IV. Diagnostics

Summary

Since the discovery of the hepatitis C virus in 1988, the field of HCV diagnostics has advanced rapidly. Key tests for diagnosing chronic HCV infection, assessing prognosis, predicting and monitoring the effects of treatment, and measuring other complications of HCV infection include: HCV-antibody and RNA-PCR (viral load) tests; liver function tests (LFTs); HCV-genotypic tests; and body scanning technologies such as liver scan, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, and ultrasound surveillance for hepatocellular carcinoma (HCC). Liver biopsy is still considered the "gold standard" for assessing HCV-induced liver damage and determining the need for HCV treatment, though researchers are working intensively to discover accurate but less invasive techniques.

Diagnosis of Acute Hepatitis C Infection. Most individuals will develop antibodies to hepatitis C virus (HCV) six weeks to six months after they have been infected, although in some instances antibodies to HCV will not appear for years after infection (M. J. Alter 1992; Beld 1999). The EIA-3 (the latest version of the HCV antibody test) has a sensitivity of 97%; however, it has a positive predictive value of only 25% in low-risk individuals (Gretch 1997). Therefore, any positive EIA-3 results, especially in individuals at low risk or individuals with rheumatoid factor or high immunoglobulin levels, need verification with confirmatory testing using either a recombinant immunoblot assay (RIBA, another test for antibodies to hepatitis C) or HCV-RNA testing (which identifies the presence of viral RNA and hence the actual virus). Because people who have achieved spontaneous viral clearance of HCV may remain HCV-antibody-positive, confirmatory testing for the presence of HCV RNA is needed to distinguish resolved infections from active, current infections. HCV-infected individuals may be intermittently viremic (Beld 1999; Villano 1999). In order to confirm or rule out current HCV infection, it is recommended that people with undetectable HCV-RNA levels have a follow-up HCV-RNA test six months later.

Individuals with acute HCV infection may receive a false negative antibody test result prior to seroconversion (when blood tests change from antibody-negative to antibody-positive). Some people may not be able to produce antibodies to hepatitis C. Transplant recipients, active injection drug users, and HIV-positive individuals—especially those with CD4 counts below 200—may require confirmatory HCV-RNA testing (Beggren 2001; Beld 1999; Busch 2001; H. H. Lin 2002; Thomas 1995).

Distinguishing Acute from Chronic Infection. Acute hepatitis C virus infections do not inevitably develop into chronic infections. Proper diagnosis of acute HCV infection with appropriate diagnostic follow-up can distinguish acute infections from resolved or chronic infections. Acute HCV infection may be diagnosed using information from several tests that rule out acute infection with other hepatitis viruses, measure alanine aminotransferase (ALT) levels, and look for the presence of HCV antibodies and HCV RNA. Chronic hepatitis C infection may be diagnosed by confirming positive EIA-3 results with RIBA or HCV-RNA testing.

HCV-RNA Testing. Hepatitis C RNA is detectable between one and two weeks after exposure (H.J. Alter 1991). The amount of hepatitis C virus in a person's blood varies among individuals,

but mode of transmission, length of infection, coinfection with hepatitis B, age, amount of alcohol consumption, and HIV status may have an effect on HCV-RNA levels (Oshita 1994; Pessione 1998; Sawada 1993; Thomas 2000). HIV-positive individuals who are coinfected with HCV have higher levels of HCV RNA than individuals with HCV alone (Cribier 1995; Di Martino 2001; Eyster 1994; Sherman 1993; Sulkowski 2002; Thomas 2001; Zylberberg 1996). Although HCV RNA is not predictive of disease progression or indicative of liver injury, individuals with HCV-RNA levels below 2,000,000 copies/mL or 800,000 international units (IU)/mL have better responses to interferon treatment (Fried 2002; Lau 1993; McHutchison 1998; Poynard 1998; Rumi 1997).

Two different types of RNA tests, quantitative and quantitative, are available. Qualitative HCV-RNA tests measure the presence or absence of HCV RNA, giving a positive or negative result, while quantitative tests measure the amount of HCV RNA per milliliter (mL) of blood. Qualitative testing is usually used to diagnose HCV infection, and may be used to measure response to HCV treatment (when quantitative test results are below the threshold of detection). Quantitative testing is used to establish the baseline amount of HCV RNA in a person's blood or tissue, to assess response to treatment and, sometimes, to diagnose HCV.

Liver Panel. The liver panel comprises a number of different blood tests and is used to provide diagnostic information for acute and chronic HCV infection as well as to assess the liver's capacity to metabolize drugs. Liver panel testing can identify possible liver damage and provide some information about response to HCV treatment (see A Guide to the Liver Panel at the end of this chapter). Frequent monitoring of liver enzyme levels is especially important for HIV-coinfected individuals in order to serially evaluate for evidence of HAART-associated hepatotoxicity.

Alanine Aminotransferase (ALT; a liver enzyme). Alanine aminotransferase (ALT) levels may remain normal, or become transiently or persistently elevated during chronic HCV infection. More than two-thirds of HCV-infected individuals have abnormally elevated ALT levels (Dufour 2000); However, ALT elevations do not predict disease progression (Herve 2001; Jamal 1999; Persico 2000). Some researchers have found less liver damage in individuals with persistently normal ALT levels, while others have observed liver damage despite persistently normal ALT levels (Herve 2001; Jamal 1999; Persico 2000; Puoti 1997).

Genotypic Tests. There are at least six different genotypes (viral strains) of HCV, and many subtypes. The genotype is the most important prognostic indicator of response to interferon treatment. Most HCV infections in the United States are genotype 1. Genotypes 1, 4, and 5 do not respond as well to interferon as do genotypes 2 and 3 (Fried 2002; Germer 2001; Mondelli 1999; Neumann 2000; Nousbaum 1998; Poynard 1998; Rosenberg 2001; Zein 1996b). A 48-week course of treatment is recommended for individuals with genotypes 1, 4, and 5; only 24 weeks of treatment are recommended for individuals with genotypes 2 and 3 (McHutchinson 2002; Poynard 2000; Soriano 2002). It is possible to become re-infected with HCV, or to have a mixed infection with more than one genotype (Accapezzato 2002; De Socio 1996; García- Samaniego 1997; Jarvis 1994; Tuveri 1997), although it is not clear if mixed infection can accelerate HCV progression.

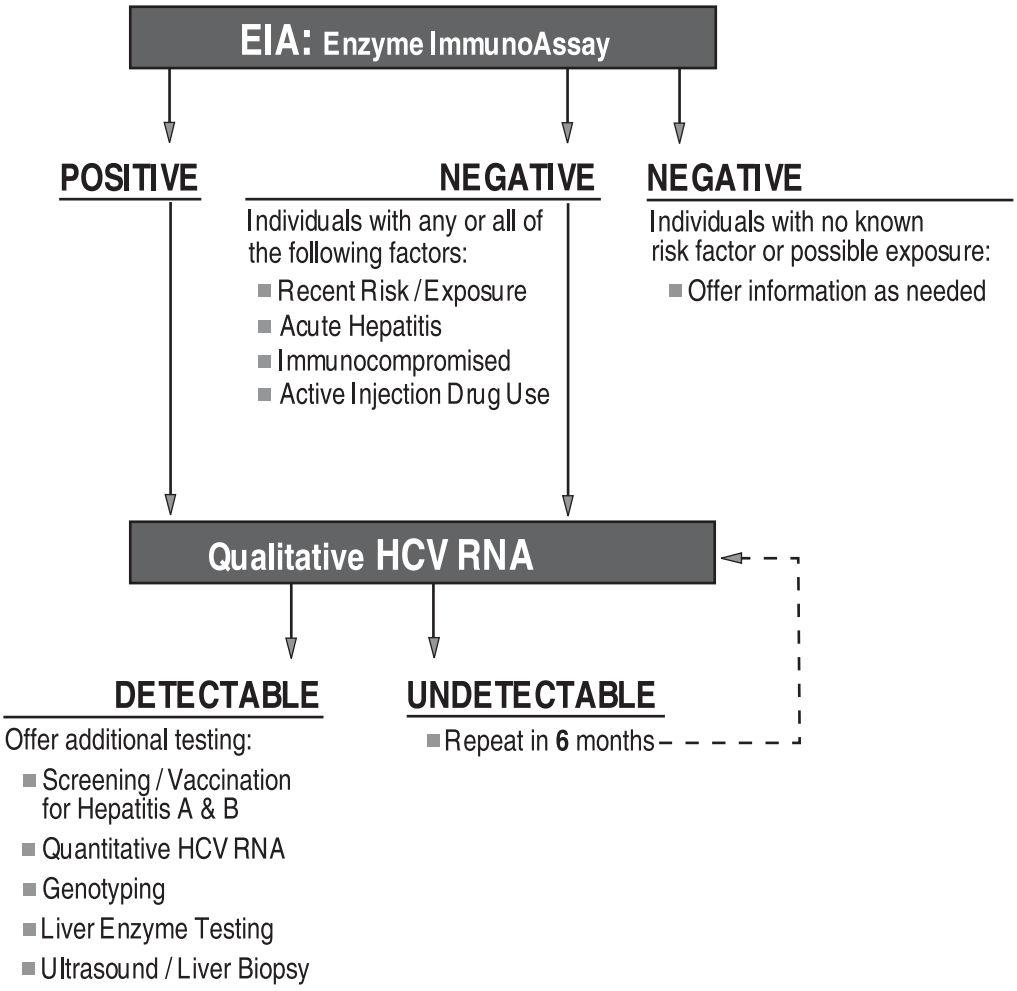
Scans. Additional information about the condition of the liver can be obtained from tests such as liver scans, CT scans, MRI, and ultrasonography, but these tests cannot replace biopsy. A combination

of blood tests has shown promising results as an alternative to biopsy in some situations, but more research is needed to identify and validate additional alternatives to biopsy (Boeker 2002; Forns 2002; Guechot 1994; Imbert-Bismut 2001; Leroy 2004; Mehta 2004; Myers 2002; Patel 2003; Sud 2004; Wai 2003).

Liver Biopsy. Liver biopsy is the only way to assess the condition of the tissue in an individual's liver. Information from liver biopsy is used to grade disease activity (assess the amount of inflammation and cell death) and stage the amount of damage to the liver; to identify any other causes of liver injury; and to make treatment decisions. Liver biopsy is usually performed in a hospital. A thin needle is quickly inserted between the ribs to remove a tiny sample of liver tissue. Liver biopsy can be painful and, occasionally, complications such as hemorrhage or puncture of adjoining organs may occur. Fatalities from biopsy are very rare (0.01–0.1%). Having a biopsy performed by an experienced physician using ultrasound guidance may reduce the risk of pain, complications, and sampling errors (Cadranel 2000; Pokorny 2002).

Ultrasound. Ultrasound surveillance for HCC in cirrhotic individuals (who are at higher risk for HCC than other HCV-infected individuals) increases early detection, but has not been proven to increase survival (Larcos 1998; Solmi 1996).

Figure 1. HCV Diagnostic Algorithm



Hepatitis C Antibody Testing

Antibodies to hepatitis C usually develop six weeks to six months after infection, although some individuals will not seroconvert (change from antibody-negative to antibody-positive) until 94 months after infection (Alter 1992; Beld 1999). Some individuals never develop antibodies to HCV, even when HCV RNA is detected in their blood (Durand 2000). Others may lose their antibodies to HCV years after infection, although they have detectable HCV RNA (M. J. Alter 1992; Beld 1999).

The sensitivity of the EIA-3 is 97%, but in low-prevalence populations, it has a positive predictive value of only 25% (Thomas 1997). False negative antibody test results may occur among people who are acutely infected with HCV and have not yet seroconverted. Immunocompromised individuals, such as transplant recipients and HIV-positive persons, may receive false negative results due to an inability to produce antibodies to hepatitis C. In some cases, injection drug users may not have antibody responses to HCV, although they have detectable HCV RNA (Beld 1999; Thomas 1995). Confirmatory HCV-RNA testing may be used to diagnose HCV infection in current injection drug users.

Because individuals who have had a recent exposure to HCV, as well as immunocompromised individuals who have been at risk for HCV infection in the past, may receive a false negative antibody test result, confirmatory testing for HCV RNA (testing that looks for the actual virus) is recommended. False positive results to HCV antibody testing may occur, especially among people with rheumatoid factor or high immunoglobulin levels. In order to properly diagnose hepatitis C infection, a positive antibody test result needs to be confirmed by recombinant immunosorbent assay (RIBA) or by testing for HCV RNA.

The RIBA has been used as a supplemental test, but the EIA-2 and the EIA-3 are more sensitive than the RIBA-2 and the RIBA-3 (Vrielink 1997). The RIBA test is useful for ruling out false positive antibody test results in low-risk groups. In individuals with a recent exposure to HCV, a negative RIBA does not rule out current HCV infection; these individuals need HCV-RNA testing to confirm or rule out current HCV infection.

The serum ELISA assay for HCV antibodies has been modified for oral fluid testing. The oral fluid ELISA was tested in 109 individuals who were HCV-antibody-positive by serum ELISA and 107 individuals who were HCV-antibody-negative by serum ELISA. The sensitivity of oral ELISA fluid testing for HCV antibodies was 98.2% and the specificity was 99.1% (Sherman 1994). This is comparable to the sensitivity (99%) and specificity (99.8%) of the serum EIA-3 (Abdel-Hamid 2002). Despite the accuracy and convenience of oral fluid testing for HCV antibodies, the oral fluid test has not been marketed.

Many current and former injection drug users have poor venous access. In addition, they may not seek HCV testing in clinical settings. An oral fluid test will facilitate individual testing in non-traditional venues such as syringe exchange programs and facilitate epidemiological surveys.

Diagnosis of Acute HCV Infection

Individuals with acute HCV do not invariably develop chronic HCV infection. Proper diagnosis of acute HCV with appropriate diagnostic follow-up is necessary to distinguish between resolved or chronic infections. Some preliminary research has indicated that treatment of acute HCV may reduce the likelihood of chronic infection (see Chapter V, Hepatitis C Treatment).

Acute hepatitis C infection may be identified by a combination of tests, beginning with screening to rule out acute infection with hepatitis A (HAV) and hepatitis B (HBV). If HAV and HBV test results are negative, acute HCV may be diagnosed by the presence of alanine aminotransferase levels (ALT) >7 times the upper limits of normal, combined with HCV antibody testing and confirmatory HCV-RNA (viral load) testing if the HCV antibody test result is positive. Identification of the seroconversion from hepatitis C antibody-negative to antibody-positive is the only reliable method to classify acute HCV infection.

Diagnosis of Chronic HCV Infection

Chronic hepatitis C infection is diagnosed by the presence of HCV antibodies, with either detectable HCV RNA on more than one occasion over a six-month interval or the presence of abnormal liver enzyme levels for at least six months.

Liver Enzymes

Measurements of enzymes, proteins, and bilirubin from a blood sample constitute a liver panel. Information from a liver panel can be used to help diagnose hepatitis C infection, identify possible liver damage, assess the liver's capacity to metabolize drugs, and partially evaluate the response to HCV treatment. A liver panel is usually comprised of measurements of the levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, albumin, and total protein. Other tests are often included, such as measurements of prothrombin time (PT), gamma-glutamyl transferase (GGT), and lactic acid dehydrogenase (LDH). For more information, see A Guide to the Liver Panel at the end of this chapter.

Liver enzyme levels in people with chronic HCV infection can be normal, periodically elevated, or persistently elevated (Herve 2001; Jamal 1999; Persico 2000). Liver enzyme levels are only rough indicators of liver disease and are not predictive of disease progression. In chronic HCV disease, ALT levels are more frequently elevated than AST levels (71% vs. 33%) (Dufour 2000).

Some studies have found less HCV-related liver damage in people with persistently normal alanine aminotransferase (ALT) levels (Jamal 1999; Persico 2000). In a two-year case-control study, Jamal and colleagues followed 75 people with normal ALT levels (defined as four consecutive normal ALT values within 12 months) and 200 people who had abnormal ALT levels. Those with persistently normal ALT levels had less severe liver disease; just 6% had cirrhosis compared to 19% of the group with elevated ALT levels ($P=0.007$). Those with persistently normal ALT levels had less fibrosis ($P<0.05$) and a slower rate of fibrosis progression ($P<0.001$), as well as significantly lower HCV-RNA levels ($P=0.02$) (Jamal 1999). In a study in chronically HCV-infected individuals comparing 80 with persistently normal ALT levels and 455 with elevated liver enzymes, Herve and

colleagues found an association with less severe liver disease, slower fibrosis progression, and persistently normal ALT levels (Herve 2001).

However, mild-to-serious liver damage has been identified in HCV-infected persons with persistently normal ALT. In a retrospective study of 864 HCV-infected persons, some degree of fibrosis, usually mild, was observed in a significant number of those with persistently normal ALT levels (Pradat 2002). Puoti and colleagues found no difference in grading and staging of liver disease between those with persistently normal levels (N=46) and those with ALT elevations (n=52) (Puoti 1997). A study of HCV-infected Japanese hemodialysis patients reported that although most patients had persistently normal ALT levels, ultrasonography revealed liver damage among 72% (Furusyo 2000).

Elevated aspartate aminotransferase (AST) levels may help to identify damaged liver tissue, although their predictive value for the development of cirrhosis is controversial (Assy 2000; T. F. Imperiale 2000; Michielsen 1997; Park 2000; Sheth 1998). Higher AST:ALT ratios have been associated with increasing grades of fibrosis (Park 2000; Sheth 1998). However, when AST:ALT ratios were examined as potential predictors of cirrhosis, the findings were inconsistent. Sheth and colleagues found an AST:ALT ratio of ≥ 1 in 25 of 47 cirrhotic individuals and in none of 92 non-cirrhotic individuals. An AST:ALT ratio of ≥ 1 distinguished 100% of cirrhotic individuals from non-cirrhotic individuals in this study (positive predictive value of 100%). The AST:ALT ratio accurately ruled out cirrhosis in 80.7% of non-cirrhotics (negative predictive value of 80.7%) (Sheth 1998). Park and colleagues found that only 5 of 123 non-cirrhotic individuals had an AST:ALT ratio of ≥ 1, while 14 of 30 cirrhotics had an AST:ALT ratio of ≥ 1. The AST:ALT ratio correctly identified cirrhosis in 73.7% and accurately ruled out cirrhosis in 88.1%. Imperiale and colleagues attempted to validate the AST:ALT ratio as a clinical decision aid. The test incorrectly identified 13 of 36 individuals (36%) as cirrhotic (T. F. Imperiale 2000).

HCV RNA

Hepatitis C RNA levels are detectable between one and two weeks after infection (H. J. Alter 1991). Those with chronic HCV infection may be intermittently viremic (Beld 1999; Villano 1999). Therefore, it is recommended that individuals who are HCV-antibody-positive but have undetectable HCV-RNA levels undergo retesting for HCV RNA in six months to confirm or rule out ongoing HCV infection.

Individual hepatitis C viral loads vary, although hepatitis C replicates rapidly; an estimated ten trillion virions (individual virus particles) are produced per day (Neumann 1998). HCV-RNA levels may be influenced by mode of transmission, duration of infection, concurrent infection with hepatitis B, age, consumption of alcohol, and HIV status. Individuals who acquired HCV from blood transfusion have higher viral loads than those infected from injection drug use or needlestick injury (Lau 1993). However, all the transfusion recipients were over 60 years of age, and age may have been a surrogate for duration of infection or infection at an older age. In a cohort of injection drug users, Thomas and colleagues found a range of serum HCV-RNA levels from <200,000 to >120 million eq/mL. Higher HCV-RNA levels were found in older participants. In this group, age was strongly correlated with duration of drug use and HCV infection, suggesting that HCV viral load levels may increase with longer duration of infection. Lower HCV-RNA levels were associated with ongoing hepatitis B infection and not sharing needles (Thomas 2000b).

Alcohol consumption increases HCV RNA (Oshita 1994; Pessione 1998; Sawada 1993). Pessione and colleagues found a strong correlation with HCV-RNA levels and alcohol consumption (P= 0.0001). They detected a dose-response relationship between HCV RNA and alcohol even in individuals with moderate intake (30 grams/day). HCV-RNA levels in HIV-coinfected individuals are higher than those found in individuals HCV monoinfection (Cribier 1995; Di Martino 2001; Eyster 1994; Sherman 1993; Sulkowski 2002; Thomas 2000b; Zylberberg 1996).

Quantitative viral load testing, which measures the amount of hepatitis C virus from a blood or liver tissue sample, provides important information for making a treatment decision and measuring the response to treatment. Although hepatitis C viral load is not a reliable surrogate marker for disease progression, it is a prognostic indicator for response to treatment. Viral loads of less than two million copies have been associated with better responses to interferon-based treatment (Fried 2002; Lau 1993; McHutchison 1998; Poynard 1998; Rumi 1997). Two different methods are used for quantitative testing of HCV RNA: bDNA and RT-PCR.

Table 1. Commonly Used Quantitative HCV-RNA Assays

Assay	Method	Range (Without sample dilution)	As International Units
Roche COBAS Amplicor HCV Monitor 2.0	RT-PCR	100 to 5 x 10^6 HCV RNA copies/mL	~40 to 2.0 x 10^6 HCV IU/mL
National Genetics Institute HCV SuperQuant	RT-PCR		600 to 8.5 x 10^5 HCV IU/mL
Bayer Quantiplex HCV RNA 2.0	bDNA	0.2 to 120 MEq/mL	~3.2 x 10^4 to 1.9 x 10^4 HCV IU/mL
Bayer Versant HCV RNA 3.0*	bDNA	2500 to 4.0 x 10^7 HCV RNA copies/mL	521 to 8.3 x 10^6 HCV IU/mL
Abbott Laboratories Lcx HCV RNA	RT-PCR		23 IU/mL to 2.3 X 10^6 IU/mL

Adapted from Germer and Zein, 2001; Leckie 2004

*The Versant 3.0 has received FDA premarket approval (an approved Premarket Approval Application (PMA) is, in effect, a private license granted to the applicant for marketing a particular medical device).

Although bDNA and RT-PCR testing are both used to measure the amount of hepatitis C virus, their results are not interchangeable, even when converted into international units (IU), the World Health Organization's standard for measuring HCV RNA. Despite strong within-test correlation between RNA copies/ml and IU, there was a wide variation between RT-PCR and bDNA results, especially in high-titer, HIV-coinfected patients (Sherman 2002c). Therefore, to ensure an accurate assessment of treatment response, it is recommended that clinicians use the same RNA test assay before and during HCV treatment, especially for those with high viral loads. After the course of treatment, more sensitive assays may be needed to detect low levels of HCV RNA.

Qualitative testing has a much lower threshold of detection than quantitative testing and has been used to identify individuals with acute HCV infection, to confirm or rule out chronic HCV infection, and to assess virologic response to HCV treatment.

Table 2. Qualitative Assays for Detection of HCV RNA

Assay	Threshold of Detection	As International Units
Bayer TMA (Transcription-Mediated Amplification)	Can detect 50 copies HCV RNA/mL	~ 5 HCV IU/mL
Roche COBAS AmpliScreen™ HCV Test		Can detect 60 HCV IU/mL

The sensitivity and specificity of testing for HCV RNA may differ, due to assay variability and a lack of standardization among laboratories. Consistent use of the same quantitative assay throughout the course of HCV treatment will increase the comparability of the results. Although consistent use of one assay is preferable, if necessary, results from different quantitative assays can be converted into IU.

Table 3. Conversion Chart for HCV RNA Assays: Copies per Milliliter (copies/mL) to International Units (IU)

Assay	Conversion Factor
Amplicor HCV Monitor v 2.0 (manual procedure)	1 IU/mL = 0.9 copies
Cobas Amplicor HCV Monitor v 2.0 (semi-automated procedure)	1 IU/mL = 2.7 copies
Versant HCV RNA 3.0 Quantitative Assay	1 IU/mL = 5.2 copies
Lcx HCV RNA Quantitative Assay	1 IU/mL = 3.8 copies
SuperQuant	1 IU/mL = 3.4 copies

Hepatitis C Support Project 2003

Hepatitis C Genotyping

There are at least six known HCV genotypes (different hepatitis C viral strains, identified by their specific genetic makeup). Different viral isolates from around the world may vary genetically by as much as one third (Davis 1999; Okamoto 1992). Before 1994, there was no consistent classification system to distinguish different HCV genotypes, making it very difficult to evaluate the role of HCV genotype in different studies. In 1994, Simmonds and colleagues suggested a system for nomenclature of hepatitis C viral genotypes, classifying them on the basis of their nucleotide sequence similarities into major genetic groups designated as genotypes. Each genotype was assigned a number, based on the order of its discovery. The most closely related strains within genotypes were designated as subtypes named in alphabetical order. All genetic variants within a single isolate of the virus are designated as quasispecies. Quasispecies are a result of viral mutations in the host; since HCV makes up to 10 trillion copies of itself on a daily basis, and replication is error-prone, quasispecies diversity can be significant.

Liver Scan, Computed Axial Tomography (CAT) Scan, Magnetic Resonance Imaging (MRI), and Ultrasound Testing

The liver scan is used to determine liver shape and function. It starts with an injection of a radioactive isotope. After injection, the person lies on a table under a scanner, which detects the pattern of the radioisotope's distribution and sends images of the area to a computer. The amount of radiation used in a liver scan is less than that of an X-ray. A liver scan is an efffective and non-invasive method used to identify portal hypertension (high blood pressure in liver blood vessels), cirrhosis, hepatocellular carcinoma, and other liver disease.

Computed axial tomography (CT or CAT) scans provide X-ray images of the body from many angles. Beams from an X-ray device that quickly rotates around the body are sent to a computer, creating a cross-sectional image. Sometimes, as part of this procedure, a person will receive an injection of dye to enhance the contrast between organs. CT scans are not painful, but the injection of dye may be painful or dangerous to individuals with iodine allergies. CT scans can detect tumors and other irregularities of liver architecture.

Magnetic resonance imaging (MRI) uses magnets and radio waves to produce images of the body. During an MRI, a person lies on a narrow slab which is slid into a large tube. Inside the tube, radio waves are broadcast towards hydrogen atoms in the body alinged with the magnetic field from the MRI scanner. These atoms reflect a signal back to the machine. MRI scans can distinguish different different body organs and tissues. Sometimes, if a sharper image is needed, small devices called body coils, which transmit and receive the radio waves, may be used. Sometimes an intravenous agent is given to increase the contrast. An MRI scan is used to detect cancers, obstructions in the flow of blood in the liver's portal vein, and liver enlargement. The procedure is not painful, but some peope find it claustrophobic.

Sonographic or ultrasound screening can identify irregularities in the shape of the liver, or screen individuals at high risk for hepatocellular carcinoma. Ultrasound testing uses very high frequency sound waves which bounce off of the body to create an image of an organ. It has the advantage of being non-invasive and painless, although it does not provide information about the condition of liver tissue and the grade and stage of liver disease.

Hepatocellular Carcinoma: Screening and Surveillance

A key feature in the clinical management of people with cirrhosis is prompt identification of hepatocellular carcinoma. HCC can be identified by measuring alpha-fetoprotein (AFP) levels and ultrasound imaging, but the value of these tests for early detection of hepatocellular carcinoma in cirrhotic individuals has not been sufficiently demonstrated. However, it is common practice for cirrhotic individuals to undergo hepatic ultrasound and alpha-fetoprotein (AFP) surveillance at six-month intervals in the hope of early detection of HCC. The sensitivity and specificity of AFP levels in the detection of HCC has varied considerably (from 39% to 64%, or 76% to 91%, respectively) in different studies (Collier 1997). Intermittent and persistent elevations in AFP levels have been observed in individuals without HCC. Some research has shown that ultrasound surveillance increases early detection of HCC without reducing mortality (Larcos 1998; Solmi 1996).

Tong and colleagues performed a seven-year prospective assessment of the predictive value of ultrasound and AFP for detecting HCC. Five hundred and twenty-six of 602 participants were biopsied, 173 (33%) of them cirrhotic. Ultrasound and AFP results were used to guide follow-up CT scans and biopsies to confirm HCC diagnosis. During surveillance, HCC was detected in 5% (31/602). All of those with HCC were cirrhotic. The mean AFP concentration was significantly higher among individuals diagnosed with HCC than those with no HCC (426.06 ± 67.23 ng/mL vs. 8.91 ± 11.34 ng/mL; $P<0.001$), although seven individuals who were diagnosed with HCC had normal AFP levels. By themselves, AFP levels alone are not sufficiently sensitive or specific for detection of HCC. Ultrasound identified nine lesions that were not HCC; these were described as false positives. HCC was identified by ultrasound in 78% of cases (positive predictive value of 78%). Despite early HCC detection, 77% (24/31) individuals died within a mean of 16.70 ± 19.40 months after diagnosis of HCC (Tong 2001).

A scoring system may help to differentiate cirrhotics at low risk from those at high risk for progressing to HCC. Demographic, clinical, and biochemical data collected from 463 cirrhotics followed for a range of 1–96 months were analyzed to determine the predictive value of each variable. In cirrhotics aged 55 or over, the presence of HCV antibodies, prothrombin activity of <75%, or a platelet count below 75,000/mm^3 were identified by multivariate analysis as independent predictors of HCC. Using a scoring range of 0 to 4.71 points, a threshold to distinguish high vs. low risk was identified, with 2.33 as the cutoff. Among 270 low-risk individuals with a score of 2.33 or less, cumulative four-year HCC incidence was 2.3% (4/270), while in the high-risk group, cumulative HCC incidence was 30.1% (34/193) ($P=0.0001$) (Velázquez 2003). This system may help direct HCC surveillance efforts towards those who are at greatest risk.

Liver Biopsy

Liver biopsy is the best way to assess the grade and stage of HCV disease. Biopsy can identify or rule out other causes of liver disease, and is the only test that provides specific information about the condition of a person's liver tissue. Biopsy results are used to inform treatment decisions. Those with no liver damage or mild damage may choose to delay treatment, while those with more serious damage may need to initiate treatment.

During liver biopsy, a thin needle is quickly inserted between the ribs, where it collects a very small sample of liver tissue. Although it is an outpatient procedure, biopsy is usually performed in the hospital. Patients usually are kept under observation for three to six hours after the biopsy to watch for complications (hemorrhage, severe abdominal pain, punctured gall bladder), which usually become clinically apparent during the observation period (Janes 1993; Piccinino 1986). The risk of death from biopsy is low, ranging from 0.01% to 0.1%. The risk of complications ranges from 125 to 278 cases per 100,000 (Piccinino 1986). McGill and colleagues collected data from 9,212 biopsies performed over 21 years. During that period there were ten fatal and nine non-fatal hemorrhages (0.11% and 0.24%, or a total incidence of 0.35%) (McGill 1990). Liver biopsy can be painful. Thirty percent of biopsy recipients report mild pain, 3% report moderate pain, and 1.5% report severe pain (Gilmore 1995; Perrault 1978; Pokorny 2002).

New technology can reduce the risk of pain and complications from liver biopsy. The use of ultrasound to guide biopsy decreases the likelihood of puncturing the gallbladder, colon, bile

ducts, or large blood vessels, while increasing the likelihood of collecting adequate specimens in one pass (Soyer 1993). Cadranel and colleagues evaluated factors which increased or decreased complications from 2,084 liver biopsies. They observed reduced incidence of complications when biopsies were performed by more experienced physicians (>150 biopsies performed), when sedation or medication was provided prior to biopsy, and when ultrasound guidance was used (Cadranel 2000). Pokorny and colleagues biopsied 251 individuals, using ultrasound or CT scans to guide the procedures. Pain at the site of the biopsy or in the right shoulder was reported as severe in 1.2% (3/ 251), moderate in 2.4% (6/ 251), and mild in 21.5% (54/ 251) of biopsy recipients. Only one complication was reported—bleeding into the liver tissue (Pokorny 2002).

To reduce the risk of pain, complications, and sampling errors, liver biopsy should be performed by experienced physicians guided by ultrasound, and pain management should be provided to those undergoing biopsy.

About Liver Architecture

The liver is composed of many small, grape-like structures called lobules. Lobules are made up of plates of hepatocytes (liver cells) radiating outward from a central vein. The lobules are squeezed together in clusters. The portal zones are found between the lobules of the liver. Liver damage from hepatitis C can cause small areas of cell death (focal necrosis). As this necrosis worsens, dense, fibrous scar tissue may develop in portal zones. As the fibrosis worsens, it may extend from one portal zone to adjoining portal zones; this is called bridging fibrosis. Bridging fibrosis is the stage before cirrhosis. Cirrhosis is characterized by serious scarring that has damaged the liver's structure and ability to function.

Evaluating Biopsy

Different systems have been used to evaluate the grade and stage of liver disease. Grading measures the amount of disease activity in a sample of liver tissue, while staging identifies the degree of liver damage from the onset of disease to the development of cirrhosis. While liver disease grading may improve or worsen, staging is often stable or progressive.

The first system for scoring biopsy samples was developed by Knodell and colleagues in 1981; it has four components. Three components grade disease activity by area and extent; they are added up with the fourth component to make up the histological activity index (HAI). The fourth component stages disease, scoring the degree of fibrosis from zero to four. The HAI system is used more frequently in research settings than in clinical practice due to its complexity. In 1994 Desmet urged that that the grading score be separated from the staging, and that periportal necrosis and bridging necrosis be evaluated separately from each other, because of differences in prognostic implications (Desmet 1994).

Table 4. The Histological Activity Index

Periportal ± Bridging Necrosis	Score	Intralobular Degeneration and Focal Necrosis	Score	Portal Inflammation	Score	Fibrosis	Score
None	0	None	0	No Portal Inflammation	0	No Fibrosis	0
Mild piecemeal necrosis	1	Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in 1/3 of lobules or nodules)	1	Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1	Fibrous portal expansion	1
Moderate piecemeal necrosis (involves <50% of the circumference of most portal tracts)	3	Moderate (involvement of 1/3-2/3 of lobules or nodules)	3	Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	3	Bridging Fibrosis (portal-portal or portal-central linkage)	3
Marked piecemeal necrosis (involves >50% of the circumference of most portal tracts)	4	Marked (involvement of >2/3 of lobules or nodules)	4	Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	4	Cirrhosis	4
Moderate piecemeal necrosis plus bridging necrosis	5						
Marked piecemeal necrosis plus bridging necrosis	6						
Multilobular necrosis	10						

Knodell 1981

A modification of the HAI described by Ishak and colleagues has supplanted the traditional HAI in many studies. It adds finer detail to the fibrosis subscore to help delineate more subtle changes.

Table 5. The Ishak Modification of the Knodell HAI: Architectural Changes, Fibrosis and Cirrhosis

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas, with occasional portal to portal bridging	3
Fibrous expansion of portal areas with marked bridging as well as portal-central	4
Marked bridging (portal to portal and/or portal-central) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
Maximum score	**6**

Ishak 1995

Table 6. The Ishak Modification of the Knodell HAI: Grading

Feature	Score
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
B. Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal-central bridging	4
Zone 3 necrosis + multiple portal-central bridging	5
Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
Absent	0
One focus or less per 10 X objective	1
Two to four foci per 10 X objective	2
Five to ten foci per 10 X objective	3
More than ten foci per 10 X objective	4
D. Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Maximum score	**18**

Ishak 1995

A newer, simpler system, the METAVIR—devised by the French research group of the same name—is more commonly used in clinical practice. Simpler grading and precise definition of pathological features in the METAVIR system may decrease observer variation. The METAVIR system evaluates liver tissue specimens for fibrosis stage and two major components of disease activity: inflammation and necrosis (cell death). The grading of disease activity is based on the severity of periportal inflammation or injury to the portal zones between the lobules of the liver—when severe, it overlaps across portal zones—and the degree of focal parenchymal necrosis (the death of small areas of liver tissue). Specimens are graded on a scale from A0 (no activity) to A3 (severe activity). The degree of fibrosis is staged from F0 (for none) to F4 (for cirrhosis).

Table 7. The METAVIR System

Histological Activity: Lobular Necrosis and Piecemeal Necrosis	Fibrosis Stage
no activity = A0	F0 = no fibrosis
mild activity = A1	F1 = portal fibrosis without septa
moderate activity = A2	F2 = portal fibrosis with rare septa
severe activity = A3	F3 = numerous septa without cirrhosis
	F4 = cirrhosis

Bedossa 1996

Other systems used to grade and stage liver disease include the Scheuer, the Sciot and Desmet (unpublished), and the Ludwig Batts with the Tsui modification.

Evaluating Cirrhosis: Child-Pugh and MELD Systems

Once an individual has been diagnosed with cirrhosis, a scoring system known as the Child-Pugh can assess its severity. The Child-Pugh system combines information from biochemical testing (bilirubin, albumin, and prothrombin time) with evaluations of hepatic encephalopathy and ascites. Another scoring system called MELD (Model of End-Stage Liver Disease) has replaced Child-Pugh scores for grading of severity during work-up and prioritization for liver transplantation. The MELD score is based on results from serum bilirubin, serum creatinine, and prothrombin time (calculated as the International Normalized Ratio).

Table 8. The Child-Pugh Score

Score	Bilirubin	Albumin	Prothrombin Time	Hepatic Encephalopathy	Ascites
1	< 2 mg/dl	> 3.5 gm/dl	1-4 seconds	None	None
2	2-3 mg/dl	2.8-3.5 gm/dl	4-6 seconds	grade 1-2	Mild (detectable)
3	> 3 mg/dl	< 2.8 gm/dl	> 6 seconds	grade 3-4	Severe (tense)

Pugh 1973

Problems with Biopsy and Alternatives

The risk of complications and possible pain involved with liver biopsy has made it an unpopular procedure with patients. Sampling errors and variation between observers also occur. Bejarano and colleagues assessed diagnostic inconsistencies from 125 biopsy specimens by comparing reports from community-based pathologists to reports from a hepatopathologist and a hepatologist. Results were classified as: major discrepancy (a diagnosis which might have resulted in inappropriate management and/or treatment decisions, misdiagnosis, and inappropriate assessments), minor discrepancy (considered unlikely to have an effect on future procedures or treatments, omissions and incomplete descriptions were included as well), and full concordant agreement. Twenty-eight percent (35/125) were categorized as major discrepancies. Failure to properly identify hepatocellular activity was identified in 28% (9/35) within this group. Eleven of these cases involved discordant

assessments of cirrhosis: six of the eleven initial reports failed to diagnose established cirrhosis, and five initial reports diagnosed cirrhosis where none had developed. Minor discrepancies were identified in 38% (47/125) of the reports (Bejarano 2001). To ensure proper diagnosis of HCV-related liver damage, biopsies should be read by pathologists who are skilled in reviewing liver biopsies.

The average biopsy specimen ranges from a few millimeters to several centimeters long, representing from one-hundred-thousandth to one thirty-thousandth of the liver (Scheuer 2003). Since liver damage is not evenly distributed throughout the liver, it is difficult to accurately assess the grade and stage of liver disease from a small biopsy sample (Bedossa 2003; Colloredo 2003). Colloredo and colleagues examined 161 biopsy samples from people with chronic hepatitis B and C. When they reduced the size of individual samples, their grading and staging scores decreased significantly; samples that were 1 mm in width were underscored for both the grade and stage of liver disease, no matter their length (Colloredo 2003). Bedossa and colleagues studied 17 large liver tissue samples from individuals with chronic hepatitis C. They used the same liver tissue to create virtual samples of differing lengths. The virtual samples were compared with the original samples to determine the minimum size for accurate liver biopsy specimens. The minimum length needed for accurate METAVIR system staging was 25 mm (Bedossa 2003).

Within an individual, the grade and stage of liver disease may vary according to the area of the liver from which the sample is obtained. Regev and colleagues compared 124 liver biopsy samples simultaneously obtained from the right and left liver lobes of 62 people with chronic hepatitis C. Samples were coded, and randomly divided between two hepatopathologists; after assessment by the Scheuer system, they were uncoded and samples from the left and right lobes of the same liver were compared to one another. The differences between right and left lobes were significant; one third of samples differed by at least one stage, and one quarter differed by at least one grade. In 14.5% (18/124) of samples from the same liver, cirrhosis was present in one of the lobes, while stage 3 fibrosis was present in the other lobe. Fifty samples were de-identified and re-examined by the hepatopathologists to assess intraobserver bias. Grading and staging of the 50 re-examined samples did not differ significantly from the first examination. Most of the differences in grading and staging were attributed to sampling (Regev 2002).

Reliable, reproducible and non-invasive biopsy alternatives are highly desirable. In a prospective study, Imbert-Bismut and colleagues assessed the predictive value of a combination of serum biochemical markers to diagnose fibrosis in people with chronic hepatitis C. They analyzed the concordance between 339 biopsy samples evaluated by the METAVIR scoring system and 11 serum biochemical markers. They selected six serum biochemical markers (α_2 macroglobulin, haptoglobulin, GGT, γglobulin, total bilirubin, and apolipoprotein-A1), and added ALT to increase diagnostic accuracy. These markers were validated in a group of 134 individuals by comparing them with liver biopsy samples. A serum biomarker index score of <0.10 predicted no fibrosis or mild fibrosis (F0 or F1) in more than 90%, while a score ≥ 0.60 accurately predicted moderate to severe fibrosis and cirrhosis (F2, F3, or F4) in more than 90%. However, more than half of the scores fell in the range between 0.10 and 0.60; these scores did not have a high enough predictive value to eliminate the need for a biopsy (Imbert-Bismut 2001).

A panel of five serum biochemical markers (α_2 macroglobulin, apolipoproteinA1, haptoglobulin, GGT, and bilirubin)— called Fibrotest— has been marketed for diagnosing fibrosis. Myers and colleagues compared Fibrotest scores from 534 people to their METAVIR biopsy scores. They

reported that a Fibrotest score below 0.50 ruled out cirrhosis in 329 of 337 individuals (98% negative predictive value). A score >0.50 predicted cirrhosis in only 55/197 (positive predictive value of 28%) (Myers 2002). Fibrotest is not as accurate for identifying mild-to-moderate liver damage.

Researchers continue to investigate non-invasive alternatives to liver biopsy (Boeker 2002; Forns 2002; Guechot 1994; Leroy 2004; Patel 2003; Sud 2004; Wai 2003). Sud and colleagues identified five independent predictors of fibrosis in people with chronic hepatitis C: age, AST, total cholesterol level, insulin resistance and past alcohol intake. These five markers accurately predicted significant fibrosis in 87% of cases among 176 individuals (Sud 2004). Wai and colleagues reported that using an AST to platelet ratio index (APRI) predicted liver damage in individuals with chronic hepatitis C. The APRI accurately predicted significant fibrosis in 51%, and cirrhosis in 81% (Wai 2003).

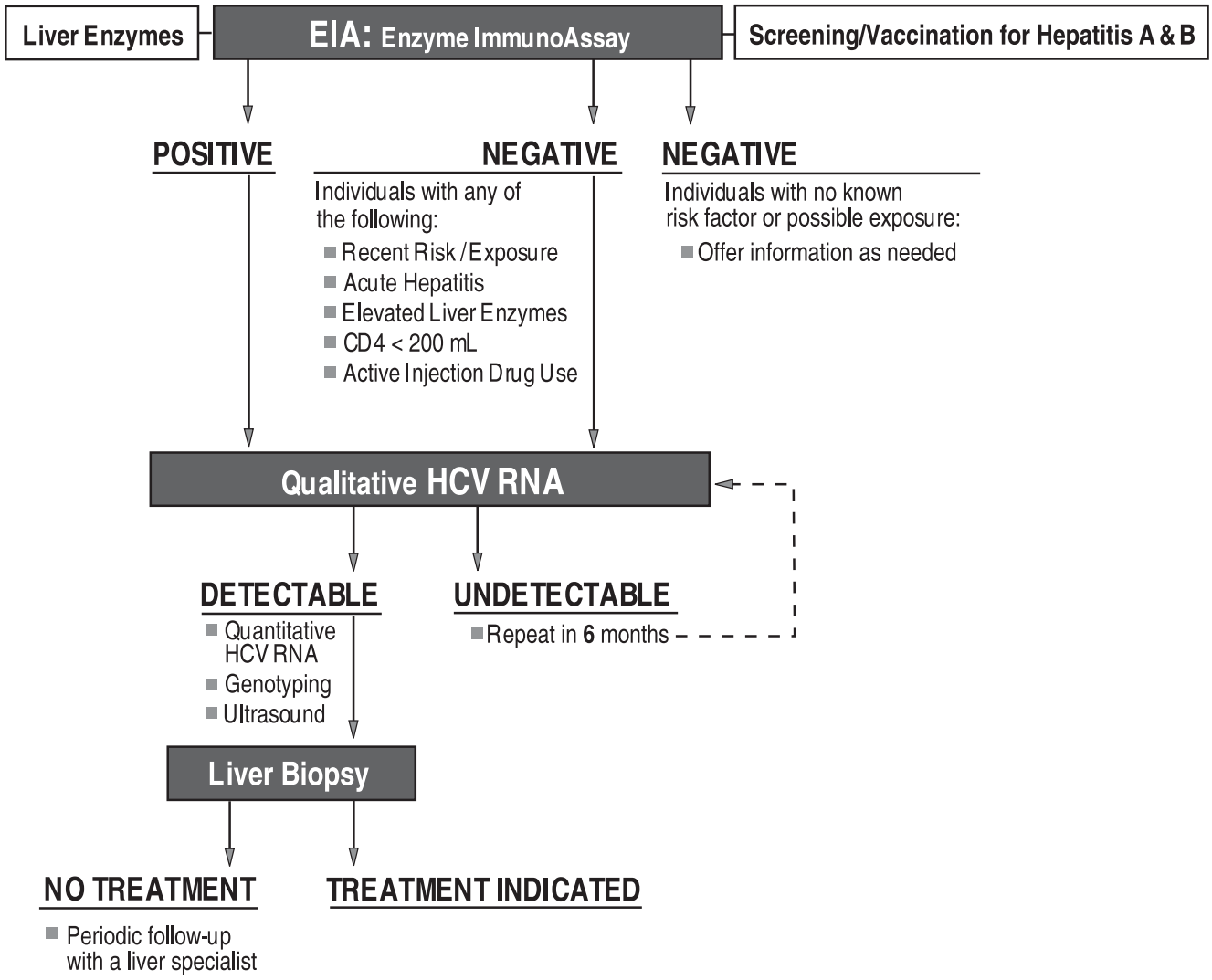
While these serum biomarkers cannot provide data on liver histology equivalent to that from a liver biopsy, these tests may be a viable first-line alternative when biopsy is contraindicated or refused. Further research is needed to determine which tests are most appropriate for diagnosing and staging fibrosis, monitoring fibrosis progression over time in untreated persons, and assessing response to HCV treatment, as well as how frequently these tests should be used.

HIV Coinfection: Diagnostic Considerations

Because of the overlap in modes of transmission, the CDC has recommended that all HIV-positive individuals undergo screening for hepatitis C infection. All positive results from HCV antibody testing should be verified with qualitative HCV-RNA testing. In addition, confirmatory testing may also be used in certain situations when antibody test results are negative but HCV infection is suspected. For example, immunodeficient individuals may not be producing HCV antibodies. Qualitative HCV-RNA testing may be needed to diagnose HCV infection in HIV-positive individuals, especially those with CD4 cell counts below 200/mm^3 (Berggren 2001; Busch 2001; H. H. Lin 2002). CD4 cell counts <100/mm^3 have been significantly associated with false-negative HCV ab (OR=49; P<0.01) (Berggren 2001). George and colleagues found a startling incidence of occult HCV infection among 131 HIV-positive individuals. Although only 31 of the HIV-positive individuals (23.7%) had antibodies to HCV, 19 of the remaining 100 (19% of the HCV-antibody-negative individuals, or 14.5% of the entire sample) had detectable HCV RNA despite being HCV-antibody-negative (George 2002).

HCV-RNA levels in coinfected individuals are higher than in those with HCV alone (Cribier 1995; Di Martino 2001; Eyster 1994; Sulkowski 2002; Thomas 2001; Zylberberg 1996). Sánchez-Quijano and colleagues found HCV-RNA levels close to ten times higher in coinfected persons. Higher HCV-RNA levels have been found in the liver and the blood of coinfected individuals (Bonacini 1999). Some studies have found HCV-RNA levels inversely correlated with CD4 cell counts (Di Martino 2001; Eyster 1994; Ghany 1996; Thomas 2001). Although no association with HCV-RNA levels and HCV disease progression has been found, HCV viral loads under 2,000,000 copies/ml (equivalent to 800,000 IU/mL) are associated with better responses to interferon-based HCV treatment.

Figure 2. Alternative HCV Diagnostic Algorithm for HIV+ Individuals



Liver Enzymes in Coinfected Individuals

Highly active antiretroviral therapy (HAART), OI prophylaxis and treatment, and medications used to manage treatment-related complications and comorbidities can adversely affect the liver. In the United States, 90% of those with an AIDS diagnosis use at least one hepatotoxic drug (Orenstein 2002). Therefore, monitoring of liver enzymes at one month after initiation of therapy and then every three months is recommended for coinfected individuals receiving HAART. More frequent monitoring is indicated for specific antiretroviral regimens, or for individuals with advanced liver disease (see Chapter VI, HIV Treatment in HIV/HCV Coinfection). HAART-mediated immune reconstitution may cause flares in ALT levels, possibly related to the restoration of HCV-specific immune responses. Severe elevations in ALT and AST levels may necessitate modification of the HIV treatment regimen in certain circumstances.

Elevations in AST have been associated with poorer survival in HIV-positive individuals (Justice 2002; Rancinan 2002). Justice and colleagues examined survival in two cohorts of HIV-positive individuals: the Collaboration in HIV Outcomes Research-US (CHORUS; n=5,985; 87% white

male MSM) and the Veterans Aging 3-Site Cohort (VACS 3; n=881; 99% male and 55% African-American, 53% infected with HIV from IDU or heterosexual exposure). There were 400 deaths in the CHORUS cohort from 1997 until 2002, and 71 deaths in the VACS 3 cohort from 1999 until 2000. In a multivariable analysis of survival, the hazard ratio of elevated AST for mortality was 6.40 (P<0.001) in the CHORUS cohort, and 2.62 (P=0.09) in the VACS 3 cohort. Although coinfection with hepatitis C was associated with elevated AST in both cohorts, (HR, 15.5; P<0.001 and HR, 6.1; P<0.001 respectively), HCV coinfection was not an independent predictor of survival (CHORUS HR, 1.53; P=0.1; VACS 3 HR, 0.24; P=0.8).

In an examination of survival in the Aquitaine cohort, (995 HIV-positive individuals; 576 of them HCV coinfected) Rancinan and colleagues found a significant association between AST elevations and poorer survival (HR for elevations >200 IU/l of 2.30; 95% CI, 1.32-4.03; P=0.004), although they did not find an association between HCV seropositivity itself and poorer survival. However, more coinfected individuals had elevated AST (15%, vs. 7% of those with HIV alone).

Liver damage may be present in coinfected individuals, regardless of persistently normal alanine aminotransferase (ALT) levels. Mendes-Corrêa and colleagues reviewed clinical information and liver biopsy samples from 195 coinfected persons, 28 with normal ALT. They found moderate to severe liver damage in biopsy samples from 32% (9/28) of coinfected individuals with normal ALT (Mendes-Corrêa 2003). Uberti-Foppa and colleagues investigated the extent of liver damage among coinfected people with persistently normal ALT (PNALT) in a retrospective examination of liver biopsies from 354 coinfected people, 26 with PNALT (three consecutive normal ALT levels over 12 months). The CD4 cell count, HIV RNA, antiretroviral regimens and HCV RNA did not differ significantly by ALT level, although HCV genotype 1 was significantly more common among those with PNALT (52% [14/26] vs. 31% [102/328]; P= 0.01). Those with PNALT had significantly lower scores for grading and staging of liver disease (4.12 ± 1.64 vs. 7.35 ± 2.7; P=0.0007 for grading; 1 ± 1.69 vs. 2.24 ± 1.79; P=0.0147 for staging).

Table 9. Fibrosis Scores in Coinfected People with Normal and Abnormal ALT

Ishak Fibrosis Score	PNALT (N=26)	Abnormal ALT (N=328)
0-1 (absent to mild)	75%	42.9%
2-3 (moderate to serious)	12.5%	33.8%
4-6 (bridging fibrosis to cirrhosis)	12.5%	23.2%

Uberti-Foppa 2004

Information on the duration of HCV and HIV infection, age, nadir CD4 cell count and duration of antiretroviral therapy was not included; any prospective study of coinfected individuals with PNALT should incorporate this information.

Although it is tempting to regard ALT measurements as a surrogate for biopsy, liver histology cannot be assessed without a liver biopsy. These data underscore the need for biopsy to ensure that liver disease is assessed accurately. Biopsy may be especially important for assessment of liver disease in coinfected persons, because it is frequently assumed that they have severe liver damage when this is not invariably the case. Merchante and colleagues performed a cross-sectional study

of liver histology in coinfected people. They examined biopsy samples collected from 152 coinfected people between November of 1989 and March of 2003. Fibrosis was absent, or mild (F0 or F1) in 37.5% (57/152) (Merchante 2003).

Genotype

García-Samaniego and colleagues investigated the influence of hepatitis C genotype on the liver histology of coinfected individuals. In a cohort of 59 HCV-infected individuals, 48 (82%) coinfected with HIV, they found significantly higher histological activity scores (a measurement of disease activity by the amount of inflammation and damage in liver tissue) and more fibrosis among individuals with HCV genotype 1b or a mixed infection including genotype 1b. The odds ratio for an association between genotype 1b infection and higher histological activity scoring was 3.5 (95% CI, 1.1–11.3; P=0.036). Genotype 1b was significantly associated with fibrosis (OR, 20.9; 95% CI, 2.8-–157.2; P=0.003). Infection with genotype 1b was significantly associated with piecemeal necrosis (liver cell death) and portal inflammation. HIV infection was also significantly associated with fibrosis, with an odds ratio of 17.9 (95% CI, 2.5–129.0; P=0.004) (García-Samaniego 1997). Because coinfection with HIV is known to accelerate HCV disease progression, and this study was finished before the HAART era, it is not possible to generalize the results to other circumstances and populations. Further study would be necessary to evaluate the influence of genotype 1b on the severity of HCV disease in coinfected individuals during the HAART era.

Alternatives to Liver Biopsy: Serum Biochemical Markers in HIV/HCV Coinfection

A panel of six serum biochemical markers—total bilirubin, γ-glutamyltranspeptidase (GGT), α_2 macroglobulin, apolipoprotein-A1 and haptoglobulin—has been evaluated for use as an alternative to liver biopsy for HIV/HCV coinfected individuals. The score from an index comprised of age, sex, and biomarker test results was compared to liver biopsy samples from 130 coinfected persons. If a score of 50 was used as a cutoff, the absence of cirrhosis would be accurately predicted in 100% of cases, and the presence of cirrhosis would be accurately predicted in 65% of cases (Myers 2003). Although this index is not equivalent to a biopsy, and does not accurately predict mild to moderate liver damage, it may be a useful screening tool for fibrosis, especially in situations where biopsy is contraindicated or refused.

Mehta and colleagues compared serum marker testing with liver biopsy results from 96 coinfected people. The threshold for each serum marker was as follows: alanine aminotranferase <93 IU/L; aspartate aminotransferase <61 IU/L; albumin >3.6 g/dL; total bilirubin <1.2 mg/dL; and hyaluronic acid <42. They found that hyaluronic acid (HA) levels were higher in coinfected persons with moderate to severe fibrosis and cirrhosis than in those with no fibrosis. A fibrosis score of >2 on the Ishak system was 12 times more likely among individuals with HA levels > 85 ng/mL (95% CI, 3.46–43.35) and almost three times more likely with HA levels from 42 to 85 (95% CI, 0.87–9.20). Lower levels of HA, albumin and AST predicted milder liver damage. Thirty-five individuals had HA, albumin and AST levels below the threshold; all had fibrosis scores of ≤ 2 (Mehta 2004).

These results merit prospective investigation of serum biochemical markers as a biopsy alternative for HIV/HCV coinfected persons. Evaluation of serum biomarkers could be incorporated into large observational studies and treatment trials.

Recommendations

Develop and market oral fluid test kits for HCV antibody testing.

Oral fluid HCV antibody testing will provide opportunities to perform initial HCV screening in individuals with poor venous access, and in venues frequented by high-risk individuals, including syringe exchange programs, methadone clinics, drug treatment facilities, shelters and correctional facilities. Oral fluid collection kits for HCV antibody testing would also increase the capacity to collect epidemiological data. The National Institutes of Health and Industry should support surveillance initiatives for oral fluid testing of hard-to-reach populations.

Identify and validate prognostic markers and effective screening methods for early diagnosis of hepatocellular carcinoma (HCC).

Hepatocellular carcinoma is a known complication of hepatitis C. In the United States, the incidence of hepatocellular carcinoma (HCC) in the general population has increased from a rate of 1.4 cases per 100,000 between 1976 and 1980, to 2.4 cases per 100,000 during the period between 1991 and 1995 (El-Serag 1999). This rise may reflect the consequences of an epidemic of increased HCV transmission that occurred decades earlier. The annual incidence of HCC in hepatitis C-infected cirrhotics ranges from 1% to 4% (Di Bisceglie 1997; Lauer 2001).

HCC can be identified by measuring alpha-fetoprotein (AFP) levels and by ultrasound imaging, but the value of these tests for early detection of HCC in cirrhotic individuals has not been sufficiently demonstrated. The sensitivity and specificity of AFP levels for detection of HCC varies considerably (from 39% to 64% and from 76% to 91%, respectively) in different studies (Collier 1997). Some research has shown that ultrasound surveillance increases early detection of HCC, but it may not reduce mortality (Larcos 1998; Solmi 1996). Mortality from hepatocellular carcinoma is extremely high, with five-year survival rates of less than 5% (El-Serag 1999). Better interventions to facilitate the early diagnosis of HCC and reduce the high fatality rate are urgently needed. The National Institutes of Health (NIH) must fund this research

Promote use of a standardized system for evaluation of liver biopsy.

A standardized system should be selected for evaluating the results of liver biopsy in research to enable cross-study analysis.

Continue research on non-invasive testing methods to replace or reduce the need for liver biopsy.

Liver biopsy is still the only way to assess the condition of liver tissue. Information from liver biopsy is used to assess the degree of inflammation, gauge hepatic cell death and damage, identify other causes of liver injury, and guide treatment decisions. Although fatalities from biopsy are extremely rare (0.01% to 0.1%), liver biopsy can be painful, and occasional complications such as hemorrhage or puncture of adjoining organs may occur. The risk of complications and the potential pain of the procedure have made liver biopsy unpopular with many patients.

Alternatives to liver biopsy using panels of serum biochemical markers are under investigation, and some have been marketed. Although these panels may serve as substitutes in cases where a biopsy is contraindicated or refused, they yield far less precise information. The identification, development, and validation of a non-invasive, cost-effective replacement for liver biopsy would be an important breakthrough and merits research from the National Institutes of Health (NIH).

Educate primary care providers about diagnosis of acute and chronic hepatitis C infection.

Acute hepatitis C infection is clinically silent for most infected people, with only 15% to 20% of individuals developing symptoms (Koretz 1993). Symptoms, when they occur—low-grade fever, fatigue, appetite loss, abdominal pain, nausea, and vomiting—are typical of many common viral infections. Chronic hepatitis C infection is also often asymptomatic, and both acute and chronic hepatitis C infections may go undiagnosed by physicians who fail to ask about risk factors (Shehab 2001; Shehab 2002; Villano 1999).

Many physicians are unaware of the proper procedures for diagnosing hepatitis C (Shehab 1997). HIV-positive individuals (especially those with fewer than 200 CD4 cells), injection drug users, and transplant recipients may harbor occult hepatitis C infection (Beggren 2001; Beld 1999; Busch 2001; H. H. Lin 2002; Thomas 1995). Routine HCV-RNA confirmatory testing following a negative HCV-antibody test result should be considered in populations who may harbor occult infection, including HIV-positive individuals and injection drug users. Provider education initiatives must be available from the Centers for Disease Control, the American Medical Association, and the AIDS Education and Training Centers.

A Guide to the Liver Panel

Alanine aminotransferase (ALT; formerly known as serum glutamic pyruvic transaminase or SGPT) is an enzyme produced in the liver that metabolizes amino acids. During acute HCV infection, ALT levels in the blood may rise to twenty times above normal. Certain medications, including some antiretroviral agents, may cause ALT level increases. ALT levels usually decrease again when these drugs are discontinued. Excess ALT seeps into the bloodstream when liver cells are injured or dying. However, if damage to the liver is widespread and the liver is incapable of producing sufficient ALT, enzyme levels in the blood may remain in a normal range. Blood ALT levels cannot reliably diagnose or predict the state or progression of HCV disease. However, a reduction in ALT levels during HCV treatment—a biochemical response—is regarded as a positive development.

The normal reference range for ALT levels usually falls between 1 and 45 U/L, although this varies by laboratory. ALT levels typically reach a peak during the afternoon and are at their lowest levels at night. Within an individual, ALT levels can vary by 10-30% from day to day. Body weight also affects ALT levels. People with a high body mass index (BMI) tend to have ALT levels 40–50% higher than thinner people. Strenuous exercise may decrease ALT levels by 20%. ALT levels vary with age; standard reference ranges may not apply to children or to people over 60. Men typically have higher ALT levels than women. Liver injury in women may be underreported if judged by reference ranges derived primarily from males.

Aspartate aminotransferase (AST; formerly known as serum glutamic oxalocetic transaminase or SGOT) is an enzyme found in the heart, kidney, pancreas, spleen, liver, lungs, skeletal muscle, brain tissue, and red blood cells. When tissues in these organs are injured, AST is released into the bloodstream. AST is neither as sensitive nor as specific to liver injury as ALT. However, AST levels may rise to twenty times above normal during acute HCV infection, and may remain elevated during chronic HCV infection. Anti-HIV medications may produce elevations in AST as the liver works to metabolize these drugs, but AST levels usually decrease when these drugs are discontinued. AST levels do not predict HCV disease progression or severity, although a decrease in AST levels during HCV treatment is considered to be a good biochemical response.

The normal reference range for blood levels of AST falls between 1-36 U/L, though this varies by laboratory. Individual AST levels can vary by 5-10% from day to day. Normal AST levels among African American males are typically 15% above the reference normal. Body weight also affects AST levels. Individuals with a high body mass index (BMI) may have AST levels 40-50% above those of thinner people. Cardiac and skeletal muscle injuries may produce significant elevations in AST. Strenuous exercise before a blood test can produce a three-fold elevation in AST.

Alkaline phosphatase (ALP) is an enzyme primarily found in the intestines, bones, and liver; some is produced and found in cells lining the bile duct and in the placenta. Obstruction in the bile duct due to liver disease can cause an accumulation of bile acids in the liver, which in turn can stimulate the production of ALP. Therefore, elevated ALP may indicate an underlying cholestatic (obstructive) liver disease. Drugs that reduce bile transport from liver cells to the bile duct can also stimulate increased production of ALP. In HCV-infected individuals, ALP elevations are associated with a type of serious liver injury called post-hepatitic cholestasis.

The normal reference range for blood ALP levels falls between 35-150 U/L for adults, 100-500 U/L for adolescents and 100-350 U/L for children. These values may vary by laboratory. Individual ALP levels can vary from 5-10% from day to day. Someone with a full stomach may have ALP increases of up to 30 U/L. Normal ALP levels are usually 15% higher than the reference range in African-American males and 10% higher in African-American females. ALP levels tend to increase in post-menopausal women. Body weight affects ALP; levels increase by 25% with higher BMI (body mass index). Smoking increases ALP by 10%. Oral contraceptives decrease ALP levels by 20%. ALP levels are increased between two-and three-fold during the third trimester of pregnancy.

Gamma-glutamyl transferase (GGT) is an enzyme produced by the bile ducts. GGT levels may be elevated in individuals with any type of liver disease, and especially in individuals with bile duct diseases. As many as 80 to 95% of individuals with acute hepatitis have elevated GGT levels. GGT levels increase with cirrhosis and hepatocellular carcinoma. Alcohol and certain medications elevate blood levels of GGT, while other medications lower GGT.

The normal reference range for GGT falls between 0-51 IU/L. These values may vary by laboratory. Within each individual, GGT levels vary by 10-15% from day to day. Among African-Americans, normal GGT levels may be two times above the reference range. Men under 50 have GGT levels 25-40% higher than those in women. Among those with moderately high body mass index (BMI), GGT levels may increase by 25%. When BMI is over 30, GGT levels may increase by 50%. Pregnancy reduces GGT levels by 25%. Smoking twenty cigarettes per day increases GGT levels by 10%; levels double with heavier smoking. GGT levels may remain elevated for weeks after heavy drinkers stop drinking.

Bilirubin is a by-product of the breakdown of red blood cells. In the spleen, hemoglobin from red blood cells is broken down and the released heme is processed into bilirubin and transported to the liver. In the liver, bilirubin undergoes further processing to become water-soluble and is excreted into the gut as part of bile, which helps to break down fats. Non-water-soluble bilirubin is called unconjugated or indirect bilirubin. Water-soluble bilirubin that has been metabolized by the liver is called conjugated or direct bilirubin. Two tests measure bilirubin levels. Total bilirubin includes both direct and indirect bilirubin levels, while direct bilirubin testing measures the amount of bilirubin processed in the liver. When both bilirubin levels are normal, it reflects a balance between bilirubin production from red blood cell breakdown and bilirubin elimination by the liver. Liver cell injury is indicated when the total bilirubin level is high while direct bilirubin is low. Hepatitis C infection can slow the processing of bilirubin in the liver, and bilirubin levels can become elevated, causing jaundice (yellowing of the skin and eyes). Some medications, including certain HIV protease inhibitors, can increase the level of total bilirubin by affecting the rate of conversion of indirect to direct bilirubin.

The normal reference range for blood levels of total bilirubin falls between 0.3-1.1 mg/dl (5.1-19.0 mmol/L in international units). The normal reference range for direct bilirubin falls between 0.1-0.4 mg/dl (1.7-6.8 mmol/L in international units). Values may vary by laboratory. Within an individual, bilirubin levels may vary by 15-30% from day to day. Overnight fasting typically increases bilirubin levels by 20-25%. Normal bilirubin levels are 33% below the reference range in African-American males, and 15% lower in African-American females. Exercise can increase bilirubin levels by 30% in males. Oral contraceptives reduce bilirubin levels by 15%. Hemolytic

anemia, a potential side effect of ribavirin, causes an increase in indirect bilirubin.

Albumin is a protein made by liver cells. It helps maintains the pressure that prevents fluids from seeping out of the bloodstream and into tissues. Albumin also carries drugs, hormones and waste products through the bloodstream. A seriously damaged liver is unable to produce sufficient albumin. Albumin levels usually remain normal during acute hepatitis. In chronic hepatitis, albumin levels may decrease gradually as progression to cirrhosis occurs. Abnormally low levels of albumin can be a prognostic marker for liver decompensation and hepatocellular carcinoma.

The normal reference range for albumin blood levels is between 3.5-5.4 gm/dl, though values may vary from lab to lab. Dehydration temporarily lowers albumin levels.

Total protein measures two major blood proteins, albumin and globulin. Approximately 60% of total protein is albumin. There are three types of globulins: alpha-, beta-, and gammaglobulins. Alpha globulins are made in the lungs and the liver. Betaglobulins, also known as low-density lipoproteins (LDLs), transport fat throughout the body. The gammaglobulins are antibodies. Total protein and globulin levels may increase with cirrhosis. When a cirrhotic liver is unable to produce sufficient albumin, the body produces extra globulin in an attempt to maintain sufficient total levels of protein.

The normal reference ranges for these tests are 6.4-8.3 g/dl for total protein, 3.5-5.4 g/dl for albumin and 2.3-3.4 g/dl for globulin. Values may vary by laboratory.

Alpha-fetoprotein (AFP) is produced in fetal liver tissue. After birth, blood AFP decreases to very low levels. AFP levels may increase in cases of acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer.

AFP levels over 50 ng/ml are considered abnormal, while levels between 11-100 ng/ml may indicate liver cell regeneration. AFP levels over 100 ng/ml may indicate hepatocellular carcinoma (HCC); levels over 1,000 usually indicate HCC.

Prothrombin Time (PT) measures the amount of time needed for blood to clot. Clotting factors are made in the liver. PT elevations may occur during acute hepatitis. In chronic hepatitis, PT usually remains normal, though abnormal PT prolongation usually accompanies progression to cirrhosis. PT is elevated in cirrhotic individuals, because a significantly damaged liver may not be able to produce enough clotting factors. Some medications increase PT. A PT in excess of three seconds above the normal range is associated with increased risk of bleeding.

The normal prothrombin time is about 11-15 seconds, though values may vary from lab to lab. Some laboratories report the international normalized ratio (INR), a standardized ratio for sample clotting time versus a control value; 1.0 represents normal.

List of Terms Used in This Chapter

Branched-chain DNA assay (bDNA): a test used to measure the amount of a virus in blood plasma.

Eq: is an abbreviation for virus equivalent. Eq is used to indicate that HCV genetic material has been measured by weight in picograms. To convert picograms, multiply the result by 1 million.

Reverse-transcriptase polymerase chain reaction (PCR) assay: a very sensitive test used to detect and measure RNA or DNA of organisms and viruses in blood plasma or tissue.