Department of Small Animals and Horses

University of Veterinary Medicine Vienna

Clinic for Small Animal Internal Medicine

(Head: Burgener Iwan, Univ.-Prof. Dr.med.vet., PhD, Dr. habil, DACVIM, DECVIM-CA)

Canine hepatobiliary diseases – diagnostics and diagnoses

Diploma thesis

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submitted by

Flora Pletzer

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Supervisor: Univ.-Prof. Dr.med.vet. Iwan Burgener, PhD, Dr. habil, Dipl. ACVIM, Dipl. ECVIM-CA, Fachtierarzt für Innere Medizin Klein- und Heimtiere

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1 Terminology

A.	Arteria
Aa.	Arteriae
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
CDmiRs	Cholangiocyte-derived mircoRNAs
cm	Centimeter
CPSSs	Congenital portosystemic shunts
CT	Computer tomography
DIC	Disseminated intravascular coagulopathy
e.g.	Exempli gratia
Fig.	Figure
FNA	Fine needle aspiration
g/l	grams per liter
GGT	Gamma-glutamyltransferase
GLDH	Glutamate-dehydrogenase
HDmiRs	Hepatocyte-derived microRNAs
IMHA	Immunmediated hemolytic anemia
Lat.	Latin
µl	Microliter
µg/dl	Microgram per deciliter
µmol/l	Micromol per liter
ml	Mililiter
ml/kg	Mililiter per kilogram

mm	Millimeter
MRI	Magnetic resonance imaging
PTT	Prothrombin time
$T_{1/2}$	Half-life
TT	Thrombin time
V.	Vena

2 Introduction

Hepatobiliary diseases are commonly encountered in dogs and can be divided into four main groups: parenchymal, biliary, vascular or neoplastic diseases. Moreover, there is a range of pathologies that can secondarily affect the liver.

They are among the leading causes of morbidity and mortality in dogs (Assawarachan et al. 2019). However, the clinical signs are usually too unspecific to determine the individual disorder and the dogs remain subclinical for a very long period of time (Poldervaart et al. 2009, Eman et al. 2018). The most prevalent disease is primary hepatitis with acute and chronic occurence. Primary hepatitis is responsible for 0.5 % of the canine referral population and chronic hepatitis prevails (Poldervaart et al. 2009, Eman et al. 2018, Lawrence et al. 2018). Chronic hepatitis in particular is a parenchymal disease that is often only diagnosed when hepatocellular injury is already very advanced, therapy is limited, and the prognosis has to be made very carefully (Dirksen et al. 2017a). It results in a repairing process that leads to fibrosis, abnormal hepatic nodules and portal-central anastomoses (Lawrence et al. 2018).

The diagnosis of hepatopathies usually depends on the interplay and interpretation of many different examination methods (Rothuizen 2006). The first step of diagnosis is usually to determine certain blood variables. Alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) are currently considered the most common enzymes to ascertain hepatocellular damage (Center 2007). Due to its localisation in the cytosol of the hepatocytes, ALT is the most significant enzyme regarding hepatocellular injury (Center 2007, Favier 2009). A study from Dirksen et al. (2016a) on labrador retrievers showed, that specifity is relatively high, but it is lacking sensitivity at only 60-70 %. Furthermore, additional indicators of liver function, such as bilirubin, bile acids and ammonia, can be measured to evaluate hepatic impairment (Schlesinger and Rubin 1993, Mery Kogika 1999, Gerritzen-Bruning et al. 2006). Even though these blood variables are the most established indicators for the detection of hepatocellular damage, they lack specificity regarding vascular diseases and the differentiation between individual disease complexes (van Straten et al. 2015, Van den Bossche and van Steenbeek 2016). For final diagnosis, more invasive examination methods such as ultrasound, computer tomography (CT), magnetic resonance imaging (MRI), fine needle aspiration (FNA) or liver biopsies are commonly necessary. A central point in the diagnostic process is the performance of a histopathological examination of liver or gallbladder tissue. The histological evaluation of hepatic tissue gained through biopsies is currently regarded as gold standard for the assessment of parenchymal

diseases such as chronic hepatitis, biliary tract diseases, and is moreover valuable for neoplastic and circulatory pathologies (Lidbury 2017, Lawrence et al. 2018). However, fine needle aspiration can also help to obtain a final diagnosis or at least narrow the differential diagnoses list (Bahr et al. 2013). It is considered to be effective in the diagnosis of diffuse vacuolar parenchymal changes (Bahr et al. 2013) and was reported to provide an accurate diagnosis in 60 % of neoplastic cases (Selmic 2017). Therefore, FNA is often a useful minimal-invasive technique for hepatobiliary diagnostics, also due to the fact that costs are limited, complication rate is low, and there is no need of general anaesthesia (Fleming et al. 2019). Ultimately, FNA or biopsies are currently almost always the decisive step that leads to a definitive and more importantly precise diagnosis for initiating further treatment.

The objective of this work was to determine the frequency of different types of canine hepatopathies in regard to the subgroups (parenchymal, biliary, vascular and neoplastic) as well as the usefulness of the different diagnostics in a large referral population over a period of eight years at the small animal clinic of the University of Veterinary Medicine Vienna, Austria.

The dataset was used to verify the following hypotheses:

1. Parenchymal liver diseases make up the largest group of hepatobiliary pathologies.
2. FNA is a sensitive and helpful technique in the diagnosis and differentiation of most hepatobiliary diseases with exception of vascular disorders.
3. FNA is not sensitive enough to reliably diagnose chronic hepatitis.

3 Theoretical background

3.1. Hepatic structure and function

The liver represents up approximately 4 % of the body weight and has, among others, exocrine and endocrine functions. Soft and brittle in consistency, its colour varies from pinkish red to brown depending on the blood amount. It is situated dorsal right in the intrathoracic section of the abdominal cavity with direct contact to the diaphragm. In the dog, the liver is composed of four different lobes: *lobus hepatis sinister* and *dexter*, which are both divided into a *lateral* and *medial* part, *lobus quadratus* beneath the *porta hepatis* and *lobus caudatus* with a *processus caudatus* and a *processus papillaris*, above the *porta hepatis* (König and Liebich 2005). The vascularisation of the liver is provided from two different vessels, the portal vein (*V. portae*) and the hepatic artery. Together with connective tissue, which is in touch with the capsule (*capsula fibrosa hepatis*), both enter the liver at its hilus. *V. portae* delivers functional venous blood from unpaired organs like stomach, pancreas, spleen and intestines, to transport the absorbed components of nutrients to the acinus (Salomon et al. 2008). Additionally, it is linked to veins of the caval system in the periphery. These connections form alternative drains for portal blood, if normal bloodstream is disabled (König and Liebich 2005). Due to the high pressure of up to 10 cm H_2O, which is much more than in the *caudal V. cava*, it is possible to surmount the resistance of the hepatic circulation. Moreover, oxygenated nutritional blood comes from the hepatic arteries (*Aa. hepaticae*) (Lahunta and Howard 2012). The liver is composed of lobules that form a structural and functional unit and consist of endodermal liver cells and mesodermal sinus capillaries. About 70 % of the cell population of the liver is made of hepatocytes. Life span of each hepatocyte is approximately 150 days; therefore, mitosis is rarely observed in liver tissue. After necrosis due to toxins or after hepatitis, the mitotic activity can increase enormously. High in regeneration capacity, even if 75 % are surgically removed the tissue can be reproduced by the organ within a few weeks (Salomon et al. 2008).

The liver ensures important metabolic processes such as the breakdown of nutrients through glycolysis, fatty acid oxidation and detoxification of metabolites to CO_2 and H_2O. It also synthesizes energy carriers and proteins through gluconeogenesis, ketogenesis, glycogen-, lipid- and lipoprotein synthesis (Engelhardt et al. 2015). The renal cortex and liver parenchyma are the only locations where gluconeogenesis can take place and with 80-85 %, the hepatocytes fulfil the majority of this process (Postic et al. 2004). One of the main tasks of the liver is the detoxification by ammonia fixation, urea formation, glutamine metabolism and

biotransformation of organic foreign substances. Since ammonia is a very competent cytotoxin, the conversion into ammonium in the liver is vital. Ammonium is introduced in the citrate cycle and metabolized to urea. Alongside glutamine and alanine, urea is the most important form of nitrogen transport from the liver to the kidney. It excretes most of the excess nitrogen accumulated in the body. Moreover, the liver ensures the metabolism of fat-soluble vitamins such as vitamin A, D, E and especially K. Bile acid synthesis as well as its formation and excretion also take place in the liver. The bile is secreted by the liver and stored in the gallbladder in which it is concentrated. Bile acids are required for lipid absorption by micelles in the small intestine and they also regulate cholesterol intake through nourishment as well as the resynthesis of cholesterol. Since proteins and lipoproteins are released into the plasma, as well as bile into the biliary system, the liver is defined as a secreting gland (Engelhardt et al. 2015). Accordingly, the liver is a central organ that is involved in almost all metabolic processes and serves as storage, detoxification and excretion organ. Though the liver can regenerate much better than other organs, it is susceptible to autoimmune, infectious, metabolic and inflammatory conditions and if irreversible damage is present, the entire organism is affected (Lejnine et al. 2014).

3.2. Pathology of liver and gallbladder

3.2.1. Parenchymal diseases

Primary hepatitis is considered as the most common parenchymal hepatic disease of dogs and can be subdivided into acute or chronic (Boomkens et al. 2004, Neumann und Danner 2012, Dirksen et al. 2016b).

Acute hepatitis primarily results from infections (e.g., canine adenovirus 1 or leptospirosis) as well as toxins or drugs (Adamus et al. 1997, Boomkens et al. 2004, Watson 2004a, Poldervaart et al. 2009). The combination of inflammation, necrosis, hepatocellular apoptosis and in some cases also regeneration characterizes the process of acute hepatitis. Abscesses of the liver are mainly caused by ascending bacterial infections. New-born dogs tend do get abscesses due to umbilical infections, whereas in adult animals *Yersinia spp*., *Nocardia asteroides* and *Actinomyces spp.* are often involved. Abscesses can also occur in association with central necrosis or hepatocellular tumours (van den Ingh et al. 2006b). The aetiology of chronic hepatitis can usually not be determined and is therefore often considered idiopathic (Webster et al. 2019a). In contrast to the changes of acute hepatitis, hepatocellular apoptosis, necrosis, mononuclear or mixed inflammatory infiltrates, regeneration and fibrosis are found in patients

with chronic hepatitis (van den Ingh et al. 2006b). Copper accumulation can cause both acute and chronic hepatitis (Hoffmann et al. 2006, Dirksen 2016). Cirrhosis is considered the end-stage of chronic hepatitis and is characterized by developing accumulation of fibrillary extracellular matrix components (Eulenberg und Lidbury 2018). This process manifests itself in fibrosis with a conversion from normal liver tissue to structurally abnormal nodules and anastomoses of the portal and central vessels (Sevelius 1995, van den Ingh et al. 2006b, Favier 2009).

Hepatic amyloidosis is mostly secondary or reactive in dogs. Amyloid is deposited after prolonged inflammation or tissue destruction because the liver produces its precursor, acute -phase-protein serum-amyloid-A (Van Winkle et al. 2006).

3.2.2. Biliary diseases

Cholestasis is a common biliary disease and can be divided into intrahepatic and extrahepatic. While intrahepatic cholestasis is found associated with many liver diseases, extrahepatic cholestasis appears through intraluminal obstructions, especially due to gall stones, mucinous cystic hyperplasia or luminal constriction caused by neoplasia or other inflammatory processes (Hadad et al. 1976, van den Ingh et al. 1986, Raweily et al. 1990). Cholangitis can be neutrophilic, lymphocytic and destructive or chronic, which may be associated with liver fluke infestation. Regarding the dog, destructive cholangitis is mainly relevant. It is characterized by the destruction and loss of the biliary tract in smaller portal areas with subsequent inflammation and ultimately portal vein fibrosis. Common causes are reactions to drugs, especially sulphonamides, but also viral infections (e.g., canine distemper) and poisoning (van den Ingh et al. 1988, 2006a, Raweily et al. 1990). Furthermore, there are diseases affecting the gallbladder itself. Cystic mucinous hyperplasia is characterized by an increased mucin production which leads to an enlargement up to a mucocele with the typical wheel spokes or kiwi shape on ultrasound (Besso et al. 2000, Holt DE et al. 2004, Pike et al. 2004, van den Ingh et al. 2006a). Solitary hepatobiliary cysts are rare in dogs, and other congenital cystic diseases of the liver, which are characterized by dilatation of segments of the intrahepatic bile ducts and fibrosis and associated with polycystic kidney disease, are more frequently found in cats than in dogs, as is cholecystitis due to bacterial infection (Bosje et al. 1998, van den Ingh et al. 2006a). Biliary atresia has only once been described in a dog (Schulze et al. 2000).

3.2.3. Vascular diseases

Congenital portosystemic shunts (CPSSs) are vascular anomalies that connect the portal venous system directly to the systemic venous circulation. A distinction can be made between intrahepatic and extrahepatic CPSSs. The failure to close the ductus venosus after birth results in an intrahepatic shunt. Commonly they originate from the left, infrequently from the right branch of the portal vein. In addition to intrahepatic shunts, extrahepatic shunts are defined as abnormal functional communications. As they arise from any part of the portal system, they drain into the caudal caval vein or the azygos vein. While intrahepatic shunts are mainly seen in large breed dogs, extrahepatic shunts are most common in small breed dogs. Secondary to the shunt, pathological changes appear within the liver. The vessel turns atrophic and as a result of extrahepatic shunts, the portal vein becomes hypoplastic downstream from the origin of the shunt. However, portal hypertension does not occur due to shunts (van den Ingh et al. 1995, Cullen et al. 2006a). Furthermore, disorders associated with outflow disturbances affecting the heart, caudal vena cava or the hepatic veins can emerge. Portosystemic hypertension with portosystemic collaterals, resulting from vascular disorders or hepatic diseases, is common in dogs. Portal vein obstruction (e.g., from thrombosis), primary hypoplasia of the portal vein as congenital disorder, as well as intravenous arterio-venous fistulas in young dogs lead to hypertension due to primary vascular disorders. Last but not least, advanced chronic liver diseases can also cause hypertension due to compression of the portal or hepatic veins (Cohn et al. 1991, Szatmári et al. 2002, Cullen et al. 2006a).

3.2.4. Neoplastic diseases

Metastatic neoplasia in the liver is much more common than primary neoplasia itself (Patnaik et al. 1980, Rothuizen et al. 2006a). Hepatocellular neoplasia includes nodular hyperplasia, adenomas and carcinomas. Especially nodular hyperplasia is very common in dogs and almost every dog over ten years of age has multiple hyperplastic nodules within the liver. Hepatocellular adenomas are pale, from friable consistency, very similar to normal liver tissue, and restricted to one or two liver lobes. In contrast, hepatocellular carcinomas are malignant neoplasias that occur as large solitary masses of the hepatocytes, or less frequently on the surface of the entire liver. They also closely resemble normal liver tissue. Metastases can be found in the liver itself, the regional lymph nodes or in other organs. By their infiltrative growth and mitotic activity, they can be distinguished from adenomas. Both carcinomas and adenomas can develop central necrosis, which in turn can lead to abscesses (Patnaik et al.

1981a, Rothuizen et al. 2006a). Cholangiocellular neoplasms can also be divided into adenomas and carcinomas. Adenomas are solitary, well-defined tumours that rarely occur in dogs. Carcinomas, on the other hand, are malignant neoplasms of the epithelium and usually originate from intrahepatic bile ducts. They can appear as large, single nodules or as multiple, irregularly shaped tumours. The distinction between the two is made on the same basis as with hepatocellular neoplasia (Trigo et al. 1982, Rothuizen et al. 2006a). Hematopoietic neoplasia of the liver is very common as part of the generalized or visceral form of the underlying disease. The liver is usually diffusely swollen, pale and affected by lobular patterns, or generally nodular infiltrated. Malignant lymphoma, originating from both B- and T- cells, is the most frequent observed type (Rothuizen et al. 2006a). Hepatic carcinoids are rare neoplasms and can occur intra- and extrahepatically. Although often described as a solitary mass, the intrahepatic carcinoids are mainly multiple nodules due to intrahepatic metastasis. The origin is thought to come from pre-existing neuroendocrine cells in the epithelium of the bile ducts and the gall bladder, but can also be attributed to hepatic progenitor cells (Patnaik et al. 1980, Trigo et al. 1982, Rothuizen et al. 2006a). In addition, hemangiosarcoma, primary vascular and mesenchymal tumours such as lymphangioma, lymphangiosarcoma, fibrosarcoma, leiomyosarcoma, malignant mesenchymoma, osteosarcoma and rhabdomyosarcoma are rarely diagnosed in dogs. They show the same properties as on other sites of the body where they are much more common (Rothuizen et al. 2006a).

3.2.5. Secondary liver diseases

Various systemic diseases or malfunctions that predominantly involve other organs can also damage the liver. A number of non-specific responses to extra-hepatic diseases or exogenous steroids are the most common causes of secondary hepatopathies (Boomkens et al. 2004, Neumann und Danner 2012, Dirksen et al. 2016b). They can be classified as reactive, vascular, toxic, immune-mediated and hormonal. Vascular obstruction can occur with thrombosis, inflammation or congenital changes and is the late common pathway of all mechanisms. It results in death of hepatocytes or cholangiocytes, which in turn leads to acute liver failure, non-cirrhotic portal hypertension, or cirrhosis itself. The innate immune pathway is started by endotoxins. This leads to inflammatory infiltration, the release of cytokines, reactive oxygen species, and finally necrosis. The acquired immune pathway is characterized by the formation of antibodies and antigen-specific cell-mediated attack on hepatocytes. However,

hepatocellular necrosis can also be caused by overnutrition which leads to hyperinsulinemia (Edwards und Wanless 2013).

The group of secondary diseases also includes reversible hepatocellular injury, such as cell swelling, steroid-induced hepatopathy, and steatosis/lipidosis. In general, in these clinical presentations the liver appears focally or diffusely swollen, with focal or diffuse paleness or yellow-brown discoloration and reduced consistency, which in turn leads to increased fragility (Burt et al. 2002, Van Winkle et al. 2006, Edwards und Wanless 2013).

Hepatocellular swelling is mainly the first recognizable sign in any form of cell injury. It appears when the cells are no longer able to maintain the ionic and fluid homeostasis and therefore accumulate water. Steroid-induced hepatopathy is a disease where glycogen is excessively accumulated in the hepatocytes. The causes are usually endogenous (hyperadrenocorticism) or exogenous glucocorticoids (iatrogenic hypercorticism), but the disease can also be caused by drugs or other steroid hormones such as progesterone and aldosterone.

The accumulation of lipid containing vacuoles in the hepatocyte cytoplasm is called hepatocellular steatosis. Steatosis/lipidosis is caused by various diseases. Due to diabetes mellitus or starvation, triglycerides are mobilized from adipose tissue and the fatty acids generated by this process are transported into the liver. Hypoxia leads to a malfunction of the hepatocytes. The reduced energy is missing for the oxidation of fatty acids. Various drugs such as tetracycline cause toxic damage to the mitochondria. In hyperadrenocorticism, the excess of insulin and glucose lead to increased esterification of the fatty acids to triglycerides. In addition, the absorption of hepatotoxins can also lead to a disturbance in the fat metabolism (Burt et al. 2002, Van Winkle et al. 2006).

3.3. Diagnostic procedures for hepatobiliary diseases

3.3.1. Enzymes

ALT

ALT is found in high concentrations within the cytoplasm and mitochondria of the hepatocytes and considered as gold-standard marker for hepatocellular injury (Lawrence and Steiner 2017). It is referred to as cytosolic liver specific enzyme, which means that damage to the cell membrane causes ALT increases (Dirksen et al. 2017a). Due to this fact it is a sensitive indicator for hepatocellular injury and is also called leakage enzyme (Dirksen et al. 2017, Schwendenwein and Moritz 2019). However, it has to be considered that membrane damage does not necessarily result in liver cell death and lots of extra-hepatic diseases can lead to

increased ALT activities (Dirksen et al. 2016, Schwendenwein and Moritz 2019). Even short-term oxygen deficiency can lead to disruption of permeability and thus an increase in ALT (Schwendenwein and Moritz 2019). Distinguishing between irreversible and reversible damage is not possible based on assessment of serum or plasma ALT activity alone (Lawrence and Steiner 2017). Lower ALT activities are also found in skeletal and cardiac muscle (Lawrence and Steiner 2017). Therefore ALT is very specific and increases are considered to be sensitive for hepatocellular injury (Lawrence und Steiner 2017). It shows a half-life ($t_{1/2}$) of two to three days (Sevelius 1995, Center 2007).

AST

AST is located in multiple tissues, such as skeletal and cardiac muscle, kidney, brain and liver, as well as in erythrocytes. Muscle damage and hemolysis can cause considerable increases in AST activity (Lawrence and Steiner 2017). Due to this fact, it is less liver-specific than the ALT but increases and decreases earlier in disease processes (Center 2007, Eman et al. 2018). If AST values are assessed in combination with other hepatic enzymes and creatinine kinase one can distinguish between increases due to hepatic damage or muscle damage (Lawrence and Steiner 2017). In addition to the cytosol, AST is also localized in the mitochondria, which implies that an AST increase is an indication of more severe liver cell damage than a pure ALT increase since mitochondria are affected only during massive damage. As it increases mainly simultaneously with ALT it is also considered to be a sensitive marker for hepatocellular damage (Lawrence and Steiner 2017). AST has a $t_{1/2}$ of five to 22 hours (Center 2007, Schwendenwein and Moritz 2019).

AP

AP is a cholestatic liver enzyme. It is located in the cell membrane, why increases in activity are caused by intrahepatic and extrahepatic cholestasis, hormones or drugs (Schwendenwein and Moritz 2019). In cholestatic diseases, it even rises long before bilirubin does. Since AP occurs not only in the liver and bile duct epithelium, but also in the intestinal and tubular epithelium, mammary glands and osteoblasts, it is not considered to be liver-specific (Center 2007, Dirksen et al. 2017). Nevertheless, the basal AP activity in adult serum is mainly determined by the liver-derived and glucocorticoid-induzed enzyme (Center 2007). In young animals, the osteoblast activity is much higher, which causes up to three times higher AP activity (Schwendenwein and Moritz 2019). Its $t_{1/2}$ in dogs is 70 hours. On the other side, the

AP fraction from the intestine and kidneys, that is mainly excreted by these organs themselves, has a short $t_{1/2}$ from under six minutes - so they hardly affect the basal activity in the blood (Center 2007).

GGT

GGT also is a cholestatic enzyme, but is not only located in the cell membrane of hepatocytes, but also in biliary membranes (Schwendenwein and Moritz 2019). It is present in several tissues, pancreas, kidneys, the intestine and the liver. However, serum enzyme increases are mainly caused due to liver GGT (Center 2007). Both AP and GGT increases are significant for cholestatic disorders (Center 2007, Schwendenwein and Moritz 2019). It has higher tissue specificity as AP but is not as sensitive. Nevertheless, the sensitivity of GGT relating cholestasis is over 90 % in addition to AP, which has a sensitivity of only 50 % (Center 2007). Both lack in specificity of differentiating parenchymal or biliary diseases and $t_{1/2}$ is 72 hours (Center 2007, Lawrence and Steiner 2017).

GLDH

Glutamate-dehydrogenase (GLDH) occurs mainly in the hepatocytes around the central vein. It is present in both, the cytosol and in mitochondria. Along with ALT and AST, an increase is the expression of high-grade liver cell damage. Solitary increases are possible during congestion of the liver secondary to cardiac insufficiency. GLDH is very liver-specific and $t_{1/2}$ is 18 hours (Schwendenwein and Moritz 2019).

3.3.2. Metabolites

Bilirubin

Bilirubin is a product of heme degradation. It mostly derives from the hemoglobin of the erythrocytes and a smaller part from the myoglobin and the heme-containing enzymes of the liver (Steiner 2010). It is degraded primarily in the liver, but also in the spleen and bone marrow of the reticuloendothelial system. As a first step, iron is extracted and recycled. What remains is a protoporphyrin ring that is broken down to bilirubin via biliverdin. This results in unconjugated bilirubin, which is bound to albumin and transported into the liver, where it is glucuronidated by liver cells. The bilirubin thus rendered water-soluble is excreted mainly via the bile but also via the urine or deconjugated in the small intestine via bacteria, then reabsorbed and reconjugated in the liver. Most of the unconjugated degradation product is

excreted as sterkobilin via the faeces, which causes it to acquire its brown color (Schwendenwein and Moritz 2019). Moreover, conjugated bilirubin gets reduced to urobilinogen by bacterial modification, reabsorbed and again cleared by liver cells and small amounts are excreted by the kidneys (Steiner 2010).

Clinically noticeable jaundice is caused by bilirubin concentrations >3-4 mg/dL. The serum is clearly yellow discolored from around 2 mg/dL (Schwendenwein and Moritz 2019). Jaundice is either a sign for hemolysis or a hepatobiliary disease and is divided into three forms. First the hemolytic or prehepatic jaundice, second the intrahepatic and third the posthepatic jaundice. In prehepatic jaundice both prehepatic bilirubin (due to hemolysis) and posthepatic bilirubin (due to anemia and associated hypoxic liver damage) increase. Therefore, the distinction between conjugated and unconjugated bilirubin makes little sense. Because of the intrahepatic and intra-canalicular swelling in hepatopathies, the transport of bile pigments is prevented, often resulting in intrahepatic jaundice. During biliary obstruction dogs quickly develop a posthepatic jaundice (Steiner 2010, Schwendenwein and Moritz 2019).

Bilirubin is not a sensitive marker for hepatic function (Steiner 2010). Moreover, jaundice is a symptome that occurs very late in the course. Furthermore, pancreatic diseases can also cause jaundice due to biliary obstruction. Therefore, AP and GGT are much more sensitive in depicting cholestasis than bilirubin (Schwendenwein and Moritz 2019).

Albumin

Albumin is synthesized in the liver and has a low molecular weight. With a $t_{1/2}$ of seven days it serves the body as important transport protein, determines the oncotic pressure of the plasma and also fulfills buffer functions. When globulin synthesis increases due to an inflammatory reaction, albumin can decrease as a so-called negative acute phase protein (Schwendenwein and Moritz 2019).

Through these functions, the liver lowers the albumin synthesis in acute inflammatory reactions to keep the oncotic pressure constant. However, the concentration of serum albumin hardly decreases. In case of severe hepatopathies, the albumin fraction also decreases, while the globulins increase due to the ingress of antigens across the portal vein. Besides all this, it is important to always keep in mind that the liver has an extremely high reserve capacity relating albumin synthesis (Steiner 2010, Schwendenwein and Moritz 2019).

Urea

The ammonia produced from the breakdown of proteins in the intestine passes directly into the liver where it is detoxified to urea. It is then excreted through the kidney, where it is first filtered into the primary urine and then largely reabsorbed within the henle's loop. Although serum urea changes are mainly associated with kidney and urinary tract disorders, they may also be diminished by decreased synthesis due to liver function disorders or portosystemic shunts (Schwendenwein and Moritz 2019).

Glucose

Glucose is an important source of energy and is therefore kept within narrow margins under physiological conditions. The carbohydrates that enter the small intestine after ingestion are split into simple sugars and absorbed. Accordingly, there may be a postprandial increase in blood glucose. Through the release of insulin, it comes to the uptake of glucose in liver, fat and muscle cells and incorporation into glycogen. The glucose uptake into erythrocytes, neurons and tubular epithelia is not dependent on insulin. To keep the blood sugar level constant, the blood glucose concentration is maintained by glycogen breakdown in liver or gluconeogenesis from fat and muscle tissue (Schwendenwein and Moritz 2019). Glycogenolysis occurs through catecholamines and glucagon, which are released when the glucose level drops. According to this, prolonged postprandial hyperglycemia occurs in severe hepatopathies because the uptake is delayed. If puppies, especially toy breeds, starve or vomit they can show central nervous symptoms due to a drop in blood glucose concentration. Moreover, severe hepatic diseases or starvation in general cause hypoglycemia because the glycogen reserves in the liver are insufficient. Hypoglycemia can occur especially in hunting dogs which are performing heavy physical work, as well as in patients suffering from sepsis or a paraneoplastic syndrome because of increased consumption either by bacteria or the tumor. In addition, endocrinological causes can also lead to reduced blood glucose levels and Persistent hyperglyemia mainly has underlying endocrinologic changes (Schwendenwein and Moritz 2019).

Cholesterol

As starting material for the synthesis of steroid hormones, bile acids and vitamin D, cholesterol is an important precursor for the cell membranes. It is important to mention that the determination of cholesterol is usually carried out for the clarification of hyperlipidemias, endocrinopathies, hepatopathies, nephropathies, enteropathies and acute pancreatitis. Accordingly, a reduction in cholesterol always has to be evaluated in conjunction with other laboratory parameters and is not specific for hepatopathies (Schwendenwein and Moritz 2019).

3.3.3. Function tests

Bile acids

Bile acids are end products of the cholesterol metabolism. They are synthesized by the liver and excreted into the small intestine through the bile, where they are used for fat digestion via emulsification (Anwer 1993, Schwendenwein and Moritz 2019). Subsequently, 95 % of the acids are reabsorbed in the ileum (Schwendenwein and Moritz 2019). Because the first pass clearance is so high, the systemic concentration is very low with approximately 2-8 µmol/l compared to the high concentrations of up to 80 µmol/l in the portal blood (Anwer and Meyer 1995). Vascular malformations and decreases of functional liver mass cause an extreme increase in postprandial bile acids, because the liver can not remove them from the portal blood anymore (Schwendenwein and Moritz 2019). Intrahepatic diseases and congenital or aquired portosystemic vascular shunts are the most common reasons for increased serum bile acid concentrations (Anwer and Meyer 1995, Schwendenwein and Moritz 2019). Due to spontaneous gallbladder contractions, transient increases in concentration can also occur in fasted animals (Schwendenwein and Moritz 2019). Consequently, both values, fasted and postprandial in combination are best for evaluation. For the reasons mentioned above, the determination of the bile acid concentration is well suited for the detection of vascular disorders, but the distinction between individual pathologies is not possible. A study found the sensitivity of fasting serum bile acids concentration for diagnosing portosystemic shunts to be 93% for dogs (Lawrence and Steiner 2017). Since the bile acids usually remain elevated even after surgical correction of the anomalies, they are rather poorly suited for therapy monitoring (Anwer and Meyer 1995, Gerritzen-Bruning et al. 2006, Schwendenwein and Moritz 2019).

Ammonia and ammonium tolerance test

Ammonia is formed in the small intestine by bacterial protein degradation and then passes through the portal blood to the liver where it is detoxified in the course of urea synthesis. Increased blood ammonia concentrations are almost always due to congenital or acquired portosystemic shunts but can also be observed in case of massive loss of functional liver tissue. In addition, of course, defects in the urea cycle can lead to increased levels, but these are extremely rare. As a result of very high increases >1000 µg/dl, hepatoencephalopathy occurs. Since ammonia affects the blood-brain barrier, it leads to disturbances in the cerebral energy balance (Meyer et al. 1978, Gerritzen-Bruning et al. 2006, Schwendenwein and Moritz 2019). While short-term slight increases in NH_4 can be a sign of physical exertion or a high

amount of protein in the diet, a significant long-term increase in this metabolite signals the development of a hepatoencephalic syndrome (Schwendenwein and Moritz 2019). It has been suggested that a greater than 70% reduction of hepatic function is required for serum ammonia concentration to be increased and that the sensitivity of plasma ammonia for the detection of congenital portosystemic shunts is 81% to 100% (Lawrence and Steiner 2017).
The ammonium tolerance test first measures the basal concentration. If the value is within the reference range, 100 mg/kg of encapsulated NH_4Cl are administered orally. After 30-45 minutes, the ammonia concentration is measured again. In healthy dogs where the enterohepatic circulation is intact, at most a minimal increase is detectable. Major sources of error include severe physical strain, congenital urea cycle disorders as observed in Irish Wolfhounds, congenital cobalamin deficiency, or when the person performing the test has previously smoked a cigarette (van Straten et al. 2015, Schwendenwein and Moritz 2019). The test has a sensitivity and specificity of 100 % detecting portosystemic shunting (Meyer et al. 1978, Rothuizen and van den Ingh 1982, Tisdall et al. 1995, Walker et al. 2001, van Straten et al. 2015).

3.3.4. Coagulation

In coagulation two systems can be distinguished, an extrinsic and intrinsic one. The prothrombin time (PT) reflects the extrinsic system and the common pathway of the plasmatic coagulation. Here, prothrombin, factor VII and factor X are particularly sensitive, while factor V and fibrinogen are also detected but less sensitively. The activated partial thromboplastin time (aPTT) is sensitive to factors XII, XI, IX and VIII and is therefore well suited for the screening of hereditary coagulation disorders or disseminated intravasal coagulopathy (DIC). The aPTT checks the intrinsic system of coagulation. Furthermore, the test also covers the prothrombin activity and fibrinogen, but unfortunately, the sensitivity is very low. Thrombin time (TT) is another parameter that can be measured in addition to the commonly collected aPTT and PT. However, this is usually only gauged when PT and aPTT are extended. It counts as screening test for suspected hypofibrinogenemia or fibrin formation disorders (Schwendenwein and Moritz 2019).
To avoid severe bleeding due to caogulopathies coagulation testing should be performed before taking liver biopsies (Rothuizen et al. 2006b). Nearly always PT, aPTT and the platelet count are measured (Badylak et al. 1983, Bigge et al. 2001, Postic et al. 2004, Zimring 2009). Coagulation parameters change very quickly in patients with liver diseases, so the samples

should not be taken more than 24 hours prior to biopsies. Reduced production of clotting factors, inadequate intestinal resorption of vitamin K, or diffuse intravascular coagulation might be reasons for coagulation problems (Rothuizen et al. 2006b). If there is a liver disease present, many dogs might have at least one abnormal coagulation test (Badylak et al. 1983, Postic et al. 2004). Vitamin K deficiency due to serious cholestasis is reflected by increased proteins induced by vitamin K absence and prolonged PT. Deficiencies in the production of clotting factors may occur due to cirrhosis, because the hepatic protein production is reduced. Necrosis of the liver, as found in hepatitis or lymphomas, lead to the activation of the clotting cascade. In such cases, DIC is the most likely cause (Rothuizen et al. 2006b). In spite of there is no information found beyond which limits its critical to take liver biopsies (Rothuizen et al. 2006b), fibrinogen is the most critical indicator. Due to this, reductions below 50 % of the 1 g/l reference level of fibrinogen is considered a contraindication for taking tissue samples. Thus, TT would be useful in this context.
Otherwise, prolonged PT, aPTT or reduced thrombocytes do not predict complications (Rothuizen et al. 2006b). If there is a change within the blood clotting parameters post biopsy bleeding should be checked with ultrasonography (Badylak et al. 1983, Mount et al. 2003, Rothuizen et al. 2006b).

3.3.5. New biomarkers

As already mentioned, the clinical signs of liver diseases are not very specific and the most common indicators for hepatobiliary diseases do not identify mechanism of underlying liver disease. In addition, they cannot distinguish whether the problem is of parenchymal, biliary, vascular or neoplastic origin (Dirksen et al. 2016a, 2017a). MicroRNAs are good biomarkers for a variety of hepatobiliary diseases in human medicine. They have a critical function in the regulation of many aspects of liver development and tend to be small noncoding RNAs which are important regulators of posttranscriptional gene expression (Krol et al. 2010, Dirksen et al. 2016a). A distinction is made between hepatocyte-derived microRNAs (HDmiRs) and cholangiocyte-derived mircoRNAs (CDmiRs). Numerous studies in human medicine have shown that in parenchymal liver diseases, such as chronic or acute hepatitis, microRNAs are sensitive biomarkers to detect the different aetiologies of the disease (Starkey Lewis et al. 2011, van der Meer et al. 2013, John et al. 2014). Even in studies of neoplastic diseases, differences in several microRNAs of healthy and diseased patients were found (Tomimaru et al. 2012). Dirksen et al. performed a study to evaluate if HDmiRs, CDmiRs and oncogenic

microRNAs were measurable in the serum of dogs with several liver pathologies. The following microRNAs were used for the study, adapted to the current human literature: miR-122 and miR148a (HDmiRs in humans), miR-21 and miR-126 (oncogenic miRs in human), and miR-200c and miR-222 (CDmiRs in humans). MiR-21, miR-122 and miR-226, miR-200c and miR-222 show a high potential in the distinction of acute hepatitis, chronic hepatitis, hepatocellular adenoma, hepatocellular carcinoma and lymphoma. Dogs with hepatocellular carcinoma showed an increased serum concentration of miR-200c, miR-21, miR-222 and miR-122. MiR-200c was even only increased in patients with hepatocellular carcinoma. MiR-21 was increased in dogs with hepatocellular carcinoma and lymphoma. Patients who had lymphoma also showed increased concentrations in miR-122. None of these microRNAs was changed in dogs with hepatocellular adenoma (Dirksen et al. 2016a). Another study of Dirksen et al. also reported, that miR-122 has a sensitivity of 84 % and a specificity of 82 % relating hepatocellular injury. Moreover they showed, that miR-122 and miR-148a increase in dogs with acute and chronic hepatitis (Dirksen 2016). In the current study, all patients with biliary tract diseases showed high serum miR-122 concentrations, due to the cell damage as result of the cholestasis. Dogs with mucoceles hat increased concentrations of miR-222. Portosystemic shunt was the only disease where none of the examined microRNAs showed significantly increased concentrations. That is because miR-122 increases upon hepatocellular injury and patients with CPSS to not show enough injuries of cells, to increase the microRNA concentration. As can be seen, circulating microRNAs show great potential to be used as biomarkers in dogs, for the evaluation of hepatobiliary diseases in the future, but need further studies in terms of detection and quantification of microRNAs (Dirksen et al. 2016a).

3.3.6. Imaging Procedures

Abdominal ultrasound

As a non-invasive method, ultrasonography is very valuable for the evaluation of hepatobiliary diseases (Assawarachan et al. 2019). The indications are manifold and range from clinical findings like jaundice, abdominal masses or ascites to suspicion of hepatomegaly and portosystemic shunts or detection of metastases. Moreover it’s the foundation for further studies such as FNA or true cut biopsy (Larson 2016). During ultrasound examination one evaluates the size of the liver, local or diffuse changes in its architecture, the diameter and wall thickness of extrahepatic and intrahepatic bile ducts, the gallbladder itself, vascular abnormalities especially of the *V. portae* and the presence of free abdominal fluid (Rothuizen

et al. 2006b). Allthough ultrasound it is very sensitive at identifying abnormalities in the liver or bile duct it lacks specificity and the probability of making the etiologic diagnosis through ultrasound imaging is low (Marolf 2017). Physiologically the liver appears as coarse and moderately echogenic. It is cranially attached to the diaphragm, caudally to the stomach and the right kidney. The liver is hypoechoic compared to the spleen and nearly equally echogenic to the right kidney. Through the parenchyma hepatic and portal veins course, which can be distinguished by more echogenic margins of the portal veins, which also show a Doppler flow towards the periphery.

The hepatic veins run towards the caudal vena cava at the hilus. The latter extends through the dorsal part of the liver and the portal vein runs immediately ventral from it (Larson 2016).

Normal gallbladder wall measurements are 2-3 mm (Spaulding 1993). It is normally filled with anechoic fluid, but gallbladder sludge is a very frequent finding. Sludge is represented as mobile, echogenic and nonshadowing sediment (Bromel et al. 1998, Larson 2016). The normal bile duct should be less than 3 mm in diameter, but is not consistenly visualized (Center 2009, Larson 2016). The volume of the gallbladder is normally equal to 1 ml/kg body weight (Larson 2016). One of the big advantages of ultrasound is that it can be performed on awake or sedated patients. Moreover it includes the ability of performing studies to detect fluid and soft tissue changes, as well as guided collection of tissue samples for cytological and histopathological interpretation (Assawarachan et al. 2019). A major disadvantage is that that the appearance of many disease processes is similar and that it is difficult to assess the complete organ if the patient is i.e. deep-chested or the stomach and intestine contain gas and overlay the liver (Marolf 2017).

Due to diffuse hepatic diseases the ultrasound of the liver may show changes in size, shape and echogenicity. If focal diseases such as nodules, metastases or abscesses are located within the liver, they have changed echogenicity and may show signs of cavitation, contain gas or have cystic appearance. Changes in gallbladder wall thicknes and echogenicity, or onionskin like apperance are sings of gallbladder pathologies. For this indication ultrasound is extremely helpful in the diagnosis of biliary diseases like inflammation, obstruction, neoplasia or abnormal contents (Larson 2016). Due to this, ultrasonography plays an important role in diagnosis of vascular liver diseases (Rothuizen et al. 2006b). There are new techniques such as the use of contrast agents and elastography. The first one uses microbubbles that create changes in the echogenicity of the parenchyma and increase the conspicuity of lesions. The second one is important for evaluating the firmness of tissues and detection of differences between normal and abnormal ones (Marolf 2017).

CT and MRI

CT and MRI offer the possibility to diagnose abdominal disorders without superimposition of other organs or gas. Due to this, it is possible to asses the whole biliary tree and liver. Multislice CT scanners allow a fast examination, so patients are more often only sedated and not under full anesthesia. In both, CT and MRI, intravenous contrast is used to show the blood flow to the tissues observed. As CT uses iodinated contrast agents and MRI gadolinium contrast agents, MRI offers the better contrast resolution of soft tissues. Furthermore, it offers multiple anatomic layers to visualize the organs.

MRI includes T1-weighted sequences where fluids are hypointens and T2-weighted sequences where fluids are hyperintense. Another technique that is often used is fat saturation, which can highlight inflammation and edema because the fat appears hypointense on T1- and T2-weighted images. The biggest disatvantage of MRI is the need of anesthesia and added expense compared to CT (Szatmári and Rothuizen 2006, Marolf 2017).

In addition, CT provides a good contrast resolution of bones and soft tissues. The reconstruction via software imaging tools serves the ability to evaluate the liver and biliary tract in unlimited angles. Attenuation is the term used to describe tissue characteristics. Due to this, bright tissues are called hyperattenuating, dark ones hypoattenuating and similar tissues isoattenuating. Fluids are generally hypoattenuating. Its disadvantage is primarily the use of ionizing radiation and the added expense (Marolf 2017, Webster et al. 2019b).

On MRI, both T1- and T2-weighted sequences show the physiological liver as uniformly hypointense in relation to the spleen. The gallbladder and bile duct are hypointense on T1-weighted and hyperintense on T2-weighted images. Due to the small size, the physiological common bile duct may not even be identified. What can be evaluated are the gallblader wall, its content, the normally smooth margins and size of the liver (Marolf 2017, Webster et al. 2019b). On CT imaging, the liver shows physiologically isoattenuating composition in relation to the spleen and the gallbladder is hypoattenuating because of the bile storage. Contrast is enhanced uniformly by the liver parenchyma. Same as in the MRI the normal common bile duct may not be seen, but gallbladder wall thickness and intraluminal biliary contents can be visible. The bile is hypoattenuating and should show no signs of internal contents. The hepatic arteries and portal veins can be evaluated with CT angiography.In this procedure, a timed bolus of intravenous contrast is followed by specifically timed scans to show the arteial, venous and delayed phases of the contrast passing through (Makara et al. 2015, Marolf 2017).

3.3.7. Sampling of liver tissue

For the diagnosis of hepatobiliary diseases, the examination of tissue is often unavoidable. There are many different techniques that can be used for obtaining tissue. FNA, needle biopsies, true cut needle, or laparoscopic and surgical biopsies can be performed (Lidbury 2017, Fleming et al. 2019). Coagulation should be tested before performing any of the mentioned methods, except FNA perhaps. It is important to know if the lesions are focal or diffuse, so a good ultrasonography should be done before taking samples of the tissue. Diffuse lesions can be obtained from random sites with all methods. The disadvantage of focal lesions is that they may be missed easily with FNA or true cut biopsies, so the samples should be taken under ultrasound guidance. Caution is advised if the lesion is suspicious to be neoplastic. In this case it is better to sample peripheral regions to avoid central necrosis or the tumours to rise (Rothuizen and Twedt 2009, Lidbury 2017). The pathologist has to be able to assess all zones of the micro architecture of the liver, so it is best to take at least two samples (Desmet and Fevery 1995). Exceptions are always possible, so if there is an inflammatory process or fibrosis, even a good puncture might miss the appropriate area. The obtained tissue is mostly fixed in 10 % neutral buffered formalin but needs specific fixation if electron microscopy or other investigations like immunohistochemistry, in-situ-hybridization or reverse-transcriptase polymerase chain reaction are performed. The latter ones are performed on snap-frozen tissue (Rothuizen et al. 2006b, Hoffmann et al. 2009). It is always good to have the patient fasted for about twelve hours due to the fact that otherwise the stomach might cover the visceral surface of the liver. Furthermore, they might get nauseous if anaesthesia is required and the glycogen amount in the hepatocytes is easier to interpret (Rothuizen et al. 2006b).

FNA

FNA is used to obtain cells, but without the histological context you get with other biopsy methods or surgery (Fleming et al. 2019). It is performed with a disposable 20-22 gauge needle. The use of longer needles is indicated for deep lesions. After aspiration, the content is put on a mounting glass and routinely stained with May-Gruenwald-Giesma. Rubeanic acid or rhodanine staining are used for intracellular copper granules (Weiss and Moritz 2002, Rothuizen et al. 2006). Usually fine needle aspirates are done under ultrasound guidance for focal lesions, but taking the samples blind might also be an option if diffuse lesions are detected (Stockhaus et al. 2004). For the blind puncture, the 10^{th} intercostal space at the level of the connection of rib to rib cartilage is used (Rothuizen und Twedt 2009a, Fleming et al. 2019). For the majority of liver diseases the histological structure is required to properly judge and

find the underlying pathology (Lidbury 2017). If the diagnosis can be made through single cells only (e.g.steatosis, steroid hepatopathy, or neoplastic diseases), FNA might also be an option. Even in the case of coagulopathy fine needle puncture is mostly safe, so blood coagulation does not need to be tested (Rothuizen et al. 2006b). Another advantage of the minimally invasive method is that in most cases no anaesthesia or sedation is required and the samples can be examined immediately after drying and staining (Stockhaus et al. 2004, Fleming et al. 2019).

The gallbladder or large bile ducts should never be damaged by wide core needles, because the risk of rupture is too high. If it is still essential, FNA under ultrasound guidance is the best option. When there is a suspicion of bile duct obstruction it is better to perform no puncture at all, because the chance of rupture or bile leakage exists (Rivers et al. 1997, Stockhaus et al. 2004, Rothuizen et al. 2006b).

True cut needle biopsies

If a histological examination is required, a true cut biopsy or a laparoscopic or surgical tissue extraction is necessary. Within the true cut needles there is an inner needle. This inner needle is first inserted and then the outer cutting needle is inserted over the inner one. Because of the very sharp edges, true cut needles should only be used with ultrasound guidance, so that no surrounding tissue is injured (Hoffmann et al. 2009, Rothuizen and Twedt 2009). Of course, true cut needles can also be used in surgeries where there is a direct view of the liver. There are three different types, the manual, the semiautomatic and the biopsy gun. The method with the gun is very good for small or firm fibrotic livers, because it is so quick, the organ cannot escape even if it is freely moving in ascites. Generally true cut needles tend to produce the best interpretable tissue samples and hepatobiliary tumours, parenchymal or biliary diseases are often diagnosed through histopathological examination (Rothuizen et al. 2006b, Lidbury 2017). The biggest disadvantage though is, that half of the lumen is filled with the inner needle, so the samples are relatively small (Rothuizen and Twedt 2009).

Laparoscopic biopsies

Additionally to the needle biopsies there is also the possibility to get tissue samples through laparoscopy (McDevitt et al. 2016). Usually the tissue is taken from local changes with forceps. A disadvantage of this method is, that the tissue comes from the subcapsular superficial region and due to that might not be representative enough. Subcapsular fibrosis that is normal may be misinterpreted and forceps often lead to compression artefacts (Geller 1994, Rothuizen et

al. 2006b). A better representation is given by deeper true cut needle samples taken with an endoscope. Due to the possibilities given by ultrasound guided needle biopsies direct sampling has nearly become obsolete (Rothuizen et al. 2006b, Lidbury 2017).

Surgical biopsies

In addition to the forceps biopsies, samples can also be taken surgically. The preferred depth is 2 cm and if the liver is diffusely affected more than one sample should be taken. Even though wedge samples are large, that is not enough indication for invasive surgery (Geller 1994, Rothuizen et al. 2006b, McDevitt et al. 2016).

4 Material and methods

4.1. Study population

In this retrospective study, all dogs presented to the Small Animal Internal Medicine Service (Small Animal Clinic, University of Veterinary Medicine, Vienna) from 01.12.2009 to 30.11.2017 and receiving an FNA of the liver were enrolled. During this period, 465 fine needle aspirations were performed at the clinical unit of diagnostic imaging. However, only 291 cases were further processed and supervised at the Small Animal Internal Medicine Service. Since the data sets of the other 174 patients were not complete and not fully in our hands, these patients were excluded from the study.

4.2. Data collection

The data of the remaining 291 cases was gathered using the electronic animal hospital information system (TIS VetWare, Agfa Health Care). A patient list was created containing the following data: date of examination, type and number of samples, animal ID, breed, age and sex. For this purpose, an EXCEL sheet (Microsoft Office 2010) was created and subsequently expanded to include all data of the diagnostic methods used. This included hepatic enzymes (ALT, AST, AP, GGT, GLDH), metabolites (bilirubin, albumin, urea, glucose, cholesterol and ammonia), function tests (bile acids, ammonia tolerance test), platelets and coagulation times (aPTT, PTT, TT) and potential other tests at initial presentation. All imaging procedures such as ultrasound, X-ray, CT and MRI were taken into account. Furthermore, FNAs and biopsies with their cytological or histopathological examination were included. Where existing, results of bacteriology, virology or parasitology of hepatic tissue or other organs were included. Finally, hormonal changes and stimulation tests, which may be related to the hepatic pathology, were added. In case of death of a patient, any existing histopathology was included.

Using this EXCEL spreadsheet, the patients were categorized into four primary groups (parenchymal, biliary, vascular, neoplastic) and one group for secondary hepatopathies in regard to their final diagnosis. To allocate the patients to the respective groups, the results of further diagnostics especially of FNA and biopsies were used. Moreover necropsy, CT, ultrasound, serology, a parasitological and bacteriological examination were decisive for allocation. Dogs suffering from primary hepatic tissue transformation in terms of acute or chronic hepatitis, cell degeneration, amyloid accumulation or abscesses were assigned to the parenchymal group. FNA, biopsies, necropsy, CT, serology and parasitology were the

diagnostic tools used to assign the diseases to this group. FNA, necropsy, ultrasound and bacteriological examinations were used to identify if the gallbladder or biliary system was affected in course of a pathology. Such cases were allocated to the group of biliary liver diseases. Patients that had abnormal vascular branches and changes in the primary vascular system were included in the vascular group. For the identification of these diseases biopsies, ultrasound and CT were used. Hepatic tumours, increased mitotic rates or abnormal cell infiltration led to the classification of the neoplastic group. They were allocated to this group because they were identified with FNA, biopsies, necropsy or CT.

There were several non-specific reactions to extrahepatic diseases or exogenous steroids found. These reactive hepatopathies were summarized in the group of secondary liver diseases and assigned to this group because of the outcome of FNA, biopsies, necropsy, serology and coombs test.

4.3. Statistical evaluation

A descriptive statistic was carried out in which the frequency of the diagnostic methods, the distribution of the patients among the groups, and the prevalence of diseases per group were calculated. Therefore, obtained categorical data like sex, groups and diagnoses is represented as absolute numbers and relative frequencies (percentage). These are shown graphically by using bar or pie charts. Metric data (e.g., age) is displayed using median value and range (minimum and maximum). Hepatic enzymes (ALT, AST, AP, GGT, GLDH), metabolites (bilirubin, albumin, urea, glucose, cholesterol and ammonia), function tests (bile acids, ammonia tolerance test), platelets and coagulation times (aPTT, PTT, TT) were compared with the physiological range and then displayed together with the deviations using bar charts. AP and ALT were also tested for their sensitivity in the individual groups. ALT was considered as gold-standart for hepatocellular injury. All diagnostic tools that lead to a final diagnosis were compared to each other in percentages and pie charts.

For the comparison of FNA and biopsies significance was tested using a chi^2-test in EXCEL (Microsoft Office 2010). P-values <0.050 were considered significant. Both examination methods were also tested for overall sensitivity and sensitivity regarding the individual parenchymal diseases and the neoplastic and secondary group.

5 Results

Out of the 291 patients, sixteen patients were excluded from the study because there was insufficient information, or the available data did not allow a clear interpretation. Another twenty-one dogs were excluded because they suffered from more than one hepatobiliary disease. Therefore, only 254 dogs remained for further analysis. The median age of the patients at the time of initial presentation was 10.31 years. The youngest dog was 0.19 and the oldest one 16.69 years old. The population included 58 (22.83 %) intact males, 39 (15.35 %) intact females, 63 (24.80 %) castrated males and 94 (37.01 %) spayed females. Most common breeds were mixed breeds (79), Golden Retriever (17), German Shepherd (9), Labrador Retriever (9), Maltese (8), Havanese (7), Chihuahua (6), Rottweiler (6), West Highland White Terrier (6), Beagle (5), Cocker Spaniel (5), Shih Tzu (5), Terrier (5), Yorkshire Terrier (5), Border Collie (4) and Magyar Vizsla (4). The dogs were assigned to the primary and the secondary groups as displayed in Tab. 1.

Table 1: Distribution of groups

Parenchymal	Biliary	Vascular	Neoplastic	Secondary
147 (57.87 %)	6 (2.36 %)	3 (1.18 %)	79 (31.10 %)	19 (7.48 %)

5.1. Epidemiological data

The five groups were compared in terms of age, gender, sexual status and breed.

5.1.1. Age

The comparison of minimum, maximum and median age of the four primary and the secondary group is displayed in Tab. 2. Patients suffering from vascular changes were on average seven years younger than the dogs of the other groups.

Table 2: Comparison of age of the groups

	Minimum	Median Age	Maximum
Parenchymal	0.19	10.62	16.69
Biliary	4.89	10.32	13.30
Vascular	0.44	1.24	6.67
Neoplastic	3.37	9.72	14.06
Secondary	8.72	10.01	15.00

5.1.2. Gender

Regarding the parenchymal and biliary group, the majority of dogs were neutered females. In contrast, the gender ratio was relatively balanced in the neoplastic and secondary group, with overall more neutered canines. All patients in the vascular group were female. The results are summarized in Tab. 3

Table 3: Comparison of gender of the groups

	Male	Male/neutered	Female	Female/neutered
Parenchymal	37 (25.17 %)	32 (21.77 %)	18 (12.24 %)	60 (40.82 %)
Biliary	2 (33.33 %)	1 (16.67 %)		3 (50.00 %)
Vascular			2 (66.67 %)	1 (33.33 %)
Neoplastic	17 (21.52 %)	23 (29.11 %)	16 (20.25 %)	23 (29.11 %)
Secondary	2 (10.53 %)	7 (36.84 %)	3 (15.79 %)	7 (36.84 %)

5.1.3. Breed

The majority of dogs (79; 31.10 %) were mixed breeds. Forty-seven of them suffered from parenchymal, 21 from neoplastic, eight from secondary and three from biliary diseases. A particularly large number of Golden Retrievers (11/16) are in the neoplastic group, whereas Labrador Retrievers (7/9), Maltese (6/8) and Havanese dogs (5/7) were mostly diagnosed with parenchymal diseases. All breeds in comparison to the groups are displayed in Tab. 4.

Table 4: Comparison of breeds of the groups

	Parenchymal	Biliary	Vascular	Neoplastic	Secondary
Airedale Terrier				1	
Alaskan Malamute	1				
American Cocker Spaniel	1				
American Staffordshire Terrier				1	1
Australian Shepherd	1			1	
Austrian Black and Tan Hound				1	
Beagle	4			1	
Berger Blanc Suisse	1			1	
Bernese Mountain Dog					1
Bloodhound				1	
Border Collie	2			2	
Boxer	1			1	

Briard	1				
Bullmastiff				1	
Cairn Terrier	1				
Chihuahua	4			1	1
Cocker Spaniel	2			3	
Dachshund	3				
Dalmatian	1			1	
Doberman	2			1	
Drentse Patrijshond	1				
English Setter	1				
Entlebuch Mountain Dog	1				
Flat Coated Retriever	3				
Fox Terrier Wirehaired Pointer	2				
Foxterrier Smoothhaired Pointer			1		
French Bulldog				1	
Galgo Español				1	
German Pinscher	1				
German Shepherd	3	1		4	1
German Shorthaired Pointer	1			2	
Golden Retriever	4	1		11	
Havanese	5			2	
Irish Terrier	1				
Irish Wolfhound				1	
Jack Russel Terrier	2			1	
Labrador Retriever	7			1	1
Leonberger	1				
Lhasa Apso		1			
Magyar Vizsla	3				1
Maltese	6			2	
Miniature Schnauzer	1				
Mixed breeds	47	3		21	8
Other Dogs					1
Papillion	1		1		
Parson Russell Terrier	1				
Pekingese	1				
Pinscher					1
Poodle	2				
Pug	1			1	
Retriever	1				
Rhodesian Ridgeback				1	
Rottweiler	1		1	3	1
Roughhaired Dachshund	2			1	
Samoyede				1	
Scottish Terrier	1				
Shar-Pei	1				
Shepherd Dog	1				
Shih Tzu	4			1	
Shorthaired Dachshund				1	
Small Münsterländer	1				
Spaniel	1			1	
St. Bernhard Dog	1				
Terrier	4			1	
Tervueren				1	
Tibet-Spaniel					1
Weimaraner	1				
West Highland White Terrier	3			2	1
Yorkshire Terrier	4			1	

5.2. Groups

The group of parenchymal pathologies included 147 cases (57.87 %) and six different diseases (hepatocellular degeneration, acute hepatitis, regeneration nodules, chronic

hepatitis, amyloidosis and abscesses). FNA, biopsies, necropsy, CT, serology and parasitology were used for the allocation of these diseases to the parenchymal group. The group of biliary pathologies involved only six patients (2.36 %) with four different diseases (cholecystitis, bile duct obstruction, cholestasis and cysts). FNA, necropsy, ultrasound and bacteriological examinations were used to identify these diseases. Vascular changes were only found in three patients (1.18 %) with three different disorders (extra- and intrahepatic shunt, microvascular dysplasia). Biopsies, ultrasound and CT were used to allocate these diseases to the vascular group. Seventy-nine patients (31.10 %) patients were assigned to the neoplastic group, which were subsequently divided into the five subgroups epithelial, mesenchymal, round cell and neuroendocrine tumours or hepatic neoplasia without further differentiation. In total, 13 different tumour types were recorded and they were identified with FNA, biopsies, necropsy and CT. Last but not least, 19 patients (7.48 %) had a liver disease secondary to an underlying disease with four different disease complexes (excess glucocorticoid, hepatolipidosis, IMHA, infarction due to torsion). FNA, biopsies, necropsy, serology and coombs test were used to identify these diseases. Fig. 1 shows the distribution of the patients among the groups in percentages.

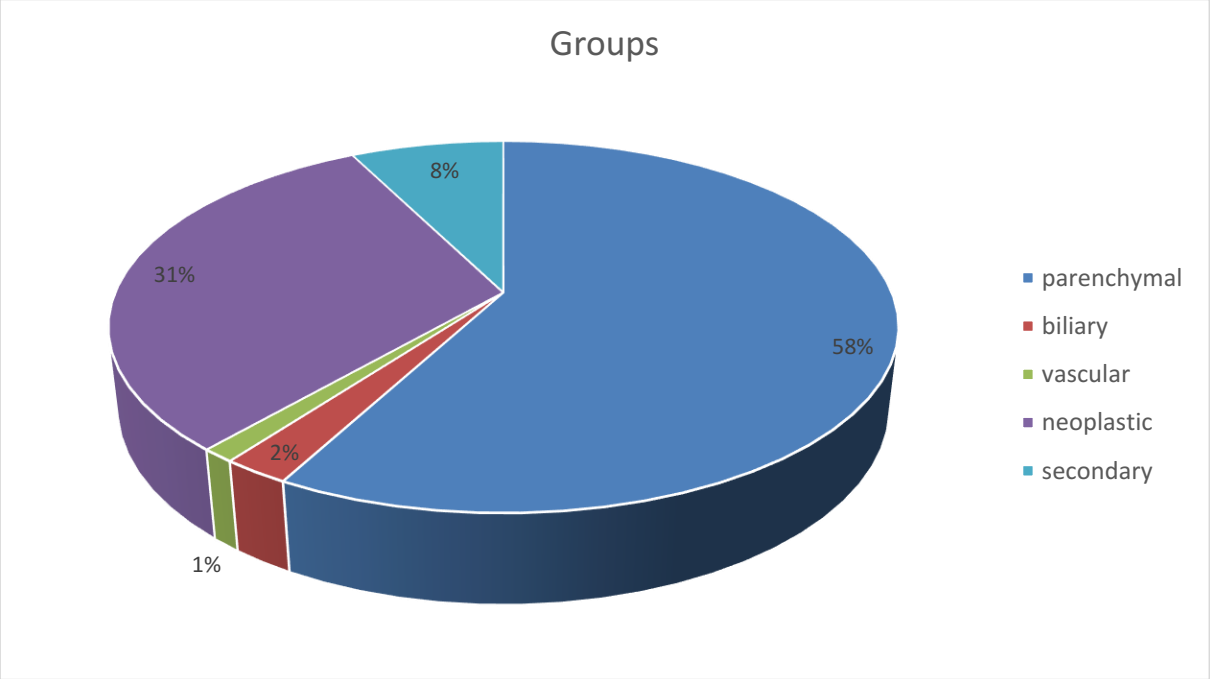

Figure 1: Groups

5.3. Diagnostics

FNA was performed for all 254 cases but only led to a final diagnosis in 178 patients (70.08 %). One dog was diagnosed through the combination of FNA and Coombs test. In 65 cases (25.59 %), liver biopsies and a histopathological examination was performed leading to a final diagnosis in 55 patients (four of them only confirmed the already correct diagnoses from FNA). Therefore, liver biopsies led to a final diagnosis in 55/65 patients (84.61 %). Another patient was diagnosed with the combination of histology and a parasitological examination.

Out of the 9/65 (13.84 %) where liver biopsies did not generate the final diagnosis, the conclusive results were obtained with FNA (5), necropsy (2), or CT (1). One sample contained not enough tissue for histopathological examination.

A post-mortem histopathological examination was necessary for the diagnosis of twelve dogs (4.72 %). Five more patients (1.97 %) were diagnosed by CT and three more (1.18 %) by ultrasound. One disease (0.39 %) could be identified using ultrasound and bacteriological examination together. Finally, two patients (0.79 %) got their diagnosis through a serologic test. Fig. 2 summarizes the diagnostic tools leading to the final diagnosis.

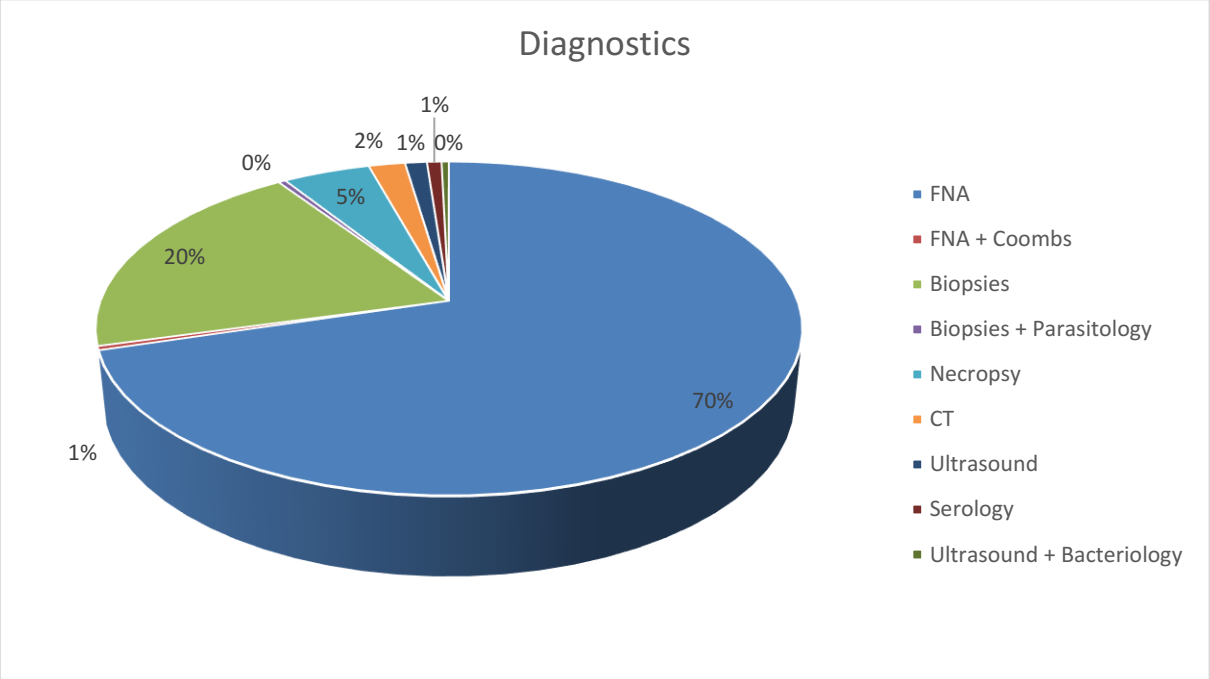

Figure 2: Diagnostic tools leading to the final diagnosis

There was a significant difference between the frequency of the correct outcome of FNA and biopsies (p=0.018). The sensitivity of FNA was 70.08 % (178/254) and 84.61 % (55/65) for biopsies. The sensitivities of FNA and biopsies in relation to parenchymal as well as neoplastic diseases and the secondary group are compared in Tab. 5.

Table 5: Sensitivity of FNA and biopsies regarding parenchymal, neoplastic and secondary changes

Sensitivity						
	Parenchymal				Neoplastic	Secondary
	Acute hepatitis	Chronic hepatitis	Hepatic cell degeneration	Regeneration nodule		
FNA	84.90 %	50.00 %	92.86 %	66.67 %	55.70 %	42.11 %
Biopsies	62.50 %	66.67 %	100.00 %	100.00 %	93.75 %	85.71 %

Furthermore, selected values of blood chemistry (bilirubin, albumin, urea, glucose, creatinine, total protein), all liver enzymes (ALT, AST, AP, GGT, GLDH), function tests (bile acids, ammonia), electrolytes and minerals (potassium, sodium, chloride, calcium, phosphate) as well as the coagulation status (platelets, PT, aPTT, TT) were examined for deviations from the physiological range. Regarding ALT and AP, the sensitivities overall and in relation to the five subgroups were evaluated and listed in Tab. 6. All parameters are discussed in more detail in course of the individual groups in chapter 5.4.

Table 6: Sensitivity of ALT and AP overall and regarding the groups

Sensitivity						
	Overall	Parenchymal	Biliary	Vascular	Neoplastic	Secondary
ALT	75.53 %	76.09 %	83.34 %	50.00 %	73.98 %	77.78 %
AP	73.01 %	72.18 %	50.00 %	0.00 %	76.81 %	81.25 %

5.4. Subgroups

5.4.1. Parenchymal diseases

One hundred forty-seven out of 254 cases (57.87 %) were assigned to the group of parenchymal disorders (Fig. 3). Hepatic cell degeneration without further differentiation was the final diagnosis of 70 patients (47.62 %). Fifty-three dogs (36.05 %) suffered from acute hepatitis, two of them (1.36 %) were of infectious origin (leptospirosis and dirofilariosis, respectively). Regenerative nodules were found in twelve cases (8.16 %). Chronic hepatitis was diagnosed in ten patients (6.80 %), where one had obvious signs of copper toxicity. One animal (0.68 %) was diagnosed with amyloidosis and one (0.68 %) with liver abscesses.

FNA led to the final diagnosis in 124 cases (84.35 %). Of these, 65 emerged as hepatic cell degeneration, 45 as acute hepatitis, eight as regeneration nodules, five as chronic hepatitis

and one case as amyloidosis. Sixteen dogs (10.88 %) got their diagnosis with the help of a histopathological examination after biopsies (four cases each with acute hepatitis, liver cell degeneration, regeneration nodules, and chronic hepatitis (one of which with copper toxicity). One of the patients (0.68 %) with acute hepatitis suffered from dirofilariasis, which was determined by histology in combination with a parasitological examination. In four dogs (2.72 %), the cause of disease was found in necropsy. Two of them suffered from acute and one from chronic hepatitis, another patient from hepatocellular degeneration. The suspected diagnosis of liver abscesses was made in one case (0.68 %) using a CT scanner. One (0.68 %) case of acute hepatitis (leptospirosis) was diagnosed with serologic testing using a micro agglutination test. Fig. 4 shows which diagnostic aids were used to make the individual diagnoses of the parenchymal group.

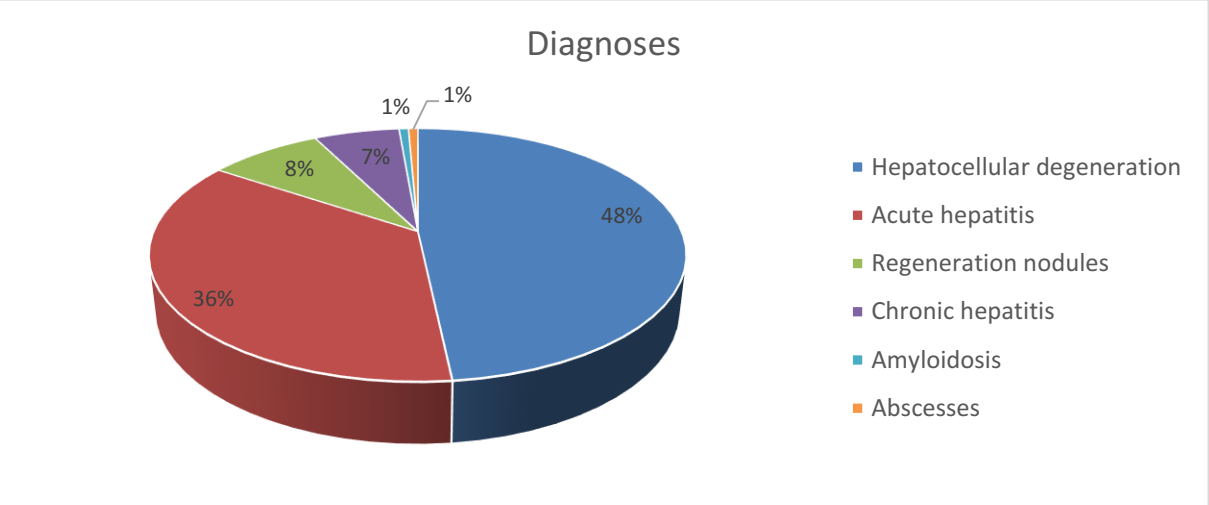

Figure 3: Diagnoses of the parenchymal group

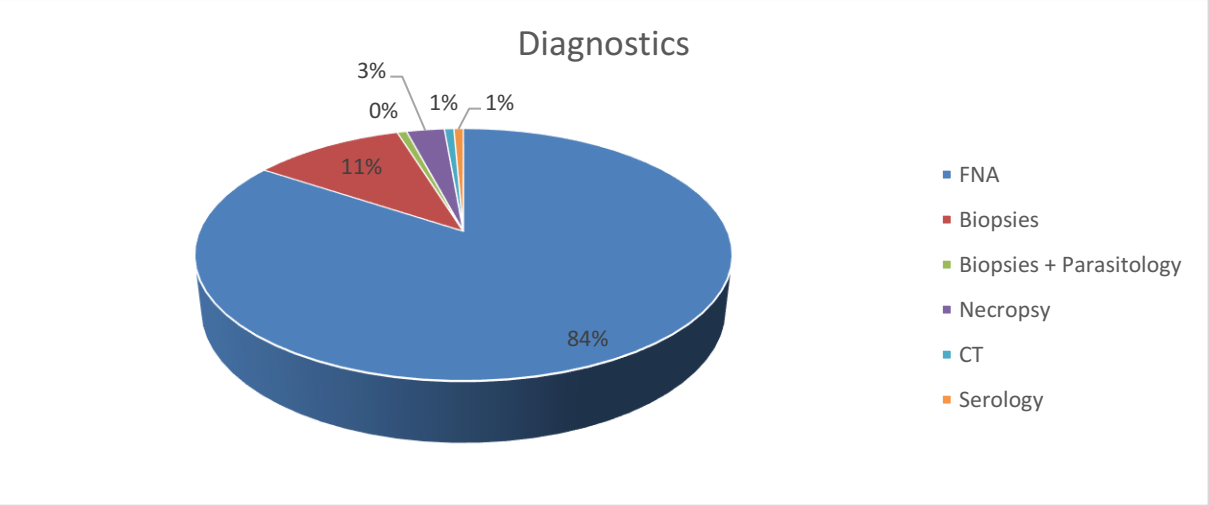

Figure 4: Diagnostic tools used for diagnosis of the parenchymal group

Due to the retrospective nature of this study, not all data sets were complete with all diagnostic measures (see individual Figs.). The parameters of blood chemistry are shown in Fig. 5. Glucose was the only value that deviated from the normal range in a large number of parenchymal cases. Overall, it was increased in 58 (49.57 %) of 117 evaluated data sets. In regard to the liver enzymes, ALT was increased in 105/138, AP in 96/133, GGT in 30/48 times and GLDH in 46/83 and thus in a majority of the patients (Fig. 6). Therefore, the sensitivity of ALT was 76.09 %, of AP 72.18 %, of GGT 62.50 %, and of GLDH 55.42 %. The evaluated data sets in relation to functional tests, electrolytes, minerals and coagulation status are explained in more detail in Fig. 7, 8, and 9.

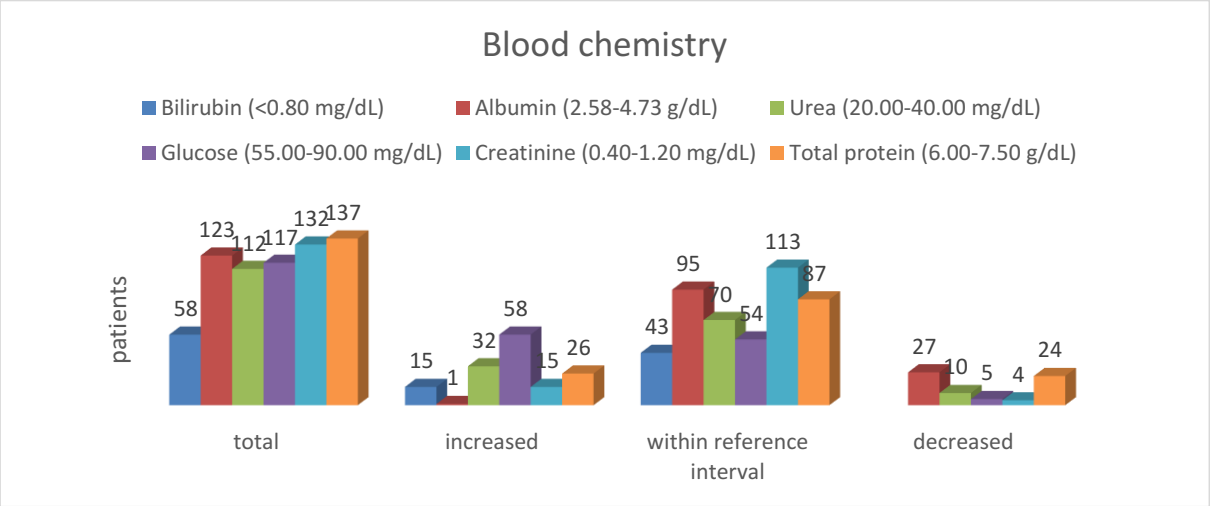

Figure 5: Values of blood chemistry in the parenchymal group

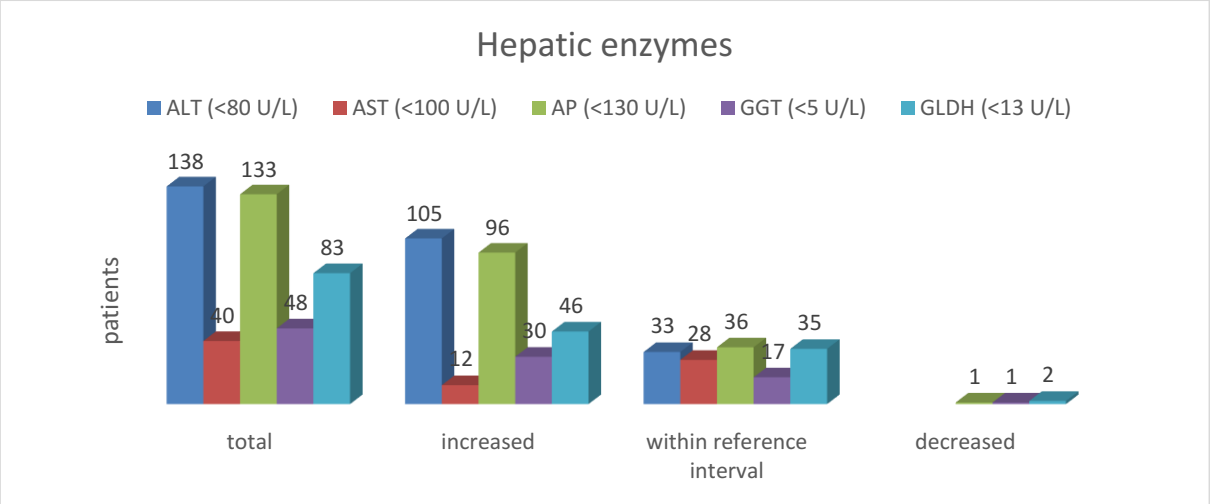

Figure 6: Values of hepatic enzymes in the parenchymal group

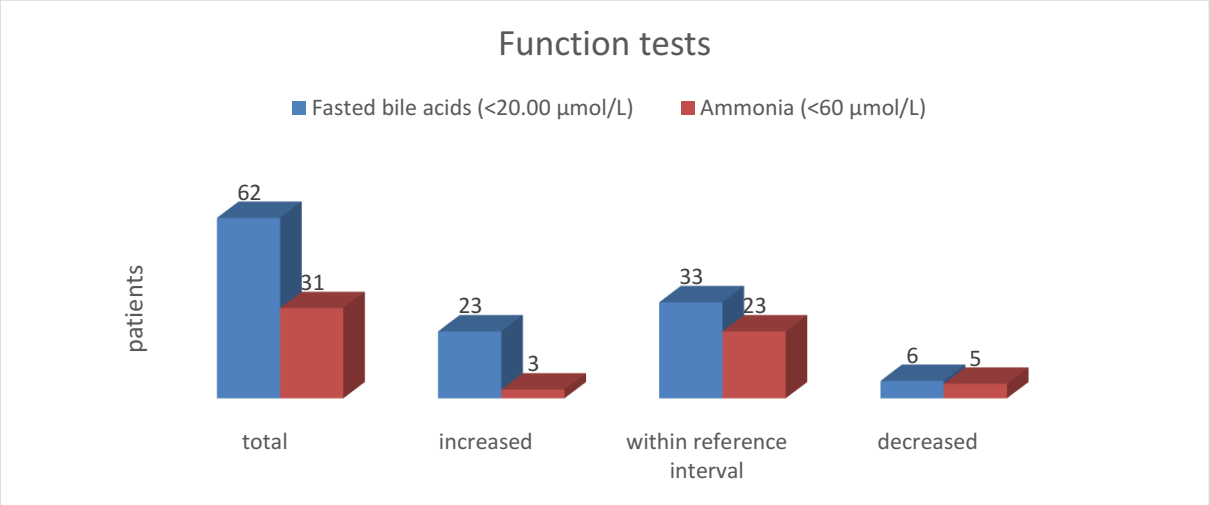

Figure 7: Values of function tests in the parenchymal group

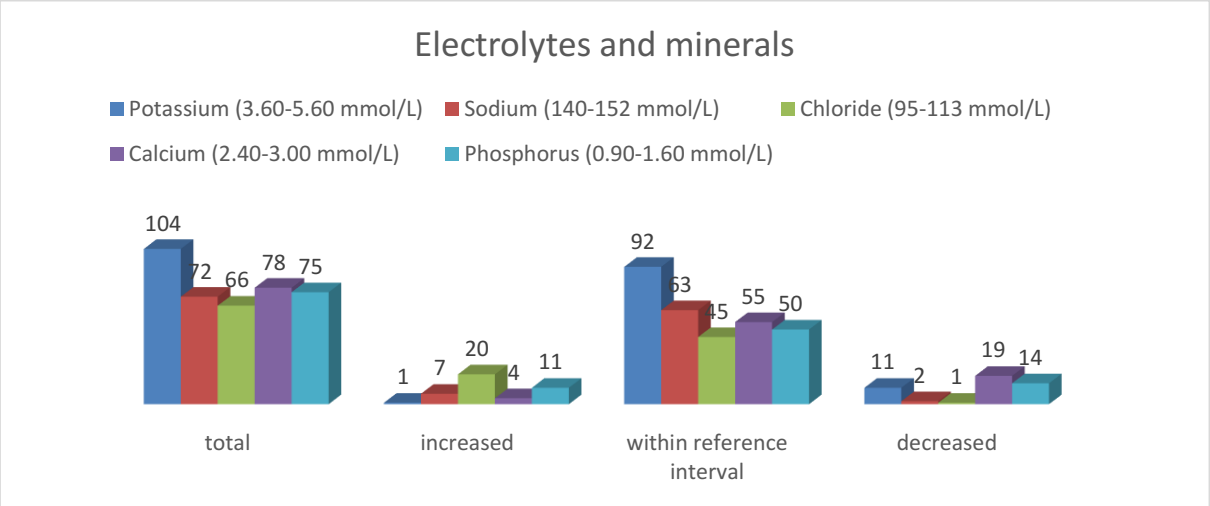

Figure 8: Values of electrolytes and minerals in the parenchymal group

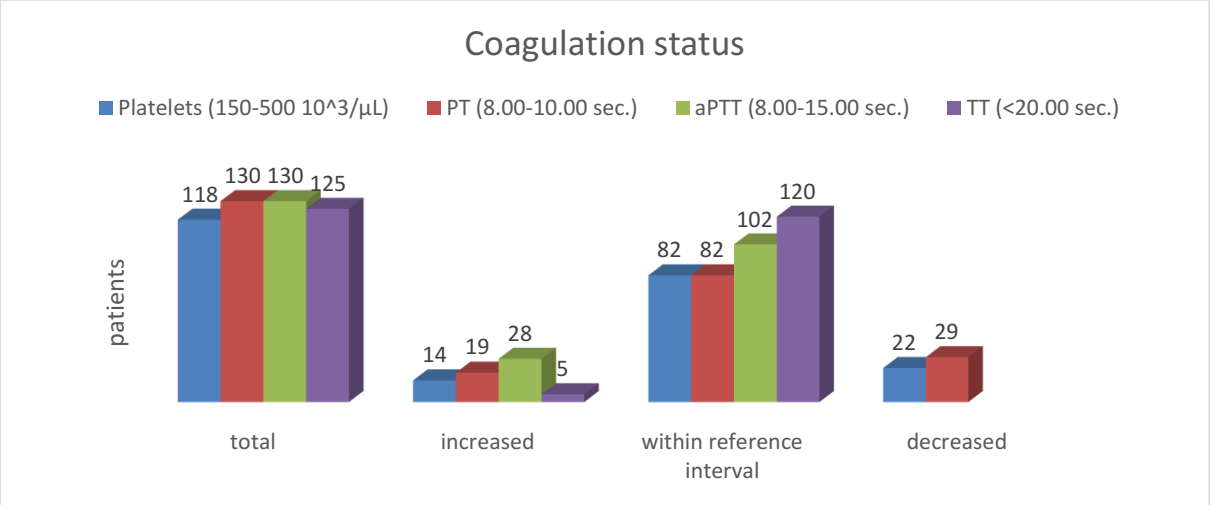

Figure 9: Values of coagulation in the parenchymal group

5.4.2. Biliary diseases

The biliary group included six out of 254 cases (2.36 %). Two (33.33 %) of them were diagnosed with cholecystitis, two (33.33 %) had underlying bile duct obstruction, and one patient each (16.67 %) had cholestasis and liver cysts, respectively (Fig. 10).

Cholestasis and liver cysts were the two cases (33.33 %) diagnosed using FNA (Fig. 11). Although the cysts were already visible on ultrasound, the fluid clarified as cystic on FNA. One case (16.67 %) of obstructed bile ducts was found in necropsy and the second one on ultrasound. The two patients suffering from cholecystitis also got their diagnosis through ultrasound, one of the two was confirmed with a positive bacteriological culture.

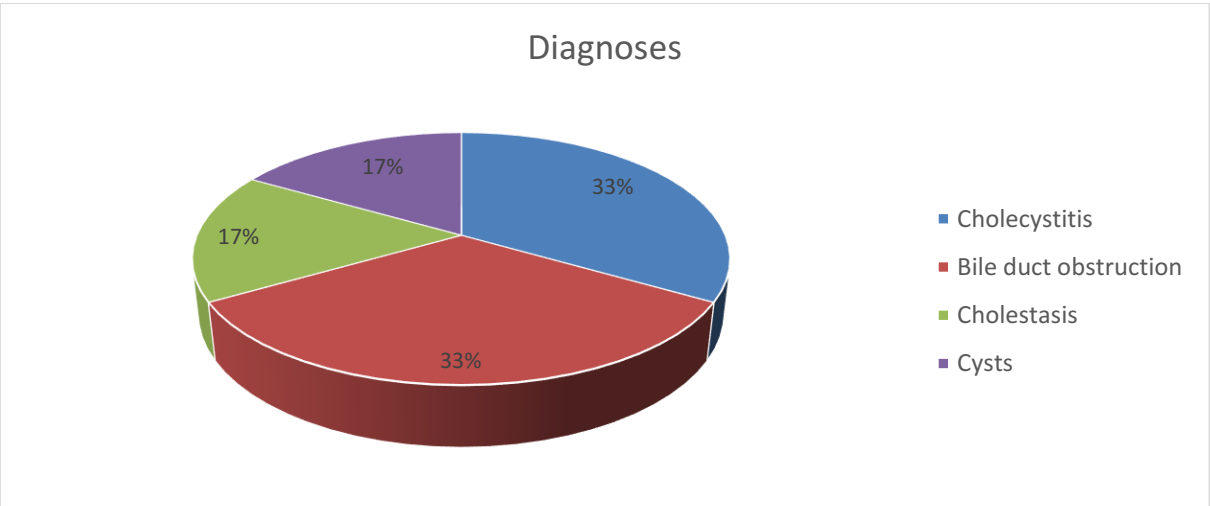

Figure 10: Diagnoses of the biliary group

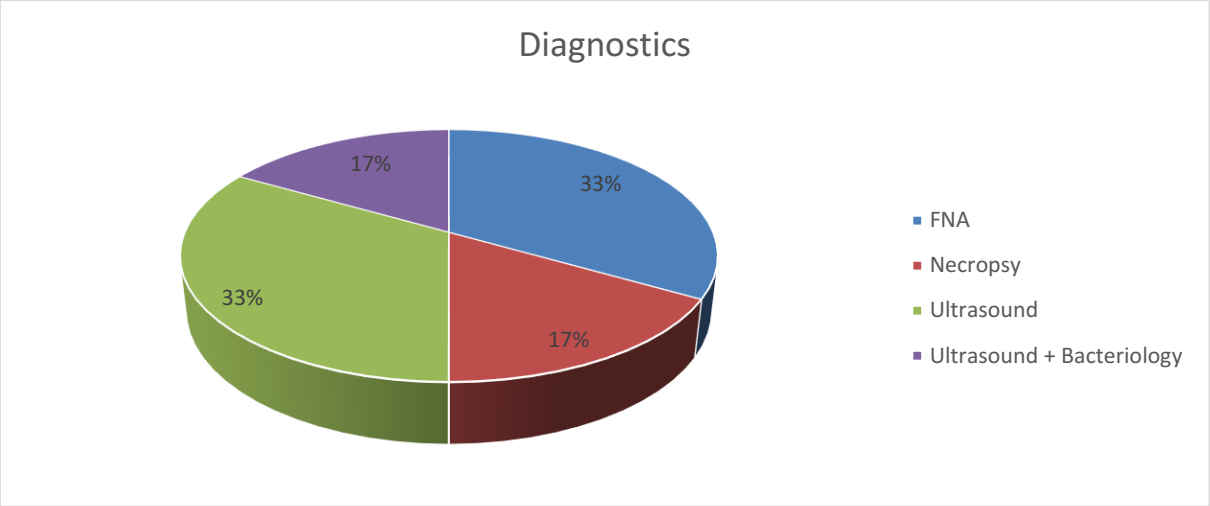

Figure 11: Diagnostic tools used for diagnosis of the biliary group

Due to the retrospective nature of this study, not all data sets were complete with all diagnostic measures (see individual Figs.). ALT was increased in 5/6 cases and thus in the majority of patients. The sensitivity of ALT regarding biliary diseases was 83.33 %, the one of AP 50.00 % (see Fig. 13). GGT was only tested in two patients but was elevated in both of them. Therefore, the sensitivity of GGT was 100.00 % for the biliary group. Chloride was elevated in three of the five cases (60.00 %) measured (Fig. 15). Figs. 12, 14 and 16 show the deviations of the tested values for blood chemistry, the functional tests and the coagulation in relation to the biliary group.

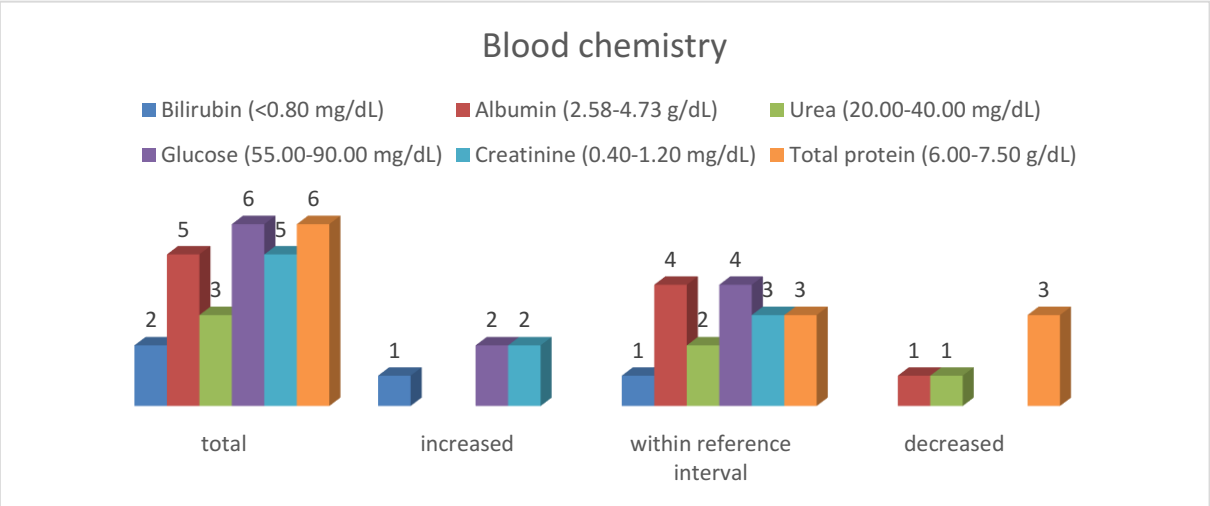

Figure 12: Values of blood chemistry in the biliary group

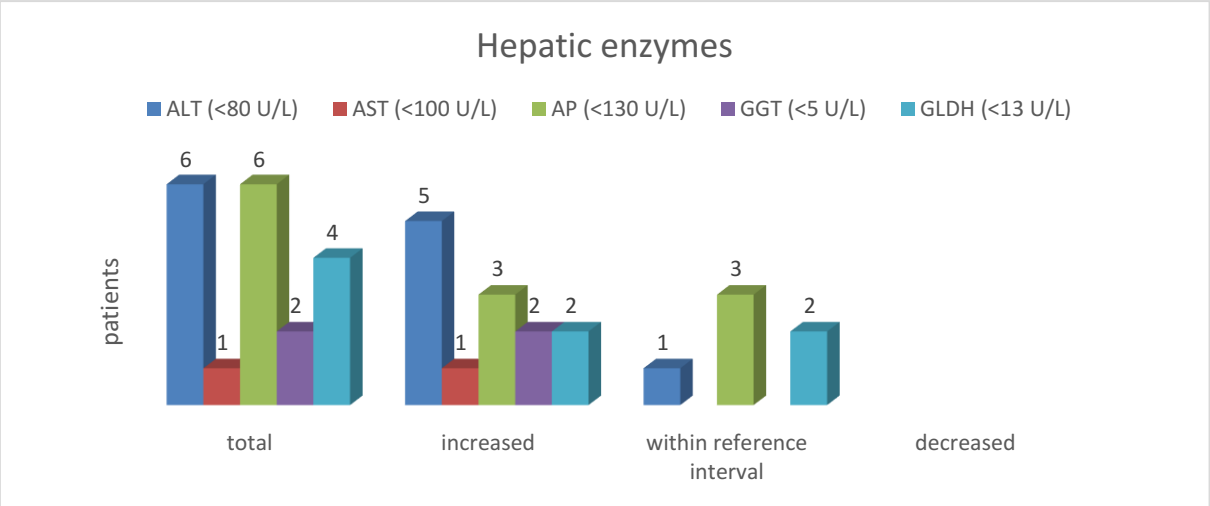

Figure 13: Values of hepatic enzymes in the biliary group

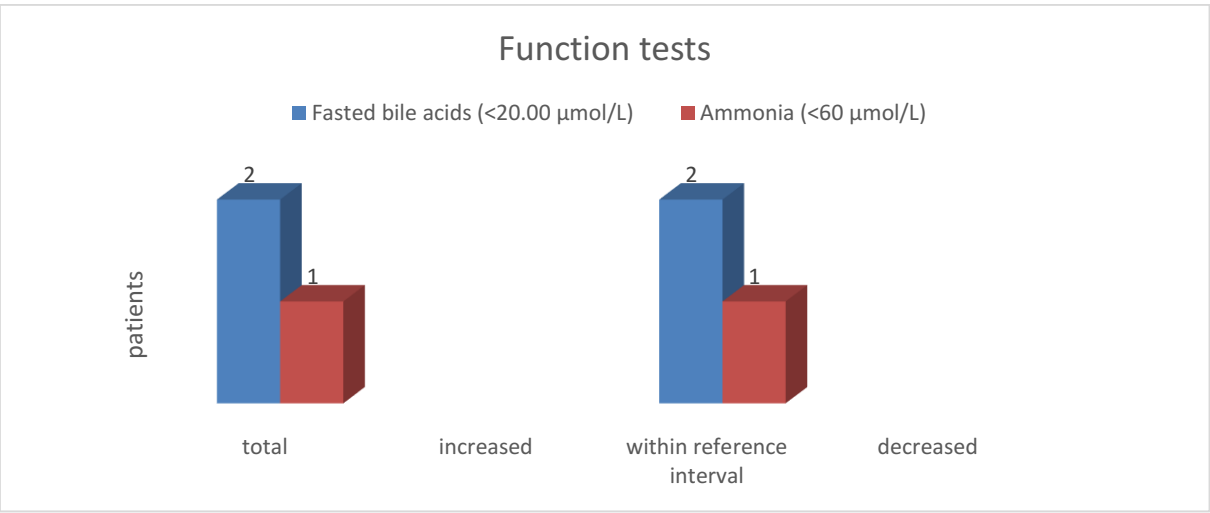

Figure 14: Values of function tests in the biliary group

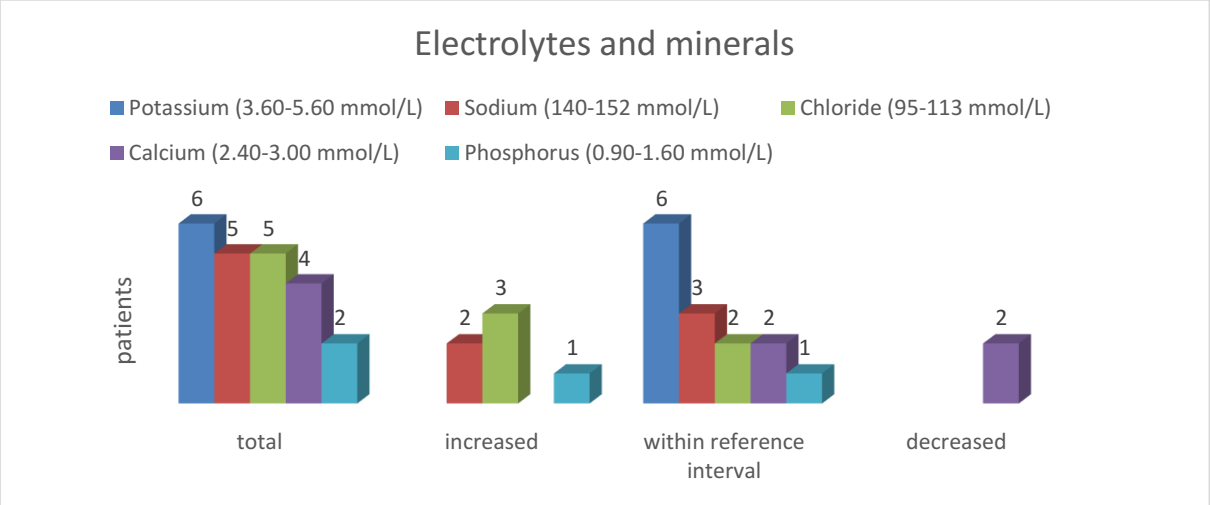

Figure 15: Values of electrolytes and minerals in the biliary group

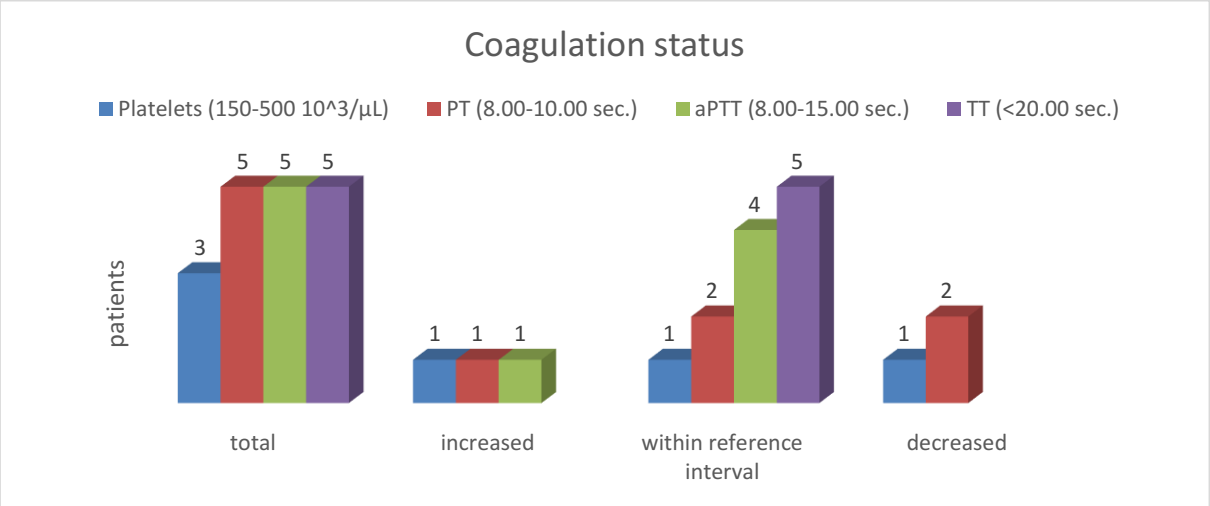

Figure 16: Values of coagulation in the biliary group

5.4.3. Vascular diseases

Only three out of 254 patients (1.18 %) suffered from vascular diseases. Two (66.67 %) had a portosystemic shunt, one intra- and one extrahepatic, respectively (Fig. 17). The third patient (33.33 %) had microvascular dysplasia proven by histopathological examination of biopsies (33.33 %), whereas the shunts were both diagnosed with imaging procedures (extrahepatic with ultrasound and intrahepatic with CT, respectively; Fig. 18).

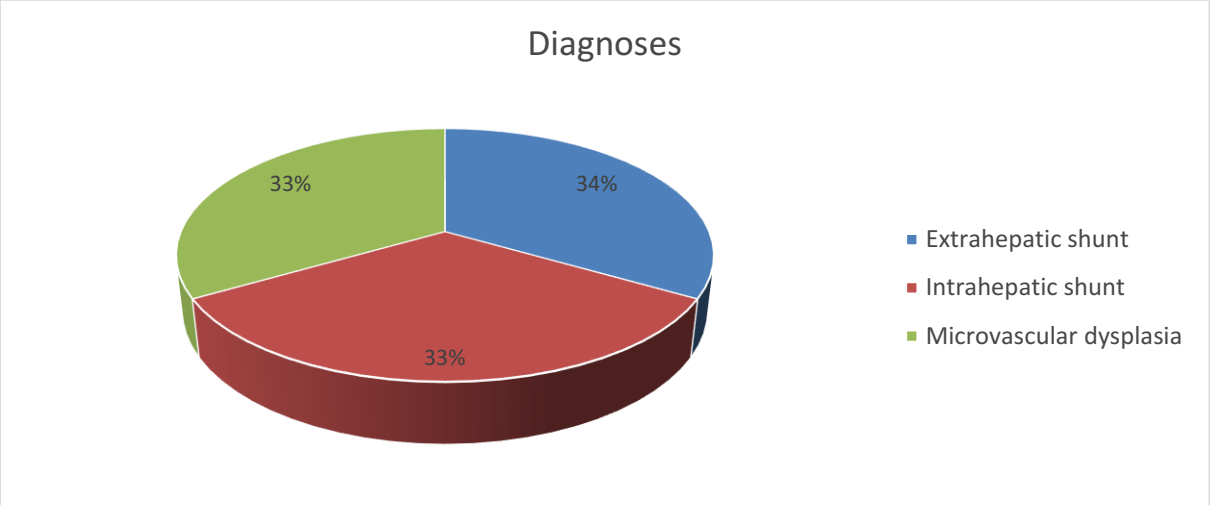

Figure 17: Diagnoses of the vascular group

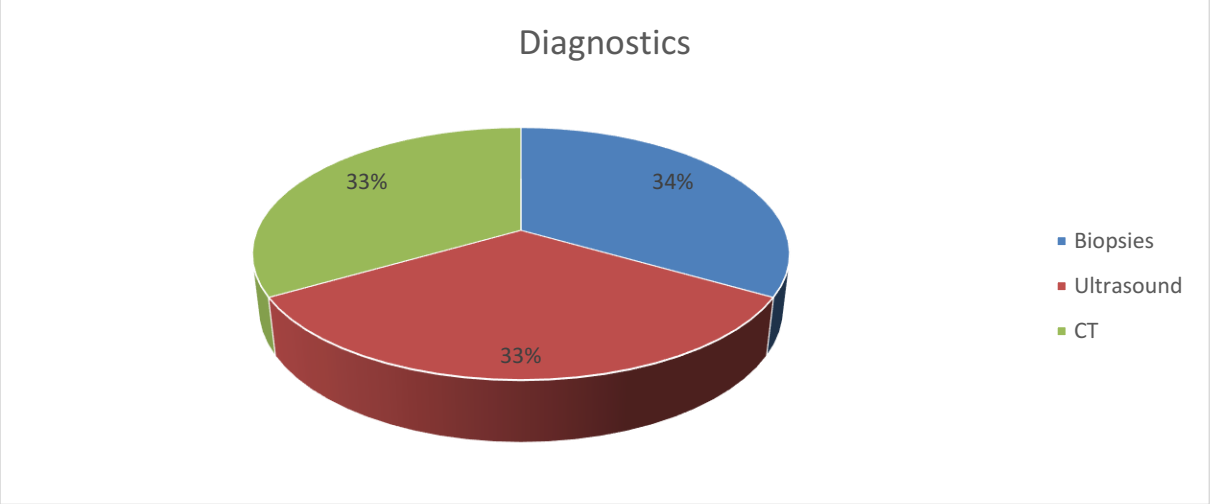

Figure 18: Diagnostic tools used for diagnosis of the vascular group

Due to the retrospective nature of this study, not all data sets were complete with all parameters (see individual Figs.). Glucose was elevated in both dogs tested (100.00 %), whereas total protein was decreased in both dogs tested (100.00 %; Fig. 19). Hepatic enzymes were mostly normal where tested (Fig. 20) which only ALT being increased in 1 out of 2 dogs. Functional tests are shown in Fig. 21. As shown in Fig. 22, two out of three patients (66.66 %) yielded increased chloride concentrations. Interestingly enough, two out of three dogs (66.66 %) revealed a thrombocytopenia (Fig. 23).

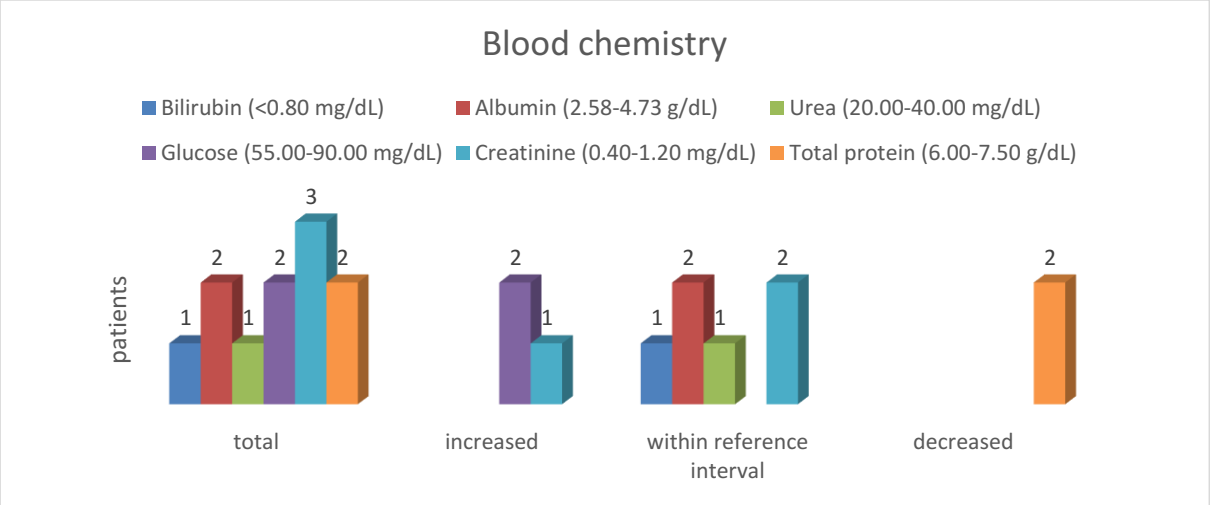

Figure 19: Values of blood chemistry in the vascular group

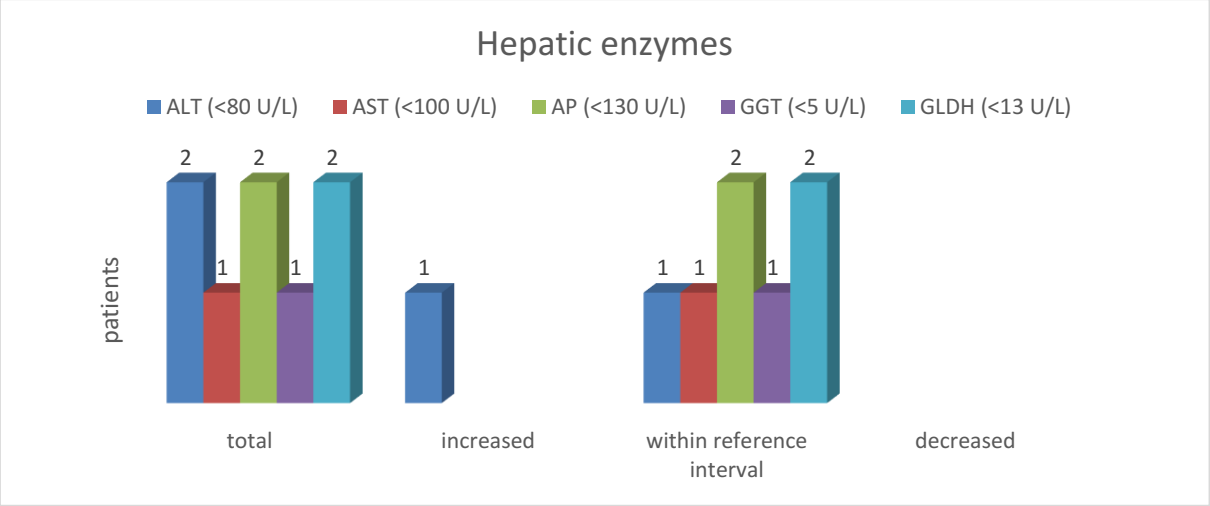

Figure 20: Values of hepatic enzymes regarding the vascular group

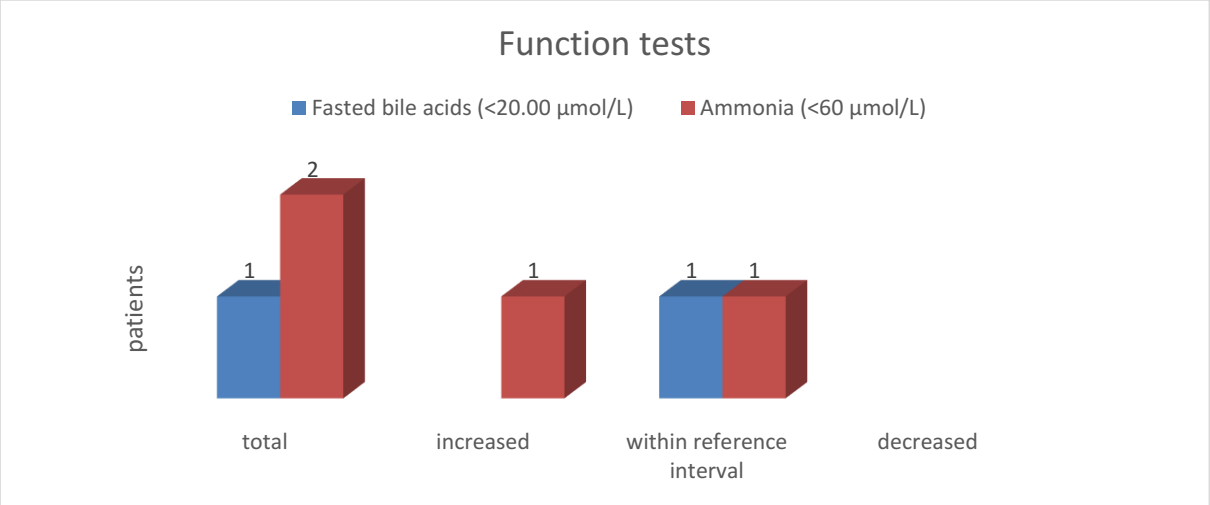

Figure 21: Values of function tests in the vascular group

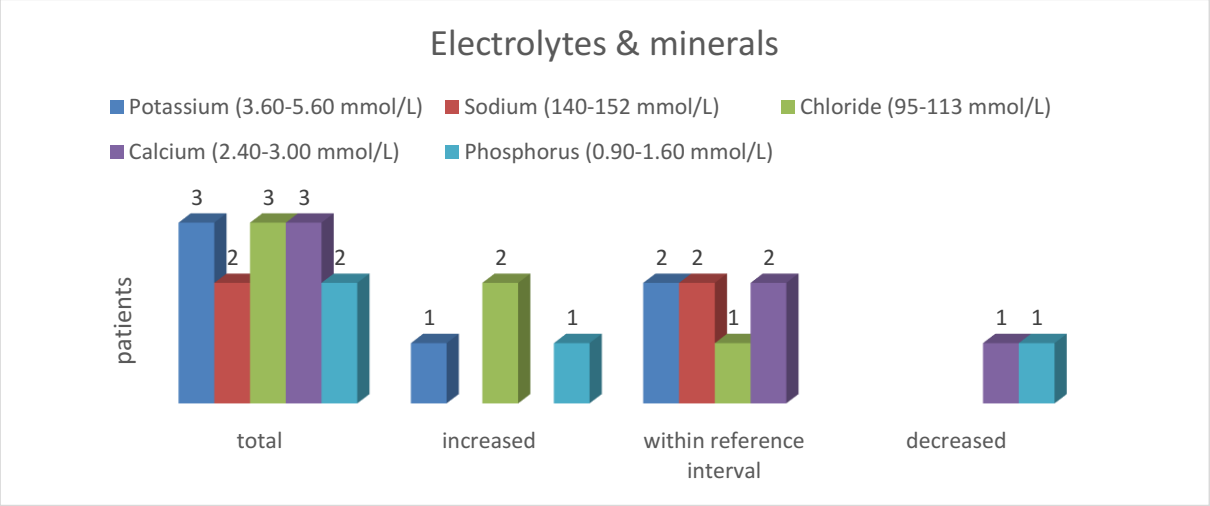

Figure 22: Values of electrolytes and minerals in the vascular group

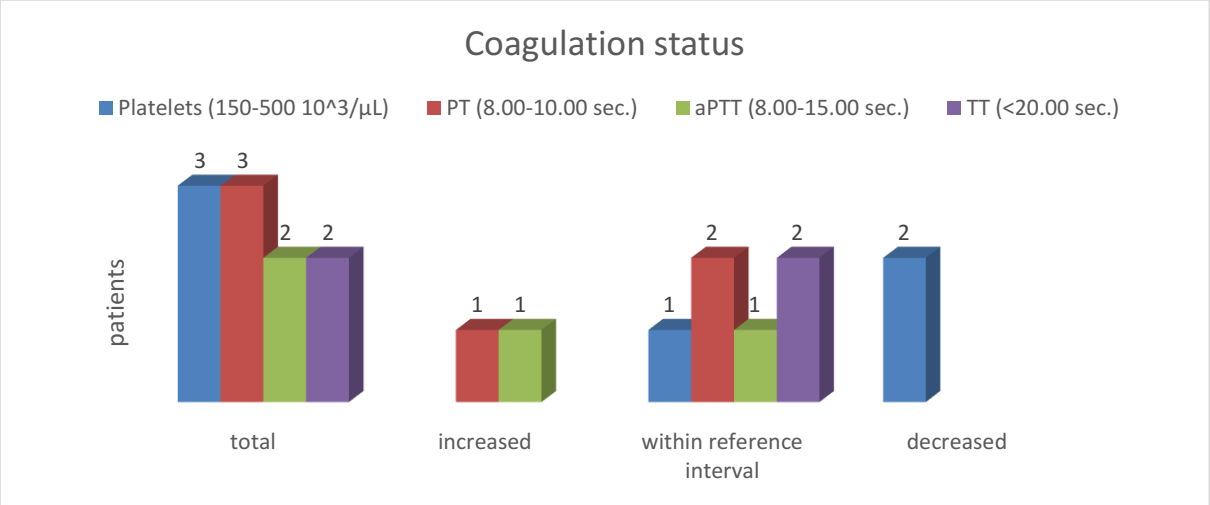

Figure 23: Values of coagulation in the vascular group

5.4.4. Neoplastic diseases

The second largest group was the one with neoplastic diseases (79/254 patients; 31.10 %; see Fig. 24). Twenty-eight cases (35.44 %) turned out to be epithelial tumours. Whereas four cases from this group were not further differentiated, 15 were classified as carcinomas (whereof one anaplastic, three adenocarcinomas, one malignant carcinoma starting from the pancreas), four hepatic adenomas/adenocarcinomas, four bile duct tumours (two bile duct carcinomas, one bile duct cystadenoma, and one biliary cystadenocarcinoma), and one case turned out to be a mesothelioma. Twenty-four patients (30.38 %) were assigned to the subgroup of round cell tumours. Of these, 16 suffered from lymphoma, four from histiocytosis (one malignant fibrous histiocytoma), three from myeloma (one plasma cell tumour), and one patient was diagnosed with a mast cell tumour. While fourteen patients (17.72 %) developed hepatic neoplasia without further differentiation (eight metastases of a primary tumour in another organ), ten dogs (12.66 %) were assigned to the subgroup of mesenchymal tumours. Eight of these developed hemangiosarcoma (one hemangioendothelioma) and two sarcomas. Finally, three dogs (3.80 %) were diagnosed with a neuroendocrine tumour.

FNA led to the final diagnosis in 44 cases (55.70 %; see Fig. 25). It made the diagnosis of nine carcinomas from the group of epithelial tumours, four epithelial tumours without further differentiation, two hepatic adenomas/adenocarcinomas, one bile duct tumour, and one mesothelioma. Within the round cell tumours, seven lymphomas, three histiocytosis, one myeloma, and one mast cell tumour were diagnosed via FNA. FNA also provided the diagnosis

of five mesenchymal tumours. In eight cases, liver neoplasia without further differentiation was found as well as two neuroendocrine tumours.

A histopathological examination after biopsies revealed the final diagnosis in 28 neoplastic cases (35.44 %; see Fig. 25). The epithelial tumours affected were seven carcinomas (whereof one anaplastic and one malignant starting from the pancreas), two liver adenomas/adenocarcinomas, and two bile duct tumours (one biliary cystadenocarcinoma and one bile duct cystadenoma each). From the round cell subgroup, five lymphomas, two myelomas, and one histiocytosis (malignant fibrous histiocytoma) were found during histological examination. In addition, four hemangiosarcomas (one hemangioendothelioma) and one sarcoma of the mesenchymal subgroup, three liver neoplasias (two metastases), and one neuroendocrine tumour were diagnosed this way.

Four lymphomas (5.06 %) within the round cell tumour subgroup were diagnosed during necropsy. With the help of CT, three liver neoplasias (3.80 %) without further differentiation were found (see Fig. 25).

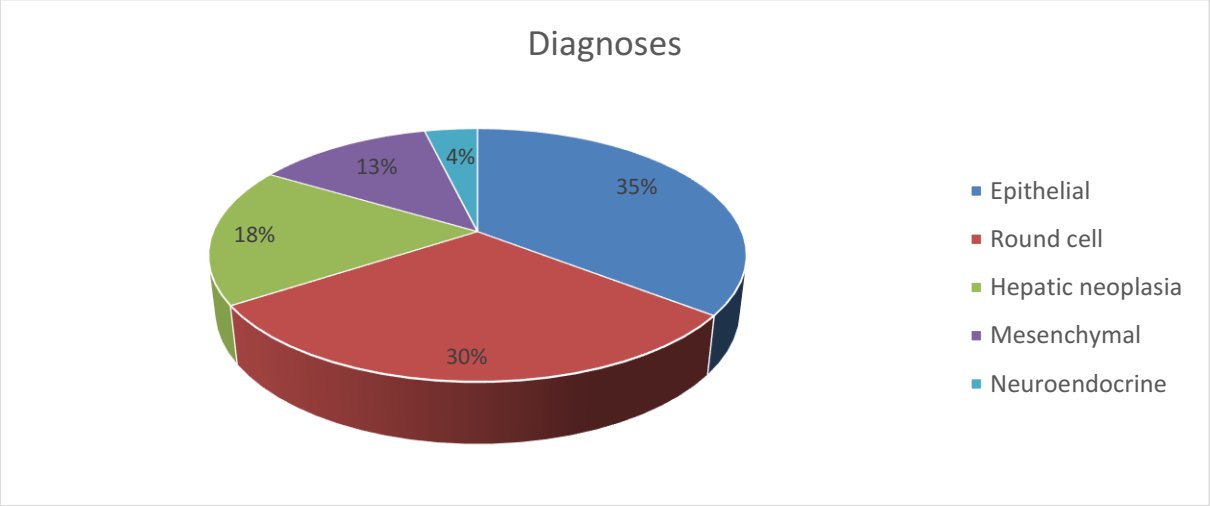

Figure 24: Diagnoses of the neoplastic group

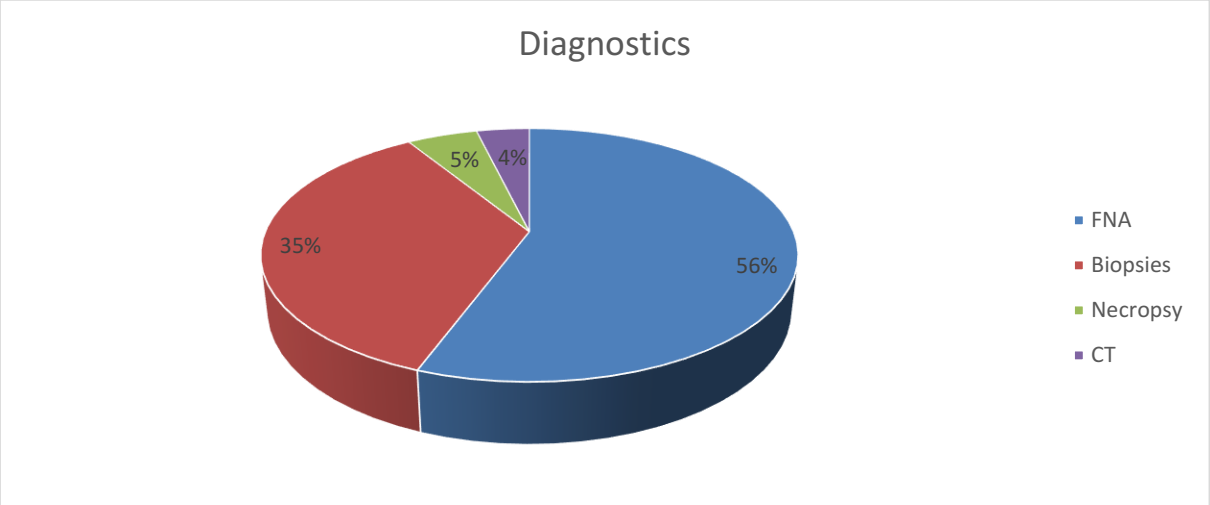

Figure 25: Diagnostic tools used for diagnosis of the neoplastic group

Due to the retrospective nature of this study, not all data sets were complete with all parameters (see individual Figs.). The most important chemistry values are summarized in Fig. 26. As shown in Fig. 27, ALT, AP, GGT and GLDH measurements were elevated in the majority of cases. ALT was increased in 54/73 tested cases (sensitivity 73.97 %), AP in 53/69 (sensitivity 76.81 %), GGT in 26/35 (sensitivity 74.29 %), and GLDH in 32/48 (sensitivity 66.67 %). Figs. 28, 29 and 30 reveals the evaluated data sets of function tests, electrolytes, minerals, and coagulation in more detail.

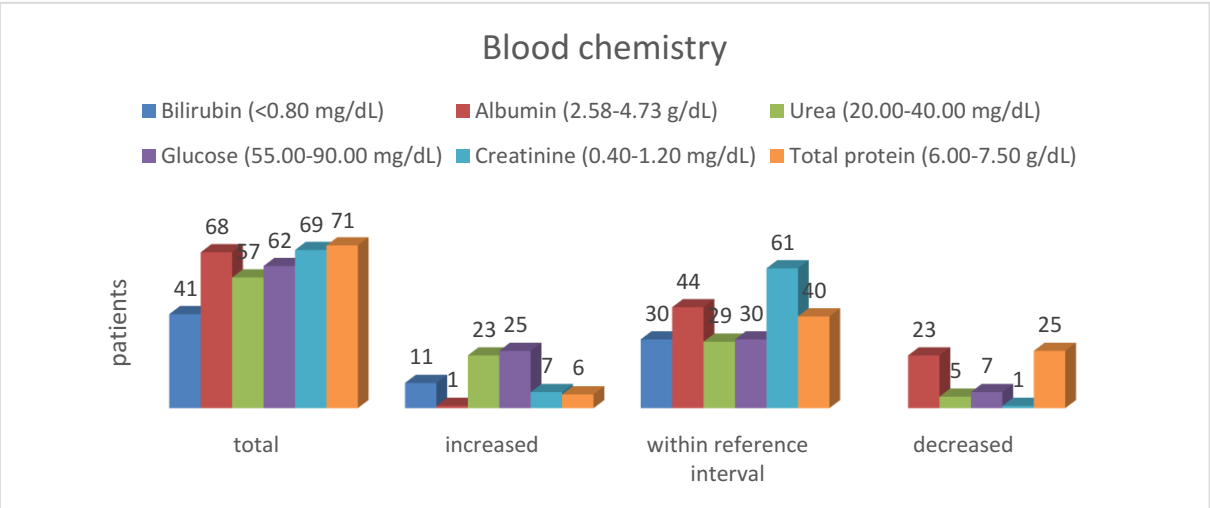

Figure 26: Values of blood chemistry in the neoplastic group

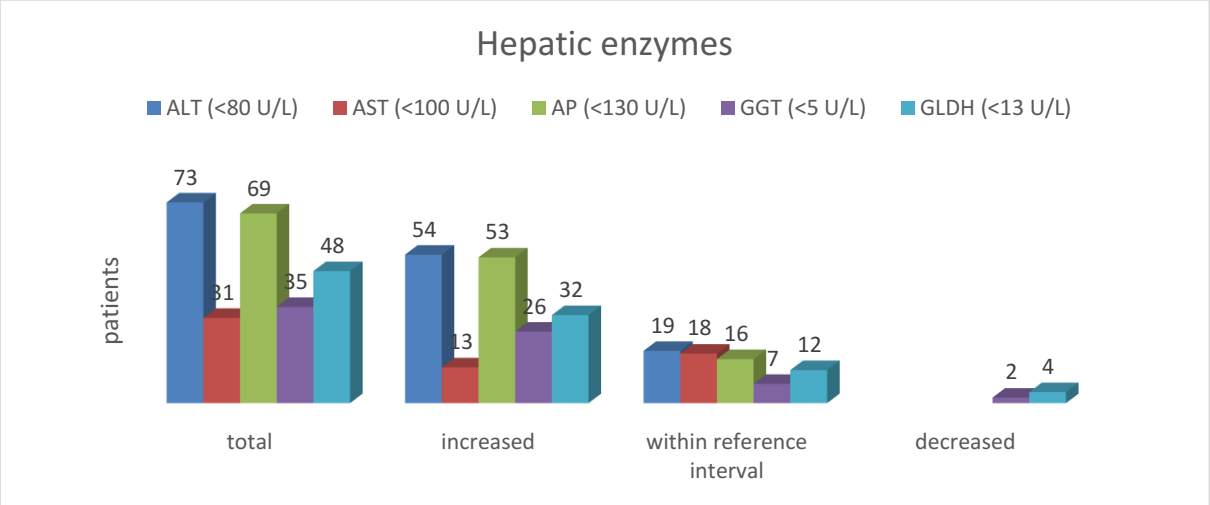

Figure 27: Values of hepatic enzymes in the neoplastic group

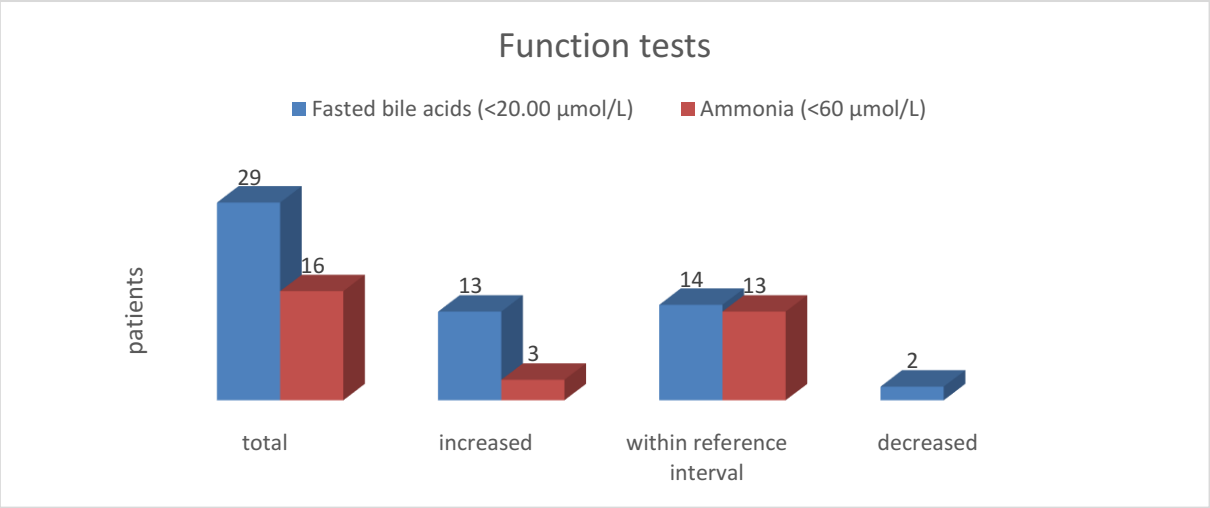

Figure 28: Values of function tests in the neoplastic group

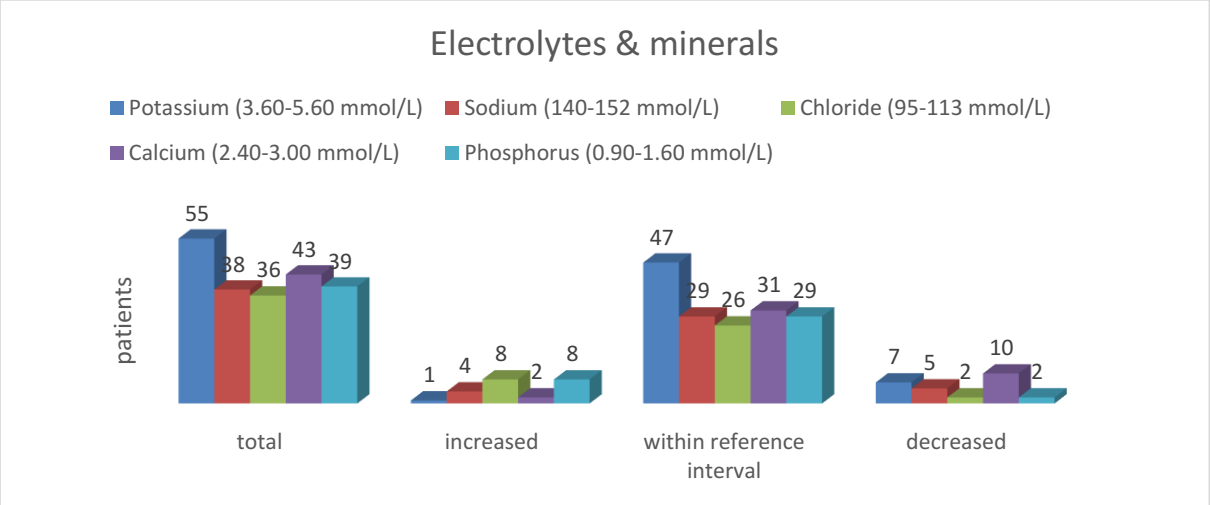

Figure 29: Values of electrolytes and minerals in the neoplastic group

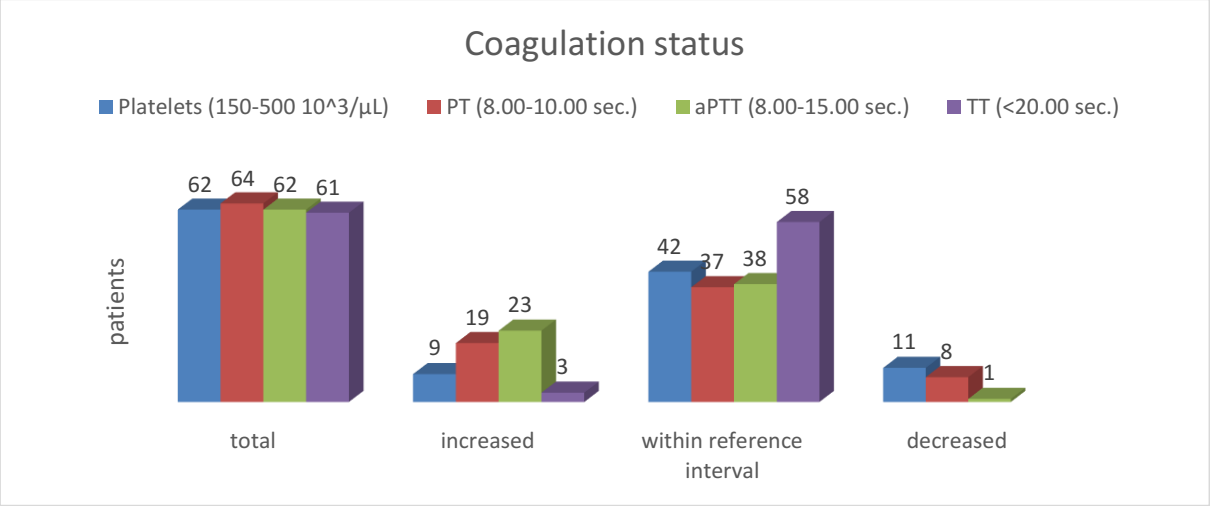

Figure 30: Values of coagulation in the neoplastic group

5.4.5. Secondary diseases

Nineteen out of 254 cases (7.48 %) were assigned to the group of secondary diseases (Fig. 31). Thirteen (68.42 %) were characterized by excess glucocorticoid, whereof nine emerged due to glucocorticoid therapy, three because of hyperadrenocorticism, and one due to steroid hepatosis. The diagnosis of hepatolipidosis was made in four patients (21.05 %). One dog (5.26 %) was affected by immunmediated hemolytic anemia (IMHA) and one (5.26 %) by infarction due to liver torsion.

As shown in Fig. 32, FNA led to the final diagnosis in eight cases (42.11 %). Six of them suffered from excess glucocorticoid (four due to glucocorticoid therapy, one because of

hyperadrenocorticism, and one in case of steroid hepatosis) and two from hepatolipidosis. One case (5.26 %) of IMHA was diagnosed with FNA in combination with a Coombs test. Histopathology of biopsies provided six diagnoses (31.58 %) with all six suffering from excess glucocorticoid (five due to glucocorticoid therapy, one because of hyperadrenocorticism). Necropsy supplied information for three final diagnoses (15.79 %; two hepatolipidosis and one infarction due to torsion, respectively). Ultimately, one case (5.26 %) of excess glucocorticoid (hyperadrenocorticism) was detected with the help of a serological examination using a combination of ACTH stimulation test and low dose dexamethasone suppression test.

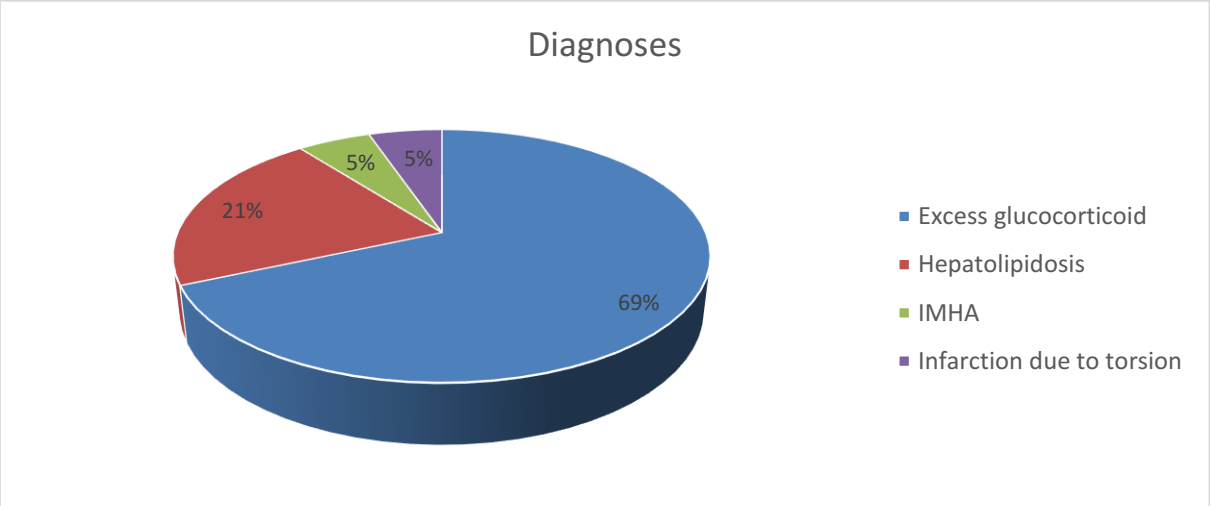

Figure 31: Diagnoses of the secondary group

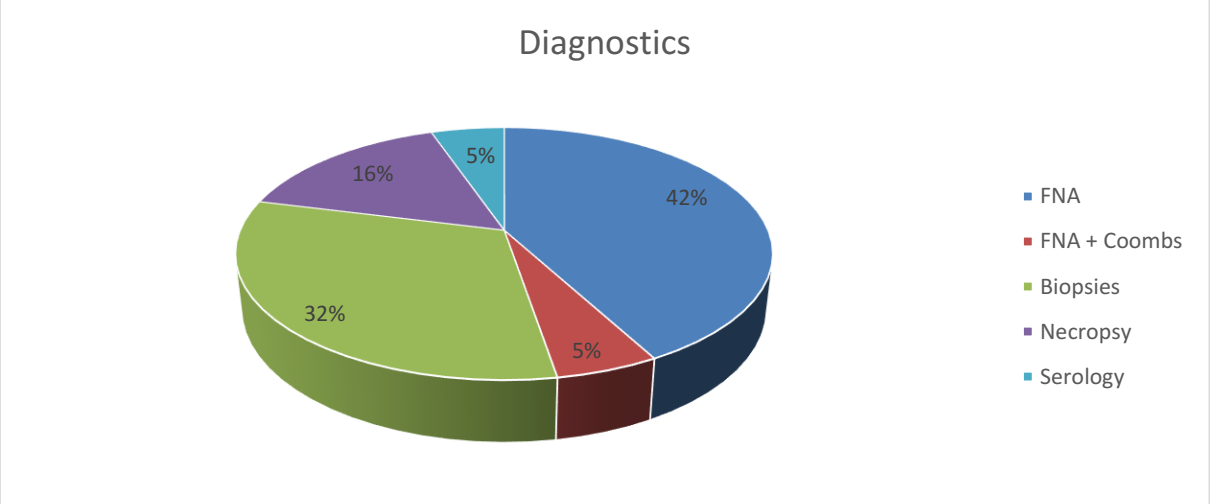

Figure 32: Diagnostic tools used for diagnosis of the secondary group

Due to the retrospective nature of this study, not all data sets where complete with all parameters (see individual Figs.). Eleven of the 14 glucose concentrations (78.57 %) were elevated (Fig. 33). As illustrated in Fig. 34, 14/18 tested cases yielded an increased ALT

(sensitivity 77.78 %), 13/16 an elevated AP (sensitivity 81.25 %), and 4/5 increased GLDH (sensitivity 80.00 %) and 7/8 abnormal high GGT concentrations (sensitivity 87.50 %). The evaluated data sets in relation to functional tests, electrolytes, minerals and coagulation status are shown in more detail in Figs. 35, 36 and 37.

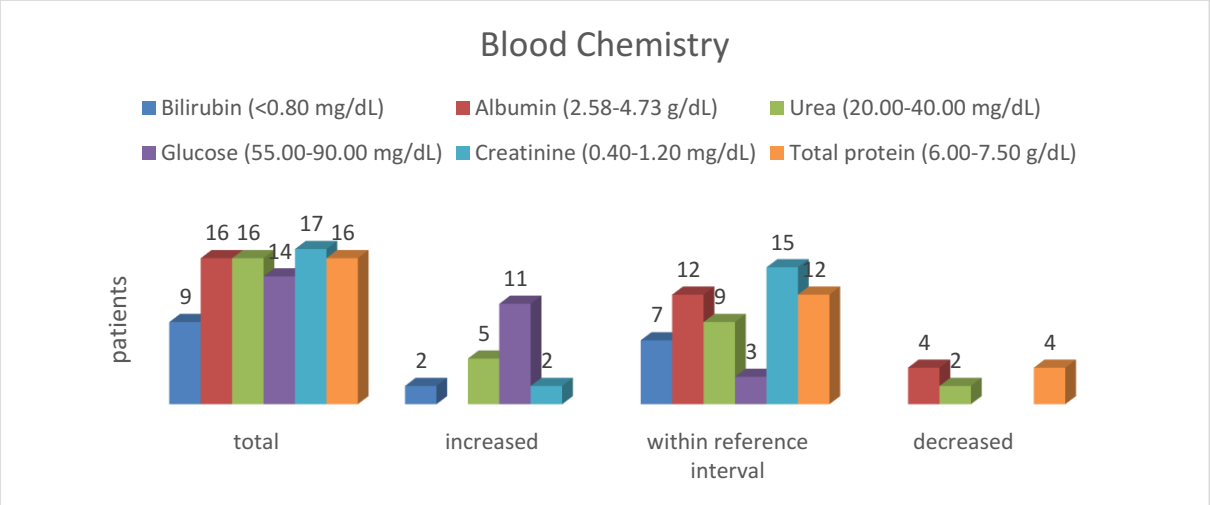

Figure 33: Values of blood chemistry in the secondary group

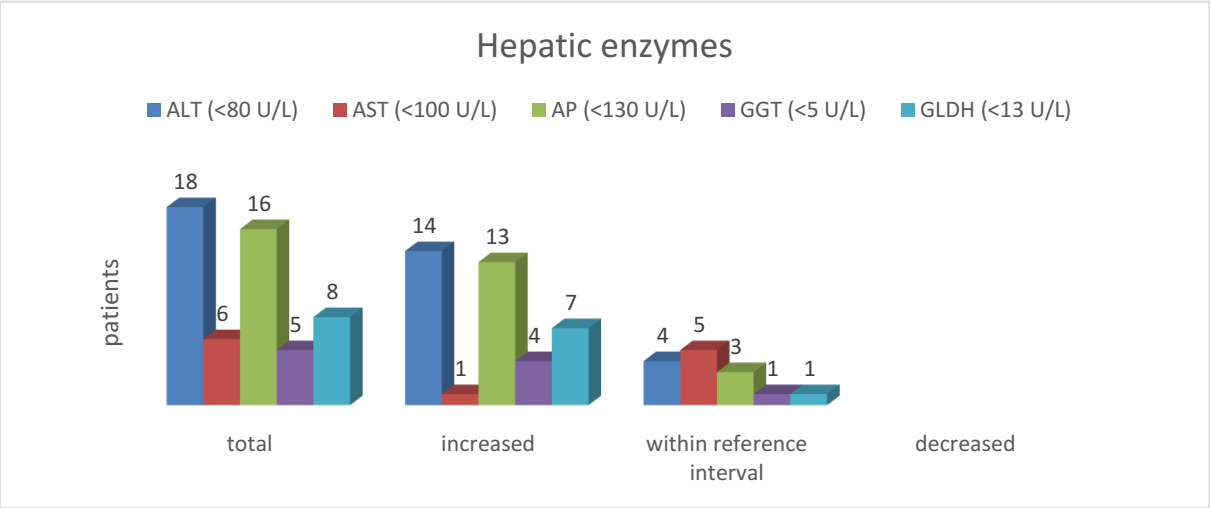

Figure 34: Values of hepatic enzymes in the secondary group

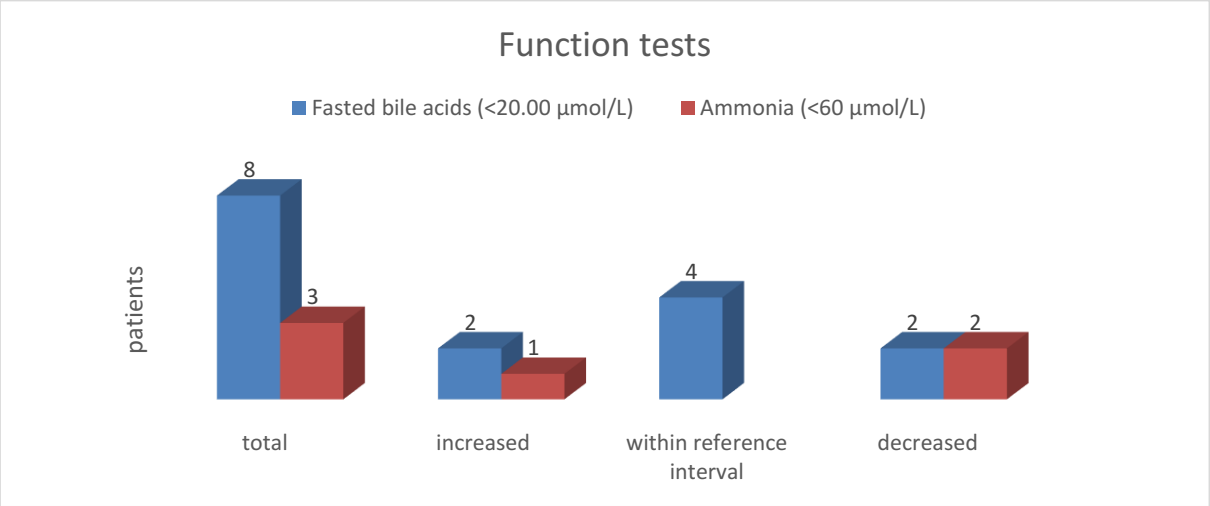

Figure 35: Values of function tests in the secondary group

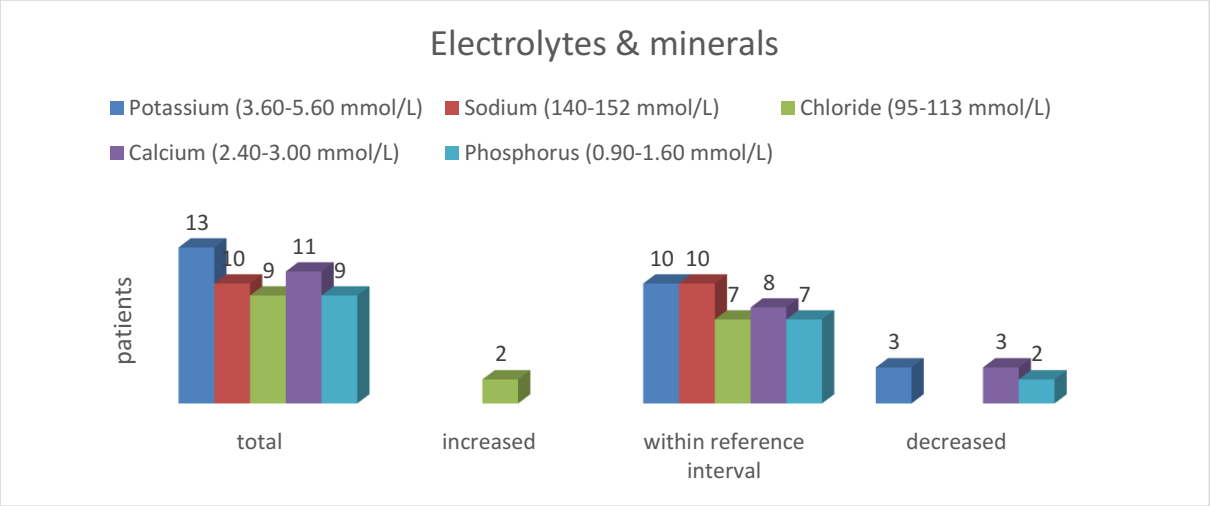

Figure 36: Values of electrolytes and minerals in the secondary group

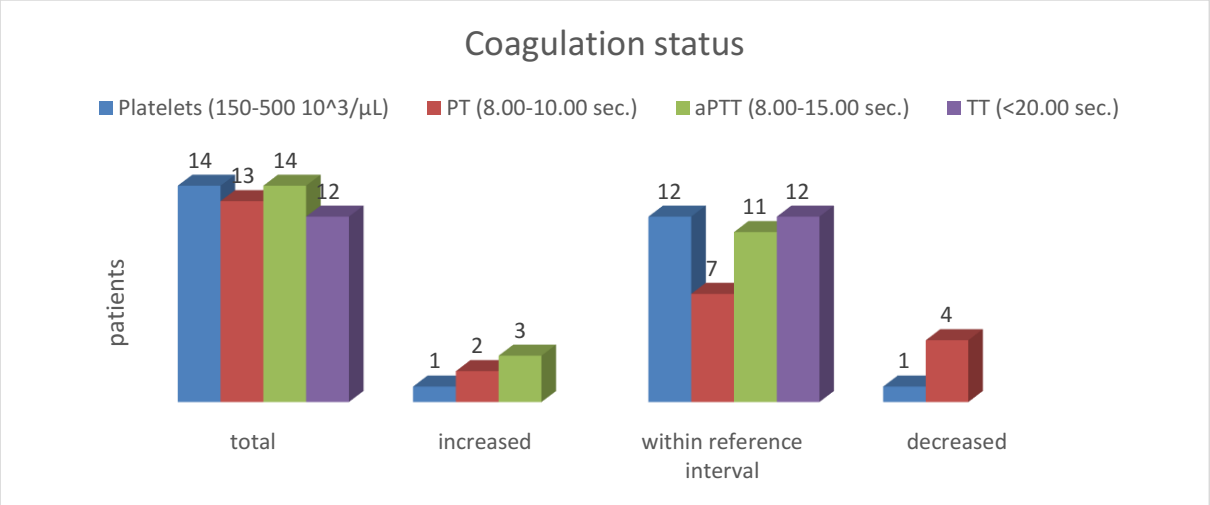

Figure 37: Values of coagulation in the secondary group

6 Discussion

In this study, the frequency of various hepatopathies in a referral dog population in Austria was evaluated. The aim of this work was to determine the frequency of different types of canine hepatopathies in regard to the subgroups (parenchymal, biliary, vascular and neoplastic) as well as the usefulness of the different diagnostics over a period of eight years at a veterinary teaching hospital. Due to the large number of dogs that were covered by this retrospective study, the reality of everyday clinics is reflected very well.

The dogs in this study had a median age of 10.31 years. Regarding the individual groups, only the dogs suffering from vascular diseases were significantly younger with a median age of 1.24 years. This is probably due to the fact, that these were congenital malformations. The dog that suffered from extrahepatic portosystemic shunt was 6.67 years old. Nevertheless, only one shunt vessel and no ascites were found on ultrasound suggesting congenital disease despite the relatively old age of the patient. As studies from Liptak et al. (2004) showed, the majority of canines suffering from hepatobiliary neoplasias are older than 10 years. However, other studies from Patnaik et al. (1980) and Balkman (2009) found, that affected dogs are diagnosed when they are younger than 10 years, what goes along with the findings of this study, where dogs suffering from tumours were on average 9.35 years with a median age of 9.72 years. The gender distribution of the subjects was very balanced overall. Only in the parenchymal group, female neutered dogs were obviously overrepresented with 60/147 (40.82 %).

Our study showed that a variety of crossbreeds and purebred dogs are affected by any type of hepatic pathology. Most patients regarding all five groups, with exception of the vascular one, were crossbreeds. According to studies from Watson (2017) and Poldervaart et al. (2009), Dobermann Pinschers, Labrador Retrievers and West Highland White Terriers are predisposed for chronic hepatitis. In our study only one Dobermann Pinscher suffered from chronic hepatitis, but four Labrador Retrievers and two West Highland White Terriers from acute hepatitis. This could be explained by the fact, that chronic changes do not receive FNAs, but rather biopsies right away and so a selection bias regarding might cause this circumstance. However, in a study of Poldervaart et al. (2009), Labrador Retrievers and West Highland White Terriers were also affected by acute hepatitis, although not in large numbers. Furthermore, our study confirmed the breed predisposition for Fox Terriers and Jack Russel Terriers for acute hepatitis as already showed by Poldervaart et al. (2009). 11/79 patients in the neoplastic group

were Golden Retriever, supporting several studies such as the one from Kent et al. (2018), where Golden Retrievers have an increased prevalence for neoplastic diseases compared to other breeds. No other pedigree dog breed was presented in the neoplastic group to such a large extent.

By dividing all pathologies into the five groups parenchymal, biliary, vascular, neoplastic and secondary as described by Rothuizen (2006), parenchymal liver changes were by far the most dominant group with 57.87% (147/254) of the cases. Regarding this parenchymal group and the overall population of the study, hepatic cell degeneration was by far the most commonly diagnosed change with 27.56 % (70/254). Since hepatic cell degeneration is not a separate disease per se, these cases probably represent patients suffering from other processes, e.g. acute hepatitis, that lead to the hepatocyte changes. Retrospectively it was not possible to define all diseases that really played a role in developing the degeneration and to potentially place them into other groups. Acute hepatitis with 52/254 (20.47 %) dogs was the second most common disease within the parenchymal group. This contradicts several studies, among others the one by Poldervaart et al. (2009), which state that acute hepatitis is less common than e.g. the chronic form. Although they also stated that due to their inclusion criteria the number of dogs referred may not reflect the true prevalence of acute hepatitis in the Netherlands, the selection bias with FNA as inclusion criterium of our study would also be an explanation. The large proportion of acute hepatitis in our study population could also be biased due to the fact that dogs are more likely to show symptoms in the acute stage of disease than during chronic processes and are therefore more often presented to veterinarians. As already mentioned, several studies (e.g., Watson 2004b) showed that chronic hepatitis is remarkably common in dogs. Watson et al. (2010) even found a prevalence of up to 12 % at post-mortem histopathological examinations. However, within our group of subjects, only 10/254 dogs suffered from this condition. As already mentioned, FNA was our main inclusion criterium. Based on personal decision, chronic diseases less likely undergo diagnostic clarification and if so, the changes are probably sampled right away. Therefore, such cases do not even appear in our study. If all 254 dogs of the study had been subjected to a histopathological examination, the result would probably show a different outcome. Nodular regeneration was diagnosed in 12/254 dogs. Regeneration nodes are a sign of fibrosis, cirrhosis, or end-stage liver as shown by Webster et al. (2019b). Finally, Cullen et al. (2006b) revealed that hepatic amyloidosis and abscesses are rare conditions in dogs. This goes along with our findings that only one dog each suffered from these diseases.

Biliary diseases only made up 2.36 % (6/254) of the study population. Cholecystitis is a condition that is reported to rarely occur in dogs (Lawrence et al. 2015), whereas Tamborini et al. (2016) revealed that it occurs more frequently than suggested by literature so far. Nevertheless, the proportion of cholecystitis cases in our study population was relatively low with only 2 out of 254 cases (0.79 %). It should be mentioned that 18/21 dogs that were excluded because they suffered from more than one disease at a time had obvious signs of cholecystitis. Furthermore, biliary diseases are more likely to be diagnosed on ultrasound and/or CT and do not undergo FNA. Last but not least, gallbladder mucocele, one of the most frequent biliary diseases in old dogs, is also mainly diagnosed using ultrasound in combination with bile acids (Besso et al. 2000, Pike et al. 2004, Larson 2016). An FNA of a distended gall bladder is contraindicated and therefore these cases did also not meet our inclusion criteria.

The vascular group, with 1.18 % (3/254) of the population, was only barely represented in the study. This is most likely due to the fact that imaging procedures in combination with bile acids and ammonia are the diagnostic method of choice for vascular changes and FNA are hardly ever required (van Straten et al. 2015). In our study only the patient suffering from microvascular dysplasia got fasted bile acids and ammonia measurements within the normal range. This is in accordance with Webster et al. (2019b), who described that most hepatic diseases regarding the vascular system are evaluated with the help of diagnostic imaging.

According to Selmic (2017) canine hepatobiliary neoplasias are uncommon. However, in this study, the neoplastic group was the second largest with 31.10 % (79/254). Primary neoplasia is thought to be less common than metastatic hepatobiliary neoplasia (Selmic 2017). Within this study, only 8/79 patients suffered from confirmed metastatic neoplasia. This could be explained by the fact that in dogs diagnosed with a primary tumor in the gastrointestinal tract, spleen, pancreas or elsewhere, FNAs of the metastases in another organ are rarely performed. Apart from the comparison between primary and secondary neoplasia, it was striking that most dogs suffered from epithelial tumours (28/79) reflecting Patnaik et al. (1981b), who showed that primary liver cancer mainly refers to hepatocellular carcinoma. Lymphoma was with 16/79 cases the most common round cell tumour in our study representing two thirds (16/24) of this group. Vail et al. (2012) also described that lymphomas are very common, and the liver can be involved in the multicentric, alimentary or hepatosplenic subgroup of lymphoma. Within the group of mesenchymal tumours (10/79) there were a striking number of hemangiosarcomas

(8/10). Hemangiosarcoma is a very aggressive and common form of cancer in dogs, however, the primary tumour is mainly found in the spleen (Kim et al. 2015). Only three of the dogs of our study were assumed to have hemangiosarcoma within the spleen. Neuroendocrine tumors are thought to be not that common in dogs (Selmic 2017) and this was also confirmed with this study (only 3/79 cases). Last but not least, there were six tumours that were not subjected to any further differentiation.

Not surprisingly the main secondary problem that led to liver changes was excess glucocorticoids (13/19). This also fits with studies by Gilor and Graves (2011), which show that hypercortisolism leads to hepatic changes. Roth (2001) found that FNA and biopsies are both very good for diagnosing fatty liver. In our study all four cases of hepatolipidosis were diagnosed through FNA (2/4) or biopsies (2/4). For IMHA no diagnostic gold standard exists, but the interpretation of blood works together with positive saline agglutination or coombs test usually lead to the diagnosis (Garden et al. 2019). With FNA as inclusion criteria it is not surprising that IMHA (1/19) was minimally represented in our study. Downs et al. (1998) states that torsions of liver lobes are rare in dogs and the necrosis of the lobe can result in shock and death. This also fits our study, where only one patient was affected by liver lobe torsion. In addition, due to the acute and severe course of this disease, FNA is most likely not performed, so the cases would not be included in this study anyway.

Since the selection of the patients in our study was made on the basis of a performed FNA, it is not surprising that most of the cases (178/254) were finally diagnosed with this diagnostic method. With the help of FNA, most parenchymal diseases (124/147) were diagnosed as well as the majority of the patients who suffered from neoplasia (44/79). Most cases that had hepatic cell degeneration as a final diagnosis (65/70) were determined by a FNA. The sensitivity of the FNA regarding this diagnosis was 92.86 %. As a study of Bahr et al. (2013) demonstrates, FNA is limited in diagnostic performance and the sensitivity and positive predictive value for inflammation is low. This is not in line with the results of our work, where acute hepatitis (45/53) was the second most common diagnosis made by FNA with a relatively high sensitivity of 84.91 %. Webster et al. (2019b) stated that FNA is not sufficient enough for the diagnosis of chronic hepatitis. However, half (5/10) of the chronic hepatitis cases of this study were finally diagnosed through cytology following FNA. Nevertheless, sensitivity is still lacking at only 50.00 %. The same applies to regeneration nodules, which were diagnosed most frequently with FNA, but also only with a sensitivity of 66.67 % (8/12). The sensitivity of

FNA in relation to the neoplastic group was only 55.70 % (44/79) overall, but related to individual tumour types like lymphoma, this sensitivity would certainly be higher. Selmic (2017) described that FNA is helpful for differentiation between benign and malignant tumours and the accuracy of diagnosis is up to 60.00 %. Furthermore, Rothuizen and Twedt (2009b) stated that neoplasia and diffuse vacuolar disorders (e.g., lipidosis or steroid hepatopathy) are often diagnosed using FNA. According to studies from Starkey and Murphy (2010), especially lymphoma represents a tumour type that is frequently diagnosed with the help of a cytological examination following FNA. Although 9/16 dogs were subjected to further diagnostic examination, 12/16 lymphomas in our study were at least suspected in the cytological examination of FNA. This in combination with already published studies suggests that FNAs may represent an appropriate tool for the diagnosis of hepatic lymphoma due to the exfoliative growth of round cell tumors. In our study, FNA diagnosed 8/19 secondary diseases. Six of them were excess glucocorticoid and two hepatolipodosis. Since both changes are diffuse, it is not surprising that they could be diagnosed well with the help of FNA (Rothuizen and Twedt, 2009a). Nevertheless, the sensitivity of FNA for the diagnosis of secondary diseases was only 42.11 %.

Numerous studies have already shown that a histopathological examination of biopsied tissue is the gold standard for the diagnosis of hepatobiliary pathologies (Stockhaus et al. 2004, Saunders 2007, Dirksen 2016). Nevertheless, only 65/254 cases of our study underwent liver biopsies. This is likely due to the fact, that biopsies are more invasive and expensive than a FNA, as well as that the tissue preparation and examination takes longer. Since a FNA is carried out quickly, it can be assumed that in most cases the minimally invasive method is preferred and biopsies only follow if the FNA does not deliver a definitive result. Considering that 55/65 (84.61 %) of the patients received their correct diagnosis with the help of biopsies, one can extrapolate what the result would have been if all 254 patients had received a histopathological examination. The comparison of the sensitivities of FNA and biopsies led to the result of 70.08 % for FNA and 84.61 % for biopsies. Previous studies showed a variation from 14 % to 86 % agreement between findings of FNA and biopsies (Roth 2001, Wang et al. 2004). This supports the hypothesis that an examination of biopsied tissue provides more meaningful results and thus more often leads to the final diagnosis. Our study yielded significant differences ($p=0.018$) in the chi^2-test. It should be noted that in this retrospective design a bias is definitely present due to the selection of all liver FNAs. The decision whether to perform a FNA or biopsies or both is the subjective judgement of the treating veterinarian.

The result is therefore falsified to the extent that not all patients on whom an FNA was performed were also biopsied. Again, if the experimental settings were different and the same number of subjects were submitted to both types of examination, this would most likely lead to even more significant results in this comparison.

Regarding the biopsies, only 9/65 results were inconsistent with the final diagnosis (one patient was diagnosed in combination of histopathology and a parasitological examination), whereof five cases were diagnosed with FNA, but not on histopathological examination. 2/5 cases had chronic hepatitis but were assumed to be hepatic cell degeneration and age-related changes on histopathology. This contradicts current literature from Webster et al. (2019a) that claims that a histopathological examination is essential for the diagnosis of chronic hepatitis. 1/5 cases had acute hepatitis and was falsely diagnosed as cell edema with biopsies. Furthermore, 2/5 cases were identified as carcinoma with FNA, but were thought to be cases of chronic hepatitis and cirrhosis on histopathology. Regarding these two cases it is possible that the carcinomas were simply missed with the biopsies. In addition, two cases were finally diagnosed during necropsy. 1/2 cases was acute hepatitis but histopathology following biopsies was unclear. 1/2 was infarction due to torsion, which was only dubbed hepatitis during examination of the biopsies. In the end the suspected diagnosis of abscesses was made with CT, but histopathology showed hepatic cell degeneration. One sample was simply too small for examination confirming Lidbury (2017) who determined that biopsies have many limitations and the success can be possibly reduced at any stage of the process, e.g. at taking the sample. In contrast, the histopathological examination showed great success in the diagnosis of parenchymal (18/25) and neoplastic diseases (30/32). That supports the study from Rothuizen and Twedt (2009b), where liver biopsies were indeed valuable for diagnosing parenchymal diseases (e.g., chronic hepatitis) and vascular and neoplastic pathologies. Although thought to be indispensable (Webster et al. 2019b) for the diagnosis of chronic hepatitis, the sensitivity was only 66.67 % according to our findings. Regarding acute hepatitis, the sensitivity was even worse with only 62.50 %. In contrast, the sensitivity in relation to neoplastic diseases was very good with 93.75 %. Several studies (among others Rothuizen and Twedt 2009b) already showed that histopathological examination is very valuable for the diagnosis of tumours. Especially for adenomas and carcinomas biopsies are thought to be essential. This also fits to our findings, where 2/4 adenomas and 7/15 carcinomas were determined by histopathological examination. In addition, also the case with microvascular dysplasia was diagnosed with the help of histopathological examination as already shown by Christiansen et al. (2000).

Other diagnostic tools that were helpful for diagnosing several cases were post-mortem histopathological investigation, CT and ultrasound. Since an examination after death offers completely different options than the ones on living animals, it is not surprising that sometimes the definitive diagnosis is only made during this type of investigation. However, our 12/254 cases (two acute and one chronic hepatitis, one hepatic cell degeneration, one bile duct obstruction, four lymphomas, two hepatolipidosis and one infarction due to torsion) were not diseases that could only be diagnosed post-mortem, which suggests that further examination was not desired due to poor clinical condition or prognosis. Imaging procedures are thought to be very helpful for diagnosing vascular and biliary diseases, as well as for detecting tumours (Larson 2016). Our study also proved this with five cases being diagnosed with CT (one case with hepatic abscesses, an intrahepatic shunt, three hepatic neoplasias) and three with abdominal ultrasound (an extrahepatic shunt, two biliary disorders).

Although in a study it would be desirable that the same blood values are tested in all patients, this is simply not possible in the course of a retrospective analysis. Given the facts that the 254 animals were treated by different clinicians, that not every parameter is always useful to workup various disease, and that the owners are often limited in cost, led to not always complete data sets with various parameters in different numbers of cases. The most commonly used indicators for hepatobiliary diseases are ALT and AP (Center 2007). In our study too, the majority of dogs of all groups except the vascular one had increased ALT and AP values. The sensitivity of ALT overall was 75.53 % (179/237) and 73.01 % (165/226) of AP. 43/53 acute hepatitis cases in this study showed increased ALT concentrations and even 41/53 dogs had increased AP values. Therefore, the sensitivity of ALT for acute hepatitis was 81.13 % and 77.35 % of AP. Regarding chronic hepatitis, even 10/10 dogs who had evaluated ALT levels within their medical record were increased and 8/9 AP measurements were too high. That results in sensitivities for chronic hepatitis of 100.00 % for ALT and 88.89 % for AP. However, Dirksen et al. (2017a) found, that the sensitivity of ALT and AP is low for detecting acute (ALT 45 %, AP 15 %) and chronic (ALT 71 %, AP 35 %) hepatitis in asymptomatic dogs. According to Center (2007), GGT is thought to be sensitive in detection of biliary diseases like cholestasis. Within our study, only 2/6 dogs who suffered from biliary pathologies had GGT measurements within their data set, however, both were increased. Furthermore, GGT was increased in most dogs from all groups, except the vascular one. GLDH was also elevated in most dogs from the parenchymal and neoplastic group. Dirksen et al. (2017a) showed that bile acids are not

sensitive enough in the detection of acute, chronic, or reactive hepatitis. These findings match ours with none of the groups showing increased bile acids in the majority of dogs. Van Straten et al. (2015) showed that fasting serum ammonia and bile acids are very specific as diagnostic tools for portosystemic shunt, which was also proven in our cases.

Due to its retrospective design our study had major limitations. Some cases were lost because they did not meet the inclusion criteria, either through incomplete data sets or unclear final diagnosis. Some other cases suffered from more than one disease at a time and therefore could not clearly be assigned to one group. Furthermore, to compare the individual diagnostic tools to each other, all patients should have ideally undergone all diagnostic methods. However, this was not the case in this retrospective study. Our study evaluated hepatobiliary diseases in dogs in terms of incidence, distribution, and diagnostic tools, especially FNA and biopsies. Not surprisingly, with FNA as inclusion criteria parenchymal pathologies (147/254) was the highest represented group. Together with neoplastic diseases (79/254), they made up 88.98 % of all disorders. Hepatic cell degeneration (70/254) and acute hepatitis (53/254) were the most common diagnoses. Furthermore, FNA was sufficient for definitive diagnosis in 70.08 % of all dogs, whereas liver biopsies had a higher sensitivity where performed (55/65 cases; 84.61 %). Last but not least, increased ALT and AP activity were frequently observed in all but vascular disorders.

7 Summary

Hepatobiliary diseases are commonly encountered in dogs and can be divided into four primary groups: parenchymal, biliary, vascular, or neoplastic diseases. They are among the leading causes of morbidity and mortality in dogs (Assawarachan et al. 2019). However, the clinical signs are usually too unspecific to determine the individual disorder, and the dogs remain subclinical for a very long period of time (Poldervaart et al. 2009, Eman et al. 2018). The definitive diagnosis depends on the interplay and interpretation of many different examination methods (Rothuizen 2006).

In a period of eight years, 254 dogs were subjected to FNA and further diagnostic methods due to hepatobiliary diseases at the Small Animal Clinic of the University of Veterinary Medicine in Vienna, Austria. The data sets of these dogs were retrospectively examined to determine the frequency of different types of canine hepatopathies regarding the four subgroups (parenchymal, biliary, vascular, neoplastic) as well as secondary hepatopathy and to analyse the usefulness of the different diagnostic tools.

The mean age of the dogs was >9 years in all subgroups, besides vascular, where dogs were on average seven years younger. Spayed females and crossbreeds were overrepresented. Golden Retrievers were the most prominent breed in the neoplastic group and Labrador Retrievers in the parenchymal group. Most dogs suffered from parenchymal liver diseases (147/254). Hepatic cell degeneration (70/254) was the most commonly diagnosed change and acute hepatitis (53/254) the most frequently observed disorder. Within our group of patients chronic hepatitis was not a common disease. Furthermore, biliary disorders and vascular changes were barely seen – most likely due to the inclusion criteria of FNA. The neoplastic group was the second largest cohort of this study. 28/79 dogs suffered from epithelial tumours. However, the most common tumour type was lymphoma (16/79) as the most predominant representative of the subgroup of round cell tumours. The disorders of the secondary group were mainly caused by excess glucocorticoid (13/19). 178/254 (70.08 %) of the cases got their final diagnosis using fine needle aspiration (124 parenchymal cases, 44 neoplastic, eight secondary, and two biliary). The overall sensitivity of fine needle aspiration was 70.08 %. Moreover, fine needle aspiration showed good sensitivity for identification of acute hepatitis (84.90 %) and a lack of sensitivity regarding chronic hepatitis (50.00 %). Biopsies were only performed in 65 cases and had an overall sensitivity of 84.62 % (55/65). It was often helpful in finding the final diagnosis of parenchymal (18/25) and neoplastic (30/32) disorders, several times in the diagnosis of secondary (6/7) and once in the detection of a vascular pathology.

There was a significant difference between the frequency of the correct outcome of FNA and biopsies ($p=0.018$). Increased ALT, AP and GGT activity were frequently observed in all but vascular disorders. GLDH was also elevated in most dogs from the parenchymal and neoplastic group.

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9 Appendix

9.1. Abstract for ECVIM congress, Barcelona 2020

Canine hepatobiliary diseases – diagnostics and diagnoses

F. Pletzer[1], I. Schwendenwein[2], I.A. Burgener [1]

[1]Small Animal Internal Medicine, Department for Companion Animals and Horses, and [2]Central Laboratory, Department for Pathobiology, VetMedUni Vienna, Vienna, Austria

Hepatobiliary diseases are among the leading causes of morbidity and mortality in dogs. However, the diagnosis usually depends on many different examination methods. Therefore, the aim of this retrospective study was to determine the frequency of different types of canine hepatopathies concerning the subgroups parenchymal, biliary, vascular, neoplastic and secondary as well as the usefulness of the different diagnostics in a large referral population.
All dogs with a suspicion of hepatobiliary disorders with blood work, imaging, and fine needle aspiration (FNA) carried out between 12/2009 and 11/2017 were enrolled in this retrospective study. During this period, a total of 465 FNAs were performed. However, only 291 cases were supervised by the Small Animal Internal Medicine Service. 37 cases were excluded due to insufficient data (16) or difficulties to clearly classify in one of the five subgroups (21), leaving 254 cases for final analysis.
The mean age of the dogs at presentation was above 9 years in all subgroups besides vascular (mean overall 9.6 years (range 0.2-16.7), vascular 2.8 years (0.4-6.7)). Spayed females were overrepresented with 37.0%. Golden Retrievers were the most prominent breed in the neoplastic group and Labrador Retrievers in the parenchymal group.
Most dogs (N=147;57.9%) suffered from parenchymal diseases, with hepatic cell degeneration (70) and acute hepatitis (53) being the most important diagnoses (12 regenerative nodules, 10 chronic hepatitis, 1 amyloidosis, 1 abscess). Neoplastic disorders were the second largest group with 31.1% (N=79). 28 epithelial tumours and 24 round cell tumors (16 lymphomas, 4 histiocytosis, 3 myeloma, 1 mast cell tumor) were the most important subgroups (14 hepatic neoplasia without further differentiation, 8 hemangiosarcomas, 2 sarcomas, 3 neuroendocrine tumours). Biliary (N=6;2.4%) and vascular changes (N=3;1.2%) were only rarely diagnosed. 19 cases (7.5%) yielded secondary changes with excess glucocorticoids being by far the most common aetiology. FNA revealed a sensitivity of 70.1% (178/254), with parenchymal diseases

(124/147;84.4%) performing better than neoplastic (44/79;55.7%) or secondary (8/19;42.1%). Biopsies were necessary in 66 cases (sensitivity 85%). ALT and AP were increased in 75.5% and 73.0% of all cases.

With FNA as inclusion criteria, parenchymal disorders were responsible for more than half of all cases. Together with neoplastic diseases, they made up to 90% of all disorders. FNA was sufficient for diagnosis in 70% of all dogs, whereas liver biopsies had higher sensitivity where performed. Increased ALT and AP activity were frequently observed in all but vascular disorders.