

# Practical Interpretation of Liver Biopsy, Volume 1



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Edited by

Xiuli Liu, Jinping Lai and Nirag Jhala

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## PREFACE

Our understanding of liver diseases has expanded in the last several decades as a result of advances in epidemiology, virology, immunology, and molecular biology. New revolutionary therapeutic agents such as anti-viral agents for hepatitis B and hepatitis C provide a cure for such patients. Recent clinical trials for non-alcoholic steatohepatitis reveal promising results. Liver pathology interpretation has played an essential role in all these aspects of *Hepatology* by providing information on the severity and stage of liver diseases and by pinpointing the etiology in some cases and narrowing down differential diagnoses in many other cases.

The spectrum of liver diseases undergoes dynamic changes. Liver pathology practice seems a daunting task for many pathologists including general pathologists, junior gastrointestinal and liver pathologists, in part, due to the complexity of anatomy, sophistication of biochemicals and metabolic functions, and multi-faceted clinical presentations of many liver diseases. In many cases, a liver biopsy only represents a snapshot of the liver disease and does not allow a pathologist to get a whole picture regarding the clinical course and reversibility of disease. A long-term clinical and histological follow-up provides the most meaningful liver pathology education in many cases. However, this type of information is difficult to acquire during our pathology residency and even liver pathology fellowship.

In this book, we include many common liver diseases with a brief clinical presentation, laboratory findings, and histological features. We also include histologic findings corresponding to treatment responses in some entities where specific therapies exist, notably, anti-viral agents in hepatitis B and C and removal of iron and copper in hereditary hemochromatosis and Wilson's disease. For metabolic liver diseases and hereditary diseases, we also emphasize the tissue allocation, biochemical analyses, ultrastructural examination, and genetic analyses.

This book reflects our struggling and thriving journey as liver pathologists. We intend to pass what we have learned from our combined 150+ years liver pathology practice to the readers of this book; we hope this book will help them generate an accurate histologic interpretation of the liver biopsies

during their practice. In addition, we would like to dedicate this book to all patients who have given us the privilege to review their liver biopsies, to learn, to share, and to teach.

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## CHAPTER ONE

# LIVER BIOPSY INDICATIONS, TISSUE HANDLING, PROCESSING AND GENERAL APPROACHES

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### **Abstract**

Liver biopsy, a minimally invasive, diagnostic procedure, has played an essential role in understanding the pathology of various liver diseases, by sampling a small portion of the liver. Detailed guidelines developed for the clinical use of liver biopsy have undergone constant changes. Liver tissue obtained by biopsy should be handled gently and processed promptly and adequately so that sufficient information can be acquired for diagnosing and staging the liver diseases. The liver biopsy is best handled and processed by using a standard protocol in the histology lab. With appropriate preparation, liver tissue can be examined under light microscopy, immunohistochemically or biochemically. In addition, ultrastructural information of the liver tissue can be acquired by electron microscopic examination. Biochemical abnormalities can be detected in reference laboratories using snap frozen fresh tissue for cases to diagnose metabolic liver diseases. In this chapter, liver biopsy indication, biopsy adequacy, and tissue processing including special stains and commonly used immunohistochemical stains will be discussed.

**Keywords:** Liver biopsy; Hematoxylin & eosin stain; Electron microscopy

## Introduction

The value of needle biopsy as a diagnostic tool in the investigation of liver disease is well recognized.<sup>1-4</sup> Liver biopsies have been used for decades to diagnose liver disease and to evaluate the severity of liver diseases in patients with abnormal liver enzymes found on blood tests, jaundice, and unexplained enlargement of the liver, and to diagnose mass lesions found by imaging studies. Although a typical liver biopsy varies between 1 and 3 cm in length and about 1.2 to 2 mm in diameter and only represents 1/50,000 of the total mass of the liver. With synthesis of all clinical, laboratory, and radiographic information, findings in the liver biopsy can further narrow down the differential diagnoses and potentially pinpoint the etiologic diagnosis. Further, serial biopsies have been used to delineate the natural history of many liver diseases and/or assess therapeutic efficacy in a variety of liver diseases. It has been reported that liver biopsy provides an accurate diagnosis in approximately 90% of patients with unexplained abnormalities revealed on liver function tests.<sup>5</sup>

Overall, the liver biopsy currently has four major roles: (1) to diagnose disease, (2) to stage the disease by assessing the fibrosis and architectural abnormalities, (3) to assist in making therapeutic management decisions, and/or (4) to assess therapeutic effect, particularly in clinical trials. The indications for liver biopsy are outlined in **Table 1.1**.



**Table 1.1: Common indications for liver biopsy.**

<b>Indications</b>	<b>Purpose of biopsy</b>	<b>Example(s)</b>
Acute liver failure of unknown etiology	To reveal possible etiology and assess severity of disease and the need for liver transplantation	Acute liver failure
Patients with recently found abnormal liver enzymes but otherwise non-revealing serologic workup	To reveal possible etiology	Acute hepatitis with negative serology results for acute viral hepatitis
Patients with known liver disease but unusual clinical presentation or clinical course	To reveal additional pathology	Patients with acute HAV, HBV, HEV with prolonged clinical course or relapsing diseases
Patients with known chronic liver disease	To stage the disease	Nonalcoholic fatty liver disease Alcoholic liver disease
Patients with chronic viral hepatitis with questionable results on stage assessment by non-invasive modality or questionable indication for anti-viral treatment	To accurately stage the disease	Patient with chronic hepatitis C and a subset of chronic hepatitis B
Early detection of metabolic liver diseases in relatives of index patients	To evaluate the tissue iron and copper burden and liver injury	Relative of index patients with hemochromatosis and Wilson's disease with quantitative estimation of iron levels and copper levels

Systemic disease and/or evaluation of fever of unknown origin	To identify possible etiology such as infection	Fever of unknown origin
Hematologic disease	To stage the disease	Hodgkin's disease
Evaluation of the therapeutic efficacy	To document the histology improvement after treatment	Clinical trials for chronic liver diseases such as chronic viral hepatitis, autoimmune hepatitis, and steatohepatitis
Evaluation of the adverse effects of treatment regimens	To document the liver toxicity	Liver injury following methotrexate therapy for psoriasis
Evaluation of liver allograft after transplantation Evaluation of the donor liver before transplantation	Liver allograft status or etiology for allograft dysfunction Suitability of living donor liver Suitability of cadaveric donor liver for transplantation	Protocol biopsy or when there is allograft dysfunction  Liver biopsy from living donor Frozen section for donor liver
Mass lesion(s) in the liver cannot be diagnosed as HCC on the imaging study	To diagnose HCC	Cirrhosis with liver mass cannot be further classified by imaging modality.
Mass lesion(s) cannot be reliably diagnosed as focal nodular hyperplasia	To confirm the diagnosis of focal nodular hyperplasia	Focal nodular hyperplasia without central scar
Multiple liver lesions, suspicious for metastatic malignancy from sites which are difficult to obtain tissue	Confirm metastatic malignancy and obtain tissue for additional testing such as mutational analysis	Multiple lesions in patient with a pancreatic mass

Note: HAV, hepatitis A; HBV, hepatitis B; HEV, hepatitis E

## 1. Routes and devices of liver biopsy

The routes of liver biopsy depend upon the clinical scenarios. In patients with diffuse liver disease, percutaneous needle biopsy, transjugular needle biopsy, and rarely a laparoscopically guided biopsy may be used. In some patients who undergo intraabdominal surgery such as gastric bypass procedure, a wedge liver biopsy may be performed by the surgeon for the evaluation of fatty liver disease.

In patients with a focal lesion, biopsy is usually guided by some imaging modalities such as ultrasonography and computed tomography (CT) or by laparoscopy so that the local lesion can be targeted more precisely. In addition to biopsy, fine needle aspiration may be performed to obtain cells for cytologic examination which can be subjected to rapid on-site interpretation and complimentary to histologic examination to further increase the diagnostic yield with an overall good diagnostic accuracy.

### A. Percutaneous liver biopsy

For percutaneous liver biopsy, variably sized needles from 14-gauge to 18-gauge have been used to obtain liver tissue with or without ultrasound (US)-guidance.<sup>6-8</sup> Although US-guided 18- and 16-gauge core biopsies are similarly safe, about 20% and 15% of 18- and 16- gauge specimens are inadequate when American Association for the Study of Liver Diseases (AASLD) quality control adequacy thresholds are applied.<sup>7</sup>

Devices for percutaneous liver biopsy originated in the late 1800s, and proliferated and refined in the early 20<sup>th</sup> Century.<sup>2,3</sup> Two types of devices widely used for sampling diffuse parenchymal liver disease are the core-aspiration or suction needles (Menghini, Jamshidi, or Klatskin-style) (**Figs. 1.1 and 1.2**) and sheathed cutting needles (either manual, spring-loaded, or automated) (**Figs. 1.3 and 1.4**).<sup>2</sup> The core-aspiration technique relies on suction generated via a syringe in conjunction with a flat or a beveled needle tip to obtain a core of liver parenchyma (**Fig. 1.1**). The suction may cause some specimens, particularly those with advanced fibrosis, to fragment more easily. Newer automated core suction-sparing needle devices have recently emerged and may be used to obtain longer cores without fragmentation. In contrast, the cutting needle passes the liver parenchyma using a troughed needle followed by sliding of an outer sheath over to secure a core of tissue. The cutting needle is especially helpful in patients with

suspected or established cirrhosis because it limits the tendency for the specimen to shatter or fragment.



Fig 1.1 Photograph of Jamshidi suction needle with a tapered distal tip and razor-sharp edge. A core of liver parenchyma is obtained when suction is applied via a syringe.

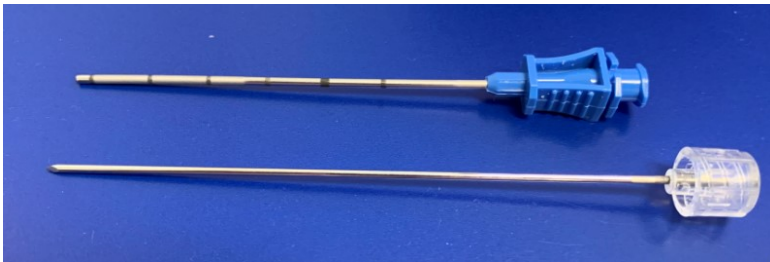


Fig 1.2 Photograph of Klatskin style suction needle.

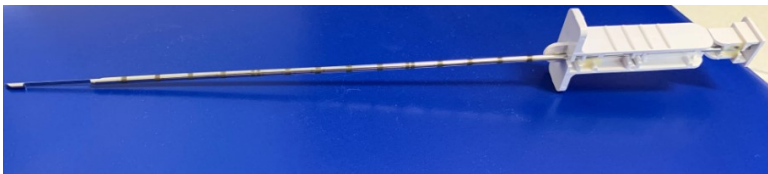


Fig 1.3 Photograph of a TruCut needle showing the obturator with the specimen notch advanced.

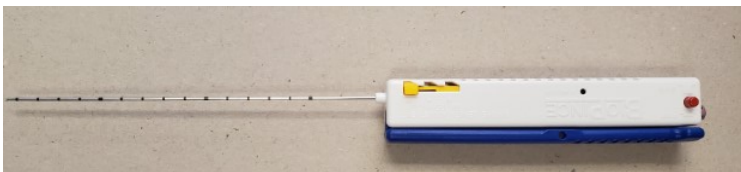


Fig 1.4 Photograph of a biopsy gun needle.

## **B. Transjugular liver biopsy**

Transjugular needle biopsy is often performed as an alternative to percutaneous liver biopsy in patients with severe coagulopathies, high volume or massive ascites, requiring ancillary vascular procedures such as simultaneous hepatic hemodynamic measurements and intrahepatic portosystemic shunting creation, suspected vascular tumor or peliosis hepatis, or failure of percutaneous liver biopsy.<sup>2,9</sup>

## **2. Complications**

Percutaneous biopsy can be performed on an outpatient basis in most cases. Ultrasound is used to mark the site for or to guide the percutaneous biopsy. Complication after liver biopsy happens but is rare.<sup>2</sup> Complications after liver biopsy depend on the route used to biopsy. In percutaneous biopsy, complications include bleeding, vasovagal reaction, pneumothorax, post-puncture anaphylactoid reaction, pain, hematoma of the liver, reduction in hemoglobin concentration without evidence of bleeding, significant perihepatic fluid, and mild bleeding.<sup>8</sup> In transjugular biopsy, complications include abdominal pain, capsular perforation, fever, neck hematoma, and hypotension associated with the procedure. In addition, extremely rare major complications such as intraperitoneal hemorrhage, retroperitoneal hemorrhage, and death have also been reported.<sup>9</sup> Common complications of liver biopsy are summarized in **Table 1.2**.

**Table 1.2: Complications after liver biopsy.**

	<b>Percutaneous liver biopsy</b>	<b>Transjugular liver biopsy</b>
Major complications (including those requiring major therapy, escalation of care, or prolonged hospitalization and those resulting in permanent adverse sequelae or death)	<p>Vasovagal reaction</p> <p>Pneumothorax</p> <p>Post-puncture anaphylactoid reaction</p> <p>Hemobilia</p> <p>Death (extremely rare, 1 in 10,000)</p>	<p>Intraperitoneal bleeding</p> <p>Intra-abdominal infection</p> <p>Cardiac arrhythmia</p> <p>Hepatic artery thrombosis</p> <p>Inadvertent biopsy of an adjacent organ</p> <p>Death (0.1% to 0.5%)</p>
Minor complications (those requiring nominal therapy with no lasting consequences or overnight hospitalization)	<p>Pain</p> <p>Vegetative symptoms</p> <p>Intrahepatic or subcapsular hematoma</p> <p>Reduction in hemoglobin concentration without evidence of bleeding</p> <p>Significant perihepatic fluid</p> <p>Mild bleeding</p>	<p>Fever</p> <p>Abdominal pain</p> <p>Hypotension</p> <p>Neck hematoma</p>

### 3. Tissue handling

#### A. Tissue allocation and process for random liver biopsy performed for liver parenchymal disease

Liver biopsy preparation starts before the procedure of liver biopsy. Depending on the clinical scenario, liver tissue obtained by biopsy may be required for several purposes. As a result, allocation of tissue should be discussed with the liver pathologist before the liver biopsy procedure and optimized at the bedside by the hepatologist or radiologist who performs the procedure. Most of the specimen should be fixed in 10% neutral buffered formalin because it usually allows the full range of stains, both biochemical and immunohistochemically. If less than 2 cm of tissue is obtained, it should be processed for histology as taking part of the specimen for uses other than routine histology may compromise standard light microscopic interpretation of the liver biopsy. If Wilson disease is strongly suspected, a quantitative analysis of hepatic copper may be of great value, so a second core may be obtained and submitted separately for copper quantitation after fixation in formalin. Alternatively, taking part of the core (at least 3 mm in length and 1 mm in width) and processing this portion to a separate block which can be used for copper quantitation. This tissue dividing process should leave adequate tissue for histology and avoid contamination that can interfere copper quantitation. Tissue copper quantitation can also be performed using the formalin fixed tissue left in the tissue block after sections for hematoxylin and eosin (H&E) staining and special stains being taken. The tissue allocation in this scenario is best handled by a protocol placed in the laboratory [**See chapter 14 for Wilson's disease of the liver**]. A similar approach can be used for cases for which hereditary hemochromatosis is highly suspected although staining for iron on routinely processed tissue has similar diagnosis efficacy as the more highly regarded quantitative assays, particularly in the era of molecular diagnosis [**See chapter 13 for iron metabolism diseases of the liver**].

In pediatric patients with a suspicion for metabolic disorders, a small portion of liver tissue (three 1-mm core transections) is fixed in glutaraldehyde for ultrastructural examination.<sup>10</sup> In addition, if a metabolic disorder is a concern, tissue is snap-frozen in liquid nitrogen in amounts sufficient for reference laboratory tests. This tissue allocation approach normally requires at least two 2-cm cores of tissue and this information should be conveyed to the hepatologist or the person who performs the liver biopsy. Approximately 20 mg of liver tissue is usually adequate for the biochemical diagnosis of most metabolic diseases.

When infection is suspected, liver biopsy tissue should be submitted for culture. In addition, snap-frozen tissue or formalin fixed paraffin embedded tissue can be used for organism identification with polymerase chain reaction (PCR).

When liver tissue has been allocated appropriately, the liver biopsy fragment destined for histology is wrapped in tissue paper as cassette sponges produce artefactual distortion of the tissue specimen which interferes with interpretation. Many types of fixatives have been used in different laboratories for liver biopsy. In our lab, liver biopsy tissue is fixed in formalin and further processed into tissue blocks. After paraffin embedding, tissue sections cut at 5- $\mu$ m are suitable for most stains.

## **B. Stains in liver biopsy specimens from patients with diffuse liver parenchymal disease**

### **a. Up-front stains**

There are variable practice patterns in terms of ordering up-front stains for liver biopsies.<sup>1,11</sup> At least 2 slides are stained for H&E and one slide for Trichrome Masson stain. Some laboratories may H&E stain 3 slides and order other special stains (periodic acid-Schiff (PAS), PAS with diastase digestion (PAS/D), and Prussian blue) as a panel of up-front stains. The advantage of this practice is a fast turn-around time. In other places, these special stains may be ordered by the pathologists as needed based on the initial impression of H&E slides.

### **b. Liver biopsy protocols used in our laboratory**

For random liver biopsy for diffuse parenchymal disease (medical liver biopsy protocol), 22 5- $\mu$ m thick sections with two sections on each slide are obtained up-front. The 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> slides are used for H&E stain. The 6<sup>th</sup>-9<sup>th</sup> slides are used for PAS, PAS/D, Trichrome Masson, and Prussian blue stains. For additional blocks, only one 5- $\mu$ m thick section is cut and stained with H&E.

### **c. Liver allograft biopsy**

For random biopsies from liver allografts (liver transplant biopsy protocol), 20 5- $\mu$ m thick sections with two sections on each slide are obtained up-front. The 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> slides are used for H&E stain. The 6<sup>th</sup>-8<sup>th</sup> slides are used for PAS, PAS/D, and Trichrome Masson stains. Some laboratories



may choose not to perform PAS and PAS/D stains in liver allograft biopsy as up-front stains.

For time 0 biopsy from liver allograft, 16 5- $\mu$ m thick sections with two sections on each slide are obtained up-front. The 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> slides are used for H&E stain. The 6<sup>th</sup> slide is used for Trichrome Masson stain.

### **C. Liver tissue allocation and process for targeted liver biopsy performed for liver mass or lesion**

A liver mass is often biopsied for the diagnosis. With increased use of molecular information for the therapeutic decision, there is a need to optimize the use of liver biopsy tissue for this purpose in addition to generating an accurate diagnosis. If there is only one core in the container, it is submitted in one cassette. If more than two tissue cores are present in the container, they are placed in two separate cassettes and processed into two blocks. If there are 3 or more cores in the container, they are submitted in two or more than two cassettes.

For the first block, 10 5- $\mu$ m thick section are obtained with one section on each slide. The 1<sup>st</sup> and 6<sup>th</sup> slides are used for H&E stain. The remaining slides are used for stains or molecular tests as needed. For the 2<sup>nd</sup> block and/or additional blocks, only one section 5- $\mu$ m thick section is cut and used for H&E stain. The remaining tissue is kept in the block(s) for additional stains and/or potential molecular testing as needed or clinically indicated.

### **D. Rush liver biopsy**

Rush liver biopsy may be performed in patients with acute liver failure or patients with liver allograft dysfunction and requires a fast tissue process and interpretation. The pathologist should be consulted and the histology laboratory informed before the liver biopsy procedure to ensure prompt and adequate process and diagnosis. Rush liver biopsy specimens are processed in an automated tissue processor for about 2 hours. The tissue is then embedded, cut into 5- $\mu$ m thick section (using the medical liver biopsy or liver transplant protocol depending upon clinical need), and stained with H&E stain. Generally speaking, H&E stained slides of a rush liver biopsy should reach the pathologist in 3.5 to 4 hours from receipt into the laboratory. For most rush liver cases, a preliminary diagnosis is rendered by the pathologist using the H&E stained slides only. Final diagnosis, of course, should be based on H&E stained slides, special stains and needed ancillary studies.

## 4. Stains used in liver biopsy specimens

### A. Routine and special stains

a. H&E stain: This stain demonstrates the structure of liver parenchyma (**Figs 1.5 and 1.6**), highlights the character of inflammatory cells, identifies bile staining and bile droplets, lipofuscin, and small deposits of hemosiderin in the cytoplasm of both hepatocytes and Kupffer cells, and small bile thrombi in the canaliculi.

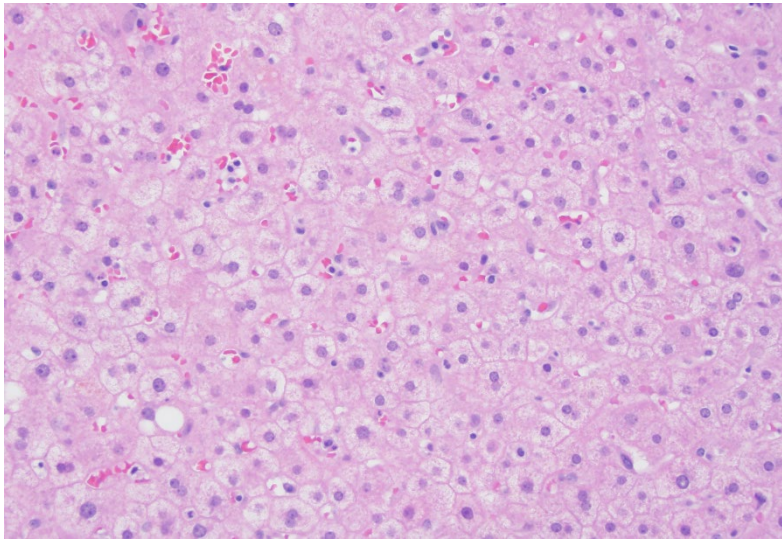


Fig 1.5 H&E stain of a liver biopsy highlights hepatocytes. Normal hepatocytes are polygonal and show eosinophilic slightly granular cytoplasm. The nucleus is relatively uniform, has fine chromatin and a single nucleolus (x200).

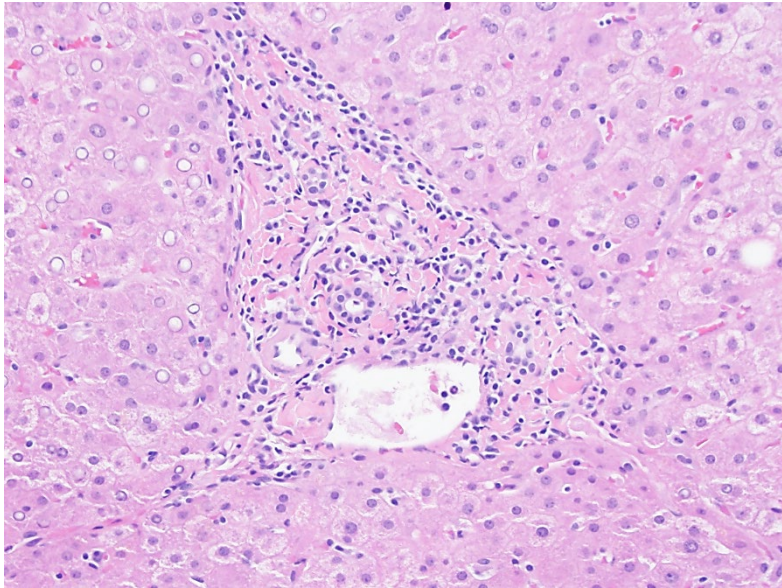


Fig 1.6 H&E stain of a liver biopsy demonstrates portal tracts. The portal tract contains one portal vein, a few bile ducts, and a few arterioles with a ratio of bile duct to arteriole near 1. A few mononuclear inflammatory cells are present.

b. Trichrome Masson stain: This stain is probably the most commonly used connective tissue stain (**Fig. 1.7**). It not only helps visualize the connective tissue, but also highlights several other features such as identification and localization of small foci of hepatocellular necrosis and dropout, hypertrophy and hyperplasia of the Kupffer cells, and small epithelioid granulomas. It also facilitates the identification of Mallory hyalines. Trichrome stain does not stain elastic tissue.

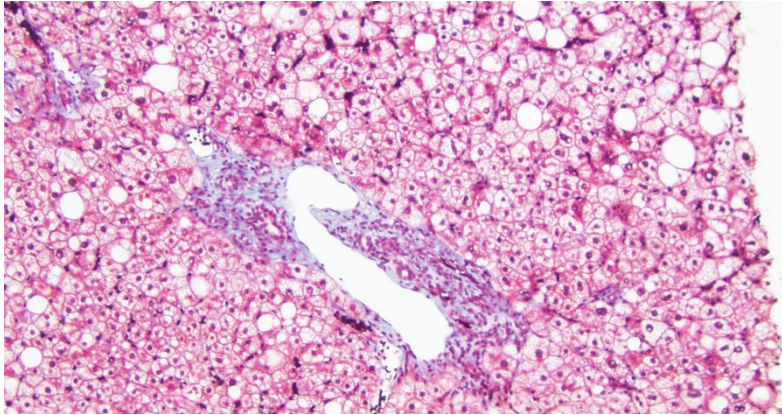


Fig 1.7 Trichrome Masson stain of liver biopsy reveals collagen in the portal tract (x200).

c. Elastic-van Gieson stain: The Verhoeff elastic stain with van Gieson counterstain highlights elastic tissue as blue-to-black and collagen as red (**Fig. 1.8**). It is used in some laboratories as the sole connective tissue stain or supplementary to Trichrome Masson stain.

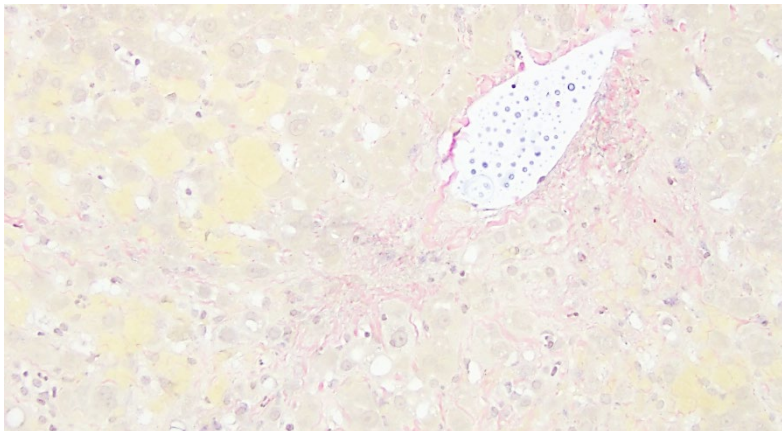


Fig 1.8 Van Gieson stain of liver biopsy reveals fibrosis (stained red) (x200).

d. PAS stain and PAS/D stain: PAS and PAS/D stains are useful in detecting the characteristic cytoplasmic globules in  $\alpha 1$  anti-trypsin deficiency (**Fig. 1.9**), which often escape detection by other stains when they are small and focal. Although PAS stain helps visualize the content and distribution of glycogen which must be confirmed by PAS/D stain of an adjacent section, these stains are not particularly helpful for the diagnosis of glycogen storage diseases or glycogenic hepatopathy as normal hepatocytes have abundant glycogen and stain brightly on PAS stain (**Fig. 1.10**).

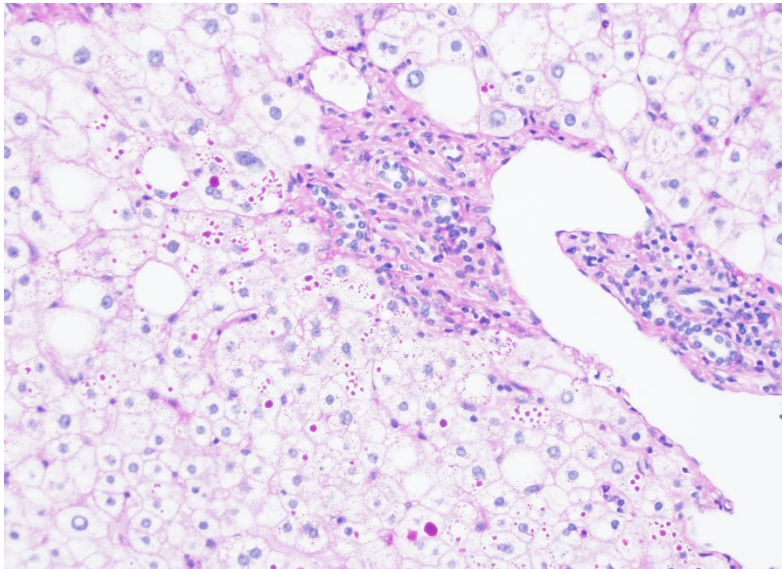


Fig 1.9 Periodic acid-Schiff stain with diastase digestion (PAS/D) of liver biopsy reveals pink intracellular globules, typical of  $\alpha 1$  anti-trypsin deficiency (x200).



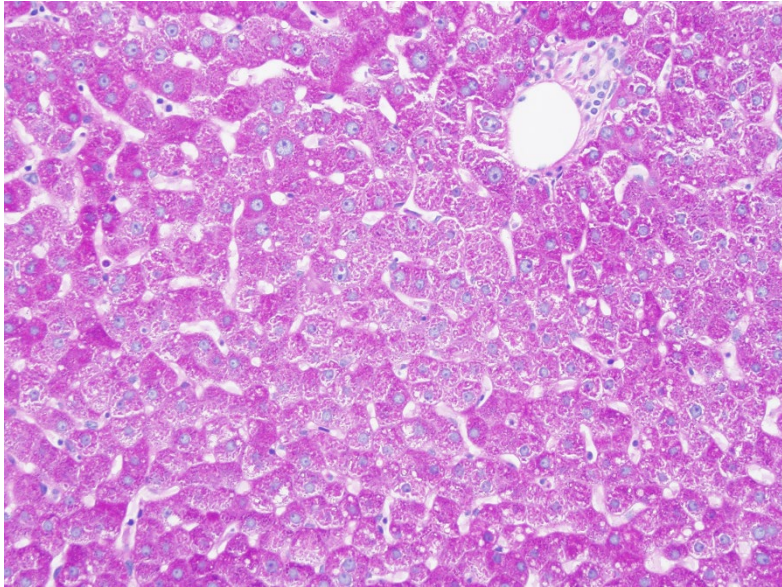


Fig 1.10 Periodic acid-Schiff (PAS) stain of the liver biopsy reveals abundant glycogen in the hepatocytes (x200).

PAS stain may be potentially useful in evaluating pseudoground-glass changes in hepatocytes as negative PAS staining suggests fibrinogen storage disease. PAS stain also helps evaluate bile duct injury by highlighting the basal lamina of bile duct. The PAS/D stains are useful in detecting and identifying lipofuscin or ceroid material in the lobular Kupffer cells and/or portal histiocytes and small bile thrombi in canaliculi. In addition, PAS/D stain may also highlight small globules of immunoglobulin in Kupffer cells including autoimmune hepatitis and primary biliary cholangitis. PAS/D stain also helps identify fungal organisms in liver biopsy and *Tropheryma whipplei* in hepatic Whipple disease.

e. Prussian blue stain: Prussian blue stain is useful in confirming and assessing the degree, zonal distribution [Fig. 1.11], and compartmentation of hemosiderin deposition in the liver tissue although heavy iron deposits are readily appreciable on H&E stain.