Diagnostic update

Liver biopsy of the dog

A valuable tool in the diagnosis and management of chronic hepatitis

Histological evaluation of a liver biopsy of the dog is often required to diagnose hepatobiliary disease, guide treatment decisions, and provide prognostic information, especially in the diagnostic workup of chronic hepatitis, one of the most frequently occurring inflammatory conditions of the hepatobiliary system.

This update summarizes how pathologists assess, score, and report histological lesions in liver biopsies from dogs suffering from chronic hepatitis. Histological scoring (grading and staging) of lesions and diagnostic aspects of copper-associated chronic hepatitis are included. Clinical aspects, such as clinical manifestations of chronic hepatitis and treatment options, are beyond the scope of this document.

How do we define chronic hepatitis?

Primary inflammatory liver disease encompasses conditions affecting the parenchyma (acute and chronic hepatitis) and conditions involving the biliary system (cholangitis). **Chronic hepatitis** in dogs is histologically characterized by hepatocyte apoptosis and/or necrosis, inflammation, regeneration and fibrosis. Chronic hepatitis can progress to cirrhosis, which is an end-stage chronic hepatitis characterized by distortion of the parenchymal architecture, fibrosis, formation of regenerative nodules and vascular anastomoses.

Non-specific reactive hepatitis is a secondary inflammatory liver condition and represents a response to a primary disease process elsewhere in the body, often involving inflammation of the gastrointestinal tract or pancreas, oral cavity or is a response to a systemic illness. The liver lesions are not the primary problem, and one should search for the presence of an extrahepatic disorder. On histology, resolution of prior injury to the liver may mimic reactive hepatitis.

Sampling and evaluation of liver tissue by fine needle capillary sampling (FNCS)

- Cytology is the simplest and fastest method of sampling the liver and is useful in the evaluation of several pathological conditions.
- As a diagnostic step in the workup of a hepatic disease, cytology should be considered since a definitive diagnosis may be established and thus avoid biopsy.

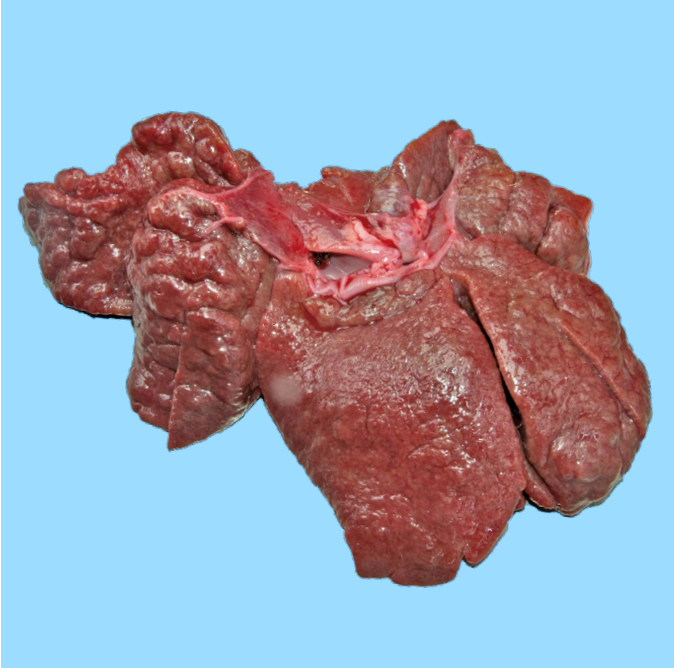

Figure 1. Chronic hepatitis in a dog. Notice variably sized hyperplastic nodules in all liver lobes (referred to as macronodular cirrhosis).

- Cytology allows a diagnosis of hepatocellular injury, cholestasis, amyloidosis, a limited number of inflammatory conditions, some biliary diseases, presence of etiological agents and recognition of neoplastic diseases.
- The cell yield is highly variable and directly related to the sampling method and skill of the clinician.
- Although useful in the recognition of cytoplasmic copper accumulation, cytology is limited by the inability of evaluating zonal copper distribution. A diagnosis of copper-associated hepatitis relies on histology by demonstrating a zonal distribution of copper, evaluating the degree of hepatocellular injury and the semi-quantitative determination of the amount of copper using special stains.

- Fibrosis is a feature of chronic hepatitis, and although recognizable by cytological examination (figure 2), overall architecture can only be correctly assessed by histologic examination of liver tissue. As such, histology and not cytology, allows staging and grading of lesions in chronic hepatitis.
- As a conclusion, although useful in the recognition of fibrosis, cytology is **not suitable for a correct and definitive diagnosis of chronic hepatitis,** where the evaluation of architectural changes in the liver, with histology, is necessary.

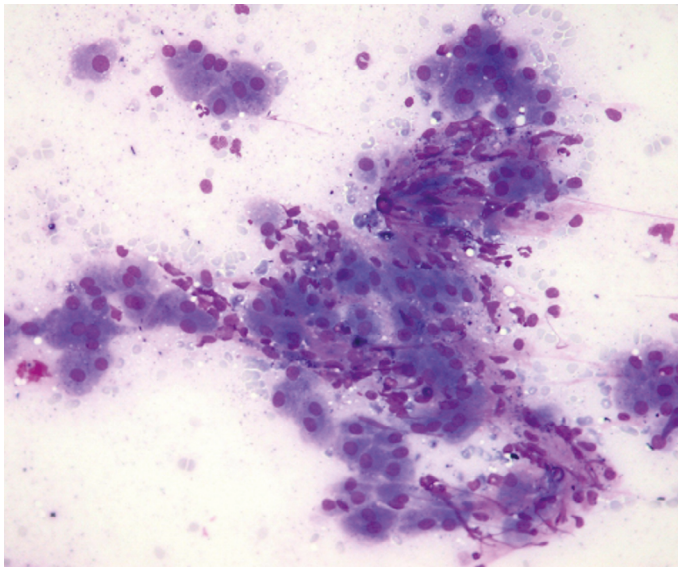

Figure 2. Cytological features of liver fibrosis: although the presence of eosinophilic bundles among the hepatocytes is highly suggestive of liver fibrosis, histological examination is needed to confirm the diagnosis of chronic hepatitis.

Liver biopsy of the dog

Common indications	Preliminary investigations	Contraindications/ bleeding complications
Increased serum liver elevation • Increased ALT activity more than 8 weeks with no response to hepatoprotective therapy • Breed predisposed to primary hepatic disease (e.g. chronic hepatitis) • Non-specific reactive hepatitis should first be ruled out **Icterus** • Intrahepatic jaundice without response to symptomatic therapy **Hepatic mass when cytological diagnosis is not achieved** **Acquired portosystemic shunts**	• Hematology (platelets) • Buccal mucosal bleeding time (BMBT) • Prothrombin time (PT)/activated partial thromboplastin time (aPTT) • Fibrinogen • If disseminated intravascular coagulation (DIC) is suspected, antithrombin III (AT III) and D-dimers	• Platelets < 50,000/µl • BMBT > 240 seconds • PT/aPTT > 1.5 x the upper reference value • Fibrinogen < 100 µg/dl • PCV < 30 % • Acute liver failure • End-stage hepatitis • Ascites (treat first)

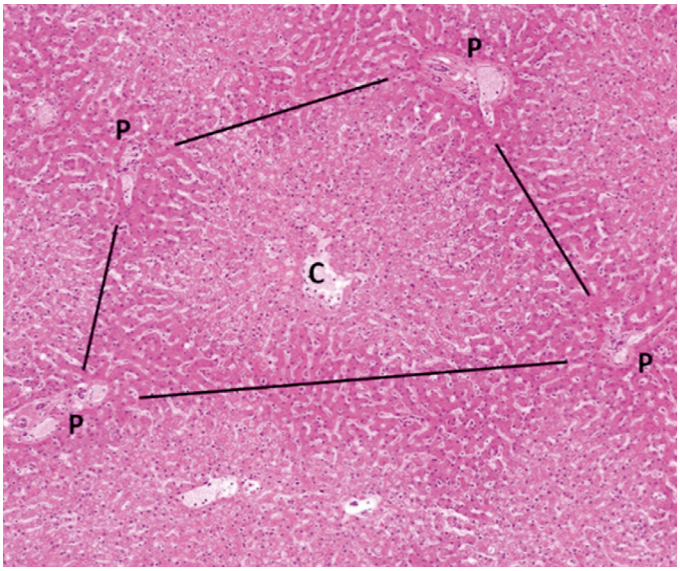

Figure 3. With light microscopy, a hepatic lobule is composed of a central vein (C) located in the center of the lobule and portal tracts (P) located at the periphery. Plates of hepatocytes are arranged radially between portal tracts and the central vein. In chronic hepatitis, the stage of the disease refers to fibrosis, which may be present around portal tracts or around central veins or may connect adjacent portal tracts (portoportal bridging fibrosis), portal tracts and central veins (portocentral bridging fibrosis) and adjacent central veins (centrocentral bridging fibrosis, see table 2).

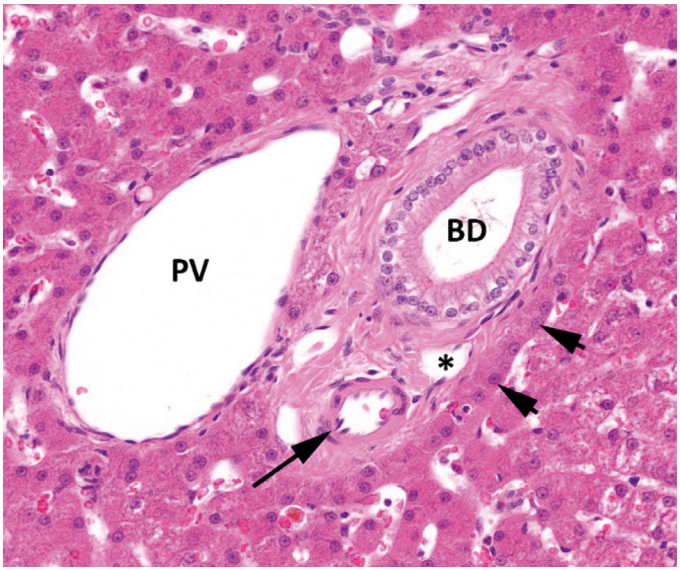

Figure 4. Normal portal tract as seen with light microscopy. The portal tract contains a bile duct (BD), branch of the hepatic artery (long arrow), branch of the portal vein (PV) and lymphatics (*). The limiting plate consists of hepatocytes joining together to form a distinct row around portal tracts (short arrows).

Microscopic structure of the liver

The classic functional subunit of the liver is the hepatic lobule, a hexagonal structure, 1 to 2 mm wide. With light microscopy, a central vein (synonym terminal hepatic venule) is located at the center of the lobule while portal tracts are situated at the angles of the lobule (see figures 3 and 4). The lobule is further subdivided into centrilobular, midzonal and periportal areas, and this terminology is used by pathologists to describe the distribution of lesions in the liver.

Sampling of liver for histopathological examination: size matters

Reliable histopathologic results are dependent upon a liver sample of adequate size and quality. The biopsy sample(s) must be representative, and at least 12–15 portal triads should be available for evaluation. The sample depth should be at least 1–2 cm, if possible, as subcapsular tissue often contains more fibrosis and non-specific inflammatory reaction. A disease process that is not diffusely distributed throughout the liver may be missed if the sampling is inadequate. Similarly, the severity of a disease process may vary considerably between liver lobes. Thus, focal lesions and/or a minimum of 3 separate liver lobes should be sampled.

The consensus statement of the American College of Veterinary Internal Medicine (ACVIM) on the diagnosis and treatment of chronic hepatitis in dogs recommends the following for hepatic biopsy sample acquisition: a minimum of 5 laparoscopic or surgical biopsy samples from at least 2 liver lobes for histopathology, aerobic and anaerobic culture, and quantitative copper analysis. If it is not possible to take samples by laparotomy or laparoscopy, then ultrasound-assisted percutaneous biopsies with a size of 14–16 G and a length of 2–2.5 cm are recommended. It should be noted that the number of 12–15 portal triads can only be achieved with this sampling technique if at least 5 samples are taken. However, it must be considered that a higher number of samples with this collection technique may be associated with an increased risk of bleeding.

Sampling of liver tissue for histopathological examination: methods

Liver tissue for histopathological examination can be obtained by different methods, each with pros and cons. Several considerations may determine the biopsy method used, such as clinical assessment of the patient, preference and technical skill of the clinician, availability of equipment and costs. Balancing the invasiveness and possible complications of the procedure versus the size of sample(s) obtained is another important consideration. The three most common hepatic biopsy techniques are ultrasound-guided percutaneous biopsy (core needle biopsy is the most widely used technique), laparoscopic biopsy, and surgical biopsy by exploratory laparotomy.

Ultrasound-guided percutaneous needle biopsy

- Least invasive
- Difficult visualization of small livers
- Small sample size and lesions may be missed in the case of focal or multifocal lesions
- Possible fragmentation of liver tissue/fibrous tissue
- Cannot observe for post-biopsy bleeding

Laparoscopic biopsy using biopsy forceps

- Less invasive than biopsy by laparotomy
- Sample size intermediate between needle biopsies and a surgical wedge biopsy
- Gross evaluation of the entire liver, biliary system and pancreas possible
- Different regions/lobes of liver can be sampled
- Can assess post-biopsy bleeding and respond

Surgical biopsy by exploratory laparotomy

- Most invasive
- Adequately sized wedge biopsies with a high diagnostic value
- Different regions/lobes of liver can be evaluated directly and sampled (see above)
- Other abdominal organs can be inspected and additional biopsies besides liver can be sampled
- Can assess post-biopsy bleeding and respond

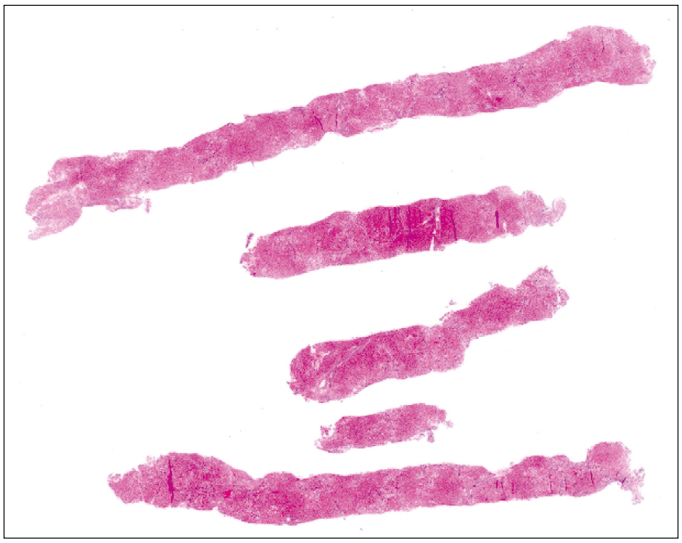

Figure 5. Photo to illustrate needle biopsies (core needle biopsies) obtained percutaneously with ultrasound guidance. The biopsies are relatively small and may be of questionable representation as significant lesions may be missed. Possible fragmentation of tissue due to fibrosis may occur.

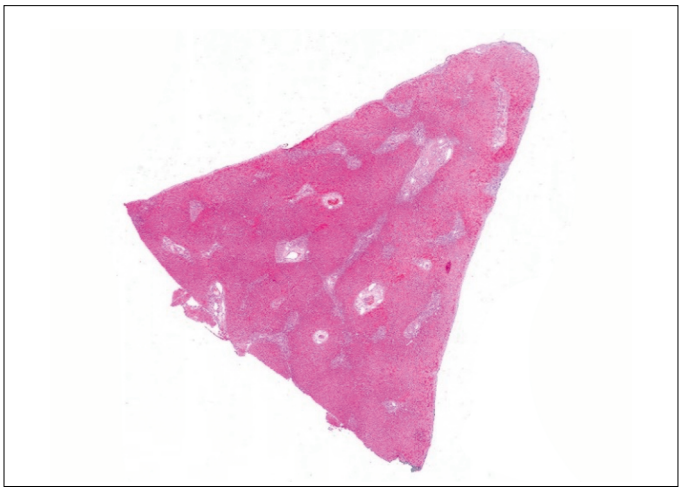

Figure 6. In diffuse liver disease, surgical biopsies harvested by exploratory laparotomy generally produce adequately sized wedge biopsies with a high diagnostic value.

Nature and extent of liver lesions matter in management and prognosis of chronic hepatitis

Most cases of chronic hepatitis are idiopathic and treatment of a specific cause, besides copper excess, is not feasible. In the absence of a specific cause, **knowledge of the nature and extent of the underlying liver lesions** may thus be helpful in optimizing **management** of the patient and judging the **prognosis** of the condition.

In the context of chronic hepatitis, several features are important:

- Type and severity of the inflammation
- Amount and extent of fibrosis
- Loss of normal architecture
- Presence of hyperplastic nodules, indicating progression to cirrhosis
- Degree of copper accumulation and its distribution
- Presence of bile stasis

The chronicity of the disease and possible prognosis can be assessed based on the presence and extent of fibrosis (bridging fibrosis) and cirrhosis. The histologic findings should also be correlated with the findings of additional diagnostics, such as bacteriological examination from liver tissue and bile (particularly any debris within the gallbladder).

Periodic histological liver examination also permits monitoring of the course of therapy. A follow-up biopsy enables the evaluation of the effect/success of therapy, a better assessment of the prognosis and, if necessary, an adjustment of therapy (e.g. in the case of copper-associated hepatitis).

Grading and staging when reporting chronic hepatitis

The World Small Animal Veterinary Association (WSAVA) International Liver Standardization Group defined a **scoring system** that consists of two components: grading and staging. **Grading** refers to quantification of the activity of the disease and is determined by the amount of inflammation and extent of hepatocellular apoptosis and necrosis (also referred to as necroinflammatory activity). The **stage** of the disease is determined by the extent and pattern of fibrosis and the possible presence of architectural distortion, including the development of cirrhosis.

In the scoring system, the grade and stage of the lesions are presented as grades 0–5 (table 1) and stages 0–4 (table 2) respectively, corresponding to the descriptive terms absent, slight, mild, moderate, marked or very marked. Grade/stage 0 (zero) corresponds to the absence of necroinflammatory activity/fibrosis and the highest grade/stage to very marked necroinflammatory activity/fibrosis. Histochemical stains for connective tissue are helpful in detecting the amount and pattern of fibrosis, particularly in early and mild disease, and should be routinely applied. Stage 4 fibrotic lesions corresponds to cirrhosis.

Copper-associated chronic hepatitis

In most cases of chronic hepatitis in the dog, the cause is unknown. Several drugs and toxins have been implicated, and current knowledge indicates that the most common toxic injury-causing chronic hepatitis in dogs is a consequence of hepatic copper (Cu) excess.

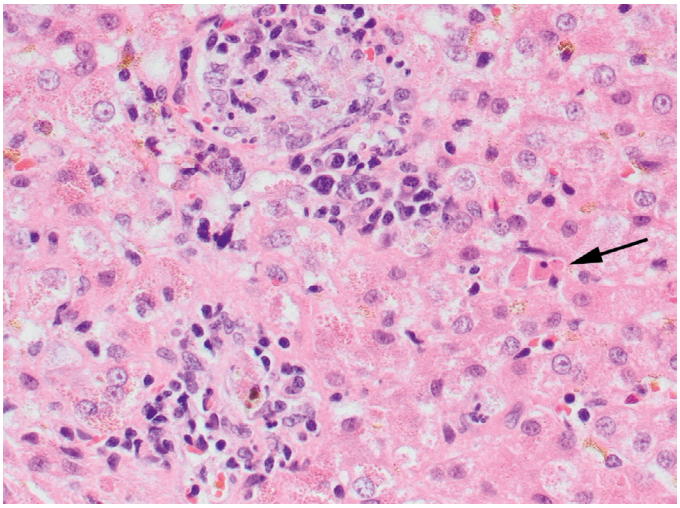

Figure 7: Two foci of inflammation within a lobule in a case of chronic hepatitis. Two apoptotic hepatocytes are seen (arrow). The number of these inflammatory foci (as well as necrosis and apoptosis of hepatocytes) is used as a criterion in the grading of necroinflammatory activity in chronic hepatitis (see table 1). For example, if there are 2–4 inflammatory foci per 10 x objective, the activity is mild, implying grade 2 activity.

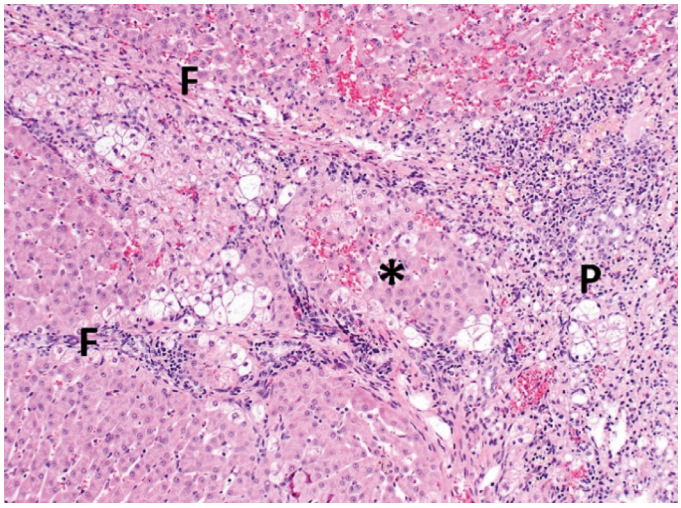

Figure 8: Chronic hepatitis characterised by extensive inflammation and bile duct proliferation (P) and bridging fibrosis (F) disturbing the normal architecture of the liver. A nodule of hepatocytes become isolated by the fibrosis (*).

Copper-associated chronic hepatitis is diagnosed semi-quantitatively by histologic evidence of hepatic copper accumulation, most of the time located in centrilobular areas of the liver, as long as lobular architecture is retained in the injured liver. For this purpose, histological examination of biopsies from dogs suffering from chronic inflammatory liver disease routinely includes evaluation of a histochemical copper stain.

The amount of **copper accumulation** in liver biopsies is scored by semi-quantitative assessment as defined by the WSAVA International Liver Standardization Group (table 3). A score of 0–5 is assigned, and the scores 0, 1 and 2 are observed in dogs for which copper is not considered a driving force for hepatocyte injury, while the scores 3, 4 and 5 are associated with pathological copper accumulation and copper-associated hepatitis.

Quantification of copper levels in liver tissue can also be determined (1 gram of liver tissue needed). Normal hepatic copper concentrations in dogs are considered below 400 mg/kg dry weight of liver tissue. In dogs affected with copper-associated hepatitis, hepatic copper concentrations are usually above 800 mg/kg dry weight liver tissue. A significant difference between the score obtained by semi-quantitative analysis and the quantitative copper level may occur, possibly due to variation in sampling. This indicates that the translation of the semi-quantitative copper score into a therapeutic approach by the clinician needs to be performed cautiously.

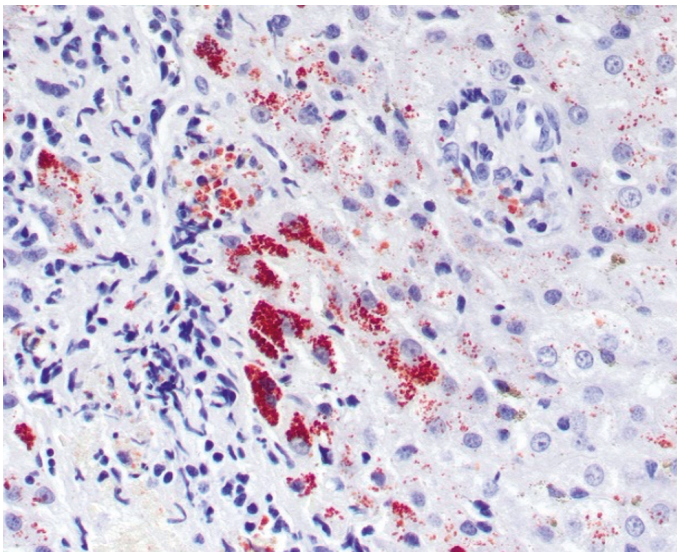

Figure 9: Liver biopsy of a dog suffering from copper-associated chronic hepatitis. The tissue is stained with rhodanine stain, and the small red intracytoplasmic granules in hepatocytes and macrophages represent copper granules. A semi-quantitative scoring system is used for the histological evaluation of copper accumulation (see table 3).

The pathology report: what to expect in a case of chronic hepatitis

The pathologist assessing the liver biopsies will use a uniform and systematic approach to describe, score and interpret morphological and inflammatory abnormalities. The report generally includes:

- Abbreviated history and mention of (suspected) clinical diagnosis.
- Number and size of biopsies.
- Artefacts and biopsy size/**quality**; report should clearly indicate where suboptimal samples (insufficient material, mechanical injury/artefacts) have limited the ability to assess the biopsies.
- **Histopathological description** of morphological and inflammatory lesions, scoring (grading and staging) of the lesions and a semi-quantitative assessment of copper granules in sections stained with hematoxylin-eosin (HE), a collagen stain, and a copper stain. Other pathological changes, such as reversible degenerative changes (glycogen accumulation and fatty change) and the presence of infectious agents, will also be described, if present.
- Morphological **diagnosis (or interpretation)**, which reflects the most significant histological changes in the biopsies (see example below).
- **Comments**, which may include a discussion of the significance of pathological changes, mention of possible cause and recommendations for further tests, if applicable.
- **Name** of the reporting pathologist, including contact details, for follow-up if questions persist.

- Example of a morphological diagnosis/interpretation in the pathology report in a case of chronic hepatitis in the dog:
  Liver: chronic hepatitis, moderate activity (activity grade 3), marked portoportal and portocentral bridging fibrosis (fibrosis stage 4), copper score 1 and marked micronodular hepatocellular regeneration; lesions morphologically consistent with cirrhosis.

Key points: chronic hepatitis in dogs

- In most cases of chronic hepatitis, the cause is unknown.
- Drugs and toxins have been implicated; the most common toxic injury is a consequence of hepatic copper excess.
- Specific criteria for the diagnosis of immune-mediated chronic hepatitis have not been developed.
- In the absence of a cause, knowledge of the nature and extent of underlying liver pathology may be helpful in optimizing management and judging prognosis.
- If not possible to sample the liver by laparotomy or laparoscopy, ultrasound-guided percutaneous biopsies with a size of 14–16 G and a length of 2–2.5 cm are recommended (in order to sample at least 12–15 portal triads).
- Ultrasound-guided percutaneous biopsies of liver have a risk of sampling error, i.e. harvesting small samples or non-diagnostic samples due to variation of histological changes among liver lobes.
- When submitting biopsies for evaluation, provide clinical history, past treatments and clinical diagnosis.
- Histological biopsies are scored according to published guidelines by the WSAVA Liver Standardization Group.
- The scoring system routinely includes grading and staging of lesions and a semi-quantitative evaluation of copper accumulation in sections stained by a copper stain.
- Fine needle aspirations of the liver may be used as a diagnostic step in the workup of a hepatic disease, but this is not a reliable technique to diagnose chronic hepatitis since architectural changes in the liver cannot be assessed by cytology.

Addendum: Tables 1–3 (see text)

Table 1: Histologic **grading** (amount of inflammation and extent of liver cell apoptosis and necrosis) in chronic hepatitis in dogs

Activity	Grade	Periportal or periseptal interface inflammation (interface hepatitis)	Focal lytic necrosis, apoptosis and focal inflammation	Confluent necrosis
Absent	0	Absent	Absent	Absent
Slight	1	Very mild (focal, few portal areas)	1 x focus or less per x 10 objective	Absent
Mild	2	Mild (focal, most portal areas)	2–4 foci per x 10 objective	Absent
Moderate	3	Moderate (continuous around < 50% of tracts or septa)	5–10 foci per x 10 objective	Absent
Marked	4	Marked (continuous around > 50% of tracts or septa)	> 10 foci per x 10 objective and/or →	Confluent or bridging necrosis
Very marked	5	Marked (continuous around > 50% of tracts or septa)	> 10 foci per x 10 objective and/or →	Bridging or panacinar/ multi-acinar necrosis

Table 2: Histologic **staging** (extent and pattern of fibrosis) in chronic hepatitis in dogs

Degree of fibrosis	Stage	Fibrosis	Bridging fibrosis	Bridging fibrosis with nodule formation
Absent	0	Absent	Absent	Absent
Mild	1	Mild fibrous expansion (periportal of central)	Absent	Absent
Moderate	2	Moderate fibrous expansion	Some bridging fibrosis	Absent
Marked	3	Marked fibrous expansion	Marked bridging fibrosis	Absent
Very marked	4	Marked fibrous expansion	Marked bridging fibrosis	Present

Table 3: Semi-quantitative histologic evaluation for hepatic **copper accumulation**

Score	Description
0	No copper detectable
1	Solitary hepatocytes in the centrilobular area containing some copper-positive granules
2	Small group of hepatocytes in the centrilobular area that contain small to moderate numbers of copper-positive granules
3	Centrilobular hepatocytes and some macrophages that contain moderate numbers of copper-positive granules (one-third of each lobule)
4	Centrilobular and midzonal hepatocytes and macrophages with marked to moderate numbers of copper-positive granules (approximately two-thirds of the hepatocytes in all lobules)
5	Panlobular or diffuse presence of hepatocytes and macrophages with marked to moderate numbers of copper-positive granules

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