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How to approach a medical liver biopsy.
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INTRODUCTION

Liver biopsy continues to play an important role in the diagnosis and management of medical liver diseases. The two main indications for liver biopsy are (i) to identify the cause of liver disease in cases where this is uncertain following other investigations and (ii) to assess disease severity in cases where the cause of liver disease has already been established. The increasing use of non-invasive markers of liver fibrosis, including serum markers and transient elastography (FibroScan), is reducing the frequency of liver biopsy to stage fibrosis in chronic liver diseases such as hepatitis C and non-alcoholic fatty liver disease (NAFLD).

This presentation aims to provide a practical diagnostic approach to the assessment of needle biopsies obtained from people who are presumed to have diseases associated with diffuse hepatic involvement. The histological assessment of focal liver lesions (usually neoplasms) will not be considered.

A SYSTEMATIC APPROACH TO LIVER BIOPSY ASSESSMENT

The following is suggested scheme for assessing liver biopsies in a systematic fashion.

1. It may be helpful to make an initial assessment of the biopsy without knowledge of the clinical history.

2. There should also be a preliminary assessment of **biopsy adequacy.** The definition of adequacy varies according to the indication for liver biopsy. For accurate assessment of disease severity, it is recommended that a biopsy should be at least 20-25mm long and contain at least 10-12 portal tracts. Thin biopsies are known to under-estimate fibrosis stage and result in incomplete sampling of portal tracts - radiologists/physicians obtaining liver biopsies should therefore be encouraged to use as wide a needle as possible, preferably at least 16-gauge.

3. The first step in the detailed examination of the liver biopsy is to assess the **liver architecture**.
    *   Are normal vascular relationships retained?
    *   Is there any evidence of fibrosis or cirrhosis?
    *   What is the pattern of fibrosis? e.g. periportal fibrosis in chronic hepatitis and chronic biliary disease, perisinusoidal/pericellular fibrosis in fatty liver disease.
    *   Are there any other subtle architectural abnormalities? e.g. atrophy or nodular regenerative hyperplasia which occur in portal venous insufficiency.

    Connective tissues stains are required to assess the liver architecture properly:

Stain	Material demonstrated	Distribution in normal liver	Changes in liver disease
Reticulin	Type III collagen fibres	Portal tracts, hepatic sinusoids	Collapse of reticulin framework in areas of recent liver cell necrosis. Thickening of cell plates in areas of nodular regeneration.
Haematoxylin Van Gieson	Type I collagen fibres	Portal tracts, walls of hepatic veins	Increased in hepatic fibrosis
Orcein	Elastic fibres	Portal tracts, walls of hepatic veins	Found in long-standing fibrosis/cirrhosis

4.   Next examine the **portal tracts and periportal regions**.
First assess the three normal portal tract components:
(a) *Bile ducts*.
- Are they present in normal numbers?
- Are there any bile duct lesions? e.g. granulomatous cholangitis in PBC, fibrous cholangitis in PSC.

(b) *Hepatic arteries*.
- Are there any hepatic arterial lesions? e.g. inflammation in polyarteritis nodosa and some drug reactions, amyloid deposits (arterial lesions are rarely seen in needle biopsies)

(c) *Portal veins*.
- Are there any portal vein lesions? e.g. obliteration and/or dilatation in obliterative portal venopathy ("non-cirrhotic portal hypertension")

Then look for the presence of other abnormal features:
(a) *Inflammatory cells* – density, distribution, composition
(b) *Interface hepatitis* ("piecemeal necrosis")
(c) *Ductular reaction* - a common reaction to many forms of liver injury, particularly those in which there is a cholestatic component.

5.   Move then to the **liver parenchyma**.
First assess the normal parenchymal components:
(a) *Hepatocytes*.
- Are there any degenerative changes, such as fatty change, ballooning or bilirubinostasis?
- Is there any evidence of liver cell death (apoptosis or necrosis)? If necrosis is present, what is the severity (spotty, confluent, bridging, panacinar)?
- Are there any inclusions -nuclear or cytoplasmic (e.g. Mallory-Denk bodies or megamitochodria in fatty liver disease, eosinophilic globules in alpha-1-AT deficiency)?
- Note whether any of the above have a zonal distribution - e.g. zone 3 Mallory-Denk bodies in fatty liver disease , zone 1 in chronic cholestasis (cholate stasis).

(b) *Sinusoidal cells*
- *Kupffer cell inclusions* may be seen in some metabolic storage diseases and certain parasitic infections
- *Kupffer cell enlargement* with cytoplasmic ceroid pigment is commonly seen as a non-specific reaction to previous hepatocellular injury.
- Transformation of *sinusoidal endothelial cells (SECs)* to a vascular phenotype (CD 34+) occurs in conditions associated with altered sinusoidal blood flow (e.g. portal venous insufficiency) and may be associated with the development of perisinsoidal fibrosis.

Then look for the presence of other abnormal features
(a) *Inflammatory cells* – density, distribution, composition.
(b) *Sinusoidal dilatation/congestion*
(c) *Deposits (e.g. amyloid)*

6.   Next examine the **hepatic veins.**
Is there evidence of endothelial inflammation?
Are veno-occlusive lesions present?
Is there inflammation in the vein wall?
Are any lesions (e.g.inflammation, hepatocyte necrosis, fibrosis) present in tissue immediately surrounding hepatic veins?

7. Then look at the **special stains**. A recommended panel is :
   - Reticulin stain to assess architecture (see table above).
   - Trichrome/ HVG to assess fibrosis (see table above).
   - Shikata's Orcein to assess HBsAg, copper associated protein and elastic fibres (see table above).
   - PAS to identify glycogen (can also be used to look for small foci of necrosis and or granulomas).
   - PAS plus diastase for recognition of ceroid pigment in Kupffer cells and alpha-1-antitrypsin globules.
   - Perls stain to assess presence of iron (haemosiderin).

8. In some cases additional **immunohistochemical stains** may be appropriate. Examples include:
   - Demonstration of viral antigens (e.g. HBsAg, HBcAg, HDV, CMV, EBV)
   - Identification/confirmation of hepatocyte inclusions (e.g. ubiquitin/CK18/p62 for Mallory's hyaline, alpha-1-antitrypsin immunohistochemistry)
   - Biliary cytokeratins (CK7, CK19, AE1) to assess bile duct loss and ductular reaction in and around portal tracts and to identify parenchymal cells with an intermediate hepatobiliary phenotype, which may be an early feature of chronic cholestasis.

9. The various features noted above should be summarised and an attempt made to **identify the main pattern(s) of damage present** (e.g steatohepatitis, acute hepatitis, chronic hepatitis, chronic biliary disease etc). In many cases a definitive diagnosis cannot be made on the basis of histology alone and the final interpretation of the biopsy will depend on correlating the histological findings with the clinical history and the results of other investigations. In some cases where a dual pathology is suspected (e.g. HCV and NAFLD), an attempt should be made to identify the main cause of liver injury.

10. Because the average needle biopsy only samples a tiny proportion of the whole liver the possibility of **sampling variation** should always be borne in mind, especially if there is a disparity between the clinical and histological findings. Disease processes which frequently have an uneven distribution include fibrosis in chronic biliary diseases, congestion and necrosis in vascular diseases and bridging/panacinar necrosis in cases of severe acute hepatitis.

11. In some cases the histological report may be supplemented by semi-quantitative **histological scoring** (e.g. grading of inflammatory activity and staging of fibrosis in chronic viral hepatitis). This approach is best used in the context of large clinical trials. There are several theoretical and practical problems with histological scoring and it should not be used as a substitute for conventional histological reporting in routine clinical practice.

12. **Electron microscopy** has a limited role to play in liver biopsy assessment. EM may be useful in the assessment of some metabolic disorders (e.g. glycogen storage disease, Niemann-Pick disease), certain viral infections (e.g paramyxovirus in giant cell hepatitis) and the identification of other hepatocyte inclusions (e.g.phospholipidosis in amiodarone toxicity).

13. Routinely processed tissue may also be used for certain **non-histological investigations**. These include biochemical measurements (e.g. liver copper or iron) or molecular analysis of extracted nucleic acids (e.g. detection of low levels of viral RNA or DNA by PCR)

CONCLUSION

The histological assessment of medical liver biopsies involves a systematic evaluation of histological abnormalities to identify one or more basic patterns of liver injury. The final interpretation of histological findings then depends on careful clinico-pathological correlation. The information provided in the concluding section of the histology report should be tailored according to the reason indicated clinically for obtaining a liver biopsy. In those cases where the information requested clinically cannot be provided histologically, this should be clearly stated. In other cases where there appears to be a disparity between the clinical and histological findings, the possibility of sampling variability should be considered.