

**Congenital Extrahepatic Portosystemic Shunts in Dogs:
Novel insights into Porto-Azygos Shunts and into the Role of Ammonia**

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Dissertation submitted in the fulfillment of the requirements for the degree of Doctor in
Veterinary Sciences (PhD), Faculty of Veterinary Medicine

Ghent University

2017

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This PhD was funded in part by the ECVS Resident research grant, awarded in 2013 and printing was supported by Alcyon.



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Faculty of Veterinary Medicine, Ghent University

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List of Abbreviations

AC	Ameroid constrictor
BA	Bile acids
BBB	Blood-brain barrier
CatDx	Catalyst Dx ^o chemistry analyzer
CB	Cellophane band
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTA	Computed tomographic angiography
EHPSS	Extrahepatic portosystemic shunts
HE	Hepatic encephalopathy
IHPSS	Intrahepatic portosystemic shunts
IV	Intravenous
MRA	Magnetic resonance imaging with angiography
MRI	Magnetic resonance imaging
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
PockBA	PocketChem BA
PRPS	Per-rectal portal scintigraphy
PSS	Portosystemic shunt
SBA	Serum bile acids
SF	Shunt fraction
Tc	Technetium
TFB	Thin film band
TSPS	Transsplenic portal scintigraphy

GENERAL INTRODUCTION

1. Introduction

A portosystemic shunt (PSS) is an anomalous vascular communication connecting the portal circulation to the venous part of the systemic circulation. As a consequence blood drained from the gastrointestinal tract bypasses the liver. Portosystemic shunting may occur within the hepatic parenchyma (intrahepatic, IHPSS) or outside the liver (extrahepatic, EHPSS) (Figure 1). Portosystemic shunts result from developmental embryogenesis anomalies (congenital) or form secondary to portal hypertension (acquired).

The origin, site of insertion, and diameter of the shunting vessel may impact the fraction of the liver that is hypoperfused and consequently the severity and progression of the clinical signs and the severity of the histopathologic changes.

This PhD thesis will be introduced by an overview of the scientific literature that was available before the onset of the PhD research, in order to better understand the rationale and the scientific aims of this thesis. This literature review will be confined to congenital EHPSSs in dogs, the topic of all studies presented in this work.

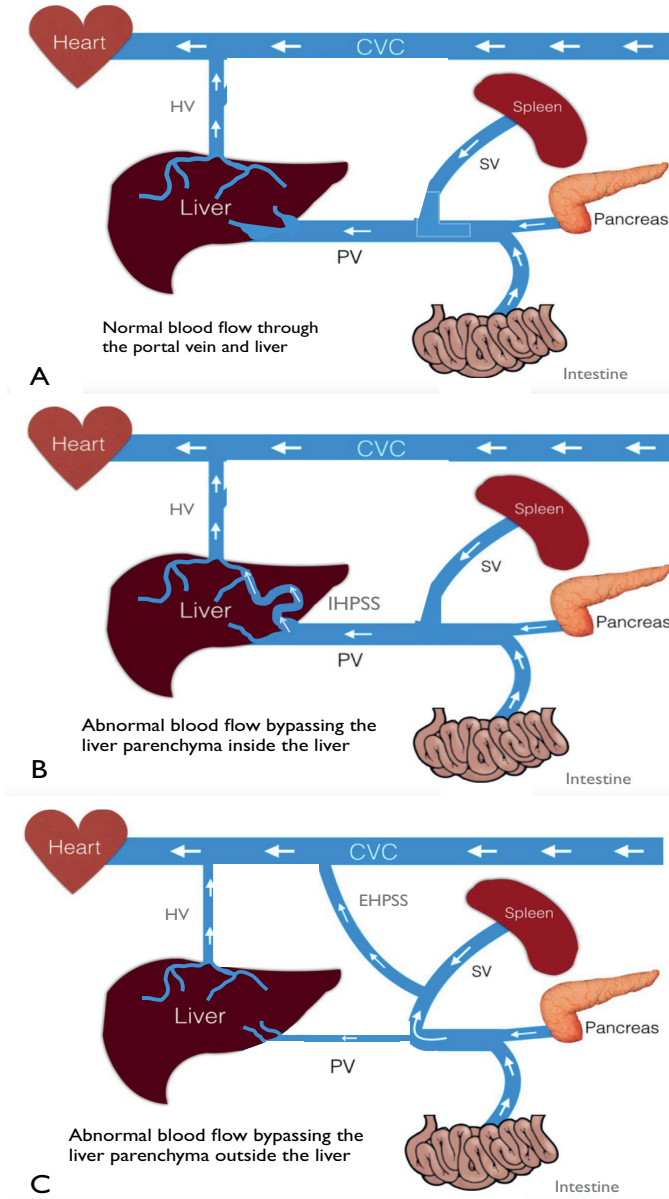


Figure 1. Portal blood flow in the dog; A, Normal blood flow through hepatic sinusoids; B, Intrahepatic portosystemic shunt (IHPSS), anomalous vascular communication, connecting portal vein to systemic circulation inside the liver; C, Extrahepatic portosystemic shunt (EHPSS), anomalous vascular communication, connecting portal to systemic circulation outside the liver. Caudal vena cava (CVC), hepatic veins (HV), portal vein (PV), splenic vein (SV) (based on <http://dx.doi.org/10.1136/vetreccr-2014-000105>)

2. Anatomy of the Normal Liver and Hepatic Vasculature in Dogs

The liver of dogs comprises five lobes (left lobe, quadrate lobe, right medial lobe, right lateral lobe and caudate lobe), two processes and a gallbladder (Figure 2). The left lobe is the largest and is subdivided into the left lateral and left medial lobes. The quadrate lobe lies almost on the midline, and its lateral aspect forms the medial side of the fossa containing the gallbladder. The right medial lobe lies laterally to the quadrate lobe; its medial aspect forms the lateral side of the gallbladder fossa. The right lateral lobe lies laterally to the medial lobe, and is usually fused with the caudate lobe, which is subdivided into the caudate and papillary processes.¹

The liver has the most complicated vasculature of any organ. The blood supply to the liver comes from two distinct sources, the hepatic artery and the portal vein. The nutrient-rich blood coming from the gastrointestinal tract flows in from the portal vein. The portal vein provides nearly 80 % of the total blood volume to the liver, and 50% of the oxygen supply. The remaining part, 20% and 50%, respectively, is supplied via the hepatic artery.² Within the liver parenchyma, drainage occurs into the hepatic veins that drain into the abdominal portion of the caudal vena cava.

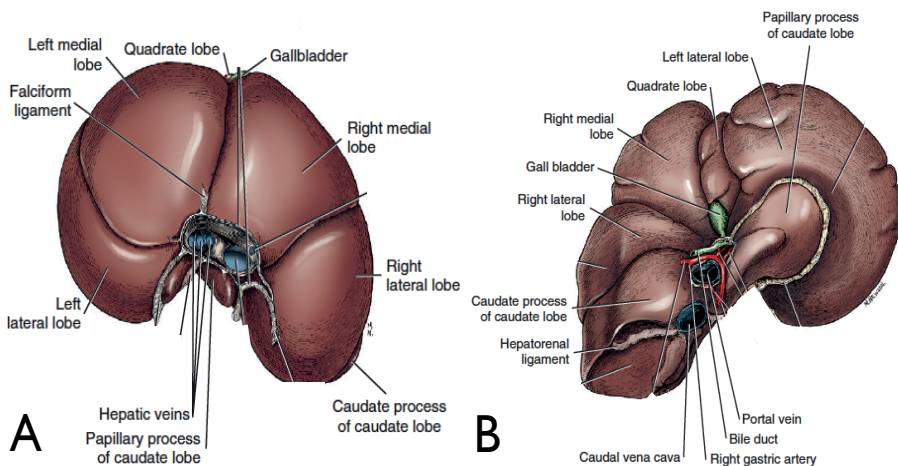


Figure 2. Anatomy of the liver lobes in the dog: A, Diaphragmatic view of the liver; B, Visceral view of the liver (From Evans HE: The digestive apparatus and abdomen. In Evans HE, deLahunta A: Miller's Anatomy of the Dog, ed 4, St Louis, 2013, Saunders/Elsevier.)

2.1 Portal Vein

The portal vein is created by the confluence of the cranial mesenteric vein, which mainly drains the small intestines, and the caudal mesenteric vein, which drains the colon and proximal rectum. Additional tributaries joining the portal vein cranially include the splenic vein, which drains blood from the spleen and part of the stomach, and, the gastroduodenal vein, which drains the pancreas, duodenum, and stomach (Figure 3).^{1,3}

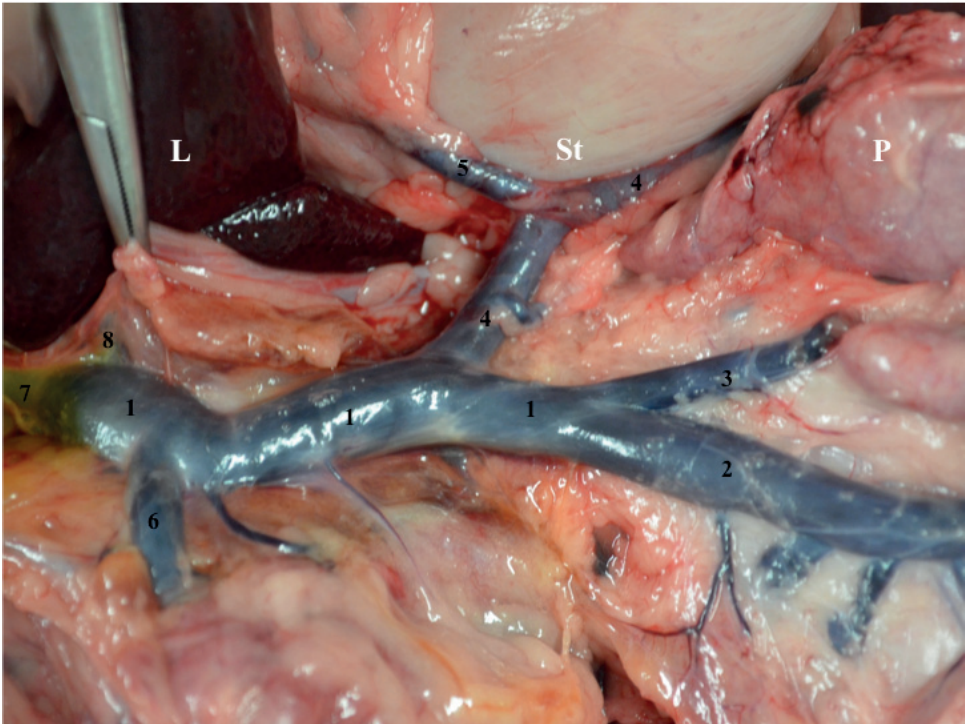


Figure 3. The portal vein with its tributaries in a canine cadaver: Ventral left oblique view after removal of the parietal leave of the omental bursa and transection of the left lobe of the pancreas (left is cranial). 1, Portal vein; 2, Cranial mesenteric vein; 3, Caudal mesenteric vein; 4, Splenic vein; 5, Left gastric vein; 6, Gastroduodenal vein; 7, Right portal vein branch; 8, Left portal vein branch; L, Liver; St, Stomach; P, Pancreas.

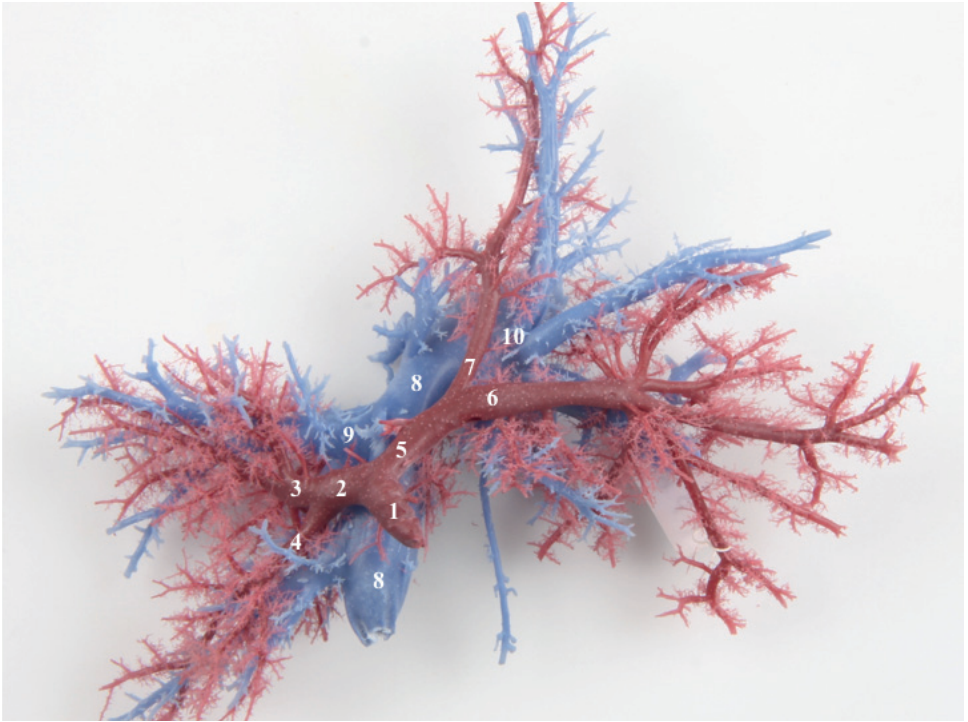


Figure 4. Vascular corrosion cast (Baston's #17) of the portal vein (red) and caudal vena cava (blue) of a normal dog: Ventral caudo-cranial view (top is cranial).

1, Portal vein; 2, Right portal vein branch; 3, Right lateral lobe branches; 4, Caudate process of caudate lobe branches; 5, Left portal vein branch; 6, Quadrate lobe, left lateral lobe and left medial lobe branches; 7, Right middle lobe and papillary process of the caudate lobe branches; 8, Caudal vena cava; 9, Right hepatic vein branches; 10, Left hepatic vein branches.

The cranial mesenteric vein is the largest tributary to the portal vein. Just cranial to the insertion point of its gastroduodenal tributary, the portal vein divides into 2 branches, the right and left portal vein (Figure 3; Figure 4). The right portal vein is shorter and smaller than its left counterpart and supplies the caudate process of the caudate lobe and the right lateral lobe of the liver. The left portal vein further divides into a central branch supplying the right medial lobe and the papillary process of the caudate lobe, and a left branch supplying the quadrate, left medial, and left lateral liver lobes.^{4,5} In 60% of dogs, the left portal vein also gives rise to a branch that supplies the dorsal part of the right lateral liver lobe.⁵

2.2 Hepatic Artery

The hepatic artery is a branch of the celiac artery, the first visceral branch of the abdominal aorta at the level of the first lumbar vertebra. The hepatic artery divides mainly into 3 branches (right lateral, right medial, and left branch), although additional branches can be present.⁵ The right lateral branch supplies the caudate and right lateral hepatic lobe.⁶ The right medial branch supplies the right medial lobe, the dorsal part of the quadrate lobe, and a portion of the left medial lobe. The left hepatic branch gives rise to the cystic artery and branches to the left lateral, the quadrate and part of the left medial lobe.⁵ The hepatic arteries are usually located on the ventral surface of the eponymous portal vein branches, although some arterial branches, such as the left hepatic branch are found dorsally.⁷

2.3 Hepatic Veins

Blood from the portal vein and from the hepatic arteries mixes within the hepatic sinusoids before collecting in central veins. These central veins merge and form the hepatic veins that drain into the abdominal part of the caudal vena cava. Dogs usually have 5 to 8 hepatic veins, but the amount of branches and topography varies.^{2,3,7-10}

3. Embryology of the Liver Vasculature in Dogs

3.1 Normal Dog

The venous system of the abdominal cavity originates from 3 main embryonic veins; the umbilical, vitelline, and cardinal veins.⁹ In dogs, the portal vein and its tributaries are formed from the embryonic vitelline veins.^{11,12} Vitelline veins also form the hepatic sinusoids. The caudal vena cava and the azygos vein are formed through several transformations of the cardinal venous system.^{9,11,12} It is suggested, but never demonstrated, that in normal dogs, numerous preexisting nonfunctional portosystemic anastomoses may open in case of sustained elevated pressures in the portal system.^{9,13}

3.2 Congenital Extrahepatic Portosystemisch Shunts

The development of abdominal vasculature in utero explains the anatomical variations observed in dogs with congenital PSS.

Congenital EHPSSs represent inappropriate functional connections between the embryonic cardinal and vitelline systems that persist postnatally.⁹ Those shunt types are thus developmental anomalies; they are not derived from pre-existing embryonic connections. Nevertheless, it has been suggested that some congenital EHPSSs result from concurrent portal microvascular underdevelopment that increases intrahepatic portal resistance, forcing residual anomalous vessels to remain open.¹⁴

A congenital EHPSS most commonly occurs as a single extrahepatic vessel that provides vascular communication between the portal venous and the systemic venous circulation, bypassing the liver. However, some animals have two or more congenital communications.^{9,14} Connections of these anomalous vessels have been found between the portal vein or its tributaries and the caudal vena cava (portocaval shunts), the azygos vein (porto-azygos shunts), the renal vein, the phrenic vein (portophrenic shunts), the internal thoracic vein, and the umbilical vein (Figure 5).^{15,16} The majority of congenital EHPSSs are portocaval.^{14,17-19} The relative incidence of the different anatomical types of congenital EHPSS, based on origin and termination of the shunting vessel, seems subjected to regional differences in dog populations.¹⁶

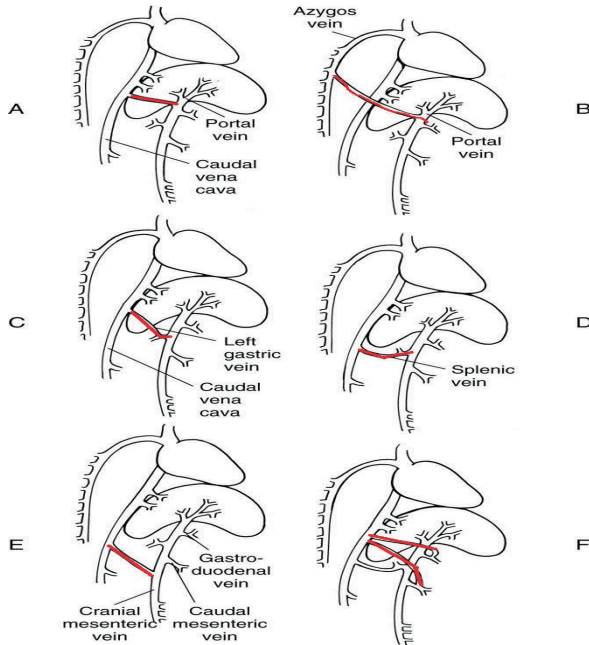


Figure 5. Types of extrahepatic portosystemic shunts in the dog: A, Portal vein to caudal vena cava; B, Portal vein to azygos vein; C, Left gastric vein to caudal vena cava; D, Splenic vein to caudal vena cava; E, Left gastric, cranial mesenteric, caudal mesenteric, or gastroduodenal vein to caudal vena cava; F Combinations of two of the above communications. (Adapted from Fossum TW, Editor: *Small Animal Surgery*, ed 3, St Louis, 2007, Mosby/Elsevier)

4. Incidence and Demographic Data in Dogs with Congenital Extrahepatic Portosystemic Shunts

Congenital PSSs are reported in 0.18% of all dogs, and in only 0.05% of mixed-breed dogs.^{20,22} Approximately 66% to 75% of all congenital PSS are extrahepatic.^{20,22}

Three-quarter of the congenital EHPSSs are identified in young dogs, but congenital shunts, especially shunts with porto-azygos or portophrenic communication, are also diagnosed in dogs older than 2 years of age.^{16,22-25} There is no clear gender predisposition.^{25,26} Extrahepatic PSSs are most commonly seen in small- or toy breed dogs.^{12,18,20,27} In several breeds a hereditary base is suspected.^{14,20} However, a genetic background for EHPSS has so far only been demonstrated in the Maltese²⁸, the Cairn terrier^{18,29}, and the Yorkshire terrier.²¹

5. Clinical Signs in Dogs with Congenital Extrahepatic Portosystemic Shunts

In few cases of dogs with a congenital EHPSS, a history of anesthetic intolerance or prolonged recovery is the only sign to suspect the presence of a PSS.^{19,27,30,31} The majority of dogs with EHPSS have intermittent episodes of dullness, lethargy, or “abnormal” behavior whereas also the gastrointestinal and urinary systems are commonly affected.

Clinical signs are considered milder in dogs with a porto-azygos shunt than in patients with a portocaval shunt; the same applies to portophrenic versus other portocaval communications. The diaphragm likely compresses porto-azygos and portophrenic shunt types during respiration or with gastric distention after meals, resulting in temporary improvement of the portal perfusion.¹⁴

Hepatic encephalopathy (HE) occurs in up to 95% of dogs with a congenital EHPSS, but the clinical signs of HE can vary between very obvious to subtle and, in the last situation, may go unnoticed by the owner or even by the attending veterinarian. Typical signs of “abnormal” behavior include stargazing, head pressing, staring into walls or corners, random barking, pacing, aggression, ataxia, unresponsiveness, circling, trembling or shaking.^{27,30,32,33} The neurological abnormalities are often episodic and may be more pronounced after eating high protein meals.^{27,33}

Dogs with a congenital EHPSS typically are undersized or reported to be the runt of the litter. Many have a history of weight loss or failure to gain weight.³⁴ They can present with gastrointestinal signs including vomiting, diarrhea, anorexia, pica, and/or signs of gastrointestinal bleeding.^{18,19,27} As a consequence of hepatic dysfunction, bile production is diminished, hindering digestion and causing maldigestion and malabsorption symptoms.³⁵ Nausea, inappetence and vomiting are also suggested to be caused by toxic substances affecting the chemoreceptor trigger zone in the brain.^{35,36}

Often lower urinary tract signs such as hematuria, stranguria, pollakiuria, or urinary tract obstruction are observed due to the presence of ammonium (bi)urate calculi formation and secondary urinary tract infection.^{18,19,33} Polydipsia with secondary polyuria is common in dogs with a congenital EHPSS. Both may be triggered by an altered renal medullary gradient due to low blood urea nitrogen (BUN), by changes in portal vein osmoreceptors, and by urinary tract disease but they may also be attributed to HE.^{14,24,37}

6. Pathophysiology of Hepatic Encephalopathy in Dogs with a Portosystemic Shunt

A healthy liver filters, neutralizes and detoxifies neurotoxic substances that are absorbed across the gastrointestinal barrier and drain into the portal system.^{12,18} In the presence of a PSS, however, a vast proportion of the portal blood bypasses the liver and those toxic byproducts of metabolism thus accumulate in the blood.³⁸ In addition, the liver lacks the nutrient supply from the blood.^{12,18} Therefore, the blood that is not shunted cannot be efficiently detoxified due to too little functional parenchyma.³⁶

Hepatic encephalopathy is a metabolic syndrome that may result from a variety of liver diseases and that is characterized by diffuse cerebral and neuropsychiatric dysfunction.^{36,39,40}

Some degree of HE is likely present in nearly every dog with a PSS, but the symptoms as well as their severity may be episodic and vary considerably. Although the syndrome is well recognized and contributing factors have been determined, the pathogenesis of HE particularly in dogs with PSS remains largely unknown. In humans, HE is mainly associated with acute or chronic liver diseases and advanced cirrhotic disease and far less with congenital portal venous bypasses, because the latter are extremely rare congenital malformations in humans.⁴¹ It also frequently develops in human patients who underwent transjugular intrahepatic portosystemic stent-shunt insertion.^{42,43}

In patients with HE, various neurotransmitter systems are affected.^{36,44} More than 20 different compounds have been found in excess in the cerebrospinal fluid (CSF) circulation of patients with HE, including ammonia, aromatic amino acids, endogenous benzodiazepines, gamma-aminobutyric acid (GABA), glutamine, short-chain fatty acids, tryptophan, and others. However, none of those substances have unambiguously been shown to be the single cause of the neurological impairment; therefore, HE can be considered a multifactorial metabolic disease.^{36,40} The mentioned substances may affect neuronal and astrocyte function, resulting in cell swelling, inhibition of membrane pumps or ion channels, an elevation in intracellular calcium concentrations, depression of electrical activity, and interference with oxidative metabolism.⁴⁵⁻⁴⁷ These effects, in addition to an altered permeability of the blood-brain barrier (BBB) in HE, impair cerebral function.^{13,45,46}

Ammonia^a is considered the most important neurotoxic substance. Increased ammonia concentrations in the brain trigger a sequence of metabolic events. These have been extensively studied in HE in rats, humans, and dogs.^{13,33,45,46,48} Dogs with PSS do have excessive accumulation of ammonia in the body since the atrophied liver is incapable of efficiently converting the blood ammonia whereas most of the blood bypasses the liver via the shunting vessel.^{36,49}

At physiological pH, most ammonia in the blood is in the form of the positively charged ammonium ions (NH_4^+) and little as gas form (NH_3).^{27,50-52} Intra- and extracellular pH has an important influence on the distribution and the form of ammonia.^{36,51} Whereas NH_3 readily diffuses across membranes, NH_4^+ does not.³⁶ NH_3 passively diffuses into the brain and is captured in the brain parenchyma as NH_4^+ due to a lower pH in the brain compared to the systemic system.⁵³⁻⁵⁵

Ammonia has both direct and indirect neurotoxic effects. It is involved in the injury to the astrocytes, which comprise about one third of the entire brain volume.^{39,56-60} The astrocytes have the key function to protect the brain from excessive neuroexcitation. They contribute to the ability of brain endothelial cells to form the BBB, a physiologic barrier that impedes passive diffusion of solutes from the blood into the central nervous system (CNS). In addition, the astrocytes are responsible for the ammonia conversion in the CNS, where glutamine is formed out of glutamate and ammonia by the glutamine synthetase reaction.³⁶ As there is no urea cycle in the brain that changes ammonia into urea, ammonia removal relies almost exclusively on glutamine synthetase, primarily localized in astrocytes.⁶¹ In case of excess ammonia in and around the brain parenchyma, there is an increase in tryptophan and glutamine, decreased ATP availability, increased glycolysis, and decreased microsomal $\text{Na}^+ \text{K}^+$ -ATPase.^{62,63}

Two main hypotheses have been proposed to better explain the role of ammonia in the pathogenesis for HE: the osmotic gliopathy theory and the Trojan horse theory. Both models challenge the traditional thought that glutamine, produced during the glutamine synthesis in astrocytes to protect the brain from blood-derived ammonia, is harmless.

^a Except when specified, ammonia refers to the sum of ammonium ions (NH_4^+) and ammonia free base (NH_3)

The ammonia-glutamine-brain swelling osmotic gliopathy hypothesis

In response to hyperammonemia, the glutamine synthesis within the astrocytes is stimulated to deal with the excess ammonia. Unfortunately, glutamine is osmotically active and therefore it is also harmful. The osmotic gliopathy theory postulates that the accumulation of glutamine within the astrocytes leads to subsequent swelling of the astrocytes (Figure 6).⁴⁷ Small increases in the water content within the altered astrocytes may lead to considerable functional neurological consequences.⁴⁴

Despite the general acceptance of this hypothesis, it is currently considered an oversimplification to assume that ammonia-related problems in the brain only include glutamine-induced astrocyte swelling.⁴⁴ Ammonia concurrently evokes mitochondrial abnormalities and triggers the generation of oxidative stress.^{59,60}

The Trojan horse theory

According to the Trojan horse theory, glutamine acts as a carrier of ammonia (Trojan horse) when excessive amounts of glutamine are transported from the cytoplasm across the mitochondrial membranes via a glutamine carrier.⁵⁹ In the mitochondria, glutamine is hydrolyzed by glutamine synthetase, resulting in the generation of ammonia and glutamate. The glutamine-derived ammonia within the mitochondria leads to excessive production of free radicals and induction of mitochondrial permeability transition (Figure 7).⁵⁹

Under normal circumstances, astrocytes efficiently protect neuronal CNS cells from the blood. However, in case of excess blood ammonia, the astrocytic glutamine synthetase process becomes overwhelmed and free ammonia reaches the neurons instead of being detoxified by the astrocytes. The resulting increase in neuronal ammonia hampers the neuronal glutaminase process, resulting in decreased production of neuronal glutamate causing neural dysfunction.³⁶

At the start of this PhD, little was known about the amount and distribution of ammonia in the CSF and/or CNS in dogs with EHPSS and about its association with clinical signs of HE. However, when dogs served as experimental model to mimic chronic HE in human patients by creation of a portocaval shunt in addition to a partial hepatectomy, ammonia levels in the CSF were elevated.⁶⁴ Those dogs all developed HE signs.

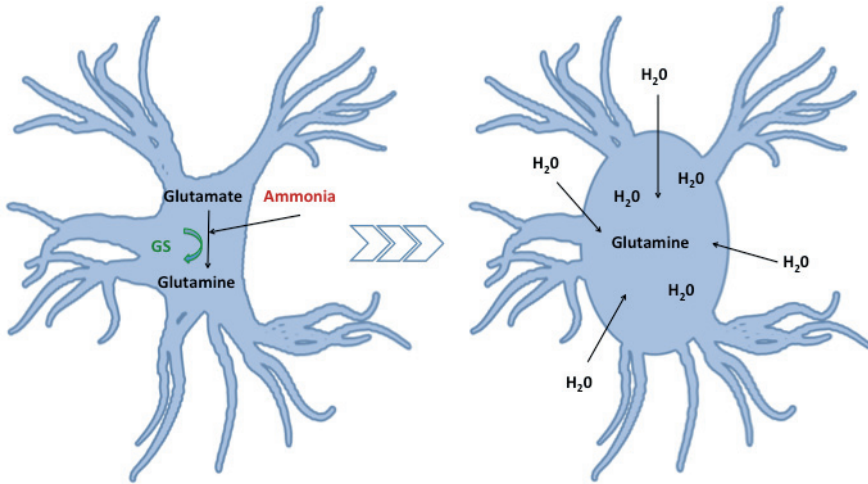


Figure 6. Schematic representation of the ammonia-glutamine-brain swelling osmotic gliopathy hypothesis. Astrocytes eliminate excess ammonia by amidation of glutamate to glutamine by glutamine synthetase (GS). The resulting glutamine is retained within the astrocytes, and its osmotic effect within the astrocyte causes it to take up water, causing it to swell.

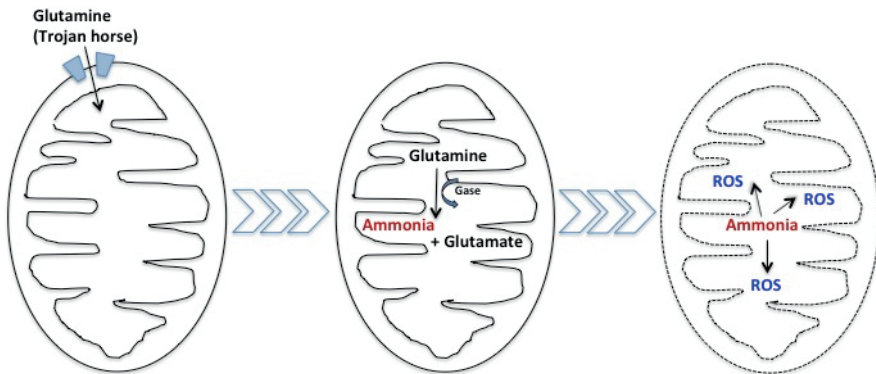


Figure 7. Schematic representation illustrating the Trojan horse hypothesis in ammonia neurotoxicity. Glutamine travels into the mitochondria via a glutamine carrier (GC). Mitochondrial glutaminase (Gase) hydrolyses glutamine and ammonia is generated. This glutamine-derived ammonia generates reactive oxygen species (ROS).

7. Diagnosis of Congenital Extrahepatic Portosystemic Shunt in Dogs

7.1 Physical Examination

Physical examination may reveal mild abnormalities, poor body condition, small body stature and ptyalisim in animals with congenital EHPSS.¹⁴

Even when the owner reports neurologic signs, the neurologic examination may fail to reveal any abnormalities. In some animals, clinical signs manifest at a later age and may be associated with other progressive or acquired conditions such as gastrointestinal or urinary pathology.

Animals with EHPSS may have other concurrent congenital defects. Cryptorchidism is reported in nearly half of the male dogs that are presented with congenital EHPSS.^{26,34}

7.2 Clinicopathologic Findings

Laboratory testing is among the first steps recommended in the diagnostic workup of dogs suspected to have an EHPSS. A complete blood count (CBC), serum biochemistry profile, fasting ammonia measurements, ammonia tolerance test, pre- and postprandial serum bile acids (SBA), coagulation tests, and urinalysis are recommended.

Abnormalities detected on blood analyses performed for other reasons unrelated to PSS diagnosis might sometimes raise the suspicion of the presence of a PSS.^{19,27,30,31}

7.2.1 Haematology, Biochemistry, and Coagulation Profiles

Changes in the CBC are observed in 60 to 70% of the dogs with congenital EHPSS and may include leukocytosis, microcytosis, and normocytic, normochromic, non-regenerative anemia and mild thrombocytopenia. Leukocytosis may be explained by the decreased bacterial clearance of the portal blood, resulting in increased antigenic stimulation.^{17,18,34,65-67} The cause of the microcytosis, and normocytic, normochromic, non-regenerative anemia is not fully understood, but has been associated with abnormalities in iron metabolism.^{66,67} Platelet counts in dogs with PSS are also lower than in normal dogs.⁶⁸

Changes in the biochemistry profile are common in dogs with congenital EHPSS.¹⁷ Due to decreased hepatic synthesis of the respective substances; hypoalbuminemia (50%), reduced

BUN (70%), hypocholesterolemia, and/or hypoglycemia are observed. As with any hepatic vascular anomaly, mild to moderate increases in serum liver enzyme activities are often present. In immature patients, alkaline phosphatase is typically higher than alanine aminotransferase.⁶⁹ Severely elevated liver enzyme activities³³ or hyperbilirubinemia⁷⁰ most likely indicate other concurrent underlying liver disease; they are not typical for the presence of a congenital EHPSS.

Since liver dysfunction will lead to impaired synthesis of clotting factors on the one hand and decreased clearance of activated clotting factors on the other hand, it is self-evident to encounter coagulation abnormalities in dogs with congenital EHPSS. The activated partial thromboplastin time (aPTT) becomes prolonged, often without prolongation in prothrombin time (PT). Spontaneous bleedings do not regularly occur but hemostatic problems may be encountered during surgical intervention.^{71,72}

7.2.2 Urinalysis

Urinalysis is recommended in all dogs suspected of congenital EHPSS. More than half of the patients are hyposthenuric or isosthenuric.^{27,30,73} As stated earlier, many factors may contribute to the low urine specific gravity; psychogenic polydipsia associated with HE, a decreased renal medullary concentration gradient and portal vein osmoreceptor alterations.^{13,14,24,38}

Another common abnormality is the presence of urinary crystals; ammonium biurate crystalluria is reported in 26% to 57% of dogs with a congenital EHPSS.^{18,23,74} This can be explained by deficiencies in both the urea and the uric acid cycle due to impaired liver function, resulting in excessive ammonia and urate excretion by the kidneys.¹⁴

Mild proteinuria can be seen in dogs with EHPSS and is suspected to be secondary to glomerular lesions.⁷⁵ In cases of more severe proteinuria, urinary tract hemorrhage secondary to urolithiasis or a concurrent urinary tract infection should be suspected.¹⁴

7.2.3 Liver Function Tests

In an animal with normal hepatic vascularity, portal blood delivers nutrients and specific hepatotropic factors to the liver.^{12,18} Therefore, the perfusion rate of the liver has a great impact on its function and on its volume. Liver volumes in dogs with congenital EHPSS are smaller than those in normal dogs.⁷⁶

Abnormalities in liver function tests are suggestive of liver dysfunction. The tests reflect accumulation of substances normally cleared by the liver. However, they cannot differentiate

the types of liver disease. The most commonly used screening tests in the diagnostic work-up of dogs suspected of EHPSS are based on either the concentration of serum bile acids (SBA) or on the concentration of ammonia in venous blood samples. The idea behind combining several liver tests is to reveal diagnostic patterns that better differentiate PSS from other liver diseases.

Pre- and Postprandial Serum Bile Acid Concentrations

The liver is the only organ where the complete bile acid (BA) biosynthesis can occur. Bile acids are synthesized and conjugated in the liver and stored in the gallbladder. After stimulation by food uptake, the gallbladder contracts and the BAs are released into the duodenum. They are then reabsorbed from the distal ileum and, in normal dogs, recirculate to the liver via the portal venous circulation by first-pass extraction.^{13,36,77,78} However, in animals with EHPSS, the BA enterohepatic cycle is disturbed because the liver is bypassed and there is little hepatic mass, resulting in accumulation of SBAs.^{8,36,69}

To perform a (paired) SBA concentration measurement test, the first venous blood sample needs to be preprandial, taken after the animal has been fasted for at least 12 hours; the second blood sample is postprandial, taken 2 hours after feeding (at least two teaspoons of commercial or homemade diets). The sensitivity and specificity of a single preprandial SBA are 78-98% and 58-87%, respectively.^{77,79,80} whereas sensitivity and even specificity rise to 100% and 89%, respectively when paired samples are evaluated in the diagnosis of PSS.⁷⁶⁷ Obviously, hepatobiliary diseases other than PSS may also lead to elevated SBA concentrations.^{69,77}

Baseline Ammonia Concentration

The vast majority of the ammonia is produced by bacterial metabolism of nitrogenous compounds in the gastrointestinal tract, mainly in the large intestine by anaerobic and coliform bacteria.^{13,30} Ammonia is also liberated from glutamine from the mesenteric arterial circulation by the enterocytes of the small and large intestines.³⁶ Ammonia is transported towards the liver by the portal blood flow. The portal blood ammonia is converted in the normal liver to urea in the periportal hepatocytes or to glutamine by the perivenous hepatocytes.^{44,49} With normal liver function, up to 85% of the ammonia from the gastrointestinal tract is deaminated via the urea cycle. In animals with PSS the conversion of ammonia to urea is inefficient, resulting in increased serum ammonia levels.³⁶

When testing blood ammonia, blood sample handling is important to avoid preanalytical

errors. The sample needs to be collected in a closed EDTA-coated tube and should either be processed immediately or be put in a cooled centrifuge with further cooling of the plasma until analysis to avoid falsely elevated ammonia levels.⁸¹

Baseline venous ammonia levels are generally not considered to be as sensitive as SBA measurements in the diagnosis of PSS in clinically affected dogs,^{18,83} although using optimal cut-off values can increase the accuracy for selective diagnosis of PSS.⁸⁰ Dogs with EHPSS in which signs of HE are successfully controlled by medical management can have normal blood ammonia values. Likewise, baseline ammonia may be within normal limits if the dog did not eat for a prolonged period.^{18,83} When liver function tests are used for population screening for the presence of congenital shunts in apparently healthy pups, ammonia measurement is preferred over SBA due to its higher sensitivity.⁷⁹

Before the onset of the PhD research, there was only one study on the comparison of arterial and venous blood ammonia levels in dogs with HE due to a variety of liver diseases, including EHPSS.³⁸ In that study, significantly higher ammonia levels were found in arterial blood compared to venous blood. However, the study did not discern between disease entities and differences in ammonia levels in EHPSS dogs were not presented separately. We know from research in human patients with HE that significantly higher arterial than venous blood ammonia levels are observed.⁵¹ In men, it is the arterial ammonia level that is used as a measure to predict intracranial hypertension and cerebral edema in case of HE.^{84,85}

Ammonia Tolerance Tests

If normal baseline venous ammonia is observed in dogs suspected of PSS, an ammonia challenge can be considered to assess the patient's ability to clear ammonia. Two ammonia tolerance tests have been described in the literature.

The first test is the postprandial ammonia tolerance test in which chicken and rice is used as a source of ammonia.⁸⁶ Six hours after the feeding, the concentration of venous ammonia peaks in dogs with hepatocellular disease. The test reaches a sensitivity of 91% in the diagnosis of congenital portosystemic vascular anomalies.⁸⁶

The second test is the oral or rectal ammonia tolerance test in which ammonium chloride is administered via an oral or rectal route, respectively. Rectal administration is often tolerated better and is easier than the oral route.⁸³ Venous blood samples are taken 20 and 40 minutes after rectal administration.⁸⁷

Although the sensitivity of these ammonia tolerance tests is 95% to 100%,^{73,83,87} it is not

safe to administer ammonia in dogs that indeed have a PSS because HE signs may be exacerbated.⁸

7.3 Diagnostic Imaging

Even in dogs with a high suspicion of the presence of a congenital EHPSS based on history, signalment, and laboratory results, achieving the definitive diagnosis of EHPSS can represent a real challenge. Fortunately, several imaging modalities are nowadays available to either document the presence or absence of a PSS or to additionally map the anatomy of the abnormal portosystemic communication.

7.3.1 Abdominal Radiographs

Plain abdominal radiographs will not help to reach the definitive diagnosis of portosystemic shunting since they do not allow visualization of the PSS itself. They may, at most, reveal secondary changes that are supportive of the presence of a PSS. The most observed abnormality registered on plain abdominal radiographs is microhepatica. Also bilateral renomegaly is often present.^{17,27} Animals with shunts may also have uroliths in the bladder, kidneys or ureters, although they might not be noticed on radiographs since ammonium urate or biurate calculi are usually radiolucent unless they also contain struvite or calcium salts.⁷⁴

7.3.2 Mesenteric portovenography

Historically, mesenteric portography was performed frequently to confirm the tentative diagnosis of PSSs. In the time, it was the “gold standard” for diagnosing portosystemic shunting.⁸⁸ It provides excellent imaging and localization of shunting vessels (Figure 8) but it is a highly invasive technique.³³ This procedure requires a laparotomy, portable fluoroscopy (C-arm or temporary closing of the abdomen after jejunal or splenic vein catheterization to transport the patient to a standing fluoroscopy unit), and intravenous contrast medium. Sensitivity of mesenteric portography to diagnose a PSS has been reported to be between 85% and 100%, the main influencing factor being patient positioning.^{17,27,33} Classically, differentiation of IHPSS from EHPSS on portography is based on the point where the shunt diverges from the portal vein.⁸⁸

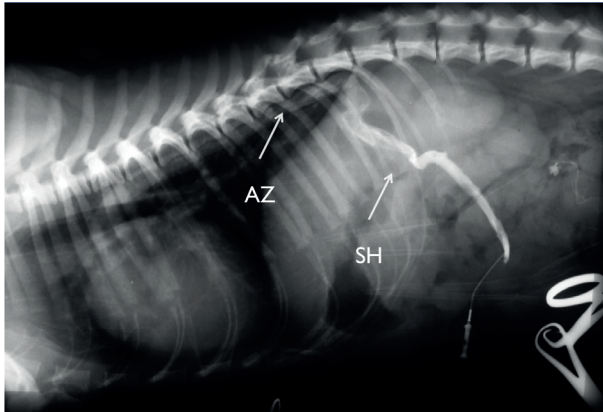


Figure 8. Mesenteric portovenogram in a dog with a porto-azygos shunt (SH), depicting the dorsal intra-abdominal course of the shunting vessel; the azygos vein (AZ) is also filled with contrast. Based on the lateral view only, the insertion of the shunt cannot be visualized. (Courtesy of Department of Veterinary Medical Imaging and Small Animal Orthopedics)

Some surgeons still prefer using mesenteric portovenography to pre-operatively diagnose PSS, despite the fact that less invasive alternatives (ultrasound, scintigraphy, CT, MRI) are available. Direct and indirect risks associated with laparotomy are not in the benefit of the dog or not acceptable to the owners when less invasive options for diagnosing PSS are available.¹⁴ Other surgeons perform mesenteric portovenography at the time of surgery, after the presence of a PSS has already been confirmed by other imaging techniques. Although this allows “real time” intraoperative imaging of the shunting vessel, the images may be difficult to interpret by the patient positioning. Furthermore, the surgeon and staff are exposed to radiation and the surgical time is prolonged, increasing the risk of infection and hypothermia.¹⁴

Alternatives to the mesenteric injection have been described; the contrast can be injected in the spleen (ultrasound-guided splenic venography), via the jugular vein (retrograde transjugular venography), or via the femoral artery (cranial mesenteric arteriography).¹⁴

7.3.3 Abdominal Ultrasonography

In contrast to radiography, ultrasonography is a valuable means to accurately diagnose congenital EHPSS in dogs. In cooperative patients, most EHPSS will be detected already by two-dimensional, grey-scale ultrasonography without the need for general anesthesia.⁸⁹ The technique is noninvasive and the equipment is available in most practices. It is currently considered the gold standard to assess the portal vascular supply without anesthetizing the patient.⁹⁰

The ultrasound examination in dogs suspected of EHPSS will not only reveal the shunting vessel. It allows evaluation of the entire abdomen, including the entire urinary tract. Whenever the combination of microhepatica, renomegaly and uroliths is present, the tentative diagnosis of the presence of a PSS becomes definitive even if the PSS cannot be seen ultrasonographically.⁹¹ Ultrasonography has a high sensitivity for detection of calculi, even when they are small and radiolucent.^{89,92}

In dogs with an EHPSS terminating in the vena cava, turbulence within the caudal vena cava can be evident; yet it can occasionally be seen in dogs without portocaval shunting. In dogs with a porto-azygos communication, the shunt often courses along the aorta in the craniodorsal abdomen but its termination in the azygos vein cannot be defined. In the majority of dogs with a congenital EHPSS, the portal vein to aorta ratio is reduced, in most cases below smaller than 0.65 (Figure 9).⁹¹

In the ultrasonographic diagnosis of congenital EHPSS, many factors may impede proper screening of the abdominal vasculature.^{89,91,92} Since congenital EHPSS is predominately seen in toy breeds, the patients are often small and also the vessel diameters may be small. Multiple ultrasonographic examinations may be needed before a confident diagnosis can be established. Portophrenic shunts are sometimes overlooked because they are situated cranial to the liver and lungs may lead to artifacts. Gas in the gastrointestinal tract may obscure the anomalous vessel connecting to the portal vein and/or the caudal vena cava.¹⁵

Because ultrasonographic detection of shunts in dog is operator- and experience-dependent, the reported sensitivity varies from 74% to 95% and the specificity from 67% to 100%.^{89,92,93} Although experienced operators will consistently detect congenital EHPSS by grey-scale ultrasound, the sensitivity of ultrasound improves by additional use of color-flow and pulse-wave Doppler, revealing changes in blood flow.^{89,92} Portal flow velocity in dogs with a congenital EHPSS is either faster than 15 cm/sec or a variable velocity is observed and the flow is not unidirectionally.²⁷

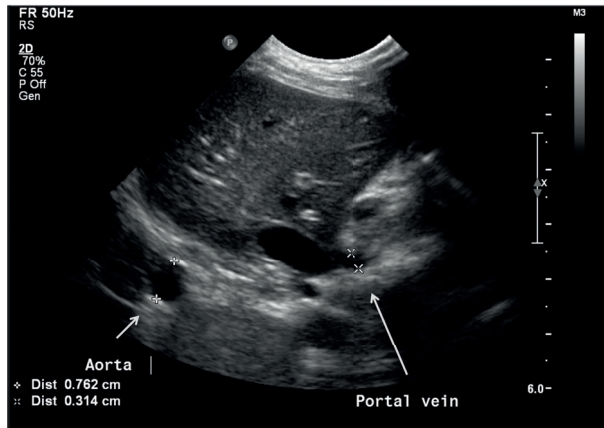


Figure 9. The diameter of the portal vein at the porta hepatis was much smaller than before a wide branch splitted off (this branch is not visible on the image). The portal vein to aorta ratio (0.41) is significantly smaller than in normal dogs (range 0.71–1.20)⁹¹ and is characteristic for animals with an EHPSS (but also in dogs with idiopathic noncirrhotic portal hypertension). (Courtesy of Department of Veterinary Medical Imaging and Small Animal Orthopedics)

More recently, the use of transsplenic injection of microbubbles (agitated saline in heparinized blood) has been evaluated to aid in the ultrasonographic diagnosis of PSS in dogs during grey-scale ultrasound.⁹⁴ The different shunting patterns could be easily and objectively identified. The microbubbles helped to identify the shunting vessel as well as its terminus in all cases of congenital EHPSS. However, for this technique dogs need to be under mild to moderate sedation.

7.3.4 Scintigraphy

Nuclear imaging is a useful, noninvasive method used in the diagnosis of PSS. In contrast to most other imaging techniques, which are anatomical, scintigraphy is a functional imaging technique that is primarily used to confirm or refute the presence of a PSS. It involves the acquisition of dynamic images with a gamma camera to evaluate the temporal uptake of a radioisotope on a nuclear portogram.

Technetium pertechnetate (^{99m}Tc pertechnetate) is the most commonly used radioisotope for this purpose.⁹² It can be administered by two different routes; via the rectum^{95,96} or within the spleen.^{97,98} For both techniques, the dogs are usually shortly anesthetized to facilitate

administration of the radioisotope and to restraint the dogs during the imaging process.

Per-rectal (transcolonic) portal scintigraphy (PRPS) involves infusion of the radioisotope via a catheter per rectum into the colon.^{95,96} The radioisotope is absorbed across the colonic mucosa and drained via the colonic veins into the caudal mesenteric vein and ultimately into the portal vein. For the transsplenic portal scintigraphy (TSPS), the radioisotope is injected under ultrasound-guidance into the splenic parenchyma. The isotope drains to the splenic vein and then into the portal circulation.^{97,98}

A nuclear portal venogram is obtained and the shunt fraction (SF) can be calculated, which represents the amount of blood that bypasses the liver.⁹⁷ In dogs without a PSS, the radioisotope first travels to the liver via the normal portal circulation before reaching the heart. Radioactivity in the liver will arrive sooner and in greater concentration than in the heart (Figure 10). On PRPS, a SF below 15% is considered normal; the normal SF in TSPS is much lower, 2-4% maximally.⁹⁸ In dogs with a PSS, the radioisotope leaves the portal vein system through the shunting vessel to travel to the heart, bypassing the liver. In these cases, the nuclear portogram will reveal the opposite situation with radioactivity that arrives first and with a higher count density in the heart and not in the liver (Figure 11). Thereafter, the radioisotope will reach the liver through the hepatic arterial circulation. In general, most dogs with congenital PSS have shunt fractions on PRPS or TSPS above 60% to 80%.⁹⁵ Based on a nuclear portogram, it is not possible to make a distinction between intra- versus extrahepatic portocaval shunts.⁹⁸

There are several arguments to favor TSPS above PRPS. A sensitivity and specificity of 88% and 67%, respectively is reached for PRPS,⁹⁶ whereas both approximate 100% for TSPS.⁹⁹ Theoretically, a caudally located EHPSS may not be visible if the portal tributary comprising the shunt is located upstream from the splenic vein, but those shunts are extremely rare.¹⁰⁰ The low count density images obtained by PRPS do not always allow anatomical description of the PSS based on the nuclear portogram. On the other hand, TSPS enables differentiation between portocaval and porto-azygos shunts and between single and multiple shunts.^{98,101} Falsely increased SFs are obtained in PRPS if the radioisotope is administered too aborally in the rectum; it will be absorbed directly into the caudal vena cava instead of being absorbed into the portal system. Far less radionuclide is required for TSPS compared to PRPS, thus leading to a decreased and shortened radiation exposure to patient, staff and owner.^{98,101}

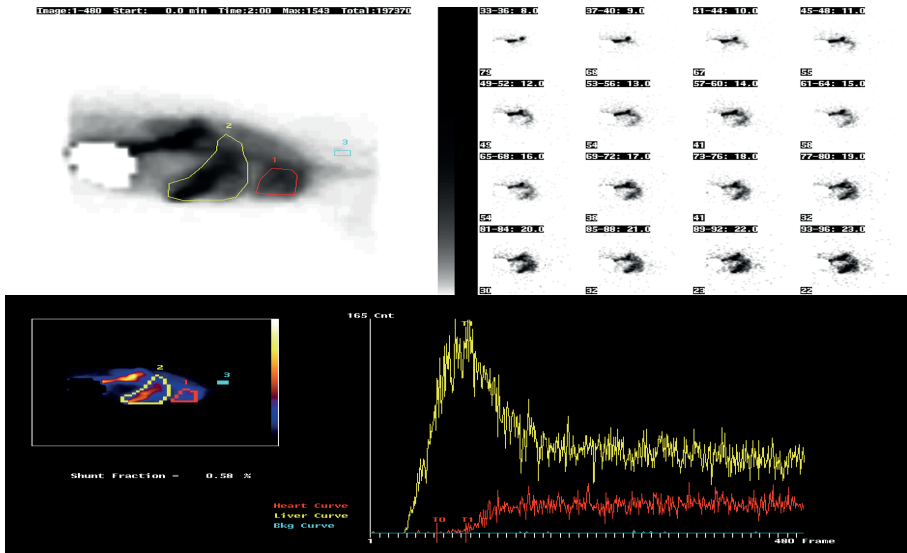


Figure 10. Transsplenic nuclear portal venogram in a dog without portosystemic shunting (top image- static images after injection of the radioactive tracer) and calculation of shunt fraction (bottom image). The regions of interest are marked in yellow (liver) and red (heart); radioactivity is first demonstrated in the liver. (Courtesy of Department of Veterinary Medical Imaging and Small Animal Orthopedics)

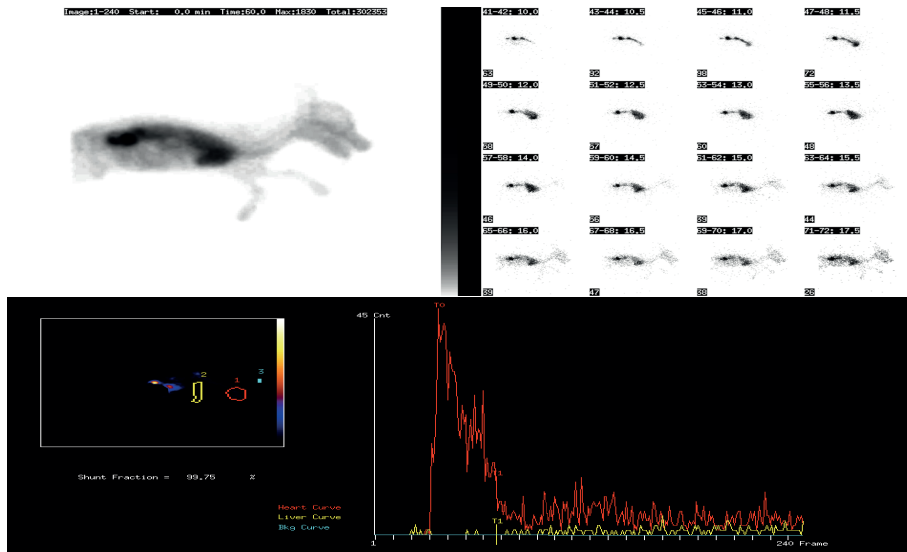


Figure 11. Transsplenic nuclear portal venogram in a dog with an extrahepatic portocaval shunt (top image- static images after injection of the radioactive tracer) and calculation of shunt fraction (bottom image). The majority of the radioactivity first arrives to the heart and only later reaches the liver. (Courtesy of Department of Veterinary Medical Imaging and Small Animal Orthopedics)

7.3.5 Computed Tomography Angiography

Computed tomography angiography (CTA) is a rather recent imaging modality that now supplants traditional mesenteric portography as the gold standard for the evaluation of the portal venous system anatomy in dogs.^{15,102} It involves an intravenous (IV) injection of an iodinated contrast medium, using single and multi-slice CT scanners and providing three-dimensional images.¹⁰³⁻¹⁰⁵ Dual-phase, arterial and portal CTA provides a complete evaluation of portal and hepatic vasculature, reducing the chance of missing small tributaries vessels, and is therefore considered superior to single-phase computed tomography.¹⁰⁴ However, dual-phase CTA demands bolus tracking with meticulous timing of the injected contrast to get a clear arterial and a portal phase images.

Computed tomographic angiography is noninvasive, fast, and provides excellent anatomic detail of portal tributaries and branches allowing the identification of the origin and insertion of the PSS (Figure 12). In contrast to ultrasonography, CTA is less operator-dependent,¹⁰⁴⁻¹⁰⁷ it can be performed in dogs of any size¹⁰⁸ and allows further manipulation of the images after the scan is completed.¹⁵ A sensitivity of 96% and a specificity of 89% have been reported.¹⁰⁷ The main disadvantage of the use of CT is the need for general anesthesia and the higher radiation burden to the patient compared to ultrasonography, MRI and even scintigraphy. The former was addressed in a recent study with a 16-slice system in which a novel CTA protocol only involved sedation without the need for test injections, delay times or timing determinations.¹⁰⁹ Specificity and sensitivity were reported to be 100%.

Anatomic details obtained by CTA reconstructions are valuable for presurgical planning.¹⁵ Thanks to CTA, the portal anatomy in a large number of dogs with EHPSS has been accurately described and nomenclature for the different type of shunts was provided.¹⁵ Yet, before this PhD research was started, there was no study in dogs on porto-azygos shunt anatomy and different types were not described.

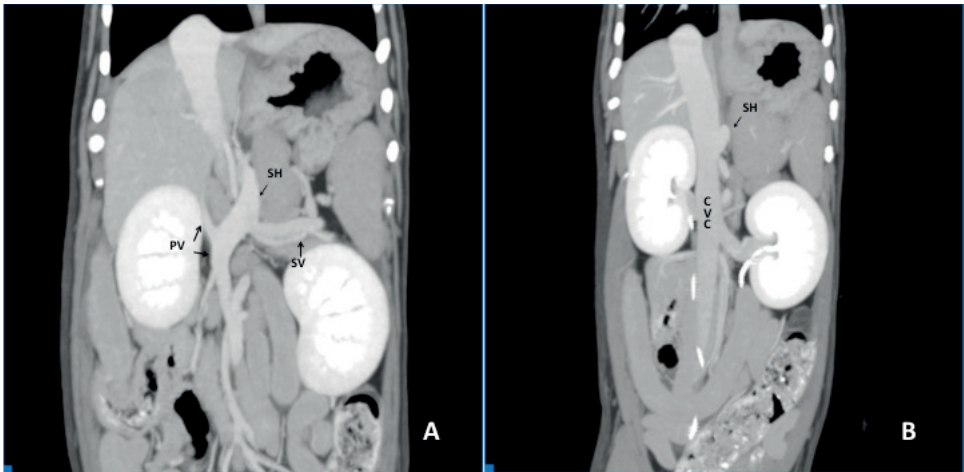


Figure 12. Computed tomographic angiography images obtained in a dog with an extrahepatic portocaval shunt, dorsal ventral views, head is pointed up. Images are taken at different levels showing the origin (A) and insertion (B) of the shunting vessel. A, The shunt (SH) leaves the portal vein (PV) caudal to the porta hepatis; the splenic vein (SV) drains into the shunt. B, The shunt (SH) enters the caudal vena cava (CVC) from the left, at the level of the cranial pole of the right kidney. (Courtesy of Department of Veterinary Medical Imaging and Small Animal Orthopedics)

7.3.6 Magnetic Resonance Angiography

Magnetic resonance imaging (MRI) with angiography (MRA) is sporadically utilized as a diagnostic technique for PSS in dogs. It involves intravenous injection of a paramagnetic contrast medium, and provides three-dimensional images.¹¹⁰⁻¹¹³ Similar to most CTA protocols, MRI requires general anesthesia. With MRA, there is no need for bolus tracking of the contrast; consequently, MRA does not require the exact timing necessary with traditional CTA. However, MRA takes longer than CTA. By utilizing positive-pressure ventilation and breath-hold techniques, MRA sequences can be obtained quickly and free from motion artifacts.^{112,113} The accurate characterization of PSSs, including the origin and insertion, can be identified with nearly 79% sensitivity and 100% specificity (Figure 13).¹¹⁰

Excellent anatomic details on shunt morphology can be obtained by MRA, which is useful for presurgical planning.^{110,112,113} Although MRA is a promising new diagnostic modality in the diagnosis of PSS, CTA provides similar detail, is performed more quickly, and is less expensive than MRA.

Although brain MRI is not routinely performed in PSS dogs with clinical HE, it has been used occasionally to assess potential brain lesions in those patients.¹¹⁴⁻¹¹⁷ Widened sulci with

a variable degree of grey matter atrophy was observed in all dogs and hyperintensity of the lentiform nuclei on the T1W images in 90%.¹¹⁴

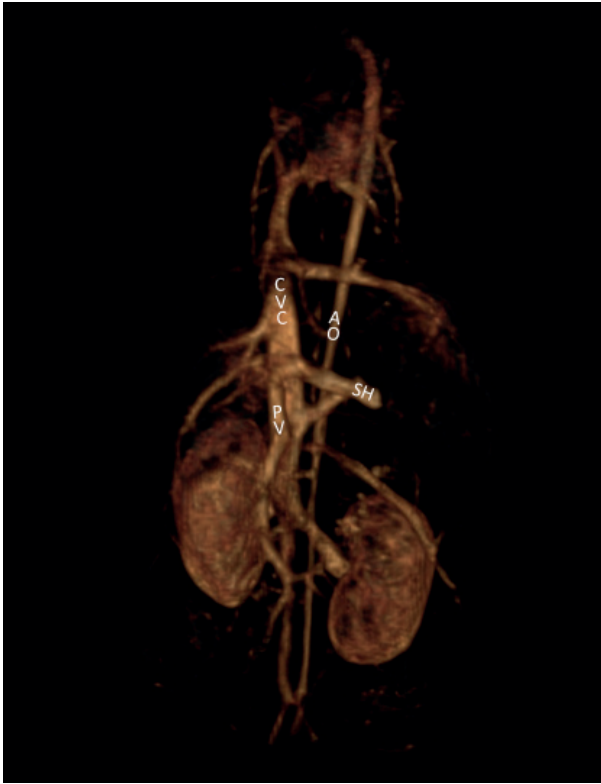


Figure 13. Magnetic resonance angiography images obtained in a dog with a portocaval shunt, dorsal maximum-intensity projection. The shunt (SH) leaves the portal vein (PV) caudal to the porta hepatis; the cranial continuation of the portal vein is not visible on this image. CVC: caudal vena cava; AO: aorta. (Courtesy of Clinic for Small Animal Surgery and Reproduction, Ludwig-Maximilians-University Munich)

Before this PhD research was started, there were no publications on other diagnostic imaging techniques that specifically focused on the brain in dogs with EHPSS. Quite recently, proton magnetic resonance spectroscopy (¹H MRS) was performed in 4 dogs with a congenital EHPSS, allowing in vivo assessment of metabolic derangements.¹¹⁷ High concentrations relative to water of the glutamine-glutamate complex were observed as well as lower myo-inositol peak areas, the latter most likely in an attempt to buffer the ammonia-induced glutamine excess.¹¹⁸

8. Treatment of Congenital Extrahepatic Portosystemic Shunts

The treatment of dogs with congenital EHPSS includes first management of clinical signs by means of medical support, and secondly surgical correction of the portosystemic shunting, if possible. Medical management will control clinical signs caused by the liver bypass but will not resolve the underlying diminished hepatic perfusion; therefore, surgery is recommended. Medical management has its role for long-term therapy when surgery is not possible or declined.

8.1 Medical Management

Medical treatment strategies in EHPSS patients aim to decrease the concentration of circulating ammonia. They can be divided into three categories; those that decrease absorption of ammonia from the gastrointestinal tract, those that improve ammonia conversion through the urea cycle, and those that increase removal of ammonia from the systemic circulation.¹¹⁹ Medical treatment generally consists of a dietary, antimicrobial, and synthetic disaccharide regimen.

Diets can be commercially available products for dogs with hepatic or gastrointestinal disease or homemade diets that contain low level high-quality, easily digestible protein,^{14,18,33,34,52,120} that are fed in small portions and at frequent intervals.⁵²

Antimicrobial treatment consists of antibiotics such as metronidazole, ampicillin, or neomycin, aiming to decrease urease-producing bacteria.³³

Disaccharides consist of oral administration lactulose (0.5–1 mL/kg/day divided into 2 to 3 doses) but, if severe HE signs are present, lactulose can also be administered transrectally.³³ Lactulose is not absorbed in the small intestines and is degraded into volatile free fatty acids by colonic bacteria. The resulting acidification gives a shift to nonabsorbable ionized ammonium, increased colon motility, and an altered and less ammonia-genic flora. The ammonia formation from glutamine metabolism in the intestinal mucosa may also be reduced by lactulose.¹⁴

Nutraceuticals such as S-adenosyl-L-methionine (SAMe), ursodeoxycholic acid, vitamin E, and milk thistle (silymarin) have been recommended as hepatic supportive therapy for a variety of liver diseases.¹²¹ However, no controlled studies have evaluated their effectiveness for treatment of animals with EHPSS.

When a patient presents with signs of HE, aggressive efforts should be instituted to stabilize the patient and decrease the ammonia concentrations. In conditions of acute, severe HE, therapy includes administration of lukewarm water enemas, oral and rectal lactulose, antibiotics, IV fluid therapy and anticonvulsant therapy, if indicated.^{19,33} Conditions that aggravate HE such as hypovolemia, hypokalemia, alkalosis, and metabolic acidosis, should be corrected and drugs like benzodiazepines, barbiturates, and methionine should be avoided. Glucocorticoids, which induce catabolism, should be also avoided if possible.^{14,119}

Seizures not caused by hypoglycemia or hyperammonemia are initially treated with a benzodiazepine.³³ Some clinicians prefer midazolam to intravenous diazepam, which contains a propylene glycol carrying agent that requires liver metabolism.^{14,119} Others feel that diazepam is the anticonvulsant of choice for immediate effect in an animal having seizures from hepatic encephalopathy associated with PSS.^{14,119} After seizures are controlled, loading doses of and continued treatment with phenobarbital, potassium bromide, sodium bromide, or levetiracetam may be considered, particularly if continued seizure activity is anticipated. Although many neurologists prefer levetiracetam, to date, there is little evidence to support the use of levetiracetam in dogs with PSS to control hepatic encephalopathy-associated seizures.¹⁴

A minimum of 2-3 weeks of medical treatment is recommended before planning any anesthetic event, implying that the definitive diagnosis of PSS might be postponed as well as the surgical intervention.^{14,119} Patients that are cachectic, that show signs of HE, or are unstable should be managed medically until they can tolerate the stress of anesthesia and surgery. Underweight patients may benefit from longer medical management to improve their body condition score before surgery. It may be tempting to owners to renounce the surgical intervention, keeping their dog on medical management indefinitely, since extremely good results are obtained after initial stabilization in the majority of the congenital EHPSS patients. However, medical treatment does not influence the fact that the liver is bypassed by nutrients and hepatotrophines;^{12,18} thus, the liver will continue to slowly deteriorate.

8.2 Surgical Attenuation

As discussed above, it is best to first install a medical treatment aimed at reducing the circulating ammonia concentration before presenting an EHPSS patient for surgery. Because

the liver is a key organ in the coagulation cascade, and liver function is impaired due to the inadequate perfusion, most surgeons will routinely administer vitamin K prior to surgery in order to avoid bleeding-related issues at the time of surgery.^{71,72}

The classical pre-operative 12-hour fast is not recommended in toy breeds and especially not in immature animals. To them, small amounts of easily digestible food can be offered until 4 to 6 hours pre-operatively.⁶⁵

The main goal of a surgical intervention in patients with PSS is to redirect portal blood flow towards the liver parenchyma. Most animals will not tolerate acute complete occlusion of shunts.^{32,82,122} Intra-operative evaluation of the portal vein may reveal portal atresia or even agenesis.⁶⁵ Some surgeons rely on the degree of opacification of the portal branches during intra-operative portovenography to assess the portal vein capacity.¹²³ Dogs with little or no demonstrable portal vasculature might not be good surgical candidates, irrespective the intended surgical modality.^{65,123} In most cases, however, gradual attenuation should be attempted to reduce the risk of postoperative complications.³²

The traditional approach was (partial) suture ligation of the shunting vessel. The degree of attenuation was based on visual inspection for evidence of portal hypertension, such as pallor or cyanosis of the intestines, increased intestinal peristalsis, cyanosis or edema of the pancreas, and increased mesenteric vascular pulsations. To be more objective, the surgeon can measure portal and central venous pressures.¹⁷ Partially attenuated shunts can be completely ligated during a subsequent surgery in 75% of animals.⁸²

Nowadays, most surgeons rely on devices other than sutures to obtain gradual and ultimately total attenuation of the shunting vessel, thus avoiding multiple surgical and anesthetic episodes.

Because PSS are considered hereditary in many breeds, neutering of affected animals is recommended if the animal is cardiovascularly stable during the anesthesia.^{20,22,29,124} In male dogs presented with cystoliths of any size or in female dogs with large bladder stones, a cystotomy is also performed during the same anaesthetic procedure.²³

8.2.1 Surgical Attenuation Devices

Options for surgical attenuation of congenital EHPSS include partial occlusion with ligatures or a more gradual attenuation of the shunting vessel with ameroid constrictor, thin film band, or hydraulic occluder.^{14,17,23,82,125,126} Most commonly used surgical options for attenuation of EHPSS include placement of an ameroid constrictor or a cellophane or other type of thin film

band.^{15,65,102,107,125,127,128}

Ameroid Constrictors

An ameroid constrictor (AC) is a ring shape commercial device, which has an inner ring of compressed casein that is surrounded by a stainless steel collar.¹²⁹ Both the casein and the steel ring have a slot to allow slippage of the device over the flattened shunting vessel after which a key is inserted to prevent dislodgment from the vessel (Figure 14). The ACs are available in different sizes; intra-operatively, an AC should be chosen that produces minimal reduction in vessel diameter after placement.^{14,129,130}

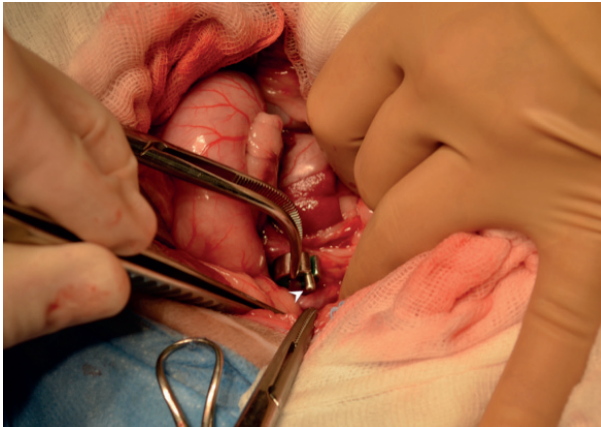


Figure 14. Placement of an ameroid constrictor on a portocaval shunt in a dog. The key (white arrowhead) has been partially inserted and will to be pushed deeper to fill the slot in the casein ring.

Casein is a hygroscopic substance; it swells by slowly absorbing body fluid, resulting in a partial reduction of the internal luminal diameter, physically compressing the shunting vessel.¹³¹ It is suggested that additional gradual shunt occlusion is obtained by implant-associated inflammation and stimulation of a fibrous tissue reaction that obliterates the vessel.^{129,131,132} Closure will occur over two or more weeks.¹³³ In some animals, thrombus formation could result in more rapid obstruction of partially attenuated shunts.¹⁴ Early attenuation of the shunt may also occur due to the sheer size and heft of the AC, due to the weight of the device when the patient is in a standing position or if the AC kinks the shunt.^{129,130}

Cellophane and other Thin Film Bands

Cellophane band (CB) is a malleable thin film band composed out of plant-derived cellulose. Only recently, it has been realized that several so-called CBs should be named thin film bands since many bands alleged to be CBs are not cellophane-based.^{127,128}

During surgery, a strip of thin film is folded lengthwise into three layers to make a thick, flexible band, which is placed around the shunt (Figure 15).¹⁴ The band is held in place by securing the ends together with surgical clips in an alternating configuration.¹³⁴ Most surgeons will place a band around the vessel to produce little immediate reduction in diameter; large shunts should not be attenuated to less than 3 mm to achieve a successful clinical outcome.¹²⁶

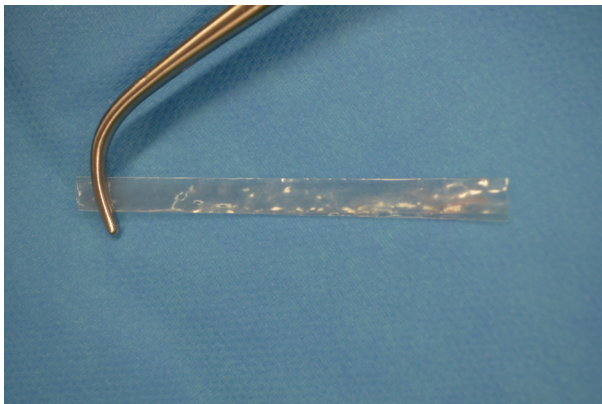


Figure 15. A strip of cellophane band is folded lengthwise into three layers; this flexible band will then be applied around the PSS.

Cellophane bands induce a chronic low-grade foreign body reaction resulting in gradual occlusion of the encircled vessel.¹³³ The attenuation process is slower than after AC placement.¹³³ Thin film bands cause fibrous tissue reaction and slow progressive shunt occlusion comparable to CB, but they have different structural and mechanical properties.^{127,128}

The main advantages of CB over AC are that the material is cheaper, not heavy nor bulky and placement requires less dissection.¹³⁰ More, Miller and Fowler introduced a less invasive approach than open surgery, consisting of laparoscopic placement of a CB around a congenital EHPSS in 2 dogs.¹³⁵

Hydraulic occluders

A hydraulic occluder is composed of a silicone cuff connected to a vascular access port by tubing to allow percutaneous control (Figure 16).^{136,137} The devices are designed specifically as an aid in the treatment of intrahepatic PSS but can be used on congenital EHPSS to obtain controlled attenuation. The cuff is positioned around the shunt, the opposing ends are secured with a non-absorbable suture and the port is implanted subcutaneously. To gradually occlude the shunt after surgery, a small amount of sterile fluid is injected transcutaneously into the port every 2 weeks. Complete shunt closure is not dependent on fibrous tissue formation and the device remains life long in place.¹³⁷

The potential advantage of a hydraulic occluder over other devices available to gradually attenuate PSSs is that complete occlusion of the vessel encircled by the cuff can be obtained. Furthermore, it is currently the only device in which attenuation can be reversed if clinically deemed necessary.

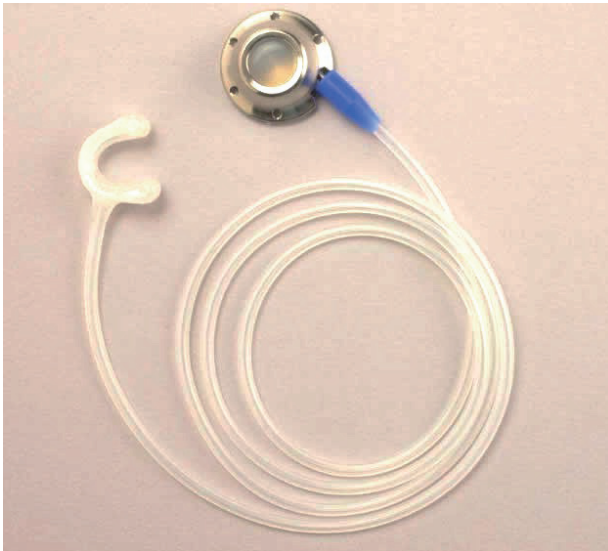


Figure 16. Canine hepatic shunt occluder with subcutaneous access port. (Image https://www.docxs.net/vet_supplies.php)

Thrombogenic coils

A thrombogenic coil is a polyester fiber-covered flexible metallic strip that stimulates thrombosis on and around the device (Figure 17). Coils were first used in the treatment of

intrahepatic PSS¹³⁸ but they can also be used in case of congenital EHPSS.^{90,139} A coil, slightly larger than the shunt diameter, is placed into the midportion of the shunt via a guide wire via a jugular or femoral vein approach. Additional coils are added until more than three-fourth of the vessel is no longer patent, as judged by venography.

The advantages of thrombogenic coils are the avoidance of invasive surgery, the relatively short anesthetic period, the faster recovery and the shorter hospital stay.^{90,139} However, in an experimental study in dogs, recanalization at the site of thrombosis was observed after complete early occlusion.¹³³ Furthermore, coil migration to the heart or lungs will be fatal to the patient.^{138,139} Other reported disadvantages are the requirement of specialized instrumentation and training and the frequent need for multiple embolization procedures.¹³⁸

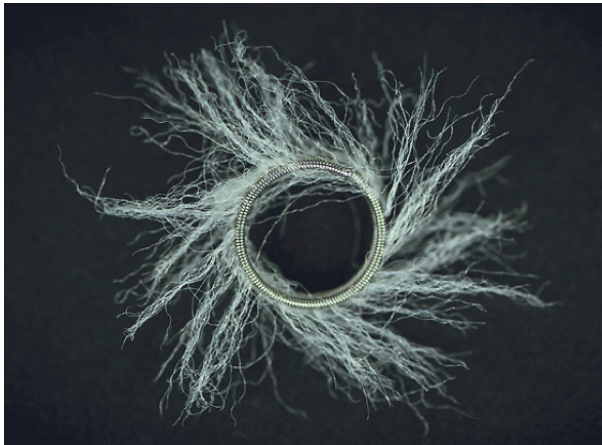


Figure 17. Coil intended to be used in dogs with a portosystemic shunt. The synthetic fibres enhance thrombus formation. (Image <http://infinitemedical.com/products/embo-coil>)

8.2.2 Intra-operative Identification of the Shunt

Portocaval Shunts

In normal dogs, there are no large vessels entering the caudal vena cava between the level of the right renal vein and the hepatic veins. This anatomical knowledge may help the surgeon to correctly identify a portocaval shunt.⁶ The portocaval communication is always reported to be at the left side of the vena cava.¹⁵ The place of insertion into the vena cava is caudal to the porta hepatis at the level of the omental foramen in the classical portocaval shunts, but

portophrenic shunts terminate cranial to the liver and caudal to the diaphragm.¹⁶ In extremely rare cases, a shunt inserts in the caudal end of the caudal vena cava in the so-called colonocaval shunts.^{16,17}

Often, identification of the insertion of portocaval shunts is possible by retracting the mesoduodenum towards the midline, disclosing the caudal vena cava. At the site of shunt insertion, the caudal vena cava may be dilated and contain turbulent blood flow.⁶ It may be necessary to gently retract the celiac artery caudally, the caudate lobe of the liver cranially, and/or the pancreas medially to see the portocaval shunt termination.⁶ If the insertion site of the shunt is difficult to reach for dissection through this approach, it may be necessary to enter the omental bursa by tearing through the superficial leaf of the major omentum.⁶ The stomach is then retracted cranially and the intestines caudally and laterally to view the left border of the caudal vena cava. The omental foramen is hereby visualized from the opposite site and shunt dissection might be easier.⁶

Portophrenic shunts are more likely to be overlooked by inexperienced surgeons. This might explain the fact that they are nowadays much more reported than in older publications.¹⁴ The distal part of the shunt is often covered by the fascia transversalis, which lines the abdominal surface of the diaphragm.

Colonocaval shunts may require a caudal extension of the abdominal incision before they can be visualized.^{16,17}

Porto-Azygos Shunts

To identify porto-azygos shunts, the omental bursa can be opened to allow examination of the dorsal bursal recesses for abnormal vessels that penetrate the diaphragmatic crura.¹³² Alternatively, porto-azygos shunts may also be found by retracting the liver and stomach to the right so the cardia and esophagus and left diaphragmatic crus are visible.¹⁴

8.2.3 Positioning of the Attenuation Device

Portosystemic shunts may have several contributing venous tributaries before they enter the systemic circulation. Therefore, although it is important to identify the shunt throughout its length, dissection should be directed to the point where the shunt connects with the systemic circulation. If the shunt is attenuated too far from its insertion and a tributary is still feeding the shunt distal to the attenuation site, continued shunting is expected.^{15,128} Hence, congenital EHPSS should be attenuated as close to their insertion sites as possible so that blood flow from all tributaries of the shunt is redirected.

Portocaval Shunts

There is widespread support to attenuate portocaval shunts at their terminus on the caudal vena cava.^{14,15,125} Dissection of the insertion site of portophrenic shunts cranial to the liver can be difficult. After the phrenic vein receives the distal gastric tributaries, a fascial layer of the diaphragm covers it. In some animals, prudent dissection is required before the shunt insertion site can be approached distal to those branches.¹⁴

Porto-Azygos Shunts

The current guidelines for attenuation of porto-azygos shunts describe dissection of the shunt at the abdominal side of the diaphragm.¹⁴ Particularly in case of porto-azygos shunts, it is important to search carefully for small branches from gastric veins that may enter the PSS just before the shunting vessel traverses the diaphragm.^{14,128} The diaphragm may be opened if more exposure is desired.¹⁴

At the start of this PhD research, publications on EHPSS only contained information on porto-azygos shunts mentioned in the same breath as portocaval shunt data. It was also striking that nowhere surgical details dedicated to porto-azygos shunts were provided. However, sticking to the general principles of successful PSS attenuation, it seems logical to also attenuate porto-azygos shunts as close to their insertion site as possible, but it was nowhere recommended.

8.2.4 Peri-operative Complications Related to Surgical Attenuation

Survival rate from the surgery is over 95% but monitoring of dogs in the immediate postoperative period remains crucial. Complications after placement of gradual attenuation devices to treat congenital EHPSS are fewer compared to (partial) ligation.

There is no need for intra-operative portal pressure measurements but although postoperative portal hypertension is uncommon, it can be fatal.^{14,65} Acute severe portal hypertension can occur in the immediate postoperative period when complete occlusion of the shunting vessel by kinking of the shunt within an AC or by thrombus formation occurs. Increased abdominal pain or diarrhea and/or vomitus that may contain fresh or digested blood can be the first signals observed before clear evidence of hypovolemic shock and abdominal distention become more obvious.^{14,65} Mild portal hypertension will induce ascites that is self-limiting in days to weeks in the majority of the cases.³³

Many dogs experience a prolonged anesthetic recovery. With adequate pre-operative imaging, pre-surgical planning can significantly shorten the surgical procedure. Nonetheless, many patients become very hypothermic because the majority are small breed dogs, often immature and/or skinny. For the same reasons, those dogs are particularly sensitive to postoperative hypoglycemia; although some researchers suggest that the main mechanism for the hypoglycemia in dogs with congenital EHPSS is an increased baseline postoperative cortisol concentration.¹⁴⁰ Blood glucose levels should be monitored regularly as long as the dog does not eat spontaneously after anesthesia and whenever the patient displays lethargy or dullness.⁶⁵

Blood pressure also needs to be assessed regularly in the immediate postoperative period to check for hypotension.⁶⁵

Anemia may be present postoperatively, even in the absence of intra-operative hemorrhage. The red blood cells sequester in the spleen or are diluted by perioperative fluid therapy.³³

Post-attenuation neurological dysfunction, unrelated to hypoglycemia or hyperammonemia, may occur in the first 3 to 6 days after surgery, likely due to changes in the concentration of certain neurotransmitters or other metabolic alterations.⁵⁰ An incidence as high as 12% has been described.¹⁴¹ The severity of the neurological signs may vary from facial twitching, muscle fasciculation, abnormal vocalization, and ataxia up to status epilepticus.^{23,50,141-143} A high mortality rate is associated with the development of postoperative status epilepticus. Pretreatment with levetiracetam is reported to potentially decrease the incidence of postoperative seizures.¹⁴⁴

8.3 Postoperative Care

Dogs are discharged on a protein-restricted diet for at least a month; also lactulose is generally continued after surgery. There are no published guidelines concerning the length of the supportive treatment. It has been studied that liver volumes in dogs with congenital EHPSS normalize within a month after successful surgery.⁷⁶ It is therefore conceivable that medical management after surgery is continued until then.

9. Prognosis in dogs with a Congenital Extrahepatic Portosystemic Shunt

9.1 Long-term Prognosis with Medical Management Alone

Prospective studies on the medical management of dogs with EHPSS have not been reported. However, there are retrospective studies that looked at this issue.^{34,52,145,146} However, none of those studies evaluated EHPSS and IHPSS separately, potentially biasing the results. In the oldest study, the owners of two-thirds of the dogs requested euthanasia after a mean treatment period of less than 10 months.³⁴ In the most recent retrospective study on 27 dogs with CPSS (21 EHPSS and 6 IHPSS) evaluated after long-term (median 1936 days) medical management alone, 89% were euthanized or died due to conditions related to PSS at a median of 836 days after diagnosis.¹⁴⁶ Dogs with EHPSS controlled by medical management alone presented with neurologic signs that were similar or less persistent than before treatment. In those retrospective studies, no correlation could be demonstrated between levels of serum bile acids, serum proteins, albumin, ALP, ALT, or MCV and survival times.^{52,146} Likewise, most of the blood parameters did not change significantly after medical management was installed.³⁴

9.2 Long-term Prognosis after Surgical Attenuation

Retrospective studies on surgical treatment of EHPSS by different attenuation devices demonstrated that surgical treatment of EHPSS resulted in significantly improved survival rate and lower frequency of persistent clinical signs compared to medical management only.^{52,145,146}

Surgical treatment aims at resolution of all clinical symptoms by gradual but complete closure of the original congenital EHPSS without concurrent development of acquired PSS. However, that is not the only possible long-term outcome observed in congenital EHPSS after surgical attenuation. Improved portal blood flow and subsequent improvement or even normalization of the clinical status can be observed after incomplete closure. Poor clinical response may also result from the presence of a second shunt, incorrect placement of the attenuation device or due to concomitant primary portal vein hypoplasia.^{19,125,128,147} At last, there are also dogs that develop multiple acquired portosystemic shunts due to portal

hypertension. In such cases, persistence or recurrence of clinical signs will be observed.^{14,32,65,119} It has been suggested that actively inducing too much reduction in shunt diameter at the time of placement of the device is associated with a poorer long-term outcome due to an increased risk of acquired shunting.¹²⁶

Many of the older studies are retrospective and categorize postoperative outcomes solely based on the evolution of the clinical signs or based on clinical signs and laboratory parameters. In more recent studies, most of the dogs are subjected to postoperative diagnostic imaging to objectively assess successful shunt closure. It became obvious that surgical outcome based on complete absence of portosystemic shunting is less favourable than outcome based on clinical improvement. Also comparison between pre- and postsurgical laboratory parameters is often inconclusive to assess absence of postoperative shunting.^{125,128,148}

The sensitivity and specificity of postoperative paired SBA measurements has not been reported. But it is obvious that accuracy is far less than when used in the diagnosis of PSS and that they are not adequate for postoperative monitoring.¹⁸ Normal postprandial SBAs have been reported despite the presence of persistent shunting or multiple acquired PSS.^{125,128} Furthermore, it is not uncommon to observe persistent abnormalities in SBA concentrations in asymptomatic dogs after successful surgical attenuation.^{18,125,128,148,149}

Similar to paired SBAs, ammonia is a popular test in the diagnosis of PSS. When the PhD research was initiated, there was no study investigating the evolution of ammonia post-attenuation.

10. Conclusion

Extrahepatic portosystemic shunts in dogs are most commonly seen as congenital communications in young small breed dogs. Neurological manifestations are most common, but yet not understood. Medical management before surgical attenuation is always recommended. Surgical attenuation should be considered in an attempt to improve perfusion of the liver and ultimately liver function. Outcome is difficult to predict since many observations before and after surgery are still poorly understood.

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SCIENTIFIC AIMS

Literature on extrahepatic portosystemic shunts (EHPSS) in dogs is far from sparse, yet shortcomings and gaps on a number of aspects of this fascinating disease inspired us for further research. Until the start of this research, there was no single study concentrating on porto-azygos shunts. The existing gap in knowledge concerning the particular morphology and the surgical approach could not rest aside our curiosity. Furthermore, many assumptions on the pathophysiology of hepatic encephalopathy (HE) in EHPSS dogs were extrapolated from what is known in human patients with liver disease exhibiting (sub)clinical HE signs. This lapse in species-specific knowledge evokes controversy and discussion about specific implementation of treatment of neurological signs observed in EHPSS dogs. Therefore, we aimed to gain more information about ammonia concentration in different body fluids including the CSF and, consequently, to demonstrate changes in the central nervous system in dogs affected by HE due to a congenital EHPSS.

The specific aims of this doctoral thesis were three-fold:

1. The first aim of this thesis was to provide a **detailed description** of various anatomical types of **porto-azygos shunts** and to **describe** and **document** a **transdiaphragmatic surgical approach** to attenuate porto-azygos shunts in dogs (Chapter 1 and 2). For this purpose, a large number of dogs with porto-azygos shunts were evaluated using Computed Tomographic Angiography (CTA) with special attention to the site of insertion of these vascular anomalies (Chapter 1). In addition, a cadaveric and clinical study was established to document a transdiaphragmatic surgical approach to attenuate porto-azygos shunts inserting in the thoracic part of the azygos vein (Chapter 2). We hypothesized that porto-azygos shunts insert into the thoracic part of the azygos vein and that a transdiaphragmatic surgical approach is suitable to attenuate these shunts and eliminates the risk of missing late tributaries to the shunting vessel.

2. The second aim of this work was to evaluate the **ammonia concentrations** in the **blood** and **cerebrospinal fluid (CSF)** of dogs with an **EHPSS** (Chapter 3 and 4), in an attempt to shed more light on the etiopathogenesis of HE in dogs with EHPSS. For this purpose, the ammonia concentrations in arterial blood, venous blood and CSF in control dogs and in dogs with a congenital EHPSS were compared on the day of diagnosis. At preset time points before and after surgery the ammonia concentrations were measured and related to the

surgical and **clinical outcome**. We hypothesized that in dogs with HE and hyperammonaemia due to the presence of an EHPSS the arterial ammonia concentration would exceed the venous level, and that the ammonia levels in the blood and the CSF would be positively correlated.

3. The third aim of this PhD was to document the alterations in the **regional cerebral blood flow (rCBF)** in EHPSS dogs with overt **HE signs**, and to determine which areas of the brain have an altered perfusion based on brain perfusion imaging scans (Chapter 5). For this purpose, SPECT scans analyses were compared between control dogs and dogs with an EHPSS with clinical signs of HE. We hypothesized that the rCBF in PSS dogs with clinical signs of HE might be altered, with the changes being observed in dogs with clinically apparent signs of neurological dysfunction and hyperammonimia.

RESEARCH STUDIES

**Determination of Porto-Azygos Shunt Anatomy in Dogs
by Computed Tomography Angiography**

Adapted from:

Determination of Porto-Azygos Shunt Anatomy in Dogs by Computed Tomography Angiography.

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Veterinary Surgery 2016, 45(8), p 1005-1012 (doi: 10.1111/vsu.12553)

1. Abstract

Objective: To describe the morphology of porto-azygos shunts in a large series of dogs using computed tomography (CT) angiography.

Study Design: Retrospective study.

Animals: Dogs (n=36) with porto-azygos shunts.

Methods: CT angiography was performed in dogs subsequently proven to have a porto-azygos shunt. The origin and insertion of the shunts were assessed on native images. The diameter of the porto-azygos shunt and the portal vein, cranial and caudal to the shunt origin, were measured. The porto-azygos shunt anatomy was studied on three-dimensional images.

Results: All porto-azygos shunts originated either in the left gastric vein (33 left gastro-azygos shunts) or the right gastric vein (3 right gastro-azygos shunts). Two left gastro-azygos shunts had concurrent caval-azygos continuation and 2 right gastro-azygos shunts had a caudal splenic loop. All shunts crossed the diaphragm through the esophageal hiatus. The majority of porto-azygos shunts (32) followed a straight pathway after traversing the diaphragm, although 4 shunts followed a tortuous route. All shunts terminated in the thoracic part of the azygos vein, perpendicular to the aorta. The shunt diameter at insertion was only 3 mm on average. The insertion site was consistently the narrowest part of the shunt.

Conclusion: CT angiography was well suited to provide anatomic details of porto-azygos shunts and comprehensively documented that all porto-azygos shunts had a thoracic terminus, after crossing the diaphragm through the esophageal hiatus. Different shunt types existed with minor variations.

2. Introduction

A portosystemic shunt is an anomalous vessel that bypasses the liver, diverting blood drainage from the venous gastrointestinal tract directly into the systemic circulation.¹ Porto-azygos shunts account for 25% of all congenital extrahepatic portosystemic shunts in dogs.^{2,3} Computed tomography (CT) angiography provides accurate details on the shunting vessels and their tributaries.⁴⁻⁶ In general, descriptions of porto-azygos shunt anatomy in dogs are vague and often it is not mentioned whether the point of insertion of the shunt into the azygos vein is situated in the abdomen or in the thorax. A preoperative description of the porto-azygos shunt insertion site facilitates the surgical approach and reduces the duration of the surgery, as well as the risk for continued shunting because of improper selection of the site of attenuation.^{4,7,8} Porto-azygos shunts that traverse the diaphragm before entering the azygos vein may have tributaries from gastric veins near the diaphragm.^{4,9-11}

Identification of such tributary vessels is often a surgical^{4,9,10} and imaging challenge¹¹ and it may be difficult to place an occluding device cranial to all gastric branches. If the device is placed too caudally, complete shunt closure will not be obtained.¹¹ To avoid incomplete closure, an option is to place the occlusive device on the thoracic portion of the shunt. To our knowledge, there has not been a study reporting porto-azygos shunt anatomy in dogs with special focus on the point of insertion of the portosystemic shunt into the azygos vein. There is therefore insufficient information to allow thoracic attenuation of such shunts. The goal of this study was to provide a detailed description of various types of porto-azygos shunts in a large number of dogs using CT angiography with special attention to the site of insertion of these vascular anomalies.

3. Materials and Methods

Dogs presenting to the Animal Medical Center of Nihon University between April 2007 and May 2015 with a porto-azygos shunt identified by CT angiography that were treated surgically were included in the study. In all dogs, surgical attenuation was performed at the abdominal part of the shunt at the level of the diaphragm (31 by ameroid ring constrictors and 5 by complete ligation).

All dogs were fasted for at least 12 hours before performing the CT scan (16 slice multi-detector CT scanner, Aquilion 16, Toshiba Medical Systems Co, Otawara, Japan). Dogs were induced at the anesthetist's discretion and anesthesia was maintained by isoflurane in oxygen. Dynamic CT angiography was performed with dogs in sternal recumbency according to the protocol described by Kutara.¹² Images were acquired using a 1-2 mm slice collimation, 120 kVp, and 150 mAs, and reconstructed using a soft tissue pass algorithm. Images were viewed using a window and level optimized for soft tissue (window 300 Hounsfield unit (HU), level 100 HU). The entire circumference of the abdomen and thorax were included in the display field of view. Before contrast medium administration, a survey CT scan was performed from the level of the thoracic inlet to the pelvic inlet. At the onset of initiating a dynamic CT scan, iodinated contrast medium (750 mg I/kg body weight; Ioverin 300, Teva Pharma Japan Inc, Nagoya, Japan) was administered at 0.3-3.0 mL/s through a peripheral catheter in the cephalic vein by an angiographic injector (Auto Enhance A-60, Nemoto-Kyorindo, Tokyo, Japan). A complete abdominal CT angiography examination was performed. To target the flow, the region of interest was placed into the aorta at the level of the 13th thoracic vertebra. When 150 HU was reached, the arterial phase was started. Images for the arterial phase were acquired every 10 seconds for a total of 700 images. The interval to maximal aortic enhancement and portal venous enhancement was calculated. During the portal vein phase images were acquired from the pelvic inlet to the thoracic inlet, 15 and 30 seconds after the arterial phase. A delayed phase was acquired 120 seconds after the beginning of the arterial phase. Images of the portal vein phase were imported into a CT image analysis program (AZE Virtual Place Plus, AZE, Tokyo, Japan) that allowed automatic selection of vessels based on HU differences. Gross anatomy of porto-azygos shunts, including the vessel of origin of the shunting vessel and its anatomic location, abdominal and thoracic pathway, and insertion point in the azygos vein (thoracic or abdominal part; level of vertebral body) was documented. If the shunt crossed the diaphragm, it was recorded through which hiatus it crossed. The diameters of the shunt vessel, portal vein, and aorta were measured on transverse images at set locations. Cranially, the portal vein diameter was measured at the hilus of the liver, caudal to the bifurcation of the vein in the right and left portal branch. On the same transverse image, the aortic diameter was measured. In addition, the diameter of the portal vein was measured immediately caudal to the origin of the shunting vessel. The shunting vessel was measured at its largest diameter, as well as at its terminus. If the shunt crossed the diaphragm, its diameter at the level of the diaphragm was defined. All measurements were performed by three independent observers (KI, KK, and KA) and mean values for each

parameter were calculated. The statistical software package SPSS Statistics (IBM, Brussels, Belgium) was used. Pearson correlation tests were performed to assess the correlation between the shunt size at insertion and the age at presentation. Significance levels were set at $P < .05$.

4. Results

A porto-azygos shunt was identified in 36 dogs on pre-operative CT images. Breeds represented were Yorkshire Terrier (7 dogs), Toy Poodle (5), Chihuahua (4), Pug (3), Papillon, Miniature Pinscher, Jack Russell Terrier, and mixed breed (2 each), Miniature Schnauzer, American Cocker Spaniel, Shih Tzu, Welsh Corgi, Bolognese, Cavalier King Charles Spaniel, Shiba Inu, Miniature Dachshund, and Norfolk Terrier (1 each). Ages at the time of CT imaging ranged from 3 months to 9.8 years (mean, 3.0 years). Bodyweight ranged from 1.5 to 8.7 kg (mean, 4.3 kg). Of the 36 dogs, 23 were female (15, intact; 8, spayed) and 13 were male (5, intact; 8, castrated).

Shunt Anatomy

All porto-azygos shunt conformations inserted into the azygos vein in the thorax. Different porto-azygos shunt conformations were identified by the CT angiography (Table 1).

Left Gastro-Azygos Shunts

Left gastro-azygos shunts (33 dogs) originated from the left gastric vein. The shunting vessels extended cranially and dorsally, crossing the diaphragm through the esophageal hiatus (Figs 1 and 2AB). The vast majority of the left gastro-azygos shunts (30) displayed a rather straight pathway, whereas 3 left gastro-azygos shunts extended craniodorsally along a curved, tortuous route after crossing the diaphragm. Two of the straight left gastro-azygos shunts had a concurrent caval-azygos continuation (Fig 3AB). In 29 dogs (85%) with a left gastro-azygos shunt the shunting vessel continued parallel and to the right of the aorta, inserting perpendicular to the azygos vein (Fig 1). However, in the remaining 4 dogs, the vessel coursed parallel to the left of the aorta until the level of the inserting point into the azygos vein. At that level, the shunt curved abruptly to the right at a 90° angle and crossed dorsal to the aorta to insert into the azygos vein (Fig 2). The left gastro-azygos shunts terminated in the thoracic part of the azygos vein at the level of T12 (13 dogs), T11 (12), T13 (6), T8 (1), and

T10 (1). The diameter of the portosystemic shunts at the point of insertion ranged from 1.6 to 5.2 mm (mean, 2.9 mm).

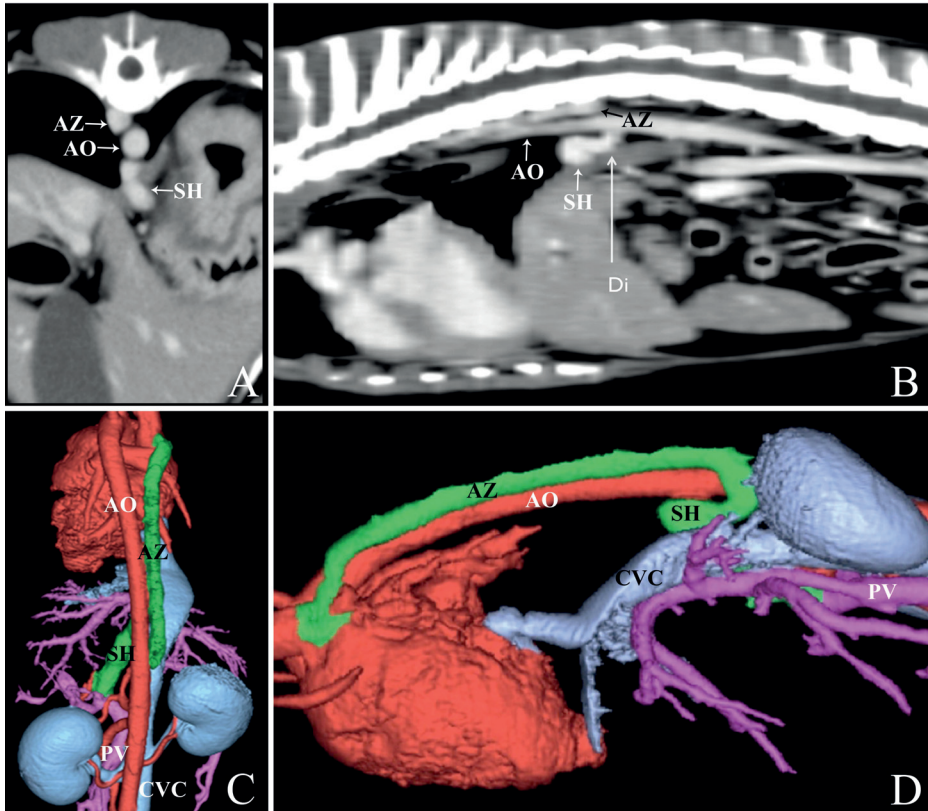


Figure 1 Left gastro-azygos shunt conformation, with a straight-lined right intra-thoracic course. (A) Transverse native image showing the shunting vessel coursing through the esophageal hiatus dorsal to the esophagus. (B) Sagittal native image showing the insertion site of the shunting vessel, situated cranial to the diaphragm. (C) Dorsoventral 3D reconstruction image showing the shunting vessel coursing to the right of the thoracic part of the aorta. (D) Sagittal 3D reconstruction image, viewed from the right side of the dog, showing the shunt coursing to the right of the thoracic part of the aorta with a characteristic L shape at insertion clearly visible. AO=aorta, AZ=azygos vein, CVC=caudal vena cava, DI=diaphragma, PV=portal vein, SH=shunting vessel,

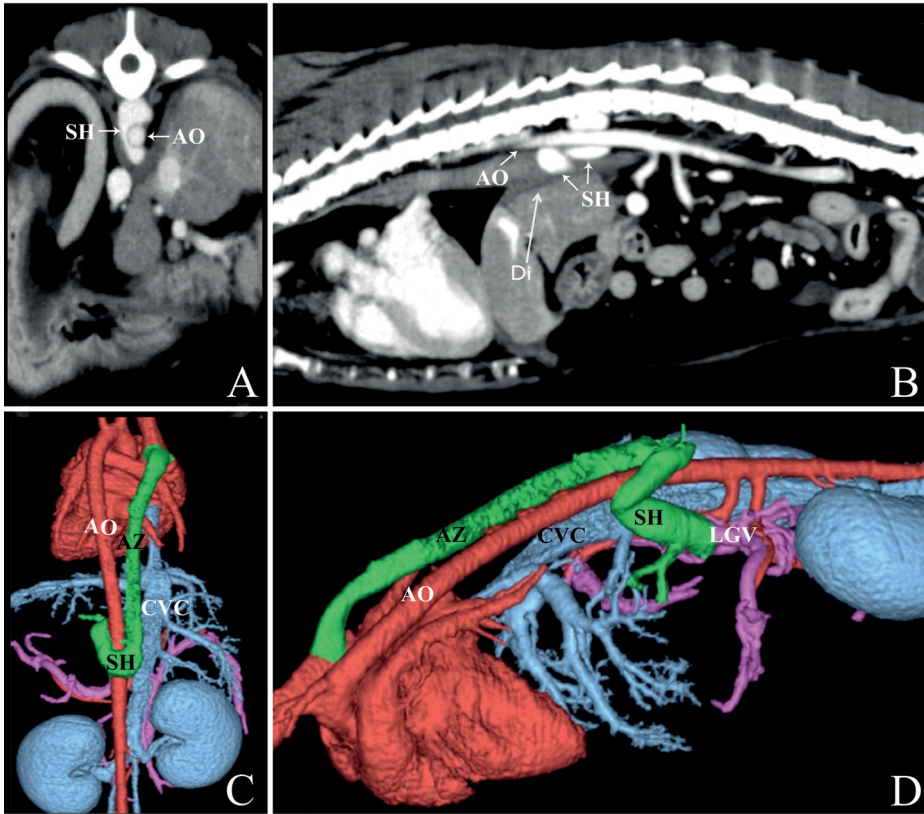


Figure 2 Left gastro-azygos shunt conformation, with a straight-lined left intra-thoracic course. (A) Transverse native image showing the shunting vessel coursing to the left of the thoracic part of the aorta. (B) Sagittal native image showing the insertion site of the shunting vessel, situated cranial to the diaphragm. (C) Dorsoventral 3D reconstruction image, showing the shunting vessel coursing to the left of the thoracic part of the aorta, passing dorsal to the aorta to inset in the azygos vein. (D) Sagittal 3D reconstruction image viewed from the left side of the dog, showing the shunt coursing to the left of the thoracic part of the aorta, passing dorsal to the aorta to insert in the azygos vein with a characteristic L shape clearly visible. AO=aorta, AZ=azygos vein, CVC=caudal vena cava, LGV=left gastric vein, SH=shunting vessel, DI=diaphragm

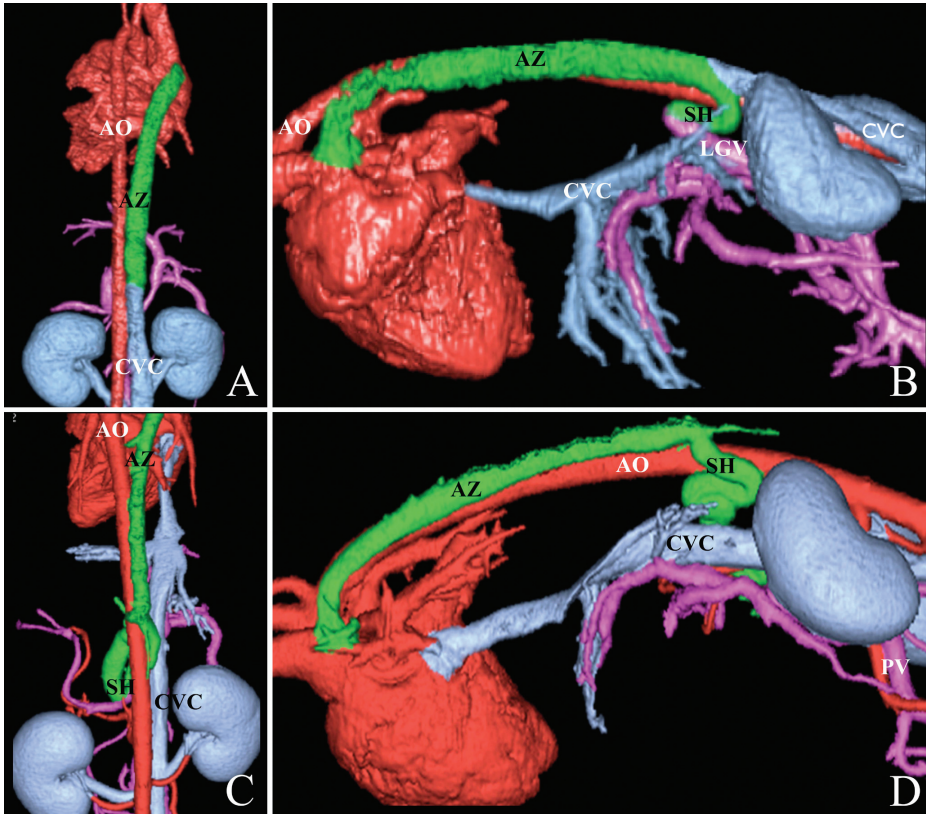


Figure 3 (A, B) Left gastro-azygos shunt conformation with a concurrent caval–azygos continuation. The pre-hepatic caudal vena cava was absent. The post-hepatic caudal vena cava, which was formed by confluence of hepatic veins, was clearly noticed. (A) Dorsoventral 3D reconstruction image. (B) Sagittal 3D reconstruction image viewed from the right side of the dog. (C, D) Left gastro-azygos shunt conformation with a tortuous intra-thoracic course. (C) Dorsoventral 3D reconstruction image. (D) Sagittal 3D reconstruction image viewed from the right side of the dog. AO=aorta, AZ=azygos vein, CVC=caudal vena cava, LGV=left gastric vein, PV=portal vein, SH=shunting vessel

Table 1 Porto-azygos shunt anatomy and relative measurements (mean [range]) of vessel diameters in 36 small breed dogs

Shunt anatomy	# dogs	Diaphragmatic passage	Relative to aorta in thorax (#)	Level of insertion ⁺	Diameter (mm) at insertion into azygos	Age (years) at presentation	Caudal		Cranial		Maximal shunt:aorta diameter
							Esophageal	hiatus	PV:aorta diameter	PV:aorta diameter	
Left gastro-azygos	33										
Straight-lined*	30	Esophageal	Right (26)	T8-T13	2.9 [1.6-5.2]	2.5 [0.3-9.8]	1.0 [0.6-1.5]	0.6 [0.4-1.0]	0.6 [0.4-1.0]	1.2 [0.7-1.6]	
			Left (4)								
Tortuous thoracic part	3	Esophageal	Right (3)	T10-T13	3.0 [1.7-3.8]	5.1 [3.1-7.1]	0.8 [0.7-0.9]	0.6 [0.5-0.7]	0.6 [0.5-0.7]	1.1 [1.0-1.2]	
Right gastro-azygos	3										
With a caudal splenic loop	2	Esophageal	Right (2)	T12	2.7 [2.0-3.4]	6.8 [6.1-7.5]	0.8 [0.8-0.8]	0.7 [0.6-0.7]	0.7 [0.6-0.7]	0.9 [0.8-1.0]	
Tortuous thoracic part	1	Esophageal	Right (1)	T12	2.3	7.3	0.9	0.7	0.7	1.1	
Total porto-azygos	36	Esophageal	Right (32)	T8-T13	3.0 [1.6-5.2]	3.0 [0.3-9.8]	0.6	1.2	1.2	1.0	
			Left (4)								

* In 2 dogs, an azygos continuation of the caudal vena cava was observed. ⁺ vertebral body; T=thoracic. PV=portal vein

Right Gastro-Azygos Shunts

The right gastro-azygos shunts (3 dogs) involved the right gastric vein and extended cranially and dorsally, similar to the left gastro-azygos shunts, and also crossed the diaphragm through the esophageal hiatus. In 2 dogs, the shunts had a fairly straight course but the splenic vein was feeding a second and larger shunting branch, which united with the right gastric vein branch before crossing the diaphragm (Fig 4AB). The shunting vessel terminated in the thoracic part of the azygos vein at the level of T12 in both dogs and the diameters at insertion were 2.0 and 3.4 mm.

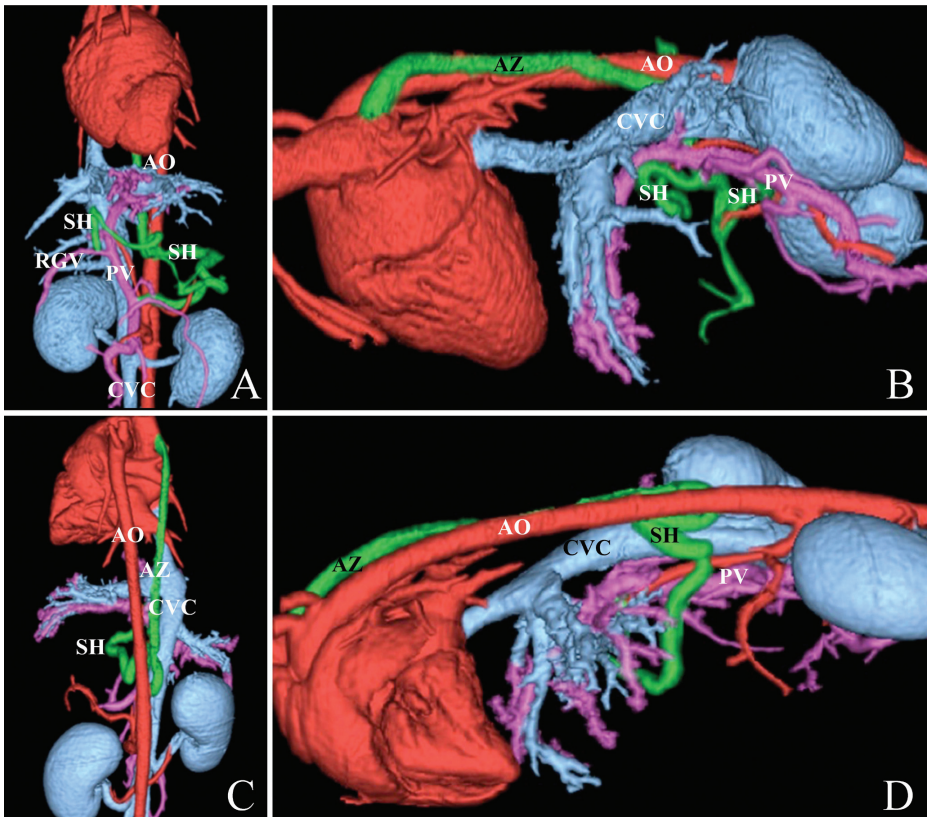


Figure 4 (A, B) Right gastro-azygos shunt conformation with a caudal splenic loop. (A) Ventrodorsal 3D reconstruction image. (B) Sagittal 3D reconstruction image viewed from the right side of the dog. (C, D) Right gastro-azygos shunt with a tortuous intra-thoracic course. (C) Dorsoventral 3D reconstruction image. (D) Sagittal 3D reconstruction image viewed from the left side of the dog. PV: Portal Vein, SH: Shunting vessel AO=aorta, AZ=azygos vein, CVC=caudal vena cava, RGV=right gastric vein, PV=portal vein

The remaining shunting vessel displayed a tortuous pathway, describing an S shape after crossing the diaphragm. The shunting vessel was situated to the right of the aorta (Fig 4CD) and terminated as a 2.3 mm vessel in the thoracic azygos vein at the level of T12.

The diameter of the portosystemic shunt at the point of insertion into the azygos vein ranged from 1.6 to 5.2 mm (mean, 3.0 ± 0.9 mm), which was consistently smaller than the diameter at the esophageal hiatus (mean, 5.0 ± 1.6 mm). In all but 5 dogs, the maximal diameter of the shunt exceeded the diameter of the portal vein immediately caudal to the origin of the shunting vessel (Table 1). Shunt size at insertion and age of the dog at presentation were not correlated ($P=.605$).

5. Discussion

To our knowledge, this is the largest study using CT angiography to determine porto-azygos shunt morphology. The study revealed 2 conformations of porto-azygos shunts in dogs, those involving the left gastric vein and those involving the right gastric vein. All porto-azygos shunts crossed the diaphragm through the esophageal hiatus and terminated in the thoracic part of the azygos vein. The maximal shunt diameter was situated in the abdominal part of the shunting vessel. At its point of insertion, the shunt was only 3 mm on average.

CT angiography supplants traditional mesenteric portography as the gold standard for the evaluation of the portal venous system anatomy in dogs^{4,10,13,14} and is far less invasive. Additionally, CT angiography provides a comprehensive overview of the entire portal vasculature, including all tributary vessels that drain into the shunt. Portography only provides visualization of the vessels through which the injected contrast agent flows.¹⁵ When performed correctly and with adequate spatial resolution, CT angiography can be performed in dogs of any size.⁷ Furthermore, the technique allows for the manipulation of the images after acquisition and for the creation of comprehensive three-dimensional models.⁴ The CT scans of the dogs with porto-azygos shunts provided the means for identifying the shunt type and detailed the size and course of the anomalous vessel.

In normal dogs, the azygos vein begins on the median plane, ventral to the body of the third lumbar vertebra.¹⁶ It runs cranially through the aortic hiatus, to the right of the aorta, terminating in the cranial vena cava. In rare cases, and considered an incidental finding, an azygos continuation of the caudal vena cava is observed. In this instance, the caudal vena

cava anastomoses with the azygos vein at the lumbar region.¹⁶⁻²³

In 25% of dogs with an extrahepatic portosystemic shunt, an abnormal communication between the portal system and the azygos vein is present.^{2,3} Based on CT angiography images of 25 dogs, Nelson and Nelson identified 4 porto-caval and 2 porto-azygos shunt types.⁴ In our study of porto-azygos communications, we also encountered right gastro-azygos shunts with a caudal splenic loop and left gastro-azygos shunts. Left gastro-azygos shunts were formerly called spleno-azygos shunts.^{4,14} Additionally, we identified a singular right gastro-azygos shunt. The assumption that a right gastro-azygos shunt without double looping might exist in dogs was briefly raised,⁴ but not previously documented.

Given the large number of dogs assessed in our study, the observed types and their variants likely represent the most common porto-azygos shunt conformations in dogs. The terminology used for naming the shunts was based on the veins that dominantly contributed to the shunt origin.^{4,14,15,24} Previous studies consistently implied that the shunting vessel in porto-azygos shunts joined the azygos vein at the level of the aortic hiatus.^{9,15} However, our study demonstrated that all shunting vessels crossed the diaphragm through the esophageal hiatus and only joined the azygos vein in its thoracic part. Despite the identification of different porto-azygos shunt types, our study identified little variation in the terminus of porto-azygos shunts on the azygos vein. The shunt diameter was consistently the smallest at the insertion site.

In 12% of dogs with left gastro-azygos shunts, the shunting vessel continued in the thorax to the left of the aorta until the level of the inserting point into the azygos vein. This could have clinical significance if a thoracic approach is used to place an occlusive device. Pre-operative CT angiography will prevent mistaking the shunting vessel for another vessel or for a hemi-azygos vein on the left side of the aorta. The curved, tortuous pathway of the shunt in the thorax, observed in the single right gastro-azygos shunt and in some of the left gastro-azygos shunts, has not been described previously and can be relevant during surgery. As previously described in portosystemic shunts, preoperative imaging can decrease the surgical time and the degree of dissection needed for shunt evaluation.^{4,8,14}

Shunts with a double looping morphology were noticed in 2 dogs with a right gastro-azygos conformation. This shunt type was previously described in a single dog.⁴ The naming convention suggests that the right gastric vein is the dominant contributor to the shunting vessel; however, we noticed that the caudal loop that communicated with the splenic vein had a larger diameter than the right gastric branch.

Dogs with an azygos continuation of the caudal vena cava in combination with a portosystemic shunt have previously been described.^{18,23,25} These dogs had a portal communication with the azygos vein in all cases^{18,25} except 1, which had a post hepatic porto-caval communication.²³ In our study, 2 left gastro-azygos shunts with concurrent caval-azygos continuations were observed. To our knowledge, this is the first documentation of this shunt conformation with CT angiography. Azygos vein distension was easily recognized on the CT images at the cranial extent of the abdomen because, in those cases, the diameter of the azygos vein exceeded the adjacent aorta diameter. The prevalence of caudal vena cava anomalies in dogs is reported to be 1-3%.^{17,23} In most cases of azygos continuation, a portosystemic shunt is not simultaneously present.^{17,23} Yet recognition of an associated shunt dictates surgical intervention, whereas an isolated vena cava anomaly does not.

Ultrasonographic measurements of the ratios between the major vessels (portal vein, shunting vessel and aorta) in normal dogs and dogs with portosystemic shunting have been reported.²⁶ Obviously, vessel diameters are easily measured on images acquired by CT angiography. Within the group of dogs with the same porto-azygos shunt type, the relative vessel diameter differences did vary. The portal vein diameter caudal to the shunt origine was smaller or similar to the shunt diameter. Likewise, the portal vein diameter at the hilus of the liver was smaller than the portal vein caudal to the origin of the shunt, which was also observed in a previous CT angiography study on different types of portosystemic shunt.⁴ The diameter of the porto-azygos shunts at the site of insertion was consistently the minimal diameter of the shunting vessel.

It was reported that porto-azygos shunts are often associated with less severe clinical signs than porto-caval shunts and are therefore mostly diagnosed in older dogs.^{7,27,28} In our study, dogs were diagnosed at a mean age of 3 years. One suggestion is that the severity of clinical signs is positively correlated with the amount of blood diverted from the portal circulation to the systemic circulation.^{7,29} Based on Poiseuille's law, there might be more resistance to blood flow in porto-azygos shunts than in porto-caval shunts, since the diameter of the azygos vein is smaller than that of the caudal vena cava.¹⁶ Moreover, porto-azygos shunts can be partially compressed by the diaphragm during respiration and by gastric distension after eating, which can result in temporary improved hepatic perfusion through the normal portal vasculature.^{7,30} Furthermore, the blood that flows through a porto-azygos shunt that inserts in the thorax, could be assumed to travel a greater distance than through most porto-caval shunts, adding to the resistance of the flow.⁷ In addition, porto-azygos shunts were found to be relatively narrow at their thoracic terminus. Statistically, however, the diameter at insertion

could not be correlated to the age at presentation.

6. Conclusion

Computed tomography angiography is well suited to provide details on porto-azygos shunt anatomy in dogs. Anatomic variations in shunt types exist, but are minor. Based on our data, the vast majority of dogs with a congenital porto-azygos shunt have a left gastro-azygos shunt, whereas other shunts involve the right gastric vein. All porto-azygos shunts included in our case series crossed the diaphragm through the esophageal hiatus and had a thoracic terminus.

7. Disclosure

The authors report no financial or other conflicts related to this report.

8. Acknowledgements

We thank Sara Kol for language editing, and Adriaan Kitshoff and Filip Clompen for assistance in composing images.

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Transdiaphragmatic Approach to Attenuate Porto-Azygos Shunts

Inserting in the Thorax

Adapted from:

Transdiaphragmatic approach to attenuate porto-azygos shunts inserting in the thorax.

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Veterinary Surgery 2016, 45(8), p 1013-1018 (doi: 10.1111/vsu.12557)

1. Abstract

Objective: To describe the surgical technique and to document the feasibility of a transdiaphragmatic approach to attenuate porto-azygos shunts inserting in the thoracic part of the azygos vein.

Study Design: Cadaveric study and prospective case series.

Animals: Canine cadavers (n=6) and client-owned dogs with porto-azygos shunts inserting in the thoracic part of the azygos vein (n=9).

Methods: In the cadavers, the azygos vein was filled with aqueous latex solution. Landmarks were established for creating a safe transdiaphragmatic approach to the caudal intra-thoracic portion of the azygos vein. In the clinical cases, porto-azygos communication was diagnosed by transsplenic portal scintigraphy. All shunts were attenuated close to their insertion site via celiotomy using a transdiaphragmatic approach. Peri-operative complications were recorded.

Results: A 3-5 cm incision, 0.5-1 cm ventral and lateral to the level of the aortic hiatus, was made in the pars lumbalis part of the diaphragm. Stay sutures at both sides of the diaphragmatic incision were placed to open up the incision and a retractor was used to push the esophagus away from the aorta. Intra-thoracic insertion of the shunt was confirmed intraoperatively. Exposure of the shunt insertion site to the azygos vein was excellent in all clinical cases. No intra- or postoperative complications were encountered.

Discussion/Conclusion: If thoracic attenuation of a porto-azygos shunt is considered, a transdiaphragmatic approach exposes the insertion site for shunt attenuation. This approach is a straightforward surgical procedure, without unnecessary abdominal organ manipulation, while the risk of missing additional contributing branches is eliminated.

2. Introduction

Surgical attenuation is the preferred method of treatment for congenital portosystemic shunts (PSS) in dogs without signs of portal hypertension.¹ It is recommended to attenuate shunting vessels as close to their insertion site as possible, so that blood flow from all tributaries of the shunt is redirected through the portal system.²

Nearly one fourth of all extrahepatic PSSs in dogs are porto-azygos shunts.^{3,4} Although the majority of those shunts traverse the diaphragm to insert in the thoracic part of the azygos vein,^{2,5} it appears that most surgeons occlude porto-azygos shunts intra-abdominally rather than intrathoracically.² Continued postoperative shunting is of concern if the occluding device is placed further away from the insertion,⁶ since porto-azygos shunts may have tributaries from gastric veins just before traversing the diaphragm.^{2,4,7,8} Identification of those tributary vessels can be a challenge as they can spasm with surgical manipulation.^{2,7,8} A recent report on CT-angiographic findings in 36 dogs with porto-azygos shunts identified 2 shunt types, either involving the left or the right gastric vein all of which had a thoracic terminus after crossing the diaphragm through the esophageal hiatus.⁵ The shunts all ran parallel to the thoracic aorta making a near 90° bend before inserting into the azygos vein, creating a characteristic “L” shape adjacent to their insertion site.⁵ Sticking to the principles of PSS attenuation,² it seems logical to only attenuate also porto-azygos shunts as close to their insertion site as possible, necessitating access to the thoracic cavity in cases where the shunting vessel terminates in the thorax.

The objective of this study was to describe and document a transdiaphragmatic surgical approach to attenuate porto-azygos shunts inserting in the thoracic part of the azygos vein.

3. Materials and Methods

A cadaveric study and a prospective case series were designed. The transdiaphragmatic approach for attenuation of intrathoracic porto-azygos shunts was evaluated in canine cadavers (n=6) and subsequently applied in clinical cases (n=9).

Six, small breed (< 10 kg) canine cadavers of different, sex, age and breed were used. Dogs were euthanized for reasons unrelated to the study. The dogs were first positioned in left

lateral recumbency. Ribs 2-6 were removed to create a thoracic window, exposing the insertion site of the azygos vein into the cranial vena cava. The azygos vein was ligated at its insertion site into the cranial vena cava using a 3-0 polyglactin 910. A 20 gauge peripheral venous catheter was placed into the azygos vein caudal to the ligature and the vein was filled by injection of 40 mL of aqueous latex (Polyester Demaere©, Belgium). The dogs were stored at 4°C for 24h to allow the latex to cure. A standard median celiotomy was then performed with the dogs in dorsal recumbency. Landmarks were established to create a safe corridor for a transdiaphragmatic approach to the caudal part of the thoracic azygos vein. These landmarks included the aorta dorsally and medially and the phrenic vein ventrally and laterally. A stab incision was made in the left side of the diaphragm 0.5-1 cm ventral and lateral to the aortic hiatus in the intermediate part of the pars lumbalis; the incision was lengthened over 3-5 cm, parallel with the direction of the muscle fibers. Stay sutures were placed through the diaphragm on both sides of the incision and the thoracic cavity was explored. All visible intrathoracic anatomical structures were recorded. Next, the ventral part of the ribs cranial to the attachment of the diaphragm on both sides of the thorax, including the associated sternum, were resected, leaving the diaphragm intact. The thoracic side of the transdiaphragmatic incision was evaluated with relation to its proximity to local thoracic structures.

The clinical case series included 9 dogs (presented from August 2013 to January 2016) with porto-azygos shunts diagnosed by transsplenic portal scintigraphy using technetium pertechnetate $^{99m}\text{TcO}_4$.⁸ All dogs underwent a transdiaphragmatic approach for shunt attenuation. All dogs received standard medical stabilization, consisting of a dietary, antimicrobial, synthetic disaccharide regimen for an initial 4-week period before surgery and vitamin K for 5 days before surgery. The intrathoracic course of the shunt was confirmed at the time of surgery. Based on the findings of the cadaveric study, a stab incision was made in the left part of the diaphragm 0.5-1 cm ventral and lateral to the aortic hiatus in the intermediate part of the pars lumbalis; the incision was lengthened over 3-5 cm, parallel to the direction of the muscle fibers. The left phrenic vein dictated the most ventral and lateral borders of the diaphragmatic window (Fig 1). All shunts were bluntly dissected from the aorta with a curved baby-Mixter forceps (Medlane[®], France) close to their insertion site into the thoracic part of the azygos vein at the level of the characteristic “L” shape⁵. A cellophane band (CB; Marlboro cigarette wrap, Belgium) was used in the first 5 cases whereas in all consecutive cases (4 first surgeries and 3 revisions), an ameroid ring constrictor (AC; Research Instruments NW, Lebanon, OR) was placed as previously described.^{2,9} The

diaphragmatic incision was closed using a 3-0 poliglecaprone 25 in a simple continuous suture pattern and negative intrathoracic pressure was restored using a 3Fr feeding tube (Teleflex, Rusch® sterile, Germany).¹⁰ The tube was inserted through the diaphragmatic incision, and, after the suture was tightened, air was removed using a 50 mL syringe connected to the tube by a three-way stopcock. As soon as negative intra-thoracic pressure was established, the tube was removed. All dogs recovered from anesthesia under direct supervision. Fluid and pain management were continued as needed. All patients were hospitalized for at least 3 days after surgery. Oral medication and diet were initiated as soon as the dogs were willing to eat. Postoperative seizures and signs of portal hypertension were evaluated daily. The dogs were discharged from the hospital on the same medicinal regimen as pre-operatively. A postoperative transsplenic portal scintigraphy was advised 12 weeks after surgery to assess shunt closure.

4. Results

The dogs included in the cadaveric study had a mean body weight of 6.0 kg (range 4.3-10.2 kg). After median celiotomy, retraction of the left liver lobes and the stomach to the right facilitated visualization of the left phrenic vein and the esophageal and aortic hiati. The position of the intrathoracic part of the aorta was identified by digital palpation through the diaphragm. The left phrenic vein dictated the most ventral and the most lateral border of the approach. Traction at the stay sutures facilitated exposure of the intrathoracic structures and allowed excellent visualization of the caudal part of the thoracic aorta in all cadavers. The sympathetic trunk was identified lateral and dorsal to the thoracic aorta at a safe distance from the incision line. The phrenic and the vagal nerves could not be visualized through the diaphragmatic window, yet both nerves were not at risk for iatrogenic damage since they coursed far ventral or medial to the diaphragmatic incision, respectively. The latex-filled azygos vein was also not visible through the diaphragmatic window. Only after extensive dissection between the psoas minor and quadratus lumborum muscles it was exposed dorsal to the thoracic aorta.

In the clinical cases, a total of 12 transdiaphragmatic approaches were performed in 9 dogs, 3 dogs underwent a second surgery due to failure of shunt closure. Data are summarized in Table 1.

Table 1 Summary data of dogs that underwent a transdiaphragmatic approach to attenuate a porto-azygos shunt in the thorax

Dog	Age (Years)	Gender	Weight (Kg)	Breed	Method of Attenuation	Post-op scintigraphy (12 weeks)
1	1.0	F	4.3	Jack Russell Terrier	CB	NA
2	4.0	M	2.2	Yorkshire Terrier	CB	Shunt patent
	4.3		2.5		AC	Shunt closed
3	1.3	F	2.2	Chihuahua	CB	Shunt patent
	1.6		2.4		AC	Shunt closed
4	4.0	F	7.8	Norwich Terrier	CB	Shunt patent
	4.6		7.9		AC	Shunt closed
5	6.0	M	12.5	Dachshund	CB	NA
6	5.4	F	2.0	Chihuahua	AC	Shunt closed
7	5.0	F	1.4	Yorkshire Terrier	AC	Shunt closed
8	0.5	F	1.6	Yorkshire Terrier	AC	Shunt closed
9	0.7	F	10.7	Border Collie	AC	(<12 weeks)

F, Female; M, Male; AC, Ameroid Constrictor; CB, Cellophane Band; NA, Not available

The approach was performed exactly as defined in the cadavers, but in addition a moistened abdominal sponge was used to cover the parietal aspect of the liver and the remaining abdominal content during the transdiaphragmatic approach and the intrathoracic dissection of the PSS (Fig 1). On the abdominal side, the left phrenic vein and the aorta were clearly visible, and the aortic pulsation permitted easy identification of the thoracic aorta by digital palpation. The left phrenic vein dictated the most ventral and the most lateral border of the approach. Traction at the stay sutures facilitated access to the thorax, but additionally a small self-retaining retractor was used to visualize the shunting vessel by pushing the esophagus ventrally (up) and to the right (medial) from the aorta (Fig 2). All the shunting vessels crossed the diaphragm through the esophageal hiatus, dorsal to the esophagus. Visualization and exposure of the characteristic “L” shape was excellent in all clinical cases. All shunting vessels terminated at the level of T10-T12. The azygos vein itself could not be visualized at any occasion. The sympathetic trunk was at a safe distance from that “L” shape and was never at risk for iatrogenic damage. The caudal left lung lobe neither obscured the shunt visibility nor interfered with placement of the CB or AC. Dissection around the shunting vessel was minimal and placement of a CB or an AC was straightforward.

The systemic arterial pressure, gastro-intestinal tract and pancreas were evaluated for signs of portal hypertension before closure of the diaphragm. There were neither technical issues nor intra-operative complications. All dogs recovered rapidly and uneventfully. All dogs were discharged 3 days after the surgery. Postoperative medical treatment was continued for 4 weeks and consisted of a liver diet, metronidazole (Stomorgyl; 7.5 mg/kg for the metronidazole fraction every 12 hours, Merial, France) and synthetic disaccharide regimen (Lactulose EG; 0.5 mL/kg every 8 hours, Eurogenerics, Belgium).

Two dogs were lost for follow-up after the one-month check-up and the most recent case did not yet reach the 3-months follow-up. All other cases (n=6) were re-evaluated at 12 weeks after surgery. All owners reported their dogs to be normal, with no clinical signs related to portosystemic shunting. Nevertheless, scintigraphy indicated persistent shunting in 3 dogs in which a CB was used. In those dogs, the pre- and or postprandial serum bile acid concentrations were above normal values and were even elevated compared to pre-surgery values. A revision surgery, including a second transdiaphragmatic approach, was proposed in order to attenuate the shunt. Two dogs were re-operated immediately whereas in the third, the surgery was delayed for another 3 months in the hope that the shunt would attenuate. In the latter, repeated serum bile acid concentrations were consistently elevated. In all 3 cases surgery was repeated due to incomplete closure of the porto-azygos shunt and the CB

replaced by an AC. No complications were experienced with the repeated transdiaphragmatic approach. There were no macroscopically visible diaphragmatic scars; no adhesions on the abdominal or thoracic side. Follow up scintigraphy 12 weeks after the second surgical intervention confirmed closure of the shunt in all dogs. Serum bile acid concentrations were reduced compared to previous measurements, almost reaching normal values.

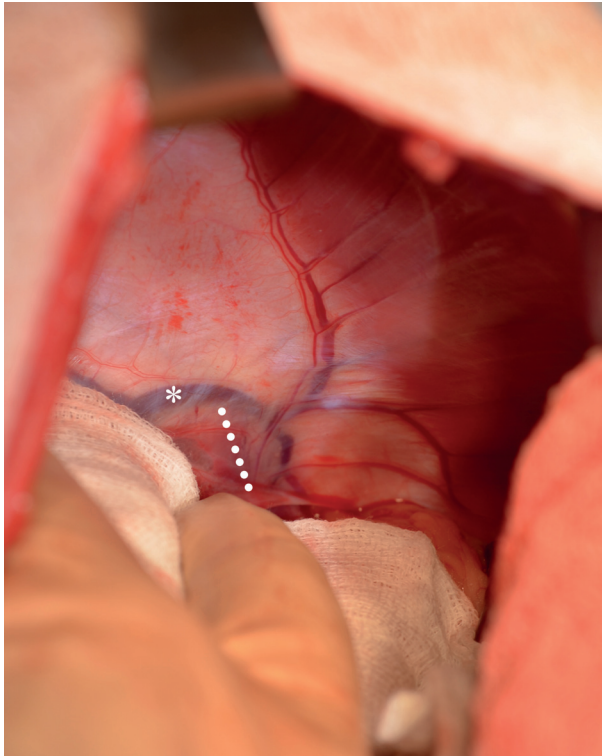


Figure 1 Transdiaphragmatic approach to expose porto-azygos shunts in the thorax of dogs, caudocranial view. The dog is positioned in dorsal recumbency with the head pointed up. The parietal aspect of the liver is covered with a moistened sponge. The left side of the diaphragm with the left phrenic vein (asterisk) is visible, dictating the most ventral and most lateral borders of the diaphragmatic window. An incision (dotted line) is started 0.5-1 cm ventral to the aorta in the intermediate part of the pars lumbalis. The incision is lengthened over 3-5 cm, along the muscle fiber alignment.

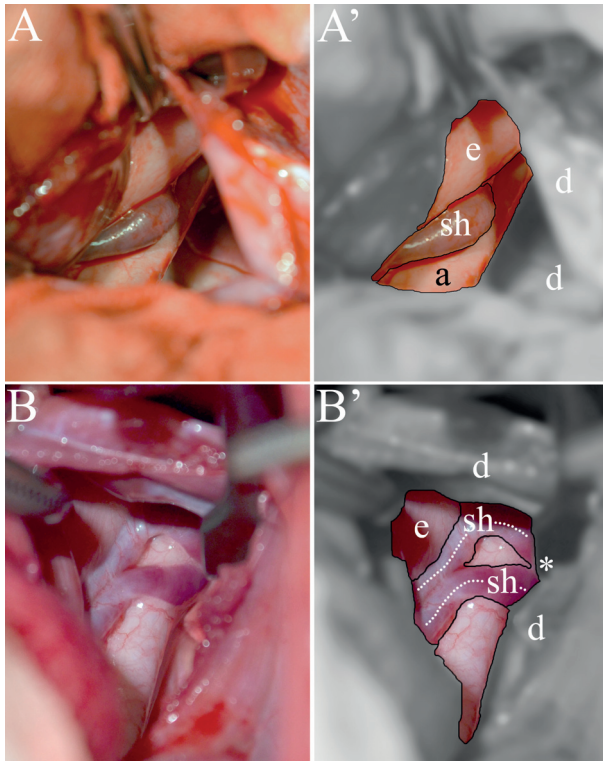


Figure 2 Anatomic variations. **(A)** Typical intra-operative appearance of a porto-azygos shunt through a transdiaphragmatic approach (dog 9). **(A')** Identical image to A, but the surroundings are discolored to better accentuate the intra-thoracic structures of interest. The shunting vessel (sh) lies adjacent and ventral to the aorta (a) and dorsal to the esophagus (e). The diaphragmatic window borders (d) are indicated. **(B)** Intra-operative image through the transdiaphragmatic approach in dog 3 reveals an unusual de duplication of the shunting vessel. **(B')** Identical image to B, but the surroundings are discolored to better accentuate the intra-thoracic structures of interest. The shunting vessel (sh), indicated by the white dotted lines, lies adjacent and ventral to the aorta (a) and dorsal to the esophagus (e). The characteristic “L” shape can be appreciated just before its insertion into the azygos vein (*). The diaphragmatic window borders (d) are indicated.

5. Discussion

The transdiaphragmatic approach to the thoracic part of a porto-azygos shunt is a straightforward surgical procedure, providing a safe corridor to the thorax with excellent exposure of the region of interest. Initial development of the approach in cadavers and subsequent validation in clinical cases have proven its feasibility to reach the insertion site in the thoracic part of the azygos vein. To the authors' knowledge, this is the first report to document a transdiaphragmatic approach to the thoracic part of porto-azygos shunts in dogs.

Transsplenic portal scintigraphy is a commonly used method for pre-operative quantitative measurement of portal bypass of the liver, allowing the diagnosis and differentiation of various shunt types (portocaval, porto-azygos or acquired).^{9,11} This imaging modality correctly distinguishes between porto-azygos and portocaval shunts in 100% of the cases, as the former shunt type displays a more dorsal pathway of the radionuclide and bolus activity that enters craniodorsally into the heart instead of caudally.⁹ In the current study, transsplenic portal scintigraphy was used to diagnose the porto-azygos shunt in all cases. Admittedly, this imaging modality cannot identify the precise insertion site of the shunt into the azygos vein. A recent study on CT evaluation of porto-azygos shunt anatomy in 36 dogs confirmed that all shunts had a thoracic terminus after crossing the diaphragm through the esophageal hiatus, dorsal to the esophagus.⁵ Therefore, a pre-operative CT-angiogram to unambiguously document the point of insertion^{5,7,12} was not considered a prerequisite by the authors.

The point of insertion of the shunting vessel is of utmost importance to the surgeon, as it is recommended to attenuate a shunt as close to its insertion site as possible, to eliminate the risk of persistent shunting by unidentified tributaries.^{2,6} Surprisingly, a leading surgical textbook² differentiates between portocaval shunts, in which that recommendation is respected, and porto-azygos shunts, in which attenuation at the abdominal side of the diaphragm is advised, even though those authors acknowledged that the shunting vessel traverses the diaphragm in the majority of cases. The authors acknowledge the lack of historical studies that are clearly suggesting that intra-abdominal occlusion of porto-azygos shunts leads to a high rate of failure to attenuate all shunting branches. Nevertheless, support for this assertion can be found in the existing literature. A recent report evaluating *in vivo* behavior of AC using postoperative CT angiography in dogs with extrahepatic PSS showed that 2 of the 5 dogs with porto-azygos shunts (attenuated at the abdominal side) had persistent shunting due to the presence of a patent tributary cranial to the AC.⁶ We hypothesized that attenuating porto-azygos shunts inserting in the thoracic part of the azygos vein at the thoracic side of the

diaphragm would have several advantages such as elimination of the risk to miss small tributaries and reduction of abdominal organ manipulation and potential morbidity during exploration of the portal vasculature, indirectly reducing surgical time.

In order to place a device on the thoracic portion of the porto-azygos shunts inserting in the thorax, a surgical approach to the thorax is needed. There is a single case report describing a right lateral thoracic approach to attenuate a porto-azygos shunt.¹² We hypothesized that a transdiaphragmatic approach would be preferable since it simultaneously allows evaluation of the gastro-intestinal tract and the pancreas before and after a CB or AC placement and it does not exclude abdominal exploration and/or concomitant intra-abdominal procedures. In addition, a lateral thoracotomy necessitates a pre-operative CT angiography to determine the side and the most ideal intercostal space. Furthermore, we argue that a thoracotomy is more invasive, more painful and more time-consuming compared to a transdiaphragmatic approach. Therefore the authors put the transdiaphragmatic approach forward as the surgical approach to a porto-azygos shunt inserting in the thorax if attenuation close to the shunt insertion is considered. The thoracic part of a porto-azygos shunt inserts perpendicular to the azygos vein, forming a characteristic “L” shape.⁵ This landmark was chosen as the target site for shunt attenuation. The proximity between the shunting vessel and the aorta guided the authors to approach the region through the left side of the diaphragm. Based on data of pre-operative CT angiography in a large number of dogs,⁵ one can presume that most (if not all) porto-azygos shunts traverse the diaphragm at the level of the esophageal hiatus. Those observations dictated the guidelines for establishing the level of the diaphragmatic incision. During the cadaveric study and subsequently in all clinical cases, the phrenic vein was found to be a reliable, easy and accurate landmark to guide the diaphragmatic incision. At any stage, the lungs did not obscure the view nor interfere with placement of an attenuation device; however, the esophagus partially covered the shunting vessel. It was necessary to retract the esophagus ventrally and to the right to provide an excellent exposure of the characteristic “L” shape of the porto-azygos shunt.

Attenuation of a porto-azygos shunt with a thoracic extension at the abdominal side of the diaphragm potentially allows small branches from gastric veins to enter the shunt just before it traverses the diaphragm.^{4,8} Such small tributaries might be missed on CT-images as well as during abdominal exploration.^{2,6} Furthermore, porto-azygos shunts are sometimes difficult to follow intra-operatively in the abdomen, because they may become nearly undetectable with vigorous retraction or dissection.^{2,7} Manipulations to identify and to follow the course of the shunt in the abdomen could lead to increased morbidity. The transdiaphragmatic approach

overcomes all the issues raised above; the approach represents a relatively easy surgical procedure, without unnecessary abdominal organ manipulation, while the risk of missing additional contributing branches is eliminated. If the insertion site of the shunting vessel lies within the thorax, it seems reasonable not to follow the course of the shunting vessel throughout the abdomen, even if no pre-operative CT is performed. After the first 3 cases, we felt comfortable to outgrow exploration of the portal system, and we immediately proceed with performing a transdiaphragmatic approach. This subjectively reduced the procedural time and reduced abdominal organ manipulation.

Three months postoperatively, persistent shunting was confirmed by scintigraphy in 3 dogs attenuated by a CB (the 2 remaining CB dogs were lost for follow-up). In all 3 cases, the radionuclide followed a pathway similar to that observed before surgery, suggesting persistent shunting rather than the formation of acquired shunts.¹¹ Although the owners of those dogs did not report clinical signs associated with portosystemic shunting, all dogs were represented for revision surgery. Repeated surgery was advised to eliminate the risk of recurrence of clinical signs since liver function (judged by serum bile acids) did not improve after the first intervention. At the time of the second surgery, failure of the CB to promote complete occlusion of the shunting vessel was confirmed. The fact that there were no adhesions at all at the CB site in all 3 cases is worth noting. This is in contrast with what is experienced by the authors at repeat celiotomy in cases with portocaval shunts attenuated by CB. The observed difference might be explained by the more limited amount of soft tissues in the thoracic cavity compared to the abdomen, causing less inflammatory and fibroblastic reaction that are typically associated with a chronic foreign body.¹³ This finding might also indicate that production of fibrosis induced by the CB is far less pronounced in the thorax. Because of these findings at re-operation, the 3 persistent cases and the more recent cases (n=6) were attenuated using an AC. All these shunts, except the most recent case, which has not yet reach the 3-months follow-up, were successfully closed after 3 months. To the authors' knowledge, there is no literature to endorse the latter suggestion, but in their experience it may be prudent to rely on AC rather than CB to occlude porto-azygos shunts in the thoracic cavity.

The authors acknowledge the limited number of patients treated by the transdiaphragmatic approach and the absence of a (historical) control group. The objective of the current study was to describe and document a novel surgical approach to attenuate porto-azygos shunts inserting in the thoracic part of the azygos vein. Due to the lack of current literature regarding outcome of porto-azygos shunts, comparison with other approaches was not attempted, but warrants further investigation.

6. Conclusion

If a thoracic insertion of a porto-azygos shunt has been identified, a transdiaphragmatic approach exposes the insertion site for shunt attenuation. This approach is a relatively easy and fast surgical procedure, without unnecessary abdominal organ manipulation, while the risk of missing additional contributing branches is eliminated.

7. Disclosure

The authors report no financial or other conflicts related to this report.

8. Acknowledgements

We wish to thank Sara Kol for the language editing, and Filip Clompen for assistance in composing the images.

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**Ammonia levels in Arterial Blood, Venous Blood and
Cerebrospinal Fluid in Dogs With and Without Extrahepatic
Portosystemic Shunting**

Adapted from:

Ammonia levels in arterial blood, venous blood and cerebrospinal fluid in dogs with and without extrahepatic portosystemic shunting

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American Journal of Veterinary Research. Accepted.*

1. Abstract

Objective: To compare ammonia levels in arterial blood, venous blood and cerebrospinal fluid (CSF) in dogs with and without extrahepatic portosystemic shunts (EHPSS).

Study Design: Prospective clinical trial.

Animals: 19 dogs with congenital EHPSS and 6 healthy control dogs.

Procedures: Transsplenic portal scintigraphy was performed to confirm the diagnosis of EHPSS. In both groups, simultaneous samples were collected during general anesthesia from the femoral artery (arterial blood), the jugular vein (venous blood) and the cerebellomedullary cistern (CSF) for ammonia measurement by two different devices, the PocketChem BA (PocBA) and the Catalyst Dx (CatDx).

Results: Ammonia concentration (arterial, venous, and CSF) were significantly higher in EHPSS dogs than in the control dogs and were comparable for both devices. Arterial ammonia levels were higher than venous levels, but this difference was only statistically significant when blood ammonia was measured by PocBA. A strong positive correlation up to 96% was observed between arterial and venous ammonia. A significant positive correlation up to 89% was demonstrated between blood (arterial or venous) and CSF ammonia levels.

Conclusion and Clinical Relevance: In this cohort of patients, ammonia levels were significantly higher in arterial than venous samples and both were significantly higher in EHPSS dogs compared to control dogs. The ammonia levels in the CSF of EHPSS dogs were significantly higher than in control dogs and were positively correlated to the arterial and venous blood ammonia levels, suggesting increased passage of ammonia across the blood-brain barrier (BBB) in EHPSS patients.

2. Introduction

Ammonia is a normal constituent of body fluids and is mainly produced in the gastrointestinal tract by bacterial metabolism of urea and glutamine.¹ Ammonia is lipid soluble and consequently easily crosses cell membranes.^{1,2} In the gut, it diffuses via the intestinal mucosa into the portal venous circulation where it is converted to ammonium NH_4^+ ; the blood is then detoxified in the liver by the urea cycle.^{1,2} An increased plasma ammonia level occurs in cases of portosystemic shunting, liver failure and urea cycle disorders.³⁻⁵

It is widely accepted that ammonia is a key factor in the pathogenesis of HE.⁶ Ammonia is widely considered to initiate HE through altered astrocyte function; astrocytes are the main cells in the brain that can metabolize ammonia. The conversion of glutamate and ammonia to glutamine results in osmotic stress leading to astrocyte swelling, cerebral edema and intracranial hypertension.⁷

Ammonia in the brain is formed in situ from the metabolism of endogenous nitrogen-containing substances.⁸⁻¹⁰ Additionally, it crosses the BBB by diffusion from the blood.⁸ Ammonia is shown to diffuse into the brain more freely in humans with severe liver disease than in normal control subjects.^{11,12} Transport of ammonia from the blood to the brain increases with increasing arterial ammonia levels.¹¹ Interestingly, increased BBB permeability results in increased diffusion of ammonia into the brain^{11,12} and can result in toxic levels even in the face of near normal arterial ammonia levels.¹¹

Dogs with PSSs are often presented with clinical signs of HE,¹³ but limited research is available on the association of PSS and HE. Two veterinary studies assessing several amino acids levels in the CSF of dogs with PSS suggested that ammonia levels in the CSF are elevated, but ammonia levels were not checked as such.^{14,15} Interestingly, in an animal model investigating different drugs to control chronic HE in human patients, a portocaval shunt was created in addition to a partial hepatectomy in experimental dogs. Ammonia levels in the CSF and blood were significantly higher in the operated group than in the sham-operated group.¹⁶ The dogs in the former group developed HE signs.¹⁶

Human patients with HE showed significantly higher arterial than venous blood ammonia levels.^{11,12} Arterial ammonia levels around 150 $\mu\text{mol/L}$ have a statistically significant association with the development of intracranial hypertension and cerebral edema.^{17,18} To our knowledge, there is only one study on the comparison of arterial and venous blood ammonia levels in canine HE.¹⁹ In that study, significantly higher ammonia levels were found in arterial blood than in venous blood in dogs with liver disease compared to control dogs, and there was

a strong correlation between the existence of HE and the presence of portosystemic collateral circulation. However, differences in ammonia levels in PSS dogs were not presented separately.¹⁹

The objective of this study was to compare ammonia levels in arterial blood, venous blood and CSF in healthy control dogs and in dogs with a congenital EHPSS in an attempt to elucidate the pathogenesis of HE in dogs with EHPSS. We hypothesized that in a given patient with hyperammonaemia due to the presence of an EHPSS the arterial ammonia level would exceed the venous level. We also hypothesized that a positive correlation exists between ammonia levels in the blood and the CSF.

3. Materials and Methods

Animal and study design

Client-owned dogs with congenital EHPSS were prospectively evaluated between July 2012 and October 2015 and their data were compared to control dogs. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (EC2012/164 and EC2013/33) and by the Belgian Deontological Committee.

Control dogs

Adult healthy Beagle dogs were used as controls. The dogs were fasted for 12 hours before testing with free access to water. A full physical examination, complete blood count, serum biochemical profile, paired serum bile acid concentration analysis, and urinalysis were performed to rule out preexisting disease. In addition, an abdominal ultrasound was obtained for health screening. The dogs were anesthetized (see below, anesthetic protocols) and a TSPS was performed to confirm the absence of portosystemic shunting. Intrasplenic injection of the tracer (sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4^-$), Drytec technetium 99mTc generator, GE Healthcare, UK) was done with ultrasound guidance and the dynamic scan was started simultaneously on a gamma camera equipped with low-energy, high-resolution collimator (Toshiba GCA7200A DI, Toshiba, Japan). Prior to the TSPS, an arterial, venous, and CSF sample was obtained for the measurement of ammonia levels (see below).

Dogs with an extrahepatic portosystemic shunt

Enrollment of dogs in the study was subject to a written owner informed consent. Before

sampling, patients were fasted for a minimum of 12 hours unless they were younger than 6 months of age (maximum of 4 hours). Patient data including age, sex, breed, body weight, and body condition were recorded. A full physical examination, complete blood count and serum biochemical profile, pre- and postprandial serum bile acid concentration analysis and arterial, venous and CSF ammonia level analysis were performed (see below). The clinical signs of HE were graded on a four-point scale (grade 0: normal; 1: reduced mobility and/or mild apathy; 2: severe apathy and/or mild ataxia; 3: salivation, severe ataxia, head pressing, apparent blindness, and/or circling; 4: seizures and/or coma) as previously described.²⁰ An abdominal ultrasound was obtained to assess the abdominal organs and to screen for potential vascular abnormalities. Transsplenic portal scintigraphy was performed in each dog to confirm the diagnosis of PSS.²¹

Collection of samples

Blood samples for paired serum bile acid concentration analysis were collected from the jugular vein. Following the collection of the preprandial blood samples, dogs were fed 2 teaspoons of a commercial highly digestible protein and fat diet (Hills a/d, Hill's Pet Nutrition NV, Brussels, Belgium) and the postprandial samples collected two hours thereafter.

Samples for determining the ammonia levels were collected while the dogs were shortly anesthetized (see below) for the TSPS procedure. Arterial blood was collected from a femoral artery using a 25-gauge needle connected to a 1 mL syringe. Arterial blood (700 μ L) was transferred to a special heparinized whole blood separator (Idexx Laboratories Europe, Hoofddorp, The Netherlands) and immediately placed on melting ice. The sample was instantly analyzed in the in-house laboratory (described below). Immediately after collection and processing of the arterial sample, a venous sample (700 μ L) was collected from a jugular vein (using a 21-gauge needle connected to a 2.5mL syringe). The processing of the venous samples was identical to that described for the arterial samples. For the CSF sample, a small area of skin (3 x 3 cm) at the atlanto-occipital region was clipped and surgically prepared to allow aseptic collection of CSF via a cisternal puncture with a 21-gauge needle. The CSF sample was collected in a tube without additives. If blood contamination occurred, a new sample was taken. The tube was immediately placed on melting ice for prompt in-house analysis.

Processing of samples

Both pre- and postprandial serum samples for bile acid analysis were centrifuged and the

serum was sent to an external laboratory for analysis.

Ammonia levels in the arterial and venous samples were measured in-house using both a portable blood ammonia analyzer (PocketChem BA, A. Menarini Diagnostics Benelux, Zaventem, Belgium; PockBA) and a non-portable chemistry analyzer (Catalyst Dx, Idexx Laboratories, Westbrook, USA; CatDx) immediately after sampling. Both devices measure the amount of gaseous ammonia (NH_3) after the ammonium ions (NH_4^+) in the sample have been converted. The samples for the PockBA were taken from the whole blood separators with a capillary tube just before its insertion into the CatDx.

Ammonia levels in the CSF samples were also measured in-house immediately after sampling. A small CSF sample (200 μL) was transferred to a sample cup (Idexx Laboratories Europe, Hoofddorp, The Netherlands) for analysis in the CatDx and the sample for analysis in the PockBA was taken from the sample cup with a capillary tube just before processing. A white blood cell count was performed on the sample remaining in the tube without additives.

Anesthetic protocols

The same anesthetic protocol was used in the EHPSS dogs and in the control dogs. First, a 22-gauge IV catheter was placed in one of the cephalic veins. Dogs were premedicated with 0.2 mg/kg butorphanol (Dolorex, Intervet NV, Oostkamp, Belgium) given IV. After 10 minutes, anesthesia was induced by administering 2-4 mg/kg propofol (Propovet Multidose, Abbott Laboratories Ltd, Maidenhead, UK) IV to effect. Following endotracheal intubation, anesthesia was maintained by a total intravenous anesthesia of propofol at a rate of 0.2-0.4 mg/kg/min. Oxygen (100%) was supplied throughout anesthesia at 1 L/min.

Statistical analyses

Statistical analysis was performed using a commercial software package (SPSS Statistics, IBM, Brussels, Belgium). Data were checked for normality by the Kolmogorov-Smirnov test with Lilliefors significance correction. For descriptive purposes, parametric data were summarized as mean \pm standard deviation (SD) whereas non-parametric data were described as median and range. To compare the continuous variables, an unpaired t-test (for normally distributed data) or the Mann-Whitney U-test (for non-normally distributed data) was used if the samples were independent whereas a paired sample t-test or a Wilcoxon signed rank test was used for paired data. Correlations were assessed by the Pearson's product-moment correlation or Spearman's rank correlation, depending on the respective normal or non-normal distribution of data. The significance level was set at 0.05.

4. Results

Study population

Six healthy dogs and 19 dogs with congenital EHPSS were studied.

The age of the dogs in the control group ranged from 36 to 54 months and their body weight from 9.1 to 16.0 kg. Three dogs were spayed females and 3 were neutered males. No abnormalities were detected on physical examination or blood analysis. Abdominal ultrasound was unremarkable and TSPS confirmed the absence of portosystemic shunting in all dogs.

The age of the EHPSS dogs at the time of diagnosis ranged from 3 to 65 months and their body weight ranged from 1.5 to 13.4 kg. Ten dogs were female (5 intact, 5 spayed) and 9 were male (7 intact, 2 castrated). Breeds represented in the study population were Yorkshire terrier (n=4), Bichon frisé, Dachshund, and Chihuahua (n=2), and Beagle, Bolonka zwetna, Cross breed, Maltese, Jack Russell Terrier, Norwich Terrier, Miniature Pincher, Miniature Schnauzer, and Scottish Collie (n=1).

Hepatic encephalopathy

None of the control dogs showed signs of HE. Hepatic encephalopathy grades in the control and EHPSS patients are represented in Table 1. The median HE grade for EHPSS dogs was 3; 15 out of the 19 dogs were presented with apathy and some degree of head pressing, ataxia, and/or circling. Seven dogs suffered from seizures (grade 4 HE), but none of them were comatose.

Serum bile acids

Bile acid concentrations are represented in Table 1. For the control dogs, all values were well within reference interval ($<19 \mu\text{mol/L}$). In EHPSS dogs, median pre-prandial bile acid levels and postprandial bile acids levels were $166 \mu\text{mol/L}$ (range, 27-381 $\mu\text{mol/L}$) and $218 \mu\text{mol/L}$ (range, 49-656 $\mu\text{mol/L}$), respectively. Pre- and postprandial bile acids levels were consistently and significantly elevated in EHPSS dogs compared to the control dogs ($P<0.001$).

Ammonia levels

Ammonia levels in the control dogs and EHPSS dogs are represented in Table 1. The mean arterial ammonia levels in the control dogs were $18.2 \pm 8.0 \mu\text{mol/L}$ (PocBA) and $16.8 \pm 8.1 \mu\text{mol/L}$ (CatDx), whereas the mean venous ammonia levels were $19.3 \pm 7.7 \mu\text{mol/L}$ (PocBA)

and 23.0 ± 9.5 $\mu\text{mol/L}$ (CatDx). The mean arterial ammonia levels for the EHPSS dogs were 173.1 ± 57.5 $\mu\text{mol/L}$ (PocBA) and 119.7 ± 69.4 $\mu\text{mol/L}$ (CatDx) and the mean venous ammonia levels were 158.0 ± 53.8 $\mu\text{mol/L}$ (PocBA) and 103.8 ± 58.3 $\mu\text{mol/L}$ (CatDx).

The blood ammonia levels were significantly higher in the EHPSS dogs than in the control dogs (arterial and venous, $P < 0.0001$). Ammonia levels in artery and vein measured by the PocBA were significantly higher than ammonia levels measured by the CatDx (arterial and venous, $P < 0.0001$). The two devices had a positive correlation of 88.4% and 81.9% for measurements taken from the artery and vein, respectively.

The arterial ammonia levels were higher than the venous levels, but statistical significance was only reached for the PocBA ($P < 0.05$). Strong positive correlations of 96.0% (PocBA) and 90.0% (CatDx) were found between the arterial and venous ammonia levels.

Correlations between preprandial bile acids and venous ammonia measurements were weak for both methods (PocBA, 46.1%, $P < 0.05$; CatDx, 48.6%, $P < 0.05$). The correlations between postprandial bile acids and venous ammonia were also weak (PocBA, 41.4%, $P < 0.05$; CatDx, 39.4%). Likewise, only weak positive correlations of 44.5% (PocBA, $P < 0.05$) and 46.1% (CatDx, $P < 0.05$) were reached between arterial measurements and the HE grade.

Macroscopically CSF samples were normal in both the control and the EHPSS dogs. White blood cell count was similar and within reference interval in both the control dogs and in EHPSS dogs (< 8 cells/ μl).

The mean CSF ammonia levels for the control dogs were 6.0 ± 5.3 $\mu\text{mol/L}$ (PocBA) and 3.7 ± 2.0 $\mu\text{mol/L}$ (CatDx) (Table 1). The mean CSF ammonia levels for EHPSS dogs were 106.6 ± 55.6 $\mu\text{mol/L}$ (PocBA) and 60.1 ± 42.9 $\mu\text{mol/L}$ (CatDx). The CSF ammonia levels were significantly higher in the EHPSS dogs than in the control dogs (PocBA, $P < 0.0001$; CatDx, $P < 0.001$). The mean ammonia levels in the CSF measured by the PocBA were significantly higher than measurements with the CatDx ($P < 0.0001$). Strong positive correlations were demonstrated between arterial ammonia levels and the CSF ammonia levels (PocBA, 88.4%, $P < 0.001$; CatDx 87.0%, $P < 0.001$). Also between venous ammonia levels and CSF ammonia levels strong positive correlations were present (PocBA, 89.2%, $P < 0.001$; CatDx 72.5%, $P < 0.001$).

No significant correlation was found between CSF ammonia measurements by PocBA and HE grade ($P = 0.09$) whereas a weak positive correlation (39.6%) between CSF ammonia levels and HE grade was obtained with borderline significance ($P = 0.05$) when the CSF ammonia level was measured by CatDx.

Table 1 Laboratory results in 6 healthy control dogs and 19 dogs with a congenital extrahepatic portosystemic shunt (EHPSS)

Dog	Grade HE (1-4)	SBA ^{pre} (μmol/L)	SBA ^{post} (μmol/L)	A-PocBA (μmol/L)	A-CatDX (μmol/L)	V-PocBA (μmol/L)	V-CatDX (μmol/L)	CSF-PocBA (μmol/L)	CSF-CatDX (μmol/L)
1	0	6	7	32	31	34	41	0	3
2	0	7	6	9	11	19	19	8	3
3	0	6	12	18	11	18	25	7	1
4	0	6	1	12	12	17	18	7	4
5	0	11	10	21	22	17	21	14	7
6	0	6	9	17	14	11	14	0	4
Median (range)	0 (0-0)	6 (6-11)	8 (1-12)						
Mean ± SD				18.2 ± 8.0	16.8 ± 8.1	19.3 ± 7.7	23.0 ± 9.5	6.0 ± 5.3	3.7 ± 2.0
1	2	381	172	199	117	144	161	102	67
2	3	27	49	160	140	130	101	94	70
3	2	133	136	255	281	272	260	195	114
4	4	254	354	89	45	70	42	11	8
5	3	98	656	189	142	169	154	184	107
6	2	345	360	123	74	134	41	66	30
7	2	192	141	242	160	214	114	219	89
8	4	178	232	98	28	76	25	36	19
9	3	30	58	154	65	179	105	101	13
10	4	75	121	159	53	168	67	81	17
11	3	78	210	149	92	141	76	92	29
12	4	47	218	244	163	246	139	144	84
13	3	323	275	237	181	181	91	93	91
14	4	273	290	209	188	154	153	100	98
15	4	104	130	206	187	170	160	84	50
16	3	168	224	135	73	107	37	130	23
17	3	166	220	248	226	220	106	176	160
18	3	71	150	104	29	121	47	68	60
19	4	200	224	88	88	105	94	49	12
Median (range)	3 (2-4)	166 (27-381)	218 (49-656)	173.1 ± 57.5	119.7 ± 69.4	158.0 ± 53.8	103.8 ± 58.3	106.6 ± 55.6	60.1 ± 42.9
Mean ± SD									

HE: hepatic encephalopathy; SBA^{pre}: preprandial serum bile acids; SBA^{post}: postprandial serum bile acids; A-PocBA: ammonia level in arterial blood measured by PocketChem; A-CatDX: ammonia level in arterial blood measured by Catalyst Dx; V-PocBA: ammonia level in venous blood measured by PocketChem; V-CatDX: ammonia level in venous blood measured by Catalyst Dx; CSF-PocBA: ammonia level in cerebrospinal fluid measured by PocketChem; CSF-CatDX: ammonia level in cerebrospinal fluid measured by Catalyst Dx

5. Discussion

Measurements of ammonia levels in arterial and venous blood and CSF of dogs demonstrated remarkably higher ammonia levels in the blood (arterial, venous) and CSF of EHPSS dogs with HE signs compared to clinically normal dogs. A strong positive correlation was documented between ammonia levels in the CSF and ammonia levels in the blood (arterial, venous).

The pathogenesis of HE is complex and although ammonia is not the sole agent involved, it has been until now the only measurable neurotoxin.²² Intra- and extracellular pH has an important influence on the distribution and the presence of ammonia or ammonium.^{1,11,12,23} At physiological pH, most ammonia is in the form of the positively charged ammonium ion (NH_4^+) and not the ammonia in gas form (NH_3).^{1,2,11,12} Ammonia passively diffuses into the brain and is captured in the brain parenchyma due to a difference between systemic and brain pH values. Normal systemic pH in humans is 7.4, but the pH of the human brain is reported to be somewhat lower.²³ Ammonia will diffuse toward a lower concentration gradient, and because the pH in the brain is lower, the ammonia will be converted to the ammonium ion. Entrapment of ammonia within the CNS occurs because the ammonium ion does not readily diffuse across cell membranes.¹¹ In addition, ammonia is shown to diffuse into the brain more freely in humans with severe liver disease than in normal control subjects.^{11,12} Ammonia toxicity results from an increase in both plasma concentration and intra- and extracellular pH.²³ The toxic effect of ammonia on the brain results from direct interaction with both excitatory and inhibitory neurotransmission.²³ The consequences of hyperammonemia in the central nervous system include amino acid disturbances, alterations in neurotransmission, cerebral energy disturbances, alterations of nitric oxide synthesis and oxidative stress, impairments of axonal and dendritic growth, and alterations in signal transduction and channel transporter activities,²⁵⁻²⁹ subsequently leading to the swelling and death of astrocytes.^{25,30,31}

In human HE patients, a significant correlation between an increase in blood ammonia levels and the severity of HE was documented.³² Arterial ammonia levels $\geq 150 \mu\text{mol/L}$ serve as a negative prognostic indicator in people.^{17,18} The previous canine study on HE also claimed a strong positive correlation between blood ammonia level and the degree of HE.¹⁹ In the current study, dogs with HE (irrespective of the grade) had significantly higher blood ammonia levels compared to dogs without HE. High plasma ammonia was predictive of HE. However, the observed correlations between the blood ammonia levels and the degree of HE

in dogs with EHPSS were rather weak.

Traditionally, in humans, arterial rather than venous blood is utilized when measuring blood ammonia.¹¹ In dogs, venous ammonia measurement is part of the work-up in dogs suspected of PSS.^{1,2} To the authors' knowledge, there is only one previous study comparing ammonia levels in arterial and venous samples in canines with HE of various etiologies.¹⁹ Unfortunately, no detailed data on the blood ammonia levels of the PSS dogs, which were a subgroup of the HE cases, were provided. In the same report, significantly higher ammonia levels were detected in the arterial compared to the venous samples. In the present study, we also observed higher arterial than venous levels; however, statistical significance was only reached when using PocBA.

Of particular interest in the present study were the high levels of ammonia in the CSF of the EHPSS dogs, which had a strong positive correlation to blood ammonia levels. Several studies have previously assumptively stated that ammonia is elevated in the CSF of congenital EHPSS dogs; however, ammonia measurements in the CSF were not performed.^{7,14} A potential reason for these lacking data is the paucity of validated techniques to perform measurements on CSF. In the present study, two different commercial devices (PocketChem BA and Catalyst Dx chemistry analyzer) were used to measure ammonia levels in the blood as well as in the CSF. Both devices have the same mode of action. Their respective slides contain a buffer in the top layer that converts the ammonium ions in the sample into gaseous ammonia. The ammonia gas passes through a selectively permeable membrane and then reacts with a pH indicator (PocBA: Bromocresol Green; CatDx: Bromophenol Blue). In both cases, the color development is measured that is proportional to the amount of ammonia. There are no previous reports validating the devices to measure the ammonia level in CSF. The values obtained by PocBA were higher than by Cat Dx. Nevertheless, the measurement results of both devices showed a high correlation, suggesting that both devices can be used in clinical practice to get an idea of ammonia levels in CSF. The authors would like to stress, however, that the CSF values obtained in this study should not be considered as absolute values but rather in a comparative way, until the devices are validated for the measurement of ammonia in CSF.

It has been previously suggested that in humans the amount of ammonia that enters the brain is not optimally reflected by its concentration in the blood.¹¹ In humans, it has also been documented that ammonia uptake in the brain increases with a rise in the arterial ammonia level, but the relative increase in the brain exceeds by far the arterial one.¹¹ The permeability of the BBB to ammonia was found to be a critical element in this non-linear increase of

ammonia levels in the brain. This implies that the impact of ammonia on the brain may be greater than that predicted on the basis of the blood ammonia levels.^{11,12,33} Similar studies have not yet been performed in dogs and there are no data on the permeability of the BBB to ammonia in PSS dogs. In the current study, the metabolic uptake of ammonia in the brain was not measured. The assumption that there is an increased permeability of the BBB to ammonia in EHPSS dogs needs further investigation. In human HE patients (including subclinical HE patients), it has been shown that the BBB was much more permeable to the gaseous form, but measurable amounts of the ionic form also crossed the BBB.^{11,34} Plasma ammonia level and CSF ammonia level in the current study were high in EHPSS dogs compared to the clinically normal control dogs, suggesting that the greater amount of ammonia in the brain potentially caused toxicity and led to severe HE signs. On the other hand, the authors could not definitively rule out the possibility that seizures and HE signs in some of the dogs were caused or triggered by another disease entity or neurodegenerative disease. Interestingly, if HE signs are indeed provoked by increases in cerebral ammonia levels, it can be suggested that a medical treatment regimen before EHPSS surgery might alter the ratio between the ionic and gaseous forms of ammonia, leading to less influx of ammonia to the CSF and brain. However, this warrants further investigation.

Currently, measurement of pre- and postprandial serum bile acid concentrations is the most commonly used liver function test in the diagnosis of PSS in dogs. The reported specificity and sensitivity of serum pre-prandial bile acids are 68-87% and 88-93%, respectively.^{2,35} In the current study, and in agreement with previous EHPSS studies,^{2,35} both pre- and postprandial serum bile acid concentrations were markedly elevated above the reference interval. Only weak positive correlations were found between pre- and postprandial bile acids and the venous ammonia levels.

The present study had a few limitations. The authors acknowledge the relatively limited number of patients with various degree of HE signs in the current study. Furthermore, ammonia is known to be unstable *ex vivo* and samples need to be processed with special care not to influence the test results. Precautions such as the use of melting ice and the nearly immediate processing of the samples (time to analysis for an individual sample was <120 sec, data not reported) and discarding CSF samples that were contaminated with blood should have minimized the risk for pre-analytical errors although they can never be completely excluded.

6. Conclusions

In conclusion, the remarkably higher levels of ammonia level in CSF of EHPSS dogs compared to clinically normal dogs and the strong positive correlation between ammonia level in the CSF and the level in the blood (arterial and venous) of EHPSS dogs might suggest increased permeability of the BBB to ammonia in EHPSS dogs. The results also demonstrated that, at least in dogs with EHPSS, venous samples can substitute for arterial samples, which are more difficult to take and require anesthesia. In addition, because of the non-linear passage of ammonia through the BBB into the brain, caution is needed not to underestimate the severity of HE or the presence of HE when interpreting ammonia levels in the blood.

The results of the current study encourage future investigation of the correlation of ammonia levels in the blood and CSF in EHPSS dogs with respect to HE signs and surgical outcome.

7. Disclosure

This study has been supported in part by the Surgeon-in-Training Research Grant - ECVS.

8. Acknowledgements

We wish to thank Sara Kol for the language editing.

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**Serial blood and cerebrospinal ammonia levels
in dogs with congenital extrahepatic portosystemic shunts
before and after surgical attenuation**

1. Abstract

Objective: To describe the evolution in ammonia levels in blood and cerebrospinal fluid (CSF) in dogs with congenital extrahepatic portosystemic shunts (EHPSS) in dogs with and without successful surgical outcome

Study Design: Prospective clinical trial

Animals: 19 dogs with congenital EHPSS

Methods: A thin film band or an ameroid ring constrictor was used for shunt attenuation. Three months postoperatively, transsplenic portal scintigraphy (TSPS) was performed to assess the surgical outcome. Dogs with patent shunts underwent a revision surgery, followed by TSPS 3 months later. Ammonia levels were assessed at the time of diagnosis (T_0 ; arterial, venous, and CSF), at the day of surgery (T_1 ; arterial and venous) and at 1 (T_2 ; venous), and 3 (T_3 ; arterial, venous, and CSF) months after surgery. Paired SBA were measured at T_0 , T_2 and T_3 . Dogs that had a revision surgery were assessed 1 (T_2') and 3 (T_3') months after the revision surgery according to the same protocol. In the dogs with complete shunt closure, paired SBA and ammonia were measured 6 months after the surgery (T_4 ; venous). Dogs were grouped based on their surgical outcome (closed, acquired, or patent group). For statistical analysis, parametric and non-parametric tests were used at a significance level of $P < .05$.

Results: Complete shunt closure was confirmed in 12 dogs (9 after first and 3 after second surgery). In total, 7 dogs developed acquired shunts (5 after first and 2 after second surgery). At T_0 , the SBA and ammonia levels were elevated in all dogs. Despite medical management and clinical improvement, ammonia levels were not lower at T_1 . At T_2 and T_2' , only 2 dogs had normalized SBA (closed group), whereas venous ammonia levels were normal in all dogs. At T_3 and T_3' , SBA was normal in 5/12 dogs (closed group) and in 1/7 dogs (acquired group). Venous ammonia levels were within normal limits in 11/12 (closed group), 4/7 (acquired group) and in 2/5 (patent group). At T_4 ,

SBA were still elevated in 5/12 dogs; venous ammonia was within reference interval in all dogs. Arterial ammonia levels and CSF ammonia levels demonstrated a strong positive correlation to the venous levels (95% and 89%, respectively; $P < .001$). CSF ammonia levels were above the reference value of venous ammonia in 14/19 dogs at T_0 . The decrease in CSF ammonia levels from T_0 to T_3 or T_3' was statistically significant in dogs with complete shunt closure.

Discussion/Conclusion: Postoperative ammonia or SBA levels are no accurate indicators of the degree of postoperative shunting. Thus, although baseline venous ammonia levels and SBA might be a useful laboratory parameter in the primary diagnosis of EHPSS, they have little value to assess attenuation after a surgical intervention.

2. Introduction

Ammonia is synthesised during amino acid metabolism and can be toxic in high concentrations. To prevent toxic build-up, the body converts ammonia to urea (via the urea cycle) or to glutamine (via glutamine synthetase) in the liver.¹ In the presence of an extrahepatic portosystemic shunt (EHPSS), a vast proportion of the portal blood bypasses the liver. This results in accumulation of ammonia and other toxic by-products of metabolism in the blood² and in reduced functional liver parenchyma due to inadequate nutrient supply.^{3,4}

In the diagnostic work-up of dogs suspected of EHPSS, measurement of paired serum bile acids (SBA) and venous ammonia level are commonly used screening tests.⁵⁻⁷ Baseline venous ammonia levels are not considered to be as sensitive as SBA measurements for the diagnosis of portosystemic shunts (PSS) in dogs,^{3,8} although using optimal cut-off values can increase its sensitivity.⁶ A recent study confirmed abnormal pre-operative arterial and venous ammonia levels but also documented significantly increased ammonia levels in the CSF of dogs with congenital EHPSS when compared to normal dogs.⁹ Given the high correlation between blood and CSF values in EHPSS dogs, the blood-brain barrier (BBB) might be more permeable to ammonia in affected dogs.⁹ To assess the outcome after shunt attenuation, paired SBA measurements are often performed but they do not seem reliable to evaluate PSS closure.^{3,10-13} Blood ammonia levels are not commonly reported in the postoperative evaluation. To the authors' knowledge, only one retrospective study specifically dealt with postoperative venous ammonia levels in dogs.¹⁴ In that study, increased ammonia in postsurgical PSS dogs was conclusive for persistent shunting but, in case of normal levels, medical imaging was required to confirm or exclude persistent shunting.¹⁴ Currently, there are no studies describing arterial or CSF ammonia levels in EHPSS dogs in the postoperative period.

The objectives of this study were to document the evolution of blood and CSF ammonia levels in dogs with congenital EHPSS before and after shunt attenuation, to compare pre-operative ammonia levels as a prognostic indicator for surgical success, and

to determine if blood and CSF ammonia levels could accurately indicate absence of persistent shunting.

3. Materials and Methods

Client-owned dogs with a congenital EHPSS were prospectively enrolled between July 2012 and October 2015. Enrolment in the study was subject to informed owner consent. The study was approved by the Ghent University Ethical Committees (EC2013/33 and EC2014/188) and by the Belgian Deontological Committee.

A dedicated owner questionnaire addressing topics like feeding, behaviour and clinical symptoms was completed. Dogs were suspected to have EHPSS based on clinical signs and SBA measurements. The presence of a PSS was confirmed by transsplenic portal scintigraphy (TSPS) after concurrent arterial, venous and CSF ammonia measurements and SBA were taken (T_0). Medical stabilization consisting of a dietary, an antimicrobial, and a synthetic disaccharide regimen was initiated and continued for at least 4 weeks before surgical intervention. At the day of surgery (T_1), a second owner questionnaire was completed. Arterial and venous samples for ammonia measurement were taken after anaesthetic induction. After surgical identification of the EHPSS, a thin film band or an ameroid ring constrictor was used for shunt attenuation. Patients were routinely hospitalized for 4-5 days postoperatively. The pre-operative medical management was continued for at least one month after surgery. The dogs were represented fasted for a follow-up appointment at 1 (T_2), 3 (T_3) and 6 (T_4) months postoperatively at which point the owners were requested to complete additional questionnaires. At these follow-up appointments, a physical examination, paired SBA and ammonia measurements (T_2 : venous; T_3 : arterial, venous, and CSF; T_4 : venous) were performed. Three months postoperatively (T_3), TSPS was repeated. In dogs with persistent shunting through the original shunt, a revision surgery was performed. The follow-up after the second surgery was identical to the one described after the first surgery (T_2 , T_3 , and T_4).

Details about the collection and processing of the samples have been described elsewhere⁹ and were identical at all occasions. Briefly, for paired SBA measurements, serum was sent to an external laboratory. The highest value obtained was used to make all calculations (reference, <19 $\mu\text{mol/L}$). The ammonia tests were run in-house immediately after blood sampling. In the current study, only the ammonia levels obtained by the portable blood ammonia analyzer Pocket ChemBA are used (reference, ≤ 70 $\mu\text{mol/L}$).

Statistical analyses were performed using a commercial software package SPSS Statistics (IBM, Brussels, Belgium). Normality was checked using the Kolmogorov-Smirnov test with Lilliefors significance correction. Descriptive parametric data were described as mean \pm standard deviation (SD) whereas descriptive non-parametric data were summarized as median and range. The SBA and ammonia data were grouped according to the result of the TSPS (closed, acquired, or patent). For normally distributed continuous variables an unpaired t-test was used; for non-normally distributed data the Mann-Whitney U-test was used if the samples were independent. Similarly, a paired sample t-test or a Wilcoxon signed rank test was used for paired data. Correlations were assessed by the Pearson's product-moment correlation (normally distributed data) or Spearman's rank correlation (non-normally distributed data). The significance level was set at .05.

4. Results

Nineteen dogs with congenital EHPSS were enrolled in this study. Patient details are summarized in Table 1. One dog (dog 4) received anti-epileptic drugs (levetiracetam and imepitoine) since 2 months before enrolment; the anti-epileptic treatment was continued postoperatively in addition to the above-mentioned preoperative medical regimen.

Table 1 Signalment and clinical features of dogs with a congenital extrahepatic portosystemic shunt that underwent surgical attenuation by thin film band or ameroid constrictor

Dog number	Age (months)	Breed	Sex	Weight (kg)	Shunt type	Attenuation (1 st / 2 nd surgery)	TSPS (after 1 st / 2 nd surgery)
1	6	Jack Russell Terrier	M	4.8	Portocaval	TFB	Closed
2	36	Bichon Frisé	F	3.7	Portocaval	TFB	Closed
3	4	Dachshund	M	3.2	Portocaval	TFB	Closed
4	12	Scottish Collie	FS	13.4	Portocaval	TFB / Suture	Patent / Closed
5	7	Maltese	M	1.9	Portocaval	TFB	Closed
6	46	Norwich Terrier	FS	8.7	Porto-azygos	TFB / AC	Patent / Closed
7	20	Yorkshire Terrier	F	2.0	Portocaval	TFB	Closed
8	14	Chihuahua	F	1.9	Porto-azygos	TFB / AC	Patent / Closed
9	4	Bolnoka Zwetna	M	2.7	Portocaval	AC	Closed
10	25	Bichon Frisé	MC	2.3	Portocaval	AC	Closed
11	8	Dachshund	MC	6.1	Portocaval	AC	Closed
12	65	Chihuahua	F	2.5	Porto-azygos	AC	Closed
13	6	Beagle	M	7.5	Portocaval	TFB	Acquired
14	23	Miniature Schnauzer	F	7.0	Portocaval	TFB	Acquired
15	47	Yorkshire Terrier	MC	1.5	Portocaval	TFB / AC	Patent / Acquired
16	32	Yorkshire Terrier	F	1.6	Portocaval	TFB	Acquired
17	3	Yorkshire Terrier	M	2.1	Portocaval	TFB	Acquired
18	6	Miniature Pincher	F	4.2	Portocaval	TFB / AC	Patent / Acquired
19	35	Terrier Cross	FS	3.7	Portocaval	AC	Acquired

AC: ameroid constrictor; F: female; FS: female spayed; M: male; MC: male castrated; TFB: thin film band; TSPS: transsplenic portal scintigraphy

At the time of surgery (T_1), all owners reported obvious clinically improvement since medical support was given to their dogs. Shunt classification was verified intra-operatively. Sixteen of the 19 dogs had a portocaval and 3 had a porto-azygos shunt (dogs 6, 8, and 12). At the time of the primary surgery, attenuation of the shunting vessel was undertaken using a thin film band in 14 dogs (12 portocaval, 2 porto-azygos) and ameroid ring constrictor in 5 dogs (4 portocaval, 1 porto-azygos). The only peri-operative complication was the development of focal seizures in one dog (dog 19) 4 days after surgery, successfully controlled by administration of levetiracetam. Only this dog and the dog that was on pre-operative anti-epileptic drugs (dog 4) were discharged on additional anti-epileptic drugs. Dietary change and the synthetic disaccharide regimen were continued until the time of repeated TSPS. At T_2 , all owners reported their dogs to have clinically improved to what they perceive to be normal for a dog. All but 2 dogs (dog 4 and 13) were perceived to be clinically normal to the owner at T_3 . However, TSPS findings revealed normal shunt fraction in only 9 out of 19 dogs. The dogs with normal shunt fraction were gradually weaned of the hepatic diet by mixing increasing amounts of normal commercial diet with decreasing amounts of hepatic diet over 1-2 weeks, and the synthetic disaccharide was discontinued. In 5 dogs (3 portocaval, 2 porto-azygos) with an abnormal shunt fraction, a patent shunt was suspected. In the remaining 5 dogs (5 portocaval), CT imaging confirmed that the abnormal shunt fraction was caused by acquired shunts, in the presence of a successfully closed congenital EHPSS. One of the dogs with acquired shunts (dog 13) was subsequently euthanized at the owners' request because of repetitive obstructive urolithiasis. The owners of the other dogs with acquired shunts were advised to continue life-long medical management.

A revision surgery was performed in the 5 dogs with patent original EHPSS. In all of these dogs initially attenuation was attempted using a thin film band. An ameroid constrictor was placed in 4 of them (2 portocaval, 2 porto-azygos) and the remaining persistent portocaval shunt (dog 4) was ligated with a polypropylene suture. No peri-operative complications occurred. In the 1-month follow-up after revision surgery (T_2'), 4 owners reported that their dog was normal; the remaining owner (dog 4) only reported clinical improvement. In the latter dog, epilepsy seemed well controlled after the revision surgery but there were episodes of dullness and intermittent anorexia; this was the dog

that was treated with anti-epileptic drugs since diagnosis. Repeated TSPS at T₃ revealed closure of all 5 congenital EHPSS. Unfortunately, in 2 dogs (2 portocaval), acquired shunts did develop. Life-long medical management was advised.

In total, 12 dogs had a successful surgical outcome although only 11 of them had a good clinical outcome. The dog that needed pre- and postoperative anti-epileptic drugs (dog 4) had recurrent seizures despite medication 5 months after the second surgery and was euthanized. The owners refused necropsy.

The correlation between SBA and venous ammonia levels was weak (48%; $P < .001$). Arterial and venous ammonia levels showed a very strong positive correlation of 95% ($P < .001$). Also between venous ammonia measurements and CSF ammonia measurements, a strong positive correlation was observed (89%; $P < .001$).

The data related to SBA measurements are presented in Table 2. At T₀, elevated SBA levels were observed in all 19 dogs; the median SBA level was 218 $\mu\text{mol/L}$ (49-656 $\mu\text{mol/L}$). There was no statistical difference between the T₀ SBA levels in dogs that ultimately were closed after surgery and those that developed acquired shunts ($P = .384$). At T₂, T₂, T₃ and T₃, SBA levels were significantly higher in dogs with acquired shunts or patent shunts compared to those with successful closure ($P < .01$ at both time points) whereas dogs with patent shunts and acquired shunts had similar SBA levels ($P = .876$ and $P = .639$, respectively). Although the SBA lowered significantly in time after successful surgery, they only returned to normal in 2/12 dogs at T₂, in 5/12 at T₃, and in 7/11 at T₄. In contrast, one dog with acquired shunts had normal SBA at T₃.

The data related to ammonia measurements are presented in Table 3. Since venous samples were available at all time points and a strong positive correlation exists with the arterial ammonia measurements, only the venous data will be displayed in detail. High ammonia levels were observed in all 19 dogs; the median venous ammonia level was 154 $\mu\text{mol/L}$ (70-272 $\mu\text{mol/L}$) at T₀ and there was no statistical difference between the venous ammonia in dogs that would have a successful outcome and those that developed acquired shunts ($P = .837$). Despite medical management and apparent clinical

improvement, the ammonia levels were not lower after the pre-operative month of medical management ($P=.587$). However, one month after surgery (T_2 and T_2'), the venous ammonia levels were within the normal reference interval in all dogs and significantly lower than at T_0 in all outcome groups (closed, $P<.01$; acquired, $P<.05$; patent, $P<.05$). The ammonia levels in the closed group were statistically lower than in the acquired ($P<.01$) as well as the patent ($P<.05$) group. At T_3 and T_3' , a significantly lower ammonia level was observed in the closed group compared to the patent group ($P<.01$) but not to the acquired group ($P=.068$). In the closed group, 11/12 dogs had normal venous ammonia levels, in the acquired group 4/7 dogs, and in the patent group 2/5 dogs. The venous ammonia levels in the 11 dogs with closed shunts that were still alive at T_4 were not statistically different from their respective values at T_3 ($P=.350$).

At T_0 , CSF ammonia levels were above the blood ammonia reference in 14 out of 19 dogs (74%); the median CSF level was 94 $\mu\text{mol/L}$ (11-219 $\mu\text{mol/L}$). There was no statistical difference between the T_0 CSF ammonia levels in the dogs that ultimately had closed shunts and those that did develop acquired shunts ($P=.398$). At T_3 and T_3' , the only statistically significant difference was observed between CSF ammonia in the closed versus the patent group ($P<.001$). When the evolution of the CSF ammonia values from T_0 to T_3 and T_3' was studied, statistically significant decreases in ammonia levels were observed in the closed group ($P<.01$) but not in the acquired ($P=.310$) nor the patent ($P=.345$) group, similar to the situation for the venous ammonia. Nevertheless, CSF ammonia levels were below the venous reference in 4 dogs with acquired shunts and in 4 dogs with patent shunt.

Table 2 Serum bile acid levels ($\mu\text{mol/L}$) before and after surgery with respect to the surgical outcome after attenuation

	T_0	T_1	T_2	T_2'	T_3	T_3'	T_4
Total	n 19 median (range) 218 (49-656)	NA	19 127 (17-873)	5 12 (17-873)	19 56 (5-639)	5 12 (5-639)	12 10 (6-118)
Closed	n 12 median (range) 186 (49-656)	NA	48 (16-312)	12 (16-312)	25 (5-163)	12 (5-163)	12 (6-118)
Acquired	n 7 median (range) 285 (190-323)	NA	285 (47-873)	7 (47-873)	289 (13-639)	7 (13-639)	NA
Patent	n NA median (range)	NA	312 (213-369)	5 (213-369)	336 (97-479)	5 (97-479)	NA

NA: not available; T_0 : diagnosis; T_1 : surgery; T_2 : 1-month after 1st surgery; T_2' : 1-month after 2nd surgery; T_3 : 3-months after 1st surgery; T_3' : 3-months after 2nd surgery; T_4 : 6-months after surgery

Table 3 Ammonia levels ($\mu\text{mol/L}$) before and after surgery with respect to the surgical outcome after attenuation

		T ₀	T ₁	T ₂	T ₂ '	T ₃	T ₃ '	T ₄
Arterial								
Total	n	19	19			19	5	
	median (range)	160 (88-255)	158 (74-high)	NA		39 (10-211)		NA
Closed	n	12	12			12		
	median (range)	160 (89-255)	162 (74-high)	NA		25 (10-98)		NA
Acquired	n	7	7			7		
	median (range)	206 (88-248)	152 (131-236)	NA		49 (low-132)		NA
Patent	n	NA	NA			5		
	median (range)	NA	NA	NA		86 (41-151)		NA
Venous								
Total	n	19	19	24	5	24	5	12
	median (range)	154 (70-272)	149 (55-high)	15 (low-62)		31 (11-231)		15 (low-67)
Closed	n	12	12	12		12		12
	median (range)	156 (70-272)	149 (114-209)	11 (low-34)		25 (11-92)		15 (low-67)
Acquired	n	7	7	7		7		
	median (range)	154 (105-220)	152 (131-236)	22 (14-62)		59 (14-231)		NA
Patent	n	NA	NA	5		5		
	median (range)	NA	NA	25 (15-62)		78 (34-135)		NA
CSF								
Total	n	19	NA	NA		19	5	
	median (range)	94 (11-219)		NA		16 (low-132)		NA
Closed	n	12	NA	NA		12		
	median (range)	98 (36-195)		NA		12 (low-56)		NA
Acquired	n	7	NA	NA		7		
	median (range)	93 (49-176)		NA		47 (low-132)		NA
Patent	n	NA	NA	NA		5		
	median (range)	NA	NA	NA		26 (16-108)		NA

NA: not available; high: above detection limit; low: below detection limit; T₀: diagnosis; T₁: surgery; T₂: 1-month after 1st surgery; T₂': 1-month after 2nd surgery; T₃: 3-months after 1st surgery; T₃': 3-months after 2nd surgery; T₄: 6-months after surgery

5. Discussion

The current study provided serial data on SBA and arterial, venous and CSF ammonia in dogs with EHPSS that underwent surgery. It was demonstrated that none of the laboratory parameters were accurate indicators of surgical success. In addition, outcome based on the owner questionnaires can be very misleading.

At diagnosis, SBA were markedly elevated in all dogs. Statistically, there was no difference between dogs that would develop acquired shunts, and dogs with successful surgical outcome. Paired SBA were assessed in all dogs at all times for it is accepted that paired SBA have a higher sensitivity and specificity compared to preprandial samples alone.^{6,15,16} Since postprandial SBA were not consistently higher than preprandial samples in the individual dogs, the highest value of the both was used for all calculations and statistical comparison between outcome groups. After successful surgery, the SBA levels did not consistently normalize and, if they did, it was often only several months after the surgery. This slow amelioration in the bile acid enterohepatic cycle is somehow surprising. Liver volumes in dogs with congenital EHPSS normalize within a month after successful surgery.¹⁷ These findings point towards the fact that the liver function recovers slower with the redirection of the portal blood flow towards the liver irrespective of liver size. Furthermore, it remains to be elucidated to which extent the liver architecture and function are able to recover from the pathological changes that occurred during the period of portosystemic shunting. Another factor potentially attributing to the persistence of abnormalities in SBA levels is the presence of concomitant primary portal vein hypoplasia (PVH).¹⁸ In dogs with PVH without macroscopic portosystemic shunting, postprandial SBA are often only mildly to moderately increased. Since PVH often remains subclinical^{19,20} and no postoperative liver biopsies were taken, it cannot be excluded that some of the dogs in the current study had persistent microscopic shunting. The sensitivity of postoperative SBA measurements to determine successful surgery is far less than when used to support the diagnosis of PSS.^{3,14} Normal postprandial SBAs have been reported in dogs with persistent shunting or in the presence of multiple acquired PSS.^{18,21} On the other hand, it is not uncommon to observe persistent hepatic dysfunction

in clinically asymptomatic dogs after successful surgery.^{3,18,21-23} Both situations were also observed in the current case series.

Besides SBA, measurement of the ammonia levels is considered a useful test in the work-up of patients suspected of PSS.^{3,6,8} Larger practices nowadays perform this laboratory test in-house, avoiding false positive results by incorrect sample handling.²⁴ Furthermore, the test requires only one small blood sample, is relatively cheap and the results are immediately available. In the current study, hyperammonaemia was present in all dogs at the time of diagnosis and levels did not differ between dogs that developed acquired shunts and dogs with successful attenuation. In the vast majority of dogs, the pre-operative medical management did not lead to venous ammonia levels within the reference interval at the time of surgery, although all owners noted noticeable clinical improvement. It was previously suggested that normal blood ammonia may be observed in dogs with EHPSS that are successfully controlled by medical management,^{3,25} but, based on the current findings, this seems exception rather than rule.

An extensive retrospective study suggested that measurement of venous ammonia levels is the first test to assess the outcome after surgical attenuation of a congenital PSS 1 month after surgery.¹⁴ In case of hyperammonaemia, it can be concluded that there is still portosystemic shunting; in case of normal ammonia levels, additional medical imaging is advised to visualize or exclude portosystemic shunting.¹⁴ In the current study, ammonia levels were within the normal range 1 month after surgery in all dogs, indicating that normalization occurred irrespective of the surgical outcome. In a study of 14 dogs with EHPSS and 9 with intrahepatic PSS,²⁶ ammonia was also re-evaluated 1 month after surgery, which consisted of 5 complete or 18 partial ligations. These authors also found overall significantly decreased ammonia levels postoperatively despite the fact that most dogs still had some degree of shunting. There is no obvious explanation why ammonia levels would drop after unsuccessful surgery and not after clinically successful medical treatment. Although not checked routinely in the current study, there is some evidence in clinical reports describing dogs with postoperative seizures that the resolution of the hyperammonaemia after surgery occurred acutely. Excessive venous ammonia is often ruled out as aetiology of in dogs with postsurgical neurologic dysfunction.^{27,28} In

the dog that developed postoperative focal seizures, venous ammonia was low at the time of seizing, whereas elevated ammonia levels were recorded 4 days earlier, at the time of surgery.

To define hyperammonaemia, the cut-off level of 70 $\mu\text{mol/L}$ was used, as previously suggested.^{16,29,30} In an earlier study of our research group,⁹ venous ammonia levels were measured in a limited number of fasted healthy beagles under identical collection and processing conditions as those for the clinical patients; the highest value was 34 $\mu\text{mol/L}$. Potentially, a more accurate cut-off level can be established. Using a cut-off as low as 45 $\mu\text{mol/L}$ as used in some other studies^{14,16,26} would not greatly influence the descriptive results of the current study, since the ammonia levels in the majority of dogs with an acquired or patent shunt would still have been considered normal at 1 month after surgery.

In 2 veterinary studies, the level of several amino acids in CSF of dogs with congenital PSS were compared to those of control dogs without signs of hepatic or neurologic dysfunction.^{31,32} Both studies suggested that ammonia levels in CSF of PSS dogs will be elevated, given the observed glutamine elevation, but ammonia was not checked as such. To the authors' knowledge, the reference interval of CSF ammonia in dogs has not been described. Ammonia is studied more extensively in human patients in studies dealing with HE. However, in contrast to PSS due to a congenital shunt, the underlying cause of HE in humans is in most instances not reversible. In human neurologic patients without liver disease, CSF ammonia levels up to 28 $\mu\text{mol/L}$ were measured using the indophenol direct method.³³ The highest value measured by the Pocket ChemBA in healthy experimental dogs was 14 $\mu\text{mol/L}$.⁹ However, prudence is called to use cut-off value in studies using other devices than the Pocket ChemBA to measure CSF ammonia. Recent validation of the device by a titration experiment demonstrated a very good correlation but a systematic underestimation of the true ammonia content in CSF samples (manuscript in preparation). For the descriptive representation of the CSF results in the current study, the same cut-off as for venous ammonia was used. Most likely, the optimal cut-off value for CSF measurement by

Pocket ChemBA is far lower than 70 or even 45 $\mu\text{mol/L}$, but before a value can be proposed, CSF of a larger group of normal dogs should be assessed with this device.

In the current study, CSF samples were obtained at the time of diagnosis and sampling was not repeated at the time of surgery; the reason being that ethical approval was obtained for one pre-operative and one postoperative sample only. This can be regretted; however, the strong correlation between CSF and venous ammonia levels leads to the assumption that venous ammonia levels are a good predictor of CSF ammonia levels.⁹

6. Conclusion

For the first time, selective changes of ammonia in the blood and CSF of dogs with congenital extrahepatic PSS, treated surgically, are demonstrated over time. Whereas SBA levels do not seem very useful in the postoperative follow-up because normalisation only occurs very slowly if at all, baseline venous ammonia is even less useful because it lowers irrespective of the surgical outcome. Presumably, a postprandial ammonia tolerance test or a rectal tolerance test would better differentiate between dogs with and without postoperative shunting. However, it might not always be safe to administer ammonia in dogs that still have portosystemic shunting since the ammonia challenge might provoke severe clinical signs of HE. Medical imaging seems preferable to correctly identify whether the reason of persistent shunting is a patent original shunt or acquired shunts.

7. Disclosure

The authors report no financial or other conflicts related to this report.

8. Acknowledgements

We thank Eline Abma for technical assistance.

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**Regional Cerebral Blood Flow Assessed by Single Photon
Emission Computed Tomography (SPECT) in Dogs With
Congenital Portosystemic Shunt and Hepatic Encephalopathy**

Adapted from:

Regional cerebral blood flow assessed by single photon emission computed tomography (SPECT) in dogs with congenital portosystemic shunt and hepatic encephalopathy

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The Veterinary Journal 2017;220:40-42

1. Abstract

Objective: To compare regional cerebral blood flow (rCBF) in dogs with congenital extrahepatic portosystemic shunt (EHPSS) and hepatic encephalopathy (HE) with rCBF in healthy control dogs.

Study Design: Prospective clinical trial.

Animals: 8 dogs with congenital EHPSS and 8 healthy control dogs.

Procedures: Regional cerebral blood flow was determined using single photon emission computed tomography (SPECT) with a ^{99m}Tc -hexamethylpropylene amine oxime (^{99m}Tc -HMPAO) tracer.

Results: SPECT scans were abnormal in all PSS dogs. Compared to the control group, rCBF in PSS dogs was significantly decreased in the temporal lobes and increased in the subcortical (thalamic and striatal) area.

Conclusion and Clinical Relevance: Brain perfusion imaging alterations observed in the dogs with EHPSS and HE are similar to those in human patients with HE. These findings suggest that dogs with EHPSS and HE have altered perfusion of mainly the subcortical and the temporal regions of the brain.

2. Introduction

Dogs with a congenital portosystemic shunt (PSS) often have neurological abnormalities, referred to as hepatic encephalopathy (HE). There are several hypotheses regarding the pathogenesis of HE and many assumptions in dogs with HE are extrapolated from human findings. The most common neurological signs in PSS dogs with HE are behavioural abnormalities, reduced consciousness, visual deficits and ataxia.¹

Functional and structural brain imaging changes have been documented in human patients with HE; both modalities have identified alterations in specific brain regions.²⁻⁴ The correlation of functional brain abnormalities with cognitive impairments is believed to yield more consistent results than correlation of structural brain alterations with cognitive impairments.⁵ Cognitive deficits are challenging to assess in dogs, since neuropsychological tests are difficult to perform in animals. Functional brain imaging in PSS dogs with HE could improve the understanding of HE and yield knowledge for future comparison with rCBF data from dogs with PSS and subclinical HE, and after surgery for PSS.

Single photon emission computed tomography (SPECT) is a functional nuclear imaging modality that uses a radiolabelled tracer. Different tracers permit the visualisation and quantification of neurotransmitter systems, as well as the regional cerebral blood flow (rCBF) in vivo in a non-invasive manner. Uptake of ^{99m}technetium hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) tracer has been used successfully to document functional alterations in specific brain regions in human patients with HE.^{5,6}

In human patients with subclinical and clinical HE, impairment of the subcortical, frontal and temporal lobes were most pronounced.^{5,6} The temporal and frontal lobes are involved in detecting, processing and integrating sensory input. The subcortical structures receive massive different inputs from the cerebral cortex and peripheral sense organs; this information is integrated and shaped to provide output, which contributes to scaling, sequencing and timing of movement, as well as learning and behavioural control.^{5,6} If the subcortical part of the brain is affected, more in particular the thalamus, sensory information will inadequately be processed and sensory confusion could result.

We hypothesized that rCBF in dogs with PSS and clinical signs of HE might also be altered and that the most prominent changes would be observed in dogs with clinical signs of neurological dysfunction. The objectives of this study were to document alterations in rCBF in dogs with PSS and overt clinical signs of HE, and to determine which areas of the brain

have altered perfusion, based on SPECT analysis. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (EC 2011/130 for healthy dogs, September 2011 and EC 2013/33 for EHPSS dogs, March 2013).

3. Materials and Methods

Eight dogs with a single congenital extrahepatic PSS, confirmed by trans-splenic portal scintigraphy (TSPS) or ultrasonography (US), exhibiting clinical signs of HE (HE group) and eight healthy beagles (control group) underwent a brain SPECT scan using ^{99m}Tc -HMPAO.⁷ Emission data were fitted to an in-house template for use in dogs,⁸ permitting semi-quantification using a predefined region map containing the following brain regions: right and left frontal, temporal, parietal and occipital lobes, the cerebellum and subcortical area. The latter includes both the thalamic and striatal structures; further subdivision was not feasible due to limited resolution of SPECT images. Regional brain perfusion indices (PI) were calculated by normalizing the regional counts per voxel to total brain counts per voxel. In addition, the clinical signs of HE were graded on a four-point scale (grade 0: normal; 1: reduced motility and/or mild apathy; 2: severe apathy and/or mild ataxia; 3: salivation, severe ataxia, head pressing, apparent blindness and/or circling; 4: seizures and/or coma).⁹ Ammonia levels in the blood and the cerebrospinal fluid (CSF) of PSS dogs and controls were determined by the portable blood ammonia analyzer PocketChem BA (A. Menarini Diagnostics). The PIs and the ammonia levels were statistically compared between the groups using the Mann-Whitney *U* test. Statistical significance was set at $P < .05$.

4. Results

Seven of the eight dogs with HE had a portocaval PSS, whereas one had a porto-azygos communication. The dogs with PSS had grade 3 ($n = 3$) or grade 4 ($n = 5$) HE signs. Ammonia levels in the blood and CSF of dogs in the HE group were significantly higher than in the control group (both $P < .05$) (Table 1).

Table 1 Age, sex, clinical signs, ammonia levels and perfusion indices in eight dogs with a single congenital extrahepatic portosystemic shunt (PSS) presenting with clinical signs of hepatic encephalopathy (HE) and in eight healthy control dogs

	PSS dogs with HE (n=8)	Control dogs (n=8)	Mann-Whitney U (P-value)
Age (months) as median (range)	19 (5-122)	60 (48-72)	.05
Gender			
Female:Male	5:3	6:2	NA
Clinical signs			
Anorexia	6/8	0/8	
Apathy	8/8	0/8	
Ataxia	8/8	0/8	
Diarrhea	3/8	0/8	
Polyuria	6/8	0/8	
Salivation	3/8	0/8	
Seizures	5/8	0/8	
Vomiting	5/8	0/8	
Ammonia levels as median (range)			
Blood ($\mu\text{mol/L}$)	153 (14-246)	27 (15-36)	.012*
CSF ($\mu\text{mol/L}$)	87 (0-154)	13 (0-25)	.045*
Regional brain PI as mean \pm SD			
Left Temporal Cortex	0.80 \pm 0.04	0.90 \pm 0.03	.002*
Right Temporal Cortex	0.79 \pm 0.08	0.88 \pm 0.03	.02*
Cerebellum	1.09 \pm 0.10	1.11 \pm 0.06	.916
Subcortical Region	1.21 \pm 0.08	1.10 \pm 0.07	.005*
Olfactory Bulb	0.70 \pm 0.35	0.52 \pm 0.13	.156
Left Frontal Cortex	1.16 \pm 0.10	1.16 \pm 0.03	.598
Right Frontal Cortex	1.14 \pm 0.07	1.11 \pm 0.07	.369
Left Occipital Cortex	1.06 \pm 0.12	1.14 \pm 0.10	.082
Right Occipital Cortex	1.05 \pm 0.11	1.14 \pm 0.08	.114
Left Parietal Cortex	1.12 \pm 0.10	1.12 \pm 0.04	.526
Right Parietal Cortex	1.11 \pm 0.09	1.07 \pm 0.03	.103

PI, perfusion index; SD, standard deviation; * $P < .05$.

Regional brain perfusion indices were significantly higher in the subcortical region of the PSS group compared to the control group ($P < 0.05$), whereas significantly lower perfusion was demonstrated in both temporal lobes in the PSS group ($P < 0.05$) (Table 1; Fig. 1). These alterations in rCBF were present in 8/8 and 7/8 of the PSS dogs, respectively.

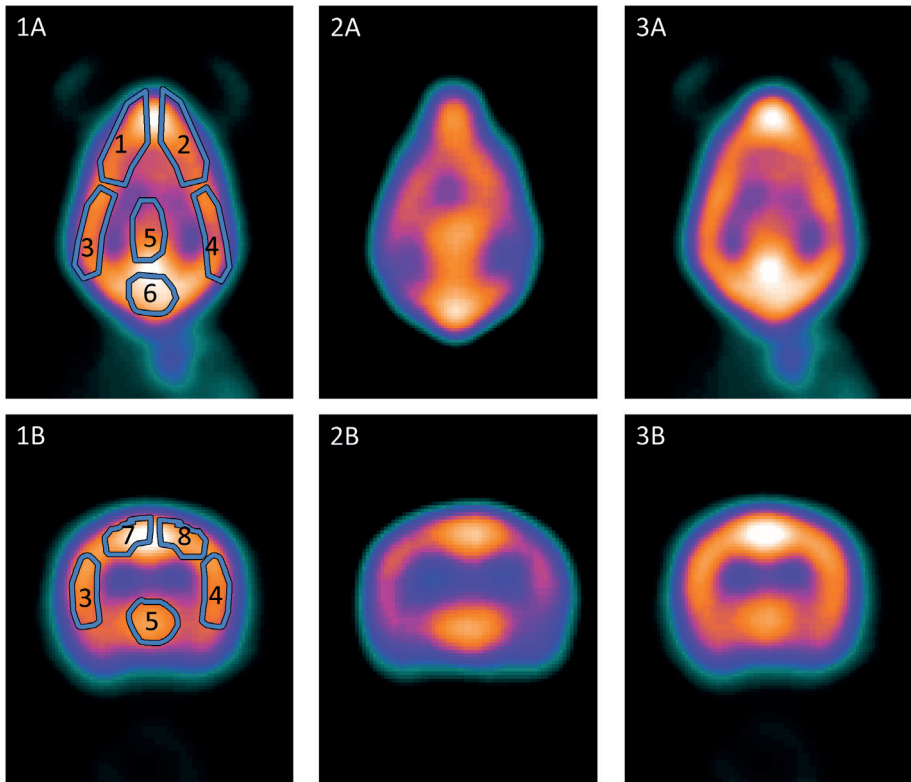


Fig. 1. Region map and acquisitions obtained with ^{99m}Tc -HMPAO SPECT in dorsal (A) and transverse (B) slices. Brain of a normal dog (1A) with regions of interest over left (1) and right (2) frontal cortices, left (3) and right (4) temporal cortices, the subcortical area (5) and the cerebellum (6). Brain of a dog with HE (2A) demonstrating decreased activity in the temporal region compared to normal regional activity in this area in a normal dog (3A). Brain of a normal dog (1B) with regions of interest over the left (3) and right (4) temporal cortices, subcortical area (5), and left (7) and right (8) parietal cortices. Brain of a dog with HE (2B) demonstrating increased activity in the subcortical area compared to the regional activity in this area in a normal dog (3B).

5. Discussion

The present study is the first brain perfusion imaging report in dogs with HE secondary to congenital PSS. Previous studies of HE in PSS dogs have mainly focused on measurement of serum and CSF chemistries. In accordance with previous data, higher ammonia levels in both blood and CSF were detected in dogs in the PSS group.

Currently, there are two case series^{9, 10} and two case reports^{11,12} describing magnetic resonance imaging (MRI) findings in dogs with PSS. In the majority of dogs with PSS in the MRI study of Torisu et al.⁹, hyperintensity in the lentiform nuclei was observed on the T1W images. In the present study, the subcortical region (including the region of the lentiform nuclei) also showed a high regional uptake of ^{99m}Tc-HMPAO. The significant differences in rCBF by SPECT in the subcortical and temporal regions in PSS dogs with HE are compatible with previously published findings in human HE patients.⁴⁻⁶

Conclusions about changes in functional brain activity in dogs with HE should be drawn cautiously, because the relationship between rCBF and functional anatomy is not completely understood in dogs. In contrast to human HE studies,⁴⁻⁶ which focus on cognitive dysfunction, there are no standardized neuropsychological tests to evaluate cognitive deficits in dogs showing HE signs. All PSS dogs in this study had overt clinical signs of HE (\geq grade 3). In humans, the neuropsychological impairment observed with subclinical HE is believed to be associated predominantly with the frontal and temporal lobes, and with the subcortical region, of the brain.⁶ There are indications to presume similarities in dogs with HE, but more canine cases should be recruited to support this.

6. Conclusion

In conclusion, we demonstrated hypoperfusion of the temporal cortex and hyperperfusion of the subcortical region in dogs with HE secondary to PSS. These data contribute to our understanding of the neuropsychological and pathophysiological mechanisms of HE in PSS dogs, suggesting predominant involvement and dysfunction of the temporal cortex and the subcortical area. Important similarities to the altered rCBF in humans with HE were found. Because of the small sample size and the lack of previous reports on SPECT studies in PSS dogs, the brain perfusion findings must be considered to be preliminary. Future studies should

examine rCBF changes in dogs with PSS with subclinical HE and should compare rCBF before and after successful PSS attenuation.

7. Disclosure

Part of this work was supported by the Resident Research Grant of the European College of Veterinary Surgeons (ECVS).

8. Acknowledgements

The authors wish to thank Filip Clompen for assistance in composing the images.

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GENERAL DISCUSSION

General Discussion

Surgery is still the recommended treatment for the majority of dogs with congenital EHPSS since dogs undergoing vascular attenuation are reported to clinically recover to a greater extent and survive significantly longer than those controlled medically only.^{1,2} Many surgical patients do indeed enjoy a presumably normal life thereafter. However, some will be asymptomatic despite persistent shunting whereas others will fail to achieve significant clinical improvement. Literature on surgical outcome after EHPSS surgery is often inconsistent in how to categorize postoperative results as excellent, good, fair or poor. This is largely due to the fact that in many institutions and in various studies, only the clinical outcome is assessed. However, clinical success in dogs with EHPSS is not always overlapping with surgical success and thus the actual postoperative shunt status remains unknown. Yet it is very important to know whether the shunt is completely closed or shunting persists and whether acquired shunts did develop or not. Even in dogs that do clinically well, the knowledge of persistent shunting is important, as drugs with a hepatic first-pass metabolism should be avoided in these dogs.³ In our case series (Chapter 4), 2 out of the 19 treated dogs were euthanatized, one because of recurrent neurological symptoms despite successful surgery and one because of obstructive urolithiasis in the presence of acquired shunts; all other dogs had an excellent clinical outcome regardless the postoperative shunt status (closed, persistent or acquired shunting).

With regard to persisting shunting, different situations have to be considered; incomplete closure of the congenital EHPSS or of one of its late tributaries, the presence of a second PSS, shunting through acquired portosystemic shunts, or concurrent primary portal vein hypoplasia.⁴⁻⁷

It has been recently reported that in 18% to 36% of dogs with EHPSS, treated with an ameroid ring constrictor or a thin band, respectively, the original EHPSS did not close completely.^{6,7} This is in agreement with the results in our patient group when surgery involved placement of a thin film band. However, when an ameroid constrictor was used, we did not experience persistent shunting (Chapter 4). In our patients, we always advised reoperation in case of non-closure of the shunt 3 to 6 months after the first surgery. A second surgical intervention has been advised when blood flow is present in the primary EHPSS in the presence of clinical signs, or if an additional congenital EHPSS is identified that was missed during the first surgery.⁸ However, debate is ongoing regarding the most appropriate

approach for patients that are still not shunt-free after surgery despite absence of clinical signs. There are currently no studies that document whether or not it is justified to wait and see if the status of the patient remains stable or not.

According to the literature, acquired shunts do occur in 10% to 29% of the dogs undergoing surgical attenuation of a single congenital EHPSS, regardless of the surgical approach.⁹⁻¹¹ Acquired shunting vessels involve pre-existing portosystemic anastomoses that become functional due to the inability of the liver to tolerate the increased portal pressure induced by the corrective surgery.¹²⁻¹⁴ Placement of a device that induces gradual shunt attenuation is meant to reduce the risk of this postoperative complication.¹² Similarly, reduction to less than 3 mm shunt diameter at the time of placement of the device should be avoided.¹¹ Despite being at least as conservative as that advice, a high percentage (37%) of acquired shunts was also observed in our case series (Chapter 4). Interestingly, but not completely surprising, all acquired shunts occurred in dogs operated for a congenital portocaval EHPSS and none in dogs that had a porto-azygos shunt. Similar to portophrenic shunts, the diameter of a porto-azygos shunt is on and off reduced due to diaphragmatic movement leading to temporary increases of blood flow towards the liver through the portal system.⁸ Another explanation can be, based on Poiseuille's law, that due to the smaller diameter of the azygos vein compared to the caudal vena cava, resistance to blood pressure is higher in this vessel.¹⁵ Furthermore, on CT images, we consistently observed a marked reduction in shunt diameter at the insertion site of porto-azygos shunts (Chapter 1), a finding that has not been documented in portocaval shunts so far. In dogs that develop acquired shunting, vena caval banding has been attempted, but turned out not to be superior to medical management,¹² a strategy that is mainly focused on avoiding clinical signs of HE by feeding a high quality low-protein diet.⁸ The dogs in our patient group that developed acquired shunts were all fed a commercial liver or kidney diet and received life-long lactulose. One dog was euthanized at the time of obstructive urolithiasis 7 months after surgery. The other dogs with acquired shunts are still alive at the time of writing, with a follow-up varying from 16 till 42 months.

Dogs with a congenital EHPSS that have concomitant primary portal vein hypoplasia might not be clinically perfect after successful closure of the shunt because microscopic shunting persists, although the clinical signs will be milder as usually not all liver lobes are affected.^{16,17} As portal vein hypoplasia cannot be cured, continuation of the dietary management that was installed prior to the shunt surgery is recommended.^{18,19} The presence of portal vein hypoplasia cannot be accurately predicted pre-operatively since the diagnosis

can only be made by histopathology after the macroscopic shunt has been completely closed (Allen et al 1999).¹⁸ It has been suggested in the literature that 10% to 58% of dogs with congenital EHPSS have concomitant primary portal vein hypoplasia.^{20,21} However, in our institution, dogs with this combination are only exceptionally diagnosed²² and, in our patient group, we did not suspect any. Some of them might be overlooked since liver biopsies are not routinely taken in the follow-up of dogs after EHPSS surgery.

To evaluate surgical outcome with objective measures, imaging or follow-up of laboratory tests may be used. Erroneously, many laboratory tests currently used for primary diagnosis of EHPSS are used for postoperative assessment, without knowledge on their sensitivity and specificity. Measurement of pre- and postprandial serum bile acid (SBA) and ammonia concentrations are currently the most commonly used liver function test in the follow-up of EHPSS in dogs.⁸ However, there is currently no biochemical test that provides accurate evidence of surgical success and normalization of SBA after surgery is not proven to be a reliable measure to evaluate shunt closure.^{6,23} We agree that normalized or reduced bile acids (<50% from initial measurements) indicate improvement in hepatic function and therefore are suggestive for (some degree of) shunt attenuation. One retrospective study concentrated on ammonia to evaluate the effect of surgical attenuation of the EHPSS.²¹ It was stated that increased basal ammonia concentration in postsurgical dogs was conclusive for persistent shunting; if fasting plasma ammonia concentration was normal, medical imaging was required to visualize or exclude persistent shunting.²¹ At one month after surgery, ammonia levels were normal in all dogs of our patient group. Three months after surgery, abnormal ammonia levels were only observed in the minority of situations with persistent shunting through the original shunt (2/5) and developed acquired shunts (3/7) (Chapter 4). In conclusion, measurements of ammonia concentrations (neither plasma nor CSF levels) did not adequately reflect the final outcome after surgery. As such, neither plasma nor CSF ammonia levels can be considered accurate to use in the follow-up of shunt patients.

During the study years (January 2014 to July 2016), the surgical protocols and postoperative evaluation for EHPSS were gradually adjusted according to the accumulated experience. This included the switch to the transdiaphragmatic approach in cases of porto-azygos shunts to avoid missing late tributaries to the shunt (Chapter 2) and renewed our preference for placing ameroid constrictors rather than thin film bands for shunt attenuation (Chapter 4). The studies that compared blood and CSF ammonia concentrations in the dogs with a congenital EHPSS (Chapters 3 and 4) were designed to be completed 6 months after

the EHPSS surgery in each individual case. However, the results of the TSPS at the 3-month follow-up were surprising and somehow disappointing. They indicated that in one fourth of the dogs that underwent shunt attenuation with a thin film band, the shunt was still patent (Chapter 4). It should be noted that “cellophane” only very recently became commercially available as Cellovet[®] and therefore the thin film we used might not have been true cellophane. None of the dogs in which an ameroid constrictor was placed had persistent shunting through the original shunt.

It is a general belief that currently only imaging modalities such as scintigraphy,^{24,25} CTA,^{6,7} or US using microbubbles²⁶ can accurately evaluate complete shunt closure. To date, we therefore strongly advise a postoperative TSPS or other imaging modality at the 3- or 6-months postoperative follow-up in all our patients as none of the available biochemical tests provides accurate evidence of shunt closure and status and the incidence of persistent shunting seems higher than expected.

Do porto-azygos shunts deserve the attention they received during this PhD research project?

Although it cannot be completely excluded that there are porto-azygos shunts that may insert at different locations, our study on CTA (Chapter 1) confirmed intra-thoracic insertion in 36 out of 36 dogs with porto-azygos shunts. In addition, all 9 dogs with porto-azygos shunt that were treated since the start of the PhD, described in Chapter 2, also had a thoracic terminus into the azygos vein. Our results demonstrated that the vast majority (if not all) of the porto-azygos shunts in dogs insert in the thorax. This is in agreement with earlier suggestions that most porto-azygos shunts traverse the diaphragm.^{8,23,27} Moreover, in the recent reports of Hunt and colleagues⁶ and Nelson and Nelson⁷ the provided CT images depict porto-azygos shunts that insert in the thorax. Yet both studies did not clearly mention this finding in the text.

To the best of our knowledge, no current reports address specifically the outcome of surgical attenuation of porto-azygos shunts. Two recent reports^{6,7} mention the outcome of porto-azygos shunts in dogs in tables and/or figures provided albeit without specific reference and discussion in the text. In the report of Hunt and colleagues, 2 out of 5 porto-azygos shunts attenuated at the abdominal side of the diaphragm, had postoperative persistent shunting whereas none of the 17 portocaval shunts attenuated near their insertion site were persistent.⁶

In the most recent study of Nelson and Nelson, 5 out of 6 porto-azygos shunts that were treated intra-abdominally had postoperative persistent shunting.⁷ In both studies, it was concluded that persistent shunting was due to misplacement of the attenuating device, which did not include cranial tributaries from the stomach. The dogs in our prospective study on porto-azygos shunts (Chapter 2) were operated via a transdiaphragmatic approach and all had a long-term follow-up including scintigraphic confirmation of shunt closure. Although some porto-azygos shunts were still patent 3 months after the first surgery due to inadequate reaction on the thin film band used for attenuation, none of the dogs did experience persistent shunting due to missed tributaries, because that was efficiently precluded by thoracic placement of the device.

The accepted recommendation for attenuating EHPSSs in general is near their insertion site, but, for unknown reasons, this guideline was not implemented in case of porto-azygos shunts. To our best knowledge, there is no obvious reason why not to assume that the same principle applies to porto-azygos shunts. We therefore challenged these guidelines for attenuation of porto-azygos shunts that describe attenuation of the shunt at the abdominal side of the diaphragm.⁸ Our preliminary findings based on a limited number of patients does support our hypothesis that thoracic attenuation will avoid the risk of persistent shunting due to missed tributaries to the shunt.

It has been reported that the abdominal dissection for attenuating porto-azygos shunt in the abdomen can be very challenging.^{8,27} In our experience, the transdiaphragmatic approach is a straightforward procedure. Although we did not perform a prospective study comparing the transdiaphragmatic approach with the classic abdominal approach, the surgical time for the former approach did not seem to be prolonged. In addition, the transdiaphragmatic approach seems to result in less morbidity because manipulation of the liver lobes and abdominal organs is minimized. Furthermore, a transdiaphragmatic approach provides a safe corridor to the thorax with excellent exposure of the most ideal site for shunt attenuation.

Alternatively, a lateral approach to the thorax would also allow placement of a device on the thoracic portion of the porto-azygos shunt. However, in comparison to a lateral thoracotomy, the transdiaphragmatic approach has many advantages. It allows simultaneously evaluation of the gastro-intestinal tract and the pancreas before and after an attenuating device is placed. In addition, it does not exclude concomitant intra-abdominal procedures. Furthermore, a pre-operative CTA is not strictly necessary whereas in case of a lateral thoracotomy, a CTA is needed to determine the side and the most ideal intercostal space.

Finally, we argue that a transdiaphragmatic approach is less invasive, less painful and less time-consuming compared to a thoracotomy.

In conclusion, in the majority of porto-azygos shunts, a thoracic terminus will be present. In those cases, a transdiaphragmatic approach exposes the insertion site for shunt attenuation. This approach is a relatively easy and fast surgical procedure, without unnecessary abdominal organ manipulation, while the risk of missing additional contributing branches is eliminated.

The controversy is not about ammonia being involved in the HE syndrome in EHPSS dogs, but how to interpret its participation ...

It has long been established that ammonia is increased in most dogs with congenital EHPSS, because conversion of ammonia to urea by the underdeveloped liver is inefficient and because a major part of the intestinal blood does not even pass through the liver.^{28,29} Furthermore, all case series report a high incidence of HE in dogs with congenital EHPSS, although the clinical signs of HE can vary between very obvious to subtle.^{10,12,30,31} Yet the role of ammonia in the development of HE in dogs is controversial and ammonia received limited attention, particularly in the context of EHPSS.²⁸

In the current PhD research, it was shown that high plasma ammonia levels are predictive for the presence of HE in dogs (Chapters 3-5). Our observations are similar to those in humans with HE.³²⁻³⁴ Ammonia is widely considered to initiate HE for the greater part through altered astrocytes function as these are the main cells in the brain that can metabolize ammonia.³⁵⁻⁴¹ However, it is important to acknowledge that several dogs with congenital EHPSS without overt HE signs (Chapter 4) still had high plasma ammonia levels after they had been on medical treatment for 4 weeks before surgery. This finding highlights that hyperammonemia is not limited to dogs with a congenital EHPSS that have overt clinical signs of HE. It suggests that there might be another explanation to link plasma ammonia levels with HE or, that other factors are involved in the pathogenesis of HE. This finding is also consistent with the observation that dogs with urea cycle enzyme deficiency have increased plasma ammonia concentrations but do not typically develop clinical signs of HE.⁴² In humans with HE, similar observations have been reported. Ammonia is frequently raised in these patients; however, there is an imperfect correlation between ammonia plasma levels and

severity of HE signs.⁴⁰ Human clinicians also tried to address the challenge to identify cases with subclinical HE. In this respect, arterial ammonia levels were found to be more sensitive and predictive for HE than venous ammonia levels.^{32,34} Functional brain imaging techniques were also used to evaluate HE.^{32,34,43}

The novel findings of this PhD dissertation concerning HE in dogs with portosystemic shunts were the documentation of high arterial and CSF ammonia levels in EHPSS dogs with HE (Chapters 3-5), and the documentation of alterations in regional cerebral blood flow in these patients (Chapter 5). Our findings are consistent with the results of studies in humans with liver disease and HE.^{32,34,43-45} In human HE patients, arterial ammonia levels over 150 $\mu\text{mol/L}$ were prognostic indicators to predict future development of intracranial hypertension and cerebral edema.^{45,46} It still needs to be established whether the same cut-off values do apply to dogs with EHPSS and HE. None of our dogs that had arterial ammonia level above 150 $\mu\text{mol/L}$ at the day of diagnosis had a history of seizures (Chapter 4). However, neither intracranial hypertension nor cerebral edema was objectively assessed in any of the dogs at the time of diagnosis. The only dog that did have a history of pre-operative seizures had relatively low blood ammonia at diagnosis.

There might be little rationale behind measuring blood ammonia in the detection of HE as ammonia exerts its impact on cerebral function only after entering the brain. The rate of ammonia entry into the brain is therefore important, not the blood levels. Brain imaging techniques have provided substantial insight into the pathophysiology of HE in humans. Neuropsychological impairment observed with subclinical and clinical HE is believed to be predominantly associated with the frontal and temporal lobes, and with the subcortical region of the brain.⁴⁷⁻⁴⁹ Also brain functional imaging studies found that the subcortical area is predominantly exposed to increased ammonia levels, resulting in astrocytic alterations and impairment of subcortical function.^{43,49} The significant alterations in regional cerebral blood flow in the subcortical and temporal regions in dogs with EHPSS and HE (Chapter 5) also suggest predominant involvement and dysfunction of these regions. Therefore, in dogs with hyperammonaemia and high CSF ammonia levels, those areas in the brain might have been more exposed to the increased ammonia levels and/or are the target regions for ammonia in the brain, as in humans. Furthermore, PET studies in human HE patients, using ammonia as a tracer, showed that the cerebral metabolism of ammonia and the permeability of the BBB for ammonia was increased in cirrhotic patients compared to healthy controls.^{32,49} The regional ammonia supply was in accordance with the regional blood flow.^{32,34,49} In our study, we did not directly assess the permeability of the BBB, but we studied the selective changes of

ammonia in the blood and CSF of dogs with EHPSS over time pre- and postoperatively (Chapter 4). The positively correlated CSF to blood ammonia levels (Chapters 3 and 4) suggest passage of ammonia across the BBB and potentially increased permeability of the BBB to ammonia in dogs with EHPSS. It explains and supports the former findings of significantly higher CSF concentrations of metabolites of ammonia such as glutamine and glutamate and other factors in the CSF of PSS dogs with HE.^{36,50}

Blood and CSF ammonia concentration in the current study (Chapters 3 and 4) were high in EHPSS dogs. Of particular interest was that the 4-week medical management before surgery resulted in decreased HE signs in all dogs while ammonia levels in the blood remained high (Chapter 4). This may suggest that medical treatment alters the proportion of blood ammonia that reaches the CNS more than the amount of blood ammonia itself. To our knowledge, this finding has not been described earlier. It is of great importance, however, since it might influence future therapeutic strategies to control HE. It has been suggested earlier that management of HE should not only be aim to reduce ammonia formation and absorption but also to reduce formation of ammonia metabolites such as glutamine and glutamate.⁵⁰ Research should therefore focus on defining the major factors that can prevent ammonia from reaching the CNS. In this respect, it is important to acknowledge that factors such as concentration gradient, pH, and BBB permeability have an important influence on the distribution and the form of ammonia.^{34,51,52} Ammonia will diffuse toward a lower concentration gradient, that is, toward the brain. In addition, the pH in the brain is reported to be lower than the blood pH;³⁴ therefore ammonia (NH_3) will be converted to ammonium (NH_4^+).^{29,34,51,52} Entrapment within the CNS occurs because the ammonium ion is not readily diffusible across cell membranes.³⁴ Furthermore, the BBB will not adequately prevent ammonia to pass in case of hyperammonaemia. It has been observed in humans HE patients that the BBB is much more permeable to the gas form but that measurable amounts of the positively charged ammonium ion (NH_4^+) also cross the BBB.³⁴

Our findings of high CSF ammonia concentrations and the previous findings of high CSF glutamate and glutamine concentrations in dogs with PSS^{36,50} raise the possibility that overstimulation of the N-methyl-D-aspartate class receptors by glutamate is involved in the disease mechanisms of HE development in dogs with PSS. Potentially, ammonia excess might also deregulate the delicate balance between glutamatergic and GABAergic systems in dogs with congenital EHPSS.

Ammonia levels in blood and CSF of dogs with pre-operative signs of HE dropped to the low normal range and HE signs were no longer observed one month after surgery (Chapter 4).

Although the results of this PhD research do not establish that ammonia initiates HE in dogs with EHPSS, ammonia definitively does play a role in the HE pathogenesis (Chapter 3-5).

Postoperative seizures are reported in 5–18% of dogs and typically occur within 72 hours of surgery.^{24,54-56} The cause of postoperative neurologic dysfunction, seizures, and other pathologic changes of the CNS in dogs with PSS are not clear. The seizures are often refractory to standard anticonvulsant drug treatment, and frequently progress to status epilepticus.^{53,54,57} Two case series on EHPSS dogs euthanized because of uncontrollable seizures report CNS lesions that were more severe than those in dogs with PSS that did not seizure.^{53,58} Yet it is not clear whether those CNS lesions represented the cause or the pathologic consequence of the seizures. In our case series, none of the dogs developed seizures in the immediate postoperative phase. However, one dog developed partial epilepsy 80 hours after an AC had been placed around its portocaval shunt. Levetiracetam successfully controlled the problem. The dog was diagnosed with acquired shunting 3 months postoperatively. The other dog with postoperative epilepsy was the only dog that also experienced pre-operative seizures. Despite successful surgery and despite medical management with imepitoin and levetiracetam, the dog's seizures could not be controlled and was finally euthanized. Since the dog had a normal postoperative scintigraphic scan as well as normalization of both ammonia and SBA values, the neurological problems might not have been directly related to portosystemic shunting. This assumption could not be assessed, since the owners declined postmortem analysis.

Histologic lesions are sometimes found in the CNS of PSS dogs at postmortem evaluation.^{28,53,58} Findings in a histologic study revealed polymicrocavitation of the brainstem, cerebellar nuclei, and sometimes polymicrocavitation at the border of the white and gray matter in the cerebral cortex, in addition to an increase in numbers of protoplasmic astrocytes.²⁸

Portosystemic shunt disease is one of those disease entities of which a lot still has to be discovered. With this PhD research, we attempted to fill in some of the gaps on EHPSS in dogs. The hypotheses that were initially proposed were all tested and novel findings already have initiated further research and will certainly continue to do so in the near future.

Limitations of the current PhD research

It should be clear to the readership that portosystemic shunt disease in dogs comprises much more than EHPSS alone. Other conditions involving portosystemic shunting in dogs are intrahepatic PSS, acquired PSS and primary portal vein hypoplasia. The relative predominance of dogs with EHPSS in our clinic population as well as the wide range and nature of portosystemic shunt disease dictated to limit the PhD research to the study of dogs with EHPSS.

Using a greater sample size in the experimental studies may have increased the reliability of the results, shedding more light on the etiopathogenesis of HE in EHPSS dogs, the role of ammonia, and the outcome of porto-azygos shunts. Choice of sample size was based on practical and ethical considerations.

Ammonia is known to be unstable *ex vivo*, and, although samples were processed with special care, pre-analytical errors cannot be completely excluded. Furthermore, ammonia is only one out of more than 20 different compounds that are known to accumulate in the systemic circulation when liver function is impaired. Several of them might also contribute to the etiopathogenesis of HE in dogs with congenital EHPSS.

Future Perspectives

Firstly, this PhD research (Chapters 1 and 2) presented a new scientific approach towards porto-azygos shunts, recommending attenuating these shunts in the thorax, at the insertion point of the shunting vessel, as opposed to former recommendations to attenuate these shunts in the abdomen. A transdiaphragmatic approach was successfully performed. However, we believe that minimal invasive approaches such as interventional radiology or thoracoscopy should also be explored for these shunt types, giving the anatomic location of the insertion point of these shunts. The feasibility, advantages and disadvantages should be studied to find out whether such approaches would be valuable alternatives.

Secondly, the results of this PhD research (Chapters 3-5) “lifted a corner of the veil” in regards to the BBB permeability and brain changes in EHPSS dogs with neurological signs, encouraging further studies to better understand and treat HE related to PSS. To further investigate the role of ammonia in EHPSS dogs with HE and to better understand the mental

changes observed in PSS dogs, further studies of brain perfusion imaging based on specific ammonia markers as well as more advanced structural imaging are needed.

There do remain some gaps in the management of portal shunt disease. A substantial bottleneck is the poor and inaccurate documentation of postoperative outcome in dogs that underwent surgical attenuation of a congenital EHPSS. Clinical outcome can be very misleading and also the currently available biochemical screening tests are not appropriate to judge postoperative shunt status. To date, clinicians should rely much more on advanced medical imaging techniques to objectively document the result of the attenuation process; complete closure of the congenital EHPSS without formation of acquired shunts, persistent shunting through the original EHPSS, or complete closure of the congenital EHPSS with formation of acquired shunts. Dogs with persistent shunting due to incomplete closure or acquired shunts will not always have obvious clinical issues, yet they are affected somehow by subclinical disease. It should be explored whether this group of patients could not benefit from some type of regenerative medicine. A recent experimental study in dogs found a high uptake of mesenchymal stem cells in the liver after transcutaneous ultrasound-guided splenic injection or jejunal vein catheterization.⁶¹ If stem cell delivery can be a key to enhanced liver regeneration, there is a potential to optimize the surgical outcome after gradual attenuation of congenital EHPSSs. In addition, the live expectancy in dogs with a congenital EHPSS that are not surgical candidates could be prolonged.

Moreover, the findings in this PhD dissertation (Chapters 3-5) provide further evidence that dogs with EHPSS are a good spontaneous model of human HE.^{59,60} Further studies into the pathogenesis of HE in dogs with a EHPSS may offer additional insights into the biology of HE in humans as well as additional insights into the biology of HE in PSS dogs, and could potentially reduce the numbers of experimental animals with induced shunts that are currently used as a model.

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SUMMARY

In the first chapters (Chapter 1 and 2) of this PhD dissertation, the gap in knowledge concerning the morphology and the surgical approach to porto-azygos shunts was addressed. In the following chapters (Chapter 3-5), the focus was on ammonia levels in blood and cerebrospinal fluid (CSF) and hepatic encephalopathy (HE) in dogs with a congenital extrahepatic portosystemic shunt (EHPSS) before and after surgical attenuation and on changes in regional perfusion of cortical and subcortical brain regions in dogs with a congenital EHPSS and overt signs of HE.

The goal of Chapter 1 was to describe the morphology of porto-azygos shunts in a large series of dogs using computed tomographic angiography (CTA), with special attention to the site of insertion of these shunts. Thirty-six dogs with porto-azygos shunt underwent CTA, and three-dimensional images were created to aid in understanding porto-azygos shunt morphology. CTA was found well suited to provide details on porto-azygos shunt anatomy in dogs. Two main conformations of porto-azygos shunts were documented, with the vast majority of dogs having a left gastro-azygos shunt whereas the other few having a right gastro-azygos communication. Anatomic variations in shunt types existed, but were minor. Of particular interest was that all porto-azygos shunts crossed the diaphragm through the esophageal hiatus and terminated in the thoracic part of the azygos vein, perpendicular to the aorta, in a characteristic “L” shape. Sticking to the general principles of shunt attenuation, it seems logical to attenuate also a porto-azygos shunt as close to its insertion site as possible, necessitating access to the thoracic cavity in cases where the shunting vessel terminates in the thorax. This led us to evaluate an alternative surgical approach toward porto-azygos shunts that insert in the thoracic part of the azygos vein (Chapter 2). In this chapter we described and documented the surgical technique and evaluated the feasibility of a transdiaphragmatic approach to attenuate porto-azygos shunts. The study comprised a cadaveric study including 6 dogs and a prospective case series including 9 dogs with insertion of the porto-azygos shunt in the thoracic part of the azygos vein. Landmarks for creating a safe transdiaphragmatic approach to the caudal intra-thoracic portion of the azygos vein were first established in the cadaveric dogs and implemented in the clinical cases. Exposure of the shunt insertion site to the azygos vein was excellent in all clinical cases, and all shunts were successfully attenuated close to their insertion site at the level of the characteristic “L” shape via this novel approach. No peri-operative complications were encountered. Thus, if thoracic attenuation of a porto-azygos shunt is considered, a transdiaphragmatic approach is safe and adequate to expose the insertion site for shunt attenuation. This approach is a straightforward surgical procedure,

without unnecessary abdominal organ manipulation, while the risk of missing additional contributing branches is eliminated. Furthermore, additional intra-abdominal procedures such as gonadectomy or cystotomy can be performed through the same approach.

For the first time in the veterinary literature, selective changes of ammonia in the blood and CSF of dogs with congenital EHPSS, treated by thin film band (TFB) or ameroid constrictor (AC), are demonstrated over time (Chapters 3 and 4). The ammonia levels in arterial blood, venous blood, and CSF were assessed on the day of diagnosis by two different devices, the portable PocketChem BA and the Catalyst Dx (Chapter 3). Those measurements were made in 6 control dogs and in 19 dogs with a congenital EHPSS. Ammonia levels in blood (arterial and venous) and CSF of dogs were significantly higher in dogs with a congenital EHPSS than in clinically normal dogs. A strong positive correlation was documented between the different ammonia levels in the different body fluids tested within each patient. The results of this study also demonstrated that, at least in dogs with EHPSS, venous samples can substitute the more cumbersome arterial samples. Of particular interest were the high levels of ammonia in the CSF of the EHPSS dogs that were strongly and positively correlated to the blood ammonia levels, suggesting permeability of the blood-brain barrier (BBB) to ammonia in case of excessive blood ammonia levels. Since PocChem and CatDx were both recently validated to perform measurements of ammonia in the CSF, the CSF ammonia levels obtained in this study should not be considered as absolute values but rather as underestimated values. In addition, the absolute values should not be used as an indicator for the diagnostic or prognostic of HE or in a comparative way, until cutoff values in a larger group of normal dogs will be evaluated.

To circumstantiate the clinical interpretation of the ammonia levels in the different body fluids, and to relate them to the surgical outcome after TFB or AC placement, ammonia levels were re-measured (Chapter 4) on the day of surgery and several points thereafter (at 1, 3 and 6 months after surgery) in 19 dogs. Transsplenic portal scintigraphy was repeated 3 months after surgery in all dogs to enable correlation of the ammonia levels to the surgical outcome. The correlations between the blood (arterial and venous) and CSF after successful surgical attenuation of the EHPSS were similar to those calculated before surgery. Ammonia levels in arterial blood, venous blood, and CSF of dogs with closed shunts were remarkably lower compared to the preoperative measurements. However, normalization of the blood ammonia levels after surgery did not necessarily indicate surgical success. In 3 of the 5 dogs that developed acquired shunts after the first surgery and in 3 out of 5 dogs that had persistent

shunting, the ammonia levels were within the normal range. Hence postoperative ammonia levels are not an accurate predictor of the shunt status (closed, patent, acquired) after surgery.

In Chapter 5, 8 dogs with a congenital EHPSS showing overt clinical signs of HE and an equal number of healthy beagles underwent a brain SPECT scan to document the alterations in regional cerebral blood flow (rCBF). The clinical signs of HE were graded and the ammonia levels in the blood and the CSF were measured. The areas of the brain with an altered perfusion were determined. The brain SPECT scans were abnormal in all EHPSS dogs. Compared to the control group, rCBF was decreased significantly in the temporal lobes while increased in the subcortical (thalamic and striatal) area in all dogs with EHPSS, cautiously suggesting the predominant involvement and dysfunction of these areas in the pathogenesis of HE due to congenital EHPSS in dogs.

SAMENVATTING

De eerste hoofdstukken (Hoofdstuk 1 en 2) van deze doctoraatsthesis beschrijven het gebrek aan morfologische kennis en chirurgische benadering van porto-azygos shunts. In de volgende hoofdstukken (Hoofdstukken 3 tot 5) wordt dieper ingegaan op het ammoniakgehalte in het bloed en cerebrospinaal vocht (CSV) alsook op hepatische encefalopathie (HE) bij honden met een congenitale extrahepatische portosystemische shunt (EHPSS) voor en na chirurgische behandeling. Ook de veranderingen in regionale perfusie van de corticale en subcorticale regio's in de hersenen bij honden met een congenitale EHPSS en symptomen van HE worden beschreven.

In het eerste hoofdstuk wordt de morfologie van porto-azygos shunts beschreven in een groot aantal honden. Hiervoor werd gebruik gemaakt van computer tomografische angiografie (CTA) waarbij speciale aandacht werd besteed aan de insertieplaats van de shunts. Zessendertig honden met een porto-azygos shunt kregen een CTA waarbij driedimensionale beelden werden gecreëerd om de morfologie van de porto-azygos shunts in beeld te brengen. CTA blijkt een goede beeldvormingsmodaliteit om gedetailleerde informatie te leveren over de morfologie van porto-azygos shunts bij honden. Twee conformaties van porto-azygos shunts werden vastgesteld, waarbij bij de grote meerderheid van de honden een linkse gastro-azygos shunt werd vastgesteld en bij een kleiner aantal een rechtse gastro-azygos verbinding. Ook minimale anatomische variaties in deze shunt types werden beschreven. Opmerkelijk was dat alle shunt types het diafragma doorkruisten door de oesofagale hiatus en eindigden in het thoracale gedeelte van de azygos vene, loodrecht ten opzichte van de aorta en in een karakteristieke "L"-vorm.

Volgens de chirurgische principes voor de behandeling van portosystemische shunts moet een shunt zo dicht mogelijk bij zijn insertieplaats onderbonden worden, waarbij het in het geval van porto-azygos shunts die eindigen in de thorax noodzakelijk zou zijn om de thoraxholte te openen. Dit gegeven resulteerde in een evaluatie van een alternatieve chirurgische benadering voor porto-azygos shunts die eindigen in het thoracale deel van de azygos vene (Hoofdstuk 2). In dit hoofdstuk beschreven en documenteerden we de chirurgische techniek en evalueerden we de haalbaarheid van een transdiafragmatische benadering voor de behandeling van porto-azygos shunts. De studie omvatte een kadaverstudie met 6 honden en een prospectieve gevallenreeks met 9 honden met een porto-azygos shunt die eindigde in het thoracale gedeelte van de azygos vene. Eerst werden

anatomische herkenningspunten voor een veilige transdiafragmatische benadering van het caudale intrathoracale deel van de azygos vene bepaald in de kadavers. Deze coördinaten werden vervolgens gebruikt in de klinische gevallen. Bij alle patiënten was de insertieplaats van de shunt in de azygos vene perfect toegankelijk en konden alle shunts dicht bij de insertieplaats op het niveau van de karakteristieke “L” vorm worden onderbonden. Er werden geen peroperatieve complicaties waargenomen.

Indien een thoracale behandeling van een porto-azygos shunt overwogen wordt, is een transdiafragmatische benadering een veilige en adequate techniek om de insertieplaats toegankelijk te maken. Deze benadering is een rechttoe rechtaan procedure zonder onnodige manipulatie van abdominale organen die daarenboven het risico op het missen van additionele kleine shunttakken minimaliseert. Daarenboven kunnen bijkomende intra-abdominale ingrepen zoals een gonadectomie of een cystotomie tijdens eenzelfde chirurgie worden uitgevoerd.

Voor het eerst in de diergeneeskundige literatuur werden de selectieve veranderingen van het ammoniakgehalte in het bloed en CSV bij honden met een congenitale EHPSS die behandeld werden met een thin film band (TFB) of een ameroid constrictor (AC) over de tijd beschreven (Hoofdstukken 3 en 4).

De ammoniak gehalten in arterieel bloed, veneus bloed en CSV werden gemeten op de dag van de diagnose met behulp van twee verschillende toestellen: de draagbare PocketChem BA en de Catalyst Dx (Hoofdstuk 3). Deze metingen werden uitgevoerd bij 6 controle honden en bij 19 honden met een congenitale EHPSS. De ammoniakgehalten in het bloed (arterieel en veneus) en CSV waren significant hoger bij honden met een congenitale EHPSS dan bij klinisch gezonde honden. Binnen eenzelfde hond werd een duidelijk positieve correlatie aangetoond tussen de gehalten aan ammoniak in de verschillende lichaamsvochten. De resultaten van deze studie toonden ook aan dat, bij de honden met een EHPSS, de veneuze bloedstalen een gelijkaardig gehalte opleverden dan de arteriële bloedstalen, die veel minder makkelijk te preleveren zijn.

Van bijzonder belang was de positieve correlatie tussen de hoge ammoniakgehalten in het CSV en de gestegen ammoniak gehalten in het bloed bij honden met een EHPSS wat doet vermoeden dat, eens er sterk gestegen ammoniakgehalten in het bloed aanwezig zijn, de bloed-hersenbarriere permeabel wordt voor ammoniak. Aangezien zowel de PocChem als de

CatDx niet gevalideerd zijn voor de metingen van ammoniak in het CSV mogen de ammoniak gehalten van het CSV die in deze studie bekomen werden niet als absolute waardes gezien worden maar eerder als vergelijkende waardes en dit tot dat de toestellen gevalideerd worden voor het meten van ammoniak in het CSV.

Bij de 19 honden werden de ammoniakgehalten opnieuw bepaald (Hoofdstuk 4) op de dag van de chirurgie en op verschillende tijdstippen nadien (1, 3 en 6 maanden na chirurgie). Het was de bedoeling om de evolutie van de ammoniakgehalten in de verschillende lichaamsvochten te evalueren alsook om de correlatie na chirurgische plaatsing van een TFB of AC te bestuderen. Om de correlatie tussen de ammoniakgehalten en het chirurgisch resultaat aan te geven, werd bij alle honden de transsplenische portale scintigrafie herhaald 3 maanden na de operatie. De correlatie tussen de ammoniakgehalten in het bloed (arterieel en veneus) en het CSV bleek zowel voor als na de chirurgische correctie van de shunt gelijkaardig. De ammoniakgehalten in het arterieel bloed, veneus bloed en CSV waren echter na de chirurgische correctie opmerkelijk lager dan voor de ingreep. Nochtans bleek normalisatie van het ammoniakgehalte na chirurgie niet noodzakelijk te duiden op chirurgisch succes gezien bij 3 van 4 honden die een verworven shunt ontwikkelden na chirurgie en bij 5 van 6 honden die een persisterende shunt hadden na chirurgie, de ammoniakgehalten ook genormaliseerd waren. Hieruit kan geconcludeerd worden dat postoperatieve ammoniakgehalten geen accurate indicator zijn voor de status van de shunt (gesloten, persistent of verworven) na chirurgie.

In Hoofdstuk 5 worden de resultaten weergegeven van 8 honden met een congenitale EHPSS en duidelijke klinische tekenen van HE en 8 gezonde beagles die een SPECT scan van de hersenen ondergingen met als doel veranderingen in de regionale cerebrale bloedvloeï (rCBV) te detecteren. De klinische tekenen van de HE werden gegradeerd en ammoniakgehalten in bloed en CSV werden gemeten. De regio's in de hersenen waar perfusieveranderingen werden vastgesteld, werden gedefinieerd. De SPECT scans van de hersenen waren afwijkend bij alle honden met een EHPSS en HE. De perfusie was significant gedaald in de temporale lobben en gestegen in de subcorticale (thalamus en striatale) regio's bij alle EHPSS honden in vergelijking met de controle groep. Uit deze resultaten kan afgeleid worden dat disfunctie van deze regio's een dominante rol speelt bij de pathogenese van HE ten gevolge van EHPSS bij honden.

CURRICULUM VITAE

Matan Or werd geboren op 26 februari 1975 in Rishon-Lezion, Israël. Hij begon in 1999 aan zijn universitaire studies aan de Hebrew University of Jerusalem. In 2002 behaalde hij zijn Bachelor of Science in Animal Science aan de Faculty of Agriculture, Magna cum Laude. Daarna vervolgde hij zijn studies aan de Koret School of Veterinary Medicine. In januari 2007 behaalde hij zijn dierenartsdiploma. Aansluitend volgde hij een Rotating internship in Small Animal Medicine and Surgery, eveneens aan de Hebrew University of Jerusalem. Even later werd hij daar voor anderhalf jaar tewerkgesteld. Daarna nam hij een Small Animal Surgery Internship op in een gerenomeerde doorverwijskliniek in Beit-Berel, Israël. In de zomer van 2011 reisde Matan naar Amerika waar hij aan de Michigan State University Veterinary Teaching Hospital een Small Animal Surgical Fellowship Program volgde. Een jaar later kwam hij naar Europa en startte een ECVS Small Animal Surgery Residency aan de Faculteit Diergeneeskunde van de Universiteit van Gent.

In 2013 verkreeg hij de Surgeon-in-Training Grant van het ECVS, waarmee een deel van zijn onderzoek gefinancierd werd. In 2016 voltooide Matan zijn residency opleiding. Inmiddels was hij auteur en mede-auteur van talrijke publicaties. Hij presenteerde ook meermaals zijn onderzoeksbevindingen op het jaarlijkse ECVS Congres. Vanaf november 2016 was Matan verbonden als assistent aan de Vakgroep Kleine Huisdieren, Faculteit Diergeneeskunde, Universiteit Gent.

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DANKWOORD

This dissertation could not have been completed without the great support that I have received from so many people. I wish to offer my most heartfelt thanks to the following people.

I would like to thank my residency supervisor and promoter, Prof. Dr. Hilde de Rooster, for teaching, guiding and supporting me over the past few years. Hilde, you have set an example of excellence as a researcher, clinician, mentor, instructor, and role model. You allowed me and inspired me to pursue research on topics for which I am truly passionate, I saw the same drive and passion in your own research efforts, and I thank you for encouraging me to do the same. I also want to thank you for the friendship and great atmosphere. I was very fortunate to be at the right place at the right time to grab this unique opportunity and I will be forever grateful.

I would like to thank my co-promoter, Prof. Dr. Kathelijne Peremans, for all the help during this PhD dissertation. I was fortunate to have the “queen of SPECT” in the team. Kathelijne, the nuclear medicine input and support in this PhD dissertation was admirable and was most important and is due to your hard work.

I would like to thank Dr. Eva Vandermeulen, the “princess of scintigraphy” for all the hard work and availability. Eva, none of the studies involving scintigraphy would have been possible without your help.

I would like to thank the members of my examination committee for all guidance through this process; your discussion, ideas, and feedback have been absolutely invaluable.

I would like to thank colleagues, technicians, administrative staff, collaborators, and the multitude of undergraduates from the Small Animal Hospital who contributed to this research. I am very grateful for your technical and scientific support. I would also like to thank you for the friendship, the great atmosphere, support, kindness and hard work, which often extended after hours. I had a great time working with you all, and I will never forget you.

Furthermore, I would like to thank my undergraduate research advisor, Prof. Shimon Harrus, for his constant enthusiasm, support and encouragement.

I would especially like to thank my family for the love, support, and constant encouragement I have gotten over the years. In particular, I would like to thank Sara Kol, my brother, Omer Or, and my sister, Keren Kol.

Finally, I would like to thank and dedicate this PhD dissertation to my parents, Sara and Jacob Or, who supported, inspired, mentored and motivated me along the way. Mom and Dad, you have taught me about hard work and respect, about persistence and about how to be independent. You are great role models of resilience, strength and character. I am grateful for you both and I am very proud of you.