



American Society for  
Clinical Pathology

**46 Medical Liver Biopsy Interpretation: A Practical Guide for  
Accurate Diagnosis and Informative Reporting**

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**AMERICAN SOCIETY FOR CLINICAL PATHOLOGY  
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## 46 Medical Liver Biopsy Interpretation: A Practical Guide for Accurate Diagnosis and Informative Reporting

This case-based session will present a guide to systematically evaluating liver biopsy specimens, including a pattern recognition based approach.

- Systematically evaluate medical liver biopsy specimens.
- Apply a pattern-recognition based approach to accurately diagnosis regularly encountered liver diseases, including entities to be considered in the differential diagnosis.
- Identify the clinically important diagnostic, therapeutic and prognostic information to be included in the Surgical Pathology report of liver biopsies for regularly encountered medical liver diseases.

### FACULTY:

Julia Iezzoni MD  
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Entire Pathology Team  
Surgical Pathology  
Surgical Pathology (GI, GU, Etc.)  
2.0 CME/CMLE Credits

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The individuals below have responded that they have no relevant financial relationships with commercial interests to disclose:

Julia C. Iezzoni, M.D.

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### Medical Liver Biopsy Interpretation:

A practical guide for accurate  
diagnosis and informative reporting

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Cleveland Clinic Foundation

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## Program Objectives

- Systematically evaluate liver biopsy specimens
- Apply a pattern-recognition based approach to accurately diagnose regularly encountered liver diseases, including entities to be considered in the differential diagnosis
- Discuss prognostically and therapeutically important liver histopathologic features to be included in surgical pathology report for regularly encountered medical liver diseases

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## Part 1: Systematic Evaluation and Informative Reporting: Overview

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## Surgical Pathology Evaluation: Tumors

- Systematically evaluate and report all tissue-based features with prognostic and therapeutic implications
- Components (diagnosis, pathologic stage, molecular markers)
- Informative SP report: Includes all tissue-based features relevant to prognosis and treatment
- Synoptic reports
  - Reinforce (and simplify) this evaluation

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### Surgical Pathology Evaluation: Liver bx

- Systematically evaluate and report all tissue-based features with prognostic and therapeutic implications
- Components
- Informative SP report: Includes all tissue-based features relevant to prognosis and treatment
- Synoptic reports
  - Not currently available for medical liver bxs
  - Goal of synoptic report is achieved

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### Liver bx: Systematic evaluation

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### Liver bx: Specimen adequacy

- Average liver bx consists of 1/ 50,000 of total hepatic mass
- No universally agreed upon standard of specimen adequacy
- Sample size can affect accuracy of histologic assessment
- At least 6-8 complete portal tracts are needed for diagnosis
  - Complete circumference and contains at least 2 portal structures
  - Total number a key factor in adequacy of biopsy
- Chronic hepatitis grading and staging bx minimum criteria
  - $\geq 20$ -25 mm long
  - $\geq 11$  complete portal tracts

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Liver bx: Adequate specimen



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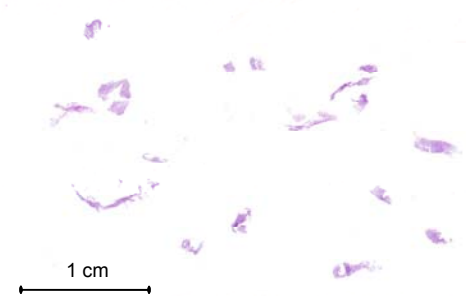
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Liver bx: Highly fragmented specimen



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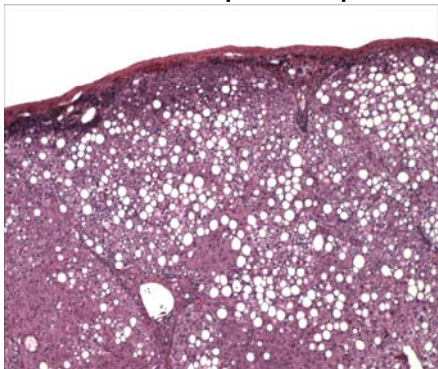
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Liver bx: Subcapsular specimen



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### Liver bx: Specimen adequacy

- Statement regarding specimen adequacy should be included in Surgical Pathology report
- "Evaluation is limited by the small size of the biopsy specimen, which includes only 3 complete portal tracts"
- "Evaluation, especially of the extent of fibrosis, is limited by the subcapsular location of the biopsy specimen"

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### Liver bx: Systematic evaluation

- Specimen adequacy - ✓
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### Histochemical stains: Purpose

Selective staining properties



Delineation of specific architectural and cellular features that are clinically important

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### Standard histochemical stains

- Trichrome (Type I collagen)
- Reticulin (Type III collagen)
- PAS with / without diastase
- Iron
- Copper (or copper binding protein)
- +/- Elastic fibers (VVG, orcein)

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### Histochemical stains: Update

- Specific role of histochemical stains has changed due to advances in diagnostic SP and medicine as a whole
  - Immunohistochemical stains (HBV)
  - Specific enzymatic tests (glycogen storage diseases)
  - Genetic tests (HFE)
- Role of liver biopsy has changed
  - Assessment for initiation and continuation of treatment
  - New patient population: Liver transplant patients
- What is role of histochemical stains in contemporary practice?

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### Histochemical stains: Update

- Trichrome stain
  - Essential for assessment of the extent of fibrosis (i.e. stage of disease)
- Other histochemical stains
  - No widely agreed upon practice by experts
- Given limited technician resources and health care dollars, is routine “pan-staining” really cost-effective and appropriate?

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**Proposed reasonable approach**

Trichrome stain  
Unstained sections



Additional stains if indicated  
after initial review

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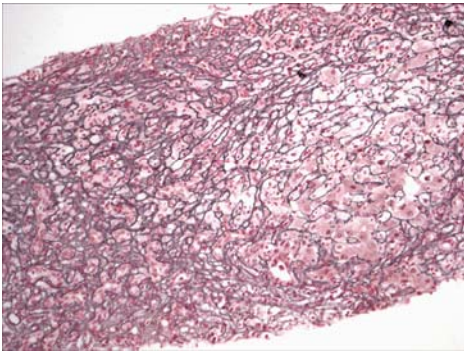
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**Reticulin: Lobular collapse**



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**Liver bx: Systematic evaluation**

- Specimen adequacy - ✓
- Histochemical stains - ✓
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### Liver: Morphologic patterns of injury

- Liver has diverse, though finite number of major histologic responses to tissue damage
  - “morphologic patterns of injury”
- Each morphologic pattern of injury is etiologically non-specific
- Morphologic pattern of injury does indicate a particular etiologic differential diagnosis
- Identification of morphologic pattern of injury is key component of the identification of etiology of liver disease

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### Morphologic patterns of injury

- Acute hepatitis
- Chronic hepatitis
- Granulomatous hepatitis
- Steatosis / steatohepatitis
- Biliary injury
- Chronic cholestasis
- Massive /submassive necrosis
- Vascular / perfusion injury
- Increased iron stores
- Increased copper stores

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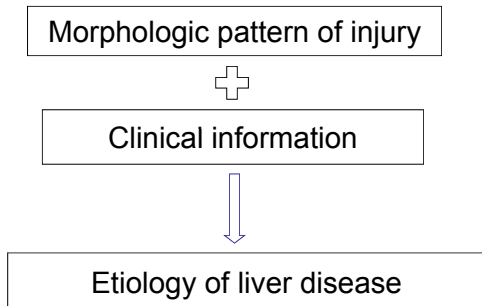
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### Etiology: Clinicopathologic correlation



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**Clinical history: Suboptimal**

- “Elevated LFTs”
- “Liver bx”
- “r/o pathology”
- “ ”

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**Clinical history: Optimal**

- LFTs: Actual values
  - ALT, AST, AP, BR
  - “hepatitic” vs “cholestatic” pattern
  - INR
- Viral serologies
- Autoantibodies
- Other – A1AT, ferritin, ceruloplasm, HFE
- Risk factors for liver disease
- Radiographic findings
- Clinical stigmata of cirrhosis

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**Liver disease:  
Final determination of etiology**

- Morphologic pattern of injury ...and...
- Clinical information: also a key requirement.

Pathologist + Hepatologist

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### Liver bx: Systematic evaluation

- Specimen adequacy - ✓
- Histochemical stains - ✓
- Etiologic diagnosis - ✓
  - Identification of morphologic pattern of injury. - ✓
  - Correlation with clinical features. - ✓
- Features of prognostic and therapeutic importance

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### Informative liver biopsy reporting

- Includes all tissue-based features relevant to prognosis and treatment.
- Varies between different disease entities.

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### AASLD: Practice guidelines

- American Association for the Study of Liver Disease (AASLD)
- Practice guidelines for diagnosis and management of liver disease
- Includes guidelines for role of liver biopsy in diagnosis, prognosis, and management of liver disease - i.e. What needs to be addressed in liver biopsy report

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**Liver bx: Systematic evaluation**

- Specimen adequacy - ✓
- Histochemical stains - ✓
- Etiologic diagnosis - ✓
  - Identification of the morphologic pattern of injury. - ✓
  - Correlation with clinical features. - ✓
- Features of prognostic and therapeutic importance - ✓

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**Medical Liver Biopsy:  
Systematic evaluation and  
informative reporting**

Synoptic report model

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**Part 2:  
Chronic Cholestatic Liver Disease:  
Acquired ductopenic diseases  
in adults**

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**Case presentation**

- 52 yo Caucasian female with fatigue, mild pruritis
- Liver biochemistries
  - ALT 63 U/L (nl: 7-45 U/L)
  - AST 71 U/L (nl: 8-43 U/L)
  - AP 410 U/L (nl: 40-150 U/L)
  - GGT 147 U/L (nl: 6-29 U/L)
- Autoantibodies
  - AMA positive
- Gamma globulins
  - IgM 603 mg/dL (nl: 50-300 mg/dL)
- Viral, metabolic, drug/alcohol etiologies ruled out during exam

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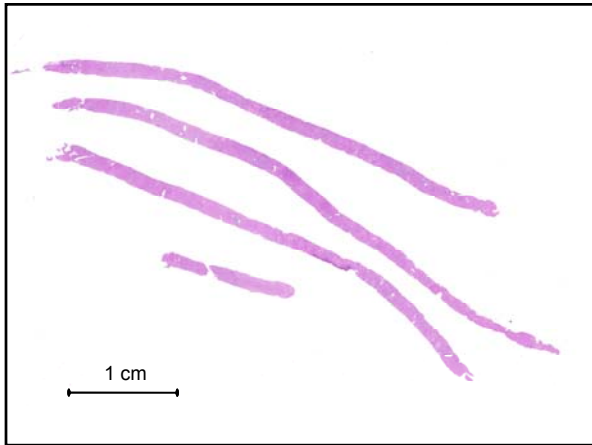
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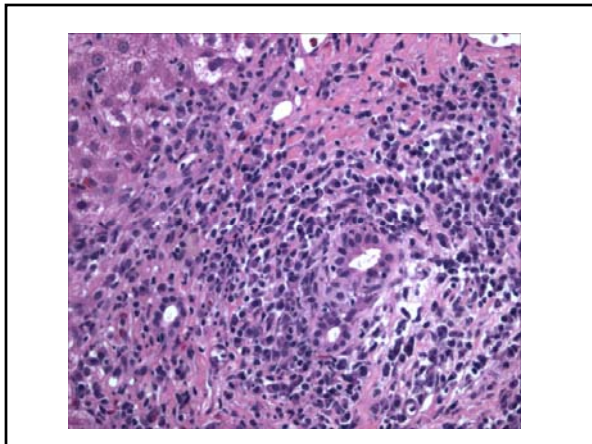
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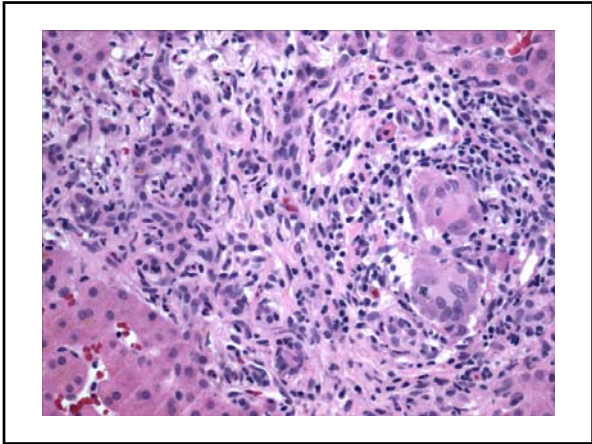
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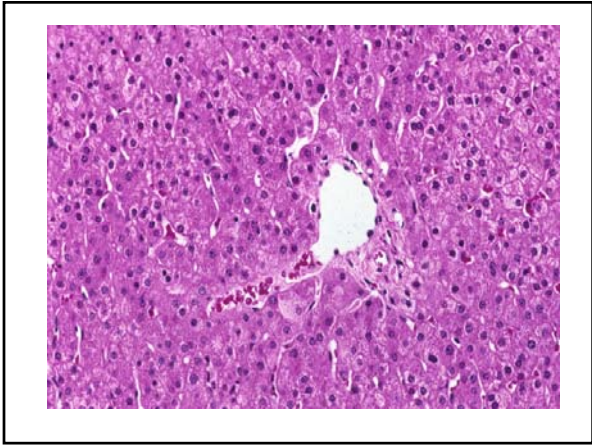
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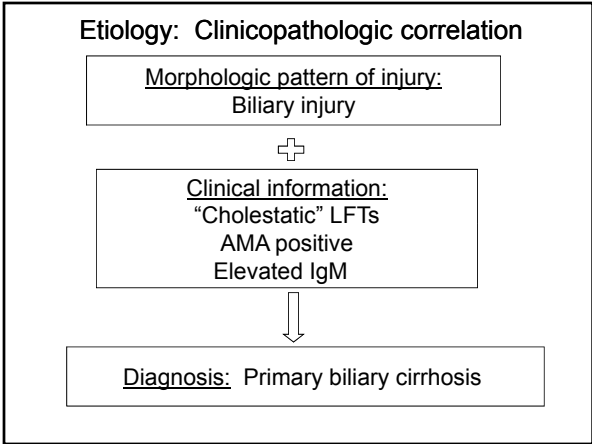
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### Cholestatic Liver Disease

- Pathophysiologic state - decrease in bile flow
  - Hepatocyte (bile formation or secretion)
  - Biliary tract (intra- or extra-hepatic)
- Small and medium-sized intra-hepatic bile ducts
  - Most common site of injury that results in chronic intra-hepatic cholestasis in adults
  - Interlobular bile ducts (< 100 µm)
  - Routinely sampled on liver biopsy
  - Not visualized by cholangiographic studies
- Surgical pathologist – bile duct loss
  - Centrally positioned to evaluate
  - Definitive evaluation can only be made by liver biopsy

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### Adult acquired ductopenic diseases: Morphologic patterns of injury

- Some histologic features vary between entities
- Common morphologic features
- Biliary injury morphologic pattern of injury
  - Earlier stage disease
  - Interlobular bile duct injury
  - Bile ductular proliferation
  - Bile duct loss – ductopenia
- Chronic cholestatic pattern of injury
  - Later stage disease
  - Histologic manifestations of chronic cholestasis

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### Ductopenia: Definition

- Descriptive morphologic term
- Absence of interlobular bile ducts from portal tracts due to any cause.
- Ductopenia: Loss of interlobular bile ducts in greater than 50% of portal tracts.
  - Duct-to-artery ratio of less than 0.5
- Specimen adequacy: At least 10 portal tracts are required (and 20 is considered preferable).
  - May be tallied on sequential biopsies.

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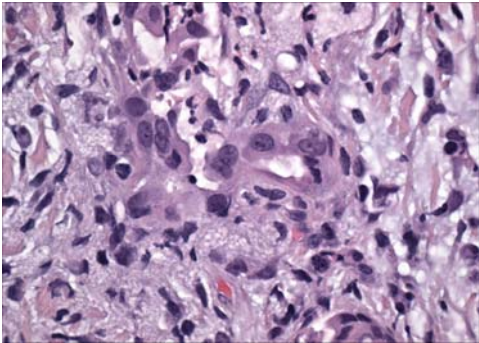
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**Biliary injury: Bile duct epithelial disarray**



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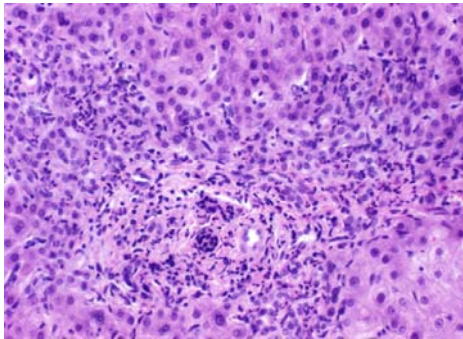
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**Biliary injury: Bile ductular proliferation**



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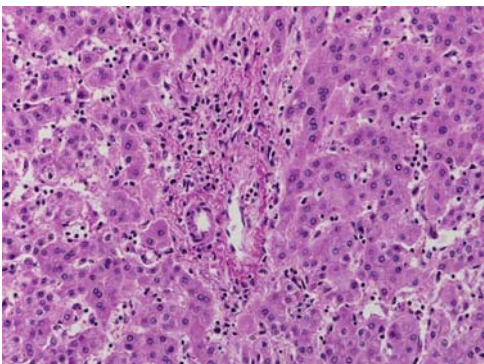
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**Biliary injury: Bile duct loss / ductopenia**



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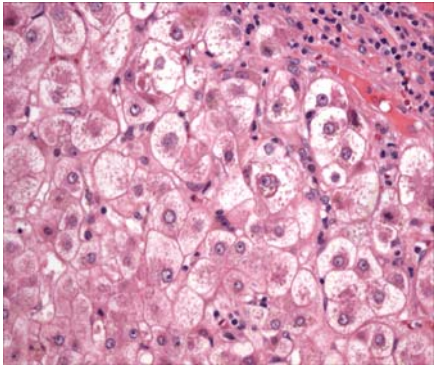
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Chronic cholestasis: Cholate stasis



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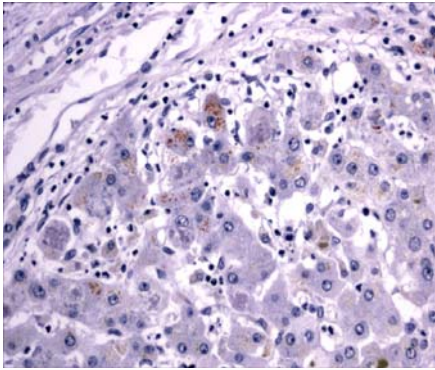
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Chronic cholestasis: Increased copper



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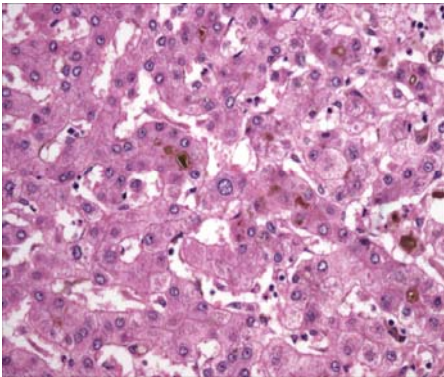
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Chronic cholestasis: Bilirubinostasis



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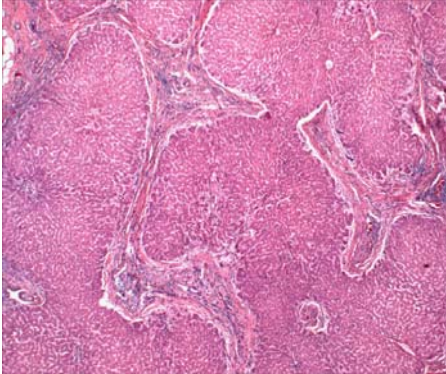
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**Biliary cirrhosis: "jigsaw puzzle pieces"**



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**Cholestatic Liver Disease:  
Informative liver biopsy reporting**

Determine whether biopsy demonstrates features of cholestatic liver disease

- Biliary injury morphologic pattern of injury
- Chronic cholestatic morphologic pattern of injury

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**Etiologies of adult acquired ductopenia**

- Immune-mediated
  - Primary biliary cirrhosis
  - Primary sclerosing cholangitis
  - Autoimmune overlap syndrome
- Infectious
  - Immunocompetent
  - Immunodeficient
- Drugs and toxins
- Ischemic
- Neoplastic
- Miscellaneous
  - Sarcoidosis
- Idiopathic

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### Primary biliary cirrhosis (PBC)

- Chronic progressive cholestatic liver disease characterized by destruction of small and medium-sized bile ducts by a non-suppurative inflammation
- Demographics:
  - Females > males (9:1)
  - Middle-aged (5th-7th decade)
- More than 60% of patients are asymptomatic at dx
  - Screening liver chemistries (elevated AP, GGT)
- Patients may remain asymptomatic for years
- Symptoms (when present): Fatigue, pruritis

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### PBC: Laboratory features

- Liver biochemistries: Cholestatic
  - Marked elevation of AP (> 2X ULN) and GGT (> 5X ULN)
  - Mild elevation of aminotransferases
- Anti-mitochondrial antibody (AMA)
  - High sensitivity (95%)
  - High specificity (97%)
  - Major diagnostic hallmark for PBC
- Other autoantibodies may be present:
  - ANA (40%) ; anti-SMA (14%)
- Gamma globulins
  - Hypergammaglobulinemia with selective elevation of IgM

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### AMA-negative PBC

- ~5% of pts with PBC are AMA-negative
- Variant of PBC or separate disease entity?
- Indistinguishable from "classic" PBC
  - Higher rate of positivity of ANA and anti-SMA
- Dx is applied in cases that are c/w PBC but lack identifiable AMA
- More sensitive tests for AMA:
  - 79% of cases AMA-neg PBC  $\implies$  AMA pos

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## PBC: Histopathology

- Progressive destruction of small and medium-sized bile ducts by a non-suppurative inflammatory process
- Specific features vary with extent of disease progression
- Morphologic patterns of injury
  - Biliary injury – earlier stage disease
  - Portal based inflammation (Chronic hepatitis)
  - Non-necrotizing granulomatous hepatitis
  - Chronic cholestasis – later stage disease

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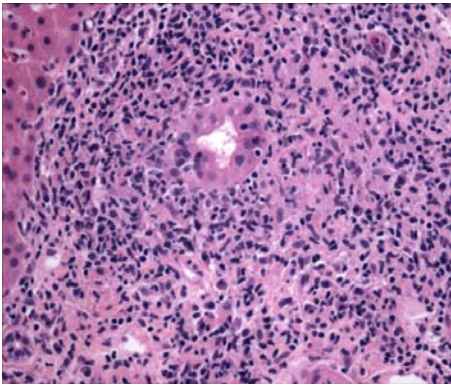
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PBC: Portal inflammation



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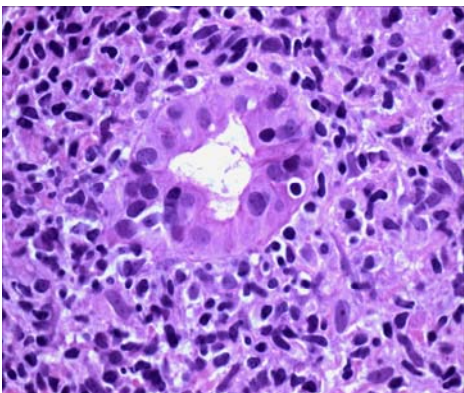
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PBC: Florid duct lesion



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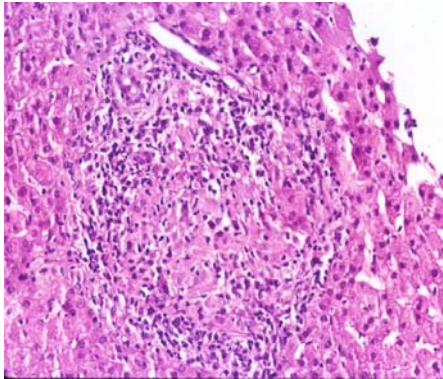
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PBC: Non-necrotizing granulomatous inflammation



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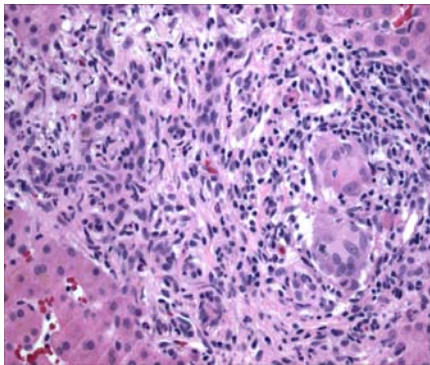
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PBC: Bile ductular proliferation



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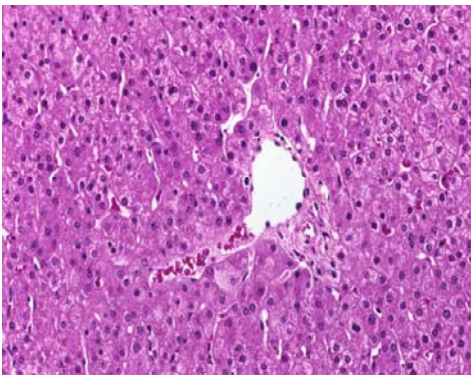
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PBC: Interlobular bile duct loss



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### PBC: Histopathology

- Within a single liver biopsy, extent of disease progression of the biliary injury may be highly variable between portal tracts
  - A "soft" diagnostic criteria
- Specimen adequacy
  - Patchy nature of characteristic findings of biliary injury
  - Size of biopsy impacts its diagnostic utility
  - Minimum of 10-15 portal tracts
  - Multiple tissue levels

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### PBC: Staging

Stage 1	Portal (changes confined to portal tracts - florid duct lesion)
Stage 2	Periportal (changes extend to periportal region)
Stage 3	Septal (portal-to-portal bridging fibrosis)
Stage 4	Cirrhosis

**Bridging fibrosis – poor prognosis**

Findings on the H&E and trichrome stained tissue sections

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### PBC: Diagnosis

- Per internationally accepted standards, diagnosis of PBC is made when 2 of following 3 features are present:
  - Positive AMA
  - Increased cholestatic liver chemistries > 6 months
  - Compatible liver histology

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### PBC: Role of histopathology in dx

- AMA-positive cases with cholestatic liver chemistries
  - Identification of etiology less dependent on liver biopsy findings.
- AMA-negative cases
  - Identification of etiology considerably more dependent of liver biopsy findings.

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### PBC: Prognosis and treatment

- PBC is progressive
  - Rate of progression is highly variable
  - Progressive fibrosis  $\rightleftarrows$  cirrhosis
- No cure
- UDCA is mainstay of therapy
  - Improves biochemical indices, delays histologic progression, and increases survival
- Corticosteroids – contraindicated
  - Not effective; significant side effects

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### PBC: Informative liver biopsy reporting

- Consistent histology
  - Biliary injury morphologic pattern of injury
  - Chronic cholestatic morphologic pattern of injury
- Stage
- Sequential biopsies
  - Disease progression
  - Treatment effect
- Concurrent liver diseases
  - Complete and accurate clinical and laboratory information
  - Adequate liver biopsy specimen

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### PBC: Liver bx report

**Liver, bx:** Biliary injury. (See note.)

**Note:** The biopsy is adequate for evaluation. It demonstrates patchy bile duct injury characterized by an admixture of florid duct lesions as well as bile ductular proliferation. Additionally, there is moderate portal inflammation with mild interface hepatitis involving some, but not all of the portal tracts. The inflammatory infiltrate is composed of lymphocytes, plasma cells and eosinophils. Periportal fibrosis is identified on trichrome stain. While the histologic features are etiologically non-specific, they are consistent with PBC, Stage 2 of 4. Clinical correlation is recommended.

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### Primary sclerosing cholangitis (PSC)

- Chronic progressive cholestatic liver disease characterized by inflammation and fibrosis of extra- and intrahepatic biliary tract
- Demographics:
  - Males > females (2:1)
  - Average age 42 yo (range 1 - 90 yo)
- ~50% of patients are asymptomatic at dx
  - Screening liver chemistries (elevated AP, GGT)
- Symptoms (when present): Fatigue, pruritis, RUQ pain
- Strong association with IBD, particularly UC

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### PSC: Laboratory features

- Liver biochemistries: Cholestatic
  - Elevation of AP and GGT
  - Mild elevation of aminotransferases
  - Normal AP does not exclude the diagnosis of PSC
- Autoantibodies are common (but not specific)
  - pANCA present in 80%
  - ANA present in 50%
  - SMA present in 15%
  - No routine role in diagnosis of PSC

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### PSC: Imaging features

- Characteristic ERC findings: “gold standard for dx”
- MRC
  - Non-invasive, no radiation exposure
  - Sensitivity 80%; 87% specificity
  - Imaging modality of first choice
- Imaging features
  - Stricturing and dilatation of biliary tract
  - “Beaded” appearance
- Diagnosis of PSC requires exclusion of secondary causes of sclerosing cholangitis
  - May mimic PSC’s characteristic imaging features

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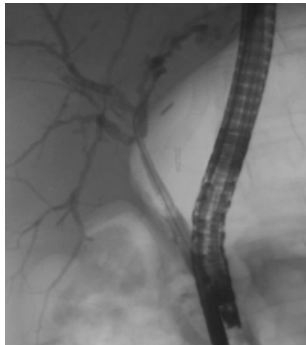
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### PSC ERC: “Beaded” appearance



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### Small duct PSC

- PSC typically affects both large (extra- and intra-hepatic) and small bile ducts.
- 15% of cases, only small ducts (<100 μm) involved
- “Small duct PSC”
  - Identical clinical & liver histologic features as routine PSC
  - Normal MRC and ERC
  - Some definitions require presence of UC
- Overall better prognosis than routine PSC and without the cholangiocarcinoma risk
- Up to 20% progress to involve large ducts; prognosis that of typical PSC.

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## PSC: Histopathology

- Progressive destruction of extra-and intrahepatic biliary tract by inflammation and fibrosis
- Large bile ducts rarely are sampled on core biopsy
- Specific features vary with extent of disease progression
- Morphologic patterns of injury
  - Biliary injury – earlier stage disease
  - Portal based inflammation (Chronic hepatitis)
  - Chronic cholestasis – later stage disease

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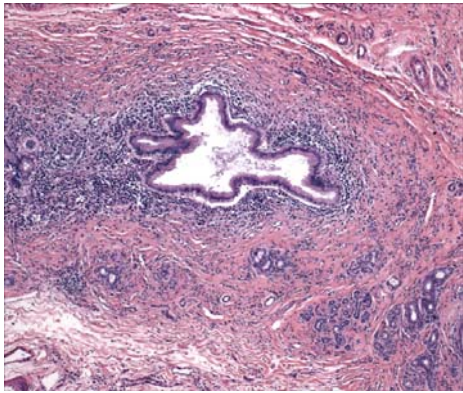
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PSC: Large duct inflammation



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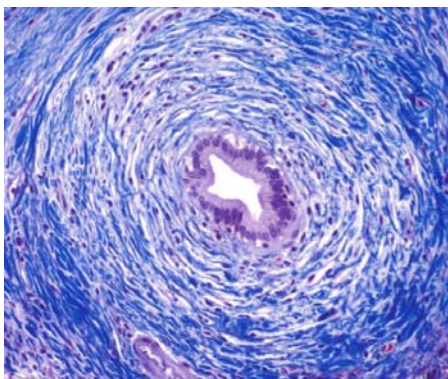
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PSC: "Onion-skinning" fibrosis



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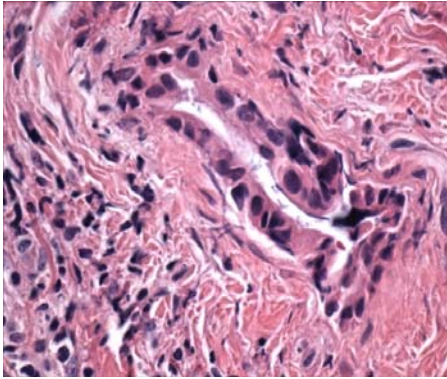
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PSC: Bile duct epithelial disarray



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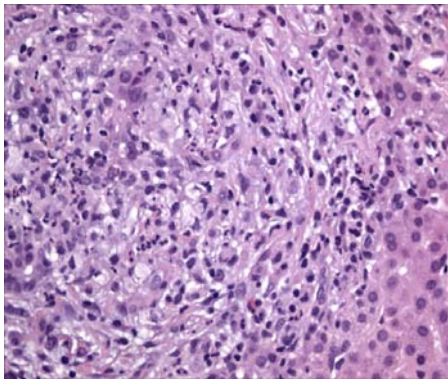
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PSC: Bile ductular proliferation



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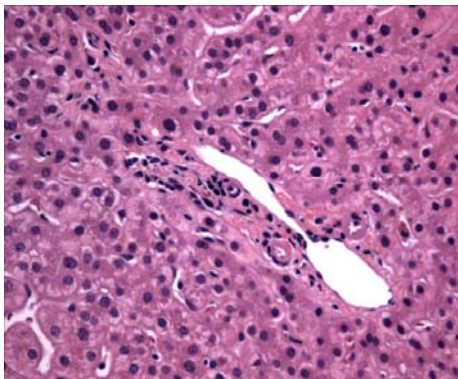
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PSC: Interlobular bile duct loss



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## PSC: Histopathology

- Disease process may be patchy
- Early in the disease, the liver biopsy may not show the characteristic changes
- Limits the utility of liver biopsy
  - Diagnosis
  - Staging

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## PSC: Staging

Stage 1	Portal (bile duct injury, portal inflammation, minimal fibrosis)
Stage 2	Periportal (changes extend to periportal region, periportal fibrosis)
Stage 3	Septal (portal-to-portal bridging fibrosis)
Stage 4	Cirrhosis

### Limited utility on liver biopsy

Findings on the H&E and trichrome stained tissue sections

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## Secondary sclerosing cholangitis (SSC)

- Assorted group of disorders that causes biliary tract strictures similar to PSC (as seen on imaging)
- Diagnosis of PSC requires exclusion of these disorders
- Some etiologies may cause ductopenia, with resultant cholestasis
- Many of etiologies are suggested by clinical history
- Other etiologies, liver biopsy may be useful in identification of causative disease

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## SSC: Etiologies

- Chronic obstructive gallstone disease
- Infections (immune status)
- Immunoglobulin G4-associated cholangitis
- Hepatic artery infusion chemotherapy
- Ischemia
- Biliary tract trauma
- Sarcoidosis
- Langerhans cell histiocytosis
- Systemic mastocytosis

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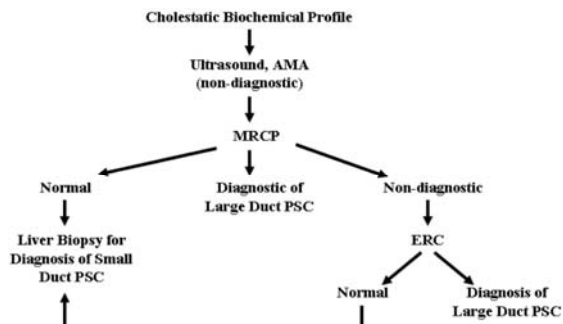
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## PSC: Diagnosis



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## PSC dx: Role of histopathology

- Cases with characteristic ERCP findings
  - Assessment for consistent histology
  - Exclusion of other etiologies of sclerosing cholangitis
  - Role of the liver biopsy (if any) in PSC?
- Small-duct PSC
  - Identification of etiology considerably more dependent of liver biopsy findings
  - Assessment for consistent histologic features

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### PSC: Prognosis and treatment

- PSC is progressive
  - Progressive  $\implies$  cirrhosis
  - Rate of progression is highly variable
  - Median time from dx to death or OLT is 8 years
- No cure
- No effective treatment
- Cholangiocarcinoma develops - 10% of cases
  - Can occur fairly early in disease course, before cirrhosis

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### PSC: Informative liver biopsy reporting

- Consistent histology
    - Biliary injury morphologic pattern of injury
    - Chronic cholestatic morphologic pattern of injury
  - Exclusion of other etiologies of sclerosing cholangitis (Secondary sclerosing cholangitis)
  - Stage
  - Sequential biopsies
    - Disease progression
  - Concurrent liver diseases
- Complete and accurate clinical, lab, radiographic information  
- Adequate liver biopsy specimen

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### PSC: Liver bx report

**Liver, bx:** Chronic hepatitis with biliary injury.  
(See note.)

**Note:** The biopsy is adequate for evaluation. It demonstrates scattered bile duct injury characterized by a bile duct epithelial disarray as well as bile ductular proliferation. Additionally, there is sparse portal inflammation with mild interface hepatitis involving some, but not all of the portal tracts. The inflammatory infiltrate is composed of predominantly of lymphocytes. Periportal fibrosis is identified on trichrome stain. While the histologic features are etiologically non-specific, they are consistent with PSC, Stage 2 of 4. Clinical correlation is recommended.

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### Autoimmune liver disease

- Three major types:
  - Autoimmune hepatitis (AIH)
  - Primary biliary cirrhosis (PBC)
  - Primary sclerosing cholangitis (PSC)
- Usually AIH, PBC, and PSC can be readily differentiated based on their characteristic clinical, biochemical, serologic, histopathologic and radiographic features

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### Autoimmune overlap syndromes

- Sizeable percentage of cases of autoimmune liver disease that generally fit into one diagnostic category will have an individual feature more characteristic of one of other types of autoimmune liver disease
- As isolated finding, does not deter from diagnosis
- When there is admixture of multiple key features (biochemical, serologic, histopathologic) of different autoimmune liver diseases, dx of “autoimmune overlap syndrome” should be considered

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### Autoimmune overlap syndromes

- Identification is challenging
  - Heterogeneous group of disorders
  - Controversial: distinct disease entities versus variants of the major autoimmune hepatopathies
  - Lack of standardization of specific diagnostic criteria and terminology
- Correct identification of a case as that of autoimmune overlap syndrome has important therapeutic implications

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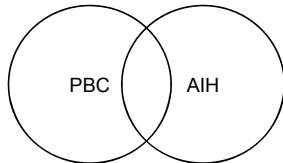
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### PBC-AIH overlap syndrome

- ~ 10% of cases of PBC or AIH have multiple key characteristics (biochemical, serologic, histopathologic) of the other disease entity
- General consensus is that these cases with multiple overlapping characteristic features qualify as PBC-AIH overlap syndrome



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### PBC-AIH overlap syndrome: Proposed diagnostic criteria

- For the diagnosis of each disease, the presence of at least 2 of the 3 following criteria are required:

PBC: 1) AP > 2X ULN or GGT > 5X ULN  
2) positive AMA  
3) florid duct lesion

AIH: 1) ALT > 5X ULN  
2) IgG > 2X ULN or positive for anti-SMA  
3) moderate to severe interface activity

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### PBC-AIH overlap syndrome

- Demographics:
  - Overwhelming female predominance (90%)
  - Majority present in 6<sup>th</sup> decade
- Majority are symptomatic at presentation
  - Fatigue and/or pruritis
- Biochemistries: cholestatic and hepatitic
- Autoantibodies
  - AMA is positive in the majority of cases
  - ANA or anti-SMA positive > 50% of cases
- Gamma globulins
  - Both IgG and IgM typically are elevated

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**PBC-AIH overlap syndrome: Prognosis and treatment**

- PBC-AIH overlap syndrome is progressive
- Progressive fibrosis  $\implies$  cirrhosis
- Faster rate and degree of progression than PBC alone
- Treatment
  - UDCA: few side effects, well-tolerated
  - Immunosuppression:
    - Improves biochemical indices, delays histologic progression
    - Significant side effects (osteoporosis)
  - UDCA + corticosteroids: current rx standard

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**PBC-AIH overlap syndrome:  
Informative surgical pathology report**

- In cases of PBC or AIH, evaluate for features suggestive of other autoimmune liver disease
  - Interface hepatitis – moderate to severe
  - Biliary injury – florid duct lesion
- Diagnosis of PBC-AIH overlap syndrome is not made on bx features alone
  - Complete and accurate clinical and laboratory information
  - Adequate liver biopsy specimen

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**PBC-AIH Overlap : Liver bx report**

**Liver, bx:** Biliary injury with moderate interface activity. (See note.)

**Note:** The biopsy is adequate for evaluation. It demonstrates patchy bile duct injury characterized by an admixture of florid duct lesions as well as bile ductular proliferation. Additionally, there is marked portal inflammation with moderate interface hepatitis involving all of the portal tracts. The inflammatory infiltrate is composed of lymphocytes, plasma cells and eosinophils. The morphologic features raise the possibility of PBC-AIH overlap syndrome. However, this diagnosis is not made based on the biopsy features alone. Clinical correlation is recommended.

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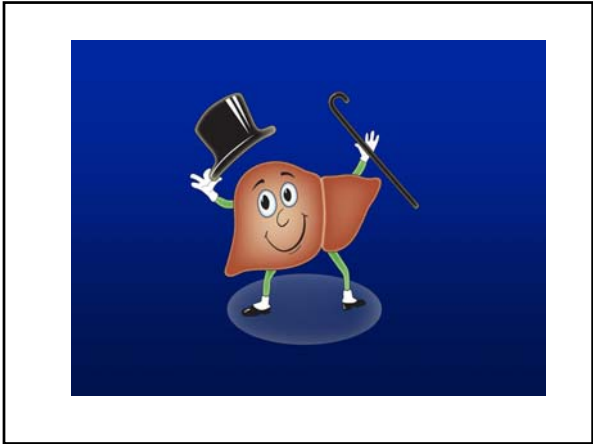
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**Part 3: Fatty Liver**

Lisa Yerian, MD  
Assistant Professor of Pathology  
Director, Hepatobiliary Pathology

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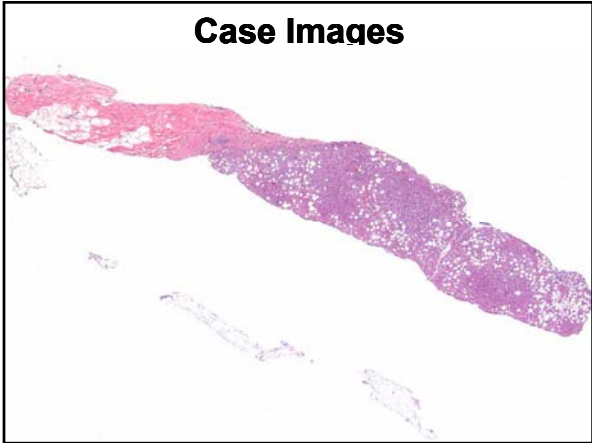
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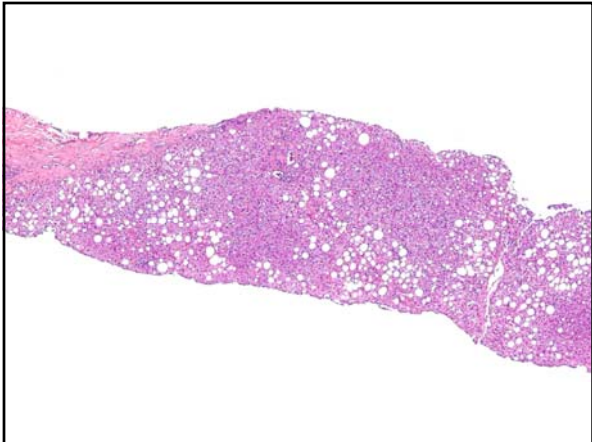
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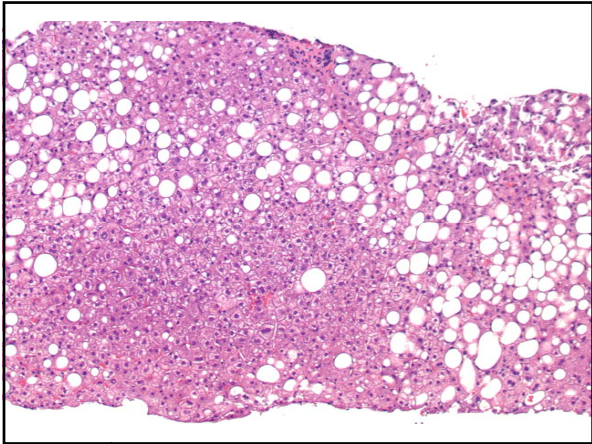
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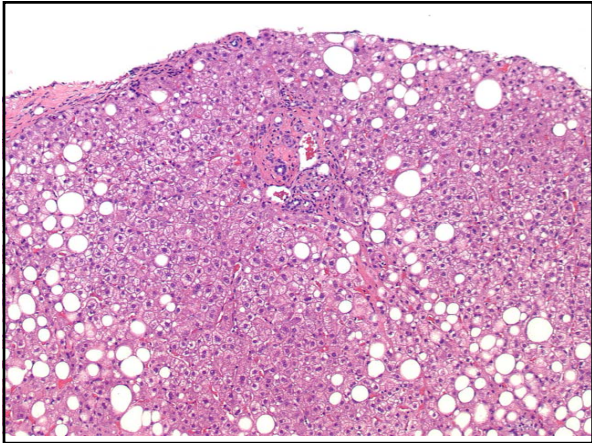
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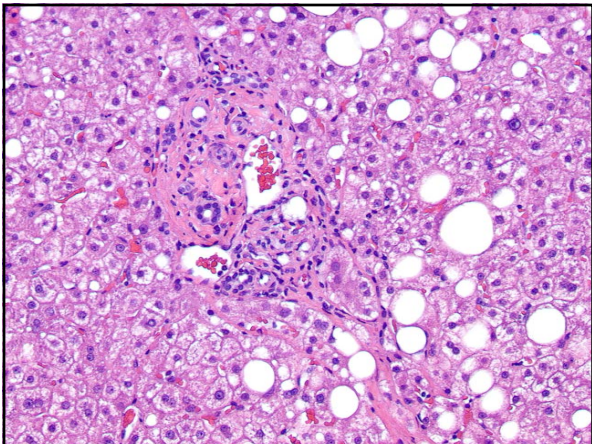
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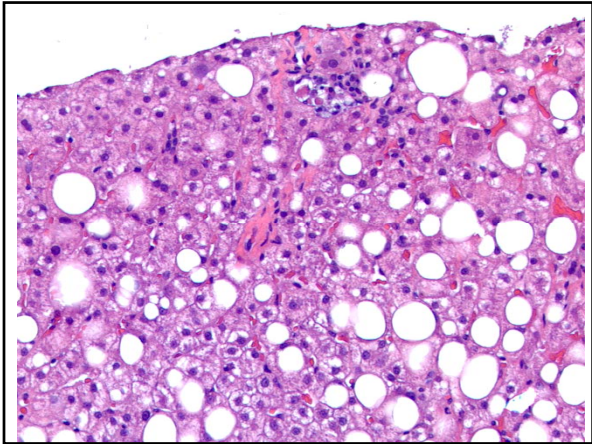
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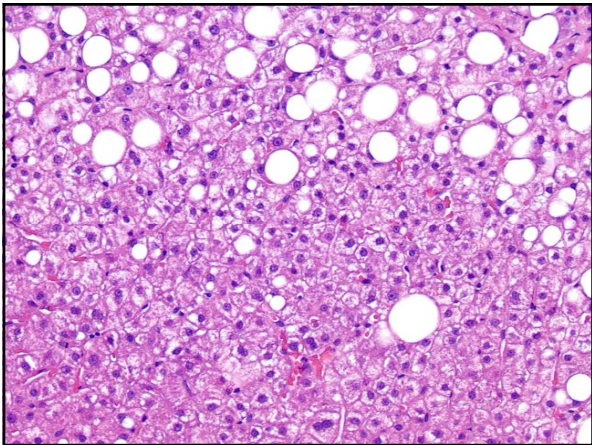
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**Liver bx: Systematic evaluation**

- **Specimen adequacy**
- **Histochemical stains**
- **Etiologic diagnosis**
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- **Features of prognostic and therapeutic importance**

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**Is this biopsy adequate?**

- Biopsy length:
  - Total: 1.5 cm
  - Liver portion: 0.4 cm
- Number of complete portal tracts: 1

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**Liver bx: Specimen adequacy**

- No universally agreed upon standard
- Sample size can affect accuracy of histologic assessment
- At least **6-8** complete portal tracts needed for diagnosis
- Chronic hepatitis grading and staging bx:
  - $\geq 20$ -25 mm long;  $\geq 11$  complete portal tracts
- Complete portal tract:
  - Complete circumference and  $\geq 2$  portal structures

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**Is this biopsy adequate?**

- Biopsy length: 0.4 cm
- Number of complete portal tracts: 1
- No
- Diagnosis: "Limited sample with ..."
- Comment: The biopsy interpretation is limited due to small sample size and only X portal tracts present for evaluation."

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**Liver bx: Systematic evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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**Trichrome stain: Essential for evaluation of fibrosis**

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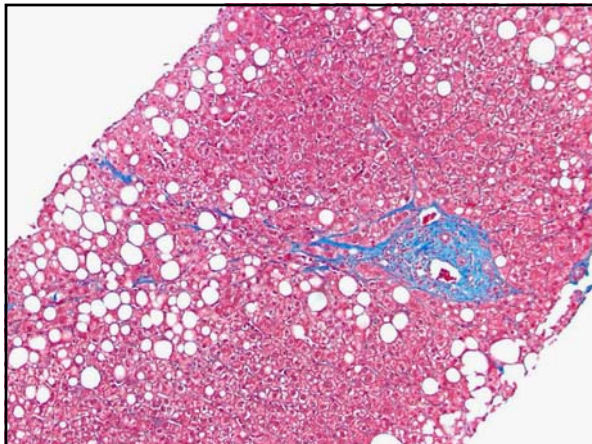
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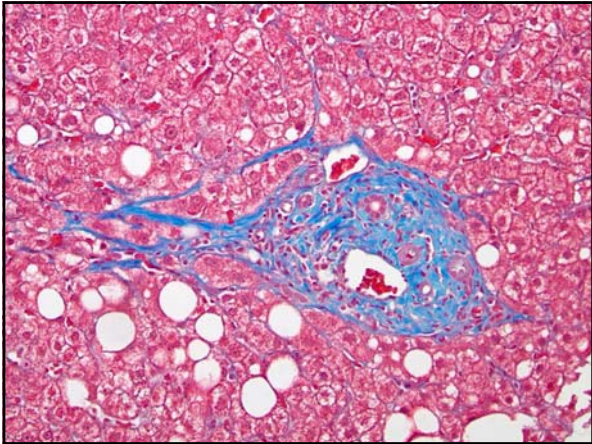
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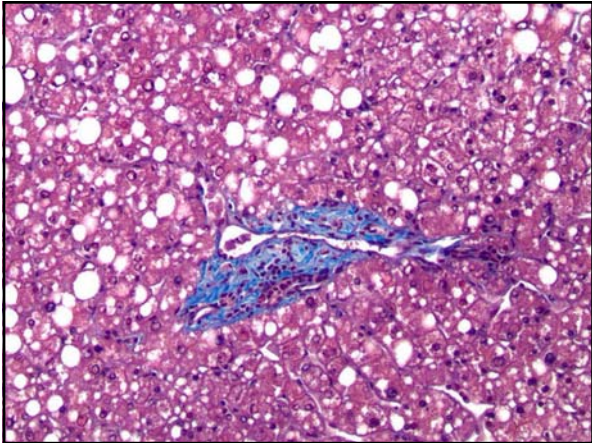
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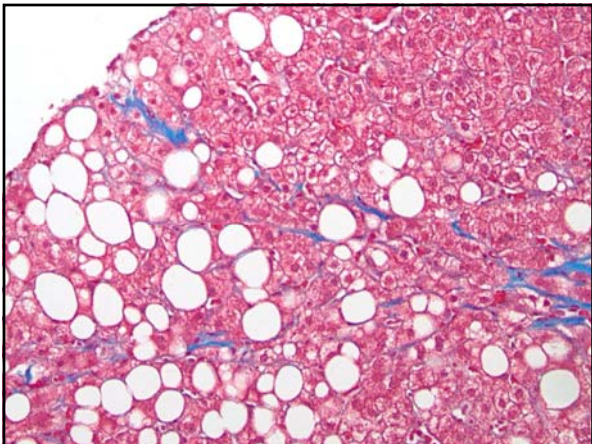
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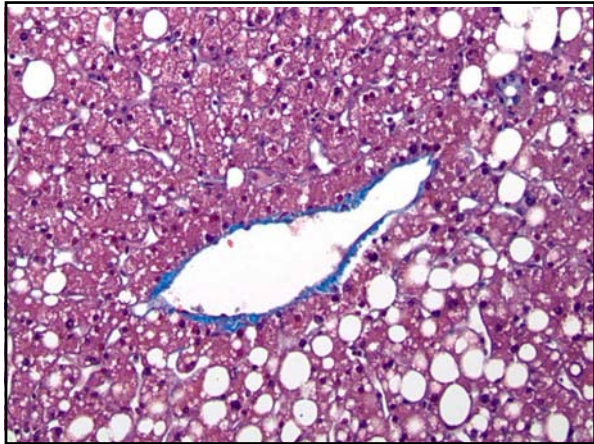
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**Trichrome Stain**

- Periportal and pericellular fibrosis
- ? Significance

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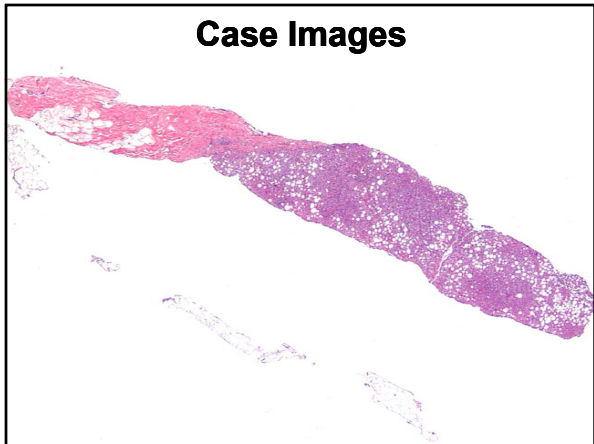
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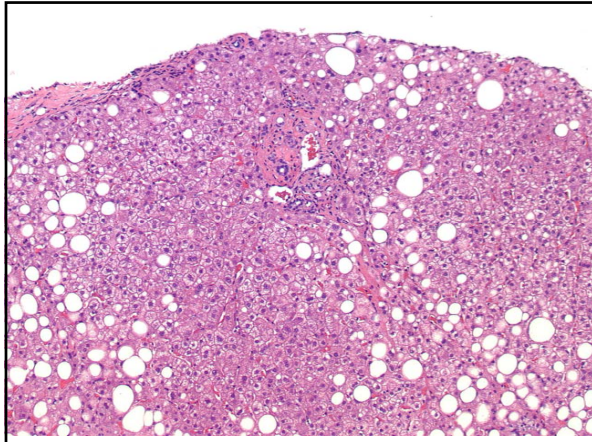
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### Trichrome Stain

- \* Subcapsular fibrous tissue may extend a few mm into parenchyma
- Interpret subcapsular biopsies with caution
- “Subcapsular sample with ..., cannot accurately stage.”

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### Liver bx: Systematic evaluation

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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**What is the morphologic pattern of injury?**

- Acute hepatitis
- Chronic hepatitis
- Granulomatous hepatitis
- Fatty liver disease / steatohepatitis
- Biliary injury
- Chronic cholestasis
- Massive /submassive necrosis
- Vascular / perfusion injury
- Increased iron stores
- Increased copper stores

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**What is “Fatty Liver Disease”?**

**Steatosis**

• Continuum of disease with steatosis, at some point

**Steatohepatitis**

- *may be less evident in cirrhosis*

**Cirrhosis**

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**Nonalcoholic Fatty Liver Disease**

**Steatosis**

This disease continuum in absence of *significant* alcohol use

**Steatohepatitis**

**Cirrhosis**

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**Alcoholic vs Nonalcoholic Steatohepatitis**

- Similarities reflected in terminology
  - Steatosis, inflammation, fibrosis pattern
  - Both progressive → cirrhosis, complications
- Cannot exclude alcohol in a given patient based on biopsy
  - Distinction made clinically

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**Differences between Alcoholic Hepatitis & NASH**

- Steatosis important for NASH, *not* always seen in alcoholic hepatitis
- Not seen in NASH:
  - Alcoholic foamy degeneration (pure microvesicular steatosis)
  - Canalicular cholestasis, bile ductular proliferation
  - Veno-occlusive lesions, Sclerosing hyaline necrosis

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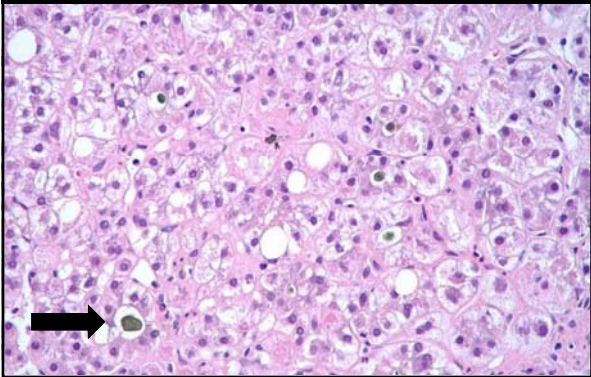
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30yo F, h/o alcoholism and binge drinking (1 pint daily x 15 yrs), admitted for worsening icterus, anemia, & cellulitis



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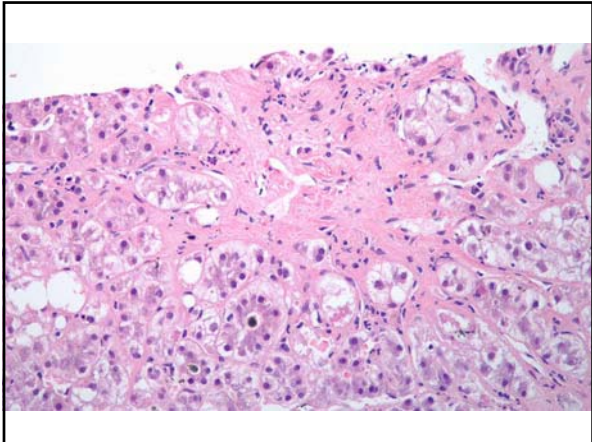
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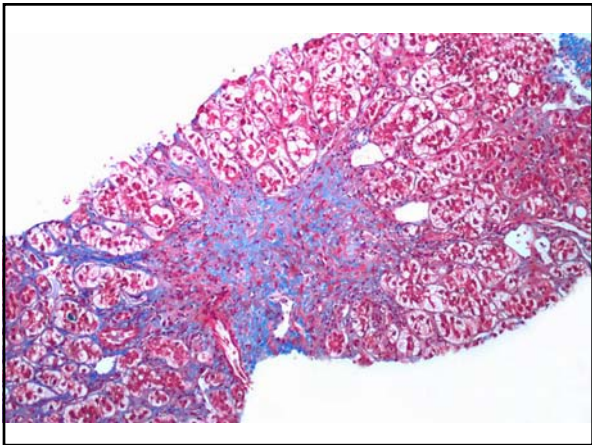
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**Nonalcoholic Fatty Liver Disease**

<b>Steatosis</b>	<p>Generally related to metabolic factors:</p> <ul style="list-style-type: none"><li>-Abdominal obesity</li><li>-Atherogenic dyslipidemia</li><li>-HTN</li><li>-Insulin resistance</li><li>-Prothrombotic or proinflammatory states</li></ul> <p>Hepatic manifestation of the metabolic syndrome</p>
<b>Steatohepatitis</b>	
<b>Cirrhosis</b>	

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### Causes of macrovesicular steatosis

- Drugs/Toxins – Alcohol, corticosteroids, others
- Hepatitis C
- Nutritional alterations: TPN, Starvation, jejunoileal bypass
- Inherited metabolic disorders
  - Galactosemia, GSD I&III, Wilson’s dz, Abetalipoproteinemia, Tyrosinemia
  - Mitochondrial defects
- Metabolic Syndrome

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### Nonalcoholic Fatty Liver Disease

<b>Steatosis</b>	• Continuum of disease with steatosis, at some point
<b>Steatohepatitis</b>	- <i>may be less evident in cirrhosis</i>
<b>Cirrhosis</b>	• 3 entities distinguished by histologic features and prognoses

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### Nonalcoholic steatosis versus steatohepatitis

- Distinct prognoses
- Steatosis alone:
  - Cardiovascular disease, stroke, HTN, DM
  - Little/no risk of progressive liver disease
- Steatohepatitis
  - 28-32% risk of progression in 5-10 yrs

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### NAFLD Progression

	132 Patients	Cirrhosis (average 8.3 yrs follow-up)	
<b>Diagnoses</b>			
Steatosis	49 (37%)	2 (4%)	<b>Lower risk</b>
Steatosis + lobular inflammation	10 (8%)	0 (0%)	
Steatosis + ballooning	19 (14%)	4 (21%)	<b>Greater risk</b>
Steatosis + ballooning + Mallory or fibrosis	54 (41%)	14 (26%)	

Matteoni et al., Gastro 1999

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### NAFLD Progression

	NAFLD Type	Cirrhosis (average 8.3 yrs follow-up)	
<b>Diagnoses</b>			
Steatosis	1	2 (4%)	
Steatosis + lobular inflammation	2	0 (0%)	
Steatosis + ballooning	3	4 (21%)	<b>Steatohepatitis</b>
Steatosis + ballooning + Mallory or fibrosis	4	14 (26%)	<b>Steatohepatitis</b>

Matteoni et al., Gastro 1999

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- ### Steatosis versus Steatohepatitis
- Distinguish on features associated with progression
  - Steatosis
  - Inflammation
  - Hepatocellular ballooning
  - Often, fibrosis
  - *\*Different pattern may be seen in children*

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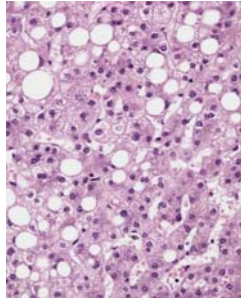
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### Steatosis

- Macrovesicular or mixed
- $\geq 5\%$
- May be centrilobular
- Good intra- and interobserver reproducibility



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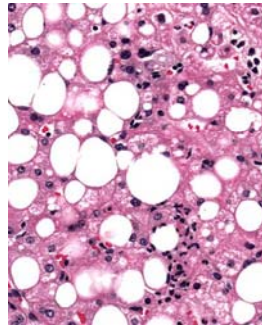
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### Inflammation

- Usually mild
- Mixture of cells
- Usually lobular,  $\pm$  portal in adults
- May be only portal in children



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### Portal Inflammation

- Severe disease of children & adults
- If portal > lobular or interface activity, consider concomitant disease (eg, AIH)
- Autoantibodies (ANA, SMA, AMA) seen in 20-40% of patients with NAFLD
- Look for interface activity, florid duct lesions, clinical features (pruritus, alkaline phosphatase)

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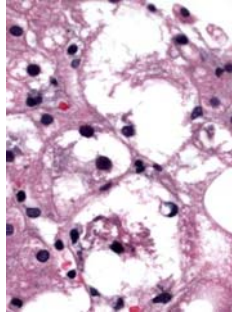
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### Ballooning hepatocyte degeneration

- Important diagnostic feature, also most difficult?
- Enlarged, pale cells with wispy or clumped cytoplasm
- Most prominent in zone 3, a/w perisinusoidal fibrosis
- May not see in pediatric NAFLD



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### Ballooned hepatocytes

- Multiple small lipid droplets
- Megamitochondria
- Dilated endoplasmic reticulum
- Mallory bodies
- Damaged cytoskeleton → Lose normal cytokeratin 8/18 pattern

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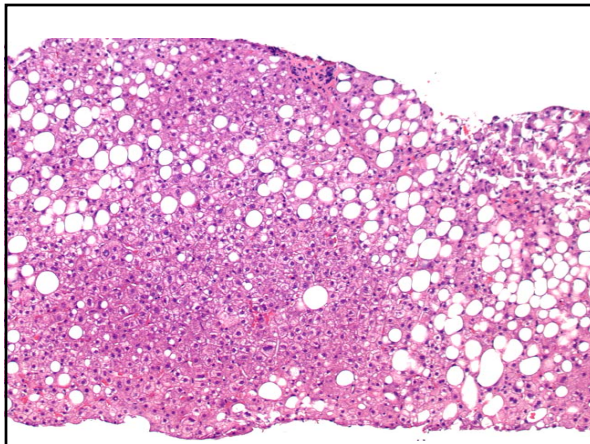
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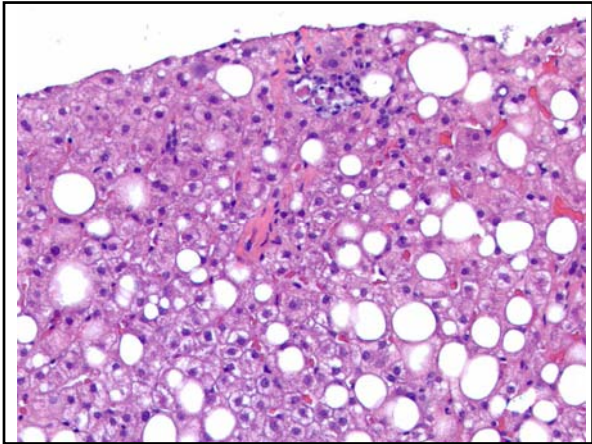
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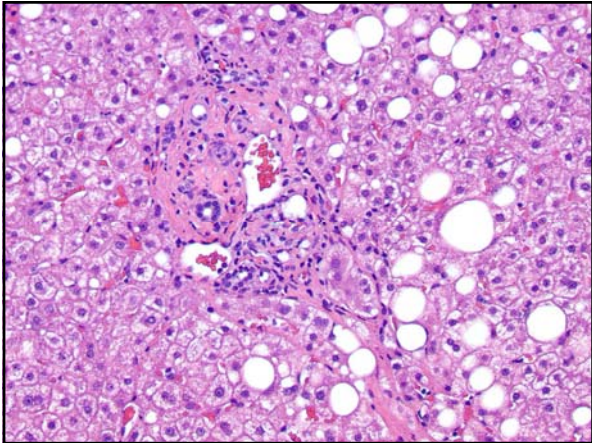
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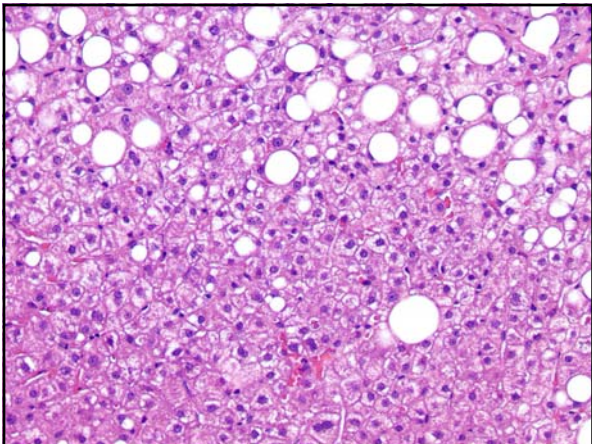
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**Etiologic diagnosis**

- Limited sample with mild steatosis,  
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- Or, (*in a case with ballooning*)
- Steatohepatitis and XX fibrosis,  
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**Liver bx: Systematic evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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**Case History**

- 58yo obese male with “elevated LFTs”
- Body mass index 32kg/m<sup>2</sup>
- Alcohol – 2 drinks/week
- AST 59; ALT 64
- Alkaline phosphatase 124
- Serologies: HepC, Hep B, ANA, SMA neg

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**Etiologic diagnosis**

- Limited sample with mild steatosis, with clinical features of nonalcoholic fatty liver disease.
  
- Or, (*in a steatohepatitis case*)
  
- Steatohepatitis with bridging fibrosis, clinically nonalcoholic steatohepatitis.

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**Liver bx: Systematic evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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**Reporting NAFLD**

- Like chronic liver diseases, separate grade and stage
  - Grade: Ongoing inflammatory activity and injury
  - Stage: Fibrosis
- Brunt et al. Am J Gastro 1999
  - Grade: Mild, moderate, severe; Stage: 0-4
- Kleiner et al. Hepatol 2005
  - NAFLD Activity Score (NAS)
  - Scoring system for NAFLD evaluation

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**NIDDK NASH CRN NAFLD Activity Score (NAS)**

<b>Activity</b> (*pattern of NAFLD)	<b>Fibrosis</b> (*Masson's trichrome)
Steatosis (0-3):	
0: <5%	1a,b: Zone 3 PSF
1: 5-33%	1c: Portal only
2: 34-66%	
3: >66%	
Lobular Inflamm (0-3):	
0: none	2: Zone 3 + portal/periportal
1: <2	
2: 2-4	
3: >4foci/20x field	
Ballooning (0-2):	
0: None	3: Bridging
1: few	
2: many/prominent	4: Cirrhosis

Kleiner et al., Hepatology 2005

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**NAFLD Activity Score**

- Intended to observe changes over time, not replace overall pathologic interpretation
- Brunt et al., Hepatology 2011: 976 Adults
  - 75% with SH had NAS ≥5
  - 86% with NAS ≥5 had SH
  - Not an acceptable threshold
  - NAS: correl w/elev ALT levels
  - NASH: correl w/fx of metabolic syndrome

Brunt et al., Hepatology 2011

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**Other prognostic factors?**

- Iron
  - Several studies, conflicting data
  - Hepatocellular iron and HFE mutations correlated with increased fibrosis risk in NAFLD (Valenti et al., Gastro 2010)
  - Reticuloendothelial system iron was a/w advanced fibrosis in NASH (Nelson et al., Hepatol 2010)

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### Steatohepatitis increases risk of liver-related mortality

	NAFLD Type	Cirrhosis (average 8.3 yrs follow-up)	
<b>Diagnoses</b>			
Steatosis	1	2 (4%)	
Steatosis + lobular inflammation	2	0 (0%)	
Steat	Increased liver related mortality (adjusted hazard ratio 9.94)		21% <b>Steatohepatitis</b>
Steat Mallo			(26% <b>Steatohepatitis</b> )

Younossi et al., Hepatology 2011

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### Treatment of Fatty Liver Disease

- Medications?
  - Vitamin E, ongoing trials
- Diet & exercise (Promrat et al., Hepatology 2010)
- Bariatric surgery

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### Pulling it all together

- Diagnosis:
- Liver, needle biopsy – Limited, subcapsular sample exhibiting mild steatosis.

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• **Comment/micro:**

• The biopsy interpretation is limited insofar as the biopsy is small, subcapsular, and contains only one portal tract for evaluation. That said, the sampling demonstrates mild steatosis (approximately 30%). Only a rare focus of lobular inflammation is present; ballooning hepatocyte degeneration is not seen. The one portal tract present harbors its usual structures. There is no bile duct injury or duct loss. The iron stain is negative. Review of the clinical history indicates mild transaminase elevations (AST: XX, ALT:YY), normal alkaline phosphatase, and negative viral hepatitis and autoimmune serologies. By report, the patient has no significant alcohol use but does exhibit risk factors for nonalcoholic fatty liver disease. Taken together, the clinical and histologic picture would be compatible with NAFLD, albeit in a limited sample. Diagnostic features of steatohepatitis are not present in the biopsy. Accurate staging is not possible on this limited and subcapsular sample, but there does not appear to be advanced fibrosis (bridging fibrosis or cirrhosis).

• *(If a study patient)* - NAFLD Activity Score: Steatosis: 1, Lobular inflammation 1, Ballooning 0. Total 2 of 8.

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**Takeaways**

- **Fatty liver disease pattern of injury**
  - Differential diagnosis
- **Steatosis versus steatohepatitis**
  - Diagnostic features
  - Prognosis
- **Reporting**
  - Description of features of injury (grade) and fibrosis (stage)

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**Questions and Discussion**

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## Part 4: Chronic Hepatitis

Lisa Yerian, MD  
Assistant Professor of Pathology  
Director, Hepatobiliary Pathology

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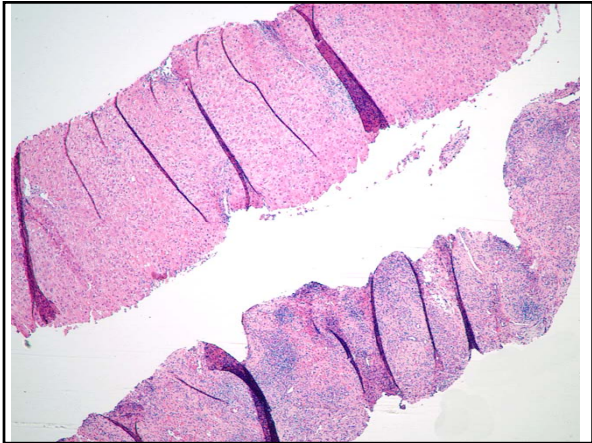
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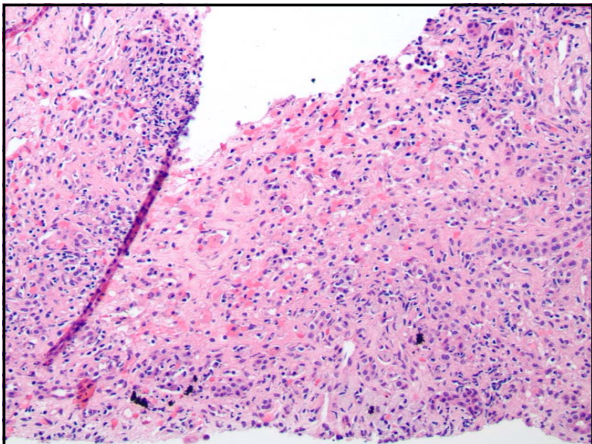
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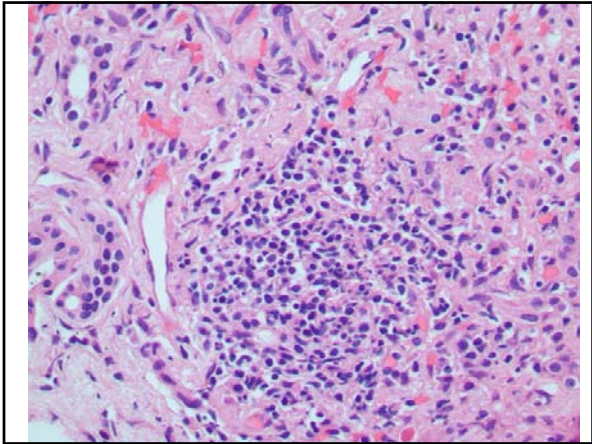
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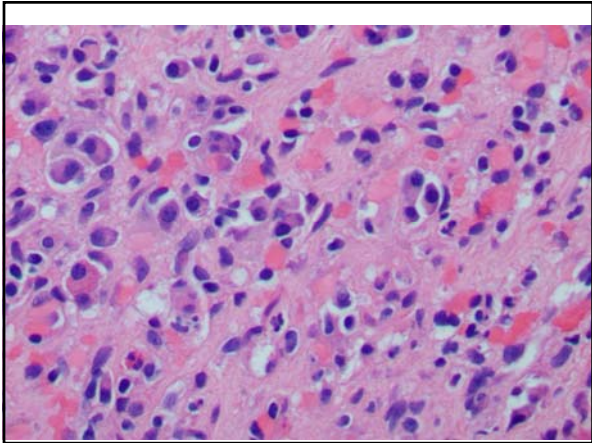
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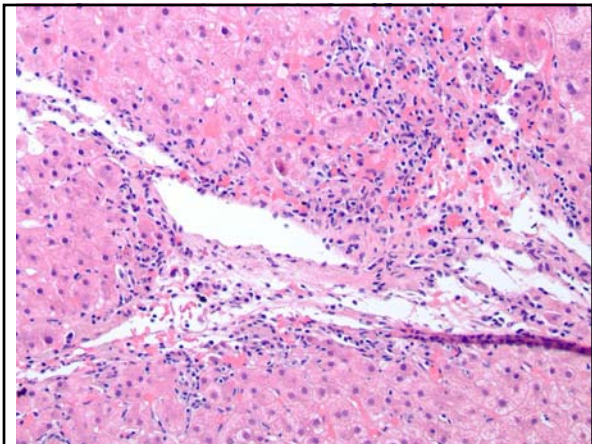
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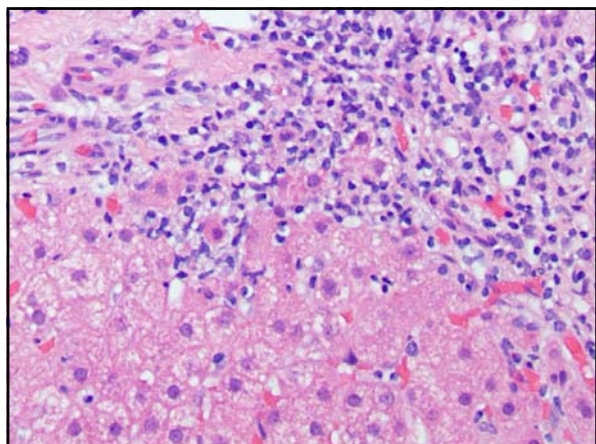
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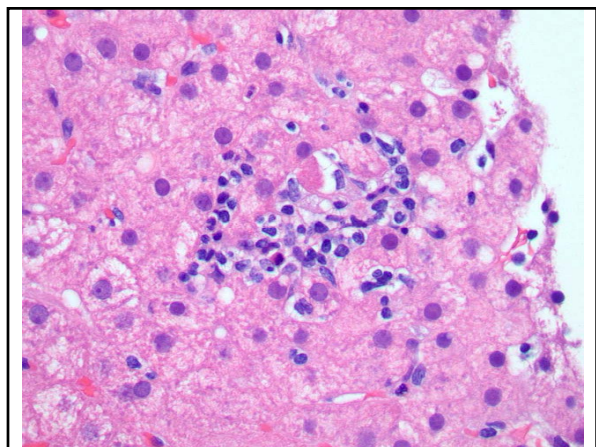
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**Liver bx: Systematic Evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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**Liver bx: Specimen adequacy**

- No universally agreed upon standard
- Sample size can affect accuracy of histologic assessment
- At least 6-8 complete portal tracts needed for diagnosis
- Chronic hepatitis grading and staging bx:
  - $\geq 20$ -25 mm long;  $\geq 11$  complete portal tracts
- Complete portal tract:
  - Complete circumference and  $\geq 2$  portal structures

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**Is this biopsy adequate?**

- Biopsy length: 1.0cm
- Number of complete portal tracts: 10
- Probably adequate for diagnosis ( $>6$ -8 portal tracts) but would like a little larger (2-2.5cm) and  $\geq 11$  portal tracts if desire grading/staging of chronic hepatitis

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**Liver bx: Systematic Evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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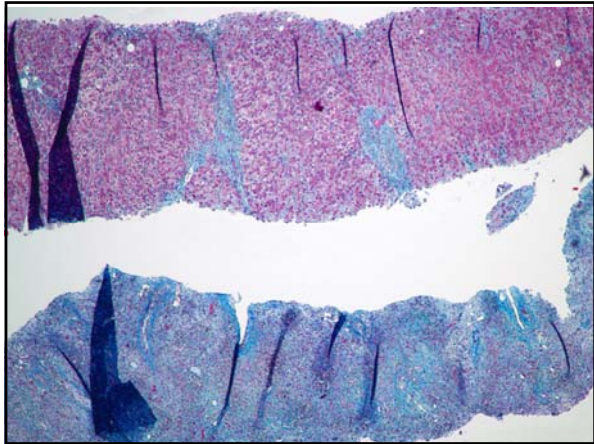
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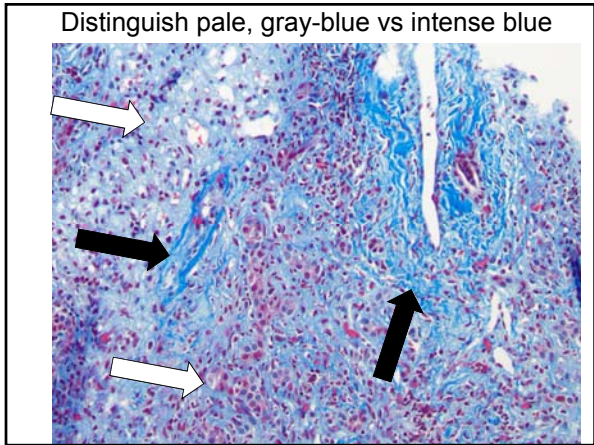
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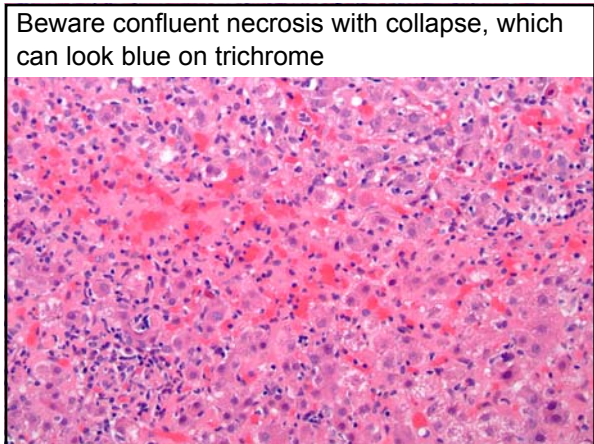
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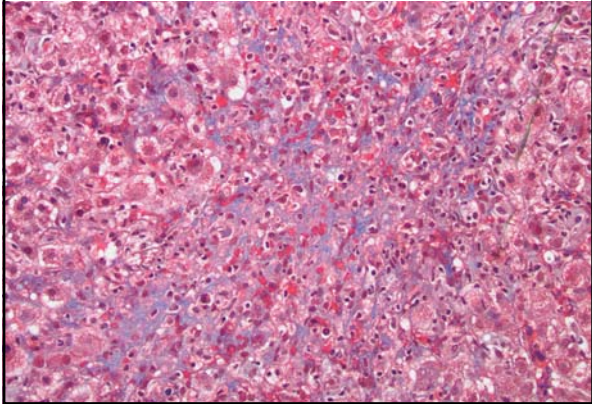
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Note the gray, pale staining in reticulin collapse



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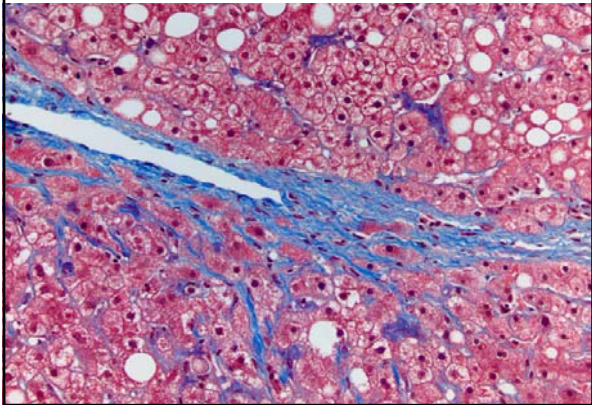
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Versus dense, bright blue of fibrosis



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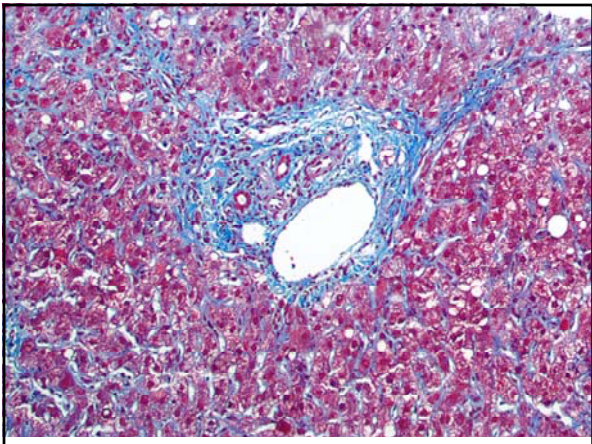
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### Trichrome stain

- Findings:
- The trichrome stain highlights areas of parenchymal collapse and also demonstrates periportal fibrosis. There is no evidence of cirrhosis.

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### Liver bx: Systematic Evaluation

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### What is the morphologic pattern of injury?

- Acute hepatitis
- Chronic hepatitis
- Granulomatous hepatitis
- Fatty liver disease / steatohepatitis
- Biliary injury
- Chronic cholestasis
- Massive /submassive necrosis
- Vascular / perfusion injury
- Increased iron stores
- Increased copper stores

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**Morphologic pattern**

- Markedly active hepatitis with bridging necrosis and plasma cells
- Periportal fibrosis; no cirrhosis
  
- Ddx:

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**What is the morphologic pattern of injury?**

- Acute hepatitis  Inflammatory
- Chronic hepatitis  Inflammatory
- Granulomatous hepatitis 
- Fatty liver disease / steatohepatitis 
- Biliary injury 
- Chronic cholestasis 
- Massive /submassive necrosis ? Necrotic
- Vascular / perfusion injury 
- Increased iron stores
- Increased copper stores ? Easy to miss

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**Morphologic pattern**

- Markedly active hepatitis with bridging necrosis and plasma cells
- Periportal fibrosis; no cirrhosis
  
- Ddx:
  - Severe chronic hepatitis
  - Acute hepatitis
  - Massive necrosis?
  - Increased copper/Wilson's disease?

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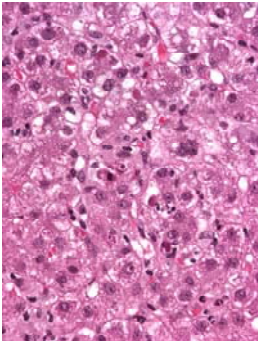
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**Acute hepatitis: Histologic features**

- Mixed lobular inflammation
- Hepatocyte swelling and injury
- Disruption of hepatocyte cords "lobular disarray"
- Acidophil bodies



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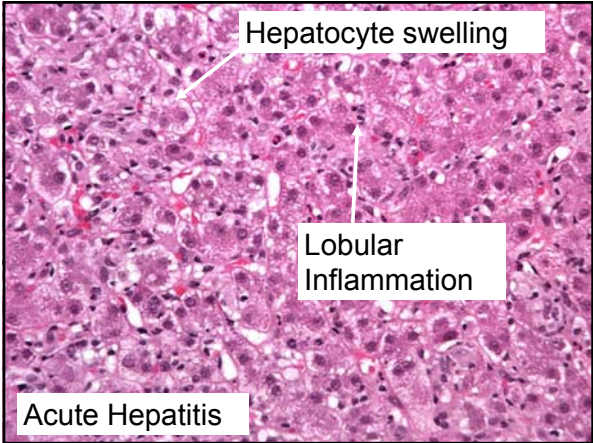
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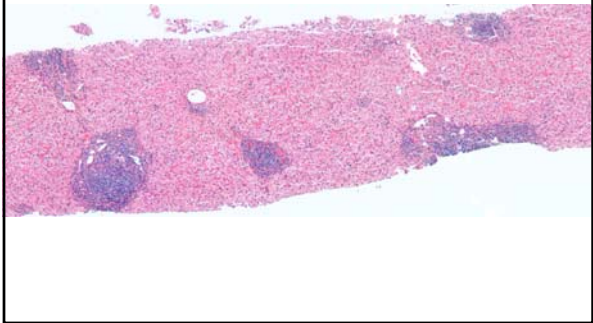
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**Chronic hepatitis: Histologic features**



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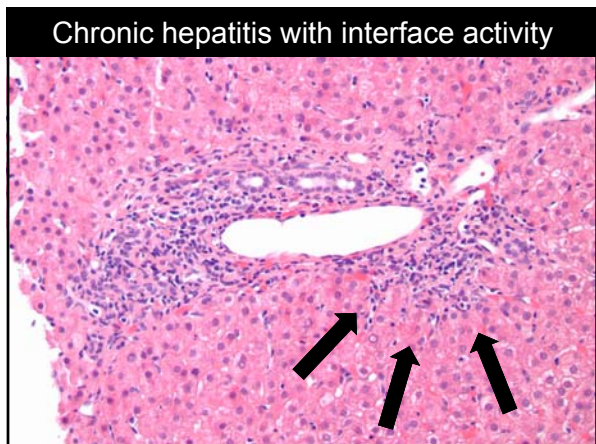
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**Morphologic pattern**

- Markedly active hepatitis with bridging necrosis and numerous plasma cells
- ? Fibrosis
  
- Ddx:
  - Severe chronic hepatitis
  - Acute hepatitis
  - Massive necrosis?
  - Wilson's disease?

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**Liver bx: Systematic Evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### Case History

- 47yo F presented to liver clinic with 2 month history of "hepatitis."

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### Morphologic pattern

- Markedly active hepatitis with bridging necrosis and numerous plasma cells
- ? Fibrosis
- Ddx:
  - Severe chronic hepatitis ←
  - Acute hepatitis ←
  - Massive necrosis? ↓
  - Wilson's disease? ↓

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### Case History

- 47yo F presented to liver clinic with 2 month history of "hepatitis." On the first day of her illness she reported headache and malaise. On d2 she was jaundiced and sought medical care. Review of LFTs over the next 2 months showed: bilirubin 13; ALT & AST in 500-600 range, Alk phos 300-400.

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### Case History

- Over the 2 months her LFTs declined but never normalized, and the patient lost 14 lbs.
- She reported no medical or surgical history, and no history of autoimmune disease.
- Meds: Calcium and a multivitamin. No other meds.

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### Physical Exam

- 5'5" tall, 123.5 lbs (BMI 20.6)
- Mildly icteric

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### Further labs

- Bili 3.7; AST 303; ALT 65; Alk phos 313
- Hep A Ab positive
- HBsAb positive
- Hep C Ab negative
- ANA negative; SMA positive
- Alpha-1 antitrypsin phenotype: MM
- Ceruloplasmin 38 (nl 21-45)

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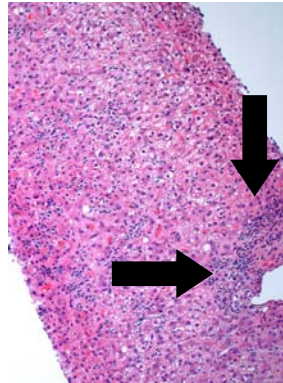
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### Acute hepatitis A

- Hepatitis A is prototype acute hepatitis pattern
- Transaminases >1000
- May also see portal inflam
- No fibrosis (may see reticulin collapse)



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### Acute hepatitis: differential diagnosis

- Acute viral hepatitis –
  - Hepatotropic - HAV, or acute presentation of HBV, HCV, HDV(+HBV), HEV
  - Nonhepatotropic viruses
- Drug-induced liver injury
- Autoimmune hepatitis

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### Biopsy for acute hepatitis

- Differential diagnosis largely clinical – we have little to add
- Serologic testing for hepatitis A, autoimmune
  - Biopsy to evaluate for AIH features, chronic changes (fibrosis), or atypical course
- Treatment supportive, most patients resolve on their own

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### Biopsy for acute hepatitis

- Hepatitis A antibody positive
  - Could be recent or distant infection
- Transaminases never >1000
- Patient has persistent LFT abnormalities after 2 months
- Fibrosis present on trichrome
- Need to consider other options

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### Chronic hepatitis: definition

- Chronic necroinflammatory diseases
  - Hepatocytes = main target of attack
- Chronic cholestatic conditions and metabolic diseases can look similar
- This does not include all things that are chronic and inflamed

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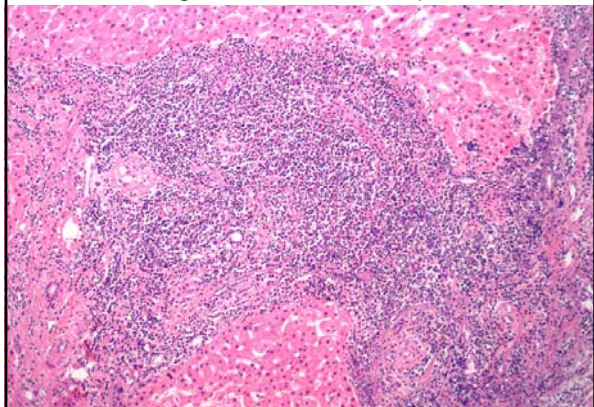
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Differential diagnosis of Chronic hepatitis



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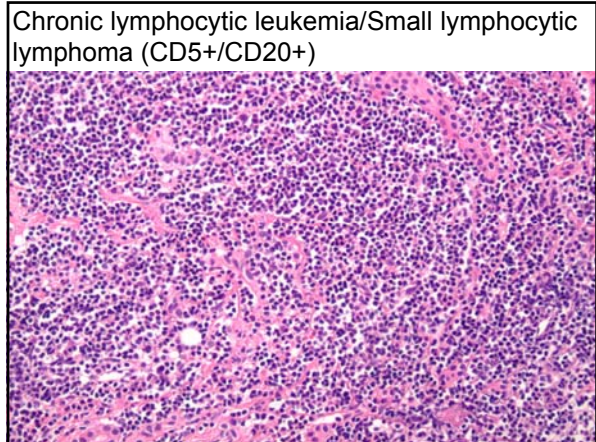
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**Acute vs. Chronic Hepatitis**

- Acute
  - Predominantly *lobular* inflammation and injury
  - No fibrosis
- Chronic
  - Predominantly *portal* inflammation and injury
  - Portal-based fibrosis

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**Chronic Hepatitis: differential diagnosis**

- Viral hepatitis: Hepatitis C, Hepatitis B
- Autoimmune hepatitis
- Drug-induced hepatitis
- Metabolic diseases: Wilson's disease, A1AT disease
- (Chronic biliary diseases: PBC)

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### Chronic viral hepatitis: HBV, HCV

- Diagnosis based on serology
- Confirm PCR testing for HBV DNA, HCV RNA
- Biopsy not used to establish diagnosis
  - Grade and stage disease
  - Assess for other forms of injury

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### Hepatitis C Virus

- Prototype chronic hepatitis pattern
  - Mild portal inflammation, interface activity, portal-based fibrosis
  - No specific histologic features
  - Serologic test - Nearly always known prior to biopsy
- Acute infection usually subclinical
- Chronic hepatitis develops in 90% infected

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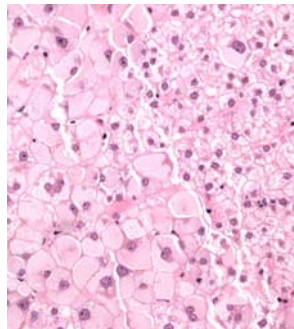
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### Hepatitis B Virus

- 5-20% → chronic
- Serology, PCR
- Ground glass hepatocytes represent actual viral replication
- Only see in chronic infection



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### Chronic hepatitis: Differential diagnosis

- Viral hepatitis: Hepatitis C, Hepatitis B
- Autoimmune hepatitis
- Drug-induced hepatitis
- Metabolic diseases: Wilson's disease, A1AT disease
- (Chronic biliary diseases: PBC)

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### Autoimmune hepatitis

- Ongoing, necroinflammatory liver disease of unknown cause, presumed autoimmune
- *A priori* chronic, but may present as acute or fulminant liver failure
- Diagnose by combination: clinical fx, autoantibodies, and histology
- Rapid response to immunosuppression

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### Autoimmune hepatitis

- Clinical picture:
  - Female
  - History autoimmune disease
- Polyclonal hypergammaglobulinemia
- Autoantibodies
  - Smooth muscle antibodies - SMA
  - Antinuclear antibodies - ANA
  - Liver-kidney-microsomal antibodies -LKM-1

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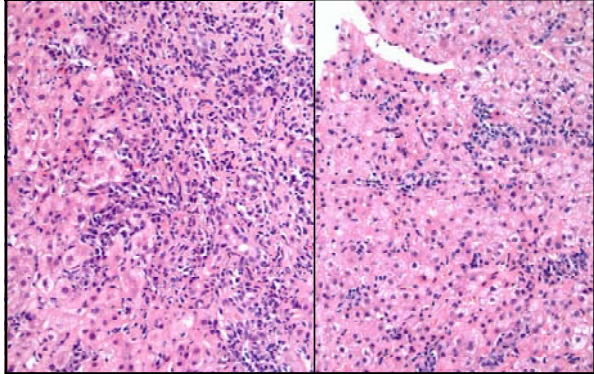
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Consider if severe chronic hepatitis, or marked lobular and portal inflamamtion/injury



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### Acute vs Chronic hepatitis

#### Acute

- Acute viral hepatitis –
- Hepatotropic viruses
- Nonhepatotropic viruses
- Autoimmune hepatitis
- Drug-induced liver injury/Toxin injury

#### Chronic

- Viral hepatitis: Hepatitis C, Hepatitis B
- Autoimmune hepatitis
- Drug-induced hepatitis
- Metabolic diseases: Wilson's disease, A1AT disease

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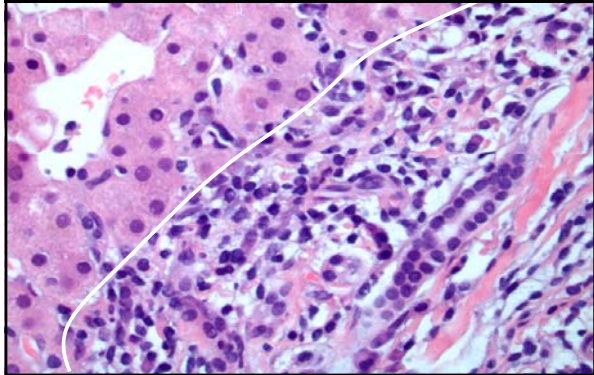
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Classic "piecemeal necrosis" of *chronic active hepatitis*



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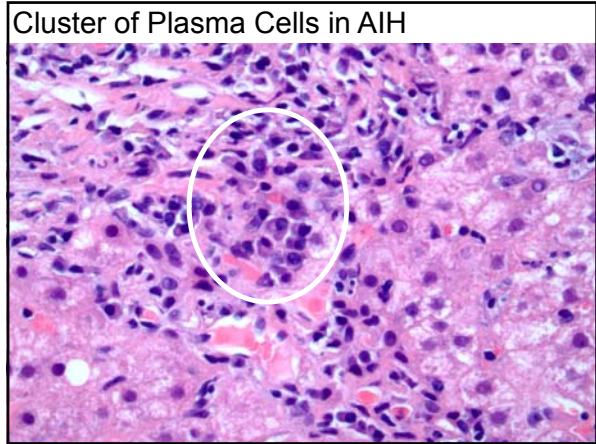
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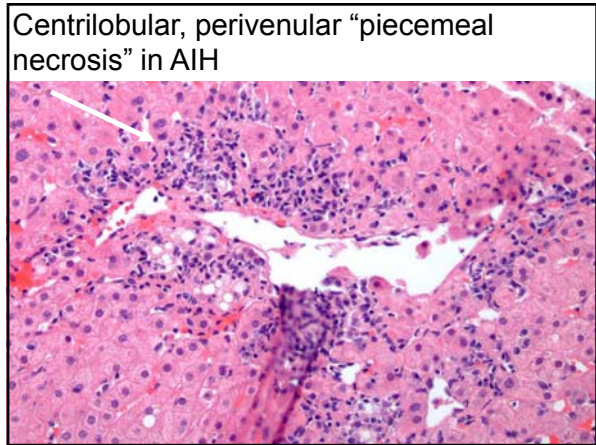
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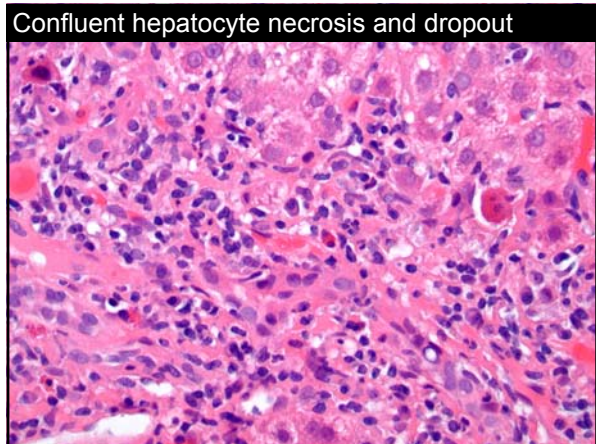
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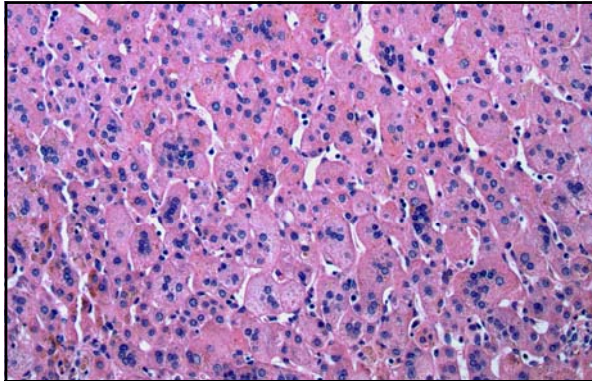
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Giant cell transformation if extensive hepatocyte injury (*"post-infantile giant cell hepatitis"*)

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### Autoimmune hepatitis vs PBC

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|---|--|
| • AST, ALT elevated                       | • AST, ALT nearly nl                   |
| • Alk phos nearly nl<br>- (high if acute) | • Alkaline phosphatase high            |
| • ANA+, SMA+                              | • AMA+                                 |
| • Ducts normal                            | • Ducts injured, lost                  |
| • Treatment:<br>- Steroids                | • Treatment:<br>- Ursodeoxycholic acid |

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### Diagnosing autoimmune hepatitis

- Principles reflected in an international group report
- Clinical, laboratory and histologic parameters included, each supporting (+) or countering (-) dx of AIH
- Score indicates likelihood of AIH
- Before rx: >15 "definite", 10-15 "probable"
- After rx: >17 "definite"; 12-17 "probable"

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### Diagnosing autoimmune hepatitis

- Actual calculation probably more useful for research than daily practice
- May not have all of data points available
- But, concepts of what argues for or against AIH is useful

Alvarez et al, J Hepatology 1999

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### Chronic hepatitis: Differential diagnosis

- Viral hepatitis
  - Hepatitis B, Hepatitis C
- Autoimmune hepatitis
- Drug-induced hepatitis
- Metabolic diseases: Wilson's disease, A1AT disease
- (Chronic biliary diseases: PBC, PSC)

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### Drug-induced hepatitis

- Always consider as a possible cause
- Eosinophils may provide clue
- Exclude other causes clinically
  - Viral hepatitis, autoimmune hepatitis
- Internet is good source of specific drug reactions

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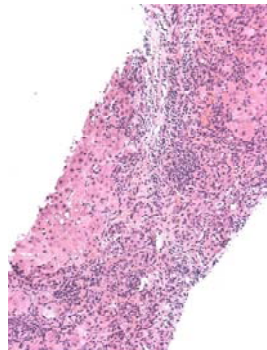
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### Wilson's disease

- Many histologic appearances
- Mild nonspecific portal inflammation, steatosis
- Chronic hepatitis/cholestatic picture
- Excluded in our patient by nl ceruloplasmin



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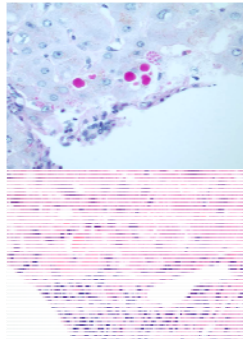
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### Alpha-1 antitrypsin deficiency

- Aut rec mut of protease inhibitor
- NI phenotype PiMM in 90% population
- PiMZ: Intermediate
- PiZZ: 10% w/clinical disease
- Can present as neonatal cholestasis or later



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### Etiologic diagnosis

- Autoimmune hepatitis with severe activity and periportal fibrosis ...
- Or,
- Chronic hepatitis with severe activity and periportal fibrosis, with clinical and histologic features most consistent with autoimmune hepatitis.

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### **Liver bx: Systematic Evaluation**

- Specimen adequacy
- Histochemical stains
- Etiologic diagnosis
  - Identification of morphologic pattern of injury
  - Correlation with clinical features
- Features of prognostic and therapeutic importance

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### **Reporting liver biopsies for chronic hepatitis**

- Confirm or establish diagnosis
- Assess inflammatory injury & fibrosis
- Evaluate concomitant disease processes
  - Example: iron, fatty liver disease
- Assess therapeutic intervention

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### **Assessing inflammatory injury and fibrosis**

- Severity of inflammatory activity
  - "Grade"
  - Based on interface activity and lobular inflammation and injury
- Extent of fibrosis
  - "Stage"
  - Portal-based fibrosis

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### Grading/Staging

- Several semiquantitative scoring systems are in use for chronic hepatitis:
  - Ishak, Knodell Histologic Activity Index
  - Metavir
  - Batts and Ludwig
- Selection based largely on preference, local convention

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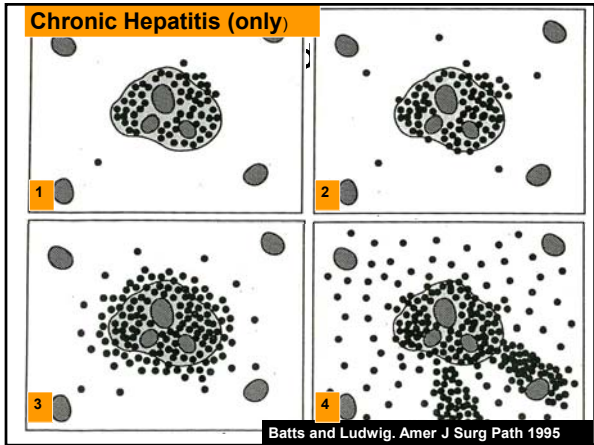
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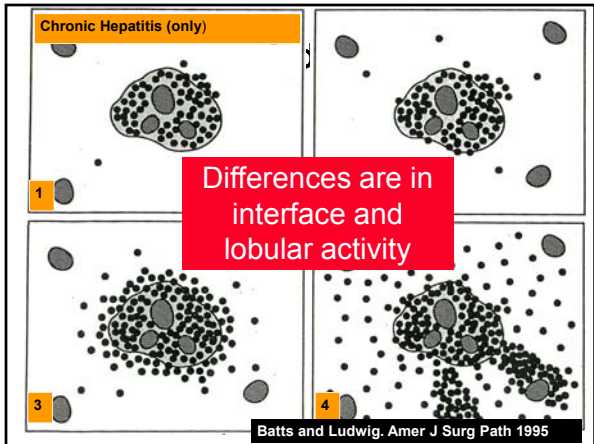
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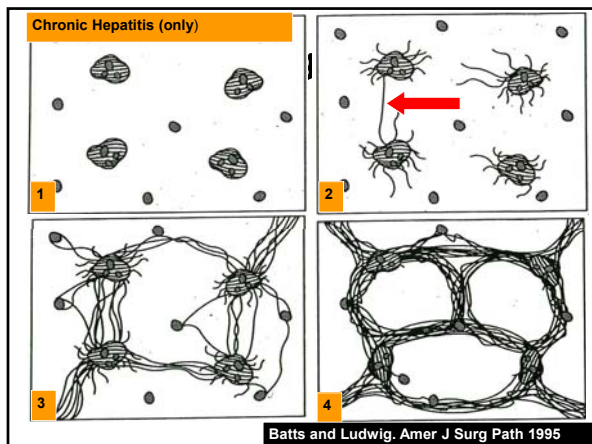
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### Pulling it all together

- Autoimmune hepatitis of severe activity with periportal fibrosis (Batts and Ludwig Grade 4 of 4, Stage 2 of 4).

Or ...

- Chronic hepatitis of severe activity with periportal fibrosis (Batts and Ludwig Grade 4 of 4, Stage 2 of 4), with clinical and histologic features most consistent with autoimmune hepatitis.

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- Chronic hepatitis of moderate activity with bridging fibrosis (Batts and Ludwig Grade 2 of 4, Stage 3 of 4), *etiology not apparent histologically.*

- Comment – Histologic features are suggestive of autoimmune hepatitis, but other causes cannot be excluded histologically. Serologic testing for viral and autoimmune markers is recommended.

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### How do grade/stage inform treatment/prognosis?

- Depends on the disease
- Hepatitis B – Biopsy less important
  - Clinical factors inform treatment
  - DNA levels more predictive of progression to cirrhosis
- Hepatitis C – More important
  - Fibrosis at initial presentation a predictive factor for progression to cirrhosis

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### Other features to report: Steatosis

- Steatosis – not uncommon, best studied in hepatitis C
- Virus may cause metabolic aberrations
- Steatosis tracks with viral
- A/w accelerated fibrosis and poor treatment response

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### Other features to report: Iron

- Chronic hepatitis C is a/w iron overload, and iron a/w increased activity in chronic hepatitis C
- Mixture of hepatocyte and reticuloendothelial cell deposition
- Iron depletion may stabilize or improve liver histology and slow disease progression
- For these reasons, I report iron and its severity

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**Takeaways**

- Most medical liver biopsies can be classified as to a pattern to aid diagnosis
- Acute hepatitis: predominantly lobular pattern, often not biopsied
- Chronic hepatitis: Biopsy aids diagnosis, and used for grading and staging, assessment before and after treatment, and evaluation for other features

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**Questions and Discussion**

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**Medical liver biopsy interpretation:**  
**A practical guide for accurate diagnosis and informative reporting**

Julia C. Iezzoni, M.D.  
University of Virginia Health System

Lisa M. Yerian, M.D.  
Cleveland Clinic Foundation

The histopathologic assessment of the liver biopsy specimen is an important part of the diagnostic evaluation, clinical management, and prognostication of patients with medical liver disease. As such, liver biopsies are regularly performed and are common specimens in most Surgical Pathology Laboratories. Despite the clinical importance and frequency of these specimens, many practicing pathologists and pathologists-in-training are uncertain on how to effectively evaluate, diagnose, and report these specimens, including common liver disease entities. Accordingly, using a case-based format, this course will: 1) present a readily applicable practical guide for systematically evaluating medical liver biopsy specimens; 2) discuss a pattern-recognition based approach for the accurate diagnosis of regularly encountered liver diseases, including entities to be considered in the differential diagnosis; and 3) identify the clinically important diagnostic, therapeutic and prognostic information to be included in the surgical pathology report of liver biopsies for each of the liver diseases discussed. As a result, the participants will learn how to systematically evaluate, accurately diagnose and effectively report medical liver biopsy specimens.

**Part 1: Systematic Evaluation and Informative Reporting of**  
**Medical Liver Biopsies: Overview**

Julia C. Iezzoni, M.D.

As a matter of routine for tumor specimens, surgical pathologists evaluate and report all tissue-based features of prognostic and therapeutic importance. Specifically, the gross and microscopic features of the case are systematically evaluated, and clinically relevant, tissue-based features are identified and reported. This standard for tumor evaluation and reporting is reinforced (and in many ways simplified) by the use of approved synoptic reports, such as those formulated by the College of American Pathologists.

Analogous to tumor specimens, the standard for liver biopsies is to evaluate and report all tissue-based features of prognostic and therapeutic importance. As with tumor specimens, this includes systematically evaluating the liver biopsy. The components of this evaluation include: 1) assessment of specimen adequacy; 2) evaluation of histochemical stain results; 3) determination of the etiology of the liver disease; and 4) identification of features of prognostic and therapeutic importance.

These components routinely should be evaluated and reported for each medical liver biopsy specimen.

Standardized, widely used, synoptic reports, analogous to those for reporting tumor specimens, currently are not available for medical liver biopsies. However, by systematically evaluating medical liver biopsy specimens, the goal of a synoptic report is achieved – specifically, all clinically relevant tissue-based features in the liver biopsy are evaluated and reported.

### **Specimen adequacy**

As with any non-excisional biopsy specimen, sample size can affect the diagnostic accuracy of medical liver biopsy specimens.<sup>1-3</sup> While there is not a clear consensus on what constitutes specimen adequacy, it has been proposed that a minimum of 6-to-8 complete portal tracts are needed for diagnosis. Preferably, the specimen is intact. Furthermore, for accurate grading and staging of chronic hepatitis, the minimum criteria for specimen adequacy is a biopsy core that is at least 20-25 mm in length and contains at least 11 complete portal tracts.<sup>4-5</sup> Of note, for these purposes, a complete portal tract is defined as one in which the complete circumference is demonstrated, and it contains at least 2 of the 3 portal structures.

Small biopsy specimens from the subcapsular region present a different limitation to biopsy evaluation. Normally, for approximately 0.2 to 0.5 cm below Glisson's capsule, there are fibrous connections between the capsule and the superficial portal tracts.<sup>6</sup> These normal features can have the appearance of septa formation and even a vague nodularity, thereby creating the misimpression of cirrhosis.<sup>7</sup> As such, small, subcapsular biopsy specimens are suboptimal for the evaluation of the extent of fibrosis.

Since the sample size or location of a liver biopsy specimen can affect the accuracy of histologic assessment, it is appropriate to include a statement regarding specimen adequacy in the surgical pathology report. This is analogous to the reporting of Pap smears, in which a statement regarding specimen adequacy routinely is included in the cytopathology report.

### **Histochemical stains**

Both historically and in present-day practice, diagnostic hepatopathology is based on the interpretation of the morphologic features demonstrated on the H & E stained tissue sections in conjunction with the findings on a series of histochemical stains.<sup>8</sup> Because of their selective staining properties, histochemical stains contribute useful information by the delineation of specific architectural and cellular features. A standard battery of histochemical stains, traditionally have been (and in many institutions, still are) performed routinely on all medical liver biopsies. Typically, these include reticulin, periodic acid-Schiff (PAS) with and without diastase, iron, and copper stains.

While histochemical stains remain an important part of the evaluation of liver tissue, their specific role has changed due to advances in diagnostic surgical pathology, as well as in medicine as a whole.<sup>8</sup> For example, the widespread availability of sensitive and specific immunohistochemical stains has replaced some of the functions previously served by histochemical stains. Specific enzymatic and genetic tests have modified and in some cases supplanted the role of histochemical stains in the diagnosis of many metabolic disorders. In addition, the role of the liver biopsy itself has changed. While

the emphasis was previously on the etiologic diagnosis of the liver disease, the liver biopsy now has important additional roles in the assessment of patients for the initiation and continuation of therapy and in the prediction of prognosis. In addition, the advent and widespread use of liver transplantation has added a new group of issues to be addressed in diagnostic hepatopathology. Due to these advances, many of the questions being asked on and of the liver biopsy (both the H&E and histochemical stained tissue sections) have changed. Accordingly, this raises the question of what is the role in contemporary practice of these standard histochemical stains?

To address this question, a brief survey of expert hepatopathologists (i.e. the membership of the Hans Popper Hepatopathology Society) on their histochemical stain practices was performed (unpublished). The results of this survey demonstrated that: 1) a trichrome stain is universally performed on all medical liver biopsy specimens, because it is considered essential to accurately determine the extent of fibrosis; and 2) there is no widely agreed upon standard for the routine use of any other histochemical stain.

The latter finding of this survey, in conjunction with the current environment of limited technician resources and health care dollars, raises the question of whether routine “pan-staining” is cost-effective and appropriate. Should we really perform reticulin, PAS with and without diastase, iron, and copper stains on all liver biopsies? While there is no universally agreed upon standard for the routine use of histochemical stains in the evaluation of medical liver biopsy specimens, a reasonable approach may be to perform a trichrome stain on all medical liver biopsies, and then, if necessary, perform additional histochemical stains as indicated after the initial review of the biopsy slides.

**Etiologic determination**

The basis for determining the etiology of medical liver disease is clinicopathologic correlation. Specifically, this consists of two components: 1) identification of the morphologic pattern of injury; and 2) correlation with the clinical features. Accordingly, the determination of the etiology of liver disease requires contributions from both the pathologist (to identify the morphologic pattern of injury) and the hepatologist (to provide complete and accurate clinical information).

The liver has a diverse, though finite number of major histologic responses to tissue damage – the so-called “morphologic patterns of injury” (Table 1). While the morphologic pattern of injury is etiologically non-specific, each pattern does indicate a particular etiologic differential diagnosis. Accordingly, the identification (by the pathologist) of the morphologic pattern of injury demonstrated on the liver biopsy is a key component for the identification of the etiology of the liver disease. Of note, some of these patterns overlap, and in some etiologies of liver disease, multiple morphologic patterns of injury may be present. For example, primary biliary cirrhosis may demonstrate the morphologic patterns of chronic hepatitis and granulomatous hepatitis, in addition to the characteristic findings of biliary injury and chronic cholestasis.

<b>Table 1: Morphologic patterns of injury</b>
Acute hepatitis
Chronic hepatitis
Granulomatous hepatitis
Steatosis / steatohepatitis
Biliary injury
Chronic cholestasis
Massive /submassive necrosis
Vascular / perfusion injury
Increased iron stores
Increased copper stores

Complete and accurate clinical information also is a key and necessary requirement to determine the etiology of medical liver disease. This includes relevant

laboratory studies, risk factors for liver disease (e.g. drug / alcohol use, obesity), and radiographic features, if indicated (e.g. endoscopic retrograde cholangiography). Unfortunately, the clinical information provided on the liver biopsy requisition form at times, is sparse, at best. In the advent of radiologists performing an increasing number of liver biopsies, communication of the clinical information from the hepatologist to the pathologist can be further hindered. If the relevant and complete clinical information is not provided with the liver biopsy specimen, the surgical pathologist (or pathology resident) should contact the clinician to obtain it.

### **Tissue-based features of prognostic and therapeutic importance**

Analogous to the surgical pathology reporting of tumor specimens, the informative medical liver biopsy report includes all tissue-based features relevant to prognosis and treatment. Similar to tumors, the clinically relevant tissue-based features of medical liver disease may vary between the different entities. Accordingly, in the following case-based sections, the therapeutically and prognostically relevant information to be included in the surgical pathology report of liver biopsies for the each of the liver diseases presented is identified and discussed.

An additional useful resource is available from the American Association for the Study of Liver Disease (AASLD), a leading organization of health care professionals and scientists specializing in liver disease ([www.aasld.org](http://www.aasld.org)). On a regular basis, the AASLD publishes Practice Guidelines, which are recommendations of the suggested preferred approaches to the diagnosis, therapy, and prevention of various liver diseases. These can be useful references for the pathologist, because they provide guidelines for the role of the liver biopsy in the diagnosis, prognostication, and management of liver.

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## **Part 2: Chronic Cholestatic Liver Disease: Acquired ductopenic diseases in adults**

Julia C. Iezzoni, M.D.

### **Introduction**

Cholestasis is a pathophysiologic state defined as a decrease in bile flow. Cholestasis may result either from a functional defect in bile formation or secretion at the level of the hepatocyte or from impairment in bile flow at any point along the biliary tract, either intra-hepatic or extra-hepatic. Accordingly, the etiologies of cholestasis include a highly diverse multitude of entities.

Within this large and heterogeneous group of diseases, the most common site of injury that results in chronic intra-hepatic cholestasis in adults is damage to the small and medium-sized intra-hepatic bile ducts (< 100  $\mu\text{m}$  in diameter), which includes the interlobular ducts.<sup>1-3</sup> The damage may result in bile duct loss, with a reduction in the number of interlobular bile ducts - so-called “ductopenia”.

Of note, these interlobular bile ducts are routinely demonstrated and readily identifiable in liver biopsies, and they are not visualized on cholangiographic studies.<sup>1</sup> Accordingly, the surgical pathologist is centrally positioned to diagnose and evaluate liver diseases characterized by ductopenia, and furthermore, the definitive identification of interlobular bile duct loss can be made only by liver biopsy examination.

### **Ductopenia – definition**

“Ductopenia” is a descriptive histologic term that refers to the absence of interlobular bile ducts from the portal tracts due to any cause. Since the interlobular bile duct usually is located in the portal tract near and parallel to a similar-sized arterial branch, loss of an interlobular bile duct is recognized in an individual portal tract when no duct is identified in proximity to hepatic artery branch. In the normal adult human liver, hepatic arteries may be unaccompanied by a bile duct of similar diameter in up to 25% of portal tracts.<sup>4</sup> Ductopenia, therefore, is defined as the loss of interlobular bile ducts in more than 50% of portal tracts (i.e. a duct-to-artery ratio of less than 0.5).<sup>5</sup> Also by definition, for the diagnosis to be made reliably, the specimen must be adequate; specifically, at least 10 portal tracts are required for accurate semi-quantitative assessment, and 20 or more is considered ideal. Bile ductular proliferation may coexist with interlobular bile duct loss, and these small ductules should not be included in the bile duct count. The size and location of the biliary elements assists in the differentiation of interlobular bile duct from bile ductules. As previously described, interlobular bile ducts are located within the portal tracts in close proximity and parallel to the hepatic



artery branch of similar size. In contrast, bile ductules typically are located close to the limiting plate with no corresponding artery branch.

**Adult acquired ductopenic diseases: Morphologic patterns of injury**

While the specific histologic features vary between specific entities or between different stages within a single entity, common to all acquired ductopenic diseases in adults is interlobular bile duct injury and loss - “biliary injury morphologic pattern of injury”. As the disease is progresses to chronic cholestasis, features of the “chronic cholestatic morphologic pattern of injury” then become apparent.

**Biliary injury:** The biliary injury morphologic pattern of injury is the characterized by damage to the bile ducts. Specifically, the bile duct epithelium demonstrates features of injury, with cytoplasmic vacuolization, disarray with irregular spacing of the nuclei, and pyknotic nuclei – so-called “bile duct epithelial disarray”. Early on, the interlobular bile duct injury may be accompanied by so-called “atypical” bile ductular proliferation, with anastomosing cord-like ductules at the periphery of the portal tracts.<sup>6</sup> As the disease progresses, however, the bile ductular proliferation subsides and typically is not prominent in late stage disease. Due to the ongoing insult, the bile ducts disappear, resulting in ductopenia. In cases associated with intense portal inflammation, immunohistochemistry for “biliary cytokeratins” (cytokeratins 7 or 19) can be performed to identify bile ducts, if present.

**Chronic cholestasis:** As a consequence of the progressive bile duct loss, histologic feature of the chronic cholestatic morphologic pattern of injury become evident.<sup>6</sup> Due to the damage caused by bile acid retention, the periportal hepatocytes become swollen with rarified cytoplasm (“cholate stasis”, “pseudoxanthomatous change”, “feathery degeneration”), and damage to the cytoskeleton results in Mallory’s hyaline. Also, copper and copper-associated protein may accumulate in the periportal hepatocytes. Bile pigment accumulation within hepatocytes (“bilirubinostasis”) may or may not be seen, and if present, is most prominent in the zone 3 hepatocytes.

**Adult acquired ductopenic diseases: Etiologies**

A variety of acquired disorders may cause ductopenia in adults (Table 1), and as a group, they are referred to as “ductopenic diseases” or “vanishing bile duct syndrome”.<sup>1,3</sup> Though the damage is caused by variety of mechanisms, including immune-mediated, ischemic, infectious, and toxic, this group of diseases share the common feature of progressive destruction and disappearance of interlobular bile ducts with resultant chronic cholestasis both clinically and morphologically. The majority of adult patients with chronic cholestasis due to acquired ductopenic disease have either primary biliary cirrhosis or primary sclerosing cholangitis. As such, this discussion will focus on these entities. In addition, primary biliary cirrhosis – autoimmune hepatitis overlap syndrome will be discussed, because the criteria for its diagnosis may not be clearly understood by either the pathologist or hepatologist.

<b>Table 1: Etiologies of Adult Acquired Ductopenia</b>	
<b>Immune-mediated</b>	
-	Primary biliary cirrhosis
-	Primary sclerosing cholangitis

### Primary biliary cirrhosis

Primary biliary cirrhosis is a form of chronic, progressive cholestatic liver disease in which there is destruction of the small and medium-sized interlobular bile ducts by a non-suppurative inflammatory process.<sup>7-9</sup> The disease is autoimmune in pathogenesis. Primary biliary cirrhosis is predominantly a disease of middle-aged women (fifth to seventh decade), with a female:male ratio of 9:1. There is an association of PBC with a variety of other autoimmune diseases, most notably Sjogren's syndrome and Raynaud's disease.<sup>8</sup> While there may be mild elevation of aminotransferases, the pattern of liver biochemistries is cholestatic, with marked elevation of alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGT). Total serum cholesterol also may be elevated. More than 60% of patients who have PBC are asymptomatic at the time of diagnosis, and typically, these patients are identified initially by screening tests that demonstrate an elevation in cholestatic liver biochemistries. When symptoms are present, the most common are fatigue and pruritis.

Serum antimitochondrial antibodies (AMA) are present up to 95% of cases of PBC, and they are quite specific (97%) for the disease.<sup>9</sup> Due to this high sensitivity and specificity, a positive AMA is a major diagnostic hallmark of PBC. Additionally, hypergammaglobulinemia with selective elevation of IgM is common and is seen in more than 70% of patients with PBC.<sup>9</sup> Of note, anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) also may be present at a prevalence of approximately 40% and up to 14%, respectively in cases of PBC.<sup>10,11</sup>

Approximately 5% of patients with PBC are AMA-negative.<sup>12</sup> This so-called "outlier syndrome" is variably referred to as "AMA-negative PBC", "autoimmune cholangitis", or "autoimmune cholangiopathy". There is a lack of consensus as to whether these cases represent a variant of PBC or alternatively, whether they represent a separate and distinct entity. The clinical, biochemical, and histologic features of AMA-negative PBC are essentially indistinguishable from those of "classic" PBC, and AMA-negative PBC has the same natural history and response to treatment as PBC. Of note, there is a significantly higher rate of positivity for anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) in patients with AMA-negative PBC than in AMA-positive disease. While there is a lack of uniform diagnostic criteria, the diagnosis of AMA-negative PBC is generally applied in cases that are consistent with PBC (clinically, biochemically, and histologically) but lack identifiable AMA. Notably, with the advent of more sensitive tests to determine the presence of AMA, as many as 79% of cases initially categorized as AMA-negative PBC are found to be AMA-positive.<sup>13</sup> This

- Autoimmune overlap syndromes
- Graft-vs-host disease (late stage)
- Chronic hepatic allograft rejection

#### Infectious

- Immunocompetent:
  - bacterial cholangitis
  - recurrent pyogenic cholangitis
- Immunodeficient:
  - cytomegalovirus
  - *Cryptosporidium parvum*
  - *Microsporidium*

#### Drugs and toxins

- Wide variety of pharmaceuticals
- Some herbal products
- Hepatic artery infusion chemotherapy

#### Ischemic

- Hepatic artery injury
- Vasculitis

#### Neoplastic

- Hodgkin's disease

#### Miscellaneous

- Sarcoidosis
- Langerhans cell histiocytosis
- Systemic mastocytosis

#### Idiopathic

- Idiopathic adulthood ductopenia

reinforces the concept that AMA-positive and AMA-negative PBC are essentially a single disease entity.

Histologically, PBC is characterized by progressive destruction of the small and medium-sized interlobular bile ducts by a non-suppurative inflammatory process.<sup>7,8</sup> Early in its course, PBC demonstrates a portal mixed inflammatory infiltrate, composed of lymphocytes, scattered eosinophils, macrophages, and a variable number of plasma cells. This inflammation is intimately associated with the bile ducts, with lymphocyte infiltration of the bile ducts (so-called “lymphocytic cholangitis”), injury to the biliary epithelium (vacuolar degeneration, disarray, and necrosis) and disruption of the bile duct basement membrane (best visualized on PAS-diastase stain). This combination of portal based inflammation, bile duct epithelial cell injury, and disruption of the bile duct basement membrane is known as the “florid duct lesion”, and it is the characteristic lesion of PBC. In addition, non-necrotizing granulomas may be present and typically are located in the portal tracts. At this early stage, the inflammation is confined to the portal tracts, and the limiting plates are intact. Of note, histologic evidence of cholestasis is not a feature of early stage PBC, and is not appreciated until later in the disease course, when liver decompensation occurs. As the disease progresses, the inflammatory infiltrate extends from the portal tracts through the limiting plate into the surrounding hepatic parenchyma. This results in interface necroinflammatory activity reminiscent of chronic hepatitis. In some portal tracts, the ongoing injury of the bile ducts is accompanied by bile ductular proliferation. In other portal tracts, the bile ducts are absent, having seemingly vanished along with the inflammatory infiltrate resulting in a “burnt out” appearance. As the disease progresses, histologic evidence of chronic cholestasis becomes apparent, with cholate stasis, periportal copper accumulation, and Mallory’s hyaline. Bile ductular proliferation subsides as the disease advances and is not prominent in late stage PBC. As a result of the progressive necroinflammatory injury at the limiting plate, periportal and portal-to-portal bridging fibrosis develops and eventually progresses to cirrhosis.

The size of the liver biopsy specimen is important in the evaluation for possible PBC.<sup>8</sup> The distribution of the characteristic bile duct changes (i.e. florid duct lesion, bile ductular proliferation, bile duct loss) typically is patchy. Accordingly, the probability of observing the biliary lesions increases with the number of portal tracts, and at least 10-15 portal tracts should be present and multiple sections should be reviewed to adequately appreciate or rule out liver histopathology consistent with PBC.

The most commonly applied staging schemes for PBC classify the histologic features into four stages, as follows: Stage 1: Portal (histologic changes are confined to the portal tract – i.e. florid duct lesion); Stage 2: Periportal (histologic changes extend to the periportal area with bile ductular proliferation and interface necroinflammatory activity); Stage 3: Septal (portal-to-portal bridging fibrosis); Stage 4: Cirrhosis.<sup>14,15</sup> Of note, this staging is based on the histopathologic features on the H&E and trichrome stained tissue sections. The value of histologic staging in PBC for predicting prognosis is somewhat limited, given the aforementioned lack of uniformity of bile duct injury and the accompanying fibrosis in this disease.<sup>9</sup> However, the presence of portal-to-portal bridging fibrosis is a poor prognostic indicator. As such, it is important to distinguish lower stage (stages 1-2) from more advanced (stage 3-4) disease.

As per accepted criteria, the diagnosis of PBC can be established when two of the following three conditions are met: 1) a positive AMA; 2) increased cholestatic liver biochemistries (usually AP) for greater than 6 months; and 3) compatible liver histology.<sup>8</sup> Accordingly, in the presence of a positive AMA and consistent biochemistries, the identification of the specific etiology of the liver disease is less dependent on the liver biopsy findings. Alternatively, in cases of AMA-negative PBC, the identification of the etiology of the liver disease is considerably more dependent on the pathologist's evaluation of the liver biopsy for consistent histologic features.

Though the rate of progression varies greatly among individual patients, PBC is progressive, with the development of cirrhosis that ultimately may result in liver failure.<sup>8</sup> While there is no cure for PBC, ursodeoxycholic acid (UDCA), an anti-cholestatic agent, improves biochemical indices, delays histologic progression and dramatically increases the survival of patients with PBC, especially those with early stage disease.<sup>7,9</sup> Accordingly, UDCA is the mainstay of therapy, and the vast majority of patients with PBC are treated with UDCA.<sup>7,8</sup> Despite the autoimmune pathogenesis of PBC, corticosteroids or other immunosuppressive medications are not effective in the treatment of PBC, and their use is additionally limited by their significant side effects.<sup>8,16</sup>

**Surgical Pathology report:** The informative surgical pathology report for liver biopsies in cases of possible PBC includes the following elements: 1) evaluation for consistent histology; 2) stage of the disease; 3) disease progression if sequential biopsies are available; 4) the effect of treatment, if administered; and 5) concurrent liver diseases. As with any liver biopsy evaluation, the interpretation requires the availability of complete and accurate clinical history and laboratory studies and an adequate liver biopsy specimen.

### **Primary sclerosing cholangitis**

Primary sclerosing cholangitis (PSC) also is a form of chronic and progressive cholestatic liver disease, and it is characterized by inflammation and fibrous obliteration of segments of the intra- and/or extra-hepatic biliary tract.<sup>7,17</sup> PSC is autoimmune in pathogenesis, and it also has a strong genetic component. Most patients with PSC are men (male-to-female ratio of 2:1). The disease typically presents during the 4<sup>th</sup> or 5<sup>th</sup> decade, the average of onset being 42 years. However, PSC also may present in children or the elderly and has an age range at presentation of 1-90 years. At the time of diagnosis, approximately 50% of patients are asymptomatic, and typically, these patients are identified initially by screening tests that demonstrate an elevation in cholestatic liver biochemistries (AP, GGT). When symptoms are present, the most common are pruritus, fatigue, and right upper quadrant pain. There is association of PSC with inflammatory bowel disease; 70% of patients with PSC inflammatory bowel disease, most often, ulcerative colitis. While there may be mild elevation of aminotransferases, the pattern of liver biochemistries in PSC is cholestatic, with elevated AP and GGT. However, a normal AP does not exclude the diagnosis.<sup>17</sup> A wide range of autoantibodies are common in patients with PSC, most at low prevalence rates and at relatively low titers.<sup>17</sup> Perinuclear antineutrophil cytoplasmic antibodies (pANCA) is present in up to 80%, ANA is present in up to 50%, and anti-SMA is present in approximately 15% of patients. These various autoantibodies are not specific for PSC and have no routine role in the diagnosis of PSC.

The “gold standard” for the diagnosis of PSC is the pattern of abnormality observed with endoscopic retrograde cholangiography (ERC). However, magnetic resonance cholangiography (MRC) is non-invasive, avoids radiation exposure, and has a sensitivity and specificity of 80% and 87%, respectively, for the diagnosis of PSC.<sup>18,19</sup> As such MRC has become of the diagnostic imaging modality of first choice when PSC is suspected.<sup>17</sup> The characteristic imaging appearance of PSC is stricturing and dilatation of the bile ducts, giving them a “beaded” appearance. As discussed below however, by definition, the diagnosis of PSC requires exclusion of secondary causes of sclerosing cholangitis, as these conditions may mimic PSC’s characteristic cholangiographic features.

While PSC typically affects both large (extra-hepatic and intra-hepatic) and small bile ducts, in 15% of cases there is involvement only of the small ducts (diameter < 100 µm), which includes interlobular bile ducts. The affected ducts are too small to be visualized on MRC or ERC. While there is lack of consensus, by most definitions, so-called “small-duct PSC” has identical clinical, liver chemistry abnormalities, and hepatic histology as typical PSC but is characterized by a normal MRC and ERC.<sup>20-22</sup> Some other definitions additionally require the presence of ulcerative colitis. In comparison to typical PSC, small-duct PSC is less rapidly progressive and has a significantly better long-term prognosis than typical PSC, and is not associated with an increased risk of cholangiocarcinoma. However, up to 20% of cases of small-duct PSC reportedly progress to involve the large ducts; in these cases, the disease course, prognosis, and cholangiocarcinoma risk assume that of typical PSC.

In PSC, the histologic findings of the large bile ducts are characteristic, with a sparse mononuclear inflammatory infiltrate, epithelial atrophy and progressive concentric periductal fibrosis (“onion-skinning”) with eventual obliterative sclerosis of the duct.<sup>7,23</sup> A rounded scar may mark the site of the destroyed duct. However, these large bile ducts rarely are sampled on the needle core biopsy. Furthermore, while this pattern of fibrosis is characteristic of PSC, it is not specific for it, and can be seen with some of the secondary etiologies of sclerosing cholangitis.<sup>7</sup> The hepatic parenchyma that typically is biopsied demonstrates features of various stages of bile duct injury, some with including vacuolar degeneration of the bile duct epithelium with irregular spacing of the nuclei (bile duct epithelial disarray), others with bile ductular proliferation, and additional portal tracts in which the interlobular bile duct appears to have vanished without a trace. Typically, only a sparse to mild portal inflammatory infiltrate is present, and interface activity is common but generally is mild. The inflammatory infiltrate is composed of mononuclear cells admixed with scattered eosinophils. With progressive disease, histologic features of chronic cholestasis are common. With ongoing injury, there is progressive portal-based fibrosis and eventual cirrhosis.

Of note, in PSC the distribution of the disease process within the liver may be patchy, and early in the disease, the small intrahepatic bile ducts sampled on the liver biopsy may not show the characteristic changes.<sup>7</sup> This significant sampling effect limits the utility of the liver biopsy in establishing the diagnosis and staging the disease.<sup>24</sup> Additionally, this significant sample variability may limit the value of serial liver biopsies for assessment of disease progression.<sup>24</sup>

<b>Table 2: Secondary sclerosing cholangitis etiologies</b>
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The staging scheme for PSC is similar to that for PBC, and it classifies the histologic features into four stages, as follows<sup>25</sup>: Stage 1: Portal (histologic changes are confined to the portal tract with bile duct injury, portal inflammation, and minimal portal fibrosis); Stage 2: Periportal (histologic changes extend to the periportal area with bile ductular proliferation, additional inflammation and periportal fibrosis ); Stage 3: Septal (portal-to-portal bridging fibrosis); Stage 4: Cirrhosis. Of note, this staging is based on the histopathologic features on both the H&E and trichrome stained tissue sections.

Chronic obstructive gallstone disease
Infections (immune status)
Immunoglobulin G4-associated cholangitis
Hepatic artery infusion chemotherapy
Ischemia
Biliary tract trauma
Sarcoidosis
Langerhans cell histiocytosis
Systemic mastocytosis

By definition, the diagnosis of PSC requires exclusion of other causes of sclerosing cholangitis.<sup>17</sup> So-called secondary sclerosing cholangitis (SSC) designates an assorted and diverse group of entities that cause biliary tract strictures similar to those in PSC, as seen on imaging.<sup>17,26</sup> Of note, some of these diseases also result in progressive ductopenia with associated clinical and histologic cholestasis. Many of the etiologies of SSC are suggested by the clinical history (e.g. history of hepatic artery infusion chemotherapy). Alternatively, for other etiologies (e.g. Langerhans cell histiocytosis), the liver biopsy findings may be useful in the identification of the causative disease.

The major criteria for the diagnosis of PSC are 1) compatible liver biochemical abnormalities; 2) characteristic ERCP findings; and 3) exclusion of other causes of sclerosing cholangiopathy.<sup>17</sup> As such, in the appropriate clinical setting and in the presence of an abnormal cholangiogram, a liver biopsy is not required to establish the diagnosis of large duct PSC, and current recommendations are for a liver biopsy not to be performed.<sup>17</sup> A liver biopsy, however, may help to exclude causes of secondary sclerosing cholangitis.<sup>27</sup> A liver biopsy, however, is considered essential for diagnosis in cases of suspected small duct PSC.<sup>17</sup> In this setting, the diagnosis is dependent on the pathologist’s evaluation of the liver biopsy for consistent histologic features, because by definition, the “gold standard” for diagnosis (i.e. the characteristic ERCP findings) is absent.

In PSC, the median time from diagnosis to death or liver transplantation is 8 years, however the severity of symptoms and rate of progression to cirrhosis are highly variable.<sup>7,17</sup> Cholangiocarcinoma develops in 10% of patients and can occur relatively early in the disease course and before the onset of cirrhosis. While UDCA treatment leads to improvement in the liver chemistries, it does not improve the histology, cholangiographic appearance, or survival, and currently, there is no effective therapy for PSC.

**Surgical Pathology report:** The informative surgical pathology report for liver biopsies in cases of possible PSC includes the following elements: 1) evaluation for consistent histology; 2) exclusion of other etiologies (secondary sclerosing cholangitis); 3) stage of the disease; 4) disease progression if sequential biopsies are available; and 5) concurrent liver diseases. As with any liver biopsy evaluation, the interpretation requires the availability of complete and accurate clinical history, laboratory studies, and radiologic studies and an adequate liver biopsy specimen.

### **Autoimmune overlap syndromes**

As described above, it is generally accepted that both PBC and PSC have, at least in part, an autoimmune basis. Together with autoimmune hepatitis (AIH), PBC and PSC form the category of autoimmune liver diseases. Usually, these entities can be readily differentiated on the basis of their characteristic clinical, biochemical, serologic, histopathologic, and in the case of PSC, radiographic features. Within this, a sizeable percentage of cases of autoimmune liver disease that generally fit into one diagnostic category will have an individual feature more characteristic of another type of autoimmune liver disease.<sup>7</sup> In cases with otherwise classic features for one disease, an isolated finding of another autoimmune liver disease typically does not deter from the diagnosis. However, when there is an admixture of multiple key features (biochemical, serologic, histopathologic, and / or radiographic) of the different autoimmune liver diseases, the diagnosis of a so-called “autoimmune overlap syndrome” should be considered.<sup>7,28,29</sup>

Autoimmune overlap syndromes are a heterogeneous group of disorders, and their identification is even more challenging because of the lack of standardization of the specific diagnostic criteria and terminology.<sup>28</sup> Furthermore, there is controversy as to whether these overlap syndromes are distinct disease entities or are simply variants of the major autoimmune hepatopathies.<sup>28</sup> Despite these challenges, the correct identification of a case as that of an autoimmune overlap syndrome has important therapeutic implications. While PSC-AIH overlap syndrome is well described, this discussion will focus on PBC-AIH overlap syndrome, because it is the most common of the autoimmune overlap syndromes.

In clinical practice, PBC and AIH usually can be readily differentiated by their distinguishing features.<sup>30</sup> However, approximately 10% of cases of PBC or AIH have multiple key characteristics (biochemical features, serologic profile, and / or histopathology) of the other disease entity.<sup>7</sup> Although investigators disagree over the exact classification, the general consensus is that these cases with multiple overlapping features qualify as PBC-AIH overlap syndrome.<sup>7,28</sup> Furthermore, while diagnostic criteria of PBC-AIH overlap syndrome varies among studies, one major study required that documentation of the presence of each disease (i.e. PBC and AIH) depended on the findings of key characteristic features in a least two of three categories: biochemical, serologic, or histopathologic (Table 3).<sup>31</sup>

While selection criteria vary, these different investigations have identified characteristic features of PBC-AIH overlap syndrome.<sup>30-34</sup> Notably, there is an overwhelming female predominance (approximately 90%), and the majority present in the 6<sup>th</sup> decade. Most, but not all patients are symptomatic at presentation, with fatigue and / or pruritis being the most common findings. The biochemistries typically are both hepatitic and cholestatic, with significant elevations of transaminases and as well as AP. In the majority of cases, AMA is positive, and greater than 50% of cases are positive for either ANA or anti-SMA, while anti-SLA is found in a minority of patients. Additionally, both IgG and IgM typically are elevated. While in the majority of cases of PBC-AIH overlap syndrome the key characteristics of the two diseases occur simultaneously, on occasion they may present sequentially, with time elapsed between

<p><b>Table 3: Proposed diagnostic criteria for PBC-AIH overlap syndrome</b></p>
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the two diagnoses ranging from 6 months to 13 years.<sup>35,36</sup> Histopathologically, PBC-AIH overlap syndrome is characterized by a lymphoplasmacytic inflammatory infiltrate with moderate to severe interface activity in the overwhelming majority of cases. Additionally, lymphocytic cholangitis and /or florid duct lesions are seen in the vast majority of cases. Ductopenia and non-necrotizing granulomas also may be present. Fibrosis typically is present, most commonly periportal or bridging.

Clinically, PBC-AIH overlap syndrome is a progressive disease and may develop into cirrhosis and liver failure if not treated.<sup>28</sup> While one study described no difference in the clinical behavior, including survival rate, between cases of PBC and PBC-AIH overlap syndrome, other studies demonstrated a significantly faster rate and degree of disease progression, including death or orthotopic liver transplantation due to cirrhosis and liver failure, in cases of PBC-AIH overlap syndrome in comparison to those of PBC alone.<sup>37,38</sup> The treatment of PBC-AIH overlap syndrome is controversial, specifically whether immunosuppressive treatment is required in addition to UDCA.<sup>28</sup> This is not a trivial decision; while UDCA has few side effects and is generally well-tolerated, corticosteroids are associated with multiple side effects including osteoporosis, a disease that PBC patients are already at a risk to develop.<sup>37</sup> The majority of studies have demonstrated significant improvement in biochemical and histologic response, including no further increase of fibrosis, in patients with PBC-AIH overlap syndrome when treated with UDCA and immunosuppression (corticosteroids with or without azathioprine) versus either UDCA alone.<sup>31,32,39</sup> As such, the AIH component of PBC-AIH overlap syndrome seems to respond to immunosuppressive therapy. While additional studies are needed and treatment should be individualized, the addition of immunosuppression to the regimen of UDCA appears to be the most effective therapeutic approach for patients with PBC-AIH overlap syndrome.<sup>39</sup>

**Surgical Pathology report:** While questions remain regarding PBC-AIH overlap syndrome, several practical points about the informative surgical pathology report are evident, as follows<sup>29</sup>: 1) when evaluating a liver biopsy for PBC or AIH, also evaluate for features of the other autoimmune liver disease. If the histopathologic features in conjunction with the clinical findings suggest PBC-AIH overlap syndrome, this possibility should be addressed in the surgical pathology report. However, since the diagnosis of PBC-AIH overlap syndrome is based on the admixture of multiple key features of the both PBC and AIH, an isolated histopathologic finding of one of the diseases in an otherwise classic case of the other, by itself, does not fulfill the diagnostic criteria of overlap syndrome. For example, the surgical pathologist should not overuse

<b>PBC: 2 of the 3 following criteria must be fulfilled</b>
1) AP levels > 2X ULN or GGT levels > 5X ULN
2) Positive for AMA
3) Liver biopsy demonstrates florid duct lesions
<b>AIH: 2 of the 3 following criteria must be fulfilled</b>
1) ALT levels > 5X ULN
2) IgG levels > 2X ULN or positive for anti-SMA
3) Liver biopsy demonstrates moderate or severe periportal or periseptal lymphocytic piecemeal necrosis
From Chazouilleres <i>et al</i> <sup>31</sup> Abbreviations: Upper limit of normal ULN; Primary biliary cirrhosis PBC; Autoimmune hepatitis AIH; Alkaline phosphatase (AP); gamma-glutamyltranspeptidase GGT; antimitochondrial antibodies AMA; alanine aminotransferase ALT; Serum immunoglobulin G IgG; smooth muscle antibodies SMA



the diagnosis of PBC-AIH overlap by applying it to an otherwise typical case (clinically, biochemically, serologically, and histopathologically) of PBC in which there is prominent interface activity<sup>7</sup>; and 2) if the morphologic features raise the possibility of PBC-AIH, it is important to include a discussion that states that the diagnosis of PBC-AIH overlap syndrome is not based on the histopathologic features alone, and correlation with the clinical, biochemical, and serologic features is necessary to establish this diagnosis. Of note, the multiplicity of features (clinical, biochemical, serologic, histopathologic) on which the diagnosis of PBC-AIH overlap syndrome is based underscores the importance of the complete clinical information and clinicopathologic correlation in the evaluation of the liver biopsy in cases of possible PBC-AIH overlap syndrome. In addition, as with any liver biopsy evaluation, the interpretation requires an adequate liver biopsy specimen.

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### **Part 3: Fatty Liver Disease**

Lisa Yerian, M.D.

#### **Case: See Images Provided**

Using the provided case as an example, I would like to apply the systematic approach to biopsy evaluation outlined earlier in this course.

#### **Specimen adequacy: Is this biopsy adequate?**

This biopsy measures 1.5 centimeters in total length; the liver parenchyma portion of the biopsy measures 0.4 centimeters. There is only one complete portal tract present. As discussed earlier, sample size is an important factor because it can affect accuracy of the histologic assessment. At least 6-8 complete portal tracts should be included for diagnosis, and for grading and staging of chronic hepatitis, the biopsy should be at least 2 – 2.5 centimeters in length with at least 11 complete portal tracts. According these criteria this present specimen is not adequate. In such cases my line diagnosis indicates the limited nature of the sample and this is explained further in the comment field. An example might read:

Diagnosis: Liver, needle biopsy – Limited sample with .... (insert findings observed).

Comment: The biopsy interpretation is limited due to small sample size and only one portal tract present for evaluation.

#### **Histochemical stains**

Although various institutions use differing medical liver biopsy staining protocols, a trichrome stain is considered essential for evaluation of fibrosis. Some pathologists prefer to examine the trichrome stain first in order to evaluate the hepatic architecture prior to assessment of the H&E. In this example a trichrome stain was performed and showed foci of periportal and perisinusoidal fibrosis. However, this finding must be interpreted with caution given the nature of the biopsy. The sampled portal tract appears to be near to a large fibrous structure and adipose tissue, suggesting it may be derived very near to the

hilum and/or the capsule. One should interpret any potential fibrosis with caution in subcapsular biopsies, as there may be accentuation of fibrous tissue in these areas. At this point, the presence, extent and location of fibrosis should be noted.

**Etiologic diagnosis: Identification of morphologic pattern of injury**

A useful approach to medical liver biopsy diagnosis is to assign the findings to an overall morphologic pattern of injury. Each pattern is characterized by a combination of histologic findings. Once the pattern has been determined, key features can be considered to further move toward the best diagnosis. It is worth noting at this point that some liver diseases can present in various patterns. An example is autoimmune hepatitis, which will be discussed further in the last section of this course.

The most prominent histologic feature of the present case is steatosis, and therefore I would place this biopsy in the fatty liver disease pattern of injury. There is insufficient lobular injury or “lobular disarray” to consider acute hepatitis, and both the portal infiltrates and portal fibrosis of chronic hepatitis are lacking. No biliary or cholestatic features are seen. Similarly, the remaining patterns are excluded.

The term “fatty liver disease” represents not one disease but rather a continuum of disease characterized by the presence of steatosis, at least at some point during their course. This feature may be less evident with progression to cirrhosis. The term “nonalcoholic fatty liver disease” (NAFLD) refers to this disease continuum occurring in the absence of significant alcohol use. Of course, this raises the question of how much alcohol use is significant. Various alcohol limits have been applied for research purposes, but amounts used for a large NIH-funded NAFLD database (the NASH Clinical Research Network, or NASH CRN) are 20grams per day for men and 10 grams per day for women. A European Association for the Study of Liver Disease (EASL) position statement published in 2009 uses slightly higher cutoffs: 30grams/day for men and 20grams/day for women [1]. As a point of reference, there are approximately 10 grams of alcohol in one 12 ounce beer or glass of wine.

The histologic similarities between alcoholic and nonalcoholic are reflected in the naming of nonalcoholic steatohepatitis, or “NASH” [2]. Both lesions are characterized by steatosis, inflammation, and a common pattern of fibrosis. Both diseases are progressive, having the propensity to cause progressive scarring, cirrhosis, and the complications of end-stage liver disease. Due to the histologic similarities alcoholic hepatitis cannot be reliably excluded in a given patient based on biopsy alone – this distinction is made clinically. That said, there are some notable differences between NASH and alcoholic liver disease. For example, steatosis is an important feature of NASH but is not always seen in alcoholic hepatitis, especially in patients with severe acute alcoholic hepatitis. Furthermore, some lesions seen in alcoholic hepatitis are not seen in NASH including canalicular cholestasis, bile ductular proliferation, veno-occlusive lesions, sclerosing hyaline necrosis, and alcoholic foamy degeneration (pure microvesicular steatosis).

Whereas alcoholic liver disease is caused by alcohol, NAFLD and is attributed to metabolic factors that are associated with increased risk of cardiovascular disease and

death known as the metabolic syndrome. These factors include abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance, and prothrombotic or proinflammatory states [3,4]. The liver disease associated with these factors, NAFLD, is considered the hepatic manifestation of the metabolic syndrome. Beyond these metabolic factors and alcohol, however, there are numerous other causes of fatty change involving the liver.

The three main forms of NAFLD (steatosis, steatohepatitis and cirrhosis) are distinguished based on their histologic features and prognoses. Steatosis is the most common form of NAFLD and carries the lowest risk of progressive disease. Steatosis without other features of hepatic injury or fibrosis is often referred to as “simple steatosis” or “benign steatosis” to convey the nonprogressive nature of the condition. However, the natural history for steatosis alone remains unclear. The histologic finding of steatosis alone is associated with cardiovascular disease, stroke, hypertension and diabetes. Steatohepatitis is more worrisome because it is associated with progressive fibrosis and, in some patients, cirrhosis and complications of end-stage liver disease. It is estimated that 28-32% of patients with NASH will progress within 5-10 years.

Matteoni et al. showed an increased risk of cirrhosis for patients with steatosis and other histologic features (lobular inflammation, ballooning, and Mallory hyaline or fibrosis) as compared to those with only steatosis [5]. Based on this and other studies, the relatively stable nature of steatosis and greater propensity for steatohepatitis to progress to significant fibrosis or cirrhosis has been confirmed, and cases bearing the histologic features that were associated with greater risk of cirrhosis (steatosis, inflammation, hepatocellular ballooning, and often, fibrosis) are now accepted as steatohepatitis [5-7]. A distinct histologic picture may be seen in patients with cirrhosis and in children [8-10].

The histologic features of steatohepatitis have been outlined [11-12]. *Steatosis* is necessary for a diagnosis of steatosis or steatohepatitis. It should affect at least 5% of the biopsy (rare steatotic cells not sufficient). A zone 3 accentuation is often found in adults, but the steatosis may also be uniformly distributed throughout the biopsy (panacinar) or patchy and variable in its location (azonal). The steatosis is exclusively or predominantly macrovesicular or mixed. Microvesicular steatosis may be present as scattered cells or in contiguous patches. Steatosis itself is a fairly reliable and reproducible feature when classified as none (<5%), mild (5-33%), moderate (34-66%) or severe (>66%).

*Inflammation* is the second feature of steatohepatitis in adults. The inflammation includes a mixture of neutrophils, lymphocytes, plasma cells and macrophages and may be lobular and/or portal. Lobular inflammation is usually more prominent in adults, whereas children may exhibit predominantly or exclusively portal inflammation. Portal inflammation also correlates with more severe disease in adults [13]. If portal inflammation exceeds the extent of lobular inflammation, the possibility of co-existing chronic hepatitis or other liver disease should be considered, particularly when interface activity is seen. However, it is worth noting that autoantibodies (ANA, SMA, AMA) are identified in 2-40% of individuals with NAFLD, and portal inflammation does not correlate with this finding nor with serum ALT. When portal inflammation and

autoantibodies are both present, one should look for interface activity, florid duct lesions, and markers of other chronic liver diseases.

The third and perhaps most challenging feature of steatohepatitis is *hepatocyte injury*, usually manifest as *ballooning hepatocyte degeneration*. Ballooned hepatocytes are those that stand out from the background hepatocytes based on their large size and pale, irregular wispy or clumpy cytoplasm. The affected cells tend to be most prominent in zone 3 where they are associated with perisinusoidal fibrosis and steatotic hepatocytes. Although an important feature of adult NASH, ballooning hepatocyte degeneration may not be seen in pediatric NASH. Ballooned hepatocytes have been found to contain multiple small lipid droplets, megamitochondria, dilated endoplasmic reticulum, Mallory bodies, and damaged cytoskeletal elements. Immunohistochemical staining for cytokeratins 8 and 18 may aid identification, as the normal cytoplasmic expression of these keratins is reduced or lost in ballooned hepatocytes [14].

Based on these features, a diagnosis of steatosis or steatohepatitis can be established. Accordingly, an etiologic diagnosis might read:

Liver, needle biopsy – Limited sample with mild steatosis.

Or, in another case with features of steatohepatitis (steatosis, inflammation, ballooning hepatocyte degeneration), the diagnosis might read:

Liver, needle biopsy – Steatohepatitis with XX fibrosis.

### **Etiologic diagnosis: Correlation with clinical features**

At this point the clinical history is reviewed. Key factors I like to have include the patient's age and gender, reason for the biopsy, body mass index (weight in kilograms divided by the height in meters squared), liver function tests, platelets (if available), and any serologic test results. If a transjugular biopsy was obtained I like to view the pressure measurements to assess for portal hypertension. Once these data have been reviewed, greater detail might be added to the diagnosis. For example, a diagnosis might read:

Liver, needle biopsy – Limited sample with mild steatosis, with clinical features of nonalcoholic fatty liver disease.

Or, in another case with features of steatohepatitis (steatosis, inflammation, ballooning hepatocyte degeneration), the diagnosis might read:

Liver, needle biopsy – Steatohepatitis with bridging fibrosis, clinically nonalcoholic steatohepatitis.

### **Features of prognostic and therapeutic importance**

The reporting of NAFLD is similar to that done for other chronic liver diseases insofar as grade (ongoing inflammatory activity and injury) is reported separately from stage (extent of fibrosis). The initial proposal for grading and staging included a global assessment of severity reported as mild, moderate or severe and stage 0-4 [15]. In 2005 the “NAFLD Activity Score” (NAS, see Table) was published as a system to assess and follow changes in the histologic features of NAFLD [16]. This score was developed by the NASH Clinical Research Network, a NIH initiated multidisciplinary cooperative effort, and was intended for use in research trials to standardize reporting and assess changes in component features that might occur as a treatment response. The NAS represents the unweighted sum of steatosis, lobular inflammation, and hepatocyte ballooning scores. The score is not intended to replace one’s overall histologic interpretation and in fact does not correlate perfectly with overall interpretation in a large recent series [17].

Other prognostic factors worth noting include iron accumulation, which has been variably associated with increased fibrosis in NAFLD or NASH when present in hepatocytes [18] or the reticuloendothelial system [19]. In addition to fibrosis risk, the diagnosis of steatohepatitis has also been associated with increased liver related mortality (liver fibrosis and cirrhosis, chronic liver disease and sequelae of chronic liver disease, liver cell carcinoma, and hepatic failure) [20]. Although there are ongoing clinical trials for the treatment of NASH, diet and exercise and in selected patients, bariatric surgery remain the main treatment modalities to date [21].

#### **NASH CRN NAFLD Activity Score**

<b>Activity Score (0-8) * overall pattern of NAFLD</b>	<b>Fibrosis Score (0-4) * using Masson’s trichrome</b>
Steatosis (0-3) 0: <5% 1: 5-33% 2: >33-<66% 3: >66%	1a: zone 3 perisinusoidal fibrosis requiring trichrome 1b: zone 3 perisinusoidal fibrosis on H&E 1c: Portal fibrosis only
Lobular inflammation (0-3) 1: <2 2: 2-4 3: >4 foci per 20x field	2: zone 3 + portal/periportal
Ballooning (0-2) 0: None 1: Few 2: Many/prominent	3: Bridging fibrosis
	4: Cirrhosis

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#### **Part 4: Chronic hepatitis**

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#### **Case: See Images Provided**

Again, using a case as an example, we will apply the systematic approach to biopsy evaluation outlined earlier in this course to a different morphologic pattern of injury

#### **Specimen adequacy: Is this biopsy adequate?**

This biopsy measures 1.0 centimeter in length and, unlike the prior case, consists entirely of liver parenchyma. There are 10 complete portal tracts present. Using the criteria presented earlier in this course, the biopsy is probably going to be adequate for diagnosis (at least 6-8 complete portal tracts) but slightly suboptimal for grading and staging of chronic hepatitis (ideally a biopsy used for grading and staging should be at least 2–2.5 centimeters with at least 11 complete portal tracts).

#### **Histochemical stains**

A trichrome stain highlights the difference in the two biopsy cores. First, let us focus on the lower, more blue core. Although at first glance this core may appear to be severely fibrotic due to the diffuse blue color, closer examination reveals that most of the core does not exhibit the intense blue collagen fibers but rather a pale gray blue amorphous background in which small residual nuclei and bile ductular structures are set. Reference

back to the H&E reminds us that this core exhibits extensive confluent necrosis. In the setting of extensive necrosis, collapse of the reticulin framework can result in areas of pale, gray blue condensed reticulin fibers evident on the trichrome stain. Comparison of the staining quality of these areas to portal tracts and good control stains usually allows for distinction from fibrosis based on the appearance and staining quality.

In this case the less necrotic core does exhibit fibrous expansion of the portal tract with extension of fibrous septa beyond the tract (“periportal fibrosis”). In difficult cases it may be useful to determine whether the liver is or is not cirrhotic. I have found that this distinction often carries more clinical significance than subtle distinction of lesser degrees of fibrosis. In this case the comment I would make would be:

Comment: The trichrome stain highlights areas of parenchymal collapse and also demonstrates periportal fibrosis. There is no evidence of cirrhosis.

**Etiologic diagnosis: Identification of morphologic pattern of injury**

I would describe the pattern of injury in this case as a markedly active hepatitis with bridging necrosis and plasma cells. There is periportal fibrosis but no cirrhosis. At this point my differential diagnosis would include two forms of inflammatory liver disease – either severe chronic hepatitis or acute hepatitis, massive necrosis (based on the confluent necrosis), and increased copper/Wilson’s disease (good to consider because easy to miss).

The histologic pattern of *acute hepatitis* is characterized by mixed lobular inflammation with hepatocyte swelling and injury. These factors cause disruption of hepatocyte cords, resulting in a histologic picture termed “lobular disarray.” Acidophil bodies may be prominent. There is no fibrosis, although reticulin collapse may cause pale blue staining with trichrome stains, which can be misinterpreted as scarring. Acute hepatitis A virus infection is the prototype “acute hepatitis” pattern of injury, although the diagnosis is based on serologic testing and biopsy is of little use in this disease. Portal inflammation may also present and may cause one to consider a diagnosis of chronic hepatitis. Usually the lobular inflammation and injury exceeds the portal inflammation, allowing the observer to accurately characterize the biopsy as an acute hepatitis.

In contrast to the lobular-based injury in acute hepatitis, chronic hepatitis is characterized by predominantly portal-based inflammation and portal fibrosis. At low-power, the most prominent feature is usually the dense, blue portal inflammatory cell infiltrates, whereas the lobules are generally relatively well-preserved. The inflamed portal tracts commonly exhibit foci of periportal interface activity. Formerly known as piecemeal necrosis, this finding consists of inflammatory cells extending beyond the limiting plate, surrounding and injuring individual hepatocytes. This is a feature we will come back to later when we discuss grading chronic hepatitis.

Massive/submassive necrosis could also reasonably enter the differential diagnosis, particularly based on the areas of confluent necrosis in one of the two cores, although the other core is relatively well-preserved. Increased copper stores (Wilson’s disease) could

also be considered simply because it can present with a diverse array of patterns and is easy to miss if we do not make an effort to think of it.

### **Etiologic diagnosis: Correlation with clinical features**

At this point, I think we have gotten about as far as we can with histology, and review of the clinical history may provide some additional help. The patient is a 47 year-old female who presented to the liver clinic with a two-month history of “hepatitis.” At this point chronic hepatitis remains on the differential diagnosis. Most causes of acute hepatitis would develop rapidly and begin resolving in this time frame, but it is possible that this is a protracted course. Massive/submassive necrosis seems unlikely because the patient is ambulatory and due to the protracted course. Wilson’s disease seems less likely due to the patient’s age, although patients presenting above this age have been reported.

Further review of the history indicates that the patient had initially presented with headache, malaise, and jaundice. Over the two months she was followed and had bilirubin as high as 13, ALT and AST in the 500-600 range, and alkaline phosphatase in the 300-400 range. Over the 2 months her LFTs gradually declined but never normalized. The patient reported a 14 pound weight loss. She reported no surgical or medical history, and no history of autoimmune disease. She was taking calcium and a multivitamin but no other medications. On physical exam she was of normal weight and mildly icteric. Additional laboratory tests were performed: bilirubin 3.7; AST 303; ALT 65; alkaline phosphatase 313. The patient was hepatitis A antibody positive; hepatitis B surface antibody positive; and hepatitis C antibody negative. Serologies were ANA negative and SMA positive. Her alpha-1 antitrypsin phenotype was MM. Ceruloplasmin was 38 (nl 21-45).

Now let’s reconsider our differential diagnosis. Given the hepatitis A antibody positivity, it is worth looking at acute hepatitis again. Hepatitis A virus infection is the prototype acute hepatitis pattern of injury – lobular inflammation and injury. There may be portal inflammation, but it usually pales in comparison to the lobular injury. There is no fibrosis, although one may see reticulin collapse as mentioned earlier. The diagnosis is based on serologic testing. We would expect to see transaminase levels >1000 in patients with acute hepatitis A virus infection. Treatment is supportive, and most patients resolve the infection on their own; a small percentage develop a relapsing pattern of disease. Affected patients are generally not biopsied because histology plays little role in the diagnosis or management of infected patients. This is true for most causes of acute hepatitis, in fact, because the differential diagnosis is largely clinical: acute viral hepatitis (hepatotropic or nonhepatotropic viruses), drug-induced liver injury, and autoimmune hepatitis. Histology adds to inform the patient’s care in this setting. If autoimmune hepatitis is a consideration, we can confirm or exclude autoimmune hepatitis features, or assess for background chronic liver disease. Patients may also be biopsied if their course is atypical. In considering acute hepatitis A virus infection for our patient with positive hepatitis A virus antibodies, we note that this patient’s transaminases never exceeded 1000, that the patient has persistent abnormalities after 2 months, fibrosis is present on

the trichrome. These factors argue against acute hepatitis A and cause us to consider another cause.

Chronic hepatitis remains in our differential diagnosis. Chronic hepatitis refers to chronic necroinflammatory diseases in which hepatocytes are the main target of attack. Because chronic cholestatic conditions and metabolic diseases can look similar histologically we include them in the differential diagnosis, but strictly speaking they are not forms of chronic hepatitis. This definition also does not include all chronic liver diseases that show inflammation. It is also good to avoid the pitfall of calling all biopsies with portal inflammation “chronic hepatitis.” Acute and chronic hepatitis can be distinguished histologically by the lobular versus portal-predominant inflammation and injury, and the absence or presence of fibrosis.

The major differential diagnosis for chronic hepatitis includes chronic viral hepatitis (hepatitis C and hepatitis B), autoimmune hepatitis, and drug-induced hepatitis. You may notice that autoimmune hepatitis appeared on the acute hepatitis differential diagnosis as well. Metabolic diseases in the differential diagnosis include Wilson’s disease (which is on several differential diagnoses due to the diverse set of histologic pictures) and alpha-1 antitrypsin deficiency. Also to be considered are chronic biliary diseases that may show histologic overlap (primary biliary cirrhosis and primary sclerosing cholangitis), although they do not fit the definition of chronic hepatitis because hepatocytes are not the main target of attack.

For chronic viral hepatitis, the diagnosis is based on serologic testing and confirmatory PCR to exclude false positives (as can occur in patients with high gamma globulin levels including those with autoimmune hepatitis.) Biopsy is not used to establish diagnosis in these patients but rather to grade and stage the disease and assess for other forms of injury. Hepatitis C is the prototype chronic hepatitis pattern of injury and usually presents as a relatively mild form of chronic hepatitis. There are no pathognomonic features, but the diagnosis is nearly always known prior to biopsy. Acute infection is virtually always subclinical in immunocompetent individuals, but 90% of those infected develop chronic disease. In contrast, only 5-20% of individuals infected with hepatitis B develop chronic disease. As with hepatitis C diagnosis is based on serology and PCR. Ground glass hepatocytes represent actual viral replication and therefore are only seen in patients with chronic disease.

Autoimmune hepatitis is an ongoing necroinflammatory disease of unknown cause, presumably autoimmune. It is defined as an a priori chronic disease, but may present as acute or fulminant liver failure. Diagnosis is based on a combination of clinical features, autoantibodies, and histology. Rapid response to immunosuppression is typical. The typical clinical picture is a female with a history of autoimmune disease with autoantibodies (smooth muscle antibodies, antinuclear antibodies, and/or liver-kidney-microsomal antibodies (LKM-1)), and polyclonal hypergammaglobulinemia. Histologically, autoimmune hepatitis can present as a chronic hepatitis, acute hepatitis, or cholestatic hepatitis picture. I particularly like to consider autoimmune hepatitis for cases

in which I have difficulty deciding if it is an acute or chronic hepatitis due to the severe periportal and lobular inflammation and injury.

Characteristic features of autoimmune hepatitis include the classic “piecemeal necrosis” used to diagnosis a so-called “chronic active hepatitis” in the past. Prominent plasma cell infiltrates are common but not always seen and therefore not required for diagnosis. Perivenular inflammation and necrosis is a useful feature, as this is not typical of other forms of chronic hepatitis in the immunocompetent patient. Confluent hepatocyte necrosis and dropout similarly is helpful and can result in “bridging necrosis” between portal tracts and central veins. An unusual but striking picture is that of giant cell transformation. This feature mimics that seen in neonatal hepatitis and appears to reflect extensive hepatocyte injury. A differential diagnosis I commonly face is that of autoimmune hepatitis versus primary biliary cirrhosis. I think this is due to the common patient population (both occur in middle-aged females) and histologic picture of inflamed portal tracts. Both can also be serologic marker negative. In this setting I find the clinical picture (alkaline phosphatase versus transaminase elevations) combined with the histology (bile duct versus hepatocyte injury) to be the most useful clues. The other entities in the differential diagnosis (drug-induced hepatitis, Wilson’s disease, and alpha-1 antitrypsin deficiency) can all be excluded on clinical grounds. Drug-induced liver injury essentially always enters my differential diagnosis in evaluating acute and chronic medical liver disease. In most cases it remains a diagnosis of exclusion. The internet serves as a useful resource of specific medications and reported reactions. Wilson’s disease I also make an effort to consider because it can be easily missed due to the diverse spectrum of presentations. If in question, quantitative copper testing can be obtained. Alpha-1 antitrypsin deficiency can be excluded by protease inhibitor phenotype testing or with a PAS after diastase.

At this point autoimmune hepatitis appears to be the most likely etiology of this patient’s liver disease. Depending on your level of comfort with the diagnosis, the report may be worded as:

Diagnosis:

Liver, needle biopsy - Autoimmune hepatitis with severe activity and periportal fibrosis  
...

Or,

Liver, needle biopsy - Chronic hepatitis with severe activity and periportal fibrosis, with clinical and histologic features most consistent with autoimmune hepatitis.

### **Features of prognostic and therapeutic importance**

The reporting of chronic hepatitis serves to confirm or establish the diagnosis, assess inflammatory injury and fibrosis, evaluate concomitant disease processes, and assess therapeutic intervention. The inflammatory injury and fibrosis are assessed as the “grade”

and “stage,” respectively. Several semiquantitative systems for grading and staging chronic hepatitis are in use, including the Ishak, Knodell Histologic Activity Index, Metavir, and Batts and Ludwig. Each of these systems have its own strengths and purposes. The choice of which to use in your practice depends on your preference and local conventions. In my hospital the hepatologists and pathologists concurred on one system to use consistently – the Batts and Ludwig. Other systems are applied as needed only when an individual patient is on a specific research protocol requiring reporting in that system. In my experience that is the most frequently used system among surgical pathologists in the U.S. The key features assessed in determination of the grade in the Batts and Ludwig system are the extent of interface activity and lobular inflammation. For each biopsy a grade of 0-4 is assigned. Stage is based on extent of scarring, ranging again from 0 (no fibrosis), to 4 (cirrhosis). I like to put the grade and stage into my diagnostic line, and to indicate which system I am applying. So the diagnosis for a chronic hepatitis case would look like:

Liver, needle biopsy - Autoimmune hepatitis of severe activity with periportal fibrosis (Batts and Ludwig Grade 4 of 4, Stage 2 of 4).

Or ...

Liver, needle biopsy - Chronic hepatitis of severe activity with periportal fibrosis (Batts and Ludwig Grade 4 of 4, Stage 2 of 4), with clinical and histologic features most consistent with autoimmune hepatitis.

Or ...

Liver, needle biopsy - Chronic hepatitis of moderate activity with bridging fibrosis (Batts and Ludwig Grade 2 of 4, Stage 3 of 4), etiology not apparent histologically.

(In this setting I would include the following in my comment – “Histologic features are suggestive of autoimmune hepatitis, but other causes cannot be excluded histologically. Serologic testing for viral and autoimmune markers is recommended.”)

So, how do these data inform treatment? It depends on the disease. For chronic hepatitis B virus infection, severity of liver histology does not consistently correlate with progression to cirrhosis. Other viral factors, particularly high viral load, appear to be more important. In contrast, liver biopsy is helpful for making treatment decisions in hepatitis C. Extent of fibrosis at initial presentation is an important predictive factor for the development of cirrhosis.

### **Prognostic factors in chronic hepatitis**

We discussed NAFLD (nonalcoholic fatty liver disease) and the associated risk factors in the prior lecture. With risk factors for NAFLD affecting such a large proportion of our population, it is not surprising that NAFLD frequently co-exists with other liver diseases including chronic hepatitis. The overlapping picture that has been most studied is that of

FLD and hepatitis C virus (HCV) infection affecting the same patient. Hepatitis C virus appears to cause steatosis in two ways. In HCV genotype 3 the virus itself is steatogenic and steatosis is associated with viral load. For other HCV genotypes, infection is associated with insulin resistance and type 2 diabetes mellitus as compared to healthy controls and individuals with chronic hepatitis B virus infection. When present, both steatosis and metabolic aberrations are associated with accelerated fibrosis and poor treatment response. Finally, insulin resistance and steatosis may regress with viral eradication and even return with recurrence of infection, and steatosis appears to improve with improvement in metabolic parameters. For these reasons, I report the presence of steatosis and give some indication of amount because this information may indicate greater risk of disease progression and poorer treatment response. Such an association has not been found in the setting of chronic hepatitis B virus infection. A remaining question is whether one can (or should) diagnose NASH in the context of hepatitis C virus infection, and if so, how. There are no criteria for diagnosing this disease as a separate entity in the context of chronic viral hepatitis C. One potential source of guidance is the criteria applied in a recent paper by Bedossa et al. In this paper patients with hepatitis C were diagnosed as also having NASH if they exhibited steatosis, hepatocyte ballooning and perisinusoidal fibrosis.

Iron is another prognostic factor to be considered, particularly in patients with chronic hepatitis C. Chronic hepatitis C is associated with hepatic iron overload, and hepatic iron is associated with increased disease activity (worse liver histology) in patients with chronic hepatitis C. In these patients the iron appears to be a mixture of hepatocyte and reticuloendothelial cell deposition, and studies suggest that iron depletion may stabilize or improve liver histology and slow disease progression. For these reasons I report the presence, location and severity of iron in chronic hepatitis C biopsies. I get iron stains routinely (per local protocol) on medical liver biopsies performed in native livers.

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