DIAGNOSTIC HEPATOLOGY:
A clinical and pathological approach
and the interaction between clinician
and pathologist.

Ted.S.G.A.M. van den Ingh, PhD, Dipl ECVP
Utrecht, The Netherlands

Asian Veterinary Diagnostics 2018
Updated electronic version available from:
Society of Comparative Hepathology: VetVisuals International
DIAGNOSTIC HEPATOLOGY

- Clinical signs and medical history
- Biochemical and haematological markers
- Ultrasonography
- Fine needle aspiration biopsy
- Histopathology (golden standard)

DIAGNOSTIC HEPATOLOGY REQUIRES TEAMWORK BETWEEN CLINICIAN AND PATHOLOGIST
Clinical signs and medical history

- Anorexia, apathy, vomition, polyuria / polydipsia, diarrhea, weight loss, abdominal distension
- Jaundice or coffee-coloured urine
- Hepatomegaly
- Ascites
- Signs of hepatoencephalopathy
Clinical signs and medical history

HEPATO-ENCEPHALOPATHY SIGNS AND GRADING

0  normal

1  apathy, more sleeping, reduced activity, “disconnected”

2  unsteady gait, ataxia, circling, head pressing, “blind”, often salivation

3  stupor, sleeping most of the time, poor response to stimuli, hard to wake up, salivation

4  coma, non-responsive

Often increased response to sedatives and anaesthesia
Biochemical and haematological markers

- Full biochemical liver profiles versus selected biochemical tests

- Biochemical liver profiles are used in man for differentiation of various types of liver diseases, but do not have clinical significance in dogs and cats

- Biochemical liver profiles not necessary and better to use a standard set of selected tests
Biochemical and haematological markers

– Selected biochemical markers:

• Fasting serum bile acids; ref. range < 10 umol/L
• Total alkaline phosphatase (AP); ref. range < 73 U/L
• Steroid induced alkaline phosphatase 65⁰ (AP65); ref. range < 15%, only dogs
• Alanine aminotransferase (ALAT); ref. range 16-69 U/L
• Total serum protein; ref. range 53-66 g/L
• Albumin; ref. range 28-45 g/L
Coagulation markers

Blood coagulation testing is very important before taking a liver biopsy and should be performed shortly beforehand because coagulation markers may change quickly in such patients.

Therefore blood for coagulation tests should not be sampled more than 24 hours before taking the biopsy.
Biochemical and haematological markers

• Coagulation markers
  – Prothrombin time (PTT); ref. range 6.7-9.5 s
  – Activated partial thromboplastin time (APTT); ref. range 10-17.2 s
  – Fibrinogen; ref. range 1-2.8 g/L

• (Haematological markers)
Biochemical and haematological markers

Abnormal coagulation due to hepatobiliary disease can be due to reduced production of clotting factors, inadequate resorption of vitamin K from the intestine, and diffuse intravascular coagulation (DIC).

Abnormal coagulation is the most frequent reason for postponing liver biopsy.
Abnormal coagulation is the most frequent reason for postponing liver biopsy.

The Utrecht Liver Research Group has found that in dogs fibrinogen is the most critical indicator and reductions below 50% of the lower 95% reference value (<1 g/L) is a contraindication for taking a liver biopsy.
Abnormal coagulation is the most frequent reason for postponing liver biopsy.

All dogs with very low fibrinogen had hepatitis or diffuse neoplastic liver disease.

Fibrinogen had increased to above the critical level after 1 week of treatment with prednisolone in 90% of the dogs with hepatitis.
Abnormal coagulation is the most frequent reason for postponing liver biopsy.

In cats with hepatobiliary diseases, the majority had abnormal clotting predominantly related to vitamin K deficiency, which responded to vitamin K1 administration.
Biochemical and haematological markers

Hepato-encephalopathy biochemically associated with HYPERAMMONAEMIA

- Fasting ammonia; ref. range 24–45 umol/L

- Rectal ammonia tolerance test
Sources of ammonia

- Bacterial degradation of nitrogenous substrates in the gastro-intestinal tract
- Degradation of proteins, amino-acids, amines and nucleic acids
- Ammonia derived from glutamate
• Ammonia comes mainly from the intestinal tract, and is normally cleared very efficiently in the liver

• Large reserve capacity; the diseased liver is usually still capable of sufficient detoxification of ammonia (except in cases of fulminant hepatic failure)
LIVER

TWO AMMONIA DETOXIFYING MECHANISMS

NH3 → GLUTAMINE SYNTHETASE → GLUTAMINE → UREA CYCLE → UREA

NORMAL
LIVER

HEPATIC FAILURE

NH3

UREA CYCLE

GLUTAMINE SYNTHETASE

NH3

- - - - - - -> UREA

- - -> GLUTAMINE

- - -> NH3
LIVER

UREA CYCLE

GLUTAMINE SYNTHETASE

NH3

UREA

GLUTAMINE

PORTO-SYSTEMIC SHUNTING
Types of Hepato-encephalopathy

• Acute HE
  Acute onset with rapid development and progression of symptoms

ASSOCIATED WITH ACUTE FULMINANT HEPATIC FAILURE

• Chronic HE
  Slow onset with gradually progressive or relapsing symptoms

ASSOCIATED WITH PORTOSYSTEMIC SHUNTING OF VENOUS BLOOD AND VARIABLE HEPATIC DYSFUNCTION
Rectal ammonia tolerance test should not be performed in acute hepatoencephalopathy / acute liver failure as this might be life threatening with coma and death.
Ammonium-urate crystals regularly present in urine in dogs with (congenital) portosystemic shunts.
ULTRASONOGRAPHY

Ultrasonography of the abdominal cavity, focusing on the liver, biliary tract and portal vein, is an essential step in diagnostic hepatology.
Systemic evaluation is required of:

- The size of the liver
- The presence of local changes or a diffusely even echodensity
- Diameter and wall thickness of extrahepatic and intrahepatic bile ducts
- The gall bladder
- Vascular changes, particularly of the portal vein
- The presence of free abdominal fluid
Cat

Dilation of the common bile duct
Dilated extrahepatic and large intrahepatic bile ducts:

- In dogs usually associated with extrahepatic cholestasis or congenital dilation of these bile ducts (Caroli syndrome)

- In cats usually associated with (chronic) cholangitis
Dog

Mucocele of the gall bladder
Dog  Intrahepatic congenital portosystemic shunt
Ultrasonography

Literature

SONOGRAPHIC EVALUATION OF THE COMMON BILE DUCT IN CATS.

ULTRASONOGRAPHIC APPEARANCE AND CLINICAL FINDINGS IN 14 DOGS WITH GALLBLADDER MUCOCCELE.

STANDARD PLANES FOR ULTRASONOGRAPHIC EXAMINATION OF THE PORTAL SYSTEM IN DOGS

Ultrasonographic identification and characterization of congenital portosystemic shunts and portal hypertensive disorders in dogs and cats.
Victor Szatmari and Jan Rothuizen WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases, Chapter 3
Colour Doppler ultrasound image. Dilated left ovarian vein (LOV) as a result of acquired spleno-renal collaterals in a cairn terrier four weeks after partial attenuation of a congenital extrahepatic spleno-caval shunt. LRV Left renal vein, CVC Caudal vena cava
Additional clinical methods (1)

- Venoportogram
Normal venoportogram
Venoportogram: underdeveloped intrahepatic portal vein branches associated with a congenital portosystemic shunt
Venoportogram in primary hypoplasia portal vein: underdeveloped intrahepatic portal vein branches and multiple collaterals
Additional clinical methods (2)

- Scintigraphy
- Computed tomography (CT)
- MRI
Fine needle aspiration biopsy (FNAB)

- Easy to perform also in cases with coagulation disorders
- Fast results
- Blind or under ultrasonographic guidance in case of (multi)focal lesions or aspiration of the gall bladder
Normal hepatocytes
Fine needle aspiration biopsy (FNAB)

Overall agreement between the histopathologic diagnosis and cytologic diagnosis was found in only 30.3% of canine cases and in 51.2% of feline cases.

Fine needle aspiration biopsy (FNAB)

• Particularly useful for diagnosis of:
  – Hepatic lipidosis
  – Steroid induced hepatopathy
  – Malignant lymphoma / mastocytosis
  – Primary or metastatic carcinomas

• Cytology is not suitable for any disease in which the histologic structure is required for proper judgment
Cat: Hepatic lipidosis
Dog: steroid induced hepatopathy
Dog: hepatocytes with cytoplasmic accumulation of pigment
Rubeanic acid stain for copper

Courtesy: Erik Teske, Small Animal University Clinic, Utrecht, The Netherlands
Dog: hepatocellular neoplasia
Dog: malignant lymphoma
Fine needle aspiration biopsy (FNAB)

Sampling of bile from the gall bladder for cytology and bacteriological culture is especially important in cats, in which cholangitis is one of the most frequent hepatobiliary diseases.
Aspirated bile from the gall bladder of a cat showing many bacteria

Courtesy: Erik Teske, Small Animal University Clinic, Utrecht, The Netherlands
Fine needle aspiration biopsy (FNAB)

- Puncture of the gall bladder and local administration of antibiotics is a very effective treatment of feline ascending / neutrophilic bacterial cholangitis
Liver histopathology is the cornerstone in the diagnosis and evaluation of liver diseases. Sampling of liver tissue is too often omitted by the clinician and their conclusions and treatment therefore are not based on solid evidence.
Liver biopsy

• Patient preparation
  – Fasting for about 12 hours
  – Blood coagulation testing less than 24 hours before taking the biopsy.

• Anesthesia
  – Cat: general anesthesia usually necessary
  – Dog: general anesthesia rarely necessary

• Ultrasonography for guided biopsies
Liver biopsy

- Liver biopsy techniques
  - Menghini needle (blind) 14 G large/medium sized dogs
  - True-cut needle 16 G small dogs /cats
  - Forceps biopsy during laparoscopy
  - Surgical wedge biopsy

- Fixation: neutral buffered formalin 10%

- Contraindications:
  - Abnormal coagulation
  - Passive congestion of the liver
Liver biopsy

- Needle biopsies versus forceps or surgical biopsies

- Needle biopsies are adequately representative and provide enough tissue for any form of histopathological examination
Recommended staining techniques for pathologists

• Standard stains:
  – hematoxylin eosin (HE)
  – reticulin stain according to Gordon and Sweet or Sirius red stain or Masson’s trichrome stain.

• Additional stains (detection or confirmation of specific substances / organisms) a.o.:
  – rubeanic acid or rhodanine (copper)
  – PAS (glycogen, fungi, yeasts) diastase PAS (ceroid / lipofuscin), ZN (lipofuscin, acid fast bacteria), Perl’s blue (iron), congo red or Stokes (amyloid), Fouchet’s (bilirubin).
Additional histopathological staining techniques

- **Immunohistochemistry**
  - Proteins
  - Micro-organisms

- **In situ hybridization**

  - Hepatocellular adenoma (glutamin synthase)
  - Necrotizing cholangitis (distemper virus)
DETERMINANTS OF DIAGNOSTIC ACCURACY

Factors related to the clinician:

- providing adequate biopsies
- assuring adequate fixation and tissue handling
- providing essential clinical information
DETERMINANTS OF DIAGNOSTIC ACCURACY

Factors related to the specimen:

- size of the specimen
  preferably 2 biopsies of 1-2 cm
- quality of the specimen (careful handling, adequate fixation, sectioning and staining)
- meaningful special stains
DETERMINANTS OF DIAGNOSTIC ACCURACY

Factors related to the interpreter:

- Experience in hepatopathology
- Interest in hepatology
- Adequate clinical information
REQUIREMENTS for OPTIMAL DIAGNOSIS

1. communicating clinician
2. adequate biopsy and fixation
3. impeccable histology
4. interested and experienced hepatopathologist
5. intelligent clinico-pathological cooperation
What may the clinician expect from the pathology report?

- Quality of the specimen (size, fragmentation, sufficient material)

- Primary liver disease or secondary (particularly non-specific reactive hepatitis)

- Primary liver disease:
  - Circulatory disorder: specified and extent of lesions
  - Biliary disorder: specified and extent of lesions
  - Parenchymal disorder: specified and extent of lesions
  - Neoplasia: specified, malignancy and prognosis.

- Possible cause
Circulatory disorders

Hypoperfusion portal vein (CPSS)

Chronic fibrous occlusion portal vein

Passive congestion

Primary hypoplasia of the portal vein
Biliary disorders

Congenital cystic disease (ductal plate anomalies)

Congenital hepatic fibrosis

Congenital dilation of the large intrahepatic bile ducts

Adult type polycystic disease
Biliary disorders (cholestasis)

Cholestasis

Extrahepatic cholestasis
Biliary disorders (cholangitis)

Destructive cholangitis associated with distemper virus infection

Neutrophilic cholangitis

Lymphocytic cholangitis

Cholangitis due to liver flukes
Parenchymal disorders (reversible hepatocellular changes)

Steroid induced hepatopathy

Hepatic steatosis (lipidosis)
Parenchymal disorders (hepatitis)

HEPATITIS IS CHARACTERIZED BY HEPATOCELLULAR APOPTOSIS AND NECROSIS, A VARIABLE MONONUCLEAR OR MIXED INFLAMMATORY INFILTRATE, REGENERATION AND FIBROSIS

THE PROPORTION AND THE DISTRIBUTION OF THESE COMPONENTS VARY WIDELY AND IT IS NECESSARY TO INCLUDE IN THE PATHOLOGY REPORT THE ACTIVITY AND STAGE OF THE DISEASE AS WELL AS THE POSSIBLE ETIOLOGY
THE ACTIVITY OF THE DISEASE IS DETERMINED BY THE QUANTITY OF INFLAMMATION AND THE EXTENT OF THE HEPATOCELLULAR APOPTOSIS AND NECROSIS

THE STAGE OF THE DISEASE IS DETERMINED BY THE EXTENT AND PATTERN OF FIBROSIS AND THE POSSIBLE PRESENCE OF ARCHITECTURAL DISTORTION (CIRRHOSIS)
Parenchymal disorders (hepatitis)

Acute hepatitis, high activity

Chronic hepatitis: high activity
Parenchymal disorders (hepatitis)

Chronic hepatitis inactive (macronodular cirrhosis) associated with superficial necrolytic dermatitis

Acute hepatitis severe due to Toxoplasma gondii (arrow) infection
HEPATITIS IN DOGS

- IDIOPATHIC

- COPPER-ASSOCIATED

Rubeanic acid
35% of canine hepatitis is copper-associated.
BREED PREDISPOSITION:

Bedlington terrier (COMMD1)
West Highland White terrier
Skye terrier
Doberman pinscher
Labrador retriever
Dalmatian
Copper associated hepatitis

• Genetic susceptibility
  – Monogenic (COMMD-1 Bedlington terrier)
  – Complex (Labrador, Dobermann)

• Alimentary copper
Alimentary copper

Dietary levels of copper and zinc at levels that are present in commercially available dog food influence liver copper and are a risk factor for the development of copper associated hepatitis in dogs, particularly in dogs with a genetic susceptibility to copper.
Alimentary copper

Low copper / high zinc diets:
1. are able to prevent copper accumulation and the secondary hepatitis in dogs with a genetic susceptibility, and
2. are able to reduce abnormal high liver copper levels and secondary hepatitis although less in older dogs with high hepatic copper levels and in dogs with a high genetic susceptibility.
Neoplasia

Hepatocellular adenoma

Hepatocellular carcinoma

Cholangiocellular carcinoma

Malignant lymphoma
Additional methods

• Biopsies for quantitative Cu-determination (copper free containers)

• Biopsies for molecular investigations
  – RNA (fixation in RNA later)
  – Proteins (-70°C)
  – DNA : total blood (EDTA) or biopsies (-70°C)
Conclusion

• Ultrasonography is the most important clinical tool in diagnostic hepatology

• Histopathology is essential for the diagnosis and follow up of most primary and secondary liver diseases

• Interactive cooperation between clinician and pathologist is fundamental in diagnostic hepatology and in the development of our knowledge on liver diseases in dogs and cats