# Chapter 3. Investigations

## 3.1 Liver Enzymes

- Liver enzymes are categorized as **Hepatocellular** or **Cholestatic**[1]
- **Hepatocellular liver tests** include the **alanine aminotransferase (ALT)** and **aspartate aminotransferase (AST)**, which are enzymes in hepatocytes that are released into the blood when the liver is inflamed or damaged
  - The ALT is more specific to hepatocytes, as the AST is also located in muscle and to a lesser extent in the kidney, brain, pancreas and red blood cells
  - The normal range for ALT and AST vary between laboratories, but it has be suggested that a ”true” normal ALT is approximately 30 (29-33) U/L for men and approximately 20 (19-25) U/L for women[2]
  - The **AST/ALT ratio** can be a clue to the etiology of the liver injury (alcohol) or a marker of the severity of the liver disease (cirrhosis)
    - In most liver diseases the ALT is higher than the AST
    - However, in alcohol related liver disease the AST is usually more than twice the ALT (AST/ALT >2)
    - In any other hepatocellular liver disease, where alcohol is excluded by history, if the AST is higher than the ALT (AST/ALT>1) it may indicate that the patient has progressed onto cirrhosis
  - **Lactate dehydrogenase (LDH)** is another hepatocellular enzyme, but it doesn’t add much to the measurement of the ALT or AST
    - As it is found in RBCs, it may be elevated in hemolysis, and it may also come from muscle, brain and kidney damage

    - It can be very high if the liver damage is due to ischemia or lack of blood flow (e.g. shock liver after cardiac arrest) or with malignant infiltration from lymphoma

- **Cholestatic liver tests** include the **alkaline phosphatase (ALP)** which comes mainly from liver or bones (the normal range is higher in children due to bone growth)
    - It can also be elevated in pregnancy, renal failure and ischemia of the bowel
    - ALP in the liver is synthesized by the canalicular membrane of the hepatocyte in response to bile duct obstruction and may take some time to rise in the blood
- **Gamma-glutamyltranspeptidase (GGT)** is neither a hepatocellular nor cholestatic enzyme (it can go up in both patterns) and in isolation is usually of little significance
    - It is a very sensitive enzyme that is easily induced by drinking alcohol, being overweight (fatty liver disease) or with certain medications (anticonvulsants or coumadin)
    - Its main use is to distinguish the source of an elevated ALP, as GGT is made in the liver, but not the bones

## 3.2 Liver Function Tests

- In addition to the "Liver Enzymes" (ALT, AST, ALP, GGT) we often describe bilirubin, albumin & INR as the "Liver Function Tests" as they provide more prognostic information about the function of the liver, rather than just whether or not the liver is inflamed (ALT, AST) or if there is a problem with bile flow (ALP, GGT)
- **Bilirubin** comes from the breakdown of hemoglobin from senescent red blood cells
    - It is measured by the lab as **direct (conjugated) bilirubin** or **total bilirubin**, which is a combination of direct and **indirect (unconjugated) bilirubin**

- ↑ bilirubin results in jaundice and may be pre-hepatic (before conjugation), hepatic (due to hepatocellular or cholestatic liver diseases) or post-hepatic (due to a blockage of extra-hepatic bile ducts) *[see Chapter 1.5]*

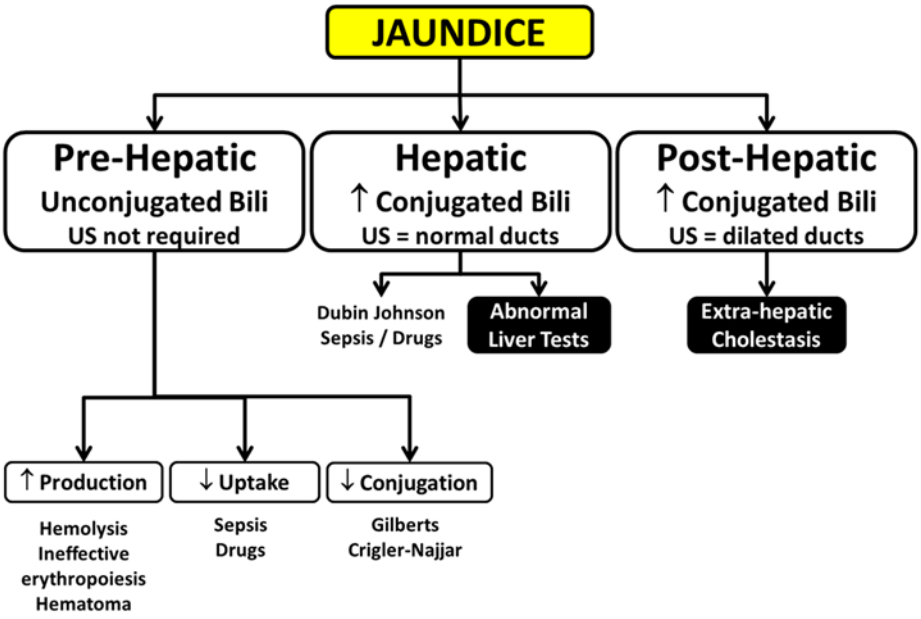
- **Albumin** is one of the main proteins manufactured by the liver and is important for transporting many things in the blood stream, including unconjugated bilirubin and drugs, while helping to maintain oncotic pressure in blood vessels
  - It has a half-life in circulation of approximately 20 days
  - ↓ albumin can be a sign of chronic liver disease but can also be seen with malnutrition or acute inflammation in the body (negative acute phase reactant)
- **Prothrombin time (PT)** or the **international normalized ratio (INR)** of PT is a measurement of clotting time
  - Factor I (fibrinogen), Factor V and the vitamin K dependent clotting factors (II, VII, IX, X) are all made in the liver
  - ↑ INR is very important for prognosis in both acute and chronic liver failure but can also be seen due to vitamin K deficiency (especially in patients with cholestatic liver disease as bile salts help us absorb fat soluble vitamins A, D, E, and K) and after prolonged use of antibiotics (as bacteria produce vitamin K in the colon)

## 3.3 Approach to Jaundice

- Jaundice is best appreciated in the sclera of the eye and usually clinically apparent when the bilirubin is >50 μmol/L
- The scheme below provides an approach to a patient with jaundice

- The first step is to examine the pattern of the bilirubin elevation as predominantly unconjugated (indirect) or predominantly conjugated (direct)
- If it is predominantly conjugated then an abdominal ultrasound (US) is needed to examine the bile ducts and determine if it is hepatic or post-hepatic jaundice




- **Pre-Hepatic jaundice** is associated with predominately **unconjugated (indirect) bilirubin** as the problem is before the conjugation of bilirubin in the hepatocyte by UGT and the other liver tests will be normal. It is due to:
  - Over-production of unconjugated bilirubin is due to hemolysis, ineffective erythropoiesis, or resorption of a large hematoma (blood clot)
  - Decreased uptake of bilirubin by transporters due to sepsis or drugs (this is rare as sepsis and drugs usually cause intrahepatic cholestasis and hepatic jaundice)
  - Abnormalities of the UGT conjugating enzymes

    - Gilbert's syndrome is seen in 5-10% of population and are due to common polymorphisms in the UGT enzymes that leads to jaundice during physiologic stress (infections) or periods of fasting
    - Rare deficiencies in UGT enzymes (Crigler-Najjar) can lead to more significant jaundice in children and kernicterus (unconjugated bilirubin induced brain dysfunction)

- If it is predominantly **conjugated bilirubin** this could either be due to a liver problem (**hepatic**) or a blockage of bile flow outside the liver (**post-hepatic**) and the next most important test is an **abdominal ultrasound** to see if the bile ducts are normal in size (hepatic jaundice) or dilated (post-hepatic jaundice)
- **Hepatic jaundice** is usually accompanied by abnormal liver tests, either hepatocellular or cholestatic *[see Abnormal Liver Tests Chapter 3.4]* and a workup for acute or chronic liver diseases should be initiated *[see Chapter 3.5]*
    - Rarely, elevated conjugated bilirubin can be seen without elevated liver tests, e.g. in Dubin Johnson syndrome (congenital) or with sepsis and some drugs
- **Post-Hepatic jaundice** is associated with dilated bile ducts and cholestatic liver test elevation *[see far right side of Abnormal Liver Tests scheme in Chapter 3.4]*
    - Magnetic Resonance Cholangio Pancreatography (MRCP) or endoscopic ultrasound (EUS) may help identify the cause of the bile duct obstruction (e.g. stricture, stones, tumours)
    - Endoscopic Resonance Cholangio Pancreatography (ERCP) can be used for both diagnosis and therapeutic interventions

## 3.4 Approach to Abnormal Liver Tests

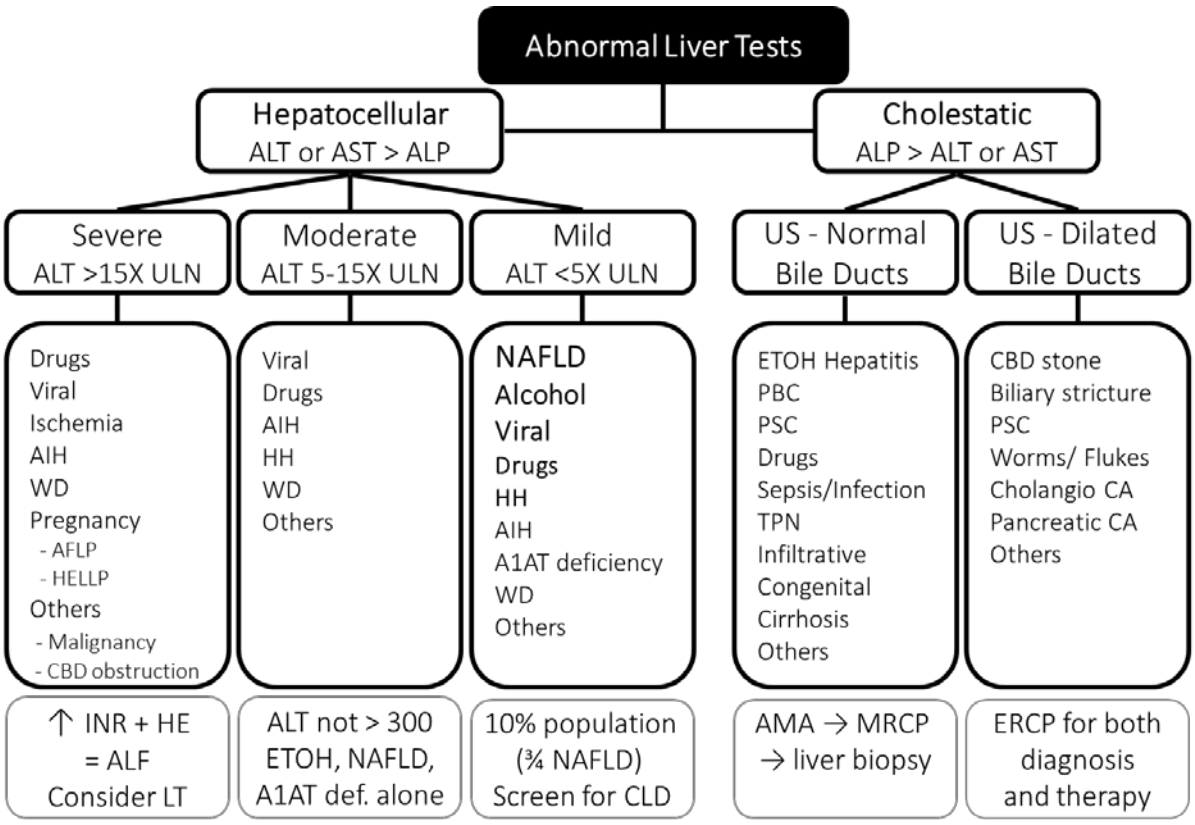
- Here is an approach to elevated liver tests based on the American Gastroenterology Association Position Paper[1] and the American College of Gastroenterology Guidelines[2]
- Tests are either predominantly **hepatocellular** or **cholestatic**, as described by the elevation of ALT or AST above the upper limit of normal (ULN) compared to the elevation of ALP above the ULN
- **If cholestatic**, the next most important test to order is an **abdominal ultrasound** (US) to examine the bile ducts
  - If bile ducts are dilated, it is **extra-hepatic cholestasis** due to intrinsic blockage (e.g. stone, stricture) or extrinsic compression of the bile duct (e.g. pancreatic cancer)
  - If the bile ducts are normal, the problem is in exporting bile out of the hepatocyte at the level of the bile duct canaliculus or intra-hepatic bile duct (**intrahepatic cholestasis**)
- The degree of the **hepatocellular** liver test elevation can help narrow the differential diagnosis
  - **Mild** elevation of ALT is very common and the differential diagnosis is wide
    - In North America, approximately 10% of the population may have an ALT or AST above the "traditional" upper limit of normal (ULN), although nearly one-third of adults will have an ALT greater than "true" ULN (~30U/L for men and ~20U/L for women) [2], with majority being due to non-alcoholic fatty liver disease (NAFLD)
  - **Moderate** elevation should not occur from alcohol, NAFLD or alpha-1 antitrypsin deficiency alone and it is uncommon for hereditary hemochromatosis to cause this level of elevation

- **Severe** elevation of ALT has a narrow differential diagnosis and when associated with significant liver dysfunction, with an elevated blood clotting times (INR) and hepatic encephalopathy (HE), is called acute liver failure (ALF) and these patients may need to be considered for liver transplant (LT)

**NOTE:** patients with chronic hepatitis can have an ALT and/or AST within the "traditional" normal range can still have significant inflammation within the liver (on biopsy)

**NOTE:** some cholestatic liver diseases also cause elevation of ALT (mixed picture), and in late stages of many hepatocellular diseases (cirrhosis) many patients become more cholestatic




Kelly W. Burak (modified 2017). References: AGA, Gastroenterology 2002; 123(4): 1367-84. ACG, Am J Gastroenterol 2017; 112: 18-35.

**Abbreviations:**

A1AT def = alpha 1 antitrypsin deficiency; AFLP = acute fatty liver of pregnancy; AIH = autoimmune hepatitis; ALF = acute liver failure; ALT = alanine aminotransferase; ALP = alkaline phosphatase; AMA = anti-mitochondrial antibody; AST = aspartate aminotransferase; CA = carcinoma; CBD = common bile duct; CLD = chronic liver disease; ETOH = alcohol; ERCP = endoscopic retrograde cholangio-pancreatography; HELLP = hemolysis elevated liver tests low platelets; HH = hereditary hemochromatosis; LT = liver transplant; MRI = magnetic resonance imaging; MRCP = magnetic resonance cholangio-pancreatography; NAFLD = non-alcoholic fatty liver disease; PBC = primary biliary cholangitis; popn = population; PSC = primary sclerosing cholangitis; TPN = total parenteral nutrition; ULN = upper limit of normal; US = ultrasound; WD = Wilsons disease.

### Summary

- So what do you do when you have a patient with abnormal liver tests?
  - If AST alone, confirm it is from the liver by doing ALT
  - If ALP alone, confirm it is from the liver by doing GGT
  - Recognize the pattern as hepatocellular or cholestatic by describing the ALT or AST and ALP in terms of upper limit of normal (ULN)
  - Perform a targeted history and physical examination
  - If liver tests are severely or are chronically elevated order specific labs tests to screen for acute or chronic liver disease *[see Chapter 3.5]* and obtain an ultrasound to look for bile duct dilatation, fatty liver infiltration or liver masses

## 3.5 Screening for Chronic Liver Disease

- There are various screening tests for the common chronic liver diseases, which should be done when investigating a patient with chronically abnormal liver tests or signs or symptoms of chronic liver disease

- The total costs of the screening tests are approximately $600 CDN, excluding the cost of the ultrasound
- One strategy is to order viral hepatitis serology first, and if this is negative, then order the other screening tests for hereditary and autoimmune liver diseases

- The screening tests, if positive, should prompt confirmatory tests
  - If viral serology is positive you must confirm the presence of the virus with polymerase chain reaction (PCR) testing
    - If HBsAg positive order HBV DNA to confirm viral replication *[Chapter 6.2]*
    - If anti-HCV positive order HCV RNA to confirm chronic infection *[Chapter 6.3]*
  - Similarly, if screening tests are positive for genetic liver diseases there are confirmatory tests to be ordered
    - Transferrin saturation >45%, but not elevated ferritin in isolation (*Choosing Wisely® Canada Recommendation*), should prompt HFE gene testing for hereditary hemochromatosis *[Chapter 8.1]*
    - If ceruloplasmin level is low then get a 24 hour urine copper measurement to rule out Wilson's Disease *[Chapter 8.2]*
    - If alpha-1 anti-trypsin level is low then phenotyping should be ordered *[Chapter 8.3]*
  - Autoantibodies can be elevated non-specifically but make you consider autoimmune diseases of the liver
    - If anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA) are positive consider autoimmune hepatitis (AIH); however, this requires a liver biopsy to establish the diagnosis *[Chapter 9.1]*

    - Anti-mitochondrial antibody (AMA) is diagnostic for primary biliary cholangitis (PBC) *[Chapter 5.2]*
  - Quantitative immunoglobulins (Ig) can give clues to the cause of the liver disease
    - ↑ IgA is seen with ETOH (remember "A is for alcohol") and can also be seen with non-alcoholic fatty liver disease (NAFLD)
    - ↑ IgG is seen with AIH
    - ↑ IgM is often seen with PBC
  - NAFLD is associated with obesity, diabetes mellitus (DM) and hyperlipidemia, therefore, it is important to get fasting glucose level (to screen for DM) and lipid profile which includes cholesterol, triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL)
- If all other tests are negative, you may consider ordering other tests to exclude thyroid disease and Celiac disease, which can sometimes present with abnormal liver tests
  - TSH (thyroid stimulating hormone) for hypo- or hyperthyroidism
  - anti-endomesial or tissue transglutaminase (TTG) antibodies as screen for Celiac disease

| Classification | Diagnosis | Screening Test | Confirmatory / Additional Tests |
|---|---|---|---|
| Viral | HBV | HBsAg | HBVDNA, HBeAg, anti-HBe |
| | HCV | Anti-HCV | HCVRNA, genotype |
| Toxin | Alcohol | History<br>*Note: AST>ALT, ↑↑ GGT, ↑IgA* | Biopsy if uncertain |
| Metabolic | NAFLD | None (obesity, DM, ↑ lipids)<br>*Note: check fasting glucose and lipids, rule out other diseases* | Biopsy if uncertain |
| Autoimmune | AIH | ANA, ASMA, ↑IgG (all non-specific) | Biopsy **required** for diagnosis |
| | PBC | AMA<br>*Note: ↑IgM* | AMA is diagnostic |
| | PSC | None<br>*Note: autoantibodies (ANCA)* | MRCP |
| Genetic | HH | Fe/TIBC (TS) >45%, ferritin >1000 | HFE gene testing (C282Y) |
| | A1AT def | A1AT level (low) | A1AT phenotype (ZZ) |
| | WD | Ceruloplasmin (low) | 24h urine copper, slit lamp (KF rings) |

**Abbreviations (diseases):**

- A1AT def = alpha 1 anti-trypsin deficiency; AIH = autoimmune hepatitis; DM = diabetes mellitus; HBV = hepatitis B virus; HCV = hepatitis C virus; HH = hereditary hemochromatosis; NAFLD = non-alcoholic fatty liver disease; PBC = primary biliary cholangitis; PSC = primary sclerosing cholangitis; WD = Wilson's disease

**Abbreviations (screening tests):**

- HBsAg = hepatitis B surface antigen
- anti-HCV = hepatitis C antibody
- IgA = immunoglobulin A
- ANA = anti-nuclear antibody
- ASMA = anti-smooth muscle antibody
- IgG = immunoglobulin G
- AMA = anti-mitochondrial antibody
- IgM = immunoglobulin M
- ANCA = anti-neutrophil cytoplasmic antibodies
- Fe (iron) / TIBC (total iron binding capacity) = TS (transferrin saturation)
- A1AT = alpha 1 anti-trypsin level

**Abbreviations (confirmatory / additional tests):**

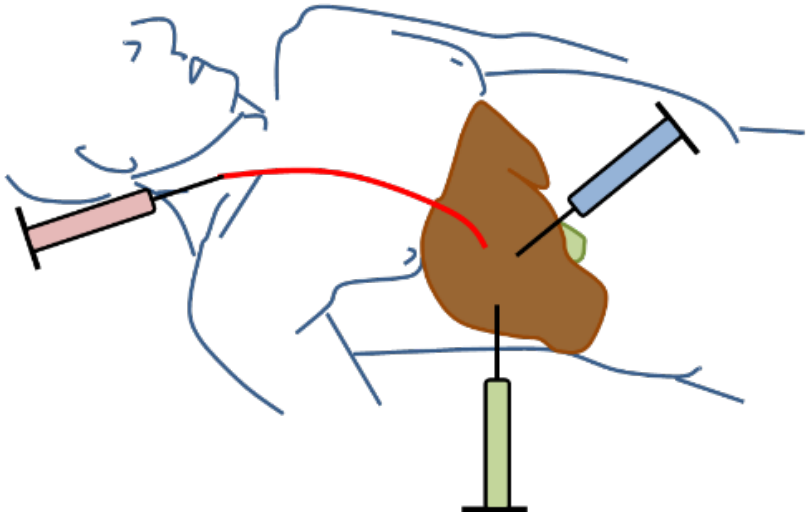
- HBVDNA = hepatitis B virus deoxynucleic acid
- HBeAg = hepatitis B “e” antigen
- Anti-HBe = hepatitis B “e” antibody
- HCVRNA = hepatitis C virus ribonucleic acid
- MRCP = magnetic resonance cholangio pancreatography
- KF = Kaiser Fleischer

## 3.6 Liver Biopsy

- A liver biopsy is predominantly done for two reasons:
  - If there remains diagnostic uncertainty after completing imaging and screening tests for chronic liver disease
  - To provide prognosis on the severity of the liver disease
- A liver biopsy can be done in one of three ways:
  - **Blind**, going between the ribs on the right side with caution taken to place the needle on the superior side of the rib to avoid the intercostal arteries
  - **Ultrasound (US) guidance**, usually done anteriorly underneath the ribs, with the US being used to guide the needle into masses or to avoid structures like the colon or gallbladder
  - A needle passed via a **transjugular route**, from the jugular vein, past the heart, into the hepatic veins, can biopsy the liver from the inside. This is done in patients with low platelets or high INR where the bleeding risk is too high to puncture the liver capsule

**Can be done blind, with US guidance, or by transjugular route**



- A liver biopsy is an invasive procedure with several risks:
  - 1:20 pain (from the liver capsule and may be felt in the RUQ or shoulder tip)
  - 1:100 hematoma (bruising on the liver)
  - 1:1000 intraperitoneal bleeding (most serious complication which can lead to need for IV fluids, blood transfusion, angiography or even surgery to stop the bleeding)
  - 1:10,000 death from bleeding (may be as high as 1:700 if biopsy done of a tumour)

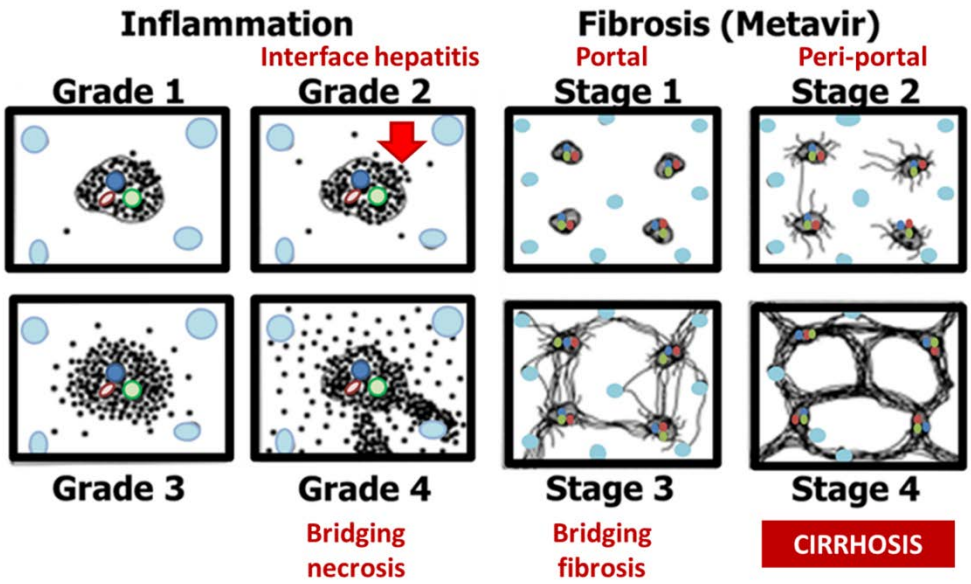
## 3.7 Pathology

- Liver pathology of the liver can be broken down into 3 parts:
- **Grade of inflammation** (shown above according to the Ludwig Batts scoring system) best assessed on hematoxylin & eosin (H&E) stain
  - Inflammation starts around portal space (grade 1)

- When it crosses the limiting plate it is known as interface hepatitis or piecemeal necrosis (grade 2)
- Grade 3 is more severe
- When inflammatory cells spread from portal space to central vein it is called bridging necrosis (grade 4)

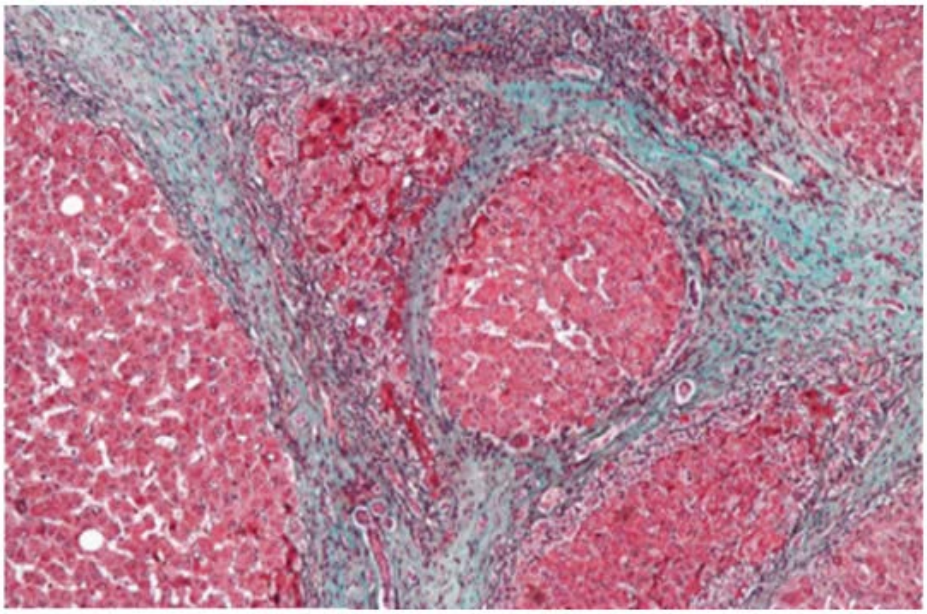
- **Stage of fibrosis** (according to the Metavir scoring system)
  - Fibrosis typically starts around portal spaces (stage 1 – portal)
  - It then starts to spread into the lobule towards other portal spaces (stage 2 – periportal)
  - Bridging fibrosis (stage 3) is more severe
  - Stage 4 is severe fibrosis, with trapped regenerating nodules of hepatocytes, and is also known as **cirrhosis**
  - Fibrosis may also line the sinusoids in certain disease like alcohol




Adapted from Batts KP, Ludwig J. Am J Surg Pathol 1995; 19: 1409-17.

- **Cirrhosis** = regenerating hepatocytes are trapped by thick bands of fibrosis forming cirrhotic nodules, is best appreciated with the use of the Mason Trichrome stain where collagen & fibrosis stains blue



**Masson Trichrome Stain** Image source: en.wikipedia.org

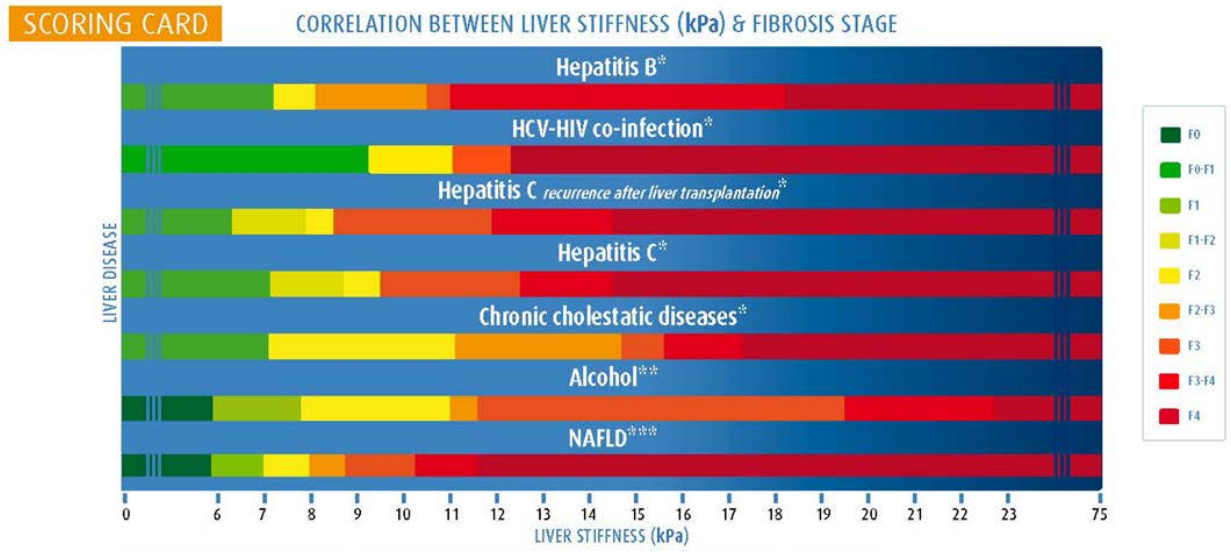
- Specific findings [some seen best on special stains] include:
  - Cholestasis = feathery degeneration and bile plugs [H&E stain]
  - Steatosis = microvesicular or macrovesicular fat [H&E stain]
  - Mallory hyaline with alcohol or NAFLD [H&E stain]
  - Iron overload [Prussian Blue stain]
  - A1AT globules [Period Acid Schiff-Diastase (PAS-D) stain]
  - Viral inclusions (e.g. CMV) [immunohistochemistry]
  - Nodular regenerative hyperplasia [reticulin stain]

## 3.8 Non-Invasive Fibrosis Testing

- We now do very few liver biopsies purely for staging of liver disease because we have non-invasive measurements of liver fibrosis, including laboratory tests or non-invasive imaging tests[3,4]
- Laboratory tests to estimate fibrosis include:
  - APRI (AST to Platelet Ratio Index)
  - Fibrosis-4 (FIB-4): age, AST, ALT, platelets
  - FibroTest®: alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, total bilirubin, ALT
  - Many others, including some developed for specific diseases, e.g. NAFLD fibrosis score (NFS): age, BMI, impaired fasting glucose/diabetes, AST/ALT ratio, platelets
- Non-invasive imaging tests to estimate fibrosis include:
  - Transient elastography (FibroScan®)
    - Measures liver stiffness in kilopascals (kPa) by bouncing sound waves into the liver (generated by percussion tap on end of probe) and measuring how fast the sound wave returns to
    - It correlates very well with biopsy findings, and is especially good for ruling in or ruling out cirrhosis
    - There are different ranges of liver stiffness for each liver disease (see below) ranging from FO-F1 (in green) to F4 or cirrhosis (in red)
    - However, liver stiffness can be falsely elevated by acute liver inflammation, cholestasis, and may be influenced by eating (should do the test fasting)




- ARFI (Acoustic Radiation Force Impulse) imaging
  - Now available on many ultrasound machines
  - Measures elastography through generation of sheer waves
  - In Calgary, Family Doctors use to risk stratify patients with NAFLD as part of the NAFLD Primary Care Pathway

    https://www.specialistlink.ca/files/NAFLD_Pathway_May2019.pdf
- MRE (Magnetic Resonance Elastography)
  - Done during MRI using a special plate over liver to generate sound waves
  - Generated a fibrosis map (elastogram) of the entire liver

**Abbreviations:**

**ALP** – alkaline phosphatase

**ALT** – alanine aminotransferase

**AMA** – anti-mitochondrial antibody

**ANA** – anti-nuclear antibody

**ANCA** – anti-neutrophil cytoplasmic antibodies

**Anti-HBe** – anti-HBe antibody

**Anti-HCV** – hepatitis C virus antibody

**APRI** – AST (aspartate aminotransferase) to platelet ratio

**ARFI** – Acoustic Radiation Force Impulse imaging

**ASMA** – anti-smooth muscle antibody

**AST** – aspartate aminotransferase

**CMV** – cytomegalovirus

**ERCP** – endoscopic retrograde cholangio pancreatography

**EUS** – endoscopic ultrasound

**Fe** – iron

**GGT** – gamma-glutamyltranspeptidase

**HBeAg** – hepatitis B "e" antigen

**HBsAg** – hepatitis B surface antigen

**HBVDNA** – hepatitis B virus deoxynucleic acid

**HCVRNA** – hepatitis C virus ribonucleic acid

**HDL** – high density lipoprotein

**HE** – hepatic encephalopathy

**Ig** – immunoglobulins

**INR** – international normalized ratio

**KF** – Kaiser Fleischer

**kPa** – kiloPascals

**LDH** – lactate dehydrogenase

**LDL** – low density lipoprotein

**MRCP** – magnetic resonance cholangio pancreatography

**MRE** – magnetic resonance elastography

**PAS-D** – Period Acid Schiff-Diastase stain

**PCR** – polymerase chain reaction

**PT** – prothrombin time

**TIBC** – total iron binding capacity

**TS** – transferrin saturation

**TSH** – thyroid stimulating hormone

**TTG** – tissue transglutaminase

**UGT** – uridine 5'-diphospho-glucuronosyltransferase

**ULN** – upper limit of normal

**US** – abdominal ultrasound

**Figure Citations**

**Pathology**

Batts KP, Ludwig J. Chronic hepatitis: An update on terminology and reporting. *Am J Surg Path* 1995; 19:1409-1417.

Cirrhosis image: https://en.wikipedia.org/wiki/Cirrhosis#/media/File:Cirrhosis_high_mag.jpg

**Non-invasive Fibrosis Testing**

http://hepatitiscnewdrugs.blogspot.ca/p/fibroscan-scoring-card-understanding.html

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