Liver Function Tests

Laboratory tests are generally effective for the following:

- Detecting hepatic dysfunction
- Assessing the severity of liver injury
- Monitoring the course of liver diseases and the response to treatment
- Refining the diagnosis

Many tests of liver biochemistry and excretory performance are called liver function tests. However, rather than assessing liver function, several tests measure liver enzymes that are released into the bloodstream (release of aminotransferases from injured liver cells or of alkaline phosphatase due to cholestasis). Only certain tests actually assess liver function by evaluating hepatobiliary excretion (bilirubin) or the liver’s synthetic capability (PT, usually reported as the INR; albumin).

The most useful laboratory tests to screen for liver disorders are serum aminotransferases (the most commonly used liver function tests), bilirubin, and alkaline phosphatase. Certain patterns of biochemical abnormalities help distinguish hepatocellular injury from impaired bile excretion (cholestasis). Tests that detect viral hepatitis, liver inflammation, or altered immunoregulation include hepatitis serologic tests and measurement of immunoglobulins, antibodies, and autoantibodies.

A few laboratory tests are diagnostic by themselves; they include the following:

- IgM antibody to hepatitis A virus (anti-HAV) for acute hepatitis A
- Hepatitis B surface antigen (HBsAg) for hepatitis B
- Antibody to hepatitis C virus (anti-HCV) and HCV-RNA for hepatitis C
- Antimitochondrial antibody for primary biliary cirrhosis
- Serum ceruloplasmin (reduced) and urinary copper (elevated) for Wilson’s disease
- Serum $\alpha_1$-antitrypsin for $\alpha_1$-antitrypsin deficiency
- $\alpha$-Fetoprotein for hepatocellular carcinoma

Table 1

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Aminotransferase Elevations</th>
<th>Alkaline Phosphatase Elevations</th>
<th>Prolongation of PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute necrosis or injury</td>
<td>Marked</td>
<td>Often present but may be mild</td>
<td>Prolonged if hepatic function is severely impaired</td>
</tr>
<tr>
<td>Chronic hepatocellular disease</td>
<td>Mild to moderate</td>
<td>Often present but may be mild</td>
<td>Prolonged if hepatic function is severely impaired</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>Often present but may be mild</td>
<td>Marked</td>
<td>Prolonged if chronic steatorrhea causes vitamin K malabsorption</td>
</tr>
<tr>
<td>Infiltration</td>
<td>Mild</td>
<td>Mild to moderate</td>
<td>Not usually prolonged</td>
</tr>
<tr>
<td>Liver failure</td>
<td>Depend on cause</td>
<td>Depend on cause</td>
<td>Prolonged but often only slightly if failure is chronic</td>
</tr>
</tbody>
</table>

Tests for Liver Injury
Aminotransferases: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) leak from damaged cells; thus, these enzymes are sensitive indicators of liver injury. Markedly high values (> 500 IU/L; normal, ≤ 40 IU/L), which indicate acute hepatocellular necrosis or injury, usually result from the following:

- Acute viral hepatitis
- Toxin- or drug-induced hepatitis
- Ischemic hepatitis or hepatic infarction

High levels continue usually for days or, in viral hepatitis, weeks. The degree of elevation may not reflect the extent of liver injury. Serial measurements better reflect severity and prognosis than does a single measurement. A fall to normal indicates recovery unless accompanied by an increase in bilirubin and in PT or INR (which indicates fulminant liver failure). Fulminant liver failure results in fewer liver cells that can leak enzymes.

Aminotransferase levels may also be markedly high in the following:

- Acute exacerbation of autoimmune hepatitis
- Reactivation of chronic hepatitis B
- Acute Budd-Chiari syndrome
- Acute fatty liver of pregnancy
- Passage of a common duct stone

Modest elevations (300 to 500 IU/L) persist in chronic liver disorders (eg, chronic hepatitis, alcoholic hepatitis) and in biliary obstruction, except where passage of a common duct stone can transiently result in markedly high levels, sometimes into the thousands.

Mild increases (< 300 IU/L) are nonspecific and often present in disorders such as

- Cirrhosis secondary to viral hepatitis
- Nonalcoholic fatty liver disease (NAFLD)
• Cholestatic liver disorders
• Hepatocellular cancer

Aminotransferases can be normal in certain liver disorders, such as

• Hemochromatosis
• Induced liver injury
• Chronic hepatitis C
• NAFLD

Elevated ALT is somewhat specific for liver injury. Because AST is present in the heart, skeletal muscle, kidneys, and pancreas, elevated AST may reflect rhabdomyolysis or injury to one of these organs. In most liver disorders, the ratio of AST to ALT is < 1. However, in alcohol-related liver disease, the ratio is characteristically > 2 because pyridoxal-5-phosphate is deficient in alcoholic patients; it is required for ALT synthesis but is less essential for AST synthesis. This deficiency also explains why elevations of ALT and AST are low (< 300 IU/L) in alcoholic patients.

Lactate dehydrogenase: LDH, commonly included in routine analysis, is present in many other tissues and is insensitive and nonspecific for hepatocellular injury. LDH is typically elevated in ischemic hepatitis and cancers that extensively infiltrate the liver.

Tests for Cholestasis

Bilirubin

Bilirubin, the pigment in bile, is produced from the breakdown of heme proteins, mostly from the heme moiety of hemoglobin in senescent RBCs. Unconjugated (free) bilirubin is insoluble in water and thus cannot be excreted in urine; most unconjugated bilirubin is bound to albumin in plasma. Bilirubin is conjugated in the liver with glucuronic acid to form the more water-soluble bilirubin diglucuronide. Conjugated bilirubin is then excreted through the biliary tract into the duodenum, where it is metabolized into
urobilinogens (some of which are reabsorbed and resecreted into bile), then into orange-colored urobilins (most of which are eliminated in feces). These bile pigments give stool its typical color.

Hyperbilirubinemia results from one or more of the following:

- Increased bilirubin production
- Decreased liver uptake or conjugation
- Decreased biliary excretion

Normally, total bilirubin is mostly unconjugated, with values of < 1.2 mg/dL (< 20 µmol/L). Fractionation measures the proportion of bilirubin that is conjugated (direct, so-called because it is measured directly, without the need for solvents). Fractionation is most helpful for evaluating neonatal jaundice and for evaluating elevated bilirubin when other liver test results are normal, suggesting that hepatobiliary dysfunction is not the cause.

**Unconjugated hyperbilirubinemia**

(indirect bilirubin fraction > 85%) reflects increased bilirubin production (eg, hemolysis) or defective liver uptake or conjugation (eg, Gilbert syndrome). Such increases in unconjugated bilirubin are usually < 5 times normal (to < 6 mg/dL [<100 µmol/L]) unless there is concurrent liver injury.

**Conjugated hyperbilirubinemia**

(Direct bilirubin fraction > 50%) results from decreased bile formation or excretion (cholestasis). When associated with other liver function test abnormalities, a high serum bilirubin indicates hepatocellular dysfunction. Serum bilirubin alone is somewhat insensitive for liver dysfunction. However, the development of severe hyperbilirubinemia in primary biliary cirrhosis, alcoholic hepatitis, and acute liver failure suggests a poor prognosis.

**Bilirubinuria**

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Reflects the presence of conjugated bilirubin in urine; bilirubin spills into urine because blood levels are markedly elevated, indicating severe disease. Unconjugated bilirubin is water insoluble and bound to albumin and so cannot be excreted in urine. Bilirubinuria can be detected at the bedside with commercial urine test strips in acute viral hepatitis or other hepatobiliary disorders, even before jaundice appears. However, the diagnostic accuracy of such urine tests is limited. Results can be falsely negative when the urine specimen has been stored a long time, vitamin C has been ingested, or urine contains nitrates (eg, due to UTIs). Similarly, increases in urobilinogen are neither specific nor sensitive.

**Alkaline phosphatase**

Increased levels of this hepatocyte enzyme suggest cholestasis. Results may not be specific because alkaline phosphatase consists of several isoenzymes and has a widespread extrahepatic distribution (eg, in the placenta, small intestine, WBCs, kidneys, and particularly bone).

Alkaline phosphatase levels increase to ≥ 4 times normal 1 to 2 days after onset of biliary obstruction, regardless of the site of obstruction. Levels may remain elevated for several days after the obstruction resolves because the half-life of alkaline phosphatase is about 7 days. Increases of up to 3 times normal occur in many liver disorders, including

- Hepatitis
- Cirrhosis
- Space-occupying lesions (eg, carcinoma)
- Infiltrative disorders (eg, amyloidosis, sarcoidosis, TB, metastases, abscesses)
- Syphilitic hepatitis (alkaline phosphatase may be disproportionately elevated compared with the modest changes in other liver tests)
Isolated elevations (when other liver test results are normal) may accompany

- Focal liver lesions (eg, abscess, tumor)
- Partial or intermittent bile duct obstruction (stone, stricture, cholangiocarcinoma)
- Syphilitic hepatitis

Occasionally, infiltrative disorders isolated elevations also occur in the absence of any apparent liver or biliary disorder, as in the following:

- Some cancers without apparent liver involvement (eg, bronchogenic carcinoma, Hodgkin lymphoma, renal cell carcinoma)
- After ingestion of fatty meals (because of an enzyme produced in the small intestine)
- Pregnancy (because of an enzyme produced in the placenta)
- Children and adolescents who are still growing (because of bone growth)
- Chronic renal failure (because of an enzyme produced in the intestine and bone)

Levels of γ glutamyl transpeptidase or 5-nucleotidase, which are more specific to the liver, can, differentiate hepatic from extrahepatic sources of alkaline phosphatase better than fractionation of alkaline phosphatases, which is technically difficult. Also, in otherwise asymptomatic elderly people, an increase in alkaline phosphatase usually originates in bone (eg, in Paget’s disease) and does not require further investigation for liver injury.

5-Nucleotidase: Increases in levels of this enzyme are as sensitive as alkaline phosphatase for detecting cholestasis and biliary obstruction but are more specific, almost always indicating hepatobiliary dysfunction. Because levels of alkaline phosphatase and 5-nucleotidase do not always correlate, one can be normal while the other is increased.
γ-Glutamyl transpeptidase (GGT): Levels of this enzyme increase in hepatobiliary dysfunction, especially cholestasis, and correlate loosely with levels of alkaline phosphatase and 5-nucleotidase. Levels do not increase because of bone lesions, during childhood, or during pregnancy. However, alcohol and certain drugs (eg, some anticonvulsants, warfarin) can induce hepatic microsomal (cytochrome P-450) enzymes, markedly increasing GGT and thus somewhat limiting its specificity.

Tests of Hepatic Synthetic Capacity

PT and INR: PT may be expressed in time (sec) or, preferably, as a ratio of the patient’s measured PT to the laboratory’s control value. The INR is more accurate than PT for monitoring anticoagulation. PT or INR is a valuable measure of the liver’s ability to synthesize fibrinogen and vitamin K–dependent clotting factors: factors II (prothrombin), V, VII, and X. Changes can occur rapidly because some of the involved clotting factors have short biologic half-lives (eg, 6 h for factor VII). Abnormalities indicate severe hepatocellular dysfunction, an ominous sign in acute liver disorders. In chronic liver disorders, an increasing PT or INR indicates progression to liver failure. The PT or INR does not increase in mild hepatocellular dysfunction and is often normal in cirrhosis.

A prolonged PT and an abnormal INR can result from coagulation disorders such as a consumptive coagulopathy or vitamin K deficiency. Fat malabsorption, including cholestasis, can cause vitamin K deficiency. In chronic cholestasis, marked hepatocellular dysfunction can be ruled out if vitamin K replacement (10 mg sc) corrects PT by ≥ 30% within 24 h.

Serum proteins: Hepatocytes synthesize most serum proteins, including α- and β-globulins, albumin, and most clotting factors (but not factor VIII, produced by the vascular endothelium, or γ-globulin, produced by B cells). Hepatocytes also make proteins that aid in the diagnosis of specific disorders:
• $\alpha_1$-Antitrypsin (absent in $\alpha_1$-antitrypsin deficiency)
• Ceruloplasmin (reduced in Wilson's disease)
• Transferrin (saturated with iron in hemochromatosis)
• Ferritin (greatly increased in hemochromatosis)

These proteins usually increase in response to damage (eg, inflammation) to various tissues, so that elevations may not specifically reflect liver disorders.

**Serum albumin**

This commonly decreases in chronic liver disorders because of an increase in volume of distribution (eg, due to ascites), a decrease in hepatic synthesis, or both. Values < 3 g/dL (< 30 g/L) suggest decreased synthesis, caused by one of the following:

• Advanced cirrhosis (the most common cause)
• Alcoholism
• Chronic inflammation
• Protein undernutrition

Hypoalbuminemia can also result from excessive loss of albumin from the kidneys (ie, nephrotic syndrome), gut (eg, due to protein-losing gastroenteropathies), or skin (eg, due to burns or exfoliative dermatitis).

Because albumin has a half-life of about 20 days, serum levels take weeks to increase or decrease.

**Other Laboratory Tests**

**Ammonia**

Nitrogen compounds that enter the colon (eg, ingested protein, secreted urea) are degraded by resident bacteria, liberating ammonia. The ammonia is then absorbed and transported via the portal vein to the liver. The healthy liver readily clears the ammonia from the portal vein and converts it to glutamine, which is metabolized by the
kidneys into urea to be excreted. In patients with portal-systemic shunting, the diseased liver does not clear ammonia, which then enters the systemic circulation, possibly contributing to portal-systemic (hepatic) encephalopathy. Elevated ammonia levels occur in hepatic encephalopathy, but levels may be falsely low or high. In advanced liver disorders, the following may increase ammonia levels:

- High-protein meals
- GI bleeding
- Hypokalemia
- Metabolic alkalosis
- Certain drugs (eg, alcohol, barbiturates, diuretics, opioids, valproate)
- High-dose chemotherapy
- Parenteral nutrition
- Renal insufficiency
- Extreme muscle exertion and muscle wasting
- Salicylate intoxication
- Shock
- Ureterosigmoidostomy
- UTI with a urease-producing organism (eg, Proteus mirabilis)

Because the degree of elevation in the ammonia level correlates poorly with severity of hepatic encephalopathy, this level has limited usefulness in monitoring therapy.

*Serum immunoglobulins*

In chronic liver disorders, serum immunoglobulins often increase. However, elevations are not specific and are usually not helpful clinically. Levels increase slightly in acute hepatitis, moderately in chronic active hepatitis, and markedly in autoimmune hepatitis. The pattern of immunoglobulin elevation adds little information, although different immunoglobulins are usually very high in different disorders:

- IgM in primary biliary cirrhosis
- IgA in alcoholic liver disease
- IgG in autoimmune hepatitis

_Antimitochondrial antibodies_

These heterogeneous antibodies are positive, usually in high titers, in > 95% of patients with primary biliary cirrhosis. They are also occasionally present in the following:

- Autoimmune hepatitis
- Drug-induced hepatitis
- Other autoimmune disorders, such as connective tissue disorders, myasthenia gravis, autoimmune thyroiditis, Addison’s disease, and autoimmune hemolytic anemia

Antimitochondrial antibodies can help determine the cause of cholestasis because they are usually absent in extrahepatic biliary obstruction and primary sclerosing cholangitis.

_Other antibodies_

Other antibodies may help in diagnosis of the following:

- Autoimmune hepatitis: Smooth muscle antibodies against actin, antinuclear antibodies (ANA) that provide a homogenous (diffuse) fluorescence, and antibodies to liver-kidney microsome type 1 (anti-LKM1) are often present.
- Primary biliary cirrhosis: Antimitochondrial antibody is key to the diagnosis.
- Primary sclerosing cholangitis: p-ANCA can help raise the index of suspicion.

Isolated abnormalities of any of these antibodies are never diagnostic and do not elucidate pathogenesis.

_α-Fetoprotein (AFP)_
AFP, a glycoprotein normally synthesized by the yolk sac in the embryo and then by the fetal liver, is elevated in neonates and hence the pregnant mother. AFP decreases rapidly during the first year of life, reaching adult values (normally, < 10 to 20 ng/mL or < 10 to 20 mg/L depending on the laboratory) by the age of 1 yr.

An increase in AFP, no matter how small, should prompt consideration of primary hepatocellular carcinoma (HCC). Serum AFP generally correlates with tumor size, differentiation and metastatic involvement. Because small tumors may produce low levels of AFP, increasing values suggest the presence of HCC, especially when > 3 cm diameter. AFP also helps predict prognosis.

Mild AFP elevations also occur in acute and chronic hepatitis, probably reflecting liver regeneration; AFP can occasionally increase to 500 ng/mL in fulminant hepatitis.

High AFP levels can occur in a few other disorders (eg, embryonic teratocarcinomas, hepatoblastomas in children, some hepatic metastases from GI tract cancers, some cholangiocarcinomas), but these circumstances are not common and usually can be differentiated based on clinical and histopathological grounds.

Sensitivity, specificity, and peak levels of AFP in patients with HCC vary by population, reflecting differences in factors such as hepatitis prevalence and ethnicity. In areas with a relatively low prevalence of hepatitis (eg, North America and Western Europe), AFP cutoff values of 20 ng/mL have a sensitivity of 39 to 64% and a specificity of 76 to 91%. However, not all HCCs produce AFP. Thus, AFP is not an ideal screening test but does have a role in detecting HCC. Levels exceeding normal (> 20 ng/mL), especially when increasing, strongly suggest HCC. In cirrhotic patients with a mass and a high value (eg, > 200 ng/mL), the predictive value is high. The combined use of AFP and ultrasonography currently provides the best surveillance
**Alanine Transferase**

Increased ALT indicates hepatic cell damage or necrosis

Alanine transferase (ALT), formerly known as SGPT is present in large quantities in the cytoplasm of canine and feline hepatocytes. This enzyme enters the blood when liver cells are damaged or destroyed and circulates for a few days.

This enzyme is a sensitive indicator of active liver damage but does not indicate the cause or reversibility of the damage. Increased serum ALT activity indicates recent or ongoing liver cell damage. An increase of at least three times normal indicates significant liver damage within the previous 2 to 5 days.

**Albumin**

Increased albumin suggests dehydration malnutrition malabsorption, enteritis, and glomerulonephritis causes decreased albumin. Severe dehydration often increases serum albumin levels.

Albumin is a serum protein that affects osmotic pressure, binds calcium, and transports fatty acids and many drugs.

Starvation, parasitism, chronic malabsorptive disease, chronic liver disease, exudative enteritis, and glomerulonephritis decrease serum albumin levels.

Hypoalbuminemia with normal serum globulin levels suggests decreased albumin production, increased loss, or sequestration. If both albumin and globulin levels are low, hemorrhage, exudation, and dilution are likely causes.

**Alkaline Phosphatase**

Alkaline phosphatase (AP) is found in both liver and bone. Elevated AP activity in the serum indicates increased production by the liver parenchyma, bile ducts, and growing bone or decreased excretion in
bile and urine. Elevated AP activity does not suggest liver or bone necrosis.

Serum AP activity increases after an episode of acute pancreatitis because of secondary cholangitis. It also increases if liver disease causes a disruption of hepatobiliary architecture with local impairment of bile flow.

Persistence of AP activity unrelated to continuing disease might be due to decreased clearance secondary to diseases such as renal failure, cirrhosis, or the formation of macroenzymes.

**Alpha-2 Acid Glycoprotein (AGP)**

Increased AGP is a very early indicator of disease

Alpha-2 acid glycoprotein (AGP) is an acute phase protein manufactured in the liver and found in the blood. Detection of elevated levels of AGP indicates illness or other stressors even though the person appears clinically normal. AGP indicates disease before antibodies are created by the immune system and before clinical symptoms are apparent. AGP is elevated by inflammation, infectious diseases, surgery, malignant tumors, autoimmune diseases, liver cirrhoses, and with all types of stress in general.

It can be used as a prognostic indicator to detect sub-clinical disease and changes in homeostasis and to monitor immune system function, chemotherapy, and vaccine efficacy. In monitoring cancer therapy, continued high levels of AGP where the level was initially high suggests that treatment is not working or not appropriate. Levels above 1000 mg/ml of serum indicate a poor prognosis, especially if subsequent measurements show increasing levels.

**Alpha Glutathione S-Transferase (GST)**

Increased GST indicates early hepatocyte injury
Alpha glutathione S-transferase (GST) is a superior marker of hepatocyte injury from toxicity, ischemia, and other liver injury. It is unique to hepatocytes, found in high concentrations, and is readily released in response to injury. It comprises 5% of the soluble protein of hepatocytes. Its rapid release into and removal from the circulation provides immediate information regarding liver status. It is a valuable tool in research evaluation of liver damage.

Amino Acid Ratio

branched-chain AA decrease with gluconeogenesis

Protein catabolism, gluconeogenesis, and increased insulin activity reduce serum levels of branched-chain amino acids. Hepatic insufficiency from portosystemic shunts or liver fibrosis (cirrhosis) increases serum levels of aromatic amino acids. The ratio of branched-chain to aromatic amino acids is termed the amino acid (AA) ratio.

Aromatic AA increase with hepatic insufficiency

The AA ratio is decreased with hepatic encephalopathy. CNS signs occur because increased levels of aromatic amino acids increase production of inhibitory neurotransmitters, while decreased levels of branched-chain amino acids decrease production of stimulatory neurotransmitters.

Normal branched-chain: aromatic AA ratio >3