

Diagnosis and Monitoring of Hepatic Injury. II. Recommendations for Use of Laboratory Tests in Screening, Diagnosis, and Monitoring

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Purpose: To review information on the use of laboratory tests in screening, diagnosis, and monitoring of acute and chronic hepatic injury.

Data Sources and Study Selection: A MEDLINE search was performed for key words related to hepatic diseases, including acute hepatitis, chronic hepatitis, alcoholic hepatitis, cirrhosis, hepatocellular carcinoma, and etiologic causes. Abstracts were reviewed, and articles discussing use of laboratory tests selected for review. Additional articles were selected from the references.

Guideline Preparation and Review: Drafts of the guidelines were posted on the Internet, presented at the AACC Annual Meeting in 1999, and reviewed by experts. Areas requiring further amplification or literature review were identified for further analysis. Specific recommendations were made based on analysis of pub-

lished data and evaluated for strength of evidence and clinical impact.

Recommendations: Although many specific recommendations are made in the guidelines, only some summary recommendations are listed here. In acute hepatic injury, prothrombin time and, to a lesser extent, total bilirubin are the best indicators of severity of disease. Although ALT is useful for detecting acute and chronic hepatic injury, it is not related to severity of acute hepatic injury and only weakly related to severity of chronic hepatic injury. Specific tests of viral markers should be the initial differential tests in both acute and chronic hepatic injury; when positive, they are also useful for monitoring recovery from hepatitis B and C.
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This reports represents a continuation of the National Academy of Clinical Biochemistry Guidelines on Use of Laboratory Tests in the Diagnosis and Monitoring of Hepatic Injury. Part 1 (1) discusses performance characteristics for laboratory tests and also describes the methodology used to develop the guidelines. Table 2 in Part 1 outlines the codes used for characterizing the recommendations contained in both parts of the Guidelines.

Hepatocyte injury is common worldwide. Despite a decreased incidence of acute viral hepatitis since introduction of vaccines for hepatitis A and B and testing of the blood supply for hepatitis C, almost 100 000 cases occur annually in the United States alone, most of them undiagnosed. An estimated 1.5% of the US population is chronically infected with hepatitis B or C virus. Individuals with chronic hepatitis are at increased risk for cirrhosis and hepatocellular carcinoma (HCC),⁷ the main causes

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⁷ Nonstandard abbreviations: HCC, hepatocellular carcinoma; AST, aspartate aminotransferase (EC 2.6.1.1); ALT, alanine aminotransferase (EC 2.6.1.2);

of death from liver disease in Western countries. Chronic liver diseases are among the more common disorders seen by physicians. We present here guidelines for the use of laboratory tests in screening, diagnosis, and monitoring of acute and chronic hepatic injury.

Acute Hepatic Injury

Acute hepatic injury can be recognized by the presence of jaundice or nonspecific symptoms of acute illness accompanied by increases in the activities of aspartate aminotransferase (AST; EC 2.6.1.1) and/or alanine aminotransferase (ALT; EC 2.6.1.2). An estimated 80% of individuals with acute viral hepatitis are never diagnosed clinically, although some may be detected by increased aminotransferases in the face of nonspecific or absent clinical symptoms. AST and ALT activities are seldom >10 times the upper reference limit in liver diseases other than acute hepatic injury. Alkaline phosphatase (ALP; EC 3.1.3.1) is more than three times the upper reference limit in <10% of cases of acute hepatic injury (2, 3). With the decreasing incidence of acute viral hepatitis, other liver diseases are more commonly encountered as causes of increased AST or ALT activities; as many as 25% of those with AST >10 times the reference limit may have obstruction as a cause (4). Approximately 1–2% of patients with bile duct obstruction have transient increases in AST and/or ALT activities of >2000 U/L (5, 6); aminotransferase activities usually fall to within reference limits by 10 days even if obstruction persists (2, 4, 5). The distribution of direct bilirubin as a percentage of total bilirubin is similar in acute hepatic injury and obstructive jaundice (7). In patients with jaundice, only 16% of those with acute hepatic injury have direct bilirubin <50% of total bilirubin; values below this suggest another cause for jaundice, such as hemolysis (7). The best discriminant values for recognizing acute hepatic injury appear to be 200 U/L for AST (sensitivity, 91%; specificity, 95%) and 300 U/L for ALT (sensitivity, 96%; specificity, 94%) (3). AST is >10 times the upper reference limit in slightly over one-half of patients at the time of presentation (3). In uncomplicated alcoholic hepatitis, AST and ALT values are almost never >10 times the upper reference limit, the AST/ALT ratio is >2 in 80% of cases, and increased ALP is present in 20% of cases (8–12). Jaundice occurs in 60–70% of cases of alcoholic hepatitis (9, 10). The frequency of jaundice in patients with acute viral hepatitis differs both by age and etiologic agent. Jaundice is rare in children with viral hepatitis, and when it is present is less severe than in adults. In one study, only 1% of children with acute

hepatitis had peak bilirubin >171 $\mu\text{mol/L}$ (10 mg/dL), whereas 27% of adults did (13). In adults, jaundice develops in 70% of cases of acute hepatitis A (14), 33–50% of cases of acute hepatitis B (15, 16), and 20–33% of cases of acute hepatitis C (17, 18). In children, there is a direct correlation between age and peak serum bilirubin; an increase of 10 years in age is associated with an average increase of 85 $\mu\text{mol/L}$ (5 mg/dL) in bilirubin. In adults, however, there is only a slight increase or no increase with increasing age (7).

Recommendation:

- Acute hepatic injury can be diagnosed by ALT >10 times the upper reference limits and ALP <3 times the appropriate upper reference limit (IIB).

MARKERS OF SEVERITY

Acute viral hepatitis A and B are usually self-limited illnesses, and most patients recover completely. In those with acute hepatitis C infection, ~85% develop chronic hepatitis. Rarely, acute hepatic injury causes severe liver damage and acute liver failure. Testing should identify patients at highest risk for liver failure. Aminotransferase activities are related more to the cause of hepatic injury than to severity (Table 1). There is weak correlation between aminotransferase activities and bilirubin in viral hepatitis (3) and none in ischemic hepatic injury (19). Peak aminotransferase activities bear no relationship to prognosis and may fall with worsening of the patient's condition (20). Prothrombin time (PT) is the most important predictor of prognosis; PT cutoff times >4 s beyond control or >20 s, or an international normalized ratio >6.5 identify patients at high risk of death (20–24). In alcoholic hepatitis, PT >5 s beyond control, bilirubin >428 $\mu\text{mol/L}$ (>25 mg/dL), or albumin <25 g/L (2.5 g/dL) in a patient >55 years of age predicts 90% likelihood of death (10, 25). In ischemic or toxic hepatic injury, prolongation of PT is common early after injury; values peak by 24–36 h after injury and then rapidly return to normal. In acetaminophen injury, marked prolongation of PT does not by itself indicate likelihood of liver failure (26, 27), but a persistent increase in or increasing PT 4 days after acetaminophen ingestion does (28). Peak bilirubin is not a good indicator of prognosis in most forms of hepatic injury (20). Total bilirubin >257 $\mu\text{mol/L}$ (15 mg/dL) or PT >3 s above the upper reference limit (29) in patients with viral hepatitis

Table 1. Patterns of laboratory tests in types of acute hepatic injury.

Disease	Peak ALT, \times URL ^a	AST:ALT ratio	Peak bilirubin, mg/dL	PT prolongation, s
Viral hepatitis	10–40	<1	<15	<3
Alcoholic hepatitis	2–8	>2	<15	1–3
Toxic injury	>40	>1 early	<5	>5 (transient)
Ischemic injury	>40	>1 early	<5	>5 (transient)

^a \times URL, times the upper reference limit.

ALP, alkaline phosphatase (EC 3.1.3.1); PT, prothrombin time; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HBe, hepatitis B virus core antigen; HBsAg, hepatitis B virus surface antigen; HDV, hepatitis D virus; AIH, autoimmune hepatitis; HEV, hepatitis E virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBeAg, hepatitis B virus e antigen; ANA, anti-nuclear antibody; A1AT, α_1 -antitrypsin; NASH, nonalcoholic steatohepatitis; ASMA, anti-smooth muscle antibody; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AMA, anti-mitochondrial antibody; Pi, protease inhibitor; HGV, hepatitis G virus; TTV, TT virus; and AFP, α -fetoprotein.

indicates severe liver injury and mandates close monitoring for encephalopathy.

Recommendations:

- Total bilirubin $>257 \mu\text{mol/L}$ (15 mg/dL) or PT >3 s above the upper reference limit in an individual with viral hepatitis, in the absence of other factors affecting results, indicates severe liver injury (IIB).
- Direct bilirubin is needed to rule out other causes of increased total bilirubin, such as hemolysis, but it does not differentiate hepatic injury from obstructive jaundice (IIB).
- With acetaminophen toxicity, a persistent increase in or increasing PT >4 days after ingestion indicates severe liver injury (IIB).

DIFFERENTIAL DIAGNOSIS

Initial laboratory evaluation of patients with acute hepatic injury should include a drug history and testing for antibodies to the hepatitis A, B, and C viruses (HAV, HBV, and HCV), using the Health Care Financing Administration-approved acute hepatitis panel [IgM against HAV (IgM anti-HAV) and the HBV core antigen (IgM anti-HBc), HBV surface antigen (HBsAg), and antibodies

against HCV (anti-HCV); Fig. 1]. Most hepatic drug reactions occur within 3–4 months of initiating treatment. However, in some cases, hepatic injury may become manifest as late as 12 months after treatment is initiated, and in a few cases injury may become evident days to weeks after the responsible drug is stopped (30). Hence, it is important to ask about all drugs the patient may have received or has continued to receive during the past year or so.

IgM anti-HAV, the diagnostic test of choice for acute HAV infection, disappears by 4–6 months (31), whereas total HAV antibodies persist for life (32) and are found in a high percentage of the population (33). Because of its brief period of transmissibility, diagnosis of acute HAV infection should be made as soon as possible after presentation, ideally within 48 h, to allow immune globulin treatment of exposed individuals. IgM anti-HBc and HBsAg are the most reliable tests for acute HBV infection (29, 34); IgG (and thus total) anti-HBc persists for many years (35). Other HBV viral markers and antibodies are not of use in the diagnosis of acute HBV infection.

There currently is no test to definitively diagnose acute hepatitis C because anti-HCV and HCV RNA can be present in both acute and chronic HCV infections. Anti-

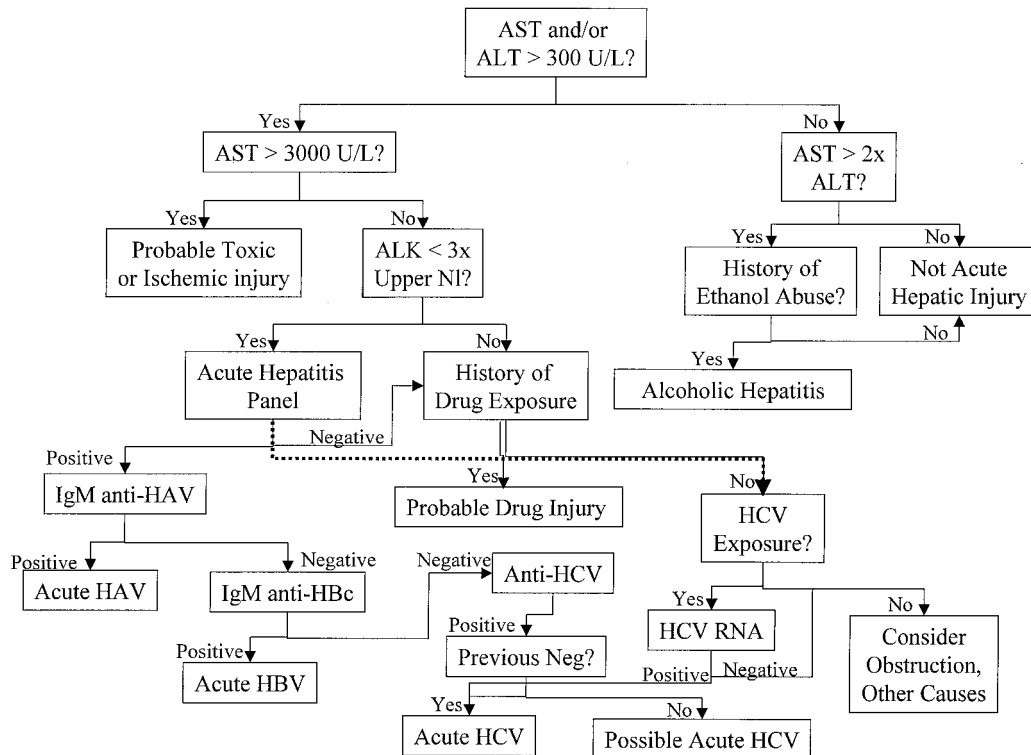


Fig. 1. Evaluation of suspected acute hepatic injury.

Initial evaluation of patients with signs and symptoms such as jaundice, fever, and right upper quadrant abdominal pain should be by measurement of aminotransferases. Marked increases (>3000 U/L) of either enzyme are usually attributable to ischemic or toxic liver injury; if history is negative for either, then the diagnostic workup should continue as in persons with smaller increases. Viral serologies are the principal tests for evaluation of acute hepatic injury, although the falling incidence of viral diseases has made other causes proportionally more common. Because both prescription and nonprescription drugs can cause acute injury, a detailed drug history is critical, particularly in those with increased ALP. In those with coexistent increases in ALP, obstruction and other viruses such as EBV and CMV must be considered as well. ALK, alkaline phosphatase; NI, normal.

HCV is detectable with second-generation enzyme immunoassays in only 57% of acute HCV cases at the time of initial increases in enzyme activities, whereas HCV RNA is positive in essentially all cases (36), although it is intermittently present in 15% (37,38). By the time of clinical presentation, 80–90% of cases have detectable anti-HCV (38). Patterns that would support a diagnosis of acute hepatitis C are negative anti-HCV but positive HCV RNA, or (if HCV RNA was not tested) anti-HCV results that convert from negative to positive within a short period. Use of antibodies to hepatitis D virus (anti-HDV) to detect delta (HDV) infection should be limited to patients with positive HBsAg, particularly if accompanied by severe acute hepatitis, high risk factors (e.g., intravenous drug abuse or hemophilia), or a biphasic pattern of illness (39). If a patient with chronic hepatitis B becomes superinfected with HDV, a clinical picture resembling severe acute hepatic injury and hepatic failure may evolve (39).

Recommendations:

- Initial evaluation of acute hepatic injury should include a detailed drug history and viral markers (IgM anti-HAV, IgM anti-HBc, HBsAg, and anti-HCV; IIB).
- Because of the need for postexposure prophylaxis, turnaround time of IgM anti-HAV should be <48 h (IIIC and IIIE).
- If cost-effective (based on prevalence), laboratories may use total antibody to HAV and anti-HBc initially, performing IgM antibodies only if one or both is positive, if the turnaround time needs can be met (IIIE).
- Diagnosis of acute HCV infection (in a patient with a clinical picture of acute hepatic injury) can be presumptively made by negative HAV and HBV markers, recent exposure, and either negative anti-HCV and positive HCV RNA or negative anti-HCV at initial presentation with development of positive anti-HCV within 1–3 months (IIIB).
- Testing for HDV should be limited to patients with positive HBsAg, atypical clinical course, and high risk for HDV infection (IIB and IIE).

WORKUP OF PATIENTS WITHOUT OBVIOUS CAUSE FOR ACUTE HEPATIC INJURY

Ischemic and toxic hepatic injury. AST or ALT values >100-fold higher than normal are rare in viral hepatitis (2,3) but common in both toxin ingestion, especially acetaminophen (27,40,41), and ischemic hepatic injury (19,26,42). In acetaminophen-induced hepatic injury, peak AST is >3000 U/L in 90% of cases (41), a value virtually never seen with acute viral hepatitis (3). Toxic or ischemic hepatic injury is the cause of >90% of cases of acute hepatic injury with AST activity >3000 U/L (43). In both ischemic and acetaminophen hepatic injury, AST and ALT activities typically peak early (often in the first 24 h after admission) with AST activity initially higher than that of ALT. After peaking, activities of both fall rapidly. AST may fall by 50% or more in the first 24 h (40,41), and it declines more rapidly than ALT because of its shorter half-life (3); AST activity reaches near normal values an average of 7 days after injury (2). PT is >4 s above the reference limits in 90% of cases (26,27) and falls rapidly after peak AST is reached (26). Bilirubin is <34 $\mu\text{mol/L}$ (2 mg/dL) in 80% of cases of toxic or ischemic injury (26,27,42). Lactate dehydrogenase (EC 1.1.1.27) activity often is higher than that of AST at presentation in toxic or ischemic hepatic injury (26,40,41), whereas it is increased on initial determination in only 55% of cases of viral hepatitis, with average values being only slightly above the upper reference limit (3). Rarely, cocaine may cause hepatic injury, usually in patients with coexisting hypotension (44).

Other causes. Rarely, Wilson disease and autoimmune hepatitis (AIH; discussed in more detail in *Chronic Hepatic Injury*) can present as acute hepatic injury (Table 2). Several viruses other than the classical agents [HAV, HBV, HCV, and hepatitis E virus (HEV)] have been associated with hepatitis, including herpesvirus, cytomegalovirus (CMV), enterovirus, coronavirus, reovirus (in neonates), adenovirus, parvovirus B6 (in pediatric populations), varicella-zoster virus, and Epstein-Barr virus (EBV). Syphilis, leptospirosis, and toxoplasmosis also

Table 2. Uncommon causes of acute hepatic injury.

Disorder	Key feature	Laboratory tests	Associated findings	References
Wilson disease	Young individuals; low ALP, high bilirubin	Low ceruloplasmin in only 40%; abnormal gene on chromosome 13	Urine, serum copper not reliable in acute Wilson disease; often associated with hemolysis and renal insufficiency	95, 96, 210–212
AIH	Young individuals; increased γ -globulins; low albumin; ascites often present	Positive ANA and/or ASMA	Other autoimmune disorders in some cases	103, 213–215
Hepatitis E	Travel to endemic area	Anti-HEV	Similar to hepatitis A	216–219
Other viruses	Clinical features of mononucleosis often present	Anti-CMV; anti-EBV	Increased ALP	220

may cause hepatic injury, as may other less common infectious agents. Rarely, other disorders, including lymphoma, Budd-Chiari syndrome, and venoocclusive disease, may present with a picture of acute hepatic injury. In general, hepatic injury associated with these etiologies either is unusual or is associated with a specific syndrome (chicken pox with varicella-zoster virus, mononucleosis with EBV, or CMV). Most patients with other infectious causes of hepatic injury have signs and symptoms that suggest a particular agent as the cause. Specific diagnosis of infection by other agents should be pursued when the etiology remains unknown after more common causes are excluded and when establishment of a specific diagnosis appears clinically indicated. Superinfection with other hepatitis viruses may occur in a patient with other forms of hepatic injury; for example, patients with chronic HCV or alcoholic hepatitis may become infected with either HAV or HBV and develop an acute hepatitis as a result of the superimposed infection. In chronic hepatitis, an acute increase in aminotransferases mimicking acute hepatic injury can occur with clearance of HBV e antigen (HBeAg) (45) or with emergence of quasispecies of HCV (46).

Recommendations:

- In patients with negative viral markers and initial AST >100 times the upper reference limit, toxic exposure or ischemia should be suspected (IIB).
- In patients with negative viral markers and enzyme concentrations 8–100 times the upper reference limit, testing must exclude the possibility of Wilson disease and AIH (IIB).
- Testing for antibody to hepatitis E is not recommended in the United States unless other viral serologies are negative and there is a history of recent travel to an endemic area (IIIE).
- Tests for other infectious agents (EBV, CMV, syphilis, toxoplasmosis) may be used if no other causes are evident (IIB).

MONITORING

Aminotransferases. Aminotransferase activities tend to increase before and peak near onset of jaundice in viral hepatitis, falling gradually from that point onward (47). Activities tend to fall slowly in viral hepatitis and alcoholic hepatitis: AST and ALT decrease, on average, 11.7% and 10.5% per day, respectively, and remain increased 22 ± 16 and 27 ± 16 days, respectively (3). In hepatitis A, a secondary increase in enzymes occurs in 5–10% of cases before activities return to baseline and is associated with circulating HAV RNA and viral particles in stool, indicating a potential for transmission of infection (48–50). As discussed above, AST and ALT fall rapidly after reaching peak activities in ischemic and toxic hepatic injury. In all forms of hepatic injury, decreasing activities occur both with recovery and with massive necrosis, making enzyme activity a poor indicator of recovery (20). Once amino-

transferases have shown a consistent pattern of decrease, they need not be checked again until the patient has clinically recovered. Return of aminotransferases to normal is not a reliable sign of recovery in hepatitis B or C. In patients with chronic HCV infection, 49% with normal ALT at the initial visit after seroconversion developed increased ALT on subsequent follow-up (51). In hepatitis B, AST and ALT may return to normal despite persistence of infection (52, 53).

Bilirubin. Bilirubin peaks later than aminotransferases, often by a week or so, and then gradually decreases. Peak bilirubin >257–342 $\mu\text{mol/L}$ (15–20 mg/dL) is unusual in viral hepatitis. Only 10–12% of patients with viral hepatitis have peak values >257 $\mu\text{mol/L}$ (15 mg/dL), and only 4% have peak values >342 $\mu\text{mol/L}$ (20 mg/dL); higher bilirubin is more common in HBV infection (3, 13). As total bilirubin declines, the proportion of δ -bilirubin increases, often reaching 70–80% of total bilirubin (54, 55). In adults with viral hepatitis, bilirubin remains increased 30.3 ± 19.7 days after peak concentrations are reached (3), but it clears more quickly in children (8); jaundice remains longer than 6 weeks in 34% of adult HBV cases but in only 15% of other forms of viral hepatitis (8). A prolonged increase in conjugated bilirubin occasionally occurs with viral hepatitis, particularly with HAV, but it does not signify a poor prognosis if synthetic function remains intact (56). Significantly increased bilirubin is uncommon in toxic and ischemic hepatic injury. Once serum bilirubin has begun to decrease, there is no reason to measure it again unless jaundice worsens clinically.

Coagulation tests. Increased PT is a common finding in ischemic and toxic hepatic injury, often with results >15 s or 4 s above the reference limit before they rapidly return to normal. There are no data on the extent to which the increase affects prognosis in ischemic hepatic injury. An increase in PT to >15 s or >4 s above reference limits in viral or alcoholic hepatitis is a marker of more severe disease (8, 10, 57).

Recommendations:

- PT >4 s above reference limits, bilirubin >257 $\mu\text{mol/L}$ (15 mg/dL), or development of encephalopathy identifies high-risk patients who require close monitoring and consideration of referral to a gastroenterologist or hepatologist (IIB).
- In patients with acute hepatitis B, repeat HBsAg measurements should be performed within 6–12 months; if negative and tests for anti-HBV surface antigen antibody (anti-HBs) are positive, no additional follow-up is needed (IIIE).
- In patients with acute hepatitis C, ALT should be measured periodically over the next 1–2 years to assure continued normal results (IIB).

Chronic Hepatic Injury

Chronic hepatic injury is a relatively common disorder with minimal symptoms but long-term risk of significant morbidity and mortality. It is defined pathologically by ongoing hepatic necrosis and inflammation of the liver, often accompanied by fibrosis. Chronic hepatic injury may progress to cirrhosis (15–20% in the case of chronic HCV) and predisposes to HCC. Most commonly, it is the result of chronic viral infection. In the United States alone, there are an estimated 2.1–2.7 million people chronically infected with HCV (58). There are also ~1–1.25 million chronic carriers of HBV in the United States. Although prevalence rates for HCV infection generally are between 0.5% and 5% in other parts of the world, prevalence rates for HBV vary markedly, and in many areas HBV is an endemic infection. The prevalence of endemic HBV in children is declining in many parts of the world because of the use of HBV vaccine. Clinical findings and laboratory investigation often are adequate to establish the most likely diagnosis, with a predictive value of 88% for alcoholic hepatitis and 81% for chronic viral hepatitis (before availability of HCV tests) compared with biopsy (59).

Recommendations:

- In the absence of liver biopsy showing chronic hepatitis, one of the following clinical definitions should be used to diagnose chronic hepatitis:
- Persistence of increased ALT for >6 months after an episode of acute hepatitis; or
- Increased ALT (without another explanation) on more than one occasion over a period of 6 months. A shorter time may be appropriate in patients with risk factors for chronic viral hepatitis, genetic causes of hepatic injury, or autoimmune liver injury, or in the presence of clinical signs or symptoms of liver disease (IIB).

Although the definition of chronic hepatic injury by increased ALT is widely accepted, 15–50% of patients with chronic hepatitis C have persistently normal ALT (51, 60–62). The likelihood of continuously normal ALT decreases with increasing number of measurements; even after three normal ALT values, 11% of patients with chronic HCV viremia subsequently developed persistently increased ALT (51). ALT often fluctuates between normal and abnormal, particularly in chronic hepatitis C; 60% of patients with multiple ALT measurements have at least occasional normal ALT values (D.R. Dufour, unpublished observations). The majority of patients with persistently normal ALT have histologic evidence of chronic hepatitis on biopsy but, in general, have milder inflammation, less fibrosis, and lower rates of progression to cirrhosis than do HCV patients with increased ALT (17, 61). Center for Disease Control and Prevention guidelines do not recommend treatment of patients with HCV and persistently normal ALT (63). Although long-term studies are needed, it appears that the clinical definition

proposed will not miss a significant group of patients who require and benefit from treatment.

It is not always possible to distinguish acute from chronic hepatic injury. Most patients with chronic hepatitis C (the most common form of chronic hepatic injury) have ALT values one to four times the upper reference limit, and 90% have maximum ALT less than seven times the upper reference limits, values lower than typically seen in acute hepatitis. In ~5% of cases, however, peak ALT may be >10 times the upper reference limit, often associated with jaundice, in a pattern similar to that seen in acute hepatic injury (D.R. Dufour, unpublished observations). In such cases, it often is necessary to do additional testing to rule out another cause of acute hepatic injury.

SCREENING

General screening of the population for chronic hepatic injury is not cost-effective and should be limited to high-risk individuals (64). These include those with a family history of genetic diseases known to affect the liver, as discussed below, or risk factors for chronic viral infection (Table 3). ALT is consistently higher than AST with all causes of chronic hepatic injury except for alcohol; AST is normal in a substantial number of cases. ALT may be normal in patients with cirrhosis, whereas AST remains increased (65–68). Total and direct bilirubin and ALP are normal in essentially all patients and are not useful in screening (59, 69–72). If increased ALT is found on routine testing, this should be confirmed by repeat testing before further evaluation. A minority of individuals with only one increased ALT value are found to have liver disease (70, 73). Patients with slightly increased ALT (one to two times the upper reference limit) are more likely to have a transient increase not attributable to disease (59, 71, 73). Approximately 30% of patients with chronic hepatic injury attributable to HCV have peak ALT activities less than two times the upper reference limit

Table 3. Risk factors for chronic viral hepatitis.^a

Established risk factors
• Injection drug use
• Chronic hemodialysis
• Blood transfusion or transplantation prior to 1992 (HCV)
• Receipt of blood (including needlestick) from a donor subsequently testing positive for HCV
• Receipt of clotting factor concentrates produced before 1987
• Asian ancestry (HBV)
• Unvaccinated healthcare workers (HBV)
• Birth to mother with chronic HBV or HCV
Possible risk factors
• Body piercing or tattooing
• Multiple sexual partners or sexually transmitted diseases
• Healthcare workers (HCV)
• Contacts of HCV positive persons

^a From CDC (63).

(D.R. Dufour, unpublished observations). Because ALT is also found in skeletal muscle, it is advisable to consider history of exercise and, if positive, to consider measurement of creatine kinase (EC 2.7.3.2) to rule out skeletal muscle as the origin of ALT (73, 74).

In patients with risk factors for chronic HBV or HCV infection (Table 3), ALT will not identify all infected individuals; HBsAg and anti-HCV should be measured to screen for chronic infection. Chronic "carriers" of HBV typically have normal ALT (53), and 15–30% of patients with chronic HCV infection have persistently or intermittently normal ALT; however, the likelihood of only normal values decreases with frequency of testing (75). Because 15–25% of individuals with anti-HCV have no detectable viremia, persons with positive anti-HCV should have qualitative HCV RNA performed to identify those with persistent infection. HCV RNA may be transiently present in the early stages of infection. If a patient has persistently increased ALT and positive anti-HCV, but negative HCV RNA, the test should be repeated.

Recommendations:

- Screening for chronic hepatitis is recommended in asymptomatic high-risk individuals (IIB and IIE).
- ALT is the most cost-effective screening test for metabolic or drug-induced liver injury; AST should also be measured with history of alcohol abuse (IIB and IIE).
- Specific viral serologies (HBsAg and anti-HCV), as well as ALT, should be used in individuals at high risk for viral hepatitis (IB).
- Confirmation of chronic HCV infection in an anti-HCV-

positive individual should be made by HCV RNA tests; if negative and ALT is increased, HCV RNA should be repeated (IIB).

DIFFERENTIAL DIAGNOSIS

If the clinical history suggests alcohol abuse and/or AST activity is greater than ALT (especially if more than twofold higher than ALT activity), the most likely diagnosis is alcoholic hepatitis. Virtually no other form of chronic hepatic injury causes AST to be higher than ALT unless cirrhosis develops (69–72). Although the majority of cases of chronic hepatic injury are caused by viruses, drugs, or ethanol, several other disorders may produce chronic hepatic injury. Additional tests are not needed if initial evaluation is consistent with hepatitis B or C or alcoholic hepatitis (71, 76). Prescription drugs may cause persistently increased ALT, most commonly with drugs such as sulfonamides, cholesterol-lowering agents, and isoniazid (30). In one study from an area with a low prevalence of viral hepatitis, a history of prescription drug use was common in those with chronic hepatic injury and no recognizable etiology despite extensive laboratory testing (77). In patients with increased ALT, negative viral markers, and a negative history for drug or alcohol ingestion, the workup should include less common causes of chronic hepatic injury (Table 4).

Recommendations:

- Initial evaluation should include a detailed drug history along with measurement of HBsAg and anti-HCV. If anti-HCV is positive, chronic infection should be con-

Table 4. Other causes of chronic increases of ALT and/or AST.

Cause	Key features	Screening test	Confirmatory test	References
NASH	Most common cause other than viral, alcoholic	None	Biopsy	59, 68, 69, 78
Hemochromatosis	Autosomal recessive trait; 1:200 among Northern European ancestry	Transferrin saturation 45%	HFE gene analysis for C282Y mutation	83–88, 90, 93
Wilson disease	Autosomal recessive trait; 1:30 000 individuals; hemolytic anemia, renal injury	Low ceruloplasmin in 65–95% of homozygotes, 20% of heterozygotes	Genetic analysis; low serum copper, high urine copper	95–100
AIH	Up to 18% of non-viral hepatitis, mainly in young women; increased γ -globulins	ANA and ASMA; false-positive anti-HCV common	Biopsy	101–105
PBC	Middle-aged women; usually mainly increase of ALP, often associated with Sjogren syndrome	AMA	Biopsy	106–110
Sclerosing cholangitis	Young to middle-aged men; usually mainly increase in ALP; often associated with inflammatory bowel disease	Anti-neutrophil cytoplasmic antibodies; ASMA, ANA may also be positive	Bile duct imaging	111–114
A1AT deficiency	Autosomal recessive trait; 1:1000 to 1:2000; controversial whether it causes chronic liver disease in adults	A1AT phenotyping		115–123

firmed by qualitative HCV RNA measurement (IIB and IIE).

- With persistently increased ALT and negative viral markers, the workup should include anti-nuclear antibodies (ANAs) and iron and iron-binding capacity (or unsaturated iron-binding capacity; IIB).
- In patients under age 40, ceruloplasmin should also be measured (IIB).
- In patients negative for these markers, α_1 -antitrypsin (A1AT) phenotype may be of use (IIB).
- If these tests are negative or inconclusive, diagnostic liver biopsy should be performed (IIB).

WORKUP OF PATIENTS WITHOUT OBVIOUS CAUSE FOR CHRONIC HEPATIC INJURY

Nonalcoholic steatohepatitis (NASH). The occurrence of chronic liver disease histologically resembling alcoholic hepatitis in patients without alcohol abuse has been termed NASH. It is the most common cause of chronic hepatic injury other than viruses and alcohol and the most common cause of cryptogenic cirrhosis (59, 69, 70, 78). Although NASH occurs most commonly in middle-aged women with obesity and/or diabetes, it also occurs in men and in patients without these risk factors (78). Patients with NASH commonly have abnormal lipid profiles, although normal results do not rule out this disease. It differs from alcoholic hepatitis in that the ALT activity is higher than AST (except in patients with cirrhosis) (79–81). Weight loss may cause significant improvement in enzyme results; in one study, a 1% reduction in weight produced a 8.1% decrease in ALT activity (82).

Recommendation:

- Biopsy is necessary to establish the diagnosis of NASH (IIB).

Hemochromatosis. An autosomal recessive trait, hemochromatosis is the most common inherited genetic defect in persons of northern European ancestry (~1:200 to 1:300 in the United States) (83). The vast majority of cases are produced by one of two point mutations of the *HFE* gene on chromosome 6. The majority (60–90%) of affected individuals are homozygous for the C282Y (845A) mutation, whereas a minority has compound heterozygosity for this mutation and the H63D (187G) mutation (84, 85). Screening involves detection of increased transferrin saturation (saturation = serum iron \times 100/total iron-binding capacity) (86) or low unsaturated iron-binding capacity (87). A transferrin saturation cutoff of $\geq 45\%$ or unsaturated iron-binding capacity cutoff $\leq 28 \mu\text{mol/L}$ (155 $\mu\text{g/dL}$) has a sensitivity of 90–100% for homozygosity for the C282Y mutation; if fasting specimens are used, the specificity is 43% (88, 89). A recent consensus conference recommended that definitive diagnosis be made by genetic analysis (90). Although several recent publications

have shown the feasibility of hemochromatosis screening using transferrin saturation, most organizations and researchers do not currently recommend screening because of unresolved issues regarding their ability to convince young adults to be tested, the specificity and reproducibility of screening tests, and questions about natural history of untreated disease (90–92). Screening has been advocated by the College of American Pathologists (93), and has been estimated to save \$3.19 per blood donor screened (94).

Recommendations:

- Initial evaluation for hemochromatosis should be by fasting serum transferrin saturation or unsaturated iron-binding capacity (IIB).
- Transferrin saturation $\geq 45\%$ or unsaturated iron-binding capacity $\leq 28 \mu\text{mol/L}$ (155 $\mu\text{g/dL}$) should be followed by analysis for *HFE* gene mutations (IIB).
- Screening of the population may be beneficial but is not currently recommended pending clarification of screening benefits (IIB and IIE).

Wilson disease. An autosomal recessive disorder, Wilson disease occurs in ~1 in 30 000 individuals in Europe and North America. It is caused by a mutation of a gene on chromosome 13 coding for an ATPase needed for copper transport (95). Wilson disease may present as liver disease, neurologic problems, or with psychiatric symptoms, almost always before age 40. Most patients who present with liver disease do not have neurologic manifestations (96). The most common diagnostic finding is low plasma ceruloplasmin. Low concentrations also occur with malnutrition, protein loss, and advanced liver disease, and falsely normal values can occur with pregnancy, estrogen administration, and acute inflammation (97). Most references report low ceruloplasmin in 95% of homozygotes and 20% of heterozygotes (97–99). One study found normal ceruloplasmin in 35% of patients with chronic liver disease attributable to Wilson disease (confirmed by genetic studies in 80%) but in only 15% of patients with Wilson disease without overt liver involvement (96). Other expected findings in Wilson disease include increased serum free copper, decreased total serum copper, increased urine copper excretion, and increased liver copper content. These tests may also provide misleading results in patients with Wilson disease (96, 100). Multiple tests frequently are needed to establish the diagnosis.

Recommendations:

- Testing for Wilson disease with ceruloplasmin is indicated in patients under age 40 with chronic hepatic injury or fatty liver and negative workup for viral hepatitis, drug-induced liver injury, and hemochromatosis (IIB).
- Screening for Wilson disease in all patients with chronic hepatic injury is not indicated (IIB and IIE).

- Genetic marker testing may be useful in equivocal cases, but testing must be able to detect multiple mutations in the Wilson disease gene (IIIB).

AIH. AIH is responsible for up to 18% of chronic hepatitis not attributable to viruses or alcohol (101). Several variants of AIH have been described (102). Type 1, found primarily in young and middle-aged women, is the most common form; it is associated with high titers of ANA and/or anti-smooth muscle antibody (ASMA). Type 2, found primarily in children, is common in western Europe but rare in the United States; it is associated with antibodies to liver-kidney microsomal antigen, but rarely with positive ANA or ASMA. Many patients with type 2 also have HCV infection. Type 3, found primarily in young women, is associated with systemic autoimmune disease in many cases. Most affected individuals lack ANA, ASMA, or anti-liver-kidney microsomal antibodies, but are positive for antibodies for soluble liver antigen. Standardized diagnostic criteria and a scoring system have been defined by an international panel (103). The classic features of the most common type 1 include increased aminotransferases; minimal or no increases in ALP; polyclonal hypergammaglobulinemia (at least 1.5 times the upper reference limit); no evidence of viral infection, risk factors for viral infection, or exposure to drugs or alcohol; and positive ANA or ASMA (at least 1:80) (103). Approximately 40% of patients with chronic HCV infection have a positive ANA or ASMA, usually in low titers (102). False-positive anti-HCV results have been reported in 60% of patients with AIH when second-generation tests are used, and in 20% when third-generation assays are used (104); anti-HCV typically disappears with successful treatment (105). In equivocal cases, HCV RNA (or a recombinant immunoblot assay) can be used to establish the diagnosis (104).

Recommendations:

- AIH should be suspected in patients with chronic hepatic injury and increased immunoglobulins and absence of viral markers or risk factors for viral hepatitis (IIIB).
- The diagnosis of type 1 AIH can be clinically supported by positivity for either ANA or ASMA in high titers (IIIB).

Primary biliary cirrhosis and primary sclerosing cholangitis. Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are autoimmune diseases that cause destruction of bile ducts. Although PBC and PSC characteristically cause increases in ALP and γ -glutamyltransferase (EC 2.3.2.2), patients with these diseases may have increased AST and ALT and be considered to have chronic hepatitis. PBC is associated with destruction of intrahepatic bile ducts; it is often associated with other autoimmune disorders, particularly Sjogren syndrome

(up to 80% of cases) (106). An autoimmune marker, anti-mitochondrial antibody (AMA), is found in almost all patients with PBC; although other diseases may be associated with positive AMA, in PBC the antibody is directed against the pyruvate dehydrogenase complex (so-called M2 type of AMA), particularly to dihydrolipoamide acetyltransferase (E2) and E3-binding protein (107). Approximately 5–10% of patients have features of both PBC and AIH (108, 109). PBC often is detected in asymptomatic individuals by a finding of increased ALP. AST and ALT are increased in approximately one-half of cases, although values are more than two times the reference limit in 20% (110). PSC is associated with damage to both intra- and extrahepatic bile ducts; 70% of cases are associated with inflammatory bowel disease (Crohn disease or ulcerative colitis) (111). Perinuclear anti-neutrophil cytoplasmic antibodies are found in approximately two-thirds of all cases (112). In PSC, antibodies commonly are directed against bactericidal/permeability-increasing protein, cathepsin G, and/or lactoferrin. There appears to be no prognostic significance to the different antibody specificities, although patients with cirrhosis more commonly have antibody to multiple antigens and to antigens other than lactoferrin (113). ASMA and ANA are also present in up to 70% of cases (114).

Recommendations:

- PBC or PSC should be suspected in patients with chronic cholestasis (IIIB).
- The diagnosis can be clinically supported by positivity for AMA (PBC) or anti-neutrophil cytoplasmic antibodies (PSC) in high titers (IIIB).

A1AT deficiency. A1AT is the most important protease inhibitor; congenital deficiency occurs in ~1 in 1000 to 1 in 2000 persons of European ancestry. The gene for A1AT is located on chromosome 14 (115); deficiency is usually attributable to a single amino acid substitution that alters carbohydrate binding and impairs release from hepatocytes (116). The most important deficiency involves homozygosity for the Z variant, termed Pi (for protease inhibitor) ZZ. Deficiency is associated with emphysema and neonatal hepatitis (117); chronic hepatic injury with cirrhosis and HCC (118) have also been reported. Almost all Pi ZZ neonates have evidence of liver injury at birth; this usually resolves by age 12 years (117). In adults, 50% of Pi Z-positive individuals (either homozygotes or heterozygotes) develop cirrhosis, and 31% develop HCC (116). There is also an excess of Pi Z heterozygotes among patients referred for liver transplants, particularly among patients with cryptogenic cirrhosis, where ~25% of patients are Pi Z positive (119). There is evidence, however, that A1AT deficiency or heterozygosity for Pi Z phenotype may not directly cause liver disease, but increase susceptibility to liver damage by other agents, especially viruses. Two controlled studies found the same frequency of Pi Z (either homozygous or heterozygous) in

patients with liver disease and controls (120, 121). In a study of 164 patients with Pi Z, 40% had chronic liver disease; 87% were also positive for HCV antibodies or HBV markers, and only 11% had no other liver disease risk factors (122). Because A1AT is an acute phase reactant, quantitative concentrations may be falsely normal with infection or inflammation, and falsely low concentrations may occur with malnutrition, protein-losing states, or end stage liver disease. In one study, quantitative concentrations were normal in 42% of heterozygous Pi Z patients with liver disease (123). Testing for A1AT deficiency should use phenotype analysis rather than quantitative plasma concentration (116).

Recommendations:

- Testing for A1AT deficiency may be of benefit in patients with chronic hepatic injury and no other apparent cause, although the role of A1AT deficiency in liver disease in adults is not clearly defined (IIB).
- Testing is especially important in neonates with evidence of hepatic injury (IIB).
- Testing for A1AT variants should be performed by determination of phenotype (IIB).
- Screening patients with chronic hepatic injury for A1AT deficiency is not recommended (IIIB and IIIE).

OTHER VIRUSES

Two other viruses have been suggested as possibly involved in the pathogenesis of chronic hepatitis: hepatitis G (HGV) and TT virus (TTV). Both viruses can be transmitted by transfusion, and chronic viremia is present with both. To date, evidence has suggested that infection with these viruses is common, but there is no clear indication that they play a role in liver injury. HGV (and the related GB virus type C) are members of the flavivirus family, as is HCV. HGV was first isolated from patients following transfusion, although most showed no evidence of liver injury (124, 125). HGV can also frequently be found in chronic hepatitis (126), but it does not appear to be a common cause of cryptogenic chronic liver disease (127). This may be because HGV RNA is rarely found in the liver in chronically viremic patients (128). TTV was first identified in patients with posttransfusion hepatitis (129). TTV DNA is found in 1–7% of blood donors in the United States (130, 131). The presence of TTV DNA is no more common in persons with acute non-A-E hepatitis than in other causes of acute hepatitis or in control patients (131, 132).

Recommendation:

- Testing for HGV or TTV, in other than a research setting, is not recommended (IIIE).

MONITORING

Although ALT is the most clinically used laboratory test for monitoring liver injury, there often are considerable fluctuations in enzyme activities over time (particularly in

chronic HCV infection) (60, 61). It is important to measure ALT repeatedly in chronic HCV before concluding that ALT is normal (75); 43% of chronically infected individuals have ALT values fluctuating between normal and abnormal, and 16% of those with normal ALT on their first two visits and 11% of those with normal ALT on their first three visits subsequently develop increased ALT (51). In patients with chronic HBV infection without increased ALT (chronic carriers), ~10% will develop increased ALT on follow-up (53); ALT should therefore be measured periodically even if initially normal.

With both chronic HBV and HCV, clearance of viral markers is the most reliable method for detecting resolution of infection. In untreated hepatitis B, a small percentage of patients spontaneously clear viral antigens; in long-term studies, loss of HBeAg occurred in one-third to one-half of patients (45, 133, 134). In those that lose HBeAg, 5–10% will subsequently clear HBsAg over 10 years of follow-up (53, 135). HBeAg should be rechecked periodically if initially positive. If HBeAg is negative and anti-HBV e antigen antibody (anti-HBe) is positive, this may indicate either the beginning of viral clearance from the body or integration of HBV DNA into host DNA and loss of ability to form replicating virus. HBsAg and anti-HBs should be measured periodically to look for viral clearance because HBsAg will remain positive in those with integration of HBV DNA. In treatment of HBV, the likelihood of viral clearance is related to baseline ALT activity; patients with increased ALT are more likely to respond than those with initially normal ALT activity (136, 137). Successful treatment is associated with loss of HBV DNA, HBsAg, and HBeAg.

Although there is evidence that quantitative HBeAg correlates well with HBV DNA (138–140), quantitative HBeAg assays are not commercially available. HBeAg may disappear even in patients who show no response to therapy (141). Moreover, there is an increasing frequency of “pre-core” mutants that cannot produce HBeAg, particularly in endemic areas in Asia and the Mediterranean region (142). Patients infected with such mutants have anti-HBe but continue to have circulating HBV DNA. In infection with normal strains, HBV DNA remains detectable longer than does HBsAg in recovery (143). When viral DNA integrates into the host genome, HBsAg is still produced, although HBeAg and HBV DNA commonly are negative in plasma (144, 145). With lamivudine treatment, however, production of viral nucleic acid through reverse transcriptase is inhibited (145), although viral DNA concentrations in the hepatocytes are not changed (137). For these reasons, use of HBV DNA, HBeAg, and HBsAg may all be useful in monitoring patients with chronic HBV because no single test provides unequivocal evidence of viral clearance.

Most studies have shown that HCV RNA fluctuates over time but rarely varies by more than 1 log; in most cases, variation is <0.5 log (146–148). In patients tested repeatedly over several years, HCV RNA increases by an

average of 0.25 log/year over time without treatment (149). In some series, however, up to a 3-log difference is seen in patients with increased ALT when HCV RNA is measured monthly (150); in approximately one-third of chronically infected patients, HCV RNA can fluctuate between a mean of 10^6 copies/mL and undetectable (151).

Currently, antiviral treatment is recommended for patients with chronic HCV infection who have increased ALT and more than mild inflammatory changes on biopsy. The most effective therapy currently available is combined ribavirin and interferon. Laboratory tests have been found helpful in predicting response to varying lengths of therapy and in detecting those who are not responding to treatment and in whom therapy should probably be discontinued. In those treated with combination therapy, both viral load and genotype have been found to identify patients who may respond to 24 rather than 48 weeks of therapy (152, 153).

In a combined analysis of these two studies, five factors were found useful in predicting response (Table 5). Persons with genotype 2 or 3, along with three or four other favorable risk factors, can be treated effectively with only 24 weeks of therapy; all other patients do better with 48 weeks of therapy (154). The best indicator of viral clearance is persistent absence of HCV RNA (determined by qualitative HCV RNA assays). The absence of HCV RNA 6 months after completion of treatment is associated with a <10% likelihood of recurrent HCV viremia (155). A decrease in viral load in the absence of clearance is not reliable evidence of treatment success; however, failure of HCV RNA to decline to <400 000 copies/mL by 12 weeks of therapy is associated with 100% likelihood of persistent HCV RNA at the end of treatment (154). An approach to monitoring treatment of patients with HCV by combination therapy is outlined in Fig. 2. Some patients cannot take ribavirin; the only current treatment option for such patients is interferon monotherapy. With this form of treatment, failure of HCV RNA to fall to undetectable concentrations or failure of ALT to return to normal at 12 weeks after initiation of therapy is associated with a

>95% likelihood of treatment failure, and is considered a reason to discontinue therapy (156).

The optimal frequency of laboratory tests in patients with chronic hepatitis C has not been determined. The European Association for the Study of the Liver Consensus Conference on Hepatitis C recommends that complete blood counts and liver enzymes be performed every 6 months in untreated patients (157). The major complications of treatment with interferon are depression, thrombocytopenia, and hypothyroidism, whereas hemolytic anemia is the major complication of ribavirin therapy. The European Association for the Study of the Liver recommends complete blood counts weekly during the first 4 weeks of treatment and regular determinations after the first 4 weeks. They also recommend measurement of thyroid-stimulating hormone every 6 months during therapy.

Recommendations:

- In viral hepatitis, viral markers are the most reliable markers of resolution of hepatitis (IIB).
- HCV RNA quantification and genotype are important determinants of duration of combination therapy. To reduce the expense of testing, if feasible, specimens should be obtained before treatment and stored at -70°C pending results of treatment. If this is not possible, testing should be performed before treatment is begun (IIB and IIE).
- In patients with HCV treated with interferon and ribavirin, qualitative HCV RNA should be measured after 24 weeks of treatment to determine potential responders. If genotype and quantitative HCV RNA were not performed but specimens were frozen for their analysis before treatment, those with negative HCV RNA and favorable risk factors should have those tests performed (IB and IE).
- In patients with HCV treated with interferon monotherapy, qualitative HCV RNA and ALT should be measured after 12 weeks of treatment to determine nonresponders (IIB).
- Following treatment in those with negative HCV RNA at 24 weeks, sensitive HCV RNA measurements (currently qualitative assays) should be performed 6 months after the end of treatment to document sustained virologic remission (IIB).
- In untreated patients with HBV, HBeAg should be monitored periodically; once HBeAg is negative and anti-HBe is positive, HBsAg should be monitored periodically to determine viral clearance. With antiviral therapy, HBV DNA should also be used to document viral clearance (IIB).
- In treated patients, a complete blood count with platelets should be measured every week for the first 4 weeks, then monthly thereafter. Thyroid-stimulating hormone should be measured every 3–6 months, or sooner if symptoms of thyroid dysfunction develop.

Table 5. Favorable and unfavorable risk factors in HCV treatment with interferon and ribavirin.

Favorable factors

- Genotype 2 or 3
- Viral load < median (3.5×10^6 copies/mL)
- Female gender
- Age <40 years
- No or only portal fibrosis

Unfavorable factors

- Genotype 1, 4, 5, 6
- Viral load > median
- Male gender
- Age 40 or above
- Septal or more severe fibrosis

^a From Poynard et al. (154).

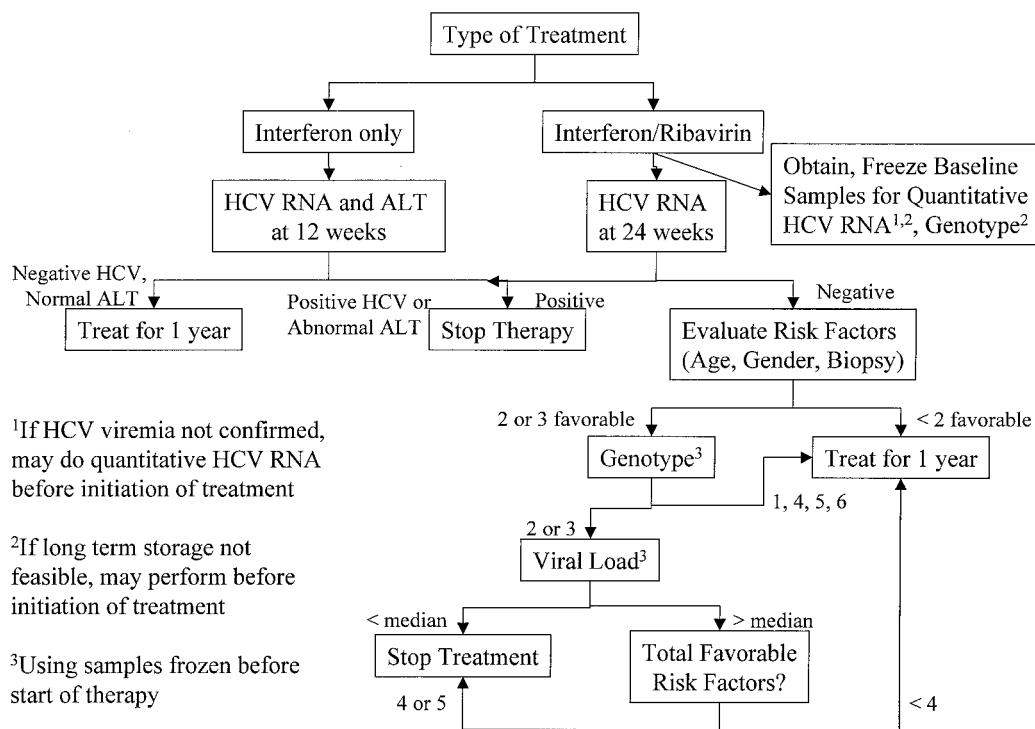


Fig. 2. Management of therapy in chronic hepatitis C: laboratory testing depends on type of treatment given.

At present, combined ribavirin-interferon is recommended for all patients without contraindications. HCV RNA should be measured at 24 weeks of treatment; if positive, treatment should be discontinued. If negative, duration of treatment is based on number of favorable risk factors present (see Table 5). If four or more factors are favorable (and genotype is 2 or 3), treatment is stopped; in other cases, treatment is continued for 48 weeks. If -70 °C storage is available, specimens for HCV genotype and viral load can be obtained before treatment and frozen until after the 24-week testing is performed; if not, testing should be performed before therapy. In those patients who cannot tolerate ribavirin, monotherapy with interferon is used. ALT and qualitative HCV RNA should be checked after 12 weeks of therapy; if ALT remains increased or HCV RNA is detectable, treatment should be discontinued. There is no benefit to determination of quantitative HCV RNA or genotype when interferon monotherapy is used.

Measurement of ALT should be performed at least monthly (IIB).

- ALT is the best marker of inflammatory activity available, but it is of limited utility in predicting degree of inflammation and of no use in estimating severity of fibrosis (IIB).

Cirrhosis

Chronic hepatitis is classified by histology based on activity of inflammation and degree of fibrosis; extent of fibrosis relates to likelihood of developing cirrhosis. Enzyme activities do not reflect severity of fibrosis, a major factor in prognosis, and there is at best a weak correlation between plasma ALT activity (158-160) or (in chronic HCV) HCV RNA concentrations (160, 161) and histological activity. At best, ALT explains only 30-50% of the variation in histologic activity, and there is considerable overlap in values in patients with mild, moderate, or severe activity (158-160), which relates to rate of progression of fibrosis (162).

Liver fibrosis is associated with deposition of several proteins in the liver. Among the proteins produced as part of fibrosis are collagen, laminin, elastin, and fibronectin; metalloproteinases and their inhibitors; and enzymes produced in collagen synthesis such as lysyl and prolyl

hydroxylase. Various proteoglycans, such as hyaluronate, are also produced in the process of fibrosis. Numerous studies of plasma concentrations of proteoglycans, proteins of fibrosis, and their precursors (163-172) have shown at best a weak correlation between marker concentrations and extent of fibrosis. Concentrations reflect degree of fibrogenesis at the time of sampling, and there is considerable overlap in values with varying degrees of fibrosis. A simple marker, calculated from routine monitoring tests, is the AST:ALT ratio. Several studies have shown that the ratio is typically <1 in patients with non-alcoholic hepatitis, but with progression to cirrhosis the ratio often increases to >1: the specificity of a ratio >1 for cirrhosis is 75-100%, with a sensitivity of 32-83% (65-67). In one study (67), the ratio increased with increasing fibrosis score. This appears to be attributable to a reduction of ALT production in damaged liver (173).

Other routine test results that predict likelihood of cirrhosis are thrombocytopenia and prolonged PT; an index using these two variables with the AST:ALT ratio has a sensitivity of 46% and a specificity of 98% for cirrhosis (65). Albumin is commonly measured in patients suspected of progressing to cirrhosis. Although it is not as sensitive as other markers, it is used as a marker of severity as part of the Child-Pugh classification of cirrho-

sis. α -Fetoprotein (AFP) is more likely to be increased as the degree of hepatic fibrosis increases (174), especially in cirrhosis; AFP $>17.8 \mu\text{g/L}$ has a sensitivity of 35%, a specificity of 98.6%, and a positive predictive value of 97.7% for cirrhosis (175).

Recommendations:

- Biopsy is the only definitive marker of progression from chronic hepatitis to cirrhosis (IIB).
- Laboratory markers of fibrosis should not be used except in research studies (IIIB and IIIE).
- Markers of hepatic function that may indicate progression to cirrhosis (AST:ALT ratio, albumin, PT, platelet count) should be measured every 3–6 months in patients with chronic hepatitis (IIIB).

HCC

Primary liver cancer (HCC) is a serious late complication of chronic hepatic injury, particularly in cirrhosis caused by HBV, HCV, and hemochromatosis. Infrequently, HCC is seen in patients with chronic HCV and in asymptomatic HBV carriers without cirrhosis. It is the fifth most common malignancy worldwide and is particularly common in Eastern Asia and Africa (176). The incidence of HCC has increased by 70% in the United States over the past 20 years, particularly among younger patients (177), and it is increasing in other parts of the world as well (176). The risk of developing HCC in cirrhosis attributable to chronic HBV or HCV infection is 1.5% per year (178, 179). In a study of 448 cases of HCC, 75% occurred in patients with cirrhosis; however, in only 30% was cirrhosis recognized clinically before HCC was diagnosed (180). These data suggest that screening programs, if instituted, must include patients with chronic hepatic injury as well as patients with diagnosed cirrhosis. In one study, however, HCC developed only in 325 patients with severe chronic hepatitis or cirrhosis, and not in any of 800 patients with mild or moderate chronic hepatitis (181). Because patients with normal ALT generally have mild inflammation on biopsy (17, 61, 63), it is reasonable to exclude from screening those persons without cirrhosis and with normal ALT or less than severe hepatitis on biopsy. Other risk factors include male gender and age >55 years.

The prognosis of patients with HCC detected by development of symptoms is grim, with few patients surviving >6 months. Detection of small tumors offers the potential for curative resection and forms the rationale for considering screening. Current practice suggests measurement of AFP and ultrasound of the liver every 6 months (182). Unfortunately, AFP interpretation is complicated by intermittent increases in AFP concentrations in 12–13% of patients with chronic HBV or HCV (183), often (but not always) associated with transient increases in ALT (184). A Consensus Development workshop recommended screening chronic HBsAg carriers at least once, and preferably twice, yearly with AFP only,

whereas patients with other risk factors (known cirrhosis, family history) should have both AFP and ultrasound (185). In chronic hepatic injury, a high risk of HCC is present in patients with hemochromatosis or with cirrhosis caused by HBV, HCV, and alcohol abuse. Other causes of chronic hepatic injury and cirrhosis have lower risks of HCC (186).

In Western countries, the predictive value of AFP is low, often in the range of 10–30%, with sensitivity of AFP between 40% and 80% (187–192). In 147 patients with cirrhosis, none of the 30 patients with HCC had AFP $>105 \mu\text{g/L}$ at the time of diagnosis and 60% had AFP $<20 \mu\text{g/L}$; however, the frequency of HCC in patients with AFP $<50 \mu\text{g/L}$ was 17% compared with 42% in those with higher AFP (192). In another study of 260 patients with cirrhosis, HCC developed in 26% of patients with initial AFP $<20 \mu\text{g/L}$, but 46% in those with higher concentrations. Moreover, those with even transient increases above $100 \mu\text{g/L}$ had a significantly higher risk of HCC than those whose AFP was consistently $<20 \mu\text{g/L}$ (193). A decision analysis on published reports of screening for HCC in Western patients with compensated cirrhosis concluded that, for patients with a likelihood of survival of 85% at 5 years, screening would likely add 3–9 months to average life expectancy at a cost of \$26 000 to \$55 000 per year of life gained, figures that compare favorably to those for colon cancer and breast cancer screening (194). In patients with a lower likelihood of survival, screening provided minimal or no gain in life expectancy and does not appear indicated. A systematic analysis of all published studies concluded that there are inadequate data to determine the benefit of screening for HCC among patients with chronic liver disease (195). If screening is used, a frequency of testing of every 6 months appears to be optimal based on observed doubling times of HCC, reported to average ~ 3 –5 months (196–198).

Des- γ -carboxy prothrombin has also been suggested as a screening test. Concentrations are increased occasionally in chronic liver disease, but there is less overlap with values seen in HCC than for AFP (199, 200). Occasional high concentrations are encountered in metastatic carcinoma to the liver, but they usually are minimally increased. Although des- γ -carboxy prothrombin appears less sensitive (50–70%) than AFP, it is more specific. There is poor correlation between AFP and des- γ -carboxy prothrombin, and some tumors are detected only by des- γ -carboxy prothrombin (199–203). Vitamin K deficiency can also cause substantial increases; repeating testing after administration of vitamin K improves specificity (199, 200, 204, 205). Recently, a more sensitive immunoassay has shown promise in detection of small HCC, with positivity in 27% of cases compared with 3% with older assays (206). Assays for des- γ -carboxy prothrombin are not widely available, in contrast to AFP assays. Other laboratory tests, including AFP variants (207) and lectin affinity chromatography of ALP (208), have been evaluated in too few patients to make definitive recommendations. A recent study identified high concentrations of

abnormal forms of γ -glutamyltransferase in 78 of 91 patients with HCC but in only 2.5% of 116 patients with other liver diseases (209).

Recommendations:

- Screening for HCC is of questionable benefit in Western populations (IIB and IIE).
- Screening should be confined to high-risk patients (those with severe chronic hepatitis or cirrhosis attributable to alcohol, HBV, HCV, or hemochromatosis) who are candidates for treatment of HCC, if detected (IIIB and IIIE).
- If screening is used, measurement of AFP and ultrasound at intervals no more frequently than every 6 months are recommended (IIB).
- There currently are few data to support the use of other tests (IIIB).

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