Laboratory tests in Liver diseases

Tushar Patel M.B., ChB
Professor of Internal Medicine
Ohio State University Medical Center

Overview
- Liver injury tests
- Liver function tests
- Disease-specific tests

Tests of Liver Injury
- Parenchymal injury
  - AST
  - ALT
  - LDH
- Hepatobiliary injury
  - Alkaline Phosphatase
  - GGT
  - 5-nucleotidase

Diagnosis of Hepatic Injury
Predominantly Parenchymal injury

Tests of Liver Injury

AST
ALT
LDH

Enzymes leak out of damaged cells

Aspartate aminotransferase (AST)
Glutamate-Oxaloacetate Transaminase

- Ubiquitous, and found in all tissues
- Highest activity in heart, with significant activity in the liver, brain, gastric mucosa, adipose tissue, skeletal muscle, and kidneys.
- In mild hepatocellular injury when the plasma but not the mitochondrial membrane is damaged, cytoplasmic form of AST is released into the blood. With more severe hepatocellular injury, mitochondrial damage may result in the release of mitochondrial AST.
- CPK or aldolase may be useful to exclude a muscle source of abnormal AST

Alanine Aminotransferase (ALT)
Glutamate Pyruvate Transaminase

- As a group, the aminotransferases catalyze the interconversion of amino acids and alpha-keto acids by transfer of amino groups.
- Highest activity in the liver, with decreasing concentrations in the kidney, heart, skeletal muscle, pancreas, spleen, and lung.
- The enzyme is mainly located in the cytoplasm and escapes into the blood circulation during the cellular injury.

Increased AST/ALT

- Increased ALT or AST usually imply hepatic parenchymal injury but can occur in other organ damage
  ✓ ALT is more “liver” specific.
- Reflect hepatic injury not hepatic function – and can be normal in advanced liver disease or cirrhosis
- Can occur with damage to other organs (myocardial infarction, muscular dystrophy, etc)
AST/ALT Ratio

- The ratio of AST to ALT activity in the blood is helpful in the diagnosis of liver diseases.
- In patients with most hepatic disorders, including acute or chronic viral hepatitis, ALT is increased more than AST.
- However, in alcoholic liver disease, AST is increased much more than ALT, and the ratio is greater than 2.

Alkaline Phosphatase

- Present in several tissues, including bile ducts, bone, kidney, intestine and placenta.
- Exists as isoenzymes that are specific for various tissues.
- The bulk of the alkaline phosphatase in normal humans is made up of liver and bone isoenzymes.

Diagnosis of Hepatic Injury

- Tests of Liver Injury
  - Predominantly Hepatobiliary injury
    - Alkaline Phosphatase
    - GGT
    - 5-nucleotidase

Increased Alkaline Phosphatase

- Identify tissue source by isoenzyme analysis, or measure either 5'-nucleotidase or gamma glutamyl transpeptidase.
- Levels may rise in persons with blood groups O and B, who are ABH secretors and Lewis antigen-positive, esp after a fatty meal. Thus, measure alkaline phosphatase in the fasting state.
- Note: Alk Phos may be normal even with extensive hepatic metastases or complete bile duct obstruction. Patients with Wilson’s disease may have normal values.
### Increased Alkaline Phosphatase

- Cholestasis
- Intrahepatic or Extrahepatic biliary obstruction (from biliary stones, cholangitis, biliary cirrhosis, cancers etc.)
- Hypernephroma and Hodgkin's disease have elevated levels in the absence of liver involvement

### γ-Glutamyl Transferase

- Present in almost all tissues, but highest activity in bile ducts, kidney and pancreas
- In the liver present in biliary tract cells

### Alk phos can be increased in non-hepatic conditions

- Some malignancies
  - Patients with malignancies may have increased levels not caused by liver or bone metastases (Regan isoenzyme)
- Bone or intestinal disease in the absence of liver disease.
  - In normal children with active bone growth, influx of enzyme from osteoid tissue may result in threefold increase in serum levels.
- Pregnancy
  - In the third trimester of pregnancy, the serum level may double as a consequence of placental contribution.

### Clinical significance of increased γGT

- Very sensitive indicator of liver disease.
- Rarely normal in the presence of hepatic disease.
- However, it is not very specific for liver diseases.
- Levels may be increased in
  - Persons who take drugs such as phenytoin, phenobarbital, or alcohol
  - Persons with renal failure, myocardial infarction, pancreatic disease etc.
- GGT is a sensitive screening test for occult alcoholism
Tests of Liver Function

Synthetic Function
- Prothrombin time
- Albumin
- Pseudocholinesterase

Excretory or Detoxifying Function
- Bilirubin (excretory)
- Ammonia (detoxifying)

**Albumin**
- 60% of the total serum protein
- The liver synthesizes 120 mg/kg of albumin daily.
- Serum half-life of about 20 days.
- Reflects relatively long term synthetic function.
- Can be lowered by renal or GI losses which exceed the capacity of hepatic synthesis even with a normal liver

**Serum Protein Electrophoresis**
- The major proteins in serum are separated in an electric field and their concentrations determined
  - Albumin, Alpha-globulins, Beta-globulins, Gamma-globulins
  - Cirrhosis: albumin may be decreased, globulins may be increased
  - Auto-immune hepatitis: gamma globulins can be markedly increased
  - Alpha-1-antitrypsin deficiency: alpha-globulins may be low

**Prothrombin Time**
- Prothrombin time is prolonged when clotting factor levels are decreased.
- Increased in chronic liver diseases if cirrhosis is present and there is fairly significant liver damage
- Useful prognostic marker in acute liver injury
  - It is increased with severe damage, and improves as the patient recovers.
- Can be increased in Vitamin K deficiency, drugs (warfarin)
**Clothing Factors**

- All coagulation factors, except factor VIII and von Willebrand factor, are made in the liver. The half-life of these factors is short (few hours to a few days).
- Factor VIII levels may be normal or increased in severe hepatic injury.
- This may help distinguish between the coagulopathy of liver disease from that due to disseminated intravascular coagulation (where factor VIII levels are decreased).
- Synthesis of several factors requires vitamin K. This fat-soluble vitamin is absorbed from the gut as a complex with bile salts. In patients with biliary obstruction, the absorption of vitamin K is inadequate which results in the decreased synthesis of these factors (II, VII, IX, X).

**Sources of Heme**

- **250-350 mg/day**
  - RBCs
  - 70%

---

**Bilirubin**

- Useful for evaluating degrees of jaundice
- Useful for the diagnosis of liver disease
- Useful for the detection of hemolytic anemia
- Bilirubin is mostly formed by the reticuloendothelial system during the destruction of red blood cells.

**Sources of Heme**

- **250-350 mg/day**
  - Hepatic Hemoproteins
  - Cytochrome p450
  - Catalase
  - 23-37%
  - RBCs
  - 70%
### Sources of Heme
250-350 mg/day

<table>
<thead>
<tr>
<th>Hepatic Hemoproteins</th>
<th>RBCs</th>
<th>Non-Hepatic Hemoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome p450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>70%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>23-37%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Hepatic Hemoproteins**
  - Cytochrome p450
  - Catalase
- **RBCs**
  - 70%
- **Non-Hepatic Hemoproteins**
  - <10%

### Unconjugated Hyperbilirubinemia
- > 80% of the total bilirubin level is unconjugated or indirect
- Consider
  - Criggler-Najjar syndrome
  - Mild changes also seen in
    - Gilbert’s disease,
    - uncomplicated hemolytic disorders
    - Congestive heart failure.
    - Neonates

### Increased Bilirubin
Conjugated (direct) or unconjugated (indirect)
Total bilirubin – direct bilirubin = Indirect bilirubin

- Virtually 100% of serum bilirubin in health is unconjugated, whereas conjugated hyperbilirubinemia occurs only in hepatobiliary conditions
- Delta bilirubin, an albumin-linked bilirubin fraction, represents a significant fraction of total serum bilirubin in hepatobiliary disorders, but not in health, neonates, or with hemolysis.
- The clearance of delta bilirubin is slow, 12 to 24 days, vs non-albumin bound conjugated bilirubin

### Gilbert’s Syndrome
- Common! Affects 3-7% of population
- Males > Females (7:1)
- Bilirubin levels increased by fasting
- Bilirubin levels decreased by:
  - Phenobarbital (UGT induction?)
  - Steroids (increased storage proteins)
Causes of Conjugated Hyperbilirubinemia

- Decreased excretion into bile ductules
- Backward leakage of the pigment

<table>
<thead>
<tr>
<th>Inherited</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubin-Johnson syndrome</td>
<td>Hepatocellular or cholestatic disease</td>
</tr>
</tbody>
</table>

Disease-specific and other tests

- Serological or virological tests for viral hepatitis
  - e.g. anti HCV, HCV RNA
- Auto-antibodies
  - e.g. ANA, AMA, ASMA
- Disease associated proteins
  - e.g. ceruloplasmin, alpha-1-antitrypsin

Ammonia

- Blood levels of ammonia are of very limited value in the evaluation of persons with known hepatobiliary disease.
- Ammonia levels are elevated in blood and CSF in hepatic coma:
  - The ammonia level correlates poorly with the degree of hepatic encephalopathy.
  - The correlation is better with arterial compared to venous levels
- The greatest use is in evaluating patients with coma or altered mental status of unclear cause. In this case, an elevated arterial ammonia would suggest hepatocellular dysfunction as an important contributing cause.

Ferritin

- Accurately reflect hepatic and total body iron stores.
- Elevated in many conditions, e.g. uremia, hemochromatosis, hepatocellular necrosis of any cause, Hodgkin’s disease, leukemia, hyperthyroidism, and rheumatoid arthritis.
- Serum iron concentration, transferrin saturation and ferritin are used to screen for hemochromatosis. However, these may be misleading in a variety of disorders such as fatty liver, alcoholism, and acute hepatitis
Ceruloplasmin

- Enzyme involved in copper metabolism.
- Low levels can occur in nephritic syndrome, protein-losing enteropathy, and in Wilson’s disease.
- In Wilson’s disease,
  - 95% of persons with Wilson’s disease have a ceruloplasmin level < 20 mg/dL.
  - The serum ceruloplasmin can be normal in up to 10% of persons with Wilsons.

Miscellaneous Tests

- Platelet count
  - Decreased in persons with cirrhosis
  - Correlates with portal hypertension and splenomegaly, and hepatic dysfunction
- Tissue transglutaminase Ab
  - Diagnosis of celiac disease, a cause of occult liver disease
- Pseudocholinesterase
  - Decreased in persons with impaired hepatic synthetic function

Alpha-1 Antitrypsin

- In alpha-1 antitrypsin deficiency, abnormal hepatic synthesis results in decreased alpha-1-antitrypsin in the blood.
  - Hereditary condition caused by the inheritance of mutated allelic genes S or Z (normal allelic gene is called M).
  - Predisposes to cholestasis in childhood, and to cirrhosis, usually in adolescence or adult life.
- A-1 antitrypsin deficiency can be detected by serum protein electrophoresis and by the specific test for anti-protease activity.
- ZZ is the pattern seen in patients with liver disease.

Patterns of Liver Test Abnormalities

Doug Levin, MD
Associate Professor
Ohio State University Medical Center
<table>
<thead>
<tr>
<th>Hepatitic Pattern</th>
<th>Very High Aminotransferases</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increased AST, ALT</td>
<td>• Acute viral hepatitis</td>
</tr>
<tr>
<td>• Increase in AST, ALT is relatively higher than increase in alkaline phosphatase</td>
<td>• Shock liver (ischemia)</td>
</tr>
<tr>
<td></td>
<td>• Toxins</td>
</tr>
<tr>
<td></td>
<td>• Acetaminophen toxicity especially with chronic alcohol use</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cholestatic Pattern</th>
<th>Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increased Alkaline Phosphatase, bilirubin</td>
<td>• ALT and AST may fall</td>
</tr>
<tr>
<td>• Increase in Alk Phos, bilirubin is relatively higher than increase in AST, ALT</td>
<td>• Probably an artifact of testing caused by interfering substances in the serum of renal failure patients</td>
</tr>
</tbody>
</table>
Other Patterns

- Vascular disease
  - High AST, ALT, LDH, relatively low alk phos
- Malignancy
  - Relatively high alk phos and LDH,
  - Relatively low AST and ALT

Liver tests with Prognostic value

- INR
- MELD
- Elevation in bilirubin in PBC
- High bilirubins in acute viral hepatitis correlate with intensity and duration of the acute illness

Acute Liver Failure

- Simultaneous elevations in AST, ALT, bilirubin, INR in acute liver failure

Isolated Increased Alk Phos

- Identify source of alk phos
  - Isoenzymes or 5'-nucleotidase or GGT
- If liver:
  - Chronic cholestatic
  - Infiltrative disease
Testing for Acute Viral hepatitis

- Hepatitis A IgM antibody
- Hepatitis B surface antigen
- Hepatitis B core IgM antibody
- Hepatitis C viral RNA

**Clinical Significance of Serological Markers for HBV Infection**

<table>
<thead>
<tr>
<th>Serological Markers</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>Acute/Chronic infection</td>
</tr>
<tr>
<td>Anti-HBc IgM</td>
<td>Acute infection</td>
</tr>
<tr>
<td>HBeAg</td>
<td>High infectivity</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Low infectivity</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Immunity</td>
</tr>
<tr>
<td>Anti-HBc IgG and HBsAg</td>
<td>Chronic infection</td>
</tr>
<tr>
<td>Anti-HBc IgG and anti-HBs</td>
<td>Resolved infection</td>
</tr>
</tbody>
</table>