EXAMINATIONS IN GASTROENTEROLOGY

Liver and Pancreas

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Outline

- Functional examination of the Liver
  - Basic liver “panel”
  - Other tests for liver diseases
- Functional examination the pancreas exocrine function

LIVER AND BILIARY SYSTEM

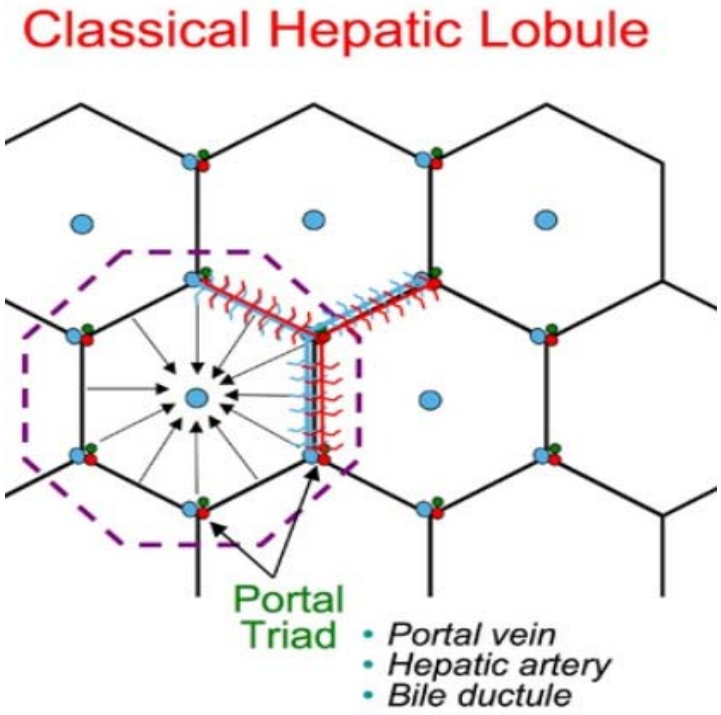
Liver Structure and Function

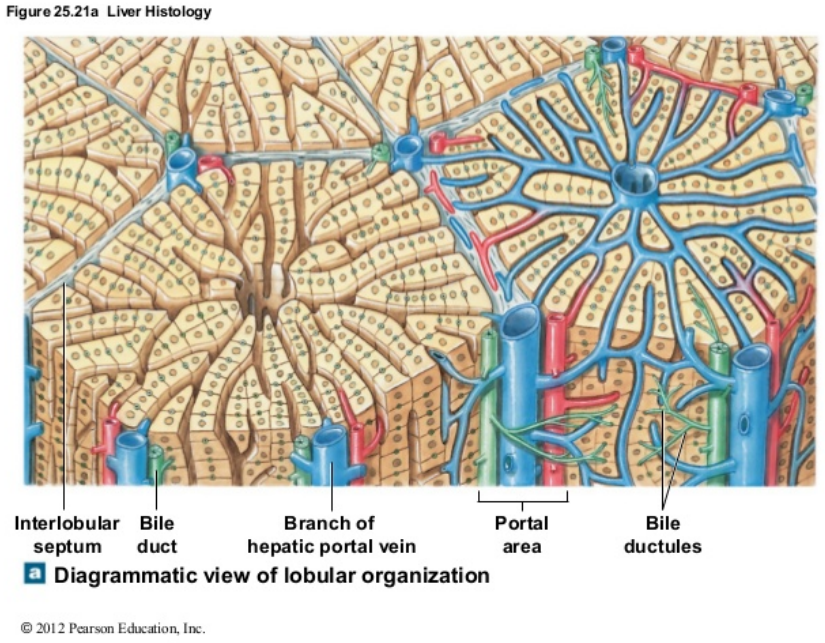
- 1–1.5 kg (1.5–2.5% of the lean body mass)
- Blood supply
  - hepatic artery - 20% oxygen-rich blood
  - portal vein - 80% nutrient-rich (toxin-rich) blood from the stomach, intestines, pancreas, and spleen

- Cell composition
  - hepatocytes (~60-70%)
  - Kupffer cells (reticuloendothelial system)
  - Stellate (Ito or fat-storing) cells
  - Endothelial cells and blood vessels
  - Bile ductular cells

Structural organization of liver tissue

- Lobules
  - portal areas at the periphery and central veins in the center of each lobule

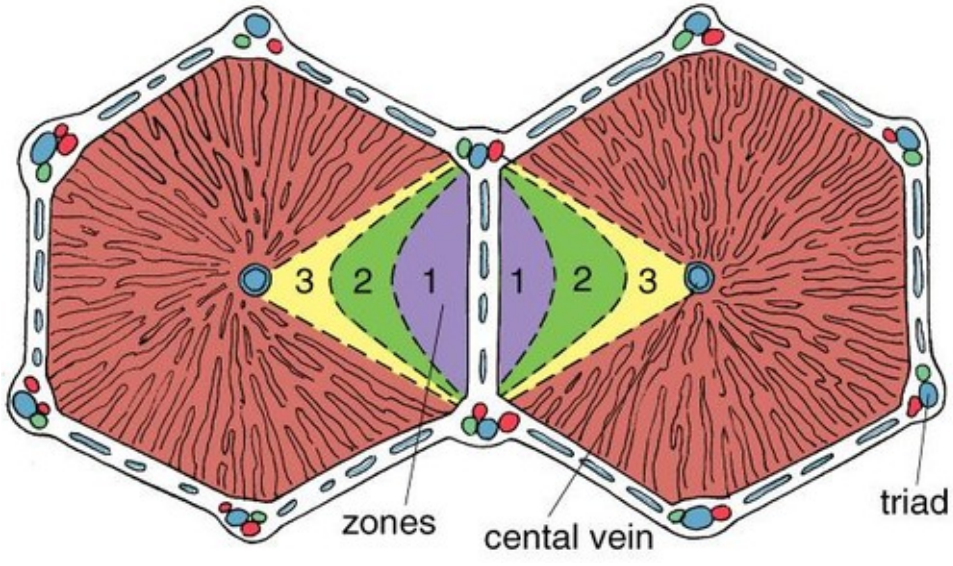


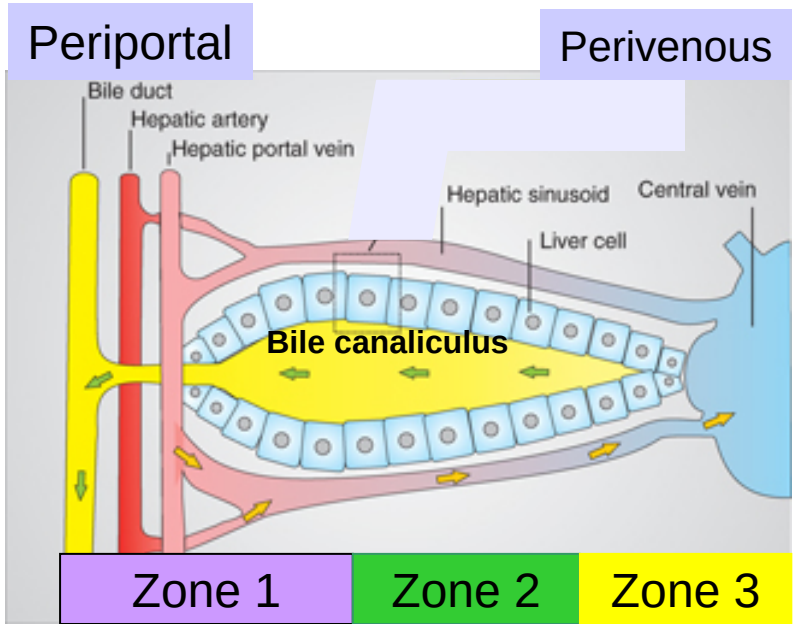


Functional organization of liver tissue

Acinus is a functional physiologic unit of the liver

- Acini
  - Hepatic arterial and portal venous blood entering the acinus from the portal areas (zone 1)
  - Intervening hepatocytes with sinusoids (zone 2)
  - Sinusoids entering the central hepatic vein (zone 3)





Liver Diseases

- Many causes of liver disease
- Present clinically in a few distinct patterns
  - Hepatocellular
    - liver injury, inflammation, and necrosis
    - e.g. viral hepatitis, alcoholic liver disease
  - Cholestatic (obstructive)
    - inhibition of bile flow
    - e.g. gallstone, malignant obstruction, primary biliary cirrhosis, some drug-induced liver diseases
  - Mixed
    - e.g. cholestatic forms of viral hepatitis and many drug-induced liver diseases

The most common causes of acute liver disease

- Viral hepatitis (particularly hepatitis A, B, and C)
- Drug-induced liver injury
- Cholangitis (infection caused by obstruction)
- Alcoholic liver disease

The most common causes of chronic liver disease

- Chronic hepatitis C
- Alcoholic liver disease
- Nonalcoholic steatohepatitis
- Chronic hepatitis B
- Autoimmune hepatitis
- Sclerosing cholangitis
- Primary biliary cirrhosis
- Hemochromatosis
- Wilson's disease

Laboratory Testing

- To distinguish hepatocellular versus cholestatic liver disease
- To decide whether the disease is acute or chronic
- To find whether cirrhosis and hepatic failure are present

Goal of evaluation of liver function

- detect the presence of liver disease
- distinguish among different types of liver disorders
- gauge the extent of known liver damage
- follow the response to treatment

BASIC LIVER PANEL

- Bilirubin (total and conjugated/direct)
- Aspartate aminotranspherase (AST)
- Alanine aminotranspherase (ALT)
- Alkaline phosphatase (ALP)
- Albumin
- Prothrombin time

Pathological results of liver tests (serum/plasma)

- **Hepatocyte damage**
  - **Increased** aminotransferases (AST, ALT), bilirubin
- **Cholestasis**
  - **Increased** bilirubin, alkaline phosphatase, cholesterol
- **Decrease** of amount of functioning hepatic **tissue** (cirrhosis, liver failure)
  - **Decreased synthesis** of proteins (e.g. albumin, clotting factors)
- Porto-systemic **shunts**
  - **Decreased uptake** of substances from blood (e.g. increased ammonia)

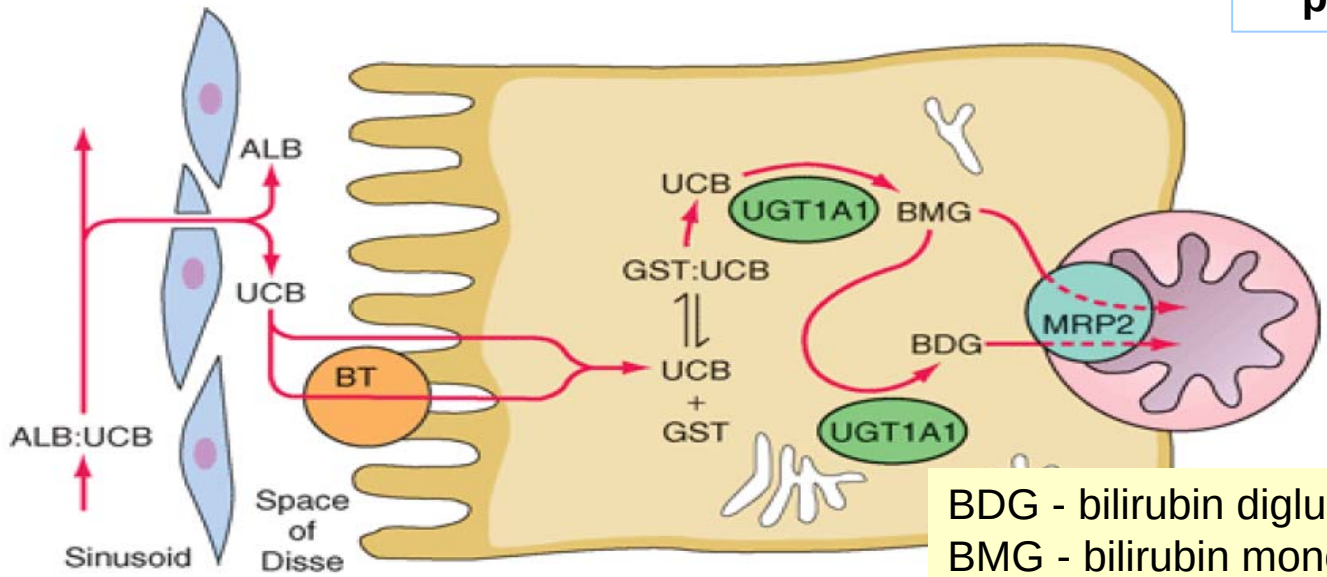
Liver Detoxification and Excretory Functions tests

- Serum Bilirubin
- Urine Bilirubin
- Blood Ammonia

Bilirubin

- Bilirubin, a breakdown product of the porphyrin ring of heme-containing proteins (70-90% from Hgb)

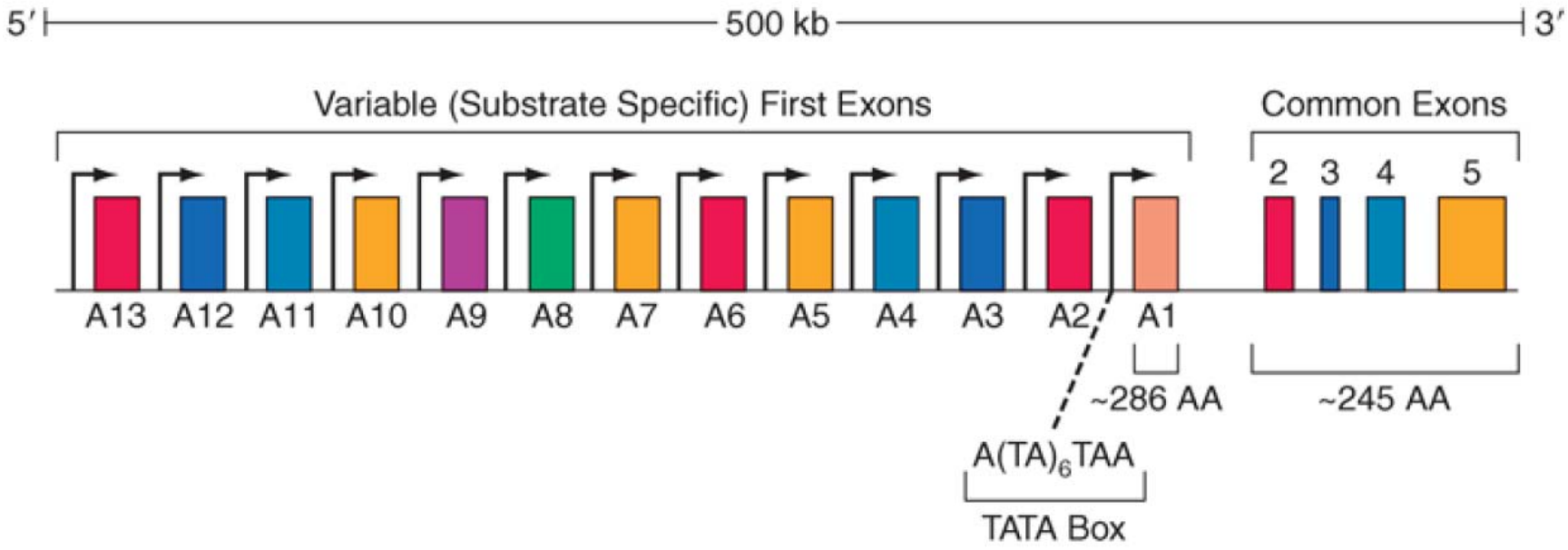
TRANSPORT			CONJUGATION	EXCRETION
Albumin-bound bilirubin	**facilitated and simple diffusion**	**bound to glutathione-S-transferases**	**bilirubin-UDP-glucuronosyl-transferase**	**multidrug resistance-associated protein 2**



BDG - bilirubin diglucuronide
BMG - bilirubin monoglucuronide

UGT1 family of UDP-glucuronosyltransferases

- Exon A1 (substrate specific) and the four common exons (2-5) encode the physiologically critical enzyme bilirubin-UDP-glucuronosyltransferase (UGT1A1)
- Mutation in one of the first exons will affect only a single enzyme isoform
- Mutation in exons 2–5 will alter all isoforms encoded by the *UGT1* gene complex



Serum bilirubin
(17-20 μmol/L, 0.3 - 1.9 mg/dL)

- Found in the blood in two fractions
  - unconjugated (indirect)
    - insoluble in water and is bound to albumin in the blood
    - The rate-limiting step in bilirubin metabolism is not conjugation of bilirubin, but rather the transport of conjugated bilirubin into the bile canaliculi
  - conjugated (direct)
    - water soluble and can be excreted by the kidney
    - if <15% of the total bilirubin is considered to all be unconjugated
    - conjugated hyperbilirubinemia almost always implies liver or biliary tract disease

Bilirubin in diseases
Causes of hyperbilirubinemia

- UNCONJUGATED HYPERBILIRUBINEMIA
  - Increased bilirubin production (e.g. hemolysis, inefective erythropoiesis)
  - Decreased hepatic uptake (e.g. Gilbert's syndrome)
  - Impaired conjugation (e.g. neonatal jaundice, Crigler-Najjar Syndrome)
- MIXED OR PREDOMINANTLY CONJUGATED HYPERBILIRUBINEMIA
  - Hepatic parenchymal disorders (e.g. acute hepatitis)
  - Obstructive disorders (e.g. bile duct stones)

Viral hepatitis - The higher the serum bilirubin, the greater the hepatocellular damage
Alcoholic hepatitis – Serum bilirubin level correlates with outcome

Urine Bilirubin

- Conjugated bilirubin (implies the presence of liver disease)
- Dipstick test give the same information as fractionation of the serum bilirubin (almost 100% accurate)
- In patients recovering from jaundice, the urine bilirubin clears prior to the serum bilirubin

Blood Ammonia

- Ammonia is produced in the body during normal protein metabolism and by intestinal bacteria (esp. colon)
  - The liver convert amonia to urea, which is excreted by the kidneys
  - Striated muscle also plays a role in detoxification of ammonia, which is combined with glutamic acid to form glutamine

- Poor correlation between the acute encephalopathy presence or severity and elevation of blood ammonia
- Poor correlation of the blood serum ammonia and hepatic function
- Useful for identifying occult liver disease in patients with mental status changes

Hepatocyte damage and cholestasis tests
Serum Liver Enzymes

- Enzymes that reflect damage to hepatocytes
- Enzymes that reflect cholestasis
- Enzyme tests that do not fit precisely into either pattern

Enzymes that reflect damage to hepatocytes

The aminotransferases (transaminases)

- aspartate aminotransferase (AST)
- alanine aminotransferase (ALT)
- Elevation ~ 100-fold of normal occur almost exclusively in disorders associated with extensive hepatocellular injury
  - viral hepatitis, ischemic liver injury, toxin- or drug-induced liver injury
- Elevation ~ 10-fold of normal are nonspecific (in any type of liver disorder)

Aspartate-aminotransferase (AST)

- 0.5-0.65 μkat/L
- Heart, muscles, brain, kidneys, pancreas, lungs, leukocytes, and ***liver***
- Severe elevation – acute viral or toxic hepatitis
- Elevation – myocardial infarction, heart failure, muscle injury, CNS diseases, and other non-hepatic diseases; ***hepatocellular damage***
- Reliable marker with good monitoring value in liver diseases (decline to normal values are observed during regeneration)

Alanine-aminotransferase (ALT)

- 0.55-0.65 μkat/L
- Primary in hepatocytes – more specific for liver diseases than AST

Ratio AST / ALT

- The pattern of the aminotransferase elevation can be helpful diagnostically
- Typically AST/ALT $\leq$ 1
- AST/ALT < 1
  - chronic viral hepatitis and non-alcoholic fatty liver disease
- AST/ALT > 1
  - cirrhosis
- AST/ALT >2 (>3)
  - alcoholic liver disease (alcohol-induced deficiency of pyridoxal phosphate - active B6 = cofactor for ALT)

Enzymes that Reflect Cholestasis

Elevated in cholestasis

- **Alkaline phosphatase**
- 5'-nucleotidase
- glutamyl transpeptidase (GGT)

Alkaline phosphates (ALP, AP)

- liver, bone, placenta, and intestine
- 2.3-2.7 μkat/L
- found in or near the bile canalicular membrane of hepatocytes
- Severe elevation
  - **cholestasis** from **intrahepatic** (prim. biliarny cirrhosis) and **extrahepatic** causes (bile duct obstruction)
- mild elevation
  - hepatocellular damage (hepatitis, cirrhosis)
- isolated elevation
  - granulomatous or focal liver lesions (abscess, tumor)
  - nonhepatic tumors (bronchogenic ca, Hodgkin's lymph.)
  - Bone diseases (metastases, osteomalatia)
  - Pregnancy

ALP

- **Elevations > 4-fold of normal**
  - cholestatic liver disorders
  - infiltrative liver diseases (cancer, amyloidosis)
  - bone conditions (e.g., Paget's disease).
- Nonspecific elevation
  - Patients over age 60 (1–1.5 times normal)
  - Individuals with blood types O and B can have an elevation of the serum alkaline phosphatase after eating a fatty meal (intestinal ALP)
  - Children and adolescents undergoing rapid bone growth (bone ALP)
  - Late in normal pregnancies (placental ALP)

- Less than 3-fold elevation can be seen in almost any type of liver disease

Glutamyl transpeptidase (GGT)

- To test whether alkaline phosphatase elevations are due to liver disease
- Rarely elevated in conditions other than liver disease
- Located in the endoplasmic reticulum and in bile duct epithelial cells
- Less specific for cholestasis than are elevations of ALP or 5'-nucleotidase
- Identify patients with occult alcohol use (nonspecific)

5'-nucleotidase

- To test whether alkaline phosphatase elevations are due to liver disease
- Found in or near the bile canalicular membrane of hepatocytes (as is ALP)

Tests that Measure Biosynthetic Function of the Liver

- Serum Albumin
- Serum Globulins
- Coagulation Factors
- The prothrombin time

Evaluation of the amount of functioning hepatic tissue (cirrhosis, liver failure)

Serum Albumin

- synthesized exclusively by hepatocytes
- long half-life
  - 18–20 days (4% degraded / day)
  - is not a good indicator of acute or mild hepatic dysfunction
- Hypoalbuminemia usually reflects severe liver damage and decreased albumin synthesis
- Is not specific for liver disease

Causes of hypoalbuminemia

- Chronic liver disease
- Protein malnutrition of any cause
- Protein-losing enteropathies
- Nephrotic syndrome
- Chronic infections (associated with prolonged increases in IL1 and/or TNF)
- Serum albumin should not be measured for screening without suspicion of liver disease
  - 12% of patients had abnormal test results (the finding was of clinical importance in only 0.4%)

Serum Globulins

- Serum globulins are a group of proteins made up of immunoglobulins produced by B lymphocytes and globulins produced primarily in hepatocytes.
- Globulins are increased in chronic liver disease
  - Increased synthesis of antibodies (gut antigen stimulation)
- Helpful in the recognition of certain chronic liver diseases
  - autoimmune hepatitis - Diffuse polyclonal increases in IgG (>100% increase)
  - primary biliary cirrhosis (IgM levels)
  - alcoholic liver disease (IgA levels)

Coagulation Factors

- Made exclusively in hepatocytes (exception F. VIII – endothelial cells)
- Serum half-lives ranging from 6 h for factor VII to 5 days for fibrinogen
- Best acute measure of hepatic synthetic function and helpful in both the diagnosis and assessing the prognosis of acute parenchymal liver disease

Prothrombin time

- May be elevated in
  - hepatitis
  - cirrhosis
  - disorders that lead to vitamin K deficiency
    - obstructive jaundice
    - fat malabsorption
- Marked prolongation of the prothrombin time (>5 s above control) and not corrected by parenteral vitamin K administration, is a poor prognostic sign in acute viral hepatitis and other acute and chronic liver diseases.

Shortcomings of liver tests

- Can be normal in patients with serious liver disease and abnormal in patients with diseases that do not affect the liver
- Rarely suggest a specific diagnosis
  - rather, they suggest a general category of liver disease (hepatocellular or cholestatic)
- Many tests do not measure liver function
  - they detect liver cell damage or interference with bile flow
  - e.g. aminotransferases or alkaline phosphatase

Other laboratory tests

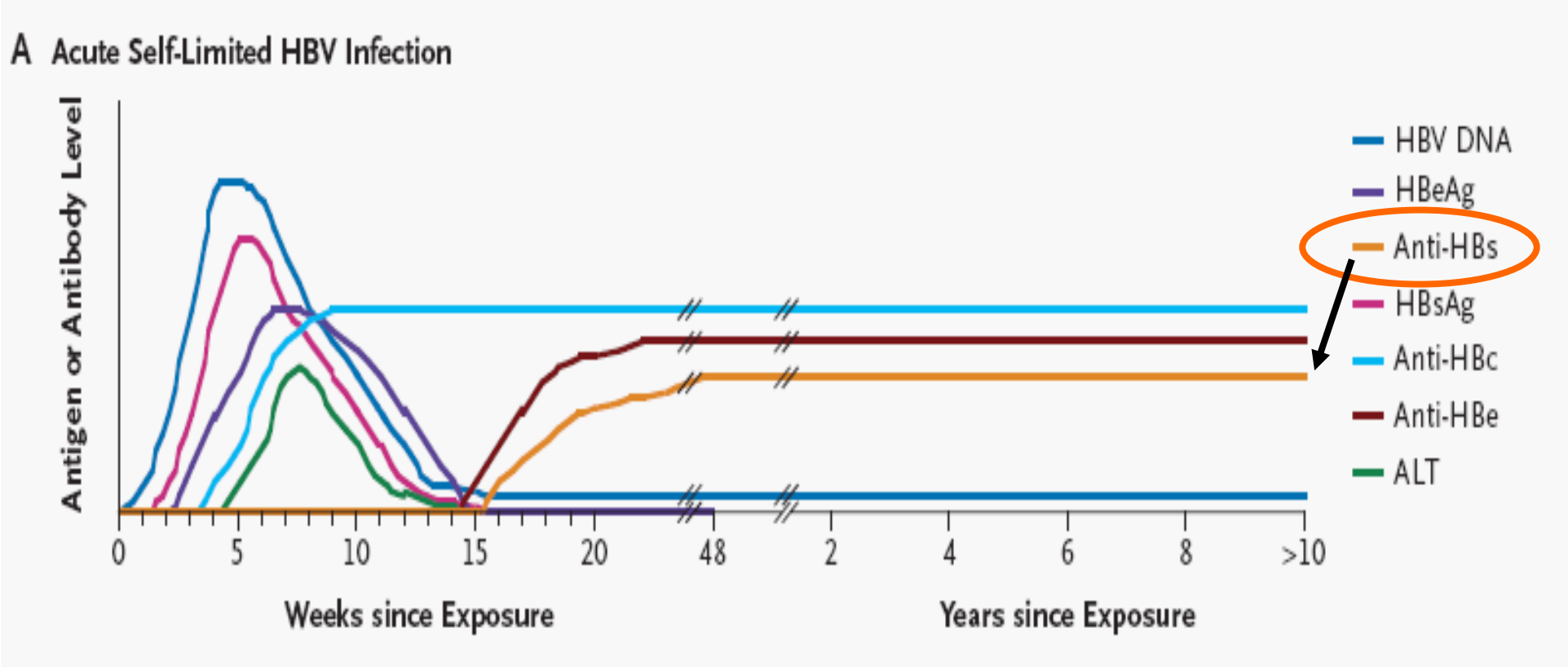
- Hepatitis serology to define the type of viral hepatitis
- Autoimmune markers

HBV infection tests

Viral antigens = Ag

- HB**s**Ag – **s**urface antigen of hepatitis B virus
  - positive 1–7 weeks before clinical manifestation of disease, during, and 1–6 weeks after and in chronic form
- HBeAg – marker of infectivity of hepatitis B
- Antigen specific antibodies (Ab):
  - antibodies IgM (acute) and IgG for hepatitis A virus
  - anti-HBs – to surface antigen
    - Positive after course of hepatitis type B and after immunization
  - anti-HBc - during acute phase of hepatitis B

Patterns of Serologic and Molecular Markers in HBV Infection



Prince AM, Lee D-H, Brotman B. Transfusion 2001;41: 329-32.

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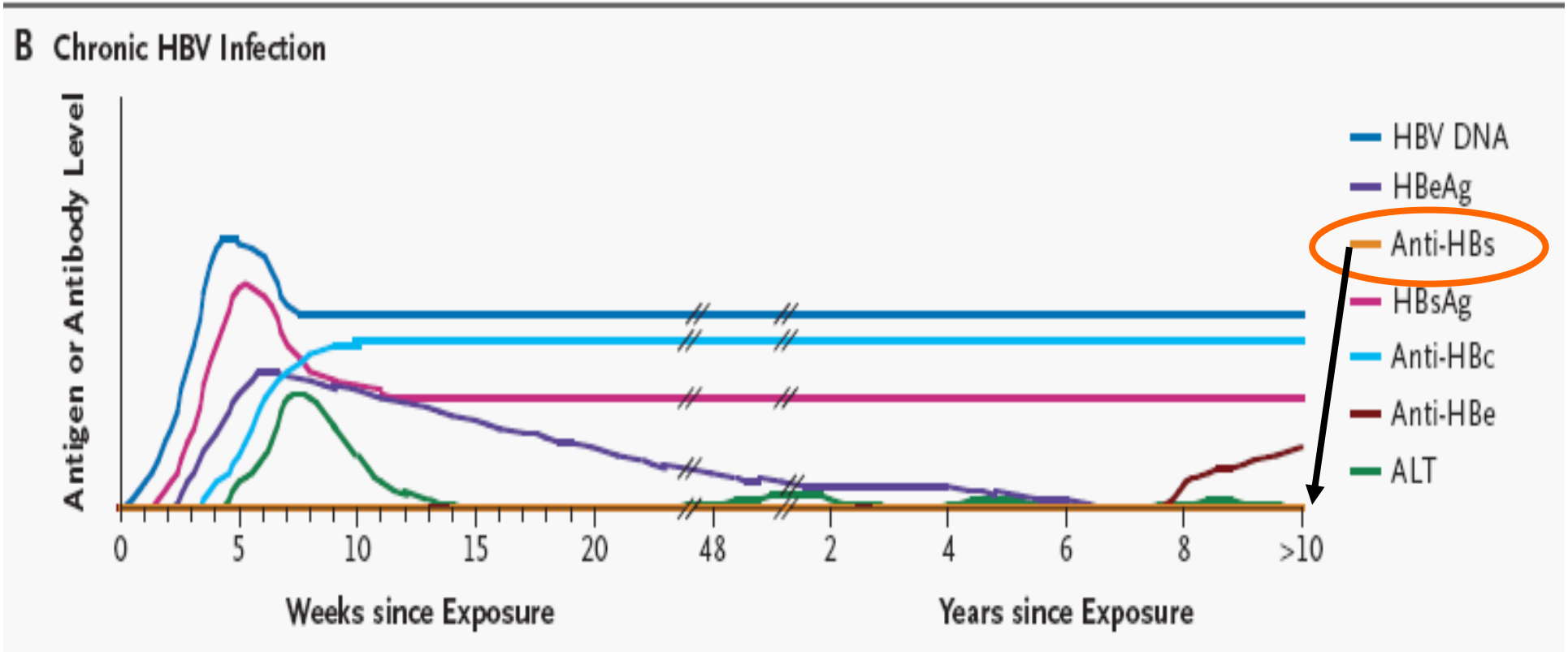

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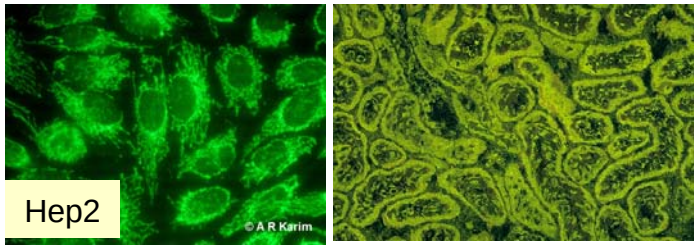
Autoimmune markers

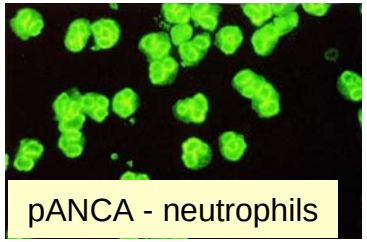
- Imunohistochemistry
- On human cells (neutrophils), cell lines (Hep2) or rodent tissues (liver, kidney, stomach)
- Detection of autoantibodies in patients serum

Autoimmune markers

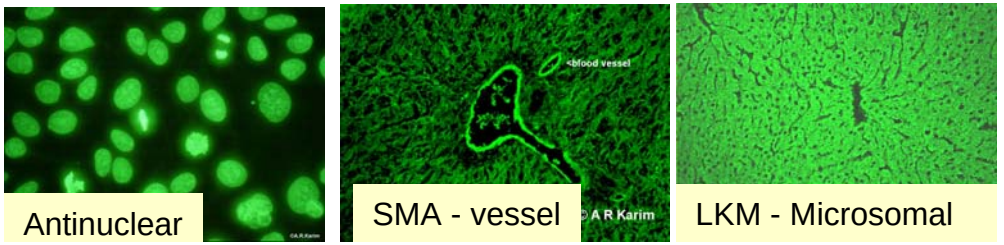
- Antimitochondrial antibody (AMA)
  - primary biliary cirrhosis
  - in 95% of patients
  - pyruvate dehydrogenase complex



- Peripheral antineutrophil cytoplasmic antibody (p-ANCA)
  - sclerosing cholangitis



- Antinuclear, smooth-muscle, and liver-kidney microsomal antibody
  - autoimmune hepatitis



pANCA	Elastase	Ulcerative colitis, Crohn's disease, primary sclerosing cholangitis, systemic lupus erythematosus
pANCA	Kathepsin G	Ulcerative colitis, primary sclerosing cholangitis, Crohn's disease
pANCA	Lysozym	Ulcerative colitis, primary sclerosing cholangitis, Crohn's disease
pANCA	Laktoferrin	Ulcerative colitis, primary sclerosing cholangitis, Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis
pANCA o. cANCA	BPI	Primary sclerosing cholangitis, ulcerative colitis, or pANCA Crohn's disease

Other Diagnostic Tests

- Percutaneous Liver Biopsy
- Ultrasonography

Percutaneous Liver Biopsy

- Performed at the bedside with local anesthesia and ultrasound guidance

Indications

- hepatocellular disease of uncertain cause
- prolonged hepatitis with the possibility of chronic active hepatitis
- unexplained hepatomegaly
- unexplained splenomegaly
- hepatic filling defects by radiologic imaging
- fever of unknown origin
- staging of malignant lymphoma.

- Most accurate in disorders causing diffuse changes

Liver biopsy

Acute liver disease (limited role)

- Drug-induced liver disease
- Establishing the diagnosis of acute alcoholic hepatitis

Chronic liver disease: important part of diagnosis of

- autoimmune hepatitis
- primary biliary cirrhosis
- nonalcoholic and alcoholic steatohepatitis
- Wilson's disease (with a quantitative hepatic copper level)

Ultrasonography

- The first diagnostic test to use in patients whose liver tests suggest cholestasis
  - presence of a dilated intrahepatic or extrahepatic biliary tree
  - identify gallstones
  - shows space-occupying lesions within the liver (cystic or solid masses)
  - Ultrasound with Doppler imaging can detect the patency of the portal vein, hepatic artery, and hepatic veins and determine the direction of blood flow.
    - The first test in patients suspected of having Budd-Chiari syndrome

Detecting alcoholism

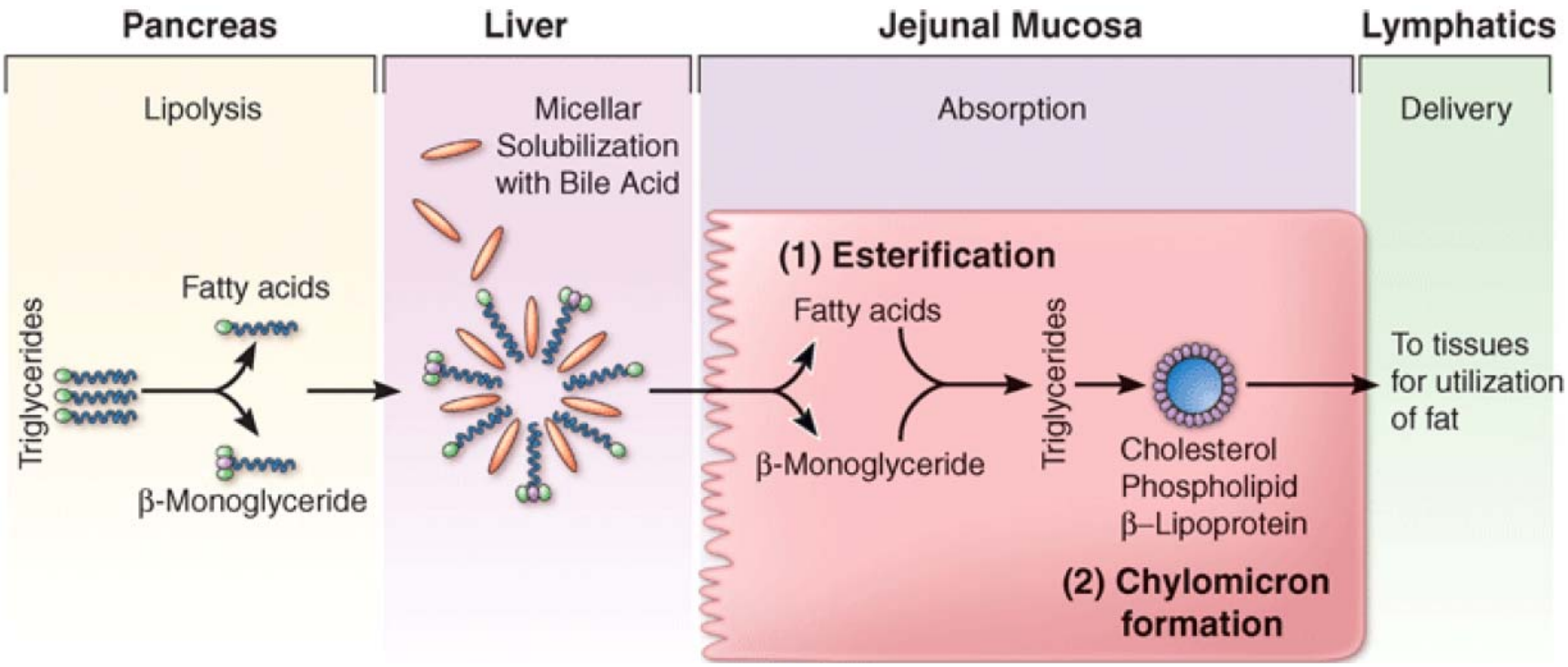
CAGE questionnaire

- The questionnaire asks the following questions:
  - Have you ever felt you needed to **C**ut down on your drinking?
  - Have people **A**nnoyed you by criticizing your drinking?
  - Have you ever felt **G**uilty about drinking?
  - Have you ever felt you needed a drink first thing in the morning (**E**ye-opener) to steady your nerves or to get rid of a hangover?

Ewing, John A. “Detecting Alcoholism: The CAGE Questionnaire” *JAMA* 252: 1905-1907, 1984

PANKREAS EXOCRINE FUNCTION

Steps in lipid digestion



Direct pancreatic stimulation or indirect stimulation by diet or test diet

- i.v. administration
  - secretin
  - secretin + Cholecystokinin (CCK)
  - ingestion of test meal

Direct pancreatic stimulation or indirect stimulation by diet or test diet

- Measurement of pancreatic secreation in duodenum (45 – 120 minut)
  - Volume (>2mL/kg/h)
  - Bicarbonate (>80 mmol/L and 10 mmol/h)
  - When S + CCK or diet
    - measurement of pancreatic enzyme activity
      - amylase, lipase, chymotrypsin, trypsin

Analysis of stool

- **Nutrient digestion**
  - standard amount of fat in diet for 72 h
  - expected digestion of more than 93% of ingested fat
  - more than 20% of ingested fat in stool = pancreatic insufficiency
- **Fecal pancreatic enzyme measurement**
  - trypsin and chymotrypsin

Pancreas function test: Bentiromide test

- Bentiromide bound on para-aminobenzoic acid (PABA) is administered orally
- Bentiromide is hydrolyzed by chymotrypsin in duodenum and free PABA is absorbed in proximal part of small intestine, conjugated in the liver and PABA metabolites are excreted in the urine
- The amount of PABA in the urine correlate with the activity of chymotrypsin
- The results may be influenced by intestinal mucosal defects, liver diseases, and kidney diseases.

Bentiromid was withdrawn in the US in October 1996

Pancreatic Enzymes In Body Fluids

- **Serum amylase**
  - pancreatic isoamylase (33% of total serum amylases)
  - screening for acute pancreatitis (pts with acute abdominal or back pain)
  - 20-40 % fals positive,
  - sensitivity 70-75%
  - values 3x of normal AP very likely
  - elevated within 24 h, up for 3-5 days
- **Serum lipase**
  - specificity is higher than amylase
  - sensitivity 70-85%

Measurement of fecal pancreatic enzymes of intraluminal digestion products

- Undigested
  - muscle fibers, stool fat, and fecal nitrogen
- Human elastase in stool
  - reflects the pancreatic output of this proteolyticenzyme.
  - To detect severe pancreatic exocrine insufficiency in patients with chronic pancreatitis and cystic fibrosis (solid stool specimen)

The End