure.[16,22,64,70] Recurrent shunting can occur through the original shunt after failure of the ligature or can be caused by a second single shunt or multiple shunts that become functional, probably induced by chronic portal hypertension.[22,64,70]

Alternative CPSS attenuating techniques

Because it was believed that only total occlusion would give a good long-term outcome, alternative techniques, such as perivascular ameroid constrictors, cellophane banding, intravascular thrombogenic coils and hydraulic occluders were introduced to achieve gradual closure of a CPSS, without acute portal hypertension.[16,32,62,65-69,82,88] Gradual occlusion may also reduce the risk of postligation seizures if this complication is related to sudden changes in the central nervous system, for example withdrawal of endogenous benzodiazepines.[66,85] However, not all studies confirm the advantage of techniques that close CPSSs gradually.[41,89]

Cellophane banding of CPSS

Breznock (1979) introduced the use of umbilical tape around an extrahepatic CPSS to cause progressive occlusion, possibly by an irritative, fibroplastic reaction. However, he doubted if a period of several weeks to achieve complete occlusion would be sufficient for development of a functional intrahepatic portal circulation, without leading to portal hypertension.[54] Another successful case of gradual occlusion using cellophane banding was reported in 1990.[90] In 1998 the technique was prospectively evaluated in 11 dogs with extrahepatic CPSSs and cellophane banding was considered to be an inexpensive, safe and relatively simple technique.[66] Cellophane bands produced complete occlusion in 10 dogs, with delayed closure in two of them. Unfortunately one dog developed postoperative seizures, but all dogs recovered without evidence of portal hypertension. It was concluded that 3 mm would be the maximum diameter of the cellophane band that can achieve complete closure. Cellophane banding with a diameter of 2.5 mm or less was advised, unless this would produce portal hypertension.[66] Also laparoscopically placed cellophane was used successfully in two dogs for extrahepatic shunt attenuation.[91]

Besides in extrahepatic shunts, cellophane can be used in banding right and left divisional intrahepatic CPSSs, provided that sufficient dissection of the shunt from surrounding liver tissue is possible.[82,92] However, most intrahepatic CPSSs are too wide to be attenuated to a diameter of 3 mm without acute portal hypertension. Further research was advised to evaluate if cellophane bands with larger diameters would still induce sufficient progressive closure.[82] Experiments in peripheral vessel occlusion with 5 mm diameter cellophane bands showed a significant reduction in diameter of the vein, but no complete occlusion.[86] However, partial attenuation of extrahepatic CPSSs to a diameter less than 3 mm with cellophane banding resulted in no better outcome than cellophane banding with a mean diameter of 5 mm. In fact, more evidence of recurrent portosystemic shunting was found in attenuated CPSS dogs, as their serum bile acid concentrations significantly increased over time in contrast to the dogs in which a cellophane band was applied without attenuating the shunt.[88]

Despite the gradual occlusion, persistent portosystemic shunting has been reported following cellophane banding, with an average of 26% in extrahepatic CPSSs. This is caused by persistent patency of the original shunt in dogs with bands between 3-6 mm and development of acquired shunts in dogs with bands < 3 mm.[66,78]

The use of ameroid constrictors

Another method for gradual CPSS occlusion is the ameroid constrictor. This implant consists of a ring of hygroscopic compressed casein clay, encased in a stainless steel or titanium ring. When exposed to fluid, the casein expands. Implanted around a CPSS, the expanding casein should gradually compress the vessel within its lumen.[65,93] In contrast to the early assumption that expansion of the material itself provided the shunt occlusion, later studies revealed that luminal area only decreased 22-32% in six weeks.[94,95] The most important factor responsible for shunt occlusion is probably the inflammatory reaction and thrombosis in the enclosed vessel that is induced by the casein material.[86,95,96] Early rapid expansion of the casein during the first two weeks after implantation was expected to be followed by two months of slow expansion. However, there is considerable and unpredictable variation in length of time before the vessel is maximally occluded.[65,95] Actually, the maximum occlusion can already be achieved within 7-9 days after implantation, which might be too short to allow adaptation of the portal vasculature to the increased flow and to prevent portal hypertension.[86,95] Petrolatum coating of the ameroid material was expected to delay occlusion, but *in vivo* experiments did not demonstrate a clinically relevant effect on the rate of closure.[94]

Compared to CPSS ligation of extrahepatic CPSSs, the placement of an ameroid constrictor decreases surgery time.[71,93] Because the ring is relatively large and heavy, care must be taken during placement and dissection to avoid kinking or collapse of the shunt due to rotation or displacement of the device.[65,66] After publications about the relatively simple placement of a constrictor around an extrahepatic CPSS, the technique was also used to occlude a left sided CPSS or the left branch of the portal vein in a dog with a left sided intrahepatic CPSS.[96,97] However, in some dogs the intrahepatic CPSS may be too large to allow placement of the largest constrictor available.[97]

Postoperative complications after ameroid constrictor placement are comparable with complications noted following ligation: generalized seizures, coagulopathy with abdominal bleeding, portal hypertension or sudden death without clinical signs or by an unknown cause.[18,65,71] A successful long-term outcome is reported in the majority of dogs with extrahepatic CPSSs treated with ameroid constrictors.[18,65] Mortality rates reported in dogs after using ameroid constrictors vary from 7 to 14%, which is not significantly different from CPSS ligation.[16,18,65,71] Although only 6% of the dogs were presented with long-term persistent clinical signs, in 17-21% of the dogs evidence of

persistent portosystemic shunting was found 6 to 10 weeks after surgery.[18,20] Nevertheless, the majority of owners of these dogs reported good to excellent outcome (81%).[18] A remarkable observation is that 4 of 18 dogs that could have been completely ligated at the time of the surgery did have persistent shunting after ameroid placement.[18] A likely explanation for persistent portosystemic shunting is that multiple portosystemic communications (acquired shunts) become functional as a result of subclinical portal hypertension when the ameroid constrictor occludes the shunt whereas the capacity of the portal vasculature to cope with the portal flow is not sufficient.[17,18,65] Also in the dogs with intrahepatic CPSSs, ameroid constrictor placement did not prevent recurrence of clinical problems and complications due to portosystemic shunting.

When ameroids and ligatures were compared with respect to attenuation of left divisional intrahepatic CPSSs, no differences were found in postoperative complications, but long-term clinical outcome was significantly better in dogs treated with partial ligation compared to dogs with ameroid constricted shunts. The ligation technique is more likely to result in a controlled attenuation, whereas closure of the shunt after using the ameroid constrictor is much less predictable.[97]

Portocaval venografts in intrahepatic CPSS surgery

During traditional intravascular closure procedures using ligatures in intrahepatic CPSSs, it is difficult to evaluate portal pressure. In human medicine, portal hypertension in cirrhosis has been controlled by surgical creation of an extrahepatic or intrahepatic portocaval shunt.[98] By creating an extrahepatic shunting venograft between the portal vein and the vena cava, portal pressure can be maintained at an acceptable level after complete closure of intrahepatic CPSSs, or when hepatic lobectomy is performed for treatment of an intrahepatic CPSS.[32,99-101] The switch of intrahepatic shunting to extrahepatic shunting also allows the use of gradual shunt occluding techniques in dogs with intrahepatic CPSSs that are difficult to dissect: the intrahepatic shunt is completely closed by ligation or resected by lobectomy and the portocaval venograft is, for example, closed with an ameroid constrictor.[17,100] However, attenuation of the venograft is not always necessary, and may even lead to a high incidence of acquired shunt formation, probably as a result of portal hypertension.[17,99] Although some dogs treated with a venograft to control portal pressure have a good outcome, severe complications with a high mortality rate may occur, also because of portal hypertension.[32,99-101]

Endovascular CPSS attenuation

An intravascular technique that attempts to achieve gradual intrahepatic CPSS occlusion is the placement of transvenous coils into the CPSS. The thrombogenic material of the coil that is introduced with a guide wire through an intravenous catheter under fluoroscopic control, will cause attenuation by progressive embolization inside the vessel over 1-2 months. A coil that is slightly larger than the shunt can be introduced from a jugular, femoral or saphenous vein, through the caudal vena cava into the shunt.[12,67,102,103] Successful cases of attenuated left and central divisional intrahepatic CPSSs have been described.[12,67,102-104] One dog with coils placed into an extrahepatic shunt was euthanized six months after partial occlusion because of hepatic insufficiency and cirrhosis.[103] Besides coil placement, also a percutaneously placed atrial septal occluder device was successfully used in one dog with an intrahepatic shunt where complete closure was deemed possible.[105] Advantages of these endovascular technique are a shorter anaesthesia time, a short hospitalization time and no necessity of an invasive surgical procedure. There is a risk of coil migration to the heart and lungs via the vena cava, but no clinical signs were seen after this complication.[102,103] Dislodgement of the coil can be avoided by placing an autoexpandable stent in the caval vein to hold migrating coils.[103] Unfortunately, sequential procedures are often needed to achieve sufficient attenuation in large diameter shunts and the shunt may still remain patent or become patent again afterwards.[67,86,102,103,105]

Hydraulic occluders

A relatively new device that is used for gradual CPSS closure is a percutaneously controlled hydraulic occluder. Hydraulic occluders consist of an inflatable silicone membrane within a polyester-reinforced, stretch-resistant cuff. The occluder was placed around the CPSS, fixated by sutures and connected to a subcutaneously implanted injection port.[68,106] The occluder was gradually inflated with sterile saline at 2, 4, 6, and 8 weeks after implantation. In a prospective study in 10 dogs with intrahepatic CPSSs with a mean follow up of 22 months, the occluder was placed around the portal vein branch leading to the shunt. The hydraulic occluder appeared a safe and effective technique to achieve gradual attenuation, but implant revision was needed in three dogs during the inflation period and one dog developed a fistulous tract associated with the injection port. Another disadvantage is the more technically demanding placement of the device. The potential advantage of this technique is the ability to make individual, non-invasive adjustments in CPSS attenuation after surgery. One year after surgery, postprandial bile acid concentrations were within reference ranges in 5 of 8 dogs.[68]

Liver transplantation
CPSS patients with atresia of the portal vein cannot be treated with the surgical techniques described above.[34] In dogs the owners will have to make a decision whether to euthanize the dog, or to treat the dog medically, as long as the dog clinically responds well. In human patients, CPSS in combination with portal atresia can be successfully treated by living donor auxilliary orthotopic liver transplantation. This technique involves a partial graft, while preserving part of the native liver. The CPSS is detached from the vena cava and reconstructed to become the portal supply of the graft.[11]

Alternatives to surgical treatment
Despite former negative recommendations,[4,56] medical management of dogs with a CPSS is still considered a realistic alternative for surgery in specific cases and may have a reasonable prognosis, especially in older dogs.[55] Medical treatment can be instituted when owners refuse surgery, when surgical treatment is not possible or in the period awaiting surgical treatment.[56] New studies are performed to optimise diet composition, for example protein source, which may improve long-term outcome in medically treated dogs with a CPSS in the future.[107]

Prognostic factors and prediction of surgical response

Prognosis after surgical CPSS attenuation can be divided in short-term and long-term prognosis. Several studies have identified factors associated with prognosis, but frequently results are controversial or are not confirmed by others. Postoperative metabolic recovery might be the most reliable predictor of long-term clinical performance.[70] To study metabolic recovery it is essential to assess the level of functional portosystemic shunting, for example by ammonia tolerance testing. Clinical recovery alone is not sufficient because a significant proportion of dogs with complete resolution of clinical signs and an excellent performance after surgery may have persistent shunting, without need for dietary restrictions or medications.[17,68,97] In contrast to clinical performance, calculated shunt fractions from trans-splenic portal scintigraphy may overestimate functional portosystemic shunting: a patent extrahepatic CPSS is not necessarily functional when it diverts mainly splenic venous blood, which contains no more toxins such as ammonia than other systemic veins.[108]

Clinical, biochemical and haematological variables
One study reported high packed cell volume (PCV) as a predictor of early postoperative mortality.[19] In another study, low PCV and low serum total protein concentration in dogs with intrahepatic shunts were associated with worse long-term

survival. Weight, total plasma protein and albumin concentrations, and blood urea nitrogen (BUN) were identified as prognostic indicators for short-term outcome.[75] Variables negatively associated with long-term outcome after ameroid placement included hypoalbuminaemia and leucocytosis.[18] Decreased total protein, albumin and PCV may be associated with hepatic insufficiency. Increased BUN as a negative prognostic indicator could be related to gastrointestinal congestion (mild portal hypertension) and bleeding (coagulopathy). Higher body weight may be associated with a better residual hepatic function, which may explain a more favourable short-term outcome.[75] Increased white blood cell count in dogs with CPSS may be associated with intestinal bacteria that enter the systemic circulation by the shunted portal blood and with impaired reticuloendothelial function, which markedly improves in many dogs within days following surgical CPSS attenuation.[109] The reason why preoperative leucocytosis is associated with long-term outcome is unknown.

After surgery, lower rectal temperatures were associated with a higher mortality. There was no statistical relation with the duration of surgery. However, the clinical significance of a lower postoperative rectal temperature was unknown and the association with mortality was not confirmed in other studies.[19,75] Finally, preoperative hepatic volume estimation was suggested as a potential prognostic marker, but data were preliminary and volumes were measured in a small number of CPSS dogs in which postoperative outcome was known.[110] Bile acid and ammonia concentrations are routinely used for diagnosis of liver dysfunction and CPSS, but plasma levels were not associated with prognosis.[69,75]

Type of shunt

Early prognosis following ligation of CPSSs was better in dogs with extrahepatic CPSSs compared with dogs with intrahepatic CPSSs.[2,37] This could be related with less surgical experience in attenuation of intrahepatic shunts.[37] Surgical attenuation of an intrahepatic CPSS is technically difficult if the shunt is completely surrounded by liver parenchyma or located near the intrahepatic caval vein, especially in right and central divisional CPSSs.[2,72] Thus, not surprisingly, the duration of the procedure was reported to be significantly longer in intrahepatic versus extrahepatic shunts.[19] Although Meyer (1999) reported no differences in long-term postoperative clinical performance between dogs with intra and extrahepatic shunts, the degree of shunt attenuation that could be achieved, was less in intrahepatic CPSSs.[41] This could be another reason for a less favourable outcome, since complete occlusion is likely to be associated with a better outcome.[69] Although left divisional intrahepatic CPSSs may be more easy to expose and often require less invasive surgical techniques,[32] there were no significant differences in survival or complication rates reported in 32 dogs with intrahepatic CPSSs between shunts from the left, central and right division.[75]

Degree of attenuation

Compared with complete closure of CPSSs, more postoperative complications were seen after partial shunt closure.[21] Partial ligation is also reported to be more often associated with persistence or recurrence of clinical signs than complete ligation.[16,58,69,70] The factors that cause or predict individual treatment response and long-term outcome after partial ligation are unknown.[32,58,59] Failure after partial attenuation is thought to be caused by insufficient attenuation of the original shunt or chronic portal hypertension resulting in the development of acquired portosystemic collaterals.[41,59,88] A persistent increased hepatic vascular resistance is suggested as an important factor for persistent shunting, but the underlying causes have not been clarified.[59] Also exaggerated attenuation of the shunts may lead to formation of acquired collaterals.[41,88]

Some findings suggest that the proportion of dogs with completely ligated CPSSs may be higher in dogs without preoperative signs of encephalopathy.[63,111] If dogs have a better prognosis after complete closure in comparison with partial attenuation, dogs without clinical signs of encephalopathy may have a more favourable prognosis. Also liver size before attenuation was greater in dogs tolerant of complete CPSS closure.[112] So, if prognosis is better in dogs after complete shunt occlusion, preoperative liver size may be a prognostic factor too. However, the correlation between degree of closure and short-term or long-term clinical outcome could not consistently be confirmed in other studies.[2,22,41,75]

Portal vasculature development

The existing hepatic portal vasculature, which can most accurately be visualized with intraoperative shunt occlusion portography, has been used to predict the degree of CPSS ligation that can be obtained and the prognosis.[111,113] More postoperative complications were seen in dogs without intrahepatic portal opacification on intraoperative mesenteric portograms compared to dogs with evidence of opacification.[21]

Long-term outcome appeared not to be correlated with arborising intrahepatic vasculature before attenuation in one study.[32] However, long-term clinical and metabolic improvement was significantly correlated with development of portal vasculature on pre- or postocclusion portograms in another study.[111] Animals that develop acquired shunts probably fail to develop an adequate intrahepatic portal system in order to reduce portal pressure after shunt attenuation.[64] Portosystemic shunt fraction before attenuation, calculated from scintigraphy, was not a reliable prognostic factor.[41,75] Unfortunately, there are currently no preoperative tests that predict whether the intrahepatic portal vasculature will expand after surgery.[64]

Immediately after extrahepatic CPSS ligation, portal flow assessed with intraoperative Doppler ultrasonography was shown to be predictive for surgical

outcome: when hepatofugal flow, caused by flow from the gastroduodenal vein, became hepatopetal immediately after shunt attenuation, outcome was excellent or good.[89] Although safe and effective in guiding CPSS ligation, intraoperative ultrasonography is not always available and this technique does not predict the outcome before surgery.

Hepatic histopathology

Liver morphology was not found to be predictive for long-term outcome after CPSS attenuation, but chronic hepatic fibrosis may lead to secondary portal hypertension and ascites.[3,55,78] Secondary hepatic fibrosis, caused by chronic persistent inflammation or hepatocellular necrosis, is poorly documented, but has been recognized in several cases.[3,18] Compared with normal livers, in livers with CPSS a mild to moderate increase in extracellular matrix or fibrosis is noted in centrilobular or portal areas, and this portal fibrosis may increase with age.[18,38] A complicating factor for postoperative recovery in dogs with CPSS may be a simultaneous occurrence of primary portal vein hypoplasia (PPVH). Histological changes of both congenital conditions are similar, but PPVH is often associated with more pronounced fibrosis.[5] The fibrosis in dogs with both diseases could explain more severe portal hypertension and a lower ability of the liver to grow after attenuation of the shunt.

Age

Lawrence (1992) reported a better prognosis after surgery in dogs less than 12 months of age, compared to dogs older than two years.[22] If so, dogs presented at an older age, may have a better prognosis with medical management.[55] However, several studies found no correlation of age with long-term outcome.[16,18,65,69,70,75]

In dogs with intrahepatic CPSSs, dogs with postoperative complications even were younger than dogs without complications.[75] Dogs with intrahepatic shunts may develop clinical signs at a younger age than dogs with extrahepatic shunts because larger fractions of blood may be shunted through the usually larger shunts in intrahepatic CPSSs.[39,75] Significantly older age at presentation for surgery has been reported in dogs with portoazygos shunts in comparison with portocaval shunts.[18,38] In one study with a large number of extrahepatic shunt dogs, age was not a risk factor.[18] However, portoazygos shunts could be more often closed completely than portocaval shunts, which may be associated with a better long-term outcome.[18] If anatomic localization of a CPSS is associated with age at surgery and surgical outcome, age may also appear to be prognostic.

Finally, Lee reported that older age was associated with a more developed intrahepatic vasculature in CPSS dogs, making older dogs more tolerant to CPSS closure, but also no clear correlation with outcome was reported.[111]

In conclusion:

Partial ligation, ameroid constrictors, cellophane bands, intravascular thrombogenic coils and hydraulic occluders have all been investigated as methods to achieve progressive CPSS attenuation. All surgical techniques have been successfully used in the majority of treated patients, but with every technique a significant number of dogs present with similar serious short-term complications and/or recurrent clinical portosystemic shunting after surgery.[18,66,82,97] Although most studies are difficult to compare because of differences in dog populations, techniques and study design, the general conclusion is that it is impossible to predict the outcome of treatment in individual patients. To date, the ideal technique does not exist and there are no preoperative clear-cut prognostic predictors.

References

1. Hickman J, Edwards JE, Mann FC. Venous anomalies in a dog; absence of the portal vein; continuity of lower part of inferior vena cava with the azygos vein. Anat Rec 1949;104:137-146.
2. Wolschrijn CF, Mahapokai W, Rothuizen J, Meyer HP, van Sluijs FJ. Gauged attenuation of congenital portosystemic shunts: results in 160 dogs and 15 cats. Vet Quart 2000;22:94-98.
3. Center SA, Magne ML. Historical, physical examination, and clinicopathologic features of portosystemic vascular anomalies in the dog and cat. Sem Vet Med Surg 1990;5:83-93.
4. Rothuizen J, van den Ingh TSGAM, Voorhout G, van der Luer RJT, Wouda W. Congenital porto-systemic shunts in sixteen dogs and three cats. J Small Anim Pract 1982;23:67-81.
5. Cullen JM, van den Ingh TSGAM, Bunch SE, Rothuizen J, Washabau RJ, Desmet VJ. Morphological classification of the circulatory disorders of the canine and feline liver. In: WSAVA Standards for clinical and histological diagnosis of canine and feline liver disease. Philadelphia, USA: Saunders Elsevier: 2006;41-59.
6. Hunt GB. Effect of breed on anatomy of portosystemic shunts resulting from congenital diseases in dogs and cats: a review of 242 cases. Aust Vet J 2004;82:746-749.
7. Kiriyama M, Takashima S, Sahara H, Kurosaka Y, Matsushita M, Akiyama T, Tomita F, Saito H, Kosaka T, Kita I, Kojima Y, Takegawa S. Case report: portal-systemic encephalopathy due to a congenital extrahepatic portosystemic shunt. J Gastroenterology Hepatology 1996;11:626-629.
8. Uchino T, Matsuda I, Endo F. The long-term prognosis of congenital portosystemic venous shunt. J Pediatr 1999;35:254-256.
9. Florio F, Nardella M, Balzano S, Giacobbe A, Perri F. Congenital intrahepatic portosystemic shunt. Cardiovasc Intervent Radiol 1998;21:421-424.
10. Akahoshi T, Nishizaki T, Wakasugi K, Mastuzaka T, Kume K, Yamamoto I, Sugimachi K. Portal-systemic encephalopathy due to a congenital extrahepatic portosystemic shunt: three cases and literature review. Hepato-gastroenterology 2000;47:1113-1116.
11. Soejima Y, Taguchi T, Ogita K, Taketomi A, Yoshizumi T, Uchiyama H, Ohno T, Shimada M, Maehara Y. Auxiliary partial orthotopic living donor liver transplantation for a child with congenital absence of the portal vein. Liver Transpl 2006;12:845-849.

12. Leveille R, Pibarot P, Soulez G, Wisner ER. Transvenous coil embolization of an extrahepatic portosystemic shunt in a dog: a naturally occurring model of portosystemic malformations in humans. Pediatr Radiol 2000;30:607-609.
13. Ivany JM, Anderson DE, Birchard SJ, Mattoon JR, Neubert BG. Portosystemic shunt in an alpaca cria. J Am Vet Med Assoc 2002;220:1696-1699.
14. Fortier LA, Fubini SL, Flanders JA, Divers TJ. The diagnosis and surgical correction of congenital portosystemic vascular anomalies in two calves and two foals. Vet Surg 1996;25:154-160.
15. Berent AC, Tobias KM. Portosystemic vascular anomalies. Vet Clin Small Anim 2009;39:513-541.
16. Winkler JT, Bohling MW, Tillson DM, Wright JC, Ballagas AJ. Portosystemic shunts: diagnosis, prognosis, and treatment of 64 cases (1993-2001). J Am Anim Hosp Assoc 2003;39:169-185.
17. Kyles AE, Gregory CR, Jackson J, Ilkiw JE, Pascoe PJ, Adin C, Samii VF, Herrgesell E. Evaluation of a portocaval venograft and ameroid ring for the occlusion of intrahepatic portocaval shunts in dogs. Vet Surg 2001;30:161-169.
18. Mehl ML, Kyles AE, Hardie EM, Kass PH, Adin CA, Flynn AK, De Cock HE, Gregory CR. Evaluation of ameroid ring constrictors for treatment for single extrahepatic portosystemic shunts in dogs: 168 cases (1995–2001). J Am Vet Med Assoc 2005;226:2020-2030.
19. Bostwick DR, Twedt DC. Intrahepatic and extrahepatic portal venous anomalies in dogs: 52 cases (1982-1992). J Am Vet Med Assoc 1995;206:1181-1185.
20. Johnson CA, Armstrong PJ, Hauptman JG. Congenital portosystemic shunts in dogs: 46 cases (1979-1986). J Am Vet Med Assoc 1987;191:1478-1483.
21. Swalec KM, Smeak DD. Partial versus complete attenuation of single portosystemic shunts. Vet Surg 1990;19:406-411.
22. Lawrence D, Bellah JR, Diaz R. Results of surgical management of portosystemic shunts in dogs: 20 cases (1985-1990). J Am Vet Med Assoc 1992;201:17.
23. Tobias KM. Portosystemic shunts and other hepatic vascular anomalies. In: Slatter D, ed. Textbook of small animal surgery. 3rd ed. Philadelphia, USA: Saunders Elsevier; 2003:727-752.
24. Payne JT, Martin RA, Constantinescu GM. The anatomy and embryology of portosystemic shunts in dogs and cats. Sem Vet Med Surg 1990;5:76-82.
25. Lamb CR, White RN. Morphology of congenital intrahepatic portacaval shunts in dogs and cats. Vet Rec 1998;142:55-60.
26. Meyer HP, Rothuizen J, Ubbink GJ, van den Ingh TSGAM. Increasing incidence of hereditary intrahepatic portosystemic shunts in Irish Wolfhounds in The Netherlands (1984 to 1992). Vet Rec 1995;136:13-16.
27. Krotschek U, Adin CA, Hunt GB, Kyles AE, Erb HN. Epidemiologic factors associated with the anatomic location of intrahepatic portosystemic shunts in dogs. Vet Surg 2007;36:31-36.
28. Tobias KM, Rohrbach BW. Association of breed with the diagnosis of congenital portosystemic shunts in dogs: 2,400 cases (1980-2002). J Am Vet Med Assoc 2003;223:1636-1639.
29. Tobias KM. Determination of inheritance of single congenital portosystemic shunts in Yorkshire terriers. J Am Anim Hosp Assoc 2003;39:385-389.
30. van Straten G, Leegwater PA, de Vries M, van den Brom WE, Rothuizen J. Inherited congenital extrahepatic portosystemic shunts in Cairn terriers. J Vet Intern Med 2005;19:321-324.
31. van Steenbeek FG, Leegwater PA, van Sluijs FJ, Heuven HC, Rothuizen J. Evidence of inheritance of intrahepatic portosystemic shunts in Irish wolfhounds. J Vet Intern Med 2009; 23:950-952.

32. White RN, Burton CA, McEvoy FJ. Surgical treatment of intrahepatic portosystemic shunts in 45 dogs. Vet Rec 1998;142:358-365.
33. Martin RA, Payne JT. Angiographic results of intrahepatic portocaval shunt attenuation. Sem Vet Med Surg 1990;5:134-141.
34. Hunt GB, Bellenger CR, Borg R, Youmans KR, Tisdall PL, Malik R. Congenital interruption of the portal vein and caudal vena cava in dogs: six case reports and a review of the literature. Vet Surg 1998;27:203-215.
35. Roskams T, Desmet VJ, Verslype C. Development, structure and function of the liver. In: Burt AD, Portmann BC, Ferrell LD eds. MacSween's pathology of the liver, 5th ed. Philadelphia, USA: Churchill Livingston Elsevier; 2007:1-73.
36. Hunt GB, Youmans KR, Sommerlad S, Swinney G, Nicholson A, Melville L, Hoffman KL, Allan GS. Surgical management of multiple congenital intrahepatic shunts in two dogs: case report. Vet Surg 1998;27:262-267.
37. Tisdall PLC, Hunt GB, Bellenger CR, Malik R. Congenital portosystemic shunts in Maltese and Australian Cattle Dogs. Aust Vet J 1994;71:174-178.
38. Baade S, Aupperle H, Grevel V, Schoon HA. Histopathological and immunohistochemical investigations of hepatic lesions associated with congenital portosystemic shunt in dogs. J Comp Pathol 2006;134:84-94.
39. Komtebedde J, Forsyth SF, Breznock EM, Koblik PD. Intrahepatic portosystemic venous anomaly in the dog, perioperative management and complications. Vet Surg 1991;20:37-42.
40. Ewing GO, Suter PF, Bailey CS. Hepatic insufficiency associated with congenital anomalies of the portal vein in dogs. J Am Anim Hosp Assoc 1974;10:463-476.
41. Meyer HP, Rothuizen J, van Sluijs FJ, Voorhout G, van den Brom WE. Progressive remission of portosystemic shunting in 23 dogs after partial closure of congenital portosystemic shunts. Vet Rec 1999;144:333-337.
42. Zaitoun AA, Path FRC, Apelqvist G, Al-Mardini H, Gray T, Bengtsson F, Record CO. Quantitative studies of liver atrophy after portacaval shunt in the rat. J Surg Res 2006;131:225-232.
43. Gerritzen-Bruning MJ, van den Ingh TSGAM, Rothuizen J. Diagnostic value of fasting plasma ammonia and bile acid concentrations in the identification of portosystemic shunting in dogs. J Vet Intern Med 2006;20:13-19.
44. Rothuizen J, van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22-25.
45. Morandi F, Cole RC, Tobias KM, Berry CR, Avenell J, Daniel GB. Use of 99mTCO4(-) trans-splenic portal scinitgraphy for diagnosis of portosystemic shunts in 28 dogs. Vet Radiol Ultrasound 2005;46:153-161.
46. Meyer HP, Rothuizen J, van den Brom WE, Voorhout G, van Sluijs FJ. Quantification of portosystemic shunting in dogs by ultrasound-guided injection of 99mTC-macroaggregates into a splenic vein. Res Vet Sci 1994;57:58-62.
47. Sura PA, Tobias KM, Morandi F, Daniel GB, Echandi RL. Comparison of 99mTcO4(-) trans-splenic portal scintigraphy with per-rectal portal scintigraphy for diagnosis of portosystemic shunts in dogs. Vet Surg 2007;36:654-660.
48. Bertolini G, Rolla EC, Zotti A, Caldin M. Three-dimensional multislice helical computed tomography techniques for canine extra-hepatic portosystemic shunt assessment. Vet Radiol Ultrasound 2006;47:439-443.
49. d'Anjou MA, Penninck D, Cornejo L, Pibarot P. Ultrasonographic diagnosis of portosystemic shunting in dogs and cats. Vet Radiol Ultrasound 2004;45:424-437.
50. Frank P, Mahaffey M, Egger C, Cornell KK. Helical computed tomographic portography in ten normal dogs and ten dogs with a portosystemic shunt. Vet Radiol Ultrasound 2003:44:392-400.

51. Holt DE, Schelling CG, Saunders HM, Orsher RJ. Correlation of ultrasonographic findings with surgical, portographic, and necropsy findings in dogs and cats with portosystemic shunts: 63 cases (1987-1993). J Am Vet Med Assoc 1995;207:1190-1193.
52. Szatmári V, Rothuizen J , van den Ingh TSGAM, van Sluijs FJ, Voorhout G. Ultrasonographic findings in dogs with hyperammonemia: 90 cases (2000–2002). J Am Vet Med Assoc 2004;224:717-727.
53. Zwingenberger A. CT Diagnosis of Portosystemic Shunts. Vet Clin North Am Small Anim Pract 2009;39:783-792.
54. Breznock EM. Surgical manipulation of portosystemic shunts in dogs. J Am Vet Med Assoc 1979;174:819-826.
55. Watson PJ, Herrtage ME. Medical management of congenital portosystemic shunts in 27 dogs – a retrospective study. J Small Anim Pract 1998;39:62-68.
56. Taboada J. Medical management of animals with portosystemic shunts. Sem Vet Med Surg 1990;5:107-119.
57. Breznock EM, Berger B, Pendray D, Wagner S, Manley P, Whiting P, Hornof W, West D. Surgical manipulation of intrahepatic shunts in dogs. J Am Vet Med Assoc 1983;182:798-805.
58. Komtebedde J, Koblik PD, Breznock EM, Harb M, Garrow LA. Long-term clinical outcome after partial ligation of single extrahepatic vascular anomalies in 20 dogs. Vet Surg 1995;24:379-383.
59. Van Vechten BJ, Komtebedde J, Koblik PD. Use of transcolonic portal scintigraphy to monitor blood flow and progressive postoperative attenuation of partially ligated single extrahepatic portosystemic shunts in dogs. J Am Vet Med Assoc 1994;204:1770-1773.
60. Smith KR, Bauer M, Monnet E. Portosystemic communications: follow-up of 32 cases. J Small Anim Pract 1995:36:435-440.
61. Mathews K, Gofton N. Congenital extrahepatic portosystemic shunt occlusion in the dog: gross observations during surgical correction. J Am Anim Hosp Assoc 1988;24:387-394.
62. Butler LM, Fossum TW, Boothe HW. Surgical management of extrahepatic portosystemic shunts in the dog and cat. Sem Vet Med Surg 1990;5:127-133.
63. Harvey J, Erb HN. Complete ligation of extrahepatic congenital portosystemic shunts in nonencephalopathic dogs. Vet Surg 1998;27:413-416.
64. Burton CA, White RN. Portovenogram findings in cases of elevated bile acid concentrations following correction of portosystemic shunts. J Small Anim Pract 2001;42:536-540.
65. Vogt JC, Krahwinkel DJ, Bright RM, Daniel GB, Toal RL, Rohrbach B. Gradual occlusion of extrahepatic portosystemic shunts in dogs and cats using the ameroid constrictor. Vet Surg 1996;25:495-502.
66. Youmans KR, Hunt GB. Cellophane banding for the gradual attenuation of single extrahepatic portosystemic shunts in eleven dogs. Aust Vet J 1998;76:531-537.
67. Partington BP, Partington CR, Biller DS, Toshach K. Transvenous coil embolization for treatment of a patent ductus venosus in a dog. J Am Vet Med Assoc 1993;202:281-284.
68. Adin CA, Sereda CW, Thompson MS, Wheeler JL, Archer LL. Outcome associated with use of a percutaneously controlled hydraulic occluder for treatment of dogs with intrahepatic portosystemic shunts. J Am Vet Med Assoc 2006;229:1749-1755.
69. Hottinger HA, Walshaw R, Hauptman JG. Long-term results of complete and partial ligation of congenital portosystemic shunts in dogs. Vet Surg 1995;24:331-336.
70. Hunt GB, Hughes J. Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 1999;77:303-307.
71. Hurn SD, Edwards GA. Perioperative outcomes after three different single extrahepatic portosystemic shunt attenuation techniques in dogs: partial ligation, complete ligation and ameroid constrictor placement. Aust Vet J 2003;81:666-670.

72. Tobias KM, Byarlay JM, Henry RW. A new dissection technique for approach to right-sided intrahepatic portosystemic shunts: anatomic study and use in three dogs. Vet Surg 2004;33:32-39.
73. Hunt GB, Bellenger CR, Pearson MR. Transportal approach for attenuating intrahepatic portosystemic shunts in dogs. Vet Surg 1996;25:300-308.
74. Wrigley RH, Macy DW, Wykes PM. Ligation of ductus venosus in a dog, using ultrasonographic guidance. J Am Vet Med Assoc 1983;183:1461-1464.
75. Papazoglou LG, Monnet E, Seim HB. Survival and prognostic indicators for dogs with intrahepatic portosystemic shunts: 32 cases (1990-2000). Vet Surg 2002;31:561-570.
76. Roy RG, Post GS, Waters DJ, Hardy RM. Portal vein thrombosis as a complication of portosystemic shunt ligation in two dogs. J Am Anim Hosp Assoc 1992;28:53-58.
77. Niles JD, Williams JM, Cripps PJ. Hemostatic profiles in 39 dogs with congenital portosystemic shunts. Vet Surg 2001;30:97-104.
78. Landon BP, Abraham LA, Charles JA. Use of transcolonic portal scintigraphy to evaluate efficacy of cellophane banding of congenital extrahepatic portosystemic shunts in 16 dogs. Aust Vet J 2008;86:169-179.
79. Matushek KJ, Bjorling D, Mathews K. Generalized motor seizures after portosystemic shunt ligation in dogs: five cases (1981-1988). J Am Vet Med Assoc 1990;196:2014-2017.
80. Tisdall PLC, Hunt GB, Youmans KR, Malik R. Neurological dysfunction in dogs following attenuation of congenital extrahepatic portosystemic shunts. J Small Anim Pract 2000;41:539-546.
81. Heldmann E, Holt DE, Brockman DJ, Brown DC, Perkowski SZ. Use of propofol to manage seizure activity after surgical treatment of portosystemic shunts. J Small Anim Pract 1999;40:590-594.
82. Connery NA, McAllister H, Skelly C, Pawson P, Bellenger CR. Cellophane banding of congenital intrahepatic portosystemic shunts in two Irish wolfhounds. J Small Anim Pract 2002;43:345-349.
83. Hardie EM, Kornegay JN, Cullen JM. Status epilepticus after ligation of portosystemic shunts. Vet Surg 1990;19:412-417.
84. Yool DA, Kirby BM. Neurological dysfunction in three dogs and one cat following attenuation of intrahepatic portosystemic shunts. J Small Anim Pract 2002;43:171-176.
85. Aronson LR, Gacad RC, Kaminsky-Russ K, Gregory CR, Mullen KD. Endogenous benzodiazepine activity in the peripheral and portal blood of dogs with congenital portosystemic shunts. Vet Surg 1997;26:189-194.
86. Youmans KR, Hunt GB. Experimental evaluation of four methods of progressive venous attenuation in dogs. Vet Surg 1999;28:38-47.
87. Roush JK. Biomaterials and surgical implants. In: Slatter D, ed. Textbook of small animal surgery. 3rd ed. Philadelphia, USA: Saunders Elsevier; 2003:141-148.
88. Frankel D, Seim H, MacPhail C, Monnet E. Evaluation of cellophane banding with and without intraoperative attenuation for treatment of congenital extrahepatic portosystemic shunts in dogs. J Am Vet Med Assoc 2006;228:1355-1360.
89. Szatmári V, van Sluijs FJ, Rothuizen J, Voorhout G. Ultrasonographic assessment of hemodynamic changes in the portal vein during surgical attenuation of congenital extrahepatic portosystemic shunts in dogs. J Am Vet Med Assoc 2004;224:395-402.
90. Harari J, Lincoln J, Alexander J, Miller J. Lateral thoracotomy and cellophane banding of a congenital portoazygous shunt in a dog. J Small Anim Pract 1999;31:571-573.
91. Miller JM, Fowler JD. Laparoscopic portosystemic shunt attenuation in two dogs. J Am Anim Hosp Assoc 2006;42:160-164.
92. Hunt GB, Tisdall PLC, Webb A, MacPherson GC, Brain P, Malik R. Congenital portosystemic shunts in toy and miniature poodles. Aust Vet J 2000;78:530-532.

93. Murphy ST, Ellison GW, Long M, Van Gilder J. A comparison of the ameroid constrictor versus ligation in the surgical management of single extrahepatic portosystemic shunts. J Am Anim Hosp Assoc 2001;37:390-396.
94. Adin CA, Gregory CR, Kyles AE, Griffey SM, Kendall L. Effect of petrolatum coating on the rate of occlusion of ameroid constrictors in the peritoneal cavity. Vet Surg 2004;33:11-16.
95. Besancon MF, Kyles AE, Griffey SM, Gregory CR. Evaluation of the characteristics of venous occlusion after placement of an ameroid constrictor in dogs. Vet Surg 2004;33:597-605.
96. Lidbetter DA, Krahwinkel DJ. Gradual occlusion of the left branch of the portal vein with an ameroid constrictor for treatment of an intrahepatic portosystemic shunt. Aust Vet Practit 2001;31:120-124.
97. Mehl ML, Kyles AE, Case JB, Kass PH, Zwingenberger A, Gregory CR. Surgical management of left-divisional intrahepatic portosystemic shunts: outcome after partial ligation of, or ameroid constrictor placement on, the left hepatic vein in twenty-eight dogs (1995-2005). Vet Surg 2007;36:21-30.
98. Wright AS, Rikkers LF. Current management of portal hypertension. J Gastrointest Surg 2005;9:992-1005.
99. White RN, Trower ND, McEvoy FJ, Garden OA, Boswood A. A method for controlling portal pressure after attenuation of intrahepatic portacaval shunts. Vet Surg 1995;25:407-413.
100. Gellasch KL, Patricelli AJ, Sicard GK, McAnulty JE. Use of portocaval venografts with ameroid constrictor placement and hepatic lobectomy for treatment of intralobular intrahepatic portocaval shunts in four dogs. J Am Vet Med Assoc 2003;222:455-460.
101. Kyles AE, Gregory CR, Adin CA. Re-evaluation of a portocaval venograft without an ameroid constrictor as a method for controlling portal hypertension after occlusion of intrahepatic portocaval shunts in dogs. Vet Surg 2004;33:691-698.
102. Gonzalo-Orden JM, Altonaga JR, Costilla S, Gonzalo-Cordero JM, Millan L, Recio AO. Transvenous coil embolization of an intrahepatic portosystemic shunt in a dog. Vet Radiol Ultrasound 2000;41:516-518.
103. Bussadori R, Bussadori C, Millán L, Costilla S, Rodríguez-Altónaga JA, Orden MA, Gonzalo-Orden JM. Transvenous coil embolisation for the treatment of single congenital portosystemic shunts in six dogs. Vet J. 2008;176:221-226.
104. Asano K, Watari T, Kuwabara M, Sasaki Y, Teshima K, Kato Y, Tanaka S. Successful treatment by percutaneous transvenous coil embolization in a small-breed dog with intrahepatic portosystemic shunt. J Vet Med Sci 2003;65:1269-1272.
105. Torisu S, Washizu M, Hasegawa D, Orima H. Brain magnetic resonance imaging characteristics in dogs and cats with congenital portosystemic shunts. Vet Radiol Ultrasound 2005;46:447-451.
106. Sereda CW, Adin CA. Methods of gradual vascular occlusion and their applications in treatment of congenital portosystemic shunts in dogs: a review. Vet Surg 2005;34:83-91.
107. Proot S, Biourge V, Teske E, Rothuizen J. Soy protein isolate versus meat-based low-protein diet for dogs with congenital portosystemic shunts. J Vet Intern Med 2009;23:794-800.
108. Szatmári V, Rothuizen J, van Sluijs FJ, van den Ingh TSGAM, Voorhout G. Ultrasonographic evaluation of partially attenuated congenital extrahepatic portosystemic shunts in 14 dogs. Vet Rec 2004;155:448-456.
109. Koblik PD, Hornof WJ. Technetium 99m sulfur colloid scintigraphy to evaluate reticuloendothelial system function in dogs with portasystemic shunts. J Vet Intern Med 1995;9:374-380.
110. Stieger SM, Zwingenberger A, Pollard RE, Kyles AE, Wisner ER. Hepatic volume estimation using quantitative computed tomography in dogs with portosystemic shunts. Vet Radiol ultrasound 2007;48:409-413.

111. Lee KC, Lipscomb VJ, Lamb CR, Gregory SP, Guitian J, Brockman DJ. Association of portovenographic findings with outcome in dogs receiving surgical treatment for single congenital portosystemic shunts: 45 cases (2000-2004). J Am Vet Med Assoc 2006;229:1122-1129.
112. Doran IP, Barr FJ, Hotston Moore A, Knowles TG, Holt PE. Liver size, bodyweight, and tolerance to acute complete occlusion of congenital extrahepatic portosystemic shunts in dogs. Vet Surg 2008;37:656-662.
113. Martin RA. Congenital portosystemic shunts in the dog and cat. Vet Clin N Am 1993;23:609-623.

Chapter 3

Prognostic Implications of the Degree of Shunt Narrowing and of the Portal Vein Diameter in Dogs with Congenital Portosystemic Shunts

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Abstract

Objective: To determine prognostic evaluation and correlation of the degree of narrowing and the diameter of the portal vein in dogs with a congenital portosystemic shunt (CPSS).
Study Design: Longitudinal prospective study.
Animals: Ninety-seven dogs with CPSS.
Methods: Shunt diameter was recorded before and after silk ligation to calculate degree of closure. Portal vein diameter was measured in 74 dogs. Short-term (30 days) and long-term (> 1 year) outcome were evaluated. Dogs with clinical signs after 1 year were re-examined to assess the degree of portosystemic shunting and compared with matched operated dogs without clinical signs. Correlations between clinical outcome, degree of closure, and portal vein diameter were statistically analyzed.
Results: Short-term and long-term mortality were 27% and 2.9% respectively. Clinical recurrence occurred in 10% of dogs. The degree of closure was significantly associated with mortality, but not with clinical recurrence. A significant correlation was found between degree of closure and the diameter of the cranial part of the portal vein. Portal vein diameter was only significantly associated with mortality in extrahepatic CPSS. Subclinical portosystemic shunting was confirmed in 3 of 10 dogs.
Conclusion: The degree of closure depended on portal development. Long-term outcome did not depend on the degree of closure or portal development at the time of surgery. This suggested that factors such as hepatic and portal regeneration after surgery may be important.
Clinical Relevance: Determination of factors that predict the outcome after surgical treatment of CPSS in dogs is important to gain insight in treatment selection or new therapeutic options.

Introduction

Congenital portosystemic shunts (CPSS) are vascular anomalies that divert blood from the portal vein to the caudal vena cava or to other systemic veins bypassing the liver parenchyma.[1] In patients with CPSS, hepatic portal perfusion depends on the volume of portal blood flowing through the shunt. If shunt flow is great, hepatic portal perfusion may be insufficient for normal function.[1] Treatment of dogs with CPSS preferably consists of complete surgical closure of the shunt.[2] However, because of hypoplasia or aplasia of the portal venous circulation cranial to the CPSS, complete closure can result in severe portal hypertension, acute shock, and death. When there is hypoplasia of the portal venous circulation cranial to the shunt, the CPSS is closed to the maximum degree of attenuation (partial ligation) that can be tolerated without fatal portal hypertension.[3-7] An alternative method is to use surgical techniques that achieve gradual progressive closure of the CPSS such as perivascular ameroid constrictors, cellophane banding, and intravascular thrombogenic coils.[8-15]

The prognosis after surgical intervention remains unpredictable and recurrence or persistence of clinical disease has been documented with all techniques used for shunt attenuation.[2,6,8-10,12,14-16] Poorly developed portal vasculature cranial to the CPSS and the degree of shunt closure have been reported to determine clinical outcome after partial shunt ligation. [2,6,17] However, not all previous reports have confirmed a relationship between degree of closure and outcome.[7,18] We hypothesized that clinical outcome was directly related to the degree of attenuation that can be achieved. Further, we hypothesized that clinical outcome and the degree of shunt attenuation that can be achieved are directly related to the degree of portal development. Accordingly, we prospectively evaluated short (30 days) and long-term (>1 year) outcome after shunt attenuation in 100 dogs.

Materials and Methods

Study Population

Between February 1996 and December 1999, 100 dogs were referred for surgical ligation of a single CPSS. Dogs had surgery consecutively by one surgeon (FJvS) using 2-0 silk ligatures, according to a technique reported by Wolschrijn et al.[7] After median celiotomy, the diameter of the shunt was estimated by placing stainless steel rods of known diameter, in increments of 0.25 mm, over the vessel. In 74 dogs, the diameter of the cranial part of the portal vein was estimated and in 24 dogs, the width of the caudal part of the portal vein was also estimated. The width of the cranial part of the portal vein was determined immediately caudal to the point where the vein enters the liver and the diameter of the caudal part was estimated directly caudal to the shunt

(extrahepatic CPSS) or directly caudal to the gastroduodenal vein if the shunt was located cranial to this vein (intrahepatic CPSS). The diameters of both parts of the portal vein were measured before ligation using the same technique described for shunt measurement.

Complete closure of the shunt was performed if during temporary (10 minutes) complete ligation of ten minutes no signs of portal hypertension were noted. Signs of portal hypertension were assessed according to documented criteria, using the color of the stomach, pancreas, and small intestine. Additional criteria included an increase in heart rate, a decrease in arterial blood pressure and end-expiratory CO_2%.[7] If the portal system could not adapt to complete closure, the shunt was partially closed to the maximum degree that was tolerated without marked signs of portal hypertension. Ligatures were tied over a rod of known diameter, which was recorded in the surgery report.

The medical records and surgery reports were reviewed for breed, type of shunt, shunt diameter before and after attenuation, diameter of the portal vein, recovery and follow-up time until the last examination 30 days after surgery. A standard questionnaire was developed to evaluate long-term follow-up (≥1 year). Special attention was given to occasionally or permanently occurring clinical signs that could be associated with portosystemic shunting. Questions were intended to reveal retarded growth or leanness, decreased mental awareness, abnormal behavior, apparent blindness, salivation, convulsions or other neurological abnormalities, urolithiasis, polyuria with polydipsia, or gastrointestinal signs such as vomiting or diarrhea. Use of medication and protein restricted diets as additive therapeutic management after surgery was recorded and other clinical events or treatments in the dog's history were noted. After 1 year, the owners of all dogs known to be alive 1 month after surgery were interviewed by telephone or, if this was not possible, contacted by mail to complete the questionnaire. After collection of data concerning clinical outcome at home, all owners that had mentioned possible signs of clinical recurrence and whose dogs were alive, were invited to return to the clinic to confirm and quantify portosystemic shunting.

Fasting plasma ammonia and plasma bile acids were routinely measured. In some dogs an ammonia tolerance test was performed to determine abnormalities in ammonia metabolism.[19] Abdominal ultrasonography was used to assess hepatic development, the site of the ligated shunting vessel, and to identify acquired collateral vessels caused by persistent portal hypertension. Elevated plasma ammonia levels (reference values, 24-45 μmol/L) or abnormalities in ammonia metabolism (abnormal ammonia tolerance testing) were considered to be evidence of portosystemic shunting. Plasma bile acids (reference values, 0-10 μmol/L) were also used as an indication of portosystemic shunting. In dogs with normal plasma ammonia and elevated bile acid levels, ammonia tolerance testing was performed to diagnose shunting.

If laboratory tests had evidence of shunting, definitive confirmation was established by portal scintigraphy using an ultrasound-guided injection of ^{99m}Tc-macroaggregates into a splenic vein.[20] This technique quantified the portosystemic collateral circulation and the fraction of portal blood bypassing the liver was expressed as the shunt index (SI). Clinical recurrence or persistence was defined as the presence of clinical signs with confirmation of portosystemic shunting by abnormal ammonia metabolism or positive SI.

After all dogs with clinical signs were rechecked, an equal number of dogs that had previous ligation of a CPSS, but without clinical signs, were invited to return to the clinic to check for subclinical portosystemic shunting (control group). Confirmation of shunting was performed as described for the suspected recurrence group. The dogs from the control group were selected by matching them to the dogs with clinical signs with respect to the type of the shunt and, if possible, breed, and length of follow-up. Portosystemic shunting was considered present in dogs that had abnormal ammonia metabolism or a positive SI. If portosystemic shunting was confirmed in dogs without clinical signs, these cases were defined as dogs with subclinical persistence. In our study all tests, including portal scintigraphy, were only performed with informed consent of the owner.

Data Analysis

Using the diameter of the shunt before and after ligation, the circumference of the shunt cross-section at the site of the ligation was calculated. The degree of closure was defined as the decrease in calculated circumference of the cross-section before and after attenuation, expressed as a percentage.

Development of the cranial part of the portal vein (referred to as portal development) was expressed in 3 ways. It was expressed as an absolute measure of the vein (the cross section of the cranial part of the portal vein). Secondly, portal development was related to the development of the shunt: it was expressed as the ratio between the calculated cross section of the portal vein and the cross section of the portosystemic shunt before closure. Lastly, in a limited number of dogs development of the cranial part of the portal vein was also related to the caudal part of the portal vein: portal development was expressed as the ratio between the cross sections of the cranial part and the caudal part of the portal vein.

Cox multiple regression was used to calculate the association between mortality and the type of the shunt, the degree of narrowing and the 3 measures for portal development described above. Multiple logistic regression was used to calculate the association between recurrence and the localization of the shunt, the degree of narrowing and the 3 measures for portal development. Spearman's rho nonparametric correlation coefficient was used to calculate the correlation between the degree of closure and the localization of the shunt and between the degree of closure and portal

development. A nonparametric correlation technique was chosen because degree of closure was not a normally distributed variable. All statistical analyses were performed for all dogs and for intrahepatic and extrahepatic CPSS separately, using a computer software program (SPSS for Windows, release 11.5.0). A *P*-value <.05 was considered significant.

Results

Of 100 dogs consecutively referred for surgery, 97 were included in this study; 3 dogs were not included because of incomplete data with respect to short-term and long-term outcome. In 1 dog, a miniature Schnauzer with a portoazygos shunt, data concerning long-term follow-up were missing and this patient was only included in the evaluation of surgery and short-term outcome. There were 58 male and 39 female dogs representing 29 different breeds and 3 mixed breed dogs. Commonly represented breeds were Yorkshire terrier (n = 13), Maltese (n = 11), Jack Russell terrier (n = 10), Cairn terrier (n = 9) and the Irish wolfhound (n = 7). Age at surgery ranged from 2 months to 7 years (median, 6.5 months).

Shunt types were: extrahepatic portocaval shunts (50 dogs), intrahepatic portocaval shunts (31 dogs) and portoazygos shunts (16 dogs). Intrahepatic shunts were located at the left hepatic division in 20 dogs and at the right hepatic division in 11 dogs. Shunt diameters before closure ranged from 3-22 millimeters (97 dogs; median, 6 mm). Portal vein width was recorded at the cranial part (median diameter, 4 mm) in 74 dogs; 51 dogs had an extrahepatic CPSS and 23 dogs an intrahepatic CPSS. In 24 of these dogs (16 extrahepatic, 8 intrahepatic CPSS), the diameter of the caudal part of the portal vein was recorded (median diameter, 4.75 mm, Table 1).

Shunt closure was complete in 16 dogs and partial in 74 dogs. Owners of seven dogs requested euthanasia during surgery, because the shunt could not be attenuated without signs of severe portal hypertension. In these dogs, aplasia or severe hypoplasia of the cranial part of the portal vein was noticed or hypoplasia of the intrahepatic portal circulation was suspected and a guarded prognosis was given. In the dogs with shunt ligation, the diameter of the ligature varied from 0 (complete closure) to 12.5 millimeters (median diameter, 2.25 mm). The mean degree of closure was similar in intrahepatic and extrahepatic CPSS (Table 2).

Table 1. Vessel diameters in dogs with attenuation of a single congenital portosystemic shunt

Variable	Number of dogs	Range	Median	Mean	Standard deviation
Diameter cranial portion PV (mm)	74	0 - 20.0	4.0	4.3	5.5
Diameter caudal portion PV (mm)	24	2.5 - 14.0	4.8	5.9	2.9
Diameter CPSS before attenuation (mm)	97	3.0 - 22.0	6.0	7.2	3.7
Diameter CPSS after attenuation (mm)	97	0.0 - 12.5	2.3	2.7	2.3
Degree closure (%)	97	0 - 100	87.2	80.0	24.6
Long-term follow-up period (years)	70	0.96 - 5.58	1.60	1.61	0.60

Abbreviations: PV, portal vein; CPSS, congenital portosystemic shunt
Degree of closure was defined as the decrease in calculated circumference of the shunt cross-section at the localization of the ligature before and after attenuation.

Table 2. Degree of narrowing and clinical outcome in dogs with attenuation of a single extrahepatic or intrahepatic congenital portosystemic shunt

Variable	Overall	Extrahepatic CPSS	Intrahepatic CPSS	P-value
Degree closure (mean) (%)	80.0 (n=97)	79.6 (n=66)	81.0 (n=31)	0.97
Mortality (%)	29 (n=28/97)	32 (n=21/66)	23 (n=7/31)	0.50
Clinical recurrence or persistence (%)	10 (n=7/70)	13 (n=6/46)	4.2 (n=1/24)	0.27

Abbreviations: CPSS, congenital portosystemic shunt; n, number of dogs
Degree of closure was defined as the decrease in calculated circumference of the shunt cross-section at the localization of the ligature before and after attenuation.

A significant correlation was found between the degree of closure and the cross section of the cranial part of the portal vein in dogs with extrahepatic CPSS (n=51, R=0.45, *P* =.001) and in dogs with intrahepatic CPSS (n=23, R=0.52, *P* =.011). Only in dogs with extrahepatic CPSS significant correlations were found between the degree of closure and the ratio between the cross sections of the cranial part and the shunt (n=51, R=0.54, *P* =.03) and the ratio between the cross sections of the cranial and the caudal part of the portal vein (n=16, R=0.32, *P* =.03). There was no significant correlation between the degree of closure and: 1) the type of shunt; 2) the ratio between the cross

sections of the cranial part of the portal vein and the shunt in dogs with intrahepatic CPSS; and 3) the ratio between the cross sections of the cranial part and the caudal part of the portal vein in dogs with intrahepatic CPSS.

Perioperative mortality (0-30 days) was 27% (26 of 97 dogs), including the 7 dogs euthanized during surgery because of severe hypoplasia of the cranial portal vein. Causes of death or euthanasia after surgery included hemorrhages from coagulopathy (7 dogs), severe postligation neurological dysfunction (6 dogs), and shock caused by portal hypertension (2 dogs). Coagulopathy was suspected in dogs with diffuse hemorrhage and in most dogs was confirmed by postoperative determination of coagulation time, fibrinogen concentration, and thrombocytes counts. Postligation neurological dysfunction consisted of progressive cerebral neurological signs (head pressing, circling, vocalizing, sopor, ataxia, tremor, anisocoria, or nystagmus), resulting in severe seizures or coma. These dogs did not improve with treatment. Portal hypertension was suspected in 2 dogs with hypovolemic shock and a painful distended abdomen, and was confirmed at necropsy.

One dog died from respiratory arrest and circulatory collapse during recovery from anesthesia and 1 dog was euthanized at home because of persistent vomiting and aspiration pneumonia. In 2 dogs the cause of death was unknown. Most dogs died or were euthanized within 5 days after surgery (17 dogs), 7 within the first 24 hours postoperatively. Necropsy was performed in 12 dogs. In the 3 intraoperatively euthanitized dogs that had a necropsy no abnormalities other than CPSS were identified. In 2 dogs with portal hypertension and 4 dogs with coagulopathy, necropsy results were consistent with the clinical diagnosis. In 2 dogs with postligation neurological dysfunction, cerebral vacuolization and laminar cortical necrosis was seen with activated endothelium and mild infiltration of macrophages. In 1 dog that died without known cause, necropsy revealed no cause of death. In the other dog that also died without known cause, no necropsy was performed at the owner's request.

During postoperative hospitalization, mild neurologic signs were seen in 13 dogs. These consisted of mild ataxia, sopor or inappropriate vocalization, and resolved completely within 30 days after surgery in all dogs. In another dog, neurologic signs were first observed at home, shortly after discharge, and responded well to phenobarbitone administration. Owners of dogs that were alive 30 days after surgery confirmed remission of clinical signs.

Long-term follow-up data were available for 70 dogs; owners of 64 dogs were interviewed by telephone and 6 owners were contacted by mail. At interview, 65 dogs were alive with follow-up ranging from 1-5.6 years (median, 1.6 years). For the 5 dogs not alive at interview, 3 had died or had been euthanized because of reasons unrelated to CPSS. Clinical signs that could be associated with portosystemic shunting were not observed before death. Two dogs were euthanized 3 and 4.5 years after surgery because of recurrence of severe clinical signs consistent with hepatic encephalopathy.

Unfortunately portosystemic shunting was not confirmed. Both dogs, a Yorkshire terrier and a West Highland white terrier, had an extrahepatic portosystemic shunt that had been attenuated for 91% and 100% respectively. Because their histories were very typical for hepatic encephalopathy, both cases were considered as clinical recurrence. Long-term mortality, therefore, was calculated to be 2.9% (2 of 70 dogs).

Table 3. Findings in 10 dogs with clinical signs of portosystemic shunting

No	Breed	Shunt	Clos. (%)	Follow-up (yr)	Signs (episodic)	NH_3 (µmol/l)	ATT	BA (µmol/l)	SI (%)	PSS
1	Leonberger	PAZ	88	3.18	sopor, tremor, vocalization	125	-	191	100	present
2	Yorkshire terrier	EPC	75	1.86	active incontinence, vomiting	178	-	84	100	present
3	Yorkshire terrier	EPC	84	1.13	apathia, vomiting, diarrhea	64	-	223	100	present
4	E.N. papillon	EPC	80	1.16	head-pressing, sopor, vomiting	206	abnormal	244	100	present
5	Hovawart	left IPC	88	0.98	mild apathia, medical management	42	abnormal	177	100	present
6	Miniature pinscher	PAZ	89	2.32	PUPD, Vomiting	19	normal	12	-	absent
7	Irish wolfhound	left IPC	96	2.38	diarrea, vomiting	13	-	7	-	absent
8	Yorkshire terrier	EPC	71	2.13	urolithiasis, apathia	28	normal	43	-	absent
9	Shetland sheepdog	PAZ	100	2.19	PUPD	19	normal	4	0	absent
10	C.K.C. spaniel	right IPC	69	1.17	ataxia, excitation	17	-	9	-	absent

Reference values NH_3: 24-45 µmol/L and BA: 0-10 µmol/L

Abbreviations: Clos., closure; yr, years; NH_3, fasting plasma ammonia concentration; ATT, ammonia tolerance test; BA, fasting plasma bile acid concentration; SI, shunt index; PSS, portosystemic shunting; E.N., Epagneul Nain; C.K.C., Cavalier King Charles; PAZ, portoazygos shunt; EPC, extrahepatic portocaval shunt; IPC, intrahepatic portocaval shunt; PUPD, polyuria/polydipsia

There was a significant association between degree of shunt closure and mortality in extrahepatic CPSS dogs (P <.001) as well as in intrahepatic CPSS dogs (P =.015). Although localization of the CPSS was not correlated with mortality (P =.50, Table 2), in extrahepatic CPSS, mortality was significantly associated with the cranial part of the portal vein (P =.048) and with the ratio between the cranial and caudal part of the portal vein (P =.015). No such association was found for intrahepatic shunts.

Of the dogs that were still alive, 11 owners reported clinical signs that could be related to portosystemic shunting (Table 3). None of the dogs without clinical signs were continued on medical or dietary management. In 11 dogs with possible clinical signs, 1 dog was treated with lactulose and protein restricted diet (dog 5), 1 dog was managed with only protein restricted diet (dog 9) and 1 dog, previously mentioned because of neurologic signs seen at home after surgery, was still treated with phenobarbitone (dog 11). In all dogs the CPSS had been attenuated partially except in dog 9). Two dogs (2, 4) had moderate signs, 2 dogs (5, 11) had no signs if treated, and the rest had only mild signs. Ten of the 11 dogs were rechecked in the clinic to confirm shunting (Table 3). In 5 dogs, portosystemic shunting could not be confirmed: plasma ammonia (range, 13-28 μmol/L) was within reference value range (24-45 μmol/l) in all 5 dogs. In 2 dogs, bile acid levels were increased to 12 and 43 μmol/L respectively (reference values, 0-10 μmol/L), but in both dogs an ammonia tolerance test had normal ammonia clearance. Ultrasonography did not reveal any portosystemic shunts in 4 dogs. A narrow portosystemic communication was still detectable in 1 dog, but was not considered to be functional because plasma ammonia and bile acid levels were within the reference range.

Portosystemic shunting was confirmed in the other 5 dogs with clinical signs (Table 3): fasting plasma ammonia levels were high in 4 dogs (range, 64-206 μmol/L). In 1 dog, fasting ammonia was only 42 μmol/L, but ammonia tolerance was abnormal. In all 5 dogs, bile acid levels were increased (84-244 μmol/L) and the SI was 100 percent. These dogs were considered to have clinical recurrence or persistence. Therefore, confirmed long-term clinical recurrence or persistence occurred in 7.7% of the surviving patients (5 of 65 dogs). Ultrasonography of these patients revealed a relatively small liver in 4 dogs and in 3 of these dogs, a single portosystemic communication was identified, which was considered to be the original congenital shunt. In 1 of these dogs, the location of the ligature itself was visible as a local narrowing of the diameter of the shunt vessel. In 2 dogs no portosystemic communication could be identified. No acquired shunts were found during ultrasonography in any of these 5 dogs.

If both dogs that were euthanized at home with severe signs of hepatic encephalopathy were also considered to have had clinical recurrence, long-term clinical recurrence was 10% (7 of 70 dogs). The average degree of shunt closure in these dogs was 87%. No significant association was found between CPSS type and clinical

recurrence (P =.27, Table 2), nor were any significant associations found between clinical recurrence and the degree of closure or portal development.

Unfortunately one owner that mentioned neurologic signs at the time of the interview (dog 11) did not want to return to the clinic for confirmation of shunting. The dog was a Yorkshire terrier that had seizures at a young age and an extrahepatic shunt was attenuated (89%) at 10 months of age. Neurologic signs persisted unchanged after surgery. Seizures completely resolved after oral phenobarbitone administration and no other clinical signs were observed. Primary epilepsy rather than portosystemic shunting was suspected to have caused neurologic problems and this dog was not classified as a recurrence.

In the group of 10 matched dogs without clinical signs, 7 dogs did not have evidence of portosystemic shunting as assessed by plasma ammonia, ammonia metabolism and bile acids (Table 4).

Table 4. Findings in 10 dogs without clinical signs of portosystemic shunting

Dog	Breed	Shunt	Closure (%)	Follow-up (yr)	NH_3 (µmol/l)	ATT	BA (µmol/l)	SI (%)	PSS
1	Bouvier	PAZ	100	3.54	7	normal	8	-	absent
2	Cairn terrier	EPC	100	2.51	< 7	-	1	-	absent
3	Cairn terrier	EPC	100	2.11	< 7	normal	1	-	absent
4	Maltese dog	EPC	88	1.71	15	normal	76	11	present
5	Hovawart	left IPC	83	0.96	80	abnormal	70	90	present
6	mixed breed	PAZ	80	2.30	16	abnormal	129	90	present
7	Irish wolfhound	left IPC	93	2.19	12	-	2	-	absent
8	Yorkshire terrier	EPC	75	1.10	8	normal	13	-	absent
9	J.R.terrier	PAZ	100	1.02	7	normal	3	-	absent
10	Bernese m.d.	right IPC	87	2.95	7	normal	4	-	absent

NOTE. Dogs were matched with the dogs in Table 3 with respect to type of shunt and listed in the same order.

Reference values NH_3: 24-45 µmol/L and BA: 0-10 µmol/L

Abbreviations: yr, years; NH_3, fasting plasma ammonia concentration; ATT, ammonia tolerance test; BA, fasting plasma bile acid concentration; SI, shunt index; PSS, portosystemic shunting; J.R., Jack Russell; m.d., mountain dog; PAZ, portoazygos shunt; EPC, extrahepatic portocaval shunt; IPC, intrahepatic portocaval shunt

In 2 dogs, abnormalities in ammonia metabolism were found, and in another dog only plasma bile acids were increased above reference values. Portal scintigraphy in these 3 dogs revealed high rates of shunting in both dogs with abnormal ammonia values (SI=90%) and a low rate of shunting in the dog with elevated bile acids (SI=11%). On ultrasonography, the liver was small in these dogs. In the first dog, the former portoazygos shunt was not identified but a second single extrahepatic portocaval shunt was found. In the second dog with a high SI, the original left intrahepatic shunt was still functional. In the third dog, with minor shunting and normal ammonia metabolism, flow was visible through the original shunt at the location of the ligature. No additional acquired shunts were identified. It was concluded that these 3 dogs had subclinical portosystemic shunting.

After completion of our study, 2 dogs with clinical recurrence were admitted for a second attempt to close a single extrahepatic portosystemic shunt surgically (Table 3; dog 3 and 4). In dog 3, the original CPSS had become functional because of suture failure caused by degeneration of the silk material. During the second surgery the shunt was partially ligated (94%) with 3-0 nylon. Postoperative recovery was good and fasting plasma ammonia, 1 month after surgery was normal with complete remission of clinical signs. Dog 4 also recovered completely after a second surgery, where a second single shunt was identified and was closed completely. Ammonia metabolism was still normal 7 months after the second surgery. The other 3 dogs with confirmed clinical recurrence have been treated conservatively with good clinical response.

Discussion

Perioperative mortality in this study (27%) was similar to another study from our clinic (29%)[7] and is high compared with other studies that have reported early mortality rates from 2.1-23%.[2-6, 21-23] On the other hand, long-term mortality (2.9%) was low in comparison with other studies that reported rates of 7% (3/41), 10% (3/29) and 16% (6/37) respectively. [2,4,5] One possible explanation for this difference was 7 dogs with a guarded prognosis were euthanized intraoperatively in our study. Surgical techniques and patient populations differ between studies, which may also have contributed to reported differences.

A significant association between degree of closure and mortality was found in both intra and extrahepatic shunts. Partial closure may account for a higher mortality or complication rate compared with complete closure,[17,24] because the latter category represents dogs with a well-developed cranial portal vein whereas the former group represents dogs with less well developed portal veins.[17]

Dogs with CPSS are usually classified into 2 groups after surgical ligation: patients with complete or partial ligation. Recurrence of clinical signs is reported to

occur more often after partial ligation.[6, 24] Hottinger (1995) advocated reoperation to attempt complete ligation for all dogs with patency of the shunt after partial CPSS ligation.[2] Others only perform a second procedure if there is overt recurrence of clinical signs.[5, 7] It is possible that the former approach may result in surgical intervention where it is not required whereas the latter approach will miss unclear recurrences. Both approaches would benefit from clear-cut criteria that predict clinical outcome.

In our study, clinical recurrence or persistence was seen in 10%, which is less than reported by Hottinger (41%) and Hunt (22%).[2, 6] No significant association was found between clinical recurrence or persistence and degree of closure, although the shunt was partially closed in 6 of 7 dogs with clinical recurrence. The only dog that had its shunt closed completely was 1 of 2 dogs that were euthanized before further examination was done. It seems logical that if the shunt remains or becomes functional after ligation, clinical signs of hepatic encephalopathy may persist or reoccur, depending on the amount of blood that is shunted (SI).[2,18] We attempted to provide an accurate description of degree of closure, because the diameter of the attenuated shunt significantly influences the residual flow. Flow (Q) is expressed universally by Ohm's law ($Q = \Delta P/R$), where ΔP is the pressure gradient and R is resistance. In a vessel with laminar flow, Q can be calculated by Poiseuille's law ($Q = c \times \Delta P \times r^4$), where r is the vessel radius and c is a factor depending on the length of the vessel and the viscosity of the blood.[25] According to these laws, vascular resistance of a shunt increases after partial ligation proportional to the fourth power of the difference in vessel radius ($R=1/[c \times r^4]$). This means that of all factors that determine residual flow through the shunt, the decrease in shunt diameter appears to be the most important. We therefore expected that our calculated degree of narrowing would correlate well with clinical recurrence or persistence, but to our surprise this was not so.

Immediate effects of shunt attenuation are a sudden rise in the portal pressure and a decrease in the central venous pressure (increased ΔP), especially in patients with a poorly developed portal vein.[17] However, the change in portal pressure is not always significantly correlated with survival.[3,4,17] In our study, mortality was significantly associated with portal development in dogs with extrahepatic CPSS, but not in dogs with intrahepatic CPSS. This may be explained by the difference in anatomy of the CPSS and the portal vein. In intrahepatic shunts, the cranial part of the portal vein and the shunt are actually the same vessel and both are generally very wide. However, extrahepatic shunts branch off from the portal vein before it reaches the hilus of the liver. In this case the size of the shunt and the portal vein are independent and a wide shunt may occur concurrently with a narrow portal vein.

Seemingly in extrahepatic shunts, the development of the cranial part of the portal vein may predict clinical outcome shortly after surgery (high short-term mortality), but not long-term clinical outcome because portal development at the time of surgery was not related to long-term persistence or recurrence. Residual flow

through the shunt and final clinical outcome may depend on the ability of the portal system and the liver to adapt to the pressure changes because of narrowing rather than the direct pressure changes themselves.[4] Expansion of the cranial part of the portal vein and hepatic regeneration reduces portal vascular resistance. After normalization of the pressure gradient, the residual flow through the shunt is theoretically decreased in proportion to the fourth power ($Q=c \times r^4$). Consequently, reduced portal vascular resistance by expansion of portal circulation may lead to reduction of portosystemic shunting postoperatively rather than perivascular fibrosis around the ligature, as is suggested in earlier publications.[6,26] In patients with sufficient expansion of the hepatic portal vasculature, complete ligation of the shunt may therefore not be necessary to accomplish complete functional closure of the shunt vessel.[5,7,16,18] In patients without expansion, chronic portal hypertension may result in a persistent functional congenital shunt or acquired multiple collaterals, which is a recognized complication after different techniques of closure. [5,6,9,12,27]

The development of the portal system before closure did not predict mortality or recurrence in dogs with an intrahepatic CPSS. The reason may be that the diameter of the cranial (extrahepatic) part of the portal vein is not a suitable variable for portal development in these dogs. An ideal variable would provide an accurate measure for portal prehepatic and intrahepatic resistance. However, in individual patients it is not always known where the bottleneck in the portal circulation is localized. Intrahepatic hypoplasia or aplasia of portal vessels remains unseen. Furthermore, measurement of vessel diameters before closure may show a poor correlation with resistance of vessels after closure because of differences in vessel elasticity or opening of collateral circulation. Despite its limitations, portal development was a good predictor for the degree of narrowing that could be achieved in extrahepatic CPSS. In our opinion, the diameter of the cranial part of the portal vein appeared to be the best measure of portal development that was available during surgery. Development of the cranial part of the portal vein may be an important factor that influences degree of narrowing and mortality in extrahepatic CPSS, but it was certainly not the only variable that predicted prognosis after surgery.

In our study, clinical outcome rather than residual flow was assessed postoperatively, and it was assumed that clinical outcome was inversely related to residual flow.[18] However, portosystemic shunting was confirmed in 3 dogs without clinical signs. These findings corroborate earlier reports of persistent portosystemic shunting in clinically normal patients.[4,12] Portal perfusion in these dogs is probably sufficient to maintain hepatic functions and to prevent clinical signs. Most owners of dogs with clinical signs described mild signs that had not precipitated consultation with their veterinarian. Confirming portosystemic shunting in dogs with mild or no signs demonstrated that determination of recurrence or persistence of shunting to assess outcome of treatment is difficult. The causes of the clinical signs in the dogs where

portosystemic shunting was not confirmed were not known, but their signs were also mild.

The frequency of persistence of portosystemic shunting in dogs without clinical signs after ligation of a CPSS cannot be predicted by extrapolation from the results of this study (3/10). One reason is that only a small number of dogs without clinical signs after shunt ligation were evaluated (only 10 dogs). Furthermore, the frequency (3/10) may be a false estimate because of the matching procedure that was used to select the dogs that were tested. By matching operated dogs without signs to patients with signs of recurrence or persistence, a group may have been selected with higher odds of subclinical persistence.

We have provided evidence that the degree of shunt narrowing that could be achieved was correlated with portal development. The degree of narrowing of a CPSS is significantly associated with mortality, but not with clinical recurrence. Apparently other factors are important to predict long-term outcome after shunt attenuation, such as hepatic regeneration or expansion of the portal vein and its branches, rather than the size of the cranial part of the portal vein or degree of closure at surgery. Future research directed at hepatic and portal expansion may yield valuable contributions to the treatment and the prognosis of CPSS.

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References

1. Rothuizen J, van den Ingh TSGAM, Voorhout G, van der Luer RJT, Wouda W: Congenital porto-systemic shunts in sixteen dogs and three cats. J Small Anim Pract 23:67-81, 1982
2. Hottinger HA, Walshaw R, Hauptman JG: Long-term results of complete and partial ligation of congenital portosystemic shunts in dogs. Vet Surg 24:331-336, 1995
3. Johnson CA, Armstrong PJ, Hauptman JG: Congenital portosystemic shunts in dogs: 46 cases (1979-1986). J Am Vet Med Assoc 191:1478-1483, 1987
4. Bostwick DR, Twedt DC: Intrahepatic and extrahepatic portal venous anomalies in dogs: 52 cases (1982-1992). J Am Vet Med Assoc 206:1181-1185, 1995
5. White RN, Burton CA, McEvoy FJ: Surgical treatment of intrahepatic portosystemic shunts in 45 dogs. Vet Rec 142:358-365, 1998
6. Hunt GB, Hughes J: Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 77:303-307, 1999
7. Wolschrijn CF, Mahapokai W, Rothuizen J, Meyer HP, van Sluijs FJ: Gauged attenuation of congenital portosystemic shunts: results in 160 dogs and 15 cats. Vet Quart 22:94-98, 2000

8. Partington BP, Partington CR, Biller DS, Toshach K: Transvenous coil embolization for treatment of a patent ductus venosus in a dog. J Am Vet Med Assoc 202:281-284, 1993
9. Vogt JC, Krahwinkel DJ, Bright RM, Daniel GB, Toal RL, Rohrbach B: Gradual occlusion of extrahepatic portosystemic shunts in dogs and cats using the ameroid constrictor. Vet Surg 25:495-502, 1996
10. Youmans KR, Hunt GB: Cellophane banding for the gradual attenuation of single extrahepatic portosystemic shunts in eleven dogs. Aust Vet J 76:531-537, 1998
11. Gonzalo-Orden JM, Altonaga JR, Costilla S, Gonzalo Cordero JM, Millan L, Recio AO: Transvenous coil embolization of an intrahepatic portosystemic shunt in a dog. Vet Radiol Ultrasound 41:516-518, 2000
12. Kyles AE, Gregory CR, Jackson J, Ilkiw JE, Pascoe PJ, Adin C, Samii VF, Herrgesell E: Evaluation of a portocaval venograft and ameroid ring for the occlusion of intrahepatic portocaval shunts in dogs. Vet Surg 30:161-169, 2001
13. Kyles AE, Hardie EM, Mehl M, Gregory CR: Evaluation of ameroid ring constrictors for the management of single extrahepatic portosystemic shunts in cats: 23 cases (1996-2001). J Am Vet Med Assoc 220:1341-1347, 2002
14. Havig M, Tobias KM: Outcome of ameroid constrictor occlusion of single congenital extrahepatic portosystemic shunts in cats: 12 cases (1993-2000). J Am Vet Med Assoc 220: 337-341, 2002
15. Connery NA, McAllister H, Skelly C, Pawson P, Bellenger CR: Cellophane banding of congenital intrahepatic portosystemic shunts in two Irish wolfhounds. J Small Anim Pract 43: 345-349, 2002
16. Murphy ST, Ellison GW, Long M, van Gilder J: A comparison of the ameroid constrictor versus ligation in the surgical management of single extrahepatic portosystemic shunts. J Am Anim Hosp Assoc 37: 390-396, 2001
17. Swalec KM, Smeak DD: Partial versus complete attenuation of single portosystemic shunts. Vet Surg 19:406-411, 1990
18. Meyer HP, Rothuizen J, van Sluijs FJ, Voorhout G, van den Brom WE: Progressive remission of portosystemic shunting in 23 dogs after partial closure of congenital portosystemic shunts. Vet Rec 144:333-337, 1999
19. Rothuizen J, van den Ingh TSGAM: Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 33:22-25, 1982
20. Meyer HP, Rothuizen J, van den Brom WE, Voorhout G, van Sluijs FJ: Quantification of portosystemic shunting in dogs by ultrasound-guided injection of 99mTC-macroaggregates into a splenic vein. Res Vet Sc 57:58-62, 1994
21. Mathews K, Gofton N: Congenital extrahepatic portosystemic shunt occlusion in the dog: gross observations during surgical correction. J Am Anim Hosp Assoc 24:387-394, 1988
22. Komtebedde J, Forsyth SF, Breznock EM, Koblik PD: Intrahepatic portosystemic venous anomaly in the dog, perioperative management and complications. Vet Surg 20:37-42, 1991
23. Harvey J, Erb HN: Complete ligation of extrahepatic congenital portosystemic shunts in nonencephalopathic dogs. Vet Surg 27:413-416, 1998
24. Smith KR, Bauer M, Monnet E: Portosystemic communications: follow-up of 32 cases. J Small Anim Pract 36:435-440, 1995
25. Guyton AC, Hall JE: Overview of the circulation; medical physics of pressure, flow, and resistance, in Guyton AC, Hall JE (eds): Textbook of Medical Physiology. Philadelphia, WB Saunders, 1996, pp 161-169
26. Youmans KR, Hunt GB: Experimental evaluation of four methods of progressive venous attenuation in dogs. Vet Surg 28:38-47, 1999
27. Burton CA, White RN: Portovenogram findings in cases of elevated bile acid concentrations following correction of portosystemic shunts. J Small Anim Pract 42:536-540, 2001

Chapter 4

Outcomes of Cellophane Banding for Congenital Portosystemic Shunts in 106 Dogs and 5 Cats

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Abstract

Objective: To report outcomes after cellophane banding of single congenital portosystemic shunts in dogs and cats.
Study Design: Retrospective study of sequential cases.
Animals: One hundred and six dogs and five cats.
Methods: Medical records were reviewed for breed, sex, age at surgery, shunt anatomy, results of pre- and postoperative biochemical analysis, development of postligation neurologic dysfunction, portal hypertension or other serious complications, and the owners' perception of their animal's response to surgery.
Results: Ninety-five dogs and all 5 cats had extrahepatic shunts. Eleven dogs had intrahepatic shunts. Six dogs (5.5%) died as a result of surgery, from portal hypertension (2 dogs), postligation neurologic dysfunction (2), splenic hemorrhage (1), and suspected narcotic overdose (1). Serious complications were more common in dogs with intrahepatic shunts than those with extrahepatic shunts (P=.002). Postligation neurologic dysfunction necessitated treatment in 10 dogs and 1 cat; 8 dogs and the cat survived. Clinical signs attributed to portosystemic shunting resolved or were substantially attenuated in all survivors. Postoperative serum bile acid concentrations or results of ammonia tolerance testing were available for 88 animals; 74 (84%) were normal and 14 (16%) were abnormal. Multiple acquired shunts were documented in two animals.
Conclusions: Cellophane banding is a safe and effective alternative to other methods of attenuation.
Clinical Relevance: Slow occlusion of portosystemic shunts using a variety of methods is being evaluated world wide. Cellophane banding is a relatively simple procedure with comparable safety and efficacy to previously reported techniques.

Introduction

A great deal of attention has been focused on methods of slowly occluding congenital portosystemic shunts (CPSS) in dogs and cats.[1-12] The rationale for slow attenuation is manifold, including reduced risk of life-threatening portal hypertension, speculation that slow occlusion may reduce the risk of post-ligation neurologic dysfunction, reduced operating time, less extensive intraoperative monitoring, and the fact that animals undergoing complete shunt occlusion have a better long-term prognosis than those undergoing partial attenuation only.[13,14] Two methods of slow occlusion using extravascular techniques have been reported: the ameroid constrictor (ameroid ring)[2-4,7-9,11,12] and cellophane bands.[1,5,10] Results of ameroid constrictor application have been encouraging, although a high incidence of multiple acquired shunts has been identified in published studies and anecdotally (RM Bright, personal communication, 2000).

Cellophane banding of a congenital portosystemic shunt in a dog was first described in 1990.[1] Rewarding results in a series of 11 dogs with congenital portosystemic shunts[5] led to cellophane banding being adopted as the procedure of choice for single extrahepatic shunts in dogs in Sydney, Australia. In time, cellophane banding was also used for CPSS in cats, and for attenuation of intrahepatic shunts where it was possible to dissect around the shunt, or the afferent branch of the portal vein.[10,15] We report outcomes after cellophane banding of extrahepatic and intrahepatic shunts in 160 dogs and 5 cats.

Materials and methods

Medical records from 106 dogs and 5 cats that had surgical attenuation, by cellophane banding, of single, congenital portosystemic shunts between March, 1993 and August, 2002 were reviewed. Ninety-four cases had surgery at the University Veterinary Centre, Sydney and the remainder were treated at two private practices in Sydney. Recorded details were: breed, sex, age at surgery, anatomy of the portosystemic shunt, results of pre- and postoperative biochemical analysis including ammonia tolerance testing (ATT), and pre and postprandial serum bile concentration (SBA), development of postoperative complications such as neurologic dysfunction or portal hypertension, and the owners' perception of the postoperative condition of their pet.

Anesthesia

Animals were anesthetized using a variety of techniques. Most were premedicated with phenobarbital (5-10 mg/kg subcutaneously) because of previous observations that perioperative phenobarbital administration may reduce the incidence and/or severity of postligation neurologic dysfunction.[16] General anesthesia was then induced by

administration of propofol (2–5 mg/kg intravenously [IV], dogs) or alphaxalone (1-2 mg/kg, IV) and maintained with inhaled isoflurane in a 1:2 combination of oxygen and nitrous oxide, supplemented by IV infusion or incremental doses of fentanyl, morphine, or methadone. Anesthetic monitoring included pulse oximetry, end-tidal isoflurane and carbon dioxide concentrations, non-invasive measurement of arterial blood pressure, electrocardiograms, and esophageal temperature. In all animals with intrahepatic shunts, catheters were inserted percutaneously for measurement of direct arterial pressure and central venous pressure. Packed cell volume, total plasma protein, and blood glucose concentrations were monitored at regular intervals.

Surgical procedure

All animals had a ventral median celiotomy that was extended to a median sternotomy in those with intrahepatic shunts. Surgical times ranged from 30-150 minutes, depending on the site of the shunt. In animals with extrahepatic shunts, surgical times were typically < 60 minutes. Portosystemic shunts were identified by abdominal exploration and classified as extrahepatic or intrahepatic. All extrahepatic shunts that entered the azygous vein were identified as portoazygos. Cellophane banding was performed in all animals with extrahepatic shunts, and in those with intrahepatic shunts where it was possible to dissect around either the shunt itself or a branch of the portal vein leading to the shunt. All animals with intrahepatic shunts and dogs weighing > 10 kg had a jejunal vein catheterized for measurement of portal venous pressure because application of a cellophane band with a recommended diameter of ≤ 3 mm[5,6] was considered likely to cause substantial and possibly dangerous shunt attenuation in these larger animals.

Cellophane bands were placed as reported previously.[5] Briefly, a strip of cellophane (10 cm long, 1.2 cm wide) was folded longitudinally to form a 3-layered strip (10 cm long, approximately 4 mm wide). The strip was passed around the shunt and tightened around both the shunt and a stainless steel pin of pre-determined size, using one or more titanium clips. In dogs < 10 kg, the size of the pin (and hence the diameter of the cellophane band) was determined by changes in heart rate, arterial pressure, intestinal color and motility, and pancreatic color when the shunt was totally occluded. Animals with minimal changes (elevation of heart rate < 10 beats/minute and reduction in arterial systolic pressure < 10 mm Hg) had a 2 mm diameter band applied, whereas those with moderate or severe changes had bands of 2.5 mm or 3 mm, respectively. In most cases, hemodynamic variables and intestinal color and motility were not substantially different to baseline once the band was applied. In dogs where portal pressure was monitored, a pin diameter was chosen that would constrict the shunt as much as possible without exceeding the maximum safe levels of attenuation described previously (a rise of portal pressure ≤ 10 cm H_2O to a final portal pressure of ≤ 20 cm H_2O).[18]

Postoperative care

Animals were monitored intensively for 24 hours after surgery, then observed for another 48 hours for signs of postligation neurologic dysfunction or portal hypertension. Phenobarbital (2-5 mg/kg every 12 hours) was administered to most animals for 2 weeks to reduce the incidence or severity of postligation neurologic dysfunction.[16] Many dogs became ataxic as a result of phenobarbital administration. Ataxia was not considered to be a manifestation of postligation neurologic dysfunction if the animal was otherwise normal and ataxia improved with reduction of the phenobarbital dose.

All animals requiring anticonvulsant therapy were administered IV phenobarbital (30 mg/kg during the first 24 hours, then 5 mg/kg every 12 hours), with or without incremental doses of midazolam and acepromazine.[16] In 6 animals, IV propofol infusion was also used to control neurologic signs.[18]

Animals were encouraged to eat on the first postoperative day; usually a combination of chicken and rice or a commercial restricted-protein diet (Hills Canine L/D, Hills, Topeka, KS). Lactulose or antibiotics were administered at the discretion of the surgeon, however, in most animals, these medications were not continued after surgery. Animals were maintained on a restricted-protein diet for at least a month. Owners were then instructed to increase the protein content by adding variable proportions of meat or commercial food with a normal protein concentration. Most animals were receiving a normal diet by 8 weeks after surgery, however, some clients chose to continue protein restriction until liver function tests were performed.

Evaluation of hepatic function after surgery

Owners were asked to return their animals for biochemical evaluation of hepatic function at least 8 weeks after cellophane banding wherever possible. Rectal ammonia tolerance testing (ATT) was performed by preference, especially in terrier-type dogs where serum bile acid (SBA) determination using a routine enzymatic test can be misleading.[19] Portosystemic shunting was considered to have resolved if ATT was normal (< 100 μmol/L after ammonia challenge) or postprandial SBA concentration was < 40 μmol/L.[19] If ammonia intolerance, or elevated SBA concentrations were encountered, results of serum biochemistry (activity of alanine aminotransferase and alkaline phosphatase, concentrations of blood urea nitrogen, cholesterol, and albumin) were compared with preoperative values as an indirect means of assessing improvement in liver function. In Maltese dogs, whose owners were unwilling to return for ATT, the results of pre and postoperative biochemical panels were compared to provide an indication of changes in hepatic function.

Owners who were unable or unwilling to return their pet for re-evaluation were surveyed as to whether they considered their pet's clinical signs to have resolved,

whether it was eating a normal diet, and whether they considered it to be otherwise healthy.

Statistical analysis

Fishers Exact test was used to compare mortality, perioperative complication rates, incidence of postligation neurologic dysfunction, results of biochemical testing between different types of shunt, and incidence of postligation neurologic dysfunction in different breeds. Ages of animals that developed postligation neurologic dysfunction or failure to recover normal hepatic function were compared with the rest of the population using the Mann-Whitney U Test. *P* values <.05 were considered significant.

Results

Cellophane banding was performed in 106 dogs and 5 cats. Breed distribution was Maltese (38; 36%), Cross bred (16; 15%), Jack Russell terriers (8; 7.6%), Miniature toy poodles (6; 5%), Silky terriers (5; 4%), Shih tzus (5; 4%), Bichon frise (4; 3%), Miniature schnauzers (4; 3%), and other individual breeds of dogs and cats. Fifty-five dogs and 2 cats were female and 51 dogs and 3 cats were male. Reported clinical signs were consistent with those previously reported.[1-16]

There were 100 extrahepatic shunts and 11 intrahepatic shunts. All 5 cats had extrahepatic shunts. Fifteen dogs had portoazygos shunts (15% of extrahepatic shunts). Thirty animals had shunts from the left gastric vein to the caudal vena cava, and 12 had shunts from the gastroduodenal vein to the caudal vena cava. In 31 animals, shunts were portocaval. In 5 animals, shunts arose from the splenic or gastrosplenic vessels. The vessel of origin of extrahepatic shunts entering the portal vein was not specifically mentioned in 2 animals.

Four dogs had right-divisional intrahepatic shunts and 6 had left-divisional shunts. The exact site of the intrahepatic shunt was not recorded in one animal. One dog had a complex shunt with an abnormal vessel arising from the left gastric vein and joining a patent ductus venosus at the termination of the left branch of the portal vein.

Cellophane band diameter was recorded in 105 cases and ranged from 2-6 mm, with most (99; 94%) being ≤ 3 mm diameter. No major intraoperative complications were reported.

Mortality rate

Six dogs (5.5%) died between 4 hours and 4 weeks postoperatively as a result of complications arising from anesthesia or surgery (Table 1). Two dogs (1.8%) died from portal hypertension and 2 from postligation neurologic dysfunction. Only 3 of 100 dogs (3%) with extrahepatic shunts died compared to 3 of 11 (27%) of dogs with intrahepatic shunts (*P*=.01). None of the cats died.

Postligation Neurologic Dysfunction

Postligation neurologic dysfunction occurred in 11 animals (10%; 10 dogs and 1 cat; Table 2), some of which were previously reported.[16] Initial signs were observed 4-72 hours after surgery (mean, 36 hours). The incidence of postligation neurologic dysfunction was not significantly different for animals with extrahepatic shunts (10/100; 10%) versus intrahepatic shunts (1/11; 9%, *P*=1). Age at surgery for the dogs and cat that had neurologic dysfunction ranged from 5-74 months (mean, 24.7 months), which was not significantly different to the rest of the population (mean, 22.1 months; range 2-96 months, *P*=.77).

No obvious predisposing factors for postligation neurologic dysfunction were identified, although 3 (38%) of the 8 Jack Russell terriers were affected. This breed was significantly over-represented for postligation neurologic dysfunction when compared to Maltese (2/36; 5.5%, *P*=.03) and all other breeds (5/62; 8%, *P*=.04). The cat that had postligation neurologic dysfunction was a Scottish Fold.

Nine of 11 animals (82%) that had postligation neurologic dysfunction survived, including all 5 animals administered phenobarbital, and 4 of 6 dogs administered phenobarbital and a propofol infusion. One dog had a cardiorespiratory arrest while being treated for status epilepticus and another dog was euthanized 4 weeks after surgery because of severe, residual neurologic deficits. Eight of 9 survivors had normal liver function at least 2 months after surgery based on rectal ATT. Two dogs remain on oral phenobarbital to control signs associated with partial motor seizures.

Postoperative liver function

Postoperative evaluation of liver function was performed at least 8 weeks after surgery (range, 2-6 months; median, 2.25 months) using rectal ATT in 27 Maltese, 42 non-Maltese dogs and 3 cats, and by determination of postprandial SBA concentrations in 14 non-Maltese dogs and 2 cats. Results indicated normal liver function in 74 of the 88 animals (71/83 dogs, 85%; 3/5 cats, 60%). Results indicated residual abnormalities in 12 dogs (15%) and 2 cats (40%) ranging from mild (post challenge serum ammonia 105 μmol/L) to severe (serum ammonia 800 μmol/L). In all cases, however, the owners reported that clinical signs had resolved or been substantially attenuated. In three animals, the owners reported that the dog seemed livelier when dietary protein

concentration was reduced, however, in none of these cases were clinical signs as severe as before surgery (Table 3).

The causes of persistent elevation in liver function tests were not determined in most animals, but may have been because of persistent shunting for reasons that were not characterized. One dog had an episode of ascites 10 days after surgery, presumably because of portal hypertension. Multiple acquired shunts were identified at repeat celiotomy in a cattle dog after application of a 4 mm cellophane band to an intrahepatic shunt. Repeat celiotomy was performed in both cats with ammonia intolerance after surgery; multiple acquired shunts were seen in one of these cats. In the other, the original shunt had failed to close. Follow up ATT and postprandial SBA determination 2 months after surgery indicated persistent hepatic dysfunction.

Six dogs where results of ATT or SBA were not available had broad-based biochemical panels performed. Five had complete resolution of preoperative abnormalities such as elevation of liver enzymes, or low concentrations of urea, cholesterol, and albumin.

In the remaining 11 dogs, no objective assessment of liver function was performed. However, follow up reports from owners between 2 months and 6 years after surgery indicated resolution of clinical signs.

Results of Cellophane Banding for extrahepatic versus intrahepatic shunts

The perioperative and early postoperative complication rate for cellophane banding of extrahepatic shunts was relatively low (13/100; 13%). Only 3 (3%) animals died. In contrast, the perioperative and early postoperative complication rate for intrahepatic shunts was significantly higher (6/11; 55%, P=.002), with 3 animals dying (27%, P=.008). One additional dog had postligation neurologic dysfunction and 2 had symptomatic but non life-threatening portal hypertension within 10 days of cellophane banding of intrahepatic shunts.

Postoperative evaluation of hepatic function was performed in 7 of 8 dogs that survived cellophane banding of intrahepatic shunts. Hepatic function was normal in 5/7 dogs (71%). By comparison, hepatic function tests were normal in 66/76 (87%) dogs with extrahepatic shunts. Hence, survival with resolution of biochemical abnormalities occurred in only 5 (50%) of 10 dogs with intrahepatic shunts compared to 66 (84%) of 79 dogs with extrahepatic shunts (P=.03).

Table 1. Cause of death in 6 dogs after cellophane banding of portocaval shunts

Breed	Age (months)	Sex	Shunt type	Cause of death
Border collie	18	F	LDIH	Portal hypertension 3 days after banding left branch of portal vein
Bichon frise	5	F	L Gastric	Portal hypertension 2 days after surgery
Miniature poodle	28	F	Portoazygos	Cardiorespiratory arrest during treatment of seizures
Maltese dog	33	M	L Gastric	Euthanized 4 weeks after surgery due to severe residual neurological deficits
Old English sheepdog	4	M	LDIH	Hemorrhage secondary to splenic rupture 2 days after surgery
Miniature poodle	84	M	RDIH	Hypothermia and respiratory arrest 4 h after surgery. Suspected narcotic overdose

Abbreviations: M, male; F, female; L, left; LDIH, left-divisional intrahepatic; RDIH, right-divisional intrahepatic

Table 2. Details of 10 dogs and 1 cat requiring treatment for postligation neurologic dysfunction

Breed	Age (months)	Sex	Hours postoperative	Outcome
Jack Russell terrier	12	M	60	Recovered fully
Jack Russell terrier	16	F	30	Recovered fully
Jack Russell terrier	7	F	18	Recovered fully
Maltese dog	74	M	48	Recovered fully
Maltese dog	33	M	36	Euthanized 4 weeks after surgery due to severe residual neurological deficits
Australian silky terrier	10	M	72	Partial seizures
Terrier cross breed	6	F	24	Recovered fully
Pug	40	F	40	Minor deficits, partial seizures
Miniature poodle	28	F	24	Cardiorespiratory arrest during treatment
Rhodesian ridgeback	8	M	40	Recovered fully
Scottish fold cat	9	M	4	Recovered fully

Abbreviations: M, male; F, female

Table 3. Details of 12 dogs and 2 cats with evidence of persistent hepatic dysfunction after cellophane banding

Breed	Age (months)	Sex	Shunt type	Band (mm)	ATT (μmol/L)	SBA (μmol/L)	Further information
Bulldog	12	F	RDIH	6	500		Ascites 10 d after surgery – suspected PH
ACD	24	F	LDIH	4	180		MAS at follow-up surgery
Pyrenean mountain dog	30	M	LDIH	6	800		NA
Siberian husky	7	M	IH	NA		105	NA
Jack Russell terrier	11	M	ileocolic	3		506	NA
Jack Russell terrier	12	M	gastroduodenal	3		97	NA
Maltese dog	17	M	L gastric	2.5	143		NA
Maltese dog	36	F	L gastric	2	299		NA
Maltese cross breed	12	F	gastroduodenal	2.5	500		NA
Maltese dog	12	M	L gastric	2.5	105		NA
Bichon frise	84	F	gastroduodenal	2.5	555		NA
Pekingese	34	M	portoazygos	3	123		NA
Himalayan	6	F	portocaval	3	337		Failure of shunt occlusion
DSH	6	M	portocaval	2.5	280		MAS at follow-up surgery

Abbreviations: ACD, Australian cattle dog; ATT, ammonia tolerance test; DSH, Domestic shorthair cat; F, Female, IH, intrahepatic; LDIH, left-divisional intrahepatic shunt; M, Male; MAS, multiple acquired shunts; NA, not available; PH, portal hypertension; RDIH, right-divisional intrahepatic shunt; SBA, postprandial serum bile acids

Result of Cellophane Banding in cats

All 5 cats survived cellophane banding. One cat developed mild neurologic dysfunction (twitching) that resolved within 7 days after surgery. Liver function normalized after surgery in three cats, whereas ammonia intolerance persisted in the other two cats. At repeat celiotomy, multiple acquired shunts were observed in one cat and failure of the cellophane band to produce fibrosis was observed in the other cat. Interestingly, the cat in which cellophane failed to promote shunt closure developed multiple acquired shunts after further attenuation using a silk ligature. The clinical condition of both cats improved substantially as a result of cellophane banding, even though portosystemic shunting persisted. Hence. although the clinical result was good to excellent in all cats, the rate of resolution of hepatic dysfunction was only 66%.

Discussion

Our results indicate that cellophane banding is an effective method of alleviating hepatic dysfunction resulting from a congenital portosystemic shunt. Mortality and morbidity rates compare favorably with reports where attenuation was achieved by use of silk ligatures or ameroid constrictors.[1-4,7-14] In particular, the incidence of life-threatening portal hypertension was very low, despite the fact that placement of cellophane bands produced substantial shunt occlusion. Three large breed dogs had signs compatible with portal hypertension within 10 days of surgery, however all responded well to symptomatic and supportive therapy. In an experimental study in dogs, cellophane banding produced up to 3 mm of occlusion in the first 6 weeks after placement around femoral veins.[6] For this reason, bands of 3 mm diameter or less were applied where possible in our study. However, wider bands can cause eventual shunt occlusion as reported in the present series and a previous report.[10] Further work is required to establish the maximum diameter of cellophane band that will produce reliable shunt occlusion.

Complete resolution of hepatic dysfunction resulting from portosystemic shunting can be expected in most animals after placement of cellophane bands. This conclusion is based on the results of postoperative liver function testing, an objective assessment of the efficacy of surgery and a sensitive predictor of long-term outcome.[14] It is hard to draw conclusions in animals where follow-up was based on reports from owners, as these may be misleading and dependent on the duration of follow-up and whether animals are still being fed a reduced protein diet and symptomatic treatment such as lactulose and antibiotics.

Some animals had continued biochemical evidence of hepatic dysfunction despite presumptive closure of the original shunt. This finding is similar to reported results of ameroid constrictor placement.[2-4,7-9,11,12,22] It is possible that in some animals

continued biochemical evidence of hepatic dysfunction resulted from persistent flow through the original shunt (as we documented in 1 cat). In other animals, it is likely that persistent shunting results from development of multiple acquired shunts, however, this was confirmed in only two animals. In some animals, other liver disorders such as microvascular dysplasia may contribute to apparent failure of surgery, even though the macroscopic abnormality was addressed. Liver biopsies were not collected routinely from animals in our study and hence this cannot be confirmed. The presence of a second, unidentified shunt must also be considered, but seems unlikely in view of the fact that hemodynamic perturbations were seen in all animals when the original shunt was occluded intraoperatively, and their clinical condition improved substantially as a result of surgery.

The incidence of postligation neurologic dysfunction we report was identical to that reported after portosystemic shunt ligation using silk,[14] suggesting that rapidity of attenuation is not major risk factor. Postligation neurologic dysfunction was no more common in animals with extrahepatic than those with intrahepatic shunts which also corroborates previous reports.[10,20] Although administration of phenobarbital does not reduce the incidence of postligation neurologic dysfunction, it does seem to reduce the severity of signs,[16] and this may explain why the survival rate in our study was higher than in some previous reports.[18,20] The apparent predisposition of Jack Russell terriers to postligation neurologic dysfunction is interesting, however, the sample size was small and this observation should not be over-interpreted.

The number of cats in this study was also small, making it difficult to draw firm conclusions about the success of cellophane banding in cats, although their behavior after shunt attenuation was consistent with other reports.[11,12,21] Two recent studies of ameroid constrictor placement in cats reported scintigraphic evidence of persistent shunting in 8/14 (57%) and 3/7 (43%) cases, respectively.[11,12] Failure of the cellophane band to promote any shunt attenuation in one of our cats was disturbing and may indicate a species difference in response to cellophane banding. Further evaluation of cats and dogs with postoperative shunting is required to determine how many have failure of occlusion of the original vessel, development of multiple acquired shunts, or both conditions.[23]

Biochemical analysis was chosen as the follow-up procedure of choice in our study for various reasons. Previously, we reported that persistent biochemical evidence of liver dysfunction was a sensitive predictor of clinical relapse in dogs undergoing partial shunt attenuation using silk.[14] Portal scintigraphy was not available at our clinic. Unfortunately, the predisposition of Maltese to congenital portosystemic shunts, the difficulty of interpreting results of bile acid determination in this breed,[19] and the restricted availability of facilities for determining blood ammonia levels in regional centres impaired our ability to adequately evaluate some cases postoperatively. Nevertheless, readers should note that liver function tests such as ATT and SBA

determination are only reliable when they are performed correctly and these tests may not detect small amounts of portosystemic shunting. Finally, animals with portosystemic shunts do not always have elevated fasting and/or postprandial ammonia levels.[24]

The reason why some animals develop multiple acquired shunts after total shunt occlusion remains unclear. No obvious risk factors have been identified. It is tempting to implicate pre-existing portal hypoplasia, however, in our experience many animals with macroscopically narrow portal veins make complete recoveries. Presumably, in animals that develop multiple acquired shunts, the primary shunt closes too rapidly for the developing hepatic vasculature to decompress the portal system. The liver's response to altered blood flow is thus the limiting factor.

Based on our observations, animals with portosystemic shunting through acquired vessels seem less likely to display severe signs of hepatic encephalopathy than those with a single, congenital vessel. The majority of animals in our study did not require medical management of hepatic encephalopathy after surgery, despite persistent shunting or biochemical abnormalities suggestive of shunting in some. Although these tests are not specific and can be affected by diet, increases in the products of hepatic metabolism such as urea and cholesterol support the contention that hepatic portal blood flow increases enough to improve hepatic function significantly, even when persistently elevated hepatic vascular resistance results in chronic portal hypertension.

Why the biological response, such as the rate and adequacy of hepatic vascular regeneration differs so much between individual dogs is the subject of ongoing debate and is worthy of further investigation. Ultimately, it is likely that adjunctive treatments to encourage hepatic regeneration and development of the hepatic microvasculature will be shown to maximize the efficacy of surgical attenuation of portosystemic shunts.

In conclusion, cellophane banding is a relatively safe, effective technique that results in resolution of biochemical abnormalities resulting from portosystemic shunting in most cases. Results of cellophane banding compare favorably with those of other techniques and it should be added to the repertoire of surgeons treating this common condition.

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References

1. Harari J, Lincoln J, Alexander J. et al: Lateral thoracotomy and cellophane banding of a congenital portoazygous shunt in a dog. J Small Anim Pract 31:571-573, 1990
2. Vogt JC, Krahwinkel DJ, Bright RM, et al: Gradual occlusion of extrahepatic portosystemic shunts in dogs and cats using the ameroid constrictor. Vet Surg 25:495-502, 1996
3. Bright RM, Vogt JC, Krahwinkel DJ, et al: Gradual occlusion of portosystemic shunts in dogs and cats using an ameroid constrictor. Vet Surg 26:253, 1997
4. Swalec KM, Seguin B, Johnston G: Surgical approaches to single extrahepatic portosystemic shunts. Compend Contin Educ Pract 20:593-601, 1998
5. Youmans KR, Hunt GB: Cellophane banding for the gradual attenuation of single extrahepatic portosystemic shunts in eleven dogs. Aust Vet J 76:531-537, 1998
6. Youmans KR, Hunt GB: Experimental evaluation of four methods of progressive venous attenuation in dogs. Vet Surg 28:38-47, 1999
7. Kyles AE, Gregory CR, Jackson J, et al: Evaluation of a portocaval venograft and ameroid ring for the occlusion of intrahepatic portocaval shunts in dogs. Vet Surg 30:161-169, 2001
8. Lidbetter DA, Krahwinkel DJ: Gradual occlusion of the left branch of the portal vein with an ameroid constrictor for treatment of an intrahepatic portosystemic shunt. Aust Vet Pract 31:120-124, 2001
9. Murphy ST, Ellison GW, Long M, et al: A comparison of the ameroid constrictor versus ligation in the surgical management of single extrahepatic portosystemic shunts. J Am Anim Hosp Assoc 37:390-396, 2001
10. Connery NA, McAllister H, Skelly C, et al: Cellophane banding of congenital intrahepatic portosystemic shunts in two Irish Wolfhounds. J Small Anim Pract 43:345-349, 2002
11. Havig M, Tobias KM: Outcome of ameroid constrictor occlusion of single congenital extrahepatic portosystemic shunts in cats: 12 cases (1993-2000). J Am Vet Med Assoc 220:337-341, 2002
12. Kyles AE, Hardie EM, Mehl M, et al: Evaluation of ameroid ring constrictors for the management of single extrahepatic portosystemic shunts in cats: 23 cases (1996-2001). J Am Vet Med Assoc 220:1341-1347, 2002
13. Hottinger HA, Walshaw R and Hauptman JG: Long-term results of complete and partial ligation of congenital portosystemic shunts in dogs. Vet Surg 24:331-336, 1995
14. Hunt GB, Hughes J: Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 77:303-307, 1999
15. Hunt GB, Tisdall PLC, Webb A, et al: Congenital portosystemic shunts in Toy and Miniature Poodles. Aust Vet J 78:530-532, 2000
16. Tisdall PLC, Hunt GB, Youmans KR, et al: Neurological dysfunction in dogs following attenuation of congenital extrahepatic portosystemic shunts. J Small Anim Pract 41:539-546, 2000
17. Swalec KM, Smeak DD: Partial versus complete attenuation of single portosystemic shunts. Vet Surg 19:406-411, 1990
18. Heldmann E, Holt DE, Brockman DJ, et al: Use of propofol to manage seizure activity after surgical treatment of portosystemic shunts. J Small Anim Pract 40:590-594, 1999
19. Tisdall PLC, Hunt GB, Tsoukalas G, et al: Post-prandial serum bile acid concentrations and ammonia tolerance in Maltese dogs with and without hepatic vascular anomalies. Aust Vet J 72:121-126, 1995
20. Yool DA, Kirby BM: Neurological dysfunction in three dogs and one cat following attenuation of intrahepatic portosystemic shunts. J Small Anim Pract 43:171-176, 2002
21. Birchard SJ, Sherding RG: Feline portosystemic shunts. Compend Contin Educ Pract Vet 14:1295-1301, 1992

22. Mehl ML, Kyles AE, Adin C, et al: Evaluation of ameroid ring constrictors for extrahepatic portosystemic shunts in 111 dogs. Vet Surg 31:490, 2002 (abstr)
23. Burton CA, White RN: Portovenogram findings in cases of elevated bile acid concentrations following correction of portosystemic shunts. J Small Anim Pract 42:536-540, 2001
24. Center SA: Liver function tests in the diagnosis of portosystemic vascular anomalies. Sem Vet Med Surg 5:94-99, 1990

Chapter 5

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

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Abstract

Background: Serious postoperative hemorrhage has been reported in dogs after closure of congenital portosystemic shunts (CPSS).
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 CPSS 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 CPSS 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 CPSS 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. CPSS dogs without shunting had improved coagulation times and increased activity of factors II, V, VII and X.
Conclusions and Clinical Importance: Dogs with CPSS 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.

Introduction

The treatment of choice of dogs with congenital portosystemic shunts (CPSS) 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 CPSS prior to 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 CPSS, 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 CPSS 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 CPSS, plasma activities of clotting factors that are produced in the liver are lower than in dogs without CPSS. As surgery can result in consumption of clotting factors, the effect of surgery on hemostasis in CPSS 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 CPSS 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 CPSS 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 CPSS was diagnosed by demonstrating the anomalous vessel with ultrasonography. In all cases the presence of a CPSS was visually confirmed during exploratory celiotomy.

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 (1μg/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 CPSS, blood was obtained from 31 healthy dogs without CPSS to measure 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, leucocytes, 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 leucocytes 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. Storage of plasma 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.

[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

To compare CPSS 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 CPSS, 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 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 CPSS 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 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 CPSS was made with ultrasonography, and a single CPSS 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 CPSS before attenuation and reference dogs were found with respect to platelet counts (lower numbers in CPSS dogs) and APTT (a higher value in CPSS dogs) (Table 1). Activities of the coagulation factors II, V, VII, and X were

significantly lower in dogs with CPSS as compared to the reference dogs. In contrast to other factors, a significantly higher activity of factor VIII was found in dogs with a CPSS. No significant differences were detected in activities of coagulation factors I, IX, and XI.

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		CPSS dogs b.s.			CPSS dogs i.a.s.			
Variable[a]	No.	Mean (SD)	No.	Mean (SD)	*P* value[1]	No.	Mean (SD)	*P* value[2]	D (%)
Platelets (x10^9/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

Abbreviations: CPSS, 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 x10^9/L, PT 6.7-9.5 seconds, APTT 10.0-17.2 seconds, fibrinogen 1.0-2.8 g/L

[b] A significant difference

In the dogs with CPSS, 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 CPSS. 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 min). 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 CPSS 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 CPSS at a median of 44 days (range 22-447 days) after surgery. Twenty-one dogs showed 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.

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

Variable[a]	OVE dogs b.s.		OVE dogs i.a.s.				
	No.	Mean (SD)	No.	Mean (SD)	*P* value[1]	D (%)	*P* value[2]
Platelets (x10^9/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

Abbreviations: 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, mean degree of decrease (-) of postoperative compared to preoperative values; *P* value[2], *P* value of postoperative values of OVE in comparison with CPSS ligation; PT, prothrombin time; APTT, activated partial thromboplastin time

[a] Laboratory reference ranges: platelets 144-603 x10^9/L, PT 6.7-9.5 seconds, APTT 10.0-17.2 seconds, fibrinogen 1.0-2.8 g/L

[b] A significant difference

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 CPSS.

Results of hemostatic analysis of recovered and non-recovered 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).

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 (x10^9/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*
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

Abbreviations: PT, prothrombin time; APTT, activated partial thromboplastin time

[a] Laboratory reference ranges: platelets 144-603 x10^9/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

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 ($P = .006$). 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.

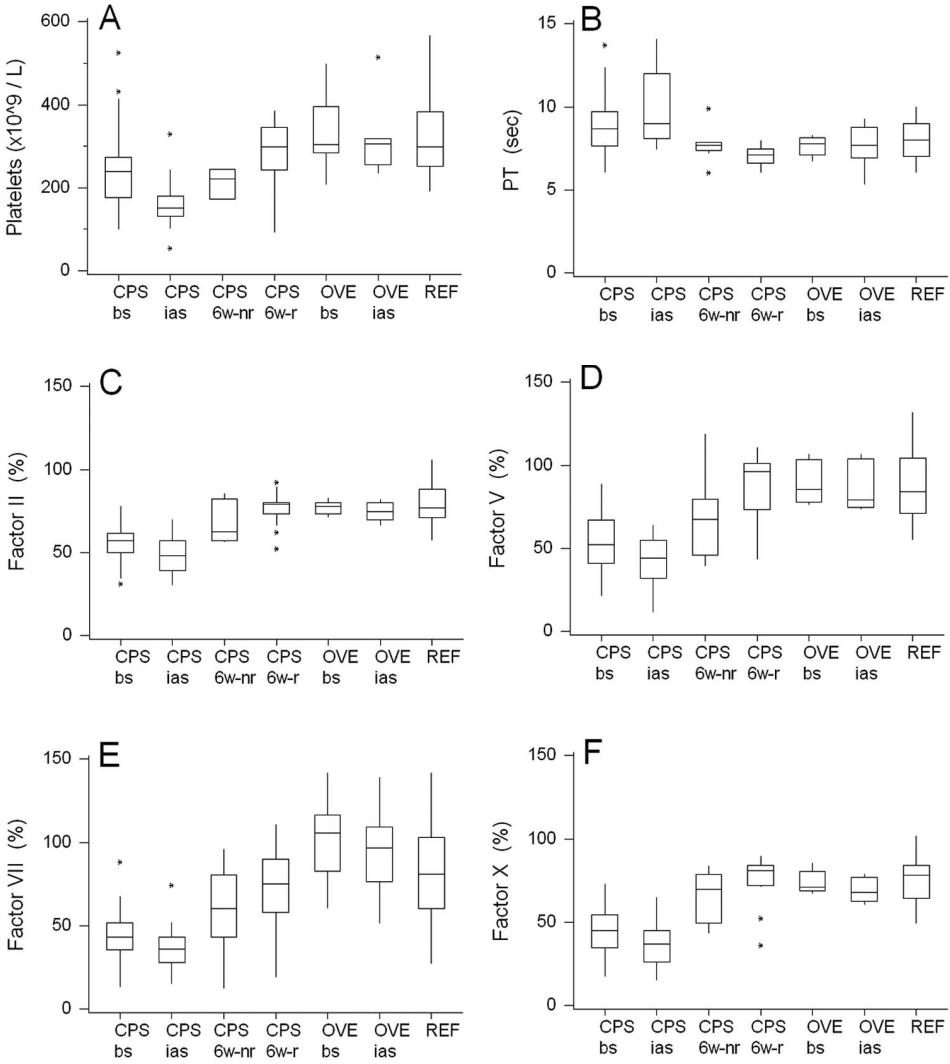

Figure 1. Box- and whiskerplots of plasma platelet counts (**A**), prothrombine time (**B**), and the activity of 4 coagulation factors (**C-F**). Hemostatic analyses were performed before surgical attenuation of a congenital portosystemic shunt (CPS bs, 33 dogs), immediately after surgical attenuation of a congenital portosystemic shunt (CPS ias, 23 dogs), approximately 6 weeks after surgical attenuation of a congenital portosystemic shunt in non-recovered 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 1.5x the interquartile range from the 1st and 3rd quartile. Outliers are plotted as *.

Preoperatively, only APTT tended to be different if recovered dogs were compared to non-recovered dogs, including the dogs that had died (P = .033). The median preoperative APTT was 17.8 seconds in recovered dogs, whereas in non-recovered 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 towards 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 CPSS dogs, control dogs, and reference dogs (Figure 1).

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

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]	CPSS dogs b.s. (n=32)	CPSS dogs i.a.s. (n=23)	CPSS dogs 6 wks, recovered (n=19)	CPSS 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

Abbreviations: CPSS, 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

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 CPSS, 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 CPSS, together with lower concentrations of several coagulation factors that are produced by hepatocytes. Although APPT 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 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 of 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 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 CPSS 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 in chronic liver disease, probably because it is the factor with the shortest half-life (4 to 6 hours). As the disease progresses, 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 CPSS, 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 CPSS dogs, both before and immediately after surgery. Additionally, both factors significantly

decreased in healthy dogs after an uncomplicated standard surgical procedure (elective ovariectomy).

There was a significantly higher concentration of factor VIII in dogs with CPSS 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 CPSS, 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 CPSS dogs.

Mean concentrations of factor I (fibrinogen) remained within normal laboratory values in the dogs in our study, although a decrease was seen during CPSS 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 CPSS 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 CPSS 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 CPSS 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 CPSS 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 CPSS, 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.

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References

1. van den Ingh TSGAM, Rothuizen J, Meyer HP. Circulatory disorders of the liver in dogs and cats. Vet Quart 1995;17:70-76.
2. Meyer HP, Rothuizen J, van Sluijs FJ, et al. Progressive remission of portosystemic shunting in 23 dogs after partial closure of congenital portosystemic shunts. Vet Rec 1999;144:133-137.
3. Hottinger HA, Walshaw R, Hauptman JG. Long-term results of complete and partial ligation of congenital portosystemic shunts in dogs. Vet Surg 1995;24:331-336.
4. Hunt GB, Hughes J. Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 1999;77:303-307.
5. Wolschrijn CF, Mahapokai W, Rothuizen J, et al. Gauged attenuation of congenital portosystemic shunts: results in 160 dogs and 15 cats. Vet Quart 2000;22:94-98.
6. Kummeling A, van Sluijs FJ, Rothuizen J. Prognostic implications of the degree of shunt narrowing and of the portal vein diameter in dogs with congenital portosystemic shunts. Vet Surg 2004;33:17-24.
7. Papazoglou LG, Monnet E, Seim HB. Survival and prognostic indicators for dogs with intrahepatic portosystemic shunts: 32 cases (1990-2000). Vet Surg 2002;31:561-570.
8. Tobias KM. Portosystemic shunts and other hepatic vascular anomalies. In: Slatter D, ed. Textbook of Small Animal Surgery. Philadelphia, PA: WB Saunders; 2003:727-752.
9. Kerwin SC, Mauldin GE. Hemostasis, surgical bleeding and transfusion. In: Slatter D, ed. Textbook of Small Animal Surgery. Philadelphia, PA: WB Saunders; 2003:44-65.
10. Niles JD, Williams JM, Cripps PJ. Hemostatic profiles in 39 dogs with congenital portosystemic shunts. Vet Surg 2001;30:97-104.

11. Prater MR. Acquired coagulopathy II: liver disease. In: Feldman BF, Zinkl JG, Jain NC, eds. Schalm's Veterinary Hematology. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:560-564.
12. Mammen EF. Coagulation defects in liver disease. Med Clin N Am 1994;78: 545-554.
13. Lisciandro SC, Hohenhaus A, Brooks M. Coagulation abnormalities in 22 cats with naturally occurring liver disease. J Vet Intern Med 1998;12:71-75.
14. Badylak SF, Dodds WJ, Van Vleet JF. Plasma coagulation factor abnormalities in dogs with naturally occurring hepatic disease. Am J Vet Res 1983;44:2336-2340.
15. Rothuizen J, van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22-25.
16. Rothuizen J. Portosystemic hepatic encephalopathy related with congenital and acquired hepatopathies in the dog. Adv Vet Sci Comp Med 1993;37:403-416.
17. Mischke R. Optimization of coagulometric tests that incorporate human plasma for determination of coagulation factor activities in canine plasma. Am J Vet Res 2001;62:625-629.
18. Mischke R, Nolte IJA. Hemostasis: introduction, overview, laboratory techniques. In: Feldman BF, Zinkl JG, Jain NC, eds. Schalm's Veterinary Hematology. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:519-525.
19. Bateman SW, Mathews KA, Abrams-Ogg ACG, et al. Evaluation of the effect of storage at -70°C for six months on hemostatic function testing in dogs. Can J Vet Res 1999;63:216-220.
20. Kemkes-Matthes B, Bleyl H, Matthes KJ. Coagulation activation in liver diseases. Thromb Res 1991;64: 253-261.
21. Kerr R. New insights into haemostasis in liver failure. Blood Coagul Fibrinolysis 2003;14: S43-S45.
22. Mammen EF. Acquired coagulation protein defects. In: Bick RL, ed. Hematology, Clinical and Laboratory Practice. St Louis,MO: Mosby; 1993:1449-1462.
23. Burns ER, Goldberg SN, Wenz B. Paradoxic effect of multiple mild coagulation factor deficiencies on the prothrombin time and activated partial thromboplastin time. Am J Clin Pathol 1993;100:94-98.
24. Littlewood JD. Disorders of secondary haemostasis. In: Day MJ, Mackin A, Littlewood JD, eds. BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine. Gloucester: British Small Animal Veterinary Association, 2000:209-215.
25. Dodds WJ. Other hereditary coagulopathies. In: Feldman BF, Zinkl JG, Jain NC, eds. Schalm's Veterinary Hematology. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:1030-1036.
26. Mammen EF. Coagulopathies of liver disease. Clin Lab Med 1994;14: 769-780.
27. Rothuizen J, Desmet VJ, van den Ingh TSGAM, et al. Sampling and handling of liver tissue. In: Rothuizen J, et al. eds. WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Disease. Edinburgh: Churchill Livingstone; 2006:5-14.

Chapter 6

Hepatic Volume Measurements in dogs with Extrahepatic Congenital Portosystemic Shunts before and after Surgical Attenuation

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Abstract

Background: In dogs with a congenital portosystemic shunt (CPSS), the ability of the hypoplastic liver to grow is considered important for recovery after surgical shunt attenuation.
Objectives: This study investigated hepatic growth after extrahepatic shunt attenuation in dogs, using MRI and CT.
Animals: 10 client owned dogs with a single extrahepatic CPSS.
Methods: Abdominal MRI and/or CT scans were performed before and eight days, one month and two months after shunt attenuation. Liver volumes were calculated from the areas of the MRI and/or CT images.
Results: Before surgery, median liver volume was 18.2 cm^3 per kg body weight. Liver volume increased significantly after surgery. Growth was highest between day 0 and day 8 and decreased afterwards. Median liver volume was 28.8 cm^3 /kg at 2 months after attenuation. No significant differences in growth were found between dogs with complete or partial shunt closure or between dogs with complete or incomplete metabolic recovery. Volumes measured from consecutively performed MRI and CT images correlated well (r=0.980), but volumes from MRI images were significantly larger than volumes from CT images (6.8%; P=0.008).
Conclusion and Clinical Importance: After shunt attenuation, a rapid normalization of liver size was observed. Hepatic growth was not decreased in dogs after partial closure of CPSS or in dogs with subclinical, persistent shunting at two months after surgery. CT is the preferred volumetric technique because of speed.

Introduction

In a congenital portosystemic shunt (CPSS), portal blood is shunted around the liver directly into the systemic circulation, resulting in macroscopic and microscopic liver hypoplasia.[1-3] In many dogs with CPSS, hepatic function is completely restored after surgical attenuation of the shunt. However, portosystemic shunting, with or without clinical disease, persists in 10 to 20% of dogs, regardless of the surgical technique employed for shunt closure.[4-6] The ability of the liver to adapt to the increased blood flow after shunt attenuation and to grow to "normal' size may contribute to recovery in individual patients.[4,5,7]

In dogs with CPSS, diagnostic imaging techniques are frequently used for pre and postoperative assessment of the anatomy of the shunt and development of the liver and the portal vein.[8-11] Liver volume was measured only in a small number of dogs before and after attenuation of the shunt. Although liver volume may not be small in all dogs with CPSS, it was suggested as a prognostic marker of hepatic function and a noninvasive method to evaluate response to therapy.[12,13] In humans, liver volume is significantly related to prognosis in hepatic diseases such as cirrhosis and fulminant liver failure.[14,15] Therefore, the aims of the present study were to describe two noninvasive methods to measure liver volume *in vivo* and to record liver growth in dogs with extrahepatic CPSS after surgical attenuation of the shunt.

Material and methods

Surgery

Dogs in which a single extrahepatic CPSS was diagnosed and that were consecutively planned for surgical shunt ligation, were entered into the study, with informed written consent of the owners. The design of the study was approved by the Ethics Committee on Animal Experimentation. The diagnosis of portosystemic shunting was based on increased plasma concentrations of bile acids and ammonia (NH_3) after 12 hours fasting (reference values: bile acids 0-10 μM; NH_3 24-45 μM) or an abnormal rectal ammonia tolerance test.[16] The presence of a single extrahepatic CPSS was confirmed with ultrasonography. All surgeries were performed by the same surgeon. After exploration of the abdominal cavity via a midline celiotomy, the extrahepatic shunt was ligated over a gauged rod to the smallest diameter that did not induce portal hypertension, using a nonabsorbable 2-0 polyester suture[a]. All dogs were kept on one commercial low protein diet during the study.

Clinical and metabolic recovery was monitored at 8 days, 1 month and 2 months after surgery. At each visit, body weight was measured and portosystemic shunting was evaluated by determining 12-hour fasting plasma bile acids and ammonia concentration

or applying a rectal ammonia tolerance test.[16] Complete recovery was defined by resolution of all clinical signs (clinical recovery) and a normal result of the rectal ammonia tolerance test (metabolic recovery).

Volume measurements

Magnetic resonance imaging (MRI) and computed tomography (CT) were performed before surgery and at 8 days, 1 month and 2 months after surgery in anesthetized dogs, positioned in dorsal recumbency. T1 weighted images (TR 560 ms, TE 15.0) of 5-mm-thick contiguous slices were made of the entire liver using a 0.2 T open MRI-scanner [b]. CT images were made of the entire abdomen with a single slice helical CT scanner[c], using 120 kVp and 280 mA settings, a collimation of 3 mm, a pitch of 1 and 0.7 seconds scanning time per rotation. Images were reconstructed to 2 mm-thick contiguous slices. CT scans were made during a state of apnea, achieved by hyperventilating or disabling intermittent positive-pressure ventilation.

MRI images were viewed with a window width of 1892 and a window center of 891; CT images were viewed with a window width of 150 and a window level of 40 Hounsfield units. Liver contours were outlined using a mouse-driven cursor on each individual image of the MRI and CT scans, excluding the gallbladder, the caudal vena cava and the portal vein, where these vessels were not completely surrounded by liver tissue. Liver surface areas were calculated on every image using the standard software programs[d,e] of the scanners. The sum of the areas of all slices per scan was multiplied with the slice thickness to calculate total liver volume. Measurements were performed twice, blinded and in a random order by one of the authors.

Statistical analysis

All statistical tests were performed using commercial software[f]. Wilcoxon signed ranks tests were used to compare first and second measurements of liver volumes, volumes calculated from simultaneous MRI and CT scans, hepatic growth, and changes in body weight. Correlations between volume measurements and imaging techniques were analyzed with linear regression. Comparisons of liver volumes with degree of intraoperative CPSS closure and postoperative recovery were performed using Mann-Whitney U tests. Spearman's rho correlation coefficients were calculated to determine correlations between age at surgery, body weight, and liver volumes. A p value <0.05 was considered significant.

[a] *Ethibond*^{tm} *excel 2-0*, Ethicon INC., Somerville, NJ, USA
[b] Magnetom Open Viva scanner (0.2 Tesla), Siemens Nederland N.V. Medical Solutions, The Hague, The Netherlands
[c] Helical CT Secura scanner, Philips Medical Systems Nederland BV, Best, The Netherlands
[d] Numaris version VB33G, Siemens Nederland N.V. Medical Solutions, The Hague, The Netherlands
[e] EasyVision release 5.1, Philips Medical Systems Nederland BV, Best, The Netherlands
[f] SPSS for Windows, release 16.0.1, SPSS, Inc, Chicago, IL, USA

Results

Twelve small breed dogs were entered into the study, but two dogs did not survive to the end of the study. One dog suddenly succumbed and died 10 hours after uncomplicated surgical shunt closure. The cause of death remained unknown. Another dog died two days after surgery because of persistent abdominal bleeding due to coagulopathy. The group with follow-up measurements consisted of 10 dogs, 7 female and 3 male dogs. Body weight at time of surgery ranged from 1.2 to 6.5 kg, with a median of 5.9 kg. Breed, age, and results of CPSS surgery of these dogs are listed in Table 1.

Table 1. Characteristics and results of surgical shunt attenuation in 10 dogs with an extrahepatic congenital portosystemic shunt

Dog	Breed	Age (mths)	Type CPSS	Closure CPSS	Recovery 2mpo
1	Jack Russell	10	splenic vein – caval vein	partial	complete
2	Cairn terrier	5.3	portal vein – azygos vein	complete	complete
3	WHW terrier	15	gastroduodenal vein – caval vein	partial	complete
4	Cairn terrier	12	splenic vein – caval vein	partial	complete
5	Shih tzu	7.6	portal vein – caval vein	partial	complete
6	mixed breed	14	portal vein – azygos vein	complete	complete
7	mixed breed	39	gastroduodenal vein – caval vein	partial	complete
8	Welsh terrier	40	portal vein – azygos vein	partial	complete
9	Yorkshire terrier	5.5	portal vein – azygos vein	partial	not complete
10	Cairn terrier	11	splenic vein – caval vein	partial	not complete

Abbreviations: CPSS, congenital portosystemic shunt; Age, age at surgery (mths, months); Recovery 2mpo, clinical and metabolic recovery of portosystemic shunting 2 months after surgery

Before surgery, median plasma bile acids concentrations were 175 μM (range 19-260 μM). All ten dogs performed clinically well immediately after surgery and returned to the clinic at exactly eight days after surgery. At 8 days after surgery, diarrhea and vomiting were observed in dog 1, and in dog 7 mild diarrhea was seen. No clinical signs were reported in the other eight dogs.

The dogs attended the visits that were scheduled at one and two months after surgery not exactly on the same postoperative day: median values were 29 and 64 days after surgery, respectively. Clinical recovery was good in all dogs: signs of hepatic encephalopathy or other clinical signs of portosystemic shunting were not reported. Two dogs retained portosystemic shunting (dog 9 and dog 10). Despite good clinical

performance in both dogs, ammonia tolerance tests were still abnormal at two months after surgery. At 20 and 40 minutes after rectal ammonia administration, plasma ammonia concentrations were >286 and 137 µM in dog 9 and >286 and 150 µM in dog 10, respectively (280 µM being the upper detection limit, and 46 µM the upper fasting reference value). Plasma bile acid concentrations in these dogs were 3.0 µM (dog 9) and 59 µM (dog 10; fasting reference value <10 µM). In the 8 recovered dogs, median plasma ammonia was 7.5 µM (range <7.0-24 µM) with normal results of tolerance testing, and median bile acids were 2.5 µM (range 0-13 µM) at 2 months after surgery. A significant increase in body weight was seen between 8 days and 1 month after surgery (P=0.005) and between 1 and 2 months after surgery (P=0.011).

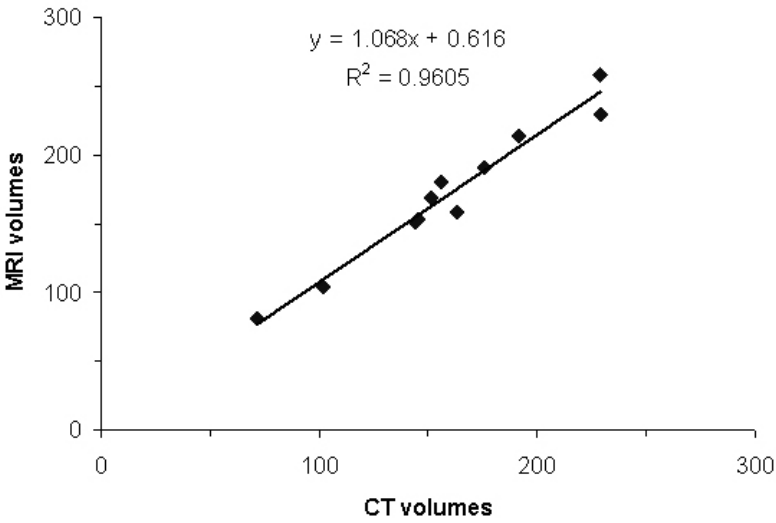


Figure 1. Average hepatic volumes (cm^3) from 11 CT and MRI scans that were consecutively performed in 3 dogs before and after attenuation of a CPSS

In the first two dogs, only MRI scans were made. In dog 3 to 5, both MRI and CT series were made and in dogs 6 to 10, only CT scanning was used to estimate liver volumes. Two consecutively planned scans in one dog were not performed (1 MRI, 1 CT) and four CT examinations were lost due to technical problems. A total amount of 46 volumetric scans were made and analyzed. The results of the volumetric measurements were normally distributed. There were no significant differences between duplicate measurements made on CT images or between duplicate measurements made on MRI images. Measurements made on MRI images were compared with consecutive measurements made on CT images in dog 3, 4 and 5 (11 CT scans and 11 MRI scans). These values correlated very well (r=0.980, Figure 1),

but a significant difference was found (P=0.008). On average, volumes estimated from CT images were 6.8% less than volumes estimated from subsequent MRI images.

For evaluation of liver growth, only values derived from CT measurements were used. In both dogs in which no CT scans were made (dog 1 and 2), the MRI derived measurements were corrected to fit CT data with a factor of 0.932. Box-and-whisker plots of liver volume and body weight after surgical attenuation of the shunt are shown in Figure 2.

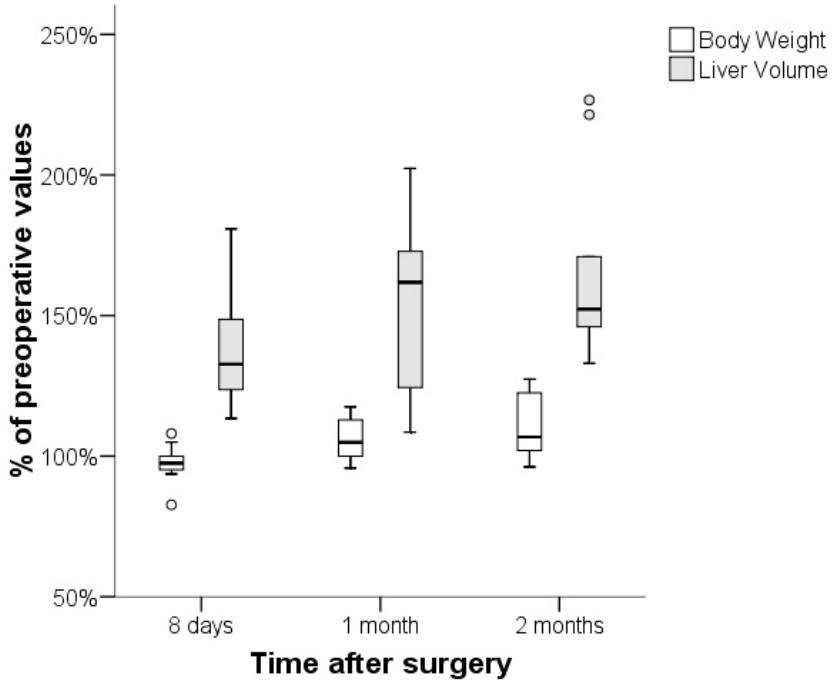


Figure 2. Box-and-whisker plots of body weight (white boxes) and hepatic volume (grey boxes) as percentages of preoperative values at the measured time points in 10 dogs after surgical attenuation of a CPSS. The horizontal line inside the box represents the median and the edges of the boxes indicate the interquartile range (the middle 50% of scores). The whiskers extend to the highest and lowest scores within 1.5x the interquartile range. Outliers are plotted as dots.

Table 2. Hepatic volumes and relative hepatic growth in 10 dogs with an extrahepatic congenital portosystemic shunt before and after shunt attenuation

	Day 0	8 days postoperative		1 month postoperative		2 months postoperative	
Dog	V (cm^3/kg)	V (cm^3/kg)	ΔV (%)	V (cm^3/kg)	ΔV (%)	V (cm^3/kg)	ΔV (%)
1	17.2	26.8	56.1	29.7	73.0	27.2	58.3
2	32.7	36.0	9.40	33.1	1.01	34.4	4.39
3	16.4	26.5	61.2	27.0	64.9	23.2	41.7
4	18.0	24.9	36.7	27.2	48.5	26.3	46.0
5	18.0	NA	NA	31.0	72.2	33.3	85.0
6	22.9	27.1	18.1	28.5	24.4	32.8	43.1
7	17.2	NA	NA	NA	NA	23.3	35.6
8	19.4	NA	NA	21.1	8.54	NA	NA
9	19.8	35.9	80.8	32.4	63.1	35.1	77.1
10	18.4	26.5	43.9	27.6	50.3	28.8	56.5
Me	**18.2**	**26.8**	**43.9***	**28.5**	**50.3***	**28.8**	**46.0***

Abbreviations: Day 0, day of surgery; V, volume; ΔV, % increase from baseline volume on day 0; Me, median; NA, not available

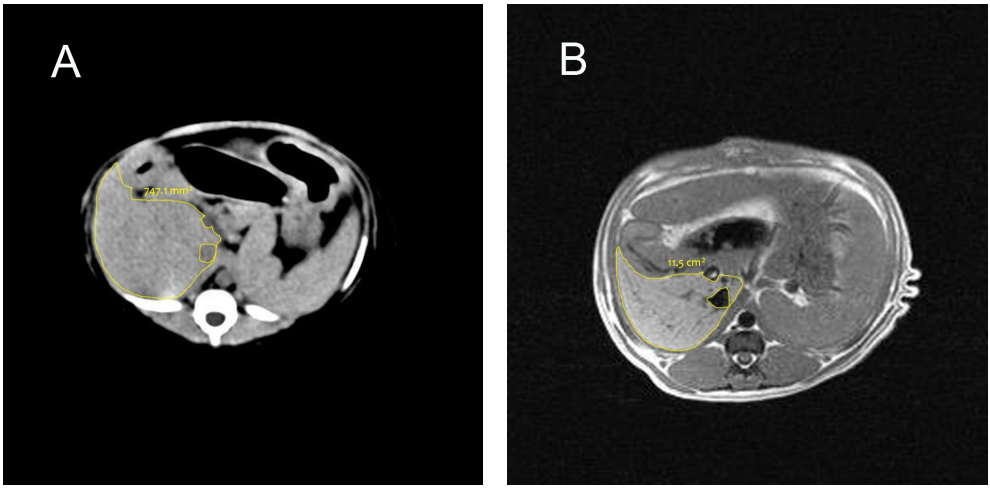
* significant growth compared to day 0

Individual liver volumes and growth were expressed in cm^3 per kg body weight (Table 2). The median liver volume before surgery was 18.2 cm^3/kg (range 16.4-32.7 cm^3/kg). The largest hepatic growth occurred in the period between day 0 and day 8. Median hepatic volumes significantly increased with 47.9 cm^3 (P=0.018) or 8.6 cm^3/kg (P=0.018) in this period. Between 8 days and 1 month postoperatively, the median hepatic growth consisted of 12.4 cm^3 (P=0.043). During this period there was no significant increase in liver volume per kilogram body weight. The gain in liver volume per kg body weight was maintained at 1 month and 2 months after surgery: the median volumes at 1 and 2 months after surgery were 28.5 cm^3/kg and 28.8 cm^3/kg respectively, which were significantly larger than before surgery (both P values were 0.008). Between 1 and 2 months after surgery, there was no significant liver growth. In two months, liver volume increased to 152% compared to pre-operative values (146% relative to body weight).

Hepatic growth in the two dogs that retained portosystemic shunting (dogs 9 and 10) was not significantly different from the eight dogs with complete metabolic recovery of portosystemic shunting at 8 days, 1 month or 2 months after surgery. Individual measurements even showed an increase of liver volume beyond the median value in these dogs (Table 2).

We found no significant differences between 2 dogs that had their shunts completely closed (dog 2 and dog 6) and 8 dogs whose shunts were only partially closed at 8 days, 1 month or 2 months after surgery.

Although before surgery no significant correlation existed between age and liver volume per kg body weight, this correlation became significant 1 month after surgery. Spearman's rho correlation coefficients were 0.95 at 1 month ($P< 0.001$) and 0.85 at 2 months postoperatively ($P=0.004$). The youngest dogs had the largest relative liver volumes after surgery. No significant correlation was found between hepatic growth and increase in body weight.



Illustrations A and B. Two images from an abdominal CT (A) and MRI (B) scan in a CPSS dog. The contours of the liver were outlined by hand, resulting in calculated liver surface areas. In each image the vena cava was excluded from the surface area.

Discussion

Performing MRI and CT in dogs with CPSS, clinical relevant results were obtained with respect to liver volume before and after shunt attenuation. Hepatic volumes estimated from consecutively performed MRI and CT images correlated well, but volumes from MRI scans were significantly larger than volumes from CT scans. Before surgery, median liver volume was 18.2 cm^3 per kg body weight. Liver growth was highest between day 0 and day 8 and decreased afterwards. Liver volume per kg body weight increased with 46% in 2 months after shunt attenuation. No significant

differences in growth were found between dogs with or without complete shunt closure or between dogs with and without complete metabolic recovery.

Liver volume measurement has been reported in dogs with CPSS using radiography, ultrasonography, and CT.[12,13,17] In abdominal radiographs, superposition of abdominal organs can make identification of the caudal border of the liver impossible.[18] However, radiographs were used to compare liver size in small dogs with CPSS and healthy dogs of the same breeds. The hepatic area in dogs with CPSS was reported to be 48% smaller than in healthy dogs.[12] In spite of differences in imaging techniques and dogs, we found a similar difference in liver volume between small sized CPSS dogs before surgery and after their recovery at 2 months postoperatively (46%).

Liver volume estimation with abdominal helical CT has been reported to be very reliable. It has been regarded as the most accurate imaging technique for *in vivo* evaluation of organ volume and is considered the gold standard.[19,20] Volume measurements with CT have been used in large numbers of human patients with liver disease, because CT is easy, safe and rapid as well.[15,21] CT is also capable of rapid and accurate measurements of liver volume and growth in small animals such as dogs.[13,22] CT volume measurements are reported to be accurate for organs larger than 10 ml.[20] The smallest liver volume in our patients was 23.8 cm^3 in a dog that weighed 1.2 kilograms (dog 9, day 0). Thus, liver volumes less than 10 ml will not often be encountered in dogs.

MRI also offers an accurate means of determining liver volume *in vivo*.[23] MRI has been used in dogs with CPSS for diagnostic purposes or to evaluate hepatic encephalopathy related brain changes before and after surgical attenuation, but liver volume measurements in dogs using this technique have not been reported before.[24-26] Compared to CT, measured MRI volumes were 6.8% larger. Differences in slice thickness (MRI 5 mm, CT 3 mm) could have attributed to less accurate measurements in MRI scans, but the most likely cause of differences between MRI and CT derived liver measurements is respiratory movement of the liver during the MRI scans.[27] During CT scanning dogs were held in apnea, which is not possible during MRI scanning because of acquisition time. Because respiratory movement during MRI can be responsible for overestimation of liver volumes and because CT is fast, CT is preferred for volumetric measurements of the liver. CT and MRI were not consistently used in all dogs. In the first two dogs the CT scanner was temporarily not available. In the next five dogs CT and MRI were used to compare both techniques. This was abandoned because performing both imaging techniques before surgery resulted in prolonged anesthesia time and severe hypothermia. The resulting increase of surgical risk was considered unacceptable. Therefore, volumetric studies in the last five dogs only used CT scanning.

In this study, larger liver volumes were found in dogs with CPSS before and after surgery than reported before. Stieger *et al.* (2007) measured a mean preoperative liver volume of 15.5 cm^3/kg in 21 CPSS dogs and postoperative volumes of 11.7 cm^3/kg in 2 intrahepatic CPSS dogs and 22.8 cm^3/kg in an extrahepatic CPSS dog, using quantitative CT.[13] The median liver volume two months after surgery (28.8 cm^3/kg) in this study is also higher than the reported volumes in normal adult dogs or humans. The average liver volume in normal dogs was 24.4 cm^3/kg and human normal adult liver size is 23.5 cm^3/kg.[13,20] In young children, up to around eight years old, relative liver volume is much larger than in adults. Mean liver volumes in children ranges from 34.1 cm^3/kg in infants, to 23.8 cm^3/kg at an age of seven years old.[28] Although postoperative liver size in this study seems more comparable with normal liver size in children, the median age in our dogs was 11 months, which is considered young adulthood in small breed dogs. However, the median age in our dogs was less than the age of the other reported extrahepatic CPSS dog that was measured after shunt ligation and the dogs that were used to determine normal liver volumes.[13] Furthermore, the significant correlation that was found in this study between age and proportional liver volumes after recovery is compatible with the age-related difference in liver volume reported in humans.[28] Besides age, different estimations of liver volumes may be a result of differences in measurement technique or breeds that were used. Although no differences were reported between dogs with intrahepatic and extrahepatic shunts before surgery, additional studies are necessary to determine shunt specific differences with respect to liver volume and growth after surgery.[13]

Regeneration of normal liver is very fast: liver volume is restored in 7 days after 2/3 hepatectomy in rats and mice.[29] Increase of liver volume in this study was also remarkably high during the first week after surgery. Body weight directly influences the results of liver volumes expressed per kg. In dogs with CPSS, retarded growth or leanness is a common finding, so after successful treatment both young and adult patients are expected to gain body weight. Although average body weight did not significantly change in the first week after surgery, in several dogs body weight had decreased at 8 days after surgery. In two dogs, this weight loss may have been caused by vomiting and diarrhea. Weight loss can also be related to the high plasma bile acid concentrations before surgery and the enormous decrease in bile acids 8 days after surgery in most dogs (Table 2). Bile acids are known to inhibit 11β-hydroxysteroid dehydrogenase type 2, an enzyme that is responsible for protecting the mineralocorticoid receptors from activation. Activation of these receptors in patients with high plasma bile acid concentrations, leads to renal sodium retention and therefore retention of fluid.[30,31] After normalization of bile acid concentrations, fluid retention is reversed and body weight may decrease. Reduced body weight increases relative liver volume. However, perfusion state has a major impact on liver volume. So, if body weight is decreased because of reversed fluid retention, the absolute liver volume (in

cm^3) is expected to be lower too. Body weight did increase significantly between 8 days and 1 month after surgery (P=0.005), which is not surprising in dogs that are still young and growing, but may also be expected as a compensating gain in weight in older dogs that recover from secondary retarded growth and leanness. Although absolute liver volumes further increased during this period, the gain of weight camouflaged hepatic growth expressed in cm^3/kg.

Stieger *et al*. (2007) suggested that dogs with a smaller hepatic liver volume before surgery have a poorer outcome. He proposed repeated CT postoperatively as a useful measure of response to therapy.[13] We found no significant differences in preoperative liver volumes or liver growth between dogs with complete recovery and dogs with persistent shunting. Recently, Doran *et al*. (2008) reported that dogs that were tolerant to full closure of the shunt during surgery, had significantly greater preoperative liver volume / body weight ratios than dogs intolerant of occlusion.[17] We did not find any significant differences between preoperative or postoperative volumetric results of dogs with completely closed shunts and dogs with shunts closed to the smallest diameter possible. The number of cases may be too small to find slight differences between dogs with complete and semi-complete recovery or between dogs with complete and partial closure. Measurements in large number of dogs, including cases with incomplete resolution of clinical signs, may help to identify such differences.

Recovery rates, and possibly also hepatic size or growth, vary among dogs after attenuation of a CPSS. This may be caused by differences in shunt localization, breed and population.[32,33] Also the surgical technique that is employed for CPSS closure may affect recovery and hepatic growth. This study was restricted to dogs with extrahepatic CPSS referred to one university clinic, using an identical ligation technique in all dogs. Although the concepts of this study may be equally valid to other CPSS dogs, results of hepatic volume and growth measurements may yield different results in different centers due to local variation of the above factors.

In conclusion, CT and MRI are both suitable non-invasive methods to measure liver volumes in dogs, but CT is preferred because of its speed. Liver volumes in dogs with extrahepatic CPSS are smaller than reported volumes in normal dogs. After attenuation of the shunt, a rapid increase of liver volume occurred within 8 days and complete normalization was measured in dogs with complete recovery of portosystemic shunting, and also in dogs with subclinical postoperative shunting. Partial closure of CPSS was sufficient to obtain complete recovery and normalization of liver volume in 6 of 8 dogs.

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References

1. Ewing GO, Suter PF, Bailey CS. Hepatic insufficiency associated with congenital anomalies of the portal vein in dogs. J Am Anim Hosp Assoc 1974;10:463-476.
2. Rothuizen J, van den Ingh TSGAM, Voorhout G, et al. Congenital portosystemic shunts in sixteen dogs and three cats. J Small Anim Pract 1982;23:67-81.
3. Zaitoun AA, Path FRC, Apelqvist G, et al. Quantitative studies of liver atrophy after portacaval shunt in the rat. J surg Res 2006;131:225-232.
4. Kummeling A, van Sluijs FJ, Rothuizen J. Prognostic implications of the degree of shunt narrowing and of the portal vein diameter in dogs with congenital portosystemic shunts. Vet Surg 2004;33:17-24.
5. Hunt GB, Kummeling A, Tisdall PLC, et al. Outcomes of cellophane banding for congenital portosystemic shunts in 106 dogs and 5 cats. Vet Surg 2004;33:25-31.
6. Mehl ML, Kyles AE, Hardie EM, et al. Evaluation of ameroid ring constrictors for treatment for single extrahepatic portosystemic shunts in dogs: 168 cases (1995–2001). J Am Vet Med Assoc 2005;226:2020-2030.
7. Szatmári V, van Sluijs FJ, Rothuizen J, Voorhout G. Ultrasonographic assessment of hemodynamic changes in the portal vein during surgical attenuation of congenital extrahepatic portosystemic shunts in dogs. J Am Vet Med Assoc 2004;224:395-402.
8. d'Anjou MA, Penninck D, Cornejo L, Pibarot P. Ultrasonographic diagnosis of portosystemic shunting in dogs and cats. Vet Radiol Utrasound 2004;45:424-437.
9. Lee KC, Lipscomb VJ, Lamb CR, et al. Association of portovenographic findings with outcome in dogs receiving surgical treatment for single congenital portosystemic shunts: 45 cases (2000-2004). J Am Vet Med Assoc 2006;229:1122-1129.
10. Zwingenberger A. CT Diagnosis of Portosystemic Shunts. Vet Clin North Am Small Anim Pract 2009;39:783-792.
11. Washizu M, Katagi M, Washizu T, et al. An evaluation of radiographic hepatic size in dogs with portosystemic shunt. J Vet Med Sci 2004;66:977-978.
12. Stieger SM, Zwingenberger A, Pollard RE, et al. Hepatic volume estimation using quantitative computed tomography in dogs with portosystemic shunts. Vet Radiol Ultrasound 2007;48:409-413.
13. Zoli M, Cordiani MR, Marchesini G, et al. Ultrasonographic follow-up of liver cirrhosis. J Clin Ultrasound 1990;18:91-96.
14. Sekiyama K, Yoshiba M, Inoue K, Sugata F. Prognostic value of hepatic volumetry in fulminant hepatic failure. Dig Dis Sci 1994;39:240-244.
15. Rothuizen J, van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22-25.
16. Doran IP, Barr FJ, Hotston Moore A, et al. Liver size, bodyweight, and tolerance vto acute complete occlusion of congenital extrahepatic portosystemic shunts in dogs. Vet Surg 2008;37:656-662.
17. Van Bree H, Jacobs V, Vandekerckhove P. Radiographic assessment of liver volume in dogs. Am J Vet Res 1989;50:1613-1615.

18. Zoli M, Pisi P, Marchesini G, et al. A rapid method for the in vivo measurement of liver volume. Liver 1989;9:159-163.
19. Geraghty EM, Boone JM, McGahan JP, Jain K. Normal organ volume assessment from abdominal CT. Abdom Imaging 2004;29:482-490.
20. Schiano TD, Bodian C, Schwartz ME, et al. Accuracy and significance of computed tomographic scan assessment of hepatic volume in patients undergoing liver transplantation. Transplantation 2000;69:545-550.
21. Moss AA, Friedman MA, Brito AC. Determination of liver, kidney and spleen volumes by computed tomography: an experimental study in dogs. J Comput Assist Tomogr 1981;5:12-14.
22. Caldwell SH, De Lange EE, Gaffey MJ, et al. Accuracy and significance of pretransplant liver volume measured by magnetic resonance imaging. Liver Transpl Surg 1996;2:438-442.
23. Seguin B, Tobias KM, Gavin PR, Tucker RL. Use of magnetic resonance angiography for diagnosis of portosystemic shunts in dogs. Vet Radiol Ultrasound 1999;40:251-258.
24. Torisu S, Washizu M, Hasegawa D, Orima H. Brain magnetic resonance imaging characteristics in dogs and cats with congenital portosystemic shunts. Vet Radiol Ultrasound 2005;46:447-451.
25. Torisu S, Washizu M, Hasegawa D, Orima H. Measurement of brain trace elements in a dog with a portosystemic shunt: relation between hyperintensity on T1-weighted magnetic resonance images in lentiform nuclei and brain trace elements. J Vet Med Sci 2008;70:1391-1393.
26. Sandrasegaran K, Kwo PW, DiGirolamo D, et al. Measurement of liver volume using spiral CT and the curved line and cubic spline algorithms: reproducibility and interobserver variation. Abdom Imaging 1999;24:61-65.
27. Noda T, Todani T, Watanabe Y, Yamamoto S. Liver volume in children measured by computed tomography. Pediatr Radiol 1997;27:250-252.
28. Michalopoulos GK. Liver regeneration. J Cell Physiol 2007;213:286-300.
29. Quattropani CQ, Vogt B, Odermatt A, et al. Reduced activity of 11β-hydroxysteroid dehydrogenase in patients with cholestasis. J Clin Invest 2001;108:1299-1305.
30. Frey FJ. Impaired 11β-hydroxysteroid dehydrogenase contributes to renal sodium activity in cirrhosis: hypothesis of fact? Hepatology 2006;44:795-780.
31. Krotschek U, Adin CA, Hunt GB, et al. Epidemiologic factors associated with the anatomic location of intrahepatic portosystemic shunts in dogs. Vet Surg 2007;36:31-36.
32. Hunt GB. Effect of breed on anatomy of portosystemic shunts resulting from congenital diseases in dogs and cats: a review of 242 cases. Aust Vet J 2004;82:746-749.

Chapter 7

Intraoperative hepatic gene expression in dogs related to outcome after attenuation of a congenital portosystemic shunt

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Abstract

Background: In dogs with a congenital portosystemic shunt (CPSS), individual outcome after surgical CPSS attenuation is difficult to predict, but is possibly associated with hepatic and portal vein proliferation.
Objectives: To evaluate intraoperative hepatic expression of 19 genes involved in hepatic and vascular growth and fibrosis in dogs with a CPSS in relation to postoperative outcome.
Animals: Forty-eight client owned dogs with a single extrahepatic (32) or intrahepatic (16) CPSS.
Methods: In liver tissue that was collected during surgical shunt attenuation, the expression of 19 gene products was measured with a quantitative real-time polymerase chain reaction assay. Univariable and multivariable data analyses were performed to evaluate the relation with outcome and possible predictive value of the gene products.
Results: The hepatic expression of methionine adenosyltransferase 2 alpha (MAT2a) and HGF activator (HGFac) was positively associated with complete recovery after CPSS attenuation. Both enzymes are involved in liver cell proliferation. Individual outcome could be predicted in 75% of dogs using MAT2a and HGFac as binary variables (high or low expression compared to cut-off values of 0.457 and 0.974, respectively). Continuous expression of MAT2a was associated with shunt localization. Other evaluated gene products were not correlated with individual outcome.
Conclusion and Clinical Importance: Increased mRNA expression of MAT2a and HGFac were associated with good recovery of CPSS. A model including both genes expressed as binary variables predicted outcome after CPSS ligation better than shunt localization. Low MAT2a may partially explain the poorer prognosis of intrahepatic CPSS.

Introduction

Prediction of the long-term outcome after surgical attenuation of a single congenital portosystemic shunt (CCPS) in dogs is difficult, even though prognostic factors have been studied extensively. Probably, an important factor influencing long-term outcome is the ability of the underdeveloped liver and portal vasculature to adapt to the increased blood flow after shunt attenuation and to grow to 'normal' size and function.[1,2] The ability of the liver to cope with the increased portal flow after shunt closure can be estimated from the size of the intrahepatic portal branches before surgery and the increase in opaficication of these vessels directly after shunt closure, visualized with intraoperative mesenteric portovenography.[3] In some dogs with severe hypoplasia or aplasia of the portal vasculature cranial to the shunt, shunt attenuation is not feasible because of acute portal hypertension during attempts to attenuate the shunt.[4] In dogs with sufficiently developed cranial portal vasculature to allow shunt attenuation, sustained intrahepatic portal vein development[3] and increase in liver volume[5] may decrease hepatic flow resistance and further increase portal flow into the liver after attenuation of the shunt. Portal blood flow is essential for hepatic development.[6] Therefore, the increasing portal flow may result in a positive spiral of hepatic development that may be responsible for the progressive decrease in portosystemic shunting over time that is seen in dogs with complete recovery after partial shunt closure.[7]

A wide variety of factors and regulatory pathways have been found to affect liver proliferation and regeneration after partial hepatectomy.[8-10] Also in various liver diseases, specific expression patterns of gene products were associated with hepatic proliferation and regeneration.[11,12] If hepatic and vascular proliferation are important for recovery after attenuation of a CPSS, hepatic expression of proliferation related genes may be related to the response to surgical treatment. Expression patterns of specific genes have been used as prognostic indicators before, but almost exclusively in malignant diseases in humans.[13,14] Proliferation related genes were differently expressed in dogs with CPSS compared to normal dogs and dogs with other liver disorders, but their relation with recovery after surgical treatment of a CPSS was unclear.[11]

We hypothesized that preoperative hepatic expression of genes involved in proliferation or apoptosis of hepatocytes, vascular growth or hepatic fibrosis are correlated with hepatic growth and growth of intrahepatic portal vasculature after attenuation of a CPSS and thus may contribute to postoperative recovery. Therefore, the aim of this study was to evaluate hepatic expression of genes involved in hepatic growth, apoptosis, vascular growth and fibrosis in relation to the outcome after surgical shunt attenuation in dogs with a CPSS.

Animals and Methods

Surgery

Dogs referred for surgical attenuation of a single CPSS were entered into the study. The study design was approved by the Ethics Committee on Animal Experimentation in compliance with Dutch legislation. The localization of the CPSS was preoperatively visualized with ultrasonography. All surgeries were performed by the same surgeon. After exploration of the abdominal cavity via a median celiotomy, the shunt was ligated over a gauged rod to the smallest diameter possible that did not induce portal hypertension, using a nonabsorbable 2-0 polyester suture[a]. Wedge biopsies of the liver were taken during surgery. After collection, the biopsies were immediately frozen in liquid nitrogen and stored at –70°C until gene expression analysis.

Postoperative recovery was assessed at one to two months after surgery in all dogs that had survived. Abdominal ultrasonography was performed to examine the site and patency of the attenuated shunt and to identify development of acquired portosystemic vessels. Functional portosystemic shunting was evaluated by determining 12-hour fasting plasma ammonia concentration (reference values 24-45 μM) and applying a rectal ammonia tolerance test.[15] Complete recovery was defined by resolution of clinical signs, finding normal fasting plasma ammonia concentrations and a normal rectal ammonia tolerance test. Dogs without complete recovery and dogs that died or were euthanized after surgery because of portal hypertension or persistent shunting were considered to be not recovered. If shunt attenuation was not feasible because of portal hypertension during attempts of shunt ligation, dogs were also considered as not recovered. Dogs that died during or after surgery from reasons unrelated to portal hypoplasia or persistent shunting and dogs in which the outcome after shunt attenuation was unclear were excluded from the study.

Gene expression

In liver tissue that was collected during surgery, the expression of 19 gene products with potential prognostic value was measured. Total RNA was extracted from each liver sample using a RNeasy Mini kit[b] according to the manufacturer's protocol, including on-column DNase digestion. RNA quality and quantity was determined spectrophotometrically using a Nanodrop ND-1000[c]. cDNA was synthesized with an MMLV-derived reverse transcriptase[d] according to the manufacturer's protocol.

The genes of interest were selected from 4 different categories of pathways involved in tissue repair: proliferation, apoptosis, angiogenesis and fibrosis (Table 1).

[a]*Ethibond*^{tm} *excel 2-0*, Ethicon INC., Somerville, NJ, USA
[b]RNeasy Mini kit, Qiagen, Venlo, The Netherlands
[c]Nanodrop ND-1000, Isogen Life Science, IJsselstein, The Netherlands
[d]iScript cDNA Synthesis Kit, Bio-Rad, Veenendaal, The Netherlands

Some genes were known to be differently expressed in CPSS dogs compared with normal dogs (HGF, HGFac, c-MET, TGF-βR2, Casp3, Bcl-2, and uPA).[11]

Table 1. Gene products for which expression was measured in liver biopsies.

Category	Gene products
Proliferation	Hepatocyte growth factor (HGF), c-MET (a tyrosine kinase HGF receptor), HGF activator (HGFac), insulin-like growth factor 1 (IGF-1), insulin-like growth factor receptor (IGF-R), insulin-like growth factor binding protein 3 (IGFBP-3), ABCG2 (a stem cell marker), BMI1 (a marker of stem cell renewal), CCND1 (a marker of progression of the cell cycle), CDC6 (a proliferative factor), and MAT2a (methionine adenosyltransferase 2 alpha, a regulatory factor of cell differentiation and proliferation).
Apoptosis	Bcl-2 and PIM-2 (both anti-apoptotic gene products), Casp3 (Caspase-3, pro-apoptotic product)
Angiogenesis	Vascular endothelial growth factor (VEGFa) and its receptor (KDR)
Fibrosis	Transforming growth factor ß1 receptor 1 and 2 (TGF-βR1, TGF-βR2) and urokinase plasminogen activator (uPA, activator of TGF-β1 and HGF).

Expression of gene products was determined by quantitative real-time polymerase chain reaction assays (qPCR) as previously described.[16] The characteristics of the dog-specific primers that were used for the qPCR measurements are described in Table 2. The maximum number of qPCR cycles performed was 45. The amount of each gene product was calculated as the ^{2}logarithm of the first cycle in the qPCR in which the cDNA product of the gene was detected (threshold cycle), which is directly correlated with the starting concentration of mRNA in the sample. For normalization, the endogenous and independent reference genes B2M, RPS5, and HPRT were selected.[16] The mRNA expression was described as the ratio between the calculated mRNA amount of each specific gene and the averaged mRNA amount of the reference genes in the sample.

Univariable statistical analyses

All statistical analyses were performed using commercial software[e]. The Kolmogorov-Smirnov test was used to evaluate Normality of data. Differences in gene expression between recovered and non-recovered dogs were evaluated by two-sample T-tests (for Normally distributed data, respectively c-MET, HGFac, ABCG2, KDR) and Mann-Whitney U tests (for data not Normally distributed, respectively HGF, IGF1, IGF-R, IGF-BP3, BMI1, CCND1, CDC6, MAT2a, Bcl-2, PIM2, Casp3, VEGFa, TGF-βR1, TGF-βR2, uPA). A p value < 0.05 was considered significant.

[e]SPSS for Windows, release 16.0.1, SPSS, Inc, Chicago, IL, USA

Multivariable statistical analyses

Logistic regression was performed to evaluate the diagnostic potential of presumptive prognostic factors in all 48 dogs, with outcome after surgery as dependent variable. Separate analyses were performed for each of the four categories of genes (proliferation, apoptosis, angiogenesis, fibrosis; Table 1). In each of these analyses, the gene products in the category studied as well as shunt type (extrahepatic versus intrahepatic) were included as independent variables. Gene products with more than one missing value were excluded from the analyses (IGF-R, CDC6, Bcl-2, KDR and TGF-βR1). For each of the four categories of genes, a final model was obtained in a backward stepwise procedure with a probability for stepwise entry of 0.05 and a probability for stepwise removal of 0.10 in the likelihood ratio test. Multicollinearity was evaluated by linear regression.[17] A tolerance <0.1, a variance inflation factor >10 or a condition index >15 were considered indicative of multicollinearity. Model fit was evaluated with the Hosmer-and-Lemeshow test. A final overall model was obtained by evaluating the expression of genes involved in apoptosis, angiogenesis and fibrosis in a backward stepwise procedure starting with a model in which HGFac and MAT2a were forced (i.e. the gene products included in the final model of the proliferation genes). The proportion of dogs with correctly predicted outcome was calculated using a classification cut-off value of 0.5. The Akaike's Information Criterion (AIC) was used to compare models.[18] In addition to the analyses including all shunt dogs, similar analyses were performed in dogs with an intrahepatic shunt and in dogs with an extrahepatic shunt, separately.

ROC analyses

For each gene product, a receiver operating characteristic (ROC) curve was plotted to determine the diagnostic sensitivity and specificity of a test to detect dogs that recover following surgery at various cut-off values between 'low' and 'high' expression of the gene.[18] Genes with an area under the ROC curve different from 0.5 at P values <0.25, were selected as binary explanatory variables ('low' versus 'high') for inclusion in a multivariate logistic regression (as described above) with outcome after surgery in all 48 dogs as dependent variable. For each selected gene, a cut-off value between 'low' and 'high' was chosen at the gene expression level that corresponded to the data point on the ROC curve with the smallest Euclidian distance to the point (1-Sp, Se) = (0, 1). Gene products with more than one missing value were excluded (IGF-R, CDC6, Bcl-2, KDR and TGF-βR1).

Table 2. Nucleotide sequences, exon locations, optimal melting temperatures, product sizes and accession numbers of dog-specific primers for real-time q-PCR

Gene	Primer	Sequence (5'-3')	Exon	MT (°C)	PS (bp)	Accession No.
ABCG2	Forward	TGCTGTCCTTTTGCTCCTG	6	58.0	192	DQ098684
	Reversed	GCTCACAATGGTAAC / CCACTG	8/7			
Bcl-2	Forward	TGGAGAGCGTCAACCGGGAGATGT	3	61.0	87	AB116145
	Reversed	AGGTGTGCAGATGCCGGTTCAGGT	3			
BMI-1	Forward	TGGACTGACAAATGCTGGAGAACT	10	68.0	139	XM_544225
	Reversed	AGGGAACTGAGGATGAGGAGACTG	10			
Casp3	Forward	ATCACTGAAGATGGATGGGTTGGT	9	58.0	140	NM_001003042
	Reversed	GAAAGGAGCATGTTCTGAAGTAGCACT	9			
CDC6	Forward	CAGTTCTGTGCCCGAAAAGTC	8	63.5	291	XM_537648
	Reversed	GAGGAGCAAAGAGCAGACCAAG	10			
c-MET[1]	Forward	TGTGCTGTGAAATCCCTGAATA / GAAATC	15/16	59.0	112	NM_001002963
	Reversed	CCAAGAGTGAGAGTACGTTTGGATGAC	16			
CCND1	Forward	ACTACCTGGACCGCT	1	58.0	150	NM_001005757
	Reversed	CGGATGGAGTTGTCA	1			
HGF[1]	Forward	AAAGGAGATGAGAAACGCAAACAG	28	58.0	92	NM_001002964
	Reversed	GGCCTAGCAAGCTTCAGTAATACC	28			
HGFac	Forward	ACACAGACGTTTGGCATCGAGAAGTAT	11	60.0	128	AY458142
	Reversed	AAACTGGAGCGGATGGCACAG	12			
IGF-1	Forward	TGTCCTCCTCGCATCTCTT	2	58.0	122	XM_848024.1
	Reversed	GTCTCCGCACACGAACTG	2			
IGF-R	Forward	CATGCCTTGGTCTCCCTGT	4	60.0	129	XM_853622
	Reversed	GGTGGTCCCAATCCCAAAG	5			

IGFBP-3	Forward	CTGCACACGAAGATGGATGT	5	61.0	127	XM_548740.2
	Reversed	TATTCCGTCTCCCGCTTGTA	5			
KDR	Forward	GGAAGAGGAAGTGTGTGACCCC	27	64.0	181	XM_539273
	Reversed	GACCATACCACTGTCCGTCTGG	29			
MAT2A	Forward	TGCTTTTGGCGGGGAGGAG	9	67.0	121	XM_532980
	Reversed	TTTAAAAGCTGCCATCTGAGGTGA	9			
PIM-2	Forward	TGATCCGCCTGCTTGACTGGTTTG	4	65.0	112	XM_548990
	Reversed	CCTAATGGGCCCTGCTCTTGTATG	4			
TGF-βR1	Forward	CAGTCACCGAGACCACAGACAAAGT	4	59.0	101	AY455799
	Reversed	TGAAGATGGTGCACAAACAAATGG	4			
TGF-βR2	Forward	GACCTGCTGCCTGTGTGACTTTG	3	61.0	116	XM_534237
	Reversed	GGACTTCGGGAGCCATGTATCTTG	4			
uPA	Forward	CTGGGGAGATGAAGTTTGAGGTGG	7	64.5	115	XM_536394
	Reversed	TGGAACGGATCTTCAGCAAGG / C	8/7			
VEGFa	Forward	CTTTCTGCTCTCCTGGGTGC	1	58.0	101	NM_001003175
	Reversed	GGTTTGTGCTCTCCTCCTGC	2			
B2M[2]	Forward	TCCTCATCCTCCTCGCT	1	62.0	85	XM_535458
	Reversed	TTCTCTGCTGGGTGTCG	2			
HPRT[2]	Forward	AG/CTTGCTGGTGAAAAGGAC	5/6	56.0	114	AY283372
	Reversed	TTATAGTCAAGGGCATATCC	7			
RPS5[2]	Forward	TCACTGGTGAG/AACCCCCT	2/3	62.5	141	XM_533568
	Reversed	CCTGATTCACACGGCGTAG	3			

Abbreviations: MT, melting temperature; PS, product size; bp, base pairs.

Note. If a primer is located on 2 exons, the junctions are shown with a dividing forward slash (/).

[1]Primers previously reported by Spee B, et al. Comp Hepatol 2005,4:7.

[2]Endogenous reference genes, primers previously reported by Brinkhof B, et al. Anal Biochem 2006;356:36-43.

Results

Forty-eight dogs were entered into the study: 32 dogs with an extrahepatic shunt and 16 dogs with an intrahepatic shunt. After surgical attenuation of the shunt, 26 dogs had recovered completely: 21 dogs with an extrahepatic shunt (66%) and 5 dogs with an intrahepatic shunt (31%). The 22 non-recovered dogs included 6 dogs where shunt attenuation was not feasible due to severe hypoplasia of the portal vein, 1 dog that probably died because of postoperative portal hypertension and 1 dog that was euthanized because of persistent portosystemic shunting without clinical improvement after shunt attenuation. In 13 of 14 surviving dogs without recovery of portosystemic shunting after CPSS attenuation, ultrasonography of the abdomen was performed after surgery. In 7 dogs the original shunt was still functional, in 2 dogs acquired shunts had developed, in 1 dog patency of the original shunt and also acquired shunts were found and in 2 dogs the location of shunting could not be visualized. These 12 dogs and the dog without postoperative ultrasonography were treated conservatively and showed no clinical problems after surgery. In 1 dog, a second single CPSS was found that had become functional after surgery. This dog made a full recovery after the second shunt was surgically closed. In 24 of 26 recovered dogs ultrasonography of the abdomen was performed after surgery. In 12 dogs the original shunt could not be detected or no flow was visible in the shunt, in 4 dogs mild reversed flow was found towards the liver, in 4 dogs mild flow was found towards the systemic circulation and in 4 dogs evaluation of the site of the original shunt was not possible. In none of the recovered dogs acquired shunts were detected.

Median age at surgery in extrahepatic shunt dogs was 9.8 months, with a range of 3 to 43 months. Age at surgery in intrahepatic shunt dogs was significantly lower (P=0.012), with a median of 6.1 months and a range of 4.1 to 12.6 months. Within both groups no differences in age existed between recovered and non-recovered dogs. In the extrahepatic shunt dogs, median body weight was 3.9 kg, ranging from 0.85 up to 18.9 kg. The extrahepatic CPSS group consisted of 29 dogs of 12 different breeds and 3 mixed breed dogs. The dogs were small sized breeds, except one Bordeaux dog, one Rottweiler and one mixed breed dog (18.9 kg). In the intrahepatic shunt dogs, median weight was 19.8 kg, ranging from 3.7 up to 34 kg. Besides one Maltese dog, the rest of this group (15 dogs) consisted of 11 medium to large-sized breeds. In both groups, with extrahepatic and intrahepatic CPSS, no significant differences in weight were noticed between dogs that had recovered or dogs that had not.

Univariable analyses

The expression of MAT2a in liver was higher (P=0.005) in recovered dogs (median, 0.62) compared to non-recovered dogs (median, 0.37). Also, in dogs with an extrahepatic shunt, the expression of MAT2a in liver was higher (P=0.02) in recovered

dogs (median, 0.64) than in non-recovered dogs (median, 0.39). In dogs with an intrahepatic shunt, there was no difference in the expression of MAT2a between recovered versus non-recovered dogs. The expression of MAT2a was plotted in Figure 1. The expression of none of the other gene products differed significantly between recovered and non-recovered dogs.

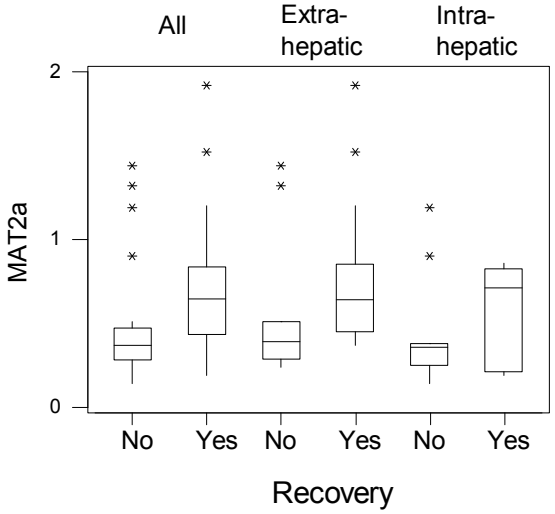

Figure 1. Box-and-whisker plot of the intraoperative mRNA expression of MAT2a in all dogs with a CPSS and the dogs with an extrahepatic or intrahepatic CPSS, with or without recovery following surgical intervention (the boxes indicate the first, second and third quartile. Whiskers extend to the lowest and highest observations within 1.5 times the inter-quartile range from the first and third quartile; outliers outside this region are plotted with asterisks).

Multivariable analyses

The expression of c-MET, IGF-1 and MAT2a was associated with type of shunt (P=0.003, P=0.015 and P=0.023, respectively). Because of multicollinearity between type of shunt and these gene products, type of shunt had to be omitted in the analysis of genes involved in proliferation (category 1). In this category, the final model for the probability of recovery included both HGFac and MAT2a. In the categories of genes for apoptosis, angiogenesis or fibrosis, none of the gene products significantly contributed to the final model. Therefore, the final model of each category only included a constant and type of shunt.

The final overall model included only HGFac and MAT2a as significant variables and a constant. None of the gene products involved in apoptosis, angiogenesis or fibrosis significantly improved this model. This model correctly predicted outcome after surgical attenuation of the shunt in 65% of the dogs. For comparison, a model including only type of shunt (and a constant) correctly predicted outcome in 67% of the study dogs. The AIC of both models was comparable (67.6 and 67.1, respectively).
None of the gene products had a significant effect in any of the separate analyses of the subset of intrahepatic shunt dogs or the extrahepatic shunt dogs.

ROC analyses
The ROC curves of CDC6, cMET, HGFac, MAT2a and TGF-βR1 had area's under the curve that were different from 0.5 at P values <0.25. In the analyses of the selected genes expressed as binary variables, there was no multicollinearity between type of shunt and the genes. Therefore, type of shunt as well as all selected genes could be analyzed in a single analysis. The final overall model included HGFac and MAT2a only and this model correctly predicted outcome in 75% of dogs (Table 3). Cut-off values of MAT2a and HGFac were 0.457 and 0.974, respectively (Figure 2). The AIC of this model was 61.4, which indicated that the model with HGFac and MAT2a as binary variables was the best model explaining the data with a minimum of parameters.

Table 3. Final overall prognostic model of intraoperative hepatic mRNA expression as binary variables ('low' or 'high') in 48 dogs with CPSS

Gene		Estimate	Standard error	Pr > Chi-square (Wald)	Odds ratio	
					Estimate	95% CI
HGFac	High	1.75	0.74	0.018	5.73	1.35; 24.3
	Low	1.00				
MAT2a	High	2.13	0.73	0.003	8.40	2.01; 35.0
	Low	1.00				
Constant		-1.60	0.60	0.008	0.20	

Abbreviations: CPSS, congenital portosystemic shunt; Pr, probability; CI, confidence interval

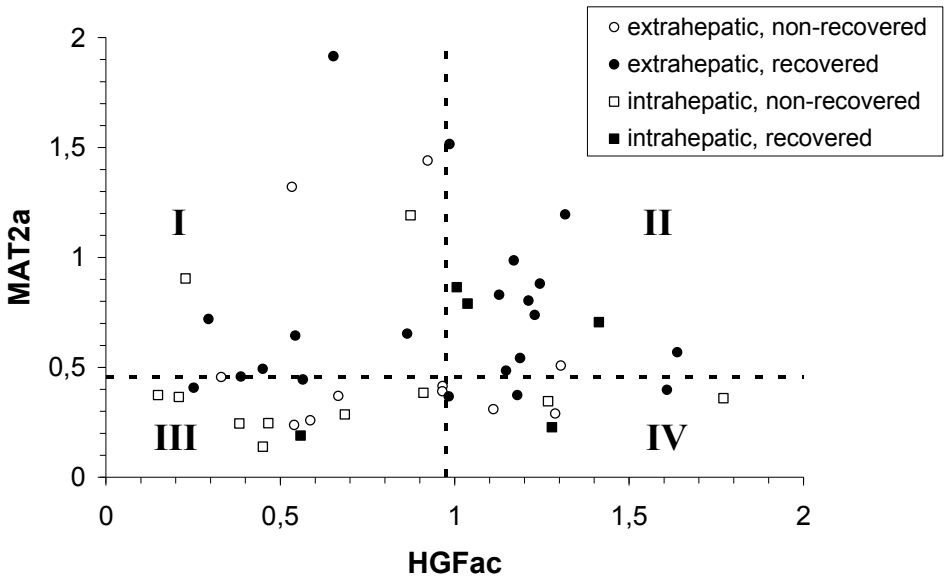

Figure 2. Intraoperative hepatic mRNA expression of MAT2a and HGFac in 48 dogs with CPSS. Cut-off values between 'low' and 'high' expression of MAT2a and HGFac are 0.457 and 0.974, respectively (interrupted lines). In each quadrant, predicted probabilities (95% confidence intervals) of recovery after attenuation of a CPSS, based on the logistic regression model with binary expression of genes, were: I) 63% (21%, 92%), II) 91% (48%, 99%), III) 17% (6%, 40%), and IV) 54% (15%, 88%).

Discussion

The preoperative hepatic expression levels of methionine adenosyltransferase 2 alpha (MAT2a) and HGF activator (HGFac) were associated with complete recovery after CPSS attenuation. Higher expression of MAT2a and HGFac mRNA in livers of dogs that recover after CPSS attenuation, fits with the role of hepatic proliferation in postoperative recovery and may predict good outcome in dogs with a CPSS.

Methionine adenosyltransferases are essential cellular enzymes in three metabolic pathways: polyamine synthesis, transmethylation and transsulfuration. In mammals, MAT1a is the form expressed in adult quiescent hepatocytes and MAT2a, predominantly expressed in fetal liver, is required for liver cell proliferation and, in adult mammals, for restoration of hepatic mass after liver injury of partial hepatectomy.[19-21] So, the ability of hypoplastic liver tissue to grow in dogs that recover well following CPSS attenuation may easily be explained by a higher expression of MAT2a. Future Western blot analysis of the active MAT2a protein in hepatic samples

will be needed to confirm its contribution to hepatic proliferation after CPSS attenuation.

HGF activator gene expression was not significantly different between recovered and non-recovered dogs in the univariate analyses (P=0.07), but in the multivariate analyses it proved to have significant prognostic value in the final models. Compared with normal dogs, the expression of HGFac was doubled in dogs with CPSS and halved in case of primary portal vein hypoplasia (PPVH), which is a non-regenerative portal vein disease.[11] HGF activator is a protease that activates single-chain HGF, which is an important mitogen in liver growth and regeneration.[22] Similarly, an increased expression of HGFac may contribute to liver growth and a favourable outcome following CPSS attenuation.

Besides HGFac, also factors Bcl-2 and uPA were reported to be differently expressed between CPSS and PPVH: in dogs with CPSS, hepatic expression of uPA and Bcl-2 was lower compared to dogs with PPVH.[11] CPSS and PPVH are both congenital hepatic diseases with reduced liver growth and portal vein hypoperfusion.[23] In contrast to PPVH, in CPSS dogs these changes are supposed to be secondary to the portosystemic shunting and potentially reversible in dogs that recover after shunt attenuation.[23,24] Theoretically, a combination of CPSS and PPVH could be responsible for failure of recovery after shunt attenuation. This theory was not supported by the results of this study as the expression of Bcl-2 and uPA was not significantly different between recovered and non-recovered dogs.

Genes that predict outcome can be used to make a preoperative decision with respect to treatment. The final prognostic model including MAT2a and HGFac as binary variables better predicted surgical outcome than anatomic localization of the shunt. Therefore, this model could potentially be useful in making a decision on individual treatment. Expression levels of MAT2a and HGFac could aid in selecting dogs with very little chance of recovery following surgical CPSS attenuation (Figure 2). With this additional knowledge, owners may prefer to treat their dogs conservatively, without the risks and costs of surgical treatment. If dogs with a MAT2a and HGFac expression below the cut-off values (<0.457 and <0.974, respectively) had been excluded from surgery, 13 of 22 dogs that failed to recover and 3 of 26 dogs that recovered would not have been operated. Thus, the recovery rate in this set of dogs would have increased from 26 of 48 dogs (54%) to 23 of 32 dogs (72%). However, before clinical application of this model will be advisable, validation of this model in an independent dataset including a larger number of dogs is essential.

The expression of MAT2a as a continuous variable was correlated with CPSS localization, preventing inclusion of both MAT2a and shunt localization in a single model. MAT2a may be a confounder for the effect of shunt localization on individual surgical outcome. However, given the biological function of MAT2a in hepatic proliferation, it seems more plausible that MAT2a is an intervening variable between

shunt localization and individual outcome. An increased preoperative expression of MAT2a in the dogs with extrahepatic shunts might be a biological explanation why the prognosis in these dogs was better than in dogs with intrahepatic shunts.

Except MAT2a and HGFac, the individual expression patterns of the other gene products measured in this study seem to be of little relevance as prognostic factors. In a previous study, mRNA expression of some growth factors, such as HGF and c-MET, were lowered in CPSS dogs as compared to healthy control animals.[11]Although the reported lower mRNA expression of growth factors in CPSS dogs compared to healthy dogs fits well with the characteristic hepatic hypoplasia in CPSS patients, the level of mRNA expression of these factors in dogs before attenuation of their shunt is not predictive for recovery. However, increasing mRNA expression and synthesis of biologically active products of these factors after attenuation of the shunt might be more important for recovery.

Many different growth factors, signaling pathways and regulatory mechanisms are involved in hepatic growth and regeneration. Despite multiple studies of liver regeneration, many aspects of this phenomenon and of other mechanisms that determine recovery in CPSS dogs remain to be further studied.[8] The selection of genes that may affect postoperative recovery is difficult and expression of unknown genes may also have prognostic value for surgical outcome after CPSS attenuation. Therefore, further studies are required to elucidate specific genes that are involved in hepatic and vascular proliferation or recovery after CPSS attenuation.

Recovery rates after attenuation of a CPSS are probably not identical among dog populations because of differences in distribution of breeds, differences within breed populations and different surgical techniques that are used.[1,2,25,26] Therefore, the prognostic value of hepatic gene expression may be population specific, resulting in differences in clinical applicability of predictive models. This study was restricted to dogs with CPSS referred to one university clinic, operated by the same surgeon using one technique. However, mechanisms of hepatic and vascular growth after surgical attenuation of the shunt are probably comparable in dogs in different populations and with different surgical techniques that are used. Therefore, the concepts of the present study might be equally valid to dog populations in other clinics and countries.

In conclusion, intraoperative expression of MAT2a and HGFac mRNA was positively related to individual outcome after CPSS attenuation in dogs, which fits well with their biological function in liver cell proliferation. A model including binary variables based on MAT2a and HGFac mRNA expression (high or low expression compared to specific cut-off values) obtained a better prediction of individual response after surgical CPSS attenuation than CPSS localization (75% and 67% respectively), and allowed identification of dogs with a poor prognosis. Future validation of this model is essential to evaluate applicability as a clinical tool in prediction of outcome after CPSS ligation, preferably in a multi-centered study.

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References

1. Hunt GB, Kummeling A, Tisdall PLC, et al. Outcomes of cellophane banding for congenital portosystemic shunts in 106 dogs and 5 cats. Vet Surg 2004;33:25-31.
2. Kummeling A, van Sluijs FJ, Rothuizen J. Prognostic implications of the degree of shunt narrowing and of the portal vein diameter in dogs with congenital portosystemic shunts. Vet Surg 2004;33:17-24.
3. Lee KC, Lipscomb VJ, Lamb CR, et al. Association of portovenographic findings with outcome in dogs receiving surgical treatment for single congenital portosystemic shunts: 45 cases (2000-2004). J Am Vet Med Assoc 2006;229:1122-1129.
4. Hunt GB, Hughes J. Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 1999;77:303-307.
5. Stieger SM, Zwingenberger A, Pollard RE, et al. Hepatic volume estimation using quantitative computed tomography in dogs with portosystemic shunts. Vet Radiol Ultrasound 2007;48:409-413.
6. Zaitoun AA, Path FRC, Apelqvist G, et al. Quantitative studies of liver atrophy after portacaval shunt in the rat. J Surg Res 2006;131:225-232.
7. Meyer HP, Rothuizen J, van Sluijs FJ, et al. Progressive remission of portosystemic shunting in 23 dogs after partial closure of congenital portosystemic shunts. Vet Rec 1999;144:333-337.
8. Michalopoulos GK. Liver regeneration. J. Cell. Physiol 2007;213:286-300.
9. Diehl AM, Rai RM. Regulation of signal transduction during liver regeneration. FASEB J 1996;10:215-227.
10. Bockhorn M, Goralski M, Prokofiev D, et al. VEGFa is important for early liver regeneration after partial hepatoectomy. J Surg Res 2007;138:291-299.
11. Spee B, Penning LC, van den Ingh TSGAM, et al. Regenerative and fibrotic pathways in canine hepatic portosystemic shunt and portal vein hypoplasia, new models for clinical hepatocyte growth factor treatment. Comp Hepatol 2005; 4:7.
12. Shackel NA, Seth D, Haber PS, et al. The hepatic transcriptome in human liver disease. Comp Hepatol 2006;5:6.
13. Wang W, Yang LY, Huang GW, et al. Genomic analysis reveals RhoC as a potential marker in hepatocellular carcinoma with poor prognosis. Br J Cancer 2004;90:2349-2355.
14. Duong C, Greenawalt DM, Kowalczyk A, et al. Pretreatment gene expression profiles can be used to predict response to neoadjuvant chemotherapy in esophageal cancer. Ann Surg Oncol 2007;14:3602-3609.
15. Rothuizen J, van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22-25.
16. Brinkhof B, Spee B, Rothuizen J, Penning LC. Development and evaluation of canine reference genes for accurate quantification of gene expression. Anal Biochem 2006;356:36-43.
17. Field A. Discovering statistics using SPSS, 2nd ed. London, UK: Sage Publications Ltd; 2005:258-263.

18. Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. Prince Edward Island, Canada: AVC Inc.;2003:104-108, 317-334.
19. Lu SC, Mato JM. S-adenosylmethionine in cell growth, apoptosis and liver cancer. J Gastroenterol Hepatol 2008;23:S73-77.
20. Pañeda C, Gorospe I, Herrera B, et al. Liver cell proliferation requires methionine adenosyltransferase 2A mRNA upregulation. Hepatology 2002;35:1381-1391.
21. Mato JM, Corrales FJ, Lu SC, Avila MA. S-adenosylmethionine: a control switch that regulates liver function. FASEB J 2002;16:15-26.
22. Miyazawa K, Shimomura T, Kitamura A, et al. Molecular cloning and sequence analysis of the cDNA for a human serine protease responsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation factor XII. J Biol Chem 1993;268:10024-10028.
23. Cullen JM, van den Ingh TSGAM, Bunch SE, et al. Morphological classification of the circulatory disorders of the canine and feline liver. In: WSAVA Standards for clinical and histological diagnosis of canine and feline liver diseases. Philadelphia: Saunders Elsevier;2006:41-59.
24. Baade S, Aupperle H, Grevel V, Schoon HA. Histopathological and immunohistochemical investigations of hepatic lesions associated with congenital portosystemic shunt in dogs. J Comp Pathol 2006;134:84-94.
25. Krotschek U, Adin CA, Hunt GB, et al. Epidemiologic factors associated with the anatomic location of intrahepatic portosystemic shunts in dogs. Vet Surg 2007;36:31-36.
26. Mehl ML, Kyles AE, Hardie EM, et al. Evaluation of ameroid ring constrictors for treatment for single extrahepatic portosystemic shunts in dogs: 168 cases (1995–2001). J Am Vet Med Assoc 2005;226:2020-2030.

Chapter 8

Comparison of hepatic gene expression profiles in dogs with different outcome after attenuation of a congenital portosystemic shunt using microarray analysis

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Abstract

Background: In dogs with a congenital portosystemic shunt (CPSS), recovery after surgical CPSS attenuation is difficult to predict, but may be associated with hepatic and portal vein proliferation.
Objectives: the aim of this study is to identify genes from that are differently expressed in liver tissue obtained during surgery from dogs that recovered and did not recover after surgical attenuation of a CPSS. Differently expression of genes may elucidate mechanisms that are important for postoperative recovery and provide classifying genes that predict outcome.
Animals: Forty-six client owned dogs with a single extrahepatic or intrahepatic CPSS.
Methods: Using 60-mer oligo microarrays, mRNA expression of 42,034 different gene probes was determined in liver tissue that was collected in dogs with a CPSS during surgical shunt attenuation and compared with a canine hepatic reference pool. The expression profiles of recovered dogs were compared with those of non-recovered dogs and a classifier was constructed.
Results: 63 genes were differently expressed between 23 recovered and 23 non-recovered CPSS dogs. In recovered dogs, higher mRNA expression was found in genes from several pathways, of which some are involved in hepatic growth, like s-adenosylmethionine metabolism. In non-recovered dogs, mRNA expression was higher in a number of genes involved in hepatic fibrosis and endothelial inflammation. A classifier provided no better prediction of postoperative outcome than type of CPSS (intrahepatic or extrahepatic).
Conclusion and Clinical Importance: Several of the differently expressed genes may affect recovery after CPSS attenuation in dogs. Although validation and confirmation of protein activity in hepatic tissue is essential, this study selected promising genes and pathways for future study in dogs with CPSS.

Introduction

In dogs with a congenital portosystemic shunt (CPSS), portal blood is shunted around the liver directly into the systemic circulation, resulting in macroscopic and microscopic liver hypoplasia.[1-3] The abnormal shunting vessel can be localized inside or outside the liver (intrahepatic or extrahepatic CPSS). In many dogs with a CPSS, hepatic function is completely restored after surgical attenuation of the shunt. However, portosystemic shunting, with or without clinical disease, persists in 10 to 20% of dogs, regardless of the surgical technique employed for shunt closure.[4-6]

Prediction of the long-term outcome after surgical attenuation of a CPSS is difficult, even though prognostic factors have been studied extensively. An important factor affecting long-term outcome is probably the ability of the liver and portal vein to adapt to the increased portal blood flow after shunt attenuation and to grow to "normal' size and function.[4,5] The ability of the liver and portal vasculature to grow after attenuation of a CPSS may be correlated with hepatic expression of genes involved in proliferation or apoptosis of hepatocytes, hepatic fibrosis or vascular growth. Intraoperative hepatic mRNA expression of 19 proliferation related genes was previously measured in 48 CPSS dogs using qPCR techniques and related to outcome after shunt attenuation. The expression levels of methionine adenosyltransferase 2 alpha (MAT2a) and hepatic growth factor activator (HGFac) were associated with complete recovery after CPSS attenuation. Both enzymes are involved in liver cell proliferation and the increased expression in dogs with good postoperative recovery fits well with their biological function. The expression patterns of the other 17 gene products were not different between recovered and non-recovered dogs.[7]

A wide variety of factors and regulatory pathways have been found to affect liver regeneration after partial hepatectomy.[8,9] Nevertheless, many aspects of this phenomenon or other mechanisms that may affect postoperative recovery in CPSS dogs remain to be understood. Different unexpected gene products may be related to postoperative recovery from this disease. In this study, it was hypothesized that preoperative differences in hepatic gene expression profiles exist between dogs with different outcome after surgical treatment. These differences may elucidate mechanisms that are important for postoperative recovery. Therefore, the primary aim of this study was to identify genes that are differently expressed between dogs that recover and dogs that do not recover after surgical attenuation of a CPSS, using microarray expression profiling in liver tissue obtained during surgery. Secondly, these differently expressed genes may be candidate classifiers that predict outcome after surgical CPSS attenuation in individual dogs.

Animals and Methods

Surgery

Dogs that were referred for surgical attenuation of a single CPSS, either intrahepatic or extrahepatic, were entered into the study without extra selection criteria. The design of the study was approved by the Ethics Committee on Animal Experimentation according to Dutch legislation. The localization of the CPSS (intrahepatic or extrahepatic) was preoperatively visualized with ultrasonography. All surgeries were performed by the same surgeon. After exploration of the abdominal cavity via a median celiotomy, the shunt was ligated over a gauged rod to the smallest diameter that did not induce portal hypertension, using a nonabsorbable 2-0 polyester suture[a]. Wedge biopsies of the liver were routinely taken during surgery for histopathology. Part of the hepatic tissue was frozen in liquid nitrogen immediately after collection and stored at –70°C until gene expression analysis.

Postoperative recovery was assessed at control visits, 1-2 months after surgery, in all dogs that had survived. Persistent portosystemic shunting was evaluated by determining 12-hour fasting plasma ammonia concentration (reference value 24-45 μM) and applying a rectal ammonia tolerance test.[10] Complete recovery was defined as resolution of all clinical signs, finding normal fasting plasma ammonia concentrations and a normal result of the rectal ammonia tolerance test. During control visits, abdominal ultrasonography was performed to examine the site and patency of the attenuated shunt and to identify development of acquired portosystemic vessels. Dogs with metabolic evidence of persistent portosystemic shunting were considered as not recovered. Dogs that died or were euthanized after surgery because of portal hypertension, hypoplasia of the portal vasculature or persistent shunting were considered as not recovered. If shunt attenuation was not feasible because of portal hypertension during attempts of shunt ligation, dogs were also considered as not recovered. Dogs that died from reasons unrelated to portal hypoplasia or persistent shunting and dogs in which the outcome after shunt attenuation was unclear were excluded from the study.

Microarray analyses

Total RNA was extracted from each liver sample using a RNeasy Mini kit[b] according to the manufacturer's protocol, including on-column DNase digestion. RNA quality and quantity was determined spectrophotometrically using a Nanodrop ND-1000[c].

[a] *Ethibond*^{tm} *excel 2-0*, Ethicon INC., Somerville, NJ, USA
[b] RNeasy Mini kit, Qiagen, Venlo, The Netherlands
[c] Nanodrop ND-1000, Isogen Life Science, IJsselstein, The Netherlands

RNA extracted from liver tissue that was obtained from 2 healthy beagle dogs without CPSS or other liver diseases, was pooled and used as reference RNA. RNA amplifications and labeling were performed as described previously on a customized ALH3000 LiquidHandling Workstation[d] with 3ug total RNA from each sample.[11]

Microarray slides used were Canine Gene Expression Microarrays V1, representing 42,034 canine-specific 60-mer probes in a 4x44K layout [e]. Hybridizations were done on a HS4800PRO system supplemented with QuadChambers[f], using 1 µg labeled cRNA per channel according to a previously published protocol.[12] Hybridized slides were scanned on an Agilent scanner[g] at 100% laser power, 30% PMT. After automated data extraction using Imagene 8.0 software[h], print-tip Lowess normalization was performed on mean spot-intensities.[13] Gene-specific dye bias was corrected based on a within-set estimate as previously described.[14]

Expression data of 42,034 different gene probes was analysed using ANOVA.[15] In a fixed effect analysis, sample, array and dye effects were modelled. P values were determined by a permutation F2-test, in which residuals were shuffled 5000 times globally. Genes with $p < 0.05$ after family wise error correction were considered significantly changed.

Classifier construction

Using the microarray results, we attempted to construct a gene set, a classifier, that permits to distinguish between CPSS dogs classed as R1 (recovery) and R0 (no recovery) using K-nearest Neighbour Classification.[16] The data was randomly divided in a training set and a test set, which was not used in the initial gene selection to avoid a selection bias.[17] In the first step, the training set was used to rank the genes based on t-test statistics. The 100 highest scoring genes were selected and the performance of this gene set was determined using the independent test set. This procedure was repeated 10,000 times using different training and test sets. In a second step the genes were ranked based on the frequency of occurrence in the 10,000 gene sets resulting from the first step. The final optimum gene set was determined using forward selection with leave-one-out cross-validation using all data. In order to investigate whether the classification was better than chance, the procedure described above was repeated using data with randomized class labels.

[d]Caliper Life Sciences NV/SA, Teralfene, Belgium
[e]Agilent Technologies, Diegem, Belgium
[f]Tecan Benelux B.V.B.A., Giessen, The Netherlands
[g]Agilent scanner G2565BA, Agilent Technologies, Amstelveen, The Netherlands
[h]Imagene 8.0, BioDiscovery, El Segundo, USA

Results

Forty-six dogs were entered into the study; 32 dogs with an extrahepatic shunt and 14 dogs with an intrahepatic shunt. After surgical attenuation of the shunt, 23 dogs had recovered completely and 23 dogs had not: 59% of the dogs with an extrahepatic shunt had recovered and 29% of the dogs with an intrahepatic shunt. Of the 19 recovered dogs with an extrahepatic shunt, 15 dogs had a portocaval shunt and 4 dogs had a portoazygos shunt. The 13 non-recovered dogs with an extrahepatic shunt included 11 dogs with a portocaval shunt and 2 dogs with a portoazygos shunt. Of the 4 recovered dogs with an intrahepatic shunt, 3 dogs had a left-sided shunt and 1 dog had a right-sided shunt. The 10 non-recovered dogs with an intrahepatic shunt consisted of 3 dogs with a left-sided shunt, 4 dogs with a right-sided shunt and 3 dogs with a shunt in the central division of the liver.

Median age at surgery in extrahepatic shunt dogs was 8.8 months, with a range of 3 months to 5.5 years. In the intrahepatic shunt dogs, median age was 6.0 months with a range of 3.8 to 17 months. No significant differences in age existed between dogs with an intrahepatic or extrahepatic CPSS or between recovered and non-recovered dogs. In the extrahepatic shunt dogs, median body weight was 3.9 kg, ranging from 0.85 up to 9.7 kg. The extrahepatic CPSS group consisted of 29 dogs of 8 different small-sized breeds and 3 mixed small breed dogs. In the intrahepatic shunt dogs, median weight was 20.6 kg, ranging from 8.2 up to 34 kg. This group consisted of 10 different medium to large-sized breeds. There were no significant differences in weight between dogs that had recovered or dogs that had not, both in the extra or intrahepatic CPSS group.

Abdominal ultrasonography was performed in all 23 recovered dogs: in 14 dogs the original shunt could not be detected or no flow was visible in the shunt, in 3 dogs mild reversed flow was visible towards the liver, in 4 dogs minor flow was noted towards the systemic circulation and in 2 dogs evaluation of the original shunt was not possible because of a full stomach. The 23 non-recovered dogs included 9 dogs in which shunt attenuation was not feasible due to severe hypoplasia of the portal vein. Of these 9 dogs, 4 dogs were euthanized intraoperatively at the owners request, 2 dogs died of postoperative portal hypertension shortly after the surgery and 3 dogs were discharged after surgery with conservative treatment. In 14 surviving dogs without recovery of portosystemic shunting, abdominal ultrasonography revealed patency of the original shunt in 9 dogs, multiple acquired shunts in 2 dogs and both abnormalities were observed in 1 dog. In 2 dogs the site of the original CPSS could not be visualized, but acquired shunting vessels were not noted. All 14 dogs were treated conservatively and showed no clinical signs after surgery.

Microarray analyses

Overall, 63 genes were identified with significantly different expression between dogs that recovered and dogs that did not recover after attenuation of the CPSS: 30 upregulated (higher expression levels in recovered versus non-recovered dogs) and 33 downregulated genes (lower expression in recovered versus non-recovered dogs). Proteins with known biological function, were previously described of 23 of the upregulated genes (Table 1) and of 23 of the downregulated genes (Table 2).

In the subset of 32 dogs with an extrahepatic CPSS, 55 genes were significantly differently expressed between recovered and non-recovered dogs. Protein products were previously described in 26 of the 32 upregulated genes (Table 3), and in 17 of the 23 downregulated genes (Table 4). In the subset of 18 intrahepatic shunt dogs, 16 genes were significantly differently expressed: 3 genes were upregulated and 13 genes were downregulated in the recovered dogs compared to the non-recovered dogs. Of the upregulated genes, 2 protein products were previously described: oxidative stress-induced growth inhibitor 1 and anaphase-promoting complex subunit 4, with M values of 0.90 and 1.04 respectively. Of the downregulated genes only one protein product was known: transthyretin precursor (prealbumin, TTR, M= -1.33).

With respect to the 30 genes that were higher expressed in recovered dogs compared to non-recovered dogs (upregulated genes, all CPSS dogs), 22 genes were differently expressed between the recovered CPSS and the reference dogs (3 genes were increased and 19 genes decreased in the CPSS recovered compared to the reference dogs) and 26 were differently expressed between non-recovered CPSS and the reference dogs (all 26 genes were decreased in the non-recovered CPSS compared to the reference dogs). With respect to the 33 genes that were expressed lower in recovered dogs compared to non-recovered dogs (downregulated genes, all CPSS dogs), 25 genes were differently expressed between the recovered CPSS and the reference dogs (20 genes were increased and 5 genes decreased in the CPSS recovered compared to the reference dogs) and 26 were differently expressed between non-recovered CPSS and the reference dogs (all 26 genes were increased in the non-recovered CPSS compared to the reference dogs). The distribution of the differently expressed gene products between dogs with successful and non-successful outcome after CPSS attenuation and the reference dogs are depicted in Figure 1.

Classifier

The mean predictive accuracy to correctly determine whether the sample was taken from a R1 sample or a R0 sample was 58.0%, with a 95% confidence interval of 57.9 - 58.1%. A two-sample t-test showed that the ability to classify samples using their gene expression profile was significantly better than the ability to classify samples with randomly assigned labels ($P < 0.001$). When the same method was applied to dogs with an extrahepatic shunt (32 dogs) the mean accuracy was only 53.9%. In comparison,

shunt type (intrahepatic or extrahepatic) had a predictive accuracy of 63.0%: 19 of 32 dogs with extrahepatic CPSS recovered, whereas 10 of 14 dogs with intrahepatic CPSS did not recover, resulting in a correct prediction in 29 of 46 dogs based on shunt type.

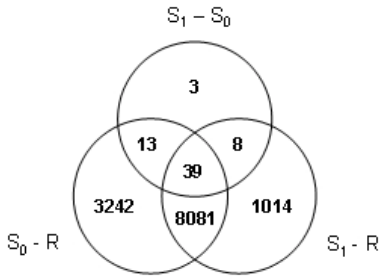

Figure 1. Venn diagram of differently expressed gene products between dogs with successful and non-successful outcome after CPSS attenuation ($S_1 – S_0$), between dogs with non-successful outcome after CPSS attenuation and healthy reference dogs ($S_0 – R$), and between dogs with successful outcome after CPSS attenuation and healthy reference dogs ($S_1 – R$).

Table 1. Upregulated genes in 23 recovered dogs compared to 23 non-recovered dogs after attenuation of a congenital portosystemic shunt

Gene ID	Gene Description	Abbreviation	P (fwer)	M
CFAB007129[1]	Glycine N-methyltransferase[2]	GNMT	.006	0.40
CFAB011870[1]	Glycine N-methyltransferase[2]	GNMT	<.0002	0.49
CFAB012960[1]	Microsomal glutathione S-transferase 2[2]	GST2	<.0002	0.83
CFAB021214	Histone-lysine N-methyltransferase, H3 lysine-79 specific[2]	HMT	.027	0.34
CFAB010956[1]	glutamate transporter[2]	GMT	.0068	0.51
CFAB017574	Glutaminase liver isoform, mitochondrial precursor[2]	GLS	.0098	0.49
CFAB034546[1]	N-acetylglutamate synthase, mitochondrial precursor[2]	NAGS	.0002	0.53
CFAB007814[1]	Cytochrome P450 2D15 (fragment)[2]	CYP2D15	<.0002	0.74
CFAB011279	Hepcidin precursor[2]	HEPC	.003	0.95
CFAB004360	Phosphatidylinositol 3-kinase regulatory subunit alpha[2]	PIK3Rα	.016	0.28
CFAB019919	Protein phosphatase Slingshot homolog 3	SSH-3L	.007	0.35
CFAB014452[1]	Retinol-binding protein III	RBP3	<.0002	0.60
CFAB013339[1]	Phospholemman precursor	FXYD1	.0006	0.51
CFAB020880	Glycerate kinase	GLYCK	.0068	0.33
CFAB014890	Transcription elongation factor A protein 3	TCEA3	.032	0.35
CFAB010977[1]	dimeric dihydrodiol dehydrogenase	DHDH	.0068	0.41
CFAB035318[1]	dimeric dihydrodiol dehydrogenase	DHDH	.012	0.48
CFAB032200[1]	17-beta-hydroxysteroid dehydrogenase 14	HSD17β14	.0002	0.43
CFAB015022	Hepatic triglyceride lipase (fragment)	LIPH	.008	0.47
CFAB014137[1]	Microfibrillar-associated protein 3-like precursor	MFAP3	<.0002	0.52
CFAB035301	Microfibrillar-associated protein 3-like precursor	MFAP3	.0024	0.30
CFAB023477[1]	Sorbitol dehydrogenase	SORD	.030	0.39
CFAB011641[1]	Small conductance calcium-activated potassium channel protein 2	SK2	<.0002	0.64

Abbreviations: P (fwer), P value after family wise error correction; M, M value=$\log_2(R/G)$; R and G (red and green) represent the background adjusted, averaged, normalized and dye-bias corrected intensity levels of the samples

[1]genes also upregulated in the subset of extrahepatic CPSS

[2]potential interesting gene products with respect to biological pathways in postoperative recovery

Table 2. Downregulated genes in 23 recovered dogs compared to 23 non-recovered dogs after attenuation of a congenital portosystemic shunt

Gene ID	Gene Description	Abbreviation	P (fwer)	M
CFAB013075[1]	Connective tissue growth factor precursor[2]	CTGF	.039	-0.78
CFAB016551[1]	Protein CYR61 precursor (IGF-binding protein 10)[2]	CYR61	<.0002	-0.82
CFAB017107	Monocyte to macrophage differentiation protein[2]	MMD	.0018	-0.33
CFAB032663	Vascular cell adhesion protein 1 precursor[2]	VCAM1	.016	-0.43
CFAB035472	Thioredoxin-interacting protein[2]	TXNIP	.0056	-0.53
CFAB031764	cAMP-dependent protein kinase inhibitor beta[2]	PKI- β	.018	-0.60
CFAB032434	Adipophilin (Adipose differentiation-related protein)[2]	ADRP	.027	-1.05
CFAB015010[1]	LysM and putative peptidoglycan-binding domain-containing protein 2	LYSMD2	<.0002	-0.40
CFAB041358[1]	Carboxypeptidase D precursor	CPD	.024	-0.30
CFAB011308	Caveolin-2	CAV2	.0006	-0.40
CFAB016786	Centrosomal protein of 192 kDa	CEP192	.016	-0.25
CFAB005422	FERM domain-containing protein 4B (GRP1-binding protein)	GRSP1	.020	-0.48
CFAB032554	Filamin-binding LIM protein 1	FBLP-1	.007	-0.35
CFAB014555[1]	Glutathione transferase omega-1	GSTO1	.005	-0.38
CFAB034095	Immunoglobulin heavy chain C gene segment	IGHV	.048	-0.28
CFAB013701	Nucleobindin-2 precursor	NUCB2	.024	-0.34
CFAB033863[1]	Protein disulfide-isomerase A4 precursor	ERp72	.0082	-0.45
CFAB029844	Proton myo-inositol cotransporter	HMIT	.039	-0.39
CFAB007171	Torsin-3A precursor	TOR3A	.0002	-0.61
CFAB010784	Transcription factor AP-2 beta	TFAP2-β	.029	-0.32
CFAB005790	Tripartite motif-containing protein 22	TRIM22	.022	-0.30
CFAB005224	XTP3-transactivated gene B protein precursor (Erlectin)	XTP3-B	.0044	-0.29
CFAB013773	Zinc finger protein DZIP1 (DAZ-interacting protein 1/2)	DZIP1	.0004	-0.46

Abbreviations: P (fwer), P value after family wise error correction; M, M value=$\log_2$(R/G); R and G (red and green) represent the background adjusted, averaged, normalized and dye-bias corrected intensity levels of the samples

[1]genes also downregulated in the subset of extrahepatic CPSS

[2]potential interesting gene products with respect to biological pathways in postoperative recovery

Table 3. Upregulated genes in 19 recovered dogs compared to 13 non-recovered dogs after attenuation of an extrahepatic congenital portosystemic shunt

Gene ID	Gene Description	Abbreviation	P (fwer)	M
CFAB007129	Glycine N-methyltransferase[1]	GNMT	.0008	0.48
CFAB011870	Glycine N-methyltransferase[1]	GNMT	.0002	0.51
CFAB012960	Microsomal glutathione S-transferase 2[1]	GST2	.003	0.76
CFAB016495	Glutathione S-transferase Mu 3[1]	GSTμ3	.03	0.67
CFAB010956	glutamate transporter[1]	GMT	.0002	0.65
CFAB034046	Glutaminase liver isoform, mitochondrial precursor[1]	GLS	.040	0.58
CFAB034546	N-acetylglutamate synthase, mitochondrial precursor[1]	NAGS	.030	0.60
CFAB007814	Cytochrome P450 2D15 (fragment)[1]	CYP2D15	<.0002	0.88
CFAB032716	Fibrinogen alpha chain (fragment)[1]	FGα	.0008	0.68
CFAB005469	Ubiquitin carrier protein (fragment)[1]	UCP	.031	0.50
CFAB014452	Retinol-binding protein III	RBP3	.049	0.55
CFAB013339	Phospholemman precursor	FXYD1	.035	0.55
CFAB017292	Transcription elongation factor A protein 3	TCEA3	.0026	0.41
CFAB010977	dimeric dihydrodiol dehydrogenase	DHDH	.0042	0.45
CFAB035318	dimeric dihydrodiol dehydrogenase	DHDH	.0002	0.54
CFAB032200	17-beta-hydroxysteroid dehydrogenase 14	HSD17β14	.027	0.42
CFAB014137	Microfibrillar-associated protein 3-like precursor	MFAP3	<.0002	0.50
CFAB023477	Sorbitol dehydrogenase	SORD	.0038	0.48
CFAB031911	Apolipoprotein C-IV precursor	ApoC-4	.0032	0.53
CFAB011641	Small conductance calcium-activated potassium channel protein 2	SK2	.013	0.63
CFAB013729	Emopamil-binding protein-like	EBPL	.045	0.37
CFAB010873	Hepatic flavin-containing monooxygenase 3	FMO3	.035	0.57
CFAB033014	Phytanoyl-CoA dioxygenase domain-containing protein 1	PHYHD1	.0006	0.62
CFAB032150	Phosphorylase b kinase regulatory subunit alpha, liver isoform	PHKA2	.025	0.43
CFAB016404	acyl-CoA synthetase medium-chain family member 4	ACSM4	.01	0.51
CFAB036157	Testis development protein NYD-SP15	NYD-SP15	.0052	0.65

Abbreviations: P (fwer), P value after family wise error correction; M, M value=$\log_2(R/G)$; R and G (red and green) represent the background adjusted, averaged, normalized and dye-bias corrected intensity levels of the samples

[1]potential interesting gene products with respect to biological pathways in postoperative recovery

Table 4. Downregulated genes in 19 recovered dogs compared to 13 non-recovered dogs after attenuation of an extrahepatic congenital portosystemic shunt

Gene ID	Gene Description	Abbreviation	P (fwer)	M
CFAB013075	Connective tissue growth factor precursor[1]	CTGF	.014	-0.71
CFAB016551	Protein CYR61 precursor (IGF-binding protein 10)[1]	CYR61	.0002	-0.87
CFAB011022	Interleukin-8 precursor[1]	IL-8	.0048	-0.81
CFAB032935	Krüppel-like factor 4[1]	KLF4	.027	-0.45
CFAB007416	Serglycin precursor (Platelet proteoglycan core protein) [1]	PPG	.0008	-0.79
CFAB038748	Mortality factor 4-like protein 1[1]	MORF4L1	.0002	-0.45
CFAB019745	Catenin delta-1 (p120 catenin)[1]	CTNND1	.001	-0.40
CFAB011157	Heat shock protein 90 kDa beta 1 (94 kDa glucose-regulated protein)	GRP94	.0086	-0.53
CFAB020668	DnaJ homolog subfamily C member 3	DNAJC3	.021	-0.46
CFAB015010	LysM and putative peptidoglycan-binding domain-containing protein 2	LYSMD2	.0026	-0.44
CFAB041358	Carboxypeptidase D precursor	CPD	.001	-0.40
CFAB014379	Rho-related GTP-binding protein Rho6 precursor	RND1	<.0002	-0.57
CFAB033863	Protein disulfide-isomerase A4 precursor	ERp72	.001	-0.56
CFAB014555	Glutathione transferase omega-1	GSTO1	.0002	-0.52
CFAB012931	HRAS-like suppressor 3	HRASLS3	.0002	-0.47
CFAB013701	Nucleobindin-2 precursor	NUCB2	.001	-0.44
CFAB011097	Signal recognition particle 54 kDa protein	SRP54	.016	-0.31

Abbreviations: P (fwer), P value after family wise error correction; M, M value=$\log_2$(R/G); R and G (red and green) represent the background adjusted, averaged, normalized and dye-bias corrected intensity levels of the samples

[1]potential interesting gene products with respect to biological pathways in postoperative recovery

Discussion

The gene products of some of the 63 differently expressed genes in the complete CPSS group (intrahepatic and extrahepatic CPSS) and of the 55 differently expressed genes in the subset of extrahepatic CPSS may have interesting biological functions with respect to recovery in CPSS. The subset of dogs with an intrahepatic CPSS did not seem to yield important genes with respect to recovery mechanisms, possibly because this group was relatively small, with a very small number of recovered dogs (4 dogs). The present conclusions are still preliminary because mRNA expression should be more accurately quantified with qPCR and activities of the protein products have to be confirmed. However, the identified genes seem to point at potential involvement of several hepatic pathways in postoperative recovery in dogs with a CPSS.

Genes upregulated in recovered compared to non-recovered CPSS dogs:
Two genes coding for glycine N-methyltransferase (GNMT) were upregulated in the dogs with a better outcome. In these recovered dogs, GNMT expression was similar to (all CPSS dogs) or higher than (extrahepatic CPSS dogs) its expression in the reference dogs, while GNMT expression was decreased in non-recovered dogs. GNMT plays a major role in hepatic methionine metabolism and in liver proliferation.[18-20] GNMT catalyzes the formation of S-adenosylhomocysteine (SAHcy) from S-adenosylmethionine (SAMe), which is synthesized from L-methionine and ATP by the enzyme methione adenosyltransferase (MAT). SAMe is important for hepatic growth, apoptosis, and sensitivity to liver injury. Exogenous SAMe administration inhibits hepatocellular growth by inhibition of HGF.[18,21] In rats, SAMe levels decrease dramatically shortly after partial hepatectomy (PH). The fall in SAMe releases the inhibition on HGF and allows the liver to respond to growth factors. [21] Hepatic SAMe is also decreased in rats after creation of a portocaval shunt.[22] Hypothetically, decreased SAMe levels could be related to an improved growth response after restoration of portal flow into the liver in dogs with CPSS. GNMT regulates the SAMe:SAHcy ratio and therefore higher GNMT activity may explain improved hepatic growth after CPSS attenuation by lower SAMe levels.

Besides regulation of SAMe levels, GNMT also participates in hepatic detoxification pathways because SAHcy is an important precursor of glutathione.[18,23] However, when SAMe is depleted, SAHcy is used to regenerate SAMe (remethylation) and less SAHcy is channeled to the trans-sulfuration pathway that forms glutathione.[21] In our study, 2 glutathione-s-transferases (GSTs) were expressed higher in recovered animals (GST2, Table 1 and 3; GSTμ3 Table 3) and one GST was expressed lower in recovered animals (GSTO1, Table 2 and 4) Glutathione synthesis and induction of GSTs are both part of the same protective pathway of the liver to hepatotoxic agents.[23,24] How this pathway may affect postoperative recovery is not clear.

Previously, we found that mRNA expression of one of the two isoforms of MAT (MAT2a) in intraoperative liver biopsies was related to complete recovery after CPSS attenuation.[7] This was not confirmed in the present study using microarray analyses. Measurement of the MAT2a protein is necessary to exclude or confirm a possible relation of hepatic MAT2a activity with recovery after CPSS attenuation.

Besides the important role in liver growth, SAMe is an important source of methyl groups in cellular reactions by at least 50 different methyltransferases, including methylation of histones.[19] Modification of histone tails in nucleosomes (acetylation, phosphorylation and possibly also methylation) is important for organization of chromatin structure and chromosome condensation during mitosis.[25] However, the role of upregulation of a histone-lysine-N-methyltransferase in recovered CPSS dogs is not clear.

Glutamate is an excitatory neurotransmitter which is increased in cerebrospinal fluid in dogs with CPSS and contributes to their neurologic clinical signs. The increase is most likely the consequence of increased ammonia removal from the brain in these animals.[26] In dogs with CPSS, plasma concentration of glutamate was also increased.[27] The uptake of glutamate is mediated by glutamate transporters (GMT). Glutaminase (GLS) splits glutamine into glutamate and ammonia, which is then incorporated in urea.[28] N-acetylglutamate synthase (NAGS) is a mitochondrial enzyme that catalyzes the formation of N-acetylglutamate from glutamate, the first and rate-limiting step in the urea cycle.[29] In this study, all three enzymes (GMT, GLS and NAGS) were upregulated in liver tissue of dogs with a good outcome after surgery, suggesting a higher hepatic metabolism of glutamate and possibly of the urea cycle in comparison with dogs with a poor postoperative prognosis. Compared to mRNA expression in the reference dogs, expression of GMT was higher and GLS and NAGS were similar in recovered CPSS dogs. It seems more likely that induction of GMT is a consequence of portosystemic shunting than an essential factor in mechanisms of recovery. However, increased glutamate metabolism in CPSS dogs with good recovery may indicate a better ability of the liver to adapt to high systemic ammonia concentrations.

Cytochrome P450 is a key component of liver enzymes involved in drug and toxin metabolism. Decreased portal flow into the liver leads to hepatic atrophy and lower expression of cytochrome P450 in rats.[30] CYP2D6, which is a fragment of cytochrome P450, was expressed lower in both recovered and non-recovered CPSS dogs compared to the reference and higher in the recovered dogs compared with the non-recovered dogs. Lower levels of cytochrome P450 in these animals probably result from altered exposure of the liver to factors that are normally present in portal blood.[30]

Hepcidin (HEPC) is an acute phase protein that is important in the regulation of iron homeostasis. HEPC is upregulated in inflammation and downregulated by hypoxia and anemia.[31] Iron status is often abnormal in dogs with CPSS as a result of portosystemic shunting.[32] In recovered CPSS dogs, the expression of HEPC was

upregulated compared to the reference and to the non-recovered CPSS dogs. Relatively higher expression of CYP2D6 and HEPC in dogs with a CPSS that recover may reflect less severe hepatic dysfunction in these animals. Like the induction of glutamate metabolism, the upregulation of CYP2D6 and HEPC in CPSS dogs that recover, may indicate a better hepatic ability to adapt to metabolic changes and predispose dogs for recovery after CPSS attenuation.

Phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3Rα) is part of a lipid kinase that plays a crucial role in insulin signal transduction and probably promotes hepatocyte growth.[33,34] The mRNA expression of this enzyme was also upregulated in recovered CPSS dogs, which fits with its biological function. Still, the expression in recovered CPSS dogs was lower than in the reference dogs.

In the subset of extrahepatic CPSS dogs, the products of two other genes that appeared to be upregulated in recovered dogs may be involved in hepatic growth or regeneration: fibrinogen, which was induced after PH in rats,[35] and ubiquitin carrier protein (UCP), which positively regulates growth in many human cancer cells.[36] Their significance for hepatic growth or recovery after CPSS attenuation remains to be clarified.

Genes downregulated in recovered compared to non-recovered CPSS dogs

Connective tissue growth factor (CTGF) and protein CYR61 (CYR61) expression levels were increased in non-recovered dogs compared to recovered dogs. This may be explained by their role in the development of hepatic fibrosis. CTGF and CYR61 are part of the IGFBP superfamily (synonyms: IGFBP8 and IGFBP10, respectively) and both proteins are important in the regulation of endothelial cell function, wound healing and angiogenesis.[37,38] CTGF is a major chemotactic and mitogenic factor for connective tissue cells and promotes collagen synthesis.[37,38] It is linked to transforming growth factor-β (TGF-β) pathways and serum concentration of CTGF can be used for clinical determination of hepatic fibrosis in human chronic liver diseases.[39,40] In all CPSS dogs, recovered and non-recovered, expression of CTGF and CYR61 was upregulated compared to the reference dogs.

The monocyte to macrophage differentiation protein (MMD) is induced during differentiation of circulating monocytes into mature tissue macrophages.[41] Recently, experimental studies indicate that hepatic recruitment of monocytes and differentiation into macrophages are critical for progression of hepatic fibrosis, but also for regression of fibrosis.[42] MMD expression was lower in recovered CPSS dogs compared to non-recovered CPSS dogs, but in both CPSS groups expression of MMD was higher than in the reference pool. The possible role of MMD in hepatic fibrosis and recovery of CPSS may be an interesting target for further research.

In human atherosclerosis, disturbed blood flow causes pathogenic features such as inflammation and oxidative stress that are characterized by expression of vascular

cell adhesion protein 1 (VCAM1) in the endothelial cells.[43] Instead, steady laminar blood flow decreases VCAM1 expression and this decrease of VCAM1 inhibits the activation of the apoptosis signal-regulating kinase (ASK1)-JNK pathway that is involved in triggering inflammation. One of the mechanisms by which blood flow inhibits the ASK1-JNK pathway is by activation of thioredoxin via downregulation of the thioredoxin-interacting protein (TXNIP).[43] The flow-dependent expression of TXNIP seems to play a pivotal role in the expression of VCAM1 and the inflammatory effects of the ASK1-JNK pathway in endothelium, also in other vascular disorders.[44] Interestingly, both VCAM1 and TXNIP are downregulated in dogs that recover after attenuation of a CPSS, compared to dogs that do not recover. In the recovered CPSS dogs VCAM1 expression was similar to its expression in reference dogs, but TXNIP expression was lower than in reference dogs. Hypothetically, the downregulation of VCAM1 and TXNIP in CPSS dogs might be associated with the inhibition of the ASK1-JNK pathway in the liver, for example in the intrahepatic portal vasculature, and reflect the degree of portal vascular development or the ability of the portal vessels to recover.

After PH, an increase in cAMP-dependent protein kinase β (PKI-β) can be detected in the late prereplicative phase of hepatic regeneration which is important for triggering DNA synthesis.[45] However, PKI-β exists in 8 or more different isoforms.[46] The specific function of each enzyme is not clear and the isoforms that are involved in the restoration of liver mass, are differently expressed in time after PH.[46,47] Therefore, the importance of downregulation of one PKI-β isoform in CPSS dogs with good recovery compared to dogs with no recovery, is not clear, but in both CPSS groups the PKI-β isoform was expressed higher than in the reference pool.

Adipophilin (ADRP) is a protein that is associated with lipid droplets and is mainly induced in the liver by fasting. Although the exact function is not known, probably the most important role of hepatic ADRP is to ensure efficient uptake and trapping of fatty acids that are released in peripheral tissues.[48] In mice, ARDP was one of the proteins that were upregulated in liver tissue after PH. Lipid storage is possibly needed for an efficient regenerative response after PH.[49] The significance of the downregulation in ADRP expression that was found in CPSS dogs with a good response after shunt surgery, is not clear. In livers of dogs with CPSS, parenchymal lipid vacuoles are frequently observed, especially in dogs older than 1 year,[50] and excessive fat accumulation in hepatocytes interferes with hepatic regeneration.[8] A correlation of these lipid droplets with ADRP expression or prognosis in dogs with CPSS is not known, but both in recovered and non-recovered dogs ADRP expression was higher than in the reference pool.

In the subset of extrahepatic CPSS dogs, the products of five other genes that appeared to be downregulated in recovered dogs may be involved in hepatic growth or regeneration: interleukin 8, which may recruit neutrophils into the liver that inhibit

regeneration after hepatic injury,[51] krüppel-like factor 4, a cell growth inhibitor in cancer cells and regulator of inflammatory responses in endothelium (possibly linked to VCAM1 expression),[52] serglycin, a proteoglycan that is also involved in endothelial inflammation,[53] mortality factor 4 protein, which is linked to cellular aging,[54] and catenin delta-1, which regulates cell apoptosis and proliferation in hepatoma cells.[55] Their effect in recovery mechanisms after CPSS attenuation remains to be clarified.

Expression patterns of specific genes, so-called gene expression signatures, have been used as prognostic indicators frequently, but almost only in human malignant diseases.[11,16,56,57] The predictive accuracies of the classifier when including all CPSS (58.0%) or only extrahepatic CPSS (53.9%) did not provide a clinically applicable tool that would predict response to surgical shunt attenuation. The predictive accuracy of 63.0% of shunt type, suggest that the predictive power of the classifier is indirect and possibly based on the effect of the shunt localization on hepatic gene expression. More hepatic samples are needed to create separate predictive classifiers for intrahepatic and extrahepatic CPSS.

Hepatic gene expression profiles and recovery rates after attenuation of a CPSS are probably not identical in different dog populations.[58] Recovery may also be affected by the surgical technique that is used for CPSS closure, although portosystemic shunting persists in 10 to 20% of dogs, regardless of the technique (ligation, cellophane banding or ameroid constrictors) that was used.[4-6] The present study was restricted to CPSS dogs referred to one university clinic, one surgeon and one technique. However, mechanisms of hepatic and vascular growth or other mechanisms of recovery after surgical attenuation of the shunt may be comparable in dogs from different populations and after application of different surgical techniques. Therefore, the concepts of the present study might be equally valid to dog populations in other clinics and countries.

We recognize that when using statistical criteria to detect differences in expression among a large number of genes, it is expected that different expression of some genes may be significant by chance. However, if there are true recovery-related genes among those evaluated, it would be more likely for their expression to be significantly different than for unrelated genes. Therefore, this microarray study attempted to select promising genes and pathways for future study in dogs with CPSS.

In conclusion, differences in intraoperative hepatic mRNA expression between dogs with and without recovery after surgical CPSS attenuation were found in 63 genes. The biological function of several of their active gene products fitted with a possible function in mechanisms involved in recovery after attenuation of a CPSS. Validation of potentially important genes and the active products is essential to confirm these findings, preferably in more dogs from different populations. A classifier based on the results of this study obtained no better prediction of individual response after surgical CPSS attenuation than CPSS type alone.

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References

1. Ewing GO, Suter PF, Bailey CS. Hepatic insufficiency associated with congenital anomalies of the portal vein in dogs. J Am Anim Hosp Assoc 1974;10:463-476.
2. Rothuizen J, van den Ingh TSGAM, Voorhout G, et al. Congenital portosystemic shunts in sixteen dogs and three cats. J Small Anim Pract 1982;23:67-81.
3. Zaitoun AA, Path FRC, Apelqvist G, et al. Quantitative studies of liver atrophy after portacaval shunt in the rat. J surg Res 2006;131:225-232.
4. Kummeling A, van Sluijs FJ, Rothuizen J. Prognostic implications of the degree of shunt narrowing and of the portal vein diameter in dogs with congenital portosystemic shunts. Vet Surg 2004;33:17-24.
5. Hunt GB, Kummeling A, Tisdall PLC, et al. Outcomes of cellophane banding for congenital portosystemic shunts in 106 dogs and 5 cats. Vet Surg 2004;33:25-31.
6. Mehl ML, Kyles AE, Hardie EM, et al. Evaluation of ameroid ring constrictors for treatment for single extrahepatic portosystemic shunts in dogs: 168 cases (1995–2001). J Am Vet Med Assoc 2005;226:2020-2030.
7. Kummeling A, Penning LC, Rothuizen J, et al. Intraoperative hepatic gene expression in dogs related to outcome after attenuation of a congenital portosystemic shunt. Submitted for Publication: chapter 7.
8. Michalopoulos GK. Liver regeneration. J. Cell. Physiol 2007;213:286-300.
9. Diehl AM, Rai RM. Regulation of signal transduction during liver regeneration. FASEB J 1996;10:215-227.
10. Rothuizen J, van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22-25.
11. Roepman P, Wessels LFA, Kettelarij N, et al. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. Nat Genet 2005;37:182-186.
12. van de Peppel J, Kemmeren P, van Bakel H, et al. Monitoring global mRNA changes with externally controlled microarray experiments. Embo Rep 2003;4:387-393.
13. Yang YH, Dudoit S, Luu P, et al. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res 2002;30:15.
14. Margaritis T, Lijnzaad P, van Leenen D, et al. Adaptable gene-specific dye bias correction for two-channel DNA microarrays. Mol Syst Biol 2009;5:266.
15. Wu H, Kerr MK, Cui X, Churchill GA. MAANOVA: a software package for the analysis of spotted cDNA microarray experiments. In: The analysis of gene expression data: methods and software. 2002; http://research.jax.org/faculty/churchill/
16. Dennis JL, Oien KA. Hunting the primary: novel strategies for defining the origin of tumours. J Pathol 2005;205:236-247.
17. Ambroise C, McLachlan GJ. Selection bias in gene extraction on the basis of microarray gene-expression data. Proc Natl Acad Sci U S A 2002;99:6562-6566.
18. Mato JM, Corrales FJ, Lu SC, Avila MA. S-adenosylmethionine: a control switch that regulates liver function. FASEB J 2002;16:15-26.

19. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. J Biol Chem 2009;284:22507-22511.
20. Varela M, Fernández D, Martínez N, et al. Impaired liver regeneration in mice lacking glycine N-methyltransferase. Hepatology 2009;50:443-452.
21. Lu SC, Mato JM. S-adenosylmethionine in cell growth, apoptosis and liver cancer. J Gastroenterol Hepatol 2008;23:S73-77.
22. Benjamin LE, Steele RD. Methionine metabolism after portacaval shunt in the rat. Am J Physiol 1985;249:G321-327.
23. Ko K, Yang H, Noureddin M, et al. Changes in S-adenosylmethionine and GSH homeostasis during endotoxemia in mice. Lab Invest 2008;88:1121-1129.
24. Ozer J, Ratner M, Shaw M, et al. The current state of serum biomarkers of hepatotoxicity. Toxicology 2008;245:194-205.
25. Tachibana M, Sugimoto K, Fukushima T, Shinkai Y. Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. J Biol Chem 2001;276:25309-25317.
26. Butterworth J, Gregory CR, Aronson LR. Selective alterations of cerebrospinal fluid amino acids in dogs with congenital portosystemic shunts. Met Brain Dis 1997;12:299-306.
27. Strombeck DR, Rogers Q. Plasma amino acid concentrations in dogs with hepatic disease. J Am Vet Med Ass 1978;173:93-96.
28. Watford M. Hepatic glutaminase expression: relationship to kidney-type glutaminase and to the urea cycle. FASEB J 1993;7:1468-1474.
29. Morizono H, Caldovic L, Shi D, Tuchman M. Mammalian N-acetylglutamate synthase. Mol Genet Metab 2004;81:S4-11.
30. Farrell GC, Zaluzny L. Portal vein ligation selectively lowers hepatic cytochrome P450 levels in rats. Gastroenterology 1983;85:275-282.
31. Lee PL, Beutler E. Regulation of hepcidin and iron-overload disease. Annu Rev Pathol Mech Dis 2009;4:489-515.
32. Simpson KW, Meyer DJ, Boswood A, et al. Iron status and erythrocyte volume in dogs with congenital portosystemic vascular anomalies. J Vet Intern Med 1997;11:14-19.
33. Hayashi T, Okamoto M, Yoshimasa Y, et al. Insulin-induced activation of phosphoinositide 3-kinase in Fao cells. Diabetologia 1996;39:515-522.
34. Khamzina L, Borgeat P. Correlation of alpha-fetoprotein expression in normal hepatocytes during development with tyrosine phosphorylation and insulin receptor expression. Mol Biol Cell 1998;9:1093-1105.
35. Fulop AK, Pocsik E, Brozik M, et al. Hepatic regeneration induces transient acute phase reaction: systemic elevation of acute phase reactants and soluble cytokine receptors. Cell Biol Int 2001;25:585-592.
36. Lim JH, Jung CR, Lee CH, Im DS. Egr-1 and serum response factor are involved in growth factors- and serum-mediated induction of E2-EPF UCP expression that regulates the VHL-HIF pathway. J Cell Biochem 2008;105:1117-1127.
37. Kim HS, Nagalla SR, Oh Y, et al. Identification of a family of low-affinity insulin-like growth factor binding proteins (IGFBPs): characterization of connective tissue growth factor as a member of the IGFBP superfamily. Proc Natl Acad Sci U S A 1997;94:12981-12986.
38. Brigstock DR. Regulation of angiogenesis and endothelial cell function by connective tissue growth factor (CTGF) and cysteine-rich 61 (CYR61). Angiogenesis 2002;5:153-165.
39. Kovalenko E, Tacke F, Gressner OA, et al. Validation of connective tissue growth factor (CTGF/CCN2) and its gene polymorphisms as noninvasive biomarkers for the assessment of liver fibrosis. J Viral Hepat 2009;16:612-620.
40. Zhang D, Wang NY, Yang CB, et al. The clinical value of serum connective tissue growth factor in the assessment of liver fibrosis. Dig Dis Sci 2009;Mar 18:epub ahead of print.

41. Rehli M, Krause SW, Schwarzfischer L, et al. Molecular cloning of a novel macrophage maturation-associated transcript encoding a protein with several potential transmembrane domains. Biochem Biophys Res Commun 1995;217:661-667.
42. Tacke F. Monocytes and Macrophages as Cellular Targets in Liver Fibrosis. Inflamm Allergy Drug Targets 2009;Sep 1:epub ahead of print.
43. Li L, Tatake RJ, Natarajan K, et al. Fluid shear stress inhibits TNF-mediated JNK activation via MEK5-BMK1 in endothelial cells. Biochem Biophys Res Commun 2008;370:159-163.
44. Yamawaki H, Pan S, Lee RT, Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. J Clin Invest 2005;115:733-738.
45. Pujol MJ, Soriano M, Coll MJ, et al. New synthesis of cyclic AMP-dependent protein kinases during liver regeneration. Cell Biochem Funct 1988;6:203-208.
46. Kumar P, Van Patten SM, Walsh DA. Multiplicity of the beta form of the cAMP-dependent protein kinase inhibitor protein generated by post-translational modification and alternate translational initiation. J Biol Chem 1997;272:20011-20020.
47. Ekanger R, Vintermyr OK, Houge G, et al. The expression of cAMP-dependent protein kinase subunits is differentially regulated during liver regeneration. J Biol Chem 1989;264:4374-4382.
48. Dalen KT, Ulven SM, Arntsen BM, et al. PPARalpha activators and fasting induce the expression of adipose differentiation-related protein in liver. J Lipid Res 2006;47:931-943.
49. Strey CW, Winters MS, Markiewski MM, Lambris JD. Partial hepatectomy induced liver proteome changes in mice. Proteomics 2005;5:318-325.
50. Isobe K, Matsunaga S, Nakayama H, Uetsuka K. Histopathological characteristics of hepatic lipogranulomas with portosystemic shunt in dogs. J Vet Med Sci 2008;70:133-138.
51. Chen YX, Sato M, Kawachi K, Abe Y. Neutrophil-mediated liver injury during hepatic ischemia-reperfusion in rats. Hepatobiliary Pancreat Dis Int 2006;5:436-442.
52. Atkins GB, Jain MK. Role of krüppel-like transcription factors in endothelial biology. Circ Res 2007;100:1686-1695.
53. Kolset SO, Tveit H. Serglycin- structure and biology. Cell Mol Life Sci 2008;65:1073-1085.
54. Pena AN, Pereira-Smith OM. The role of the MORF/MRG family of genes in cell growth, differentiation, DNA repair, and thereby aging. Ann N Y Acad Sci 2007;1100:299-305.
55. Nong CZ, Pan LL, He WS, et al. P120ctn overexpression enhances beta-catenin-E-cadherin binding and down regulates expression of survivin and cyclin D1 in BEL-7404 hepatoma cells. World J Gastroenterol 2006;12:1187-1191.
56. Wang W, Yang LY, Huang GW, et al. Genomic analysis reveals RhoC as a potential marker in hepatocellular carcinoma with poor prognosis. Br J Cancer 2004;90:2349-2355.
57. Győrffy B, Schäfer R. Meta-analysis of gene expression profiles related to relapse-free survival in 1,079 breast cancer patients. Breast Cancer Res Treat. pub online 05 December 2008. doi: 10.1007/s10549-008-0242-8
58. Krotschek U, Adin CA, Hunt GB, et al. Epidemiologic factors associated with the anatomic location of intrahepatic portosystemic shunts in dogs. Vet Surg 2007;36:31-36.

Chapter 9

Summarizing Discussion and Conclusion

Aim of this thesis

The general aim of this thesis was to identify factors associated with outcome after surgical attenuation of congenital portosystemic shunts in dogs and, consequently, to gain insight in underlying mechanisms of postoperative recovery in this disease.

Shunt attenuating techniques and recovery after CPSS attenuation

Chapters 3 and 4 evaluated surgical outcome and prognostic factors of two different surgical techniques that are commonly used in CPSS attenuation; ligation and cellophane banding. Both techniques were applied in a relatively large group of dogs (97 and 106 dogs, respectively). Comparing both techniques, similar postoperative complications were noticed. Cellophane banding (chapter 4) may be an easier technique to perform by surgeons with less experience in CPSS attenuation. Furthermore, short-term complication and mortality rates may be lower with cellophane banding, especially when bands are loosely applied around the shunt without causing constriction. These advantages seem to hold only for extrahepatic shunts, because intrahepatic shunts usually have a larger diameter and may be completely surrounded by hepatic tissue; the application of a non constricting cellophane band, preferably with a recommended diameter ($\leq$ 3mm), is therefore often difficult or even impossible around an intrahepatic shunt.[1-3] This is confirmed by significantly higher postoperative complication and mortality rates in intrahepatic shunts (55% and 27% respectively) compared to extrahepatic shunts (13% and 3%; P=.002 and P=.008 respectively) after placement of cellophane bands (chapter 4). Perioperative mortality rate after CPSS ligation was 27% and no differences in mortality rates between intrahepatic and extrahepatic shunts were found (chapter 3).

After CPSS ligation (chapter 3), achieved degree of closure was negatively associated with mortality. In dogs with extrahepatic shunts, both variables (degree of CPSS closure and postoperative mortality) were correlated with the diameter of the portal vein (portal development), which may show considerable variation. CPSS is apparently not only a congenital disease of an abnormal portosystemic communication, but also a developmental disease that affects the portal vasculature towards and probably also inside the liver. Unexpectedly, long-term outcome did not depend on the degree of CPSS closure or on portal development at the time of surgery. Both studies reported dogs with good clinical performance after surgical treatment in spite of persistent portosystemic shunting.

In conclusion, both techniques are effective methods to achieve resolution of clinical signs and hepatic dysfunction in the majority of dogs. However, with either technique a small proportion of dogs does not respond to CPSS attenuation as expected: chapter 3 reported 10% of dogs with clinical recurrence after CPSS ligation and in 16% of dogs hepatic dysfunction was reported after cellophane banding of a CPSS in chapter 4. Despite differences in study population and breed distribution between both studies, surgical technique is apparently not the most important factor that defines long-term outcome. Other factors such as the ability of the liver and the portal vasculature to enlarge after surgery may be equally, or even more important in coping with the increased portal flow into the liver and achieving a normal liver development and function.

Coagulopathy and recovery after CPSS attenuation

The study described in **chapter 5** was initiated because persistent haemorrhage caused by abnormal coagulation was an important cause of short-term postoperative death in the dogs with a CPSS described in chapter 3. The mechanisms underlying the alterations in haemostasis were not fully understood. In this prospective study, coagulation times, platelet counts and 8 coagulation factors were evaluated in 39 healthy dogs and 34 dogs with a CPSS before and after attenuation of their shunt. Dogs with a CPSS had lower preoperative platelet counts, lower activity of clotting factors II, V, VII, X, an increased activity of factor VIII and a prolonged activated partial thromboplastin time (APTT) compared to healthy dogs. Immediately after surgical attenuation of their shunt, platelet counts and the activity of clotting factors I, II, V, VII, IX, X, and XI were further decreased, and prothrombin time (PT) became prolonged.

To evaluate the effect of a median celiotomy with vessel ligation on coagulation factor activity in dogs without portosystemic shunting, coagulation profiles were measured before and after ovariectomy in 8 healthy dogs. This surgical procedure resulted in decreased activity of factors VII and X in these healthy dogs.

In dogs with complete recovery of shunting, haemostasis was normalized after 6 weeks, but in dogs with persistent portosystemic shunting at 6 weeks after surgery, no improvement of haemostatic values was observed compared to preoperative values. The findings in this study prompted us to administrate plasma transfusions before and during surgery to dogs with a CPSS and prolonged APTT and/or PT, to frequent examination of potential bleeding sites and a more intensive monitoring of platelets and coagulation times after surgery.

After the studies of chapter 3 and 5 were performed, mortality rates were expected to decrease. Because the achieved degree of closure was not correlated to long-term outcome after surgical CPSS attenuation in chapter 3, euthanasia was no longer advised in dogs in which a minimum of 50% shunt diameter reduction could not be achieved. Furthermore, we expected to find a reduction of cases with clinical bleeding after measures had been taken to prevent or detect abnormalities in coagulation in an early stage. Finally, significantly fewer dogs have been noted to develop neurological dysfunction over time and probably more dogs are currently advised to be treated conservatively after ligation of a CPSS has proved to be not possible (personal observations). Between January 1, 2006 and August 1, 2009, 77 dogs were operated on a CPSS (20 dogs with an intrahepatic and 57 dogs with an extrahepatic CPSS). Mortality in this group was still 19.2% (15 dogs), 5 dogs in the intrahepatic group (25%) and 10 dogs in the extrahepatic group (17.5%). In the dogs with extrahepatic shunts, the major cause of death was persistent bleeding because of coagulopathy (7 dogs) and in the dogs with intrahepatic shunts, all 5 dogs that died were euthanized during surgery because attempts to attenuate the shunt had failed. Mortality rates were not significantly different from the study population described in chapter 3.

Hepatic growth and recovery after CPSS attenuation

In chapter 3 and 4 it was concluded that the ability of the liver to adapt to the increased blood flow after shunt attenuation and to grow to 'normal' size and function might be an important factor for long-term recovery after shunt attenuation in individual dogs. The first step in providing further foundation of the concept that hepatic growth is related to, and possibly even essential for, postoperative recovery was performing the volumetric study described in **chapter 6**. The aim of this study was to describe objective and suitable techniques to measure liver size in dogs with a CPSS and to record hepatic growth following surgical attenuation of a CPSS.

Abdominal MRI and/or CT scans were performed before and 8 days, 1 month and 2 months after shunt attenuation in 10 dogs. Liver volumes were calculated from the areas of the MRI and CT images and related to body weight. Before surgery, median liver volume was 18.2 cm^3 per kg body weight. Hepatic growth was highest between day 0 and day 8 (median, 44%). The livers of the dogs had increased at 2 months after attenuation to a median volume of 28.8 cm^3/kg. No significant differences in growth were found between dogs with complete or partial shunt closure or between dogs with complete or incomplete metabolic recovery. Volumes measured from consecutively performed MRI and CT images correlated well (r=0.980), but volumes from MRI images were systematically larger than volumes from CT images (6.8%;

P=0.008). CT is the preferred volumetric technique because of speed and expected better accuracy.

In this study, it was concluded that liver size was normal at 2 months after attenuation of the CPSS, because the median volume 28.8 cm^3/kg matched with previously reported liver volumes in normal adult dogs (24.4 cm^3/kg) and normal adult humans (23.5 cm^3/kg).[4,5] However, ultimate liver size per kg body weight in this small group of dogs was similar in dogs with complete recovery (dogs with clinical and metabolic recovery) and dogs with incomplete recovery (dogs with clinical recovery, but metabolic evidence of persistent portosystemic shunting). Although ultimate liver size may not be a sensitive indicator for (mild) persistent portosystemic shunting after surgery, our findings do not refute the assertion that the ability of the liver to grow to a normal size is important for long-term prognosis. We only studied liver growth after CPSS ligation in dogs with clinical recovery and not in dogs without clinical improvement, in dogs with severe persistent shunting or in dogs with clinical recurrence or long-term postoperative complications related to portosystemic shunting. More studies in hepatic growth after CPSS attenuation should be conducted, preferably in dogs from different populations and with different types of shunts.

With respect to the postoperative increase of hepatic size, it would be interesting to know which factors define ultimate liver size. Attenuation of a CPSS, and thus increased portal flow into the liver, resulted in a rapid increase of liver volume, whereas the opposite (a decrease of portal blood flow into the liver, by constructing a portocaval shunt) directly leads to a significantly decreased liver volume.[6] This suggests that the amount of portal blood that flows into the liver is an important factor for liver size. This concept is supported by the observation that an increase of portal blood flow per unit liver tissue after partial hepatectomy (PH), is essential for hepatic growth after PH.[7,8] Increased portal flow may trigger a growth response by exerting a pressure effect on fenestrations in hepatic capillaries, by hypoxia and by an increase of growth factors, nutrients and cytokines from organs that feed the portal vein.[7] The ultimate hepatic size is adapted to body weight. After PH, restoration of hepatic volume is followed by a small wave of apoptosis. An increase of liver mass by administration of xenobiotics is also reversed by apoptosis.[7] And after an initial increase in hepatic proliferation and liver volume induced by exogenous HGF administration without changing portal flow, liver volume decreased to its original size within 4 weeks after the last HGF treatment.[9] These findings suggest that (excessive) liver growth is corrected by a control system or feedback loops to a predetermined hepatic volume in which portal flow, or factors transported with it, may play an essential role to maintain hepatic volume after proliferation of the liver. Other factors involved in these control mechanisms include the extracellular matrix (binding of HGF) and TGFβ1.[7]

In dogs with complete recovery after partial attenuation of a CPSS, the increase of portal flow may result in a positive spiral of hepatic and vascular development and a progressive decrease of portosystemic shunting,[10] until liver size is normalized and further hepatic growth is stopped. This progressive decrease of portosystemic shunting is not observed in all CPSS dogs after partial shunt closure. Further studies are necessary to evaluate if and how mechanisms that correct excessive liver mass may affect hepatic growth after CPSS attenuation in dogs with and without normalization of hepatic volume.

Hepatic gene expression and recovery after CPSS attenuation

Hepatic development to a 'normal' size and function after shunt ligation is a complicated process that is considered comparable to hepatic regeneration after PH.[11] The study described in chapter 6 demonstrated a rapid increase of liver volume after surgical CPSS ligation in the first eight days following surgery, which is similar to hepatic mass restoration after PH.[7] Multiple studies illustrated the enormous complexity in regenerative mechanisms in the liver after PH,[7,12-14] but the mechanisms that are actually involved in growth or recovery after CPSS attenuation have not yet been clarified.

Chapter 7 evaluated the relation between intraoperative hepatic expression of 19 genes involved in fibrosis, apoptosis, hepatic and vascular growth and complete recovery after attenuation of a CPSS in 48 dogs. These genes were selected because they were anticipated to be possibly involved in individual response after surgical treatment. The hepatic expression of methionine adenosyltransferase 2 alpha (MAT2a) and hepatocyte growth factor activator (HGFac) were positively associated with recovery. A model was constructed using both factors as binary variables (high or low expression), which predicted individual outcome in 75% in these dogs. Cut-off values between 'high' and 'low' expression of MAT2a and HGFac were 0.457 and 0.974, respectively.

In **chapter 8** the primary aim was to identify genes that are differently expressed in liver tissue collected during surgical CPSS attenuation between dogs that recover and dogs that do not recover, using microarray analyses. This technique allowed screening of a large number of gene fragments (42,034). Sixty-three genes were differently expressed between 23 recovered and 23 non-recovered dogs. Several of the products of these genes may directly affect postoperative recovery because they are involved in hepatic growth, hepatic fibrosis and endothelial inflammation. Other gene products may reflect differences in hepatic function, such as genes involved in glutamate metabolism.

Although confirmation of protein activity in hepatic tissue and validation in independent groups of CPSS dogs is essential, this study (chapter 8) yielded promising genes and pathways for future study to elucidate postoperative recovery mechanisms in dogs with CPSS. Of all hepatic pathways possibly involved in CPSS recovery, s-adenosylmethionine (SAMe) metabolism (Figure 1) appears to be the most promising for further research at this moment, because mRNA expression of gene products involved in SAMe metabolism were significantly related to recovery after CPSS attenuation in both chapter 7 (MAT2a) and chapter 8 (glycine N-methyltransferase, GNMT).

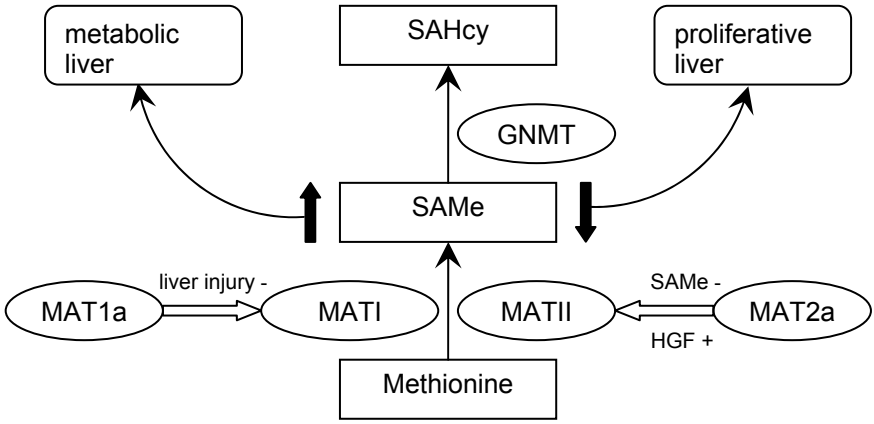

Figure 1. Regulation of liver function by s-adenosylmethionine (SAMe). SAMe is synthesized of methionine by methionine adenosyltransferase (MATI/II). Because SAMe downregulates MAT2a expression and inhibits MATII activity, the contribution of MATII in hepatic SAMe synthesis is small under normal conditions. Liver injury (i.e. PH) initiates HGF and other growth factors that inactivate MATI activity and reduces hepatic SAMe. The reduced SAMe content releases the inhibition on the proliferative activity of HGF, facilitating liver regeneration. On restoration of the original liver mass, the inhibition on MATI is released and MAT2a expression is again downregulated. In cirrhotic patients, MAT1a is progressively silenced by methylation of the gene promoter. SAMe is primarily converted to s-adenosylhomocysteine (SAHcy) by glycine-N-methyltransferase (GNMT), which is also essential to decrease SAMe contents during liver regeneration.[16,18]
Note: MATI and MATII are the active protein products of mRNA expression of the genes MAT2a and MAT1a.

Further research of gene products that directly affect recovery mechanisms after attenuation of a CPSS in dogs may yield additional treatment options in the future. Recently, oral administration of SAMe was advised in medical management of dogs with portal vascular anomalies as a hepatoprotective therapy.[15] Chronic liver diseases in humans often lead to lower hepatic SAMe levels, which was also reported in rats with portocaval shunts.[16,17] Treatment with SAMe has been shown to be beneficial and safe in several human liver diseases,[16] but the effects of SAMe administration may not be advantageous in dogs before surgical attenuation of a CPSS. Increased hepatic SAMe levels inhibit hepatic proliferation and may even inhibit angiogenesis in the liver.[16,18,19] Before SAMe treatment can be recommended in dogs with a CPSS, the effects of SAMe in this disease should be thoroughly studied.

Recovery rates depend on the criteria that are used to define recovery. Clinical performance alone is not sufficient to define postoperative recovery (chapter 2). In the last studies (chapter 7 and 8), recovery was defined by 1) feasibility of the CPSS to be attenuated without causing portal hypertension, 2) survival in the postoperative period, 3) absence of functional portosystemic shunting 1-2 months after surgery. Dogs that died because of portal hypertension, hypoplasia of the portal vasculature or persistent shunting in the postoperative period were classified as non-recovered, whereas dogs that died from other reasons were excluded from the study. These criteria were based on the hypotheses that shunt attenuation is needed to obtain an increased portal flow into the liver, which may trigger hepatic and portal vascular development, and that long-term clinical prognosis after shunt attenuation is most reliably predicted by postoperative metabolic recovery.[20] No minimum degree of attenuation was set because the degree of attenuation was not correlated with long-term recovery (chapter 3). It is debatable whether dogs that died of complications such as coagulopathy or neurological dysfunction should be excluded. One could also question whether dogs with minor persistent portosystemic shunting (for example dogs with normalization of hepatic volume and normal fasting plasma ammonia concentrations but abnormal ammonia tolerance test results) should be regarded as not recovered.

Shunt localization appeared to be a partial confounding factor in chapter 7 and 8. A confounder is an explanatory variable which is related to both the response of interest (postoperative recovery) and to one or more other explanatory variables (expression of MAT2a and other prognostic genes), so that it is impossible to separate the effects of the explanatory variables on the response.[21] In chapter 7, expression of MAT2a and shunt localization were both considered to be explanatory variables of outcome, while an intrahepatic localization of the shunt may partially explain low expression of MAT2a. This can be depicted in a causal model (Figure 2).[22]

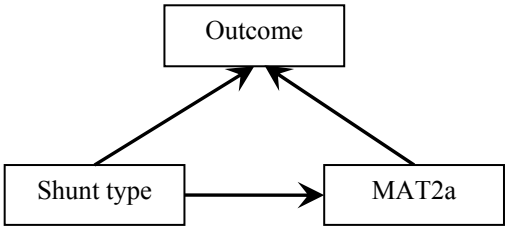

Figure 2. Hypothesized causal model of the explanatory variables 'shunt type' and 'hepatic mRNA expression of MAT2a' on outcome after CPSS attenuation

In chapter 8, the effect of localization of the shunt (intrahepatic or extrahepatic) also seemed to interfere with the expression of prognostic genes when a predictive classifier was constructed. Although the predictive value of the expression profiles of recovered and non-recovered CPSS dogs seemed to be promising at first sight, a classifier constructed from the microarray data obtained no better prediction of postoperative outcome than CPSS localization. This could also be caused by a confounding effect of shunt localization, but remarkably only 7 of the 63 gene products that were significantly differently expressed between recovered and non-recovered dogs were correlated with shunt localization. The group of dogs with an intrahepatic CPSS was relatively small (14 of 46 dogs), with a very small number of dogs that recovered (4 dogs). This may have caused an imbalance in the data from the subset of dogs with intrahepatic shunts and consequently difficulties in the construction of an applicable classifier.

A possible solution to confounding effects is stratification of the data, meaning separate analyses in the subsets of intrahepatic and extrahepatic CPSS.[23] This requires large numbers of dogs to be included in the analyses that will be easier to obtain in a multi-centered study. Multi-centered studies also have the advantage that interpretations of the results may be representative for more dog populations worldwide, because bias by restrictions in study population and surgical technique can be avoided. Stratification may also clarify possible structural differences between intrahepatic and extrahepatic CPSS (or even between subtypes of intrahepatic and extrahepatic shunts) with respect to pathways that are involved in postoperative recovery or hepatic metabolism.

The finding that CPSS type did not appear to be a prognostic variable in chapter 3 seems to be in contradiction with the findings in chapter 7 and 8 and with previous reports.[24,25] A possible explanation may be that 20/31 (65%) intrahepatic shunts described in chapter 3 were left divisional shunts, whereas only 9/16 (56%) of the

intrahepatic shunts in chapter 7 and 6/14 (43%) in chapter 8 consisted of the left divisional subtype. Although these differences are not significant, they may indicate a shift in dogs that are presented for surgery in our clinic towards breeds or subtypes of intrahepatic shunts with a worse prognosis. For example, in chapter 3 no dogs with central divisional shunts were included, while the studies in chapter 7 and 8 reported 2 and 3 dogs respectively with this subtype of shunt, none of which recovered. Another, probably more important reason may be a more precise determination of recovery at 1-2 months after surgery in the dogs that were included in the studies of chapter 7 and 8. Both studies evaluated recovery prospectively according to a well defined protocol which may have revealed more dogs with persistent portosystemic shunting in spite of good clinical recovery, compared to chapter 3.

Future studies in dogs with CPSS will primarily be aimed at the development and validation of clinically applicable tests to predict outcome in dogs after surgical shunt attenuation. It is important to consider what a test should measure before a suitable test can be designed. One should decide ahead if a test must be sensitive in the identification of individual dogs that will have a very poor prognosis or be sensitive in the identification of individuals that have a high chance to recover. The first decision will provide a less conservative test with respect to surgical treatment; such a test will advise surgical treatment to a large number of dogs that have any chance to recover, while only a few hopeless dogs will be excluded from this treatment option. The second test will be much more conservative and only advise surgery when the chance for a positive outcome is substantially high. A larger number of dogs, which would otherwise be cured with surgery, will be treated conservatively. However, the demands that are made of a test may be variable and depend on factors such as risk of surgery, costs, prognosis of alternative treatment options and subjective feelings of owners and veterinarians treating these dogs.

In the design of the final predictive model that was described in chapter 7, a compromise was reached between sensitivity (to correctly test dogs positive that will recover) and specificity (to correctly test dogs negative that will not recover). This was done by choosing cut-off values between ‘low’ and ‘high’ gene expression levels that corresponded to the data points on the receiver operating characteristic (ROC) curves with the smallest Euclidian distance to the point (1-Sp, Se) = (0%, 100%) (Figure 3).

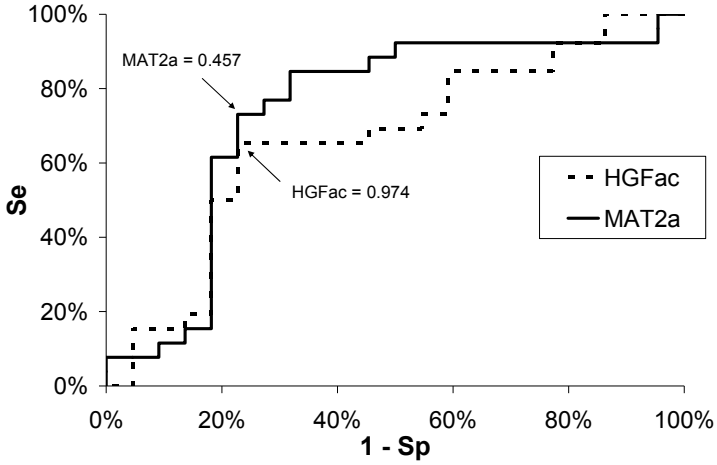

Figure 3. Receiver operating characteristic (ROC) curves of hepatic mRNA expression of MAT2a and HGFac in 48 dogs with CPSS. Cut-off values between 'low' and 'high' gene expression levels correspond to the data points on the ROC curves with the smallest Euclidian distance to the point (1-Sp, Se) = (0%, 100%), with 'lower' expression on the left and 'higher' expression on the right side of the cut-off points of both genes.
Abbreviations: HGFac, hepatic growth factor activator; MAT2a, methionine adenosyltransferase 2 alpha; Se, sensitivity; Sp, specificity

According to specific demands on probability of recovery when selecting dogs for surgery, cut-off values can be changed. Another option is to use the test differently, depending on specific demands. For example, owners of dogs that are situated in the quadrants I and IV (chapter 7, Figure 2) can be given a positive or negative advice with respect to surgical attenuation of the CPSS in their dogs, depending on individual demands and expectations.

Normally, hepatic volume after PH is restored by proliferation of mature adult hepatocytes and other hepatic cell types. If hepatocyte proliferation is blocked or fails, the formation of liver progenitor cells (LPCs) or oval cells is induced. These cells have the ability to proliferate intensely and form new hepatocytes.[7,26] Activation of LPCs was described in canine hepatic diseases such as acute hepatitis, cirrhosis, lobular dissecting hepatitis and primary portal vein hypoplasia.[27] In dogs with CPSS, future research may address the question if hepatic growth after shunt attenuation depends primarily on proliferation of adult hepatocytes or also on involvement of LPCs, especially in relation to postoperative recovery. Interesting is the fact that CTGF, which was higher expressed in non-recovered CPSS dogs compared to recovered CPSS dogs (chapter 8), also plays a role in LPC induction.[26]

The studies in chapter 7 and chapter 8 aimed to relate hepatic mRNA expression before surgical treatment to the ability of the dog to obtain a favourable long-term clinical recovery after surgical treatment. However, liver tissue was collected by performing intraoperative biopsies, because this technique is easier and carries less risk than preoperative transabdominal biopsies. Larger amounts of liver tissue are obtained and bleeding after performing the biopsy can be controlled directly. To avoid influence of the anaesthesia or the surgical procedure on hepatic mRNA expression of genes that affect recovery, the liver tissue should preferably be collected preoperatively, especially when the clinical applicability is tested of a predictive model that is aimed to aid in preoperative planning of treatment. In addition to the studies in chapter 7 and 8, further research will be performed in more dogs with CPSS. It may not be very practical and definitely bears a reasonable amount of risk to obtain preoperative hepatic biopsies in large numbers of dogs with CPSS. Another problem with liver biopsies is that biopsies obtained from different localizations may yield different results because the severity of lesions may vary in different liver lobes in CPSS dogs.[28] If available, a reliable, safe and technically easier method to determine the individual ability for postoperative recovery would be a very attractive alternative.

Low activity of serum IGF and IGFBP3 was previously measured in two dogs with CPSS and was thought to be directly related to liver dysfunction,[29] and gene expression in peripheral circulating blood leucocytes was reported to reflect mRNA expression changes in hepatic grafts.[30] It is not known if protein concentrations in plasma or mRNA expression in peripheral leucocytes reflect hepatic expression of the genes involved in postoperative CPSS recovery. However, the potential expression of these relevant genes in the peripheral circulation is a very interesting topic for further study because it would facilitate sample collection for future research and clinical purposes enormously.

Conclusion

Postoperative complications and recurrence or persistence of portosystemic shunting have been documented after all techniques used for surgical attenuation of a congenital portosystemic shunt in dogs. Therefore, adaptation of the surgical technique alone appears no solution to prevent complications or failure of long-term recovery. Future research of pathways involved in recovery may help to elucidate what causes different outcome after surgery and yield additive forms of treatment. Predictive models may become applicable clinical tools to decide what the best therapeutic options are in individual dogs. In addition to alternative treatments, surgical CPSS attenuation will probably remain necessary to increase portal flow into the liver and maintain hepatic function over time.

References

1. Youmans KR, Hunt GB. Cellophane banding for the gradual attenuation of single extrahepatic portosystemic shunts in eleven dogs. Aust Vet J 1998;76:531-537.
2. Connery NA, McAllister H, Skelly C, Pawson P, Bellenger CR. Cellophane banding of congenital intrahepatic portosystemic shunts in two Irish wolfhounds. J Small Anim Pract 2002;43:345-349.
3. Youmans KR, Hunt GB. Experimental evaluation of four methods of progressive venous attenuation in dogs. Vet Surg 1999;28:38-47.
4. Stieger SM, Zwingenberger A, Pollard RE, Kyles AE, Wisner ER. Hepatic volume estimation using quantitative computed tomography in dogs with portosystemic shunts. Vet Radiol Ultrasound 2007;48:409-413.
5. Geraghty EM, Boone JM, McGahan JP, Jain K. Normal organ volume assessment from abdominal CT. Abdom Imaging 2004;29:482-490.
6. Zaitoun AA, Path FRC, Apelqvist G, Al-Mardini H, Gray T, Bengtsson F, Record CO. Quantitative studies of liver atrophy after portacaval shunt in the rat. J Surg Res 2006;131:225-232.
7. Michalopoulos GK. Liver regeneration. J. Cell. Physiol 2007;213:286-300.
8. Marubashi S, Sakon M, Nagano H, Gotoh K, Hashimoto K, Kubota M, Kobayashi S, Yamamoto S, Miyamoto A, Dono K, Nakamori S, Umeshita K, Monden M. Effect of portal hemodynamics on liver regeneration studied in a novel portohepatic shunt rat model. Surgery 2004;36:1028-1037.
9. Arends B, Kruitwagen HS, Spee B, van den Ingh TSGAM, Penning LC, Rothuizen J. First clinical trial of HGF treatment in a dog with a congenital portosystemic shunt. In Arends B. New therapeutic strategies for canine liver disease, growth factors and liver progenitor cells. (thesis) May 2008;Cpt6:94-109.
10. Meyer HP, Rothuizen J, van Sluijs FJ, Voorhout G, van den Brom WE. Progressive remission of portosystemic shunting in 23 dogs after partial closure of congenital portosystemic shunts. Vet Rec 1999;144:333-337.
11. Spee B, Penning LC, van den Ingh TSGAM, Arends B, IJzer J, van Sluijs FJ, Rothuizen J. Regenerative and fibrotic pathways in canine hepatic portosystemic shunt and portal vein hypoplasia, new models for clinical hepatocyte growth factor treatment. Comp Hepatol 2005; 4:7.
12. Diehl AM, Rai RM. Regulation of signal transduction during liver regeneration. FASEB J 1996;10:215-227.
13. Strey CW, Winters MS, Markiewski MM, Lambris JD. Partial hepatectomy induced liver proteome changes in mice. Proteomics 2005;5:318-325.
14. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien P.A. Platelet-derived serotonin mediates liver regeneration. Science 2006;312:104-107.
15. Berent AC, Tobias KM. Portosystemic vascular anomalies. Vet Clin Small Anim 2009;39:513-541.
16. Mato JM, Corrales FJ, Lu SC, Avila MA. S-adenosylmethionine: a control switch that regulates liver function. FASEB J 2002;16:15-26.
17. Benjamin LE, Steele RD. Methionine metabolism after portacaval shunt in the rat. Am J Physiol 1985;249:G321-327.
18. Varela M, Fernández D, Martínez N, Embade N, Gómez L, Beraza N, Vázquez M, Rodríguez J, Luka Z, Wagner C, Lu SC, Martínez ML, Mato JM. Impaired liver regeneration in mice lacking glycine N-methyltransferase. Hepatology 2009;50:443-452.

19. Lu SC, Ramani K, Ou X, Lin M, Yu V, Ko K, Park R, Bottiglieri T, Tsukamoto H, Kanel G, French SW, Mato JM, Moats R, Grant E. S- adenosylmethionine in the chemoprevention and treatment of hepatocellular carcinoma in a rat model. Hepatology 2009;50:462-471.
20. Hunt GB, Hughes J. Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs. Aust Vet J 1999;77:303-307.
21. Petrie A, Watson P. Statistics for veterinary and animal science. Oxford, UK: Blackwell Science Ltd.;1999: 221-235.
22. Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. Prince Edward Island, Canada: AVC Inc.;2003:235-271.
23. Rothman KJ. Epidemiology, an introduction. New York, USA: Oxford University Press Inc.; 2002:144-167.
24. Tisdall PLC, Hunt GB, Bellenger CR, Malik R. Congenital portosystemic shunts in Maltese and Australian Cattle Dogs. Aust Vet J 1994;71:174-178.
25. Wolschrijn CF, Mahapokai W, Rothuizen J, Meyer HP, van Sluijs FJ. Gauged attenuation of congenital portosystemic shunts: results in 160 dogs and 15 cats. Vet Quart 2000;22:94-98.
26. Tanimizu N, Miyajima A. Molecular mechanism of liver development and regeneration. Int Rev Cyt 2007;259:1-48.
27. Schotanus BA, van den Ingh TSGAM, Penning LC, Rothuizen J, Roskams TA, Spee B. Cross-species immunohistochemical investigation of the activation of the liver progenitor cell niche in different types of liver disease. Liver Int 2009;29:1241-1252.
28. Baade S, Aupperle H, Grevel V, Schoon HA. Histopathological and immunohistochemical investigations of hepatic lesions associated with congenital portosystemic shunt in dogs. J Comp Pathol 2006;134:84-94.
29. Maxwell A, Hurley K, Burton C, Batt R, Camacho-Hübner C. Reduced serum insulin-like growth factor (IGF) and IGF-binding protein-3 concentrations in two deerhounds with congenital portosystemic shunts. J Vet Intern Med 2000;14:542-545.
30. Hama N, Yanagisawa Y, Dono K, Kobayashi S, Marubashi S, Nagano H, Umeshita K, Watanabe S, Uchiyama Y, Monden M. Gene expression profiling of acute cellular rejection in rat liver transplantation using DNA microarrays. Liver Transpl 2009;15:509-521.

Chapter 10

Samenvatting (summary in Dutch)

Inleiding en doel van het proefschrift
Dit proefschrift beschrijft onderzoek naar de chirurgische behandeling van honden met een congenitale portosystemische shunt (CPSS) en naar factoren die van invloed kunnen zijn op het postoperatieve herstel van deze aandoening. In **hoofdstuk 1** worden het doel en de vraagstellingen beschreven van het proefschrift en in **hoofdstuk 2** wordt een overzicht gegeven van de literatuur die betrekking heeft op het onderwerp.

Een congenitale portosystemische shunt of aangeboren levershunt is een afwijkend bloedvat dat het bloed afkomstig van organen zoals de milt, alvleesklier en darmen rechtstreeks naar de grote veneuze bloedvaten richting het hart afvoert, in plaats van de normale afvoer naar de lever via de poortader (Vena porta). Deze aandoening komt voor bij verschillende diersoorten en zeer zelden ook bij de mens. Congenitale portosystemische shunts zijn in te delen in intrahepatische shunts (shunts gelegen in de lever) en extrahepatische shunts (shunts gelegen buiten de lever). Door de sterke vermindering van de portale bloedvoorziening naar de lever, is de lever onderontwikkeld en functioneert deze slecht. Bovendien veroorzaakt de abnormale bloedafvoer van de poortader langs, in plaats van door de lever, verschillende klinische afwijkingen door een hogere concentratie van toxinen, hormonen en andere substanties in de rest van het lichaam.

De behandeling bestaat bij voorkeur uit een chirurgische vernauwing van de shunt, maar deze ingreep kent ernstige complicaties kort na operatie en leidt op lange termijn niet bij alle patiënten tot een volledig herstel van klinische verschijnselen (klinisch herstel) of tot een volledige afwezigheid van bloedstroom door de shunt (volledig herstel van de portosystemische shunting). Helaas is vooraf de uitkomst van de operatie moeilijk te voorspellen. Een volledige sluiting van de portosystemische shunt, met bijvoorbeeld een ligatuur van onoplosbaar hechtmateriaal, heeft in de meeste honden een betere prognose dan een gedeeltelijke vernauwing. Een volledige sluiting van de shunt tijdens de operatie is echter in de meerderheid van de honden niet mogelijk doordat de lever en de portale bloedvaten vaak onvoldoende ontwikkeld zijn om het extra toegevoerde bloed te kunnen verwerken. Als gevolg hiervan ontstaat een hypertensie (verhoogde bloeddruk) in de portale organen, met in ernstige gevallen de dood als gevolg. Daarom wordt tijdens het ligeren (onderbinden) van een portosystemische shunt, dit bloedvat in stappen vernauwd tot de kleinste diameter die mogelijk is zonder dat er verschijnselen van portale hypertensie ontstaan. Ondanks het feit dat met deze techniek de shunt slechts gedeeltelijk gesloten wordt, treedt in de meerderheid van de honden in de maanden na operatie een toenemende tot volledige functionele sluiting op. In een klein gedeelte van de honden blijft de lever echter slecht functioneren ten gevolge van persisterende portosystemische shunting, door de originele shunt of door vorming van multiple collaterale bloedvaten, die ook wel verkregen portosystemische shunts worden genoemd.

Om de kans op volledig herstel te vergroten zijn een aantal chirurgische technieken ontwikkeld die de shunt in de periode na de operatie geleidelijk sluiten, zoals het aanbrengen van een cellofaan band om de shunt. De lever en de portale bloedvaten hebben hierdoor meer tijd om zich aan te passen aan de extra bloedtoevoer vanuit de portale organen zonder dat hypertensie optreedt. Ook deze technieken blijken in het grootste gedeelte van de honden met een CPSS tot volledig herstel te leiden. Toch worden bij elke techniek honden beschreven die niet herstellen. Het is niet bekend waardoor herstel in deze honden uitblijft en er zijn geen duidelijke criteria bekend die voorafgaande aan een operatie de kans op herstel kunnen voorspellen.

Het doel van dit proefschrift was het identificeren van factoren die gerelateerd zijn aan de resultaten na chirurgische vernauwing van een aangeboren portosystemische shunt bij de hond, en inzicht te krijgen in het onderliggende mechanisme dat bepalend is voor postoperatief herstel bij deze aandoening.

Chirurgische technieken en herstel na CPSS vernauwing

In de **hoofdstukken 3 en 4** worden de postoperatieve uitkomsten en prognostische factoren beschreven van twee verschillende chirurgische technieken die regelmatig worden gebruikt om een aangeboren portosystemische shunt te vernauwen, namelijk het vernauwen door middel van een ligatuur of een cellofaan band. Beide technieken werden toegepast in een relatief grote groep honden (97 en 106 honden).

Bij beide technieken traden vergelijkbare postoperatieve complicaties op. Het aanbrengen van een cellofaan band om de shunt (hoofdstuk 4) lijkt een eenvoudigere procedure voor chirurgen met minder ervaring in het vernauwen van portosystemische shunts. Bovendien blijken de percentages van complicaties en mortaliteit (sterfte) kort na de operatie lager na aanbrengen van cellofaan, vooral als de bandjes relatief los om de shunt worden aangebracht. Deze voordelen lijken alleen op te gaan voor extrahepatische shunts. Omdat intrahepatische shunts meestal een grotere diameter hebben en volledig omringd kunnen zijn door leverweefsel, is het aanbrengen van een niet-vernauwende cellofaan band (met de aanbevolen maximale diameter van 3mm) om een intrahepatische shunt vaak moeilijk, of zelfs onmogelijk. Dit wordt bevestigd door de hogere complicatie- en mortaliteitscijfers bij intrahepatische shunts (55%, respectievelijk 27%) vergeleken met de cijfers bij extrahepatische shunts (13%, respectievelijk 3%) na het aanbrengen van cellofaan (hoofdstuk 4). De mortaliteit na aanbrengen van een ligatuur om een aangeboren portosystemische shunt was 27% en er werden geen verschillen gezien in mortaliteit tussen honden met intrahepatische en extrahepatische shunts (hoofdstuk 3).

Na het vernauwen van een shunt met een ligatuur (hoofdstuk 3), bleek dat een grotere mate van vernauwing tijdens de ingreep geassocieerd was met een lagere mortaliteit na de ingreep. Beide variabelen (mate van vernauwing en mortaliteit) waren gecorreleerd met de diameter van de hoofdstam van de poortader, welke een grote

variatie kent bij aangeboren portosystemische shunts. Aangeboren portosystemische shunts bleken daarom een aandoening te zijn waarbij niet alleen een afwijkend bloedvat aanwezig is, maar een aandoening waarbij naast een afwijkend bloedvat ook de portale bloedvaten naar en in de lever onderontwikkeld zijn. De prognose op lange termijn bleek echter onverwacht niet afhankelijk van de mate van operatieve vernauwing van de shunt of de diameter van de poortader op het moment van operatie. Uit beide studies bleek dat honden met blijvende portosystemische shunting na operatie meestal toch goed konden functioneren.

De conclusie was dat beide technieken effectief zijn om de klinische verschijnselen en de verminderde leverfunctie bij de meeste honden met een aangeboren portosystemische shunt te herstellen. Met beide technieken blijken er echter honden te zijn die niet herstellen. Hoofdstuk 3 beschrijft recidief van klinische verschijnselen in 10% van de honden na aanbrengen van een ligatuur om de shunt en in hoofdstuk 4 blijkt na toepassing van een cellofaan band om de shunt in 16% van de honden de lever nog niet normaal te functioneren. Ondanks verschillen in beide onderzoeken wat betreft opzet, populaties en rassen, blijkt de chirurgische techniek mogelijk niet de belangrijkste factor voor herstel op lange termijn. Andere factoren zoals het vermogen van de lever en de portale bloedvaten om zich alsnog te ontwikkelen na een operatieve vernauwing van de shunt, kunnen net zo belangrijk zijn om een normale leverfunctie te bereiken.

Stollingsstoornissen en herstel na CPSS vernauwing

De studie beschreven in **hoofdstuk 5** werd geïnitieerd omdat in de studie van hoofdstuk 3 bloedverlies ten gevolge van een afwijkende stolling een belangrijke oorzaak was van mortaliteit kort na een operatieve CPSS vernauwing. Stollingstijden, thrombocyten (bloedplaatjes) en 8 stollingsfactoren werden bepaald in 39 gezonde honden en in 34 honden met een CPSS, voor en na vernauwing van de shunt. Honden met een portosystemische shunt hadden voor de operatie een lager aantal thrombocyten, een lagere activiteit van de factoren II, V, VII, X, een verhoogde activiteit van factor VIII, en een verlengde geactiveerde partiële thromboplastine tijd vergeleken met gezonde honden. Direct na chirurgische vernauwing van de shunt, bleken het aantal thrombocyten en de activiteit van de factoren I, II, V, VII, IX en XI te zijn verminderd ten opzichte van voor de operatie, en de prothrombine tijd was verlengd.

Om het effect van een buikoperatie via de mediaanlijn in combinatie met ligatie van bloedvaten te evalueren in honden zonder portosystemische shunt, werden dezelfde bepalingen uitgevoerd in 8 gezonde honden voor en na een ovariectomie (verwijdering van de eierstokken). Deze chirurgische ingreep in gezonde honden veroorzaakte een vermindering van de activiteit van de factoren VII en X.

In de honden met een CPSS die volledig herstelden van portosystemische shunting na de operatie, waren de gevonden afwijkingen in de stolling 6 weken na de operatie volledig genormaliseerd, maar in de honden met persisterende shunting bleven de afwijkingen in de stolling bestaan.

De resultaten uit dit onderzoek hebben er toe geleid dat aan honden met een CPSS en verlengde stollingstijden plasma transfusies worden gegeven voor en tijdens de operatie en dat na de operatie stollingstijden, thrombocyten en plaatsen waar bloedingen zijn te verwachten intensief worden gecontroleerd.

Groei van de lever en herstel na CPSS vernauwing

In het volgende hoofdstuk (**hoofdstuk 6**) werden 2 beeldvormende technieken beschreven om de grootte van de lever te bepalen, CT en MRI. De grootte en de groei van de lever werd gemeten bij 10 honden met een portosystemische shunt voor en na chirurgische vernauwing, omdat de groei van de lever een rol zou kunnen spelen in de prognose op lange termijn. MRI en/of CT scans gemaakt direct voor de operatie en 8 dagen, 1 maand en 2 maanden na operatie werden gebruikt om het levervolume per kilogram (kg) lichaamsgewicht te berekenen. Vòòr de operatie was het levervolume 18,2 cm^3 per kg*. De groei van de lever was het grootst in de eerste 8 dagen na de operatie (44%*). Twee maanden na operatie hadden de honden een levervolume van 28,8 cm^3 per kg*, wat overeenkomt met een normale levergrootte. Er werden geen verschillen gevonden in groei van de lever tussen honden met een complete of gedeeltelijke operatieve sluiting van de shunt of tussen honden met een volledig of gedeeltelijk herstel van portosystemische shunting na de operatie. De levervolumes die werden bepaald met CT en MRI waren nauw gecorreleerd (r=0,98), maar de volumes van levers gemeten met MRI bleken 6,8% groter te zijn dan de volumes van dezelfde levers gemeten met CT. CT had de voorkeur boven MRI vanwege de grotere snelheid en de mogelijk betere nauwkeurigheid.

Gen expressie in de lever en herstel na CPSS vernauwing

De ontwikkeling van de lever na het vernauwen van een portosystemische shunt is een gecompliceerd proces dat mogelijk veel overeenkomsten kent met leverregeneratie na gedeeltelijke resectie van de lever. In **hoofdstuk 7** werd de mRNA expressie van 19 genen bestudeerd in leverweefsel dat werd verzameld in 48 honden tijdens de operatieve vernauwing van een CPSS. De genen werden geselecteerd omdat ze betrokken zijn bij processen die regeneratie van de lever beïnvloeden (fibrose, apoptose en de groei van lever en bloedvaten). Twee genen waren in dit onderzoek gecorreleerd met postoperatief herstel van portosystemische shunting, namelijk methionine adenosyltransferase 2 alpha (MAT2a) en hepatocyt groei factor activator (HGFac).

* mediane waarde

Er werd een model ontworpen dat beide genen gebruikt als binaire variabelen om het herstel in individuele honden te kunnen voorspellen. In dit model werd de expressie van de genen uitgedrukt als hoog of laag ten opzichte van een bepaalde grenswaarde (0,457 voor MAT2a en 0,974 voor HGFac). Vòòr de operatie kon met dit model de kans op herstel na operatie correct worden voorspeld in 75% van de honden.

Omdat niet duidelijk is welke andere genen een rol zouden kunnen spelen in het proces van herstel na vernauwing van een CPSS, werd vervolgens onderzoek gedaan om genen te identificeren met een verschil in expressie tussen honden die wel herstellen en honden die niet herstellen. De eerste resultaten van dat onderzoek zijn beschreven in **hoofdstuk 8**. Met behulp van microarray analyses werd de mRNA expressie van een groot aantal genen (42.034) gescreend in leverweefsel van 23 honden met volledig herstel en 23 zonder volledig herstel na operatieve vernauwing van een levershunt. De expressie van 63 genen bleek verschillend te zijn tussen beide groepen. Een aantal van deze genen zijn betrokken bij groei van de lever, leverfibrose of ontsteking van endotheel (vaatwanden) en kunnen daardoor heel goed direct een effect hebben op het postoperatieve herstel. Andere genen kunnen gerelateerd zijn aan de verschillen in leverfunctie tussen de beide groepen (bijvoorbeeld genen betrokken bij het metabolisme van glutamaat). Uit de resultaten van dit onderzoek kon geen bruikbaar model worden geconstrueerd om herstel voorafgaande aan de operatie te voorspellen.

Dit onderzoek heeft een aantal interessante genen opgeleverd om de vraagstellingen van dit proefschrift in de toekomst verder uit te werken. Daarvoor is onderzoek noodzakelijk om de rol van de geïdentificeerde genen in het proces van postoperatief herstel te bevestigen en de manier waarop de betreffende genen effect op de prognose hebben verder uit te zoeken. Een voorbeeld van een belangrijke richting voor nader onderzoek is het metabolisme van s-adenosyl methionine in honden met een CPSS. Genen betrokken bij dit metabole pad in de lever waren gerelateerd aan postoperatief herstel in zowel het onderzoek beschreven in hoofdstuk 7, als in hoofdstuk 8.

Conclusie

Hoofdstuk 9 van het proefschrift beschrijft naast een samenvatting en een discussie van de belangrijkste resultaten, de conclusie van dit proefschrift. Naar verwachting blijft een chirurgische vernauwing van een congenitale portosystemische shunt essentieel om een volledig herstel van portosystemische shunting te bereiken. Toekomstig onderzoek, bij voorkeur in samenwerking met verschillende klinieken, kan leiden tot een betere voorspelling van het effect van een operatieve CPSS vernauwing bij een individuele hond en tot de ontwikkeling van aanvullende behandelingen.

Curriculum vitae / List of publications

Curriculum vitae

Anne Kummeling was born in Geldrop, The Netherlands, on September 7, 1971. She attended secondary school at the Anton van Duinkerken College in Veldhoven (1983-1984) and at the Rythovius College in Eersel (1984-1989). In 1989 the author started to study veterinary medicine at Utrecht University. During her studies, she investigated causes of calf diarrhoea in the Tilarán region, Costa Rica, in a research project at the Universidad Nacional, Heredia (1993-1994). After returning to the Netherlands, the author was invited to enrol in the 'Excellent Tracé' research training program at the Veterinary Faculty in Utrecht (1994–1995). In this program, she studied two different techniques for closure of abdominal fascia and skin in median celiotomy wounds in dogs. This study was rewarded with the Master of Veterinary Research degree. The author graduated cum laude as a Doctor of Veterinary Medicine in 1998.

After experiencing life as veterinarian in a companion animal practice in Roosendaal, she returned to the Department of Clinical Sciences of Companion Animals in Utrecht to complete an internship (1998-1999) and a residency in companion animal surgery (1999-2002). In 2003, she passed the board examination of the European College of Veterinary Surgery (ECVS) in Glasgow, UK, and became a Diplomate of the ECVS. Her main professional interests are hepatic and urologic surgery. At present, the author is staff member at the Department of Clinical Sciences of Companion Animals, where she runs the Urology section. She is married to Maarten and together they have a son (Sarne, 2005) and a daughter (Marrit, 2007).

This PhD research project was started in 1999 to study surgical techniques, postoperative complications and prognostic factors in dogs that were presented with a congenital portosystemic shunt. The results were described in the present thesis, which will be defended on December 10, 2009 in Utrecht.

List of publications and conference proceedings

Pérez E**, Kummeling A**, Janssen MMH, Jiménez C, Alvarado R, Caballero M, Donado P, Dwinger RH. Infectious agents associated with diarrhoea of calves in the canton of Tilarán, Costa Rica. Preventive Veterinary Medicine 1998;33:195-205.

Kummeling A, van Sluijs JF. Closure of the rectus sheath with a continuous looped suture and the skin with staples in dogs: speed, safety, and costs compared to closure of the rectus sheath with interrupted sutures and the skin with a continuous subdermal suture. Veterinary Quarterly 1998;20:126-130.

Kummeling A. Postligation seizure syndrome in dogs and cats: 9 cases. Scientific Proceedings WSAVA/FECAVA World Congress 2000:495.

Kummeling A, van Sluijs FJ, Rothuizen J. Recurrence of hepatoencephalopathy after attenuation of congenital portosystemic shunts in dogs. Proceedings Voorjaarsdagen Conference 2002:247-248.

Kummeling A, van Sluijs FJ, Rothuizen J. Prognostic implications of the degree of shunt narrowing and of the portal vein diameter in dogs with congenital portosystemic shunts. Veterinary Surgery 2004;33:17-24.

Hunt GB, **Kummeling A**, Tisdall PLC, Marchevsky AM, Liptak JM, Youmans KR, Goldsmid SE, Beck JA. Outcomes of cellophane banding for congenital portosystemic shunts in 106 dogs and 5 cats. Veterinary Surgery 2004;33:25-31.

Kummeling A, Hunt GB. Cellophane banding for congenital portosystemic shunts in dogs and cats. Proceedings 13th Annual Scientific Meeting ECVS 2004:329-331.

Kummeling A, Rothuizen J, Penning LC, Brinkhof B, van Sluijs FJ. Prognostic factors for congenital portosystemic shunts based on gene expression profiles. Proceedings 15th ECVIM-CA Congress 2005;185-187.

Kummeling A, Teske E, Rothuizen J, van Sluijs FJ. Coagulation profiles in dogs with congenital portosystemic shunts before and after surgical attenuation. Journal of Veterinary Internal Medicine 2006;20:1319-1326.

Kummeling A, Penning LC, Rothuizen J, van Sluijs FJ. Prognostic implications for congenital portosystemic shunts in dogs of hepatic gene expression. Proceedings Voorjaarsdagen Conference 2007:198.

Kummeling A, Rothuizen J, Penning LC, Brinkhof B, Weber MF, van Sluijs FJ. Regulatory pathways predicting the prognosis of surgical intervention of portosystemic shunts. Proceedings 19th ECVIM-CA Congress 2009:125-128.

Kummeling A, Vrakking DJE, Rothuizen J, Gerritsen KM, van Sluijs FJ. Hepatic volume measurements in dogs with extrahepatic congenital portosystemic shunts before and after surgical attenuation. Journal of Veterinary Internal Medicine (accepted).

Contributions to books

Kummeling A, Klein W. Chirurgische infecties en antimicrobiële profylaxe. In: Kirpensteijn J, Klein W, eds. Leren opereren. Ripon, United Kingdom: Roman House Publishers Ltd; 2005:86-93.

Kummeling A, Klein W. Surgical infections and antimicrobial prophylaxis. In: Kirpensteijn J, Klein W, eds. The Cutting Edge. Ripon, United Kingdom: Roman House Publishers Ltd; 2006:88-95.

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Abbreviations

ABCG2	ATP-binding cassette protein, subfamily G member 2
ADRP	adipophilin
AIC	Akaike's information criterion
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
ASK	apoptosis signal-regulating kinase
ATP	adenosine triphosphate
ATT	ammonia tolerance testing
B2M	beta 2 microglobulin
Bcl-2	B-cell lymphoma 2 protein
BMI1	BMI1 polycomb ring finger oncogene protein
BUN	blood urea nitrogen
c-DNA	cyclic DNA (deoxyribonucleic acid)
c-MET	MET proto-oncogene (tyrosine kinase HGF receptor)
Casp3	caspase-3
CCND1	cyclin D1
CDC6	cell division control protein 6 homolog
CPSS	congenital portosystemic shunt
CT	computed tomography
CTGF	connective tissue growth factor
CYP2D6	cytochroom P450 2D6 fragment
CYR61	cysteine-rich angiogenic inducer 61 protein
GLS	glutaminase
GMT	glutamate transporter
GNMT	glycine N-methyltransferase
GST	glutathione-s-transferase
HEPC	hepcidin
HES	hydroxyethyl starch
HGF	hepatocyte growth factor
HGFac	hepatocyte growth factor activator
HPRT	hypoxanthine-guanine phosphoribosyltransferase
IGF	insulin-like growth factor
IGF-R	insulin-like growth factor receptor
IGFBP	insulin-like growth factor binding protein
IV	intravenous(ly)
JNK	c-jun N-terminal kinase
KDR	kinase insert domain receptor (VEGF receptor)
LPCs	liver progenitor cells

MAT (1a/2a)	methionine adenosyltransferase (1 alpha/2 alpha)
MMD	macrophage differentiation protein
MRI	magnetic resonance imaging
mRNA	messenger RNA (ribonucleic acid)
NAGS	N-acetylglutamate synthase
PCV	packed cell volume
PH	partial hepatectomy
PIK3Rα	phosphatidylinositol 3-kinase regulatory subunit alpha
PIM-2	serine/threonine-protein kinase Pim-2
PKI-β	cAMP-dependent protein kinase beta
PPVH	primary portal vein hypoplasia
PT	prothrombin time
qPCR	quantitative real-time polymerase chain reaction assay
ROC	receiver operating characteristic
RPS5	ribosomal protein S5
SAHcy	S-adenosylhomocysteine
SAMe	S-adenosylmethionine
SBA	serum bile acid concentration
SI	shunt index
Se	sensitivity
Sp	specificity
TGF-β1	transforming growth factor β1
TGF-βR	transforming growth factor β1 receptor
TTR	transthyretin
TXNIP	thioredoxin-interacting protein
uPA	urokinase plasminogen activator
UPC	ubiquitin carrier protein
VCAM1	vascular cell adhesion protein 1
VEGFa	vascular endothelial growth factor A
VWF	von Willebrand factor