Evaluation of Abnormal Liver Tests

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KEYWORDS

• Aminotransferases • Alkaline phosphatase • Hepatocellular injury • Cholestasis
• Bilirubin metabolism

KEY POINTS

- Serum aminotransferases are sensitive markers of hepatocellular injury.
- Assessing the pattern and degree of elevation in aminotransferases can help suggest the cause of liver injury.
- Elevation in serum alkaline phosphatase occurs as a result of cholestasis, which may result from intrahepatic causes, extrahepatic obstruction, or infiltrative disorders of the liver.
- Hyperbilirubinemia may occur as the result of both hepatocellular and cholestatic injury.
- Albumin and prothrombin time are true markers of liver synthetic function.

INTRODUCTION

The use of serum biochemical tests plays an important role in the diagnosis and management of liver diseases. The routine use of such tests has led to the increased detection of liver diseases in otherwise asymptomatic patients, often providing the first clue of the presence of liver pathology. Such laboratory tests, in addition to a careful history, physical examination, and imaging tests, can help clinicians determine the cause of liver disease in most cases.

The term "liver function tests" is commonly used to refer to a combination of liver biochemical tests, including serum aminotransferases, alkaline phosphatase (AP), and bilirubin. This is a misnomer, because aminotransferases and AP are markers of hepatocyte injury and do not reflect liver synthetic function. Traditionally, liver injury has been characterized as primarily hepatocellular versus cholestatic based on the degree of elevation of aminotransferases compared with AP (**Table 1**). Although such a distinction can help direct initial evaluation, there is often significant overlap in the presentation of various liver diseases, which often have a mixed pattern.[1] It is

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Table 1
Categorization of liver diseases by pattern of elevation of liver enzymes

Liver Disease Category	Aminotransferases	Alkaline Phosphatase
Hepatocellular	↑↑	↑
Cholestatic	↑	↑↑

useful to classify liver biochemical tests into the following categories[2]: (1) markers of hepatocellular injury (aminotransferases and AP); (2) tests of liver metabolism (total bilirubin); (3) tests of liver synthetic function (serum albumin and prothrombin time [PT]); and (4) tests for fibrosis in the liver (hyaluronate, type IV collagen, procollagen III, laminin, FibroTest [BioPredictive, Paris, France], and FibroScan [Echosens, Paris, France]).

Furthermore, when evaluating patients with abnormal liver enzyme or function tests, it is helpful to define the liver injury as acute versus chronic. Liver disease is considered chronic if the abnormalities in liver enzyme tests or function persist for more than 6 months.

MARKERS OF HEPATOCELLULAR INJURY

The liver contains a multitude of enzymes in high concentration, some of which are present in the serum in very low concentrations. Injury to the hepatocyte membrane leads to leakage of these enzymes into the serum, which results in increased serum concentrations within a few hours after liver injury. Serum enzymes tests can be categorized into two groups[2]: enzymes whose elevation reflects generalized damage to hepatocytes (aminotransferases); and enzymes whose elevation primarily reflects cholestasis (AP, γ-glutamyltransferase [GGT], 5′ nucleotidase [5′-NT]).

Aminotransferases

The aminotransferases (previously called transaminases) are located in hepatocytes and are sensitive indicators of hepatocyte injury. They are useful in detecting acute hepatocellular diseases, such as hepatitis.[2] They consist of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Aminotransferases catalyze the transfer of the α-amino groups from aspartate or alanine to the α-keto group of ketoglutaric acid, forming oxaloacetic acid and pyruvic acid, respectively. The enzymatic reduction of oxaloacetic acid and pyruvic acid to malate and lactate, respectively, is coupled to the oxidation of the reduced form of nicotinamide dinucleotide to nicotinamide dinucleotide. Because only nicotinamide dinucleotide absorbs light at 340 nm, this reaction can be followed spectrophotometrically by the loss of absorptivity at 340 nm, and provides an accurate method to assay aminotransferase activity.[3]

AST and ALT are present in the serum at low concentrations, usually less than 30 to 40 IU/L.[4] The normal range varies among clinical laboratories, based on measurements in specific populations. Several factors have been shown to influence ALT activity, such as gender and obesity.[5] Men tend to have a higher serum ALT activity compared with women.

ALT is found in highest concentration in hepatocytes and in very low concentrations in any other tissues. In contrast, AST is found in many other tissues including muscle (cardiac, skeletal, and smooth muscle); kidney; and brain.[2] Thus, ALT is a more specific marker for liver injury. A ratio of AST/ALT greater than five, especially if ALT is normal or slightly elevated, is suggestive of injury to extrahepatic tissues, such as skeletal muscle in the case of rhabdomyolysis or strenuous exercise.

AST is present in the cytoplasm and mitochondria, whereas ALT is only present in the cytoplasm (Fig. 1). About 80% of AST activity in the liver is derived from the mitochondrial isoenzyme, whereas most serum AST activity is derived from the cytosolic isoenzyme in healthy persons.[2] Processes leading to necrosis of hepatocytes or damage to the hepatocyte cell membrane with increased permeability result in release of AST and ALT into the blood.[6]

Assessing the pattern and degree of elevation in liver enzymes can help elucidate the cause of liver injury and direct subsequent diagnostic testing and management. Any type of liver cell injury can cause moderate elevations in serum aminotransferase levels. Levels up to 300 IU/L are nonspecific and can be seen in any type of liver disorder.[7] Massive elevations with aminotransferase levels greater than 1000 IU/L are almost exclusively seen in disorders associated with extensive hepatocellular injury, most commonly caused by (1) toxin- or drug-induced liver injury, (2) acute ischemic liver injury, or (3) acute viral hepatitis. Severe autoimmune hepatitis or Wilson disease may also cause markedly elevated aminotransferases.

ALT is present in highest concentration in periportal hepatocytes (Zone 1) and in lowest concentration in hepatocytes surrounding the central vein (Zone 3). AST, however, is present in hepatocytes at more constant levels (Fig. 2). Hepatocytes around the central vein have the lowest oxygen concentration and thus are more prone to damage in the setting of acute hepatic ischemia that can occur as the result of acute hypotension or severe cardiac disease. The ensuing centrilobular necrosis results in a rapid rise in aminotransferases, with AST value greater than ALT in the initial days of hepatic injury.

After there is no further injury to hepatocytes, the rate of decline of AST and ALT depends on their rate of clearance from the circulation. AST and ALT are catabolized by the liver, primarily by cells in the reticuloendothethlial system. The plasma half-life of AST and ALT are 17 ± 5 hours and 47 ± 10 hours, respectively.[2] Thus, AST declines more rapidly than ALT, and ALT may be higher than AST in the recovery phase of injury.

Biliary obstruction, such as that caused by a common bile duct stone causing an acute increase in intrabiliary pressure, may also lead to an acute, transient elevation in aminotransferases.

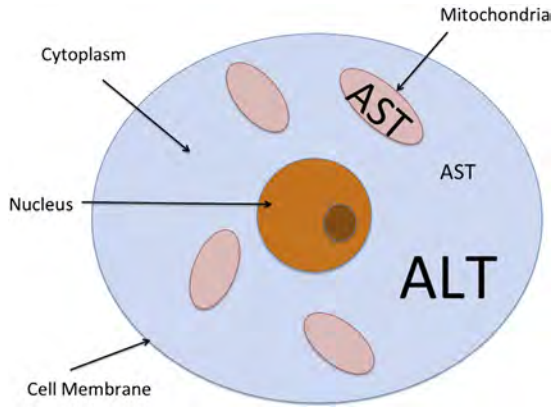

Fig. 1. Location of AST and ALT in hepatocyte. ALT is only present in the cytoplasm, whereas AST is present in both the cytoplasm and mitochondria. Eighty percent of AST activity in the liver is derived from the mitochondrial isoenzyme.

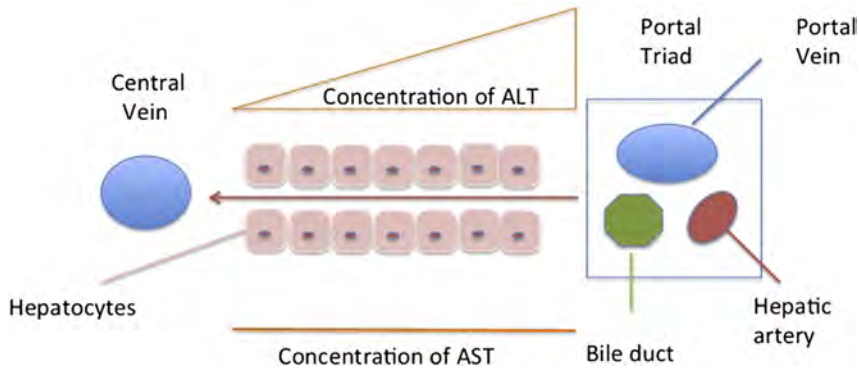

Fig. 2. Concentration of AST and ALT according to location of hepatocytes in portal triad.

A wide variety of disorders can cause chronic elevation in serum transaminases. Nonhepatic causes include thyroid diseases, celiac sprue, anorexia nervosa, Addison disease, and muscle diseases.[3] The most common hepatic causes of chronically elevated liver enzymes are chronic viral hepatitis (hepatitis C and B); alcoholic liver disease; nonalcoholic fatty liver disease; and drugs. Drugs that can cause elevated aminotransferase levels include antituberculosis drugs, such as isoniazid; antifungals, such as azole drugs; and antiepileptic drugs. Other causes of chronic hepatitis include (1) autoimmune hepatitis, which is most commonly seen in women and is associated with other autoimmune diseases; (2) inherited metabolic liver disease, such as hereditary hemochromatosis, Wilson disease, and α_1-antitrypsin deficiency; and (3) infiltrative disorders, such as granulomatous liver diseases.

Aminotransferases levels are typically less than 400 IU/L in alcoholic liver disease. An AST/ALT ratio of greater than two suggests alcoholic liver disease, whereas a ratio greater than three is strongly suggestive.[8] Low AST activity is secondary to a deficiency of pyridoxal 5′-phosphate, which is common in alcoholics.[9] Furthermore, alcohol primarily damages mitochondria, which results in the release of AST into the serum. Elevation in aminotransferase levels to greater than 1000 IU/mL is almost never caused by alcoholic liver disease alone and suggests the presence of a concomitant process, such as drug-induced liver injury or viral hepatitis. Chronic alcoholic use leads to induction of the hepatic cytochrome CYP2E1, which converts acetaminophen to the highly toxic intermediate, *N*-acetyl-*p*-benzoquinoneimine. Thus, patients who drink alcohol on a chronic basis are at increased risk of developing acetaminophen hepatotoxicity when consuming acetaminophen, even at doses less than 4 g/day.

Although ALT is more elevated than AST in most forms of chronic liver disease with the exception of alcoholic liver disease, the ratio of AST to ALT changes as fibrosis develops. As fibrosis progresses, the AST/ALT ratio increases and becomes greater than one after cirrhosis has developed in most cases. The platelet count also decreases with advancing fibrosis and cirrhosis, because of the reduction of thrombopoetin synthesis by the liver and splenic sequestration of platelets in the setting of portal hypertension. A platelet count of less than 150,000/μL in the absence of an underlying hematologic disorder is highly suggestive of cirrhosis. Thrombocytopenia may also be seen in acute alcoholic hepatitis because of bone marrow suppression from alcohol toxicity.

Tables 2 and **3** summarize the causes of acute and chronic elevations in aminotransferase levels, their pattern of AST and ALT elevation, and additional diagnostic

Table 2
Hepatic causes of acute elevation in aminotransferase levels and their patterns of liver enzyme injury

Disease	Aminotransferase Levels	Diagnostic Tests	Clinical Clues
Drug- or toxin-induced liver injury			
Acetaminophen	Often >500 IU/L	Acetaminophen level	History of ingestion
Amanita phalloides poisoning	AST > ALT	—	Wild mushroom ingestion
Acute viral hepatitis			
HAV	Often >500 IU/L	Anti-HAV IgM	Risk factors
HBV	ALT > AST	HBsAg, HBV DNA, anti-HBc	
HCV (rare)	—	HCV RNA, anti-HCV	
HDV (in setting of HBV coinfection)	—	Anti-HDV	
HEV	—	HEV IgM	
HSV	—	HSV IgM	
EBV	—	EBV IgM, EBV DNA	
CMV	—	CMV IgM, CMV DNA	
VZV	—	VZV IgM	
Parvovirus B19	—	Parvovirus B19 IgM	
Ischemic hepatitis	Often >500 IU/L AST > ALT	—	Recent hypotension
Alcoholic hepatitis	<400 IU/L	—	History of excess alcohol consumption
	AST: ALT >2		Disproportionate elevation in total bilirubin
Acute biliary obstruction	May be up to 1000 IU/L	Imaging (eg, ultrasound)	Acute onset of right upper quadrant pain
	ALT > AST	—	History of cholelithiasis

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; HSV, herpes simplex virus; VZV, varicella zoster virus.

testing indicated. An algorithm for the work-up of patients who present with a hepatocellular pattern of liver injury is outlined in Fig. 3.

Cholestasis

Cholestasis refers to the pathologic condition in which there is impairment in the liver's ability to secrete bile. Disorders that predominantly affect the biliary system are referred to as cholestatic diseases. They may affect the intrahepatic or extrahepatic bile ducts, or both. In such disorders, the elevation in AP is the predominant feature.

Alkaline phosphatase

AP refers to a group of zinc metalloenzymes that catalyze the hydrolysis of several organic phosphate esters at a neutral pH.[3] APs are found in the canalicular membrane

Table 3
Hepatic causes of chronic elevation in aminotransferase levels and their patterns of liver enzyme injury

Disease	Aminotransferase Levels	Diagnostic Tests	Clinical Clues
Chronic viral hepatitis			
HCV	<500 IU/L	Anti-HCV, HCV RNA	Risk factors
HBV	ALT > ALT	HBsAg, HBV DNA	
HDV (in setting of HBV coinfection)	—	Anti-HDV	
Alcoholic liver disease	<400 IU/L AST: ALT >2	—	History of excess alcohol consumption
Nonalcoholic fatty liver disease	<300 IU/L ALT > AST	—	History of obesity, diabetes, hyperlipidemia
Drug-induced liver injury	Up to 2000 IU/mL ALT > AST	Improvement after drug discontinuation	Inciting medication
Autoimmune hepatitis	Up to 2000 IU/L ALT > AST	ANA, antismooth muscle antibody IgG levels	Usually women, 30–50 y of age Presence of other autoimmune diseases
Hereditary hemochromatosis	<200 IU/L ALT > AST	Ferritin, iron saturation, HFE gene testing	Family history
Wilson disease	Up to 2000 IU/L ALT > AST	Serum ceruloplasmin 24 h urinary copper collection Slit-lamp examination	Age <40 y Low serum AP —
α_1-antitrypsin deficiency	<100 IU/L	Serum α_1-antitrypsin level	Family history Presence of lung disease at young age
Infiltrative liver disease	<500 IU/L ALT > AST	Imaging Liver biopsy	—
Cirrhosis of any cause	<300 IU/L AST > ALT	—	Platelet count <150,000/μL Signs of portal hypertension

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HFE.

of hepatocytes, the membrane of bone osteoblasts, the brush border of small intestinal mucosal cells, the proximal convoluted tubules of the kidney, the placenta, and white blood cells.[2] Most AP in the serum is derived from the liver, bone, and intestine. Individuals with blood type O and B have been shown to have an elevation in serum AP after consumption of a fatty meal.[10] The level of serum AP also varies by age. Individuals older than the age of 60 were found to have higher serum AP levels compared with younger adults.[11] Woman in the third trimester of pregnancy can have elevated serum AP levels because of influx from the placenta.

The first step in the evaluation of an elevated serum AP level in asymptomatic patients is to determine the origin of the elevation. The most widely available and accepted approach is to measure the activity of serum GGT or 5′-NT, liver enzymes that are released in parallel to liver AP.[7] The most precise method to determine the

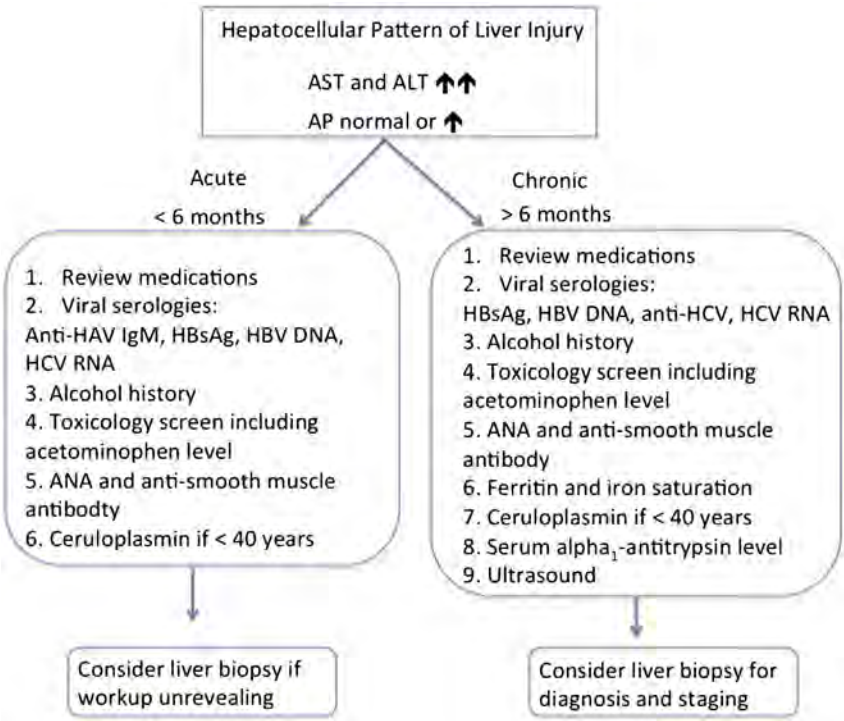

Fig. 3. Algorithm for evaluation of patients with hepatocellular pattern of liver injury. ANA, antinuclear antibodies; HAV, hepatitis A virus; HBsAG, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus.

source of AP is to fractionate AP isoenzymes by electrophoresis; however, this is not widely available in most laboratories.

Elevation in serum AP occurs when the hepatocyte canalicular membrane is disrupted, causing translocation from the canalicular membrane to the basolateral (ie, sinusoidal) surface of the hepatocyte and leakage into serum. The mechanism of the increase in AP is thought to be caused by the enhanced translation of messenger RNA (mRNA) of AP in hepatocytes, rather than the failure to excrete AP.[2] This seems to be mediated by the action of bile acids, which induce synthesis of the enzyme and may cause leakage into serum. Hence, in the setting of acute obstruction of the biliary tree caused by gallstones, serum AP may initially be normal as de novo synthesis is required, whereas marked elevation in aminotransferases may be seen.

Hepatocellular disease can lead to an increase in serum AP, which is generally less than three times the upper limit of normal. Thus, moderate elevations in AP are nonspecific and can be seen in viral hepatitis, chronic hepatitis, cirrhosis, congestive heart failure, and infiltrative diseases of the liver.

The pattern of liver injury can be characterized based on the ratio (R) of the serum ALT to AP (both expressed as multiples of the upper limit of normal): an R ratio of less than two indicating cholestatic, greater than five hepatocellular, and two to five as mixed cholestatic-hepatocellular injury.

The most common causes of cholestasis are listed in **Table 4**. Intrahepatic cholestasis is most commonly caused by medications, including certain antibiotics, antiepileptic drugs, and anabolic steroids. It can also be caused by sepsis or total parenteral

Table 4
Common causes of intrahepatic and extrahepatic cholestasis

Disease	Diagnostic Tests	Clinical Clues
Intrahepatic causes		
Primary biliary cirrhosis	Antimitochondrial antibody	Usually middle-aged women Presentation with fatigue or pruritus
Primary sclerosing cholangitis	MRCP or ERCP	Presence of ulcerative colitis
Infiltrative disorders	Imaging Liver biopsy	History of tuberculosis, sarcoidosis, amyloidosis, or malignancy Presentation with weight loss
Medication-induced injury	Improvement after medication discontinuation	Inciting medication
Sepsis	—	History of recent/current infection
TPN	—	TPN use
Extrahepatic causes		
Choledocholithiasis	Ultrasound ERCP or MRCP	History of biliary colic Acute onset of right upper quadrant pain, fever, or jaundice
Primary sclerosing cholangitis	ERCP	Presence of ulcerative colitis
Malignancy		
Pancreatic cancer Cholangiocarcinoma	Imaging (computed tomography or magnetic resonance imaging)	Presentation with jaundice and weight loss

Abbreviations: ERCP, endoscopic retrograde cholangiopancreatography; MRCP, magnetic resonance cholangiopancreatography; TNP, total parenteral nutrition.

nutrition. Several diseases cause injury to the small intrahepatic bile ducts, including primary biliary cirrhosis, primary or secondary sclerosing cholangitis, and infiltrative disorders. Infiltrative disorders of the liver, such as sarcoidosis, tuberculosis, lymphoma, amyloidosis, and metastatic disease to the liver are commonly associated with elevated alkaline phosphatase. Other causes are chronic liver allograft rejection (which leads to ductopenia) and infectious hepatobiliary disorders in patients with AIDS, such as cytomegalovirus or cryptosporidial infection (AIDS cholangioathy).

Causes of extrahepatic obstruction include benign and malignant conditions. Benign causes include choledocholithiasis and primary or secondary cholangitis, which may affect both the intrahepatic and extrahepatic biliary tree. Malignant causes include cholangiocarcinoma, pancreatic, and ampullary cancers.

Imaging of the liver with ultrasonography is indicated in the initial assessment of patients with a predominantly cholestatic pattern of liver enzyme injury to assess for the presence of biliary ductal dilatation. Dilated bile ducts suggest the presence of biliary obstruction and warrants further evaluation with additional imaging (magnetic resonance imaging, magnetic resonance cholangiopancreatography) or endoscopic retrograde cholangiopancreatography for diagnostic and possible therapeutic purposes.

Low levels of AP can present in patients with fulminant Wilson disease and is associated with hemolytic anemia.

γ-glutamyltransferase

GGT is an enzyme that catalyzes the transfer of the γ-glutamyl group of peptides, such as glutathione to other peptides or amino acids. GGT is present in the cell membranes of many tissues including the proximal renal tubule, liver, pancreas, intestine, and spleen.[3] In the liver, GGT is located primarily on biliary epithelial cells and on the apical membrane of hepatocytes. The predominant source of serum GGT is the liver. Entry of GGT into the serum may occur by solubilization and release of membrane-bound GGT or the death of biliary epithelial cells.[12]

Serum GGT is a sensitive indicator of the presence of injury to the bile ducts or liver. However, its use is limited by its lack of specificity, because many nonhepatic disorders can lead to elevation, including diabetes, hyperthyroidism, chronic obstructive pulmonary disease, and renal failure.[13] Alcohol abuse and certain medications, such as barbiturates or phenytoin, lead to induction of hepatic microsomal GGT.[14] The main clinical use of GGT is to confirm the hepatic origin of elevated AP levels, because GGT is not elevated in patients with bone disease.

5′ nucleotidase

5′-NT catalyzes the hydrolysis of nucleotides, such as adenosine 5′-phosphate and inosine 5′-phosphate, resulting in the release of free inorganic phosphate, which is most commonly measured by assays of its activity. 5′-NT is found in the liver, intestine, brain, heart, blood vessels, and pancreas.[2] In the liver, it is found bound to the canalicular and sinusoidal membrane of hepatocytes. Its activity parallels that of AP, which is likely a reflection of their similar location in the hepatocyte.[15] Most studies show that 5′-NT and AP have equal clinical use in the detection of hepatobiliary disease.[2] Like GGT, its clinical value lies in its ability to determine the origin of elevated serum AP levels, because its elevation in this setting strongly suggests a hepatic origin. The algorithm for the evaluating patients with a predominant elevation in AP is summarized in **Fig. 4**.

TESTS OF LIVER METABOLISM: TOTAL BILIRUBIN

Bilirubin is a naturally occurring pigment derived from the breakdown of heme-containing proteins. Most of the 250 to 300 mg of bilirubin produced each day is derived from the breakdown of hemoglobin in senescent red blood cells.[16] The remainder is derived from the premature destruction of erythroid cells in the bone marrow and from the turnover of heme-containing proteins in tissues in the body.[17] The liver has a high concentration of heme-containing proteins with high turnover rates, such as the cytochrome P-450 enzymes.

The formation of bilirubin occurs primarily in reticuloendothelial cells in the liver and spleen. The first step consists of the oxidation of heme by heme oxygenase to form biliverdin. The second reaction is the reduction of biliverdin by biliverdin reductase to form bilirubin. Unconjugated bilirubin is lipid-soluble and insoluble in water. Thus, to be transported in blood, unconjugated bilirubin must be bound to albumin, which occurs in a reversible, noncovalent fashion. Unconjugated bilirubin is thus not filtered by the kidney because it is always bound to albumin in the serum. Bilirubin is then transported to the liver, where it is taken up by hepatocytes by carrier-mediated membrane transport. In the hepatocyte, it is bound to glutathione-*S*-transferases. Bilirubin is then conjugated by a family of enzymes called uridine diphosphoglucuronosyltransferase (UDP-glucuronosyltransferase). Conjugated bilirubin is water-soluble and thus may be excreted by the kidney. Conjugated bilirubin is transported across the canalicular membrane into bile by active transport against a concentration using ATP. This is the rate-limiting step in bilirubin excretion.[2]

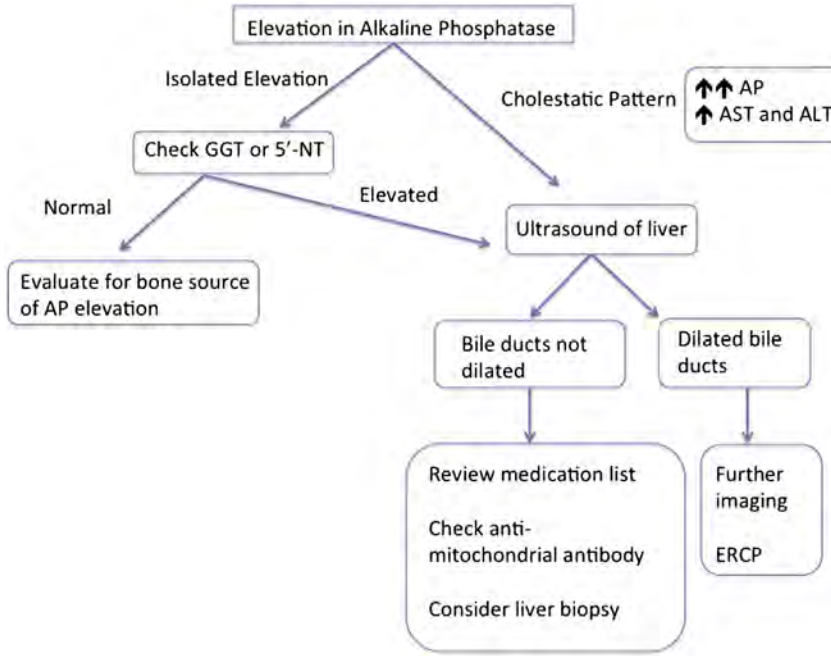

Fig. 4. Algorithm for evaluation of patients with elevated alkaline phosphatase. ERCP, endoscopic retrograde cholangiopancreatography.

The terms direct and indirect bilirubin originated from the van den Bergh method of measuring bilirubin concentration.[18] In the assay, bilirubin reacts with diazotized sulfanilic acid and divides into two dipyrrl azopigments that absorb light maximally at 540 nm. The direct fraction reacts with diazotized sulfanilic acid in 1 minute in the absence of alcohol, and provides an estimate of the concentration of conjugated bilirubin in the serum. The total serum bilirubin concentration is then ascertained by the addition of alcohol and determination of the amount that reacts in 30 minutes. The indirect fraction is thus calculated as the difference between the total and direct bilirubin concentrations. Normal total serum bilirubin concentration is less than 1 mg/dL using the van den Bergh method of bilirubin measurement. The direct fraction comprises as much as 30% or 0.3 mg/dL of the total.

Newer techniques for the measurement of serum bilirubin use high-performance liquid chromatography. These techniques have revealed that almost all of serum bilirubin in healthy persons is unconjugated. Furthermore, it seems that there is a fraction of conjugated bilirubin that is covalently bound to albumin.[19] This fraction increases in patients with cholestasis and hepatobiliary disorders, when the excretion of conjugated bilirubin is impaired, resulting in increased serum concentration of conjugated bilirubin. This explains the prolonged elevation in bilirubin seen in patients recovering from hepatobiliary injury, because the clearance rate of bilirubin bound to albumin from serum is determined by long half-life of albumin (about 21 days) and not the shorter half-life of bilirubin (about 4 hours).[19] This also explains why bilirubinuria is not present in some patients with conjugated hyperbilirubinemia during the recovery phase of their illness.

The concentration of bilirubin in the serum is determined by the balance between bilirubin production and clearance by hepatocytes. Thus, elevated serum bilirubin

levels may be caused by (1) excessive bilirubin production, which occurs in states of increased red blood cell turnover, such as hemolytic anemias or hematoma resorption; (2) impaired uptake, conjugation, or excretion of bilirubin; and (3) release of unconjugated or conjugated bilirubin from injured hepatocytes or bile ducts.[7]

The presence of unconjugated hyperbilirubinemia (defined as direct bilirubin fraction <20%) is rarely caused by liver disease. It is primarily seen in hemolytic disorders, such as sickle cell disease or hereditary spherocytosis, or in setting of hematoma resorption. In general, the total serum bilirubin is less than 5 mg/dL in such cases. If hemolysis is ruled out, the most likely cause of mild elevation in indirect bilirubin in an otherwise asymptomatic patient is Gilbert syndrome. This is the result of a genetic defect leading to a mild decrease in the activity of UDP-glucuronosyltransferase. Total bilirubin is usually in the range of 2 to 4 mg/dL. Levels increase during times of fasting or stress. No further evaluation is indicated if Gilbert disease is suspected, because there are no clinical sequelae. Crigler-Najjar syndrome type 1 is a rare, autosomal-recessive disorder that results from near complete absence of UDP-glucuronosyltransferase and leads to severe unconjugated hyperbilirubinemia and kernicterus in newborns. Crigler-Najjar syndrome type 2 results from a milder form of UDP-glucuronosyltransferase deficiency, and patients are generally asymptomatic.

Unlike unconjugated hyperbilirubinemia, the presence of conjugated hyperbilirubinemia (and hence hyperbilirubinuria) almost always signifies the existence of liver disease. Both hepatocellular and cholestatic liver injury may lead to elevated serum bilirubin levels.[20,21]

There are rare inherited disorders in which bilirubin excretion into the bile is impaired, resulting in conjugated hyperbilirubinemia, namely Rotor syndrome and Dubin-Johnson syndrome. Both conditions have a benign clinical course. The algorithm for the evaluation of patients with hyperbilirubinemia is summarized in **Fig. 5**.

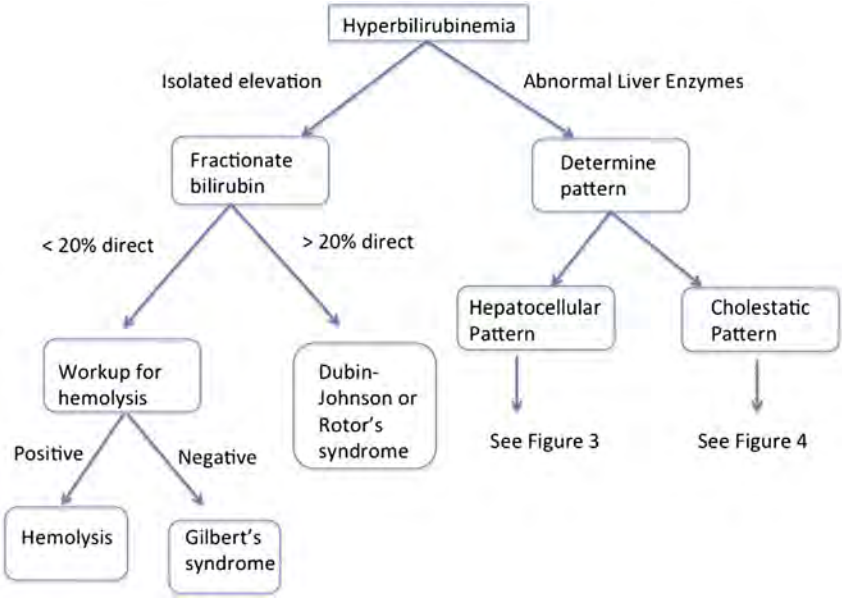

Fig. 5. Algorithm for evaluation of patients with hyperbilirubinemia.

TESTS OF LIVER SYNTHETIC FUNCTION

The liver is the exclusive site of synthesis of albumin and most coagulation factors. Thus, serum albumin and PT serve as true tests of hepatic synthetic function.

Serum albumin has a long half-life of about 21 days. About 4% is degraded per day. Because of its long half-life, serum albumin levels may not be affected in acute liver disease, such as acute viral hepatitis or drug-induced liver injury. In cirrhosis or chronic liver disease, low serum albumin may be a sign of advanced liver disease. However, low serum albumin is not specific for liver disease, and may occur in other conditions, such as malnutrition, infections, nephrotic syndrome, or protein-losing enteropathy.[7]

PT/international normalized ratio (INR) measures the activity of coagulation factors II, V, VII, and X, which are all synthesized in the liver and dependent on vitamin K for synthesis. Coagulation factors have a much shorter half-life than albumin. Thus, PT/INR is the best measure of liver synthetic function in the acute setting. Prolongation of the PT to more than 5 seconds above the control value (INR>1.5) is a poor prognostic sign in liver disease, and an important factor in priority of liver transplantation in model of end-stage liver disease score.

Elevation in PT/INR is also a predictor of high mortality in patients with acute alcoholic hepatitis. Vitamin K deficiency also causes prolongation in PT, and is associated with poor nutrition, malabsorption, and severe cholestasis with inability to absorb fat-soluble vitamins. Administration of parental vitamin K can help distinguish vitamin K deficiency from hepatocyte dysfunction, because it results in the correction of PT in the case of vitamin K deficiency but not liver dysfunction.[2] **Table 5** summarizes the general pattern of liver enzymes and liver function tests seen in the different categories of hepatobiliary disease.

NONINVASIVE MARKERS OF FIBROSIS

Noninvasive tests of hepatic fibrosis have been studied extensively in many clinical trials. Most studies of serologic markers and radiologic tests have looked at the use of these tests for staging of fibrosis in patients with chronic liver disease.[22–24]

There are two general categories of noninvasive tests for fibrosis: serologic panels of tests and radiologic tests. These include indicators of cytolysis (AST, ALT); cholestasis (GGT, bilirubin); hepatocellular synthetic function (INR, cholesterol, ApoA1, haptoglobin, N-glycans); and hypersplenism caused by portal hypertension (ie, platelet count). The most studied panels are the AST to platelet ratio, FibroTest/FibroSure (Labcorp, Burlington, USA), Hepascore (Quest Diagnostics, USA), and FibroSpect (Prometheus Alb Inc., USA).

Radiologic methods for staging hepatic fibrosis are emerging as promising tools. The methods include ultrasound-based transient elastography and magnetic resonance elastography. Ultrasound-based transient elastography using a probe (FibroScan) is the most studied radiologic method for staging hepatic fibrosis. Fibroscan was approved by Food and Drug Administration in April 2013. Using FibroScan in United States becoming more popular as a reliable noninvasive method of assessing liver fibrosis.

APPROACH TO PATIENT EVALUATION AND DIAGNOSIS

The first step in evaluating patients found to have liver enzyme abnormalities is to take a careful and thorough medical history. Risk factors for viral hepatitis including travel history, sexual practices, illicit drug use, acquisition of tattoos, body

Table 5
Patterns of liver enzymes and liver function tests in various hepatobiliary diseases

Hepatobiliary Disorder	Aminotransferases	Alkaline Phosphatase	Bilirubin	Albumin	Prothrombin Time
Hepatocellular					
Acute Toxin/drug Viral Ischemic	↑↑↑ (>500 IU/mL)	Normal or ↑ to <3 times normal	↑	Normal	Usually normal ↑ to >5 seconds above control value portends poor prognosis
Chronic	↑↑ (<300 IU/mL)	Normal or ↑ to <3 times normal	Normal to ↑	Normal or ↓	Often ↑, will not correct with parenteral vitamin K administration
Cholestatic					
Acute	Normal to ↑↑↑	Normal to ↑	Normal to ↑	Normal	Normal
Chronic	Normal to ↑↑	↑↑↑ to >4 times normal	↑	Normal or ↓	Normal or ↑, will correct with vitamin K administration
Infiltrative	Normal to ↑	↑↑↑ to >4 times normal	Normal	Normal	Normal

piercings, occupational exposure, and history of blood transfusions before 1990 should be ascertained. The presence of prodromal symptoms, such as nausea, vomiting, abdominal pain, anorexia, malaise, fevers, or chills suggestive of acute viral hepatitis, should be elicited. A history of significant weight loss raises the possibility of malignancy. Inquiry into the development of jaundice, dark urine, or light stools is important. The acute onset of right upper quadrant pain, fever, or jaundice suggests biliary obstruction caused by gallstones. History of pruritus suggests cholestasis.

Clinicians should inquire about the consumption of alcohol, including frequency, quantity, and duration. A history of obesity, diabetes mellitus, or hyperlipidemia in the absence of significant alcohol consumption suggests the possibility of nonalcoholic fatty liver disease. Meticulous review of the patient's medication list and inquiry into the use of any over-the-counter or herbal medications or nutritional supplements should be performed. A history of inflammatory bowel disease raises the possibility of primary sclerosing cholangitis. Finally, a family history of liver disease is important because it suggests possible inherited liver disorders.

The physical examination is also an important diagnostic tool in the assessment of patients with suspected liver disease. The presence of scleral icterus or jaundice should be assessed. Hepatomegaly may be present in acute viral hepatitis, alcoholic hepatitis, infiltrative liver disorders, or severe congestive hepatopathy caused by heart failure. Temporal wasting and cachexia suggest advanced liver disease or malignancy. Clinicians should assess for stigmata of chronic liver disease, including spider angiomas, palmar erythema, and gynecomastia. Splenomegaly and caput medusa suggests the presence of portal hypertension. Signs of decompensated cirrhosis include the presence of ascites, jaundice, or asterixis/encephalopathy.

If an asymptomatic patient is found to have a first-time elevation in liver enzymes, it is reasonable to observe the patient if (1) no risk factors for liver diseases are identified; (2) the elevation in liver enzymes is mild (ie, less than twice the upper limit of normal); and (3) liver synthetic function is preserved.[4] Repeat testing should be performed within 3 months. If the liver enzymes remain elevated at 3 months, work-up for liver disease should be initiated based on the pattern of liver enzyme elevation as detailed previously.

Liver biopsy remains the gold standard for the grading of inflammation and staging of fibrosis in chronic liver disease, and plays an important role in determining the need for treatment and assessing response to therapy.

SUMMARY

The routine use of serum biochemical tests allows for the detection of acute and chronic liver injury before the onset of symptoms. These tests consist of markers of hepatocellular injury (aminotransferases and APs); tests of liver metabolism (total bilirubin); and tests of liver synthetic function (serum albumin and PT). Noninvasive tests for assessment of liver fibrosis are promising tools for diagnosis and prognosis of patients with chronic liver disease. A comprehensive history, physical examination, and assessment of pattern of liver injury with additional laboratory and imaging testing establish the cause of hepatobiliary disease in most cases.

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