Diagnosis of Hepatitis C

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Currently, the second- and third-generation enzyme immunoassays (EIA-2 and EIA-3) for hepatitis C virus antibody (anti-HCV) are the most practical screening tests for the diagnosis of HCV infection. The need for and the choice of supplementary or confirmatory tests depend on the clinical setting and the likelihood of a true-positive EIA result. Detection of HCV RNA in serum by polymerase chain reaction (PCR) assay is the gold standard for the diagnosis of HCV infection. However, the lack of uniformity in current PCR assays has tarnished this standard. Confirmatory tests for the diagnosis of HCV infection are in general unnecessary in anti-HCV–positive patients who present with chronic liver disease. When indicated, the most appropriate test in this setting is a qualitative PCR assay for HCV RNA. Confirmatory tests should always be performed in anti-HCV–positive blood donors and individuals with normal aminotransferase levels. The most appropriate approach is to retest for anti-HCV using recombinant immunoblot assay (RIBA) and then test for HCV RNA using PCR assay in those who are RIBA positive or indeterminate. Liver histology is the gold standard in assessing severity of liver disease. Quantitative tests for serum HCV RNA levels do not help to determine the severity of liver disease. At the moment, HCV genotyping should be considered a research tool and not a part of the diagnostic work-up in clinical practice. The goals of treatment for chronic hepatitis C are sustained biochemical and virological response. Viral clearance should be determined by qualitative PCR assay. Quantifying serum HCV RNA level can help in predicting response to interferon treatment, but further studies using more standardized assays are needed to determine if these values can be used to select patients for treatment. (HEPATOLOGY 1997;26 (Suppl 1):48S-56S.)

Diagnosis of hepatitis C involves confirmation of the presence of hepatitis C virus (HCV) infection and assessment of the severity of liver disease. In addition, the diagnostic work-up should include investigations that may help to predict prognosis and response to treatment. This review focuses on the application of tests for antibody to HCV (anti-HCV), HCV RNA, and HCV genotypes in the diagnosis of HCV infection, assessment of the severity of liver disease, monitoring progress of liver disease, determination of the likelihood of response to interferon therapy, and monitoring of response to treatment.

DIAGNOSIS OF HEPATITIS C

Anti-HCV Tests. Currently, the second-generation enzyme immunoassays (EIA-2) for anti-HCV are the most practical screening tests for the diagnosis of HCV infection in the United States.[1,2] These assays detect antibodies to recombinant HCV antigens from the core (C22) and nonstructural regions 3 (C33) and 4 (C-100). They are easy to perform and the results are highly reproducible. Recently, third-generation EIAs (EIA-3) have been approved by the Food and Drug Administration for blood donor screening. EIA-3 differs from EIA-2 in that it incorporates additional recombinant HCV antigen from the nonstructural region 5 (NS5). EIA-3 is slightly more sensitive than EIA-2, but most of the improved sensitivity appears to be attributable to increased detection of anti-C33 and not the addition of NS5.[3-5] The second-generation recombinant immunoblot assays (RIBA-2) permit the detection of antibodies to individual recombinant HCV antigens: C22, C33, C-100, and 5-1-1 (overlaps with C-100). Patients who react to two or more HCV antigens are considered to be RIBA positive, whereas those who react to one HCV antigen only are considered to have indeterminate results.[1,2] RIBAs are technically more demanding than EIAs, but they are simpler, more standardized, and more reproducible than tests for HCV RNA. RIBAs confer increased specificity compared with EIAs. Nevertheless, RIBA positivity is not always indicative of ongoing HCV infection because patients with recovered HCV infection may remain anti-HCV positive for many years. RIBA-3, which differs from RIBA-2 in having additional recombinant proteins from NS5 and synthetic peptides from the core and NS3 antigens, has helped to resolve many of the RIBA-2 indeterminate samples, but only approximately 50% of the RIBA-3–positive blood donors are HCV RNA positive by polymerase chain reaction (PCR) assays.[6,7] EIA-3 and RIBA-3 have replaced EIA-2 and RIBA-2 in many European and Asian countries.

HCV RNA Assays. Confirmation of the diagnosis of ongoing HCV infection relies on the detection of viremia. This may be achieved by qualitative reverse-transcription PCR or branched DNA (bDNA) assays. Although the bDNA assay is technically simpler and has a lower chance of cross-contamination, PCR assays are preferred for the confirmation of HCV infection because of their increased sensitivities. The sensitivity limits of most PCR assays are in the range of 500 to 1,000 Eq/mL, whereas that of the second-generation bDNA assay is 200,000 Eq/mL.[8,9] Thus, 10% to 30% of patients with chronic hepatitis C who are HCV RNA positive by PCR assays may

Abbreviations: HCV, hepatitis C virus; anti-HCV, antibody to HCV; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction; bDNA, branched DNA signal amplification assay; ALT, alanine aminotransferase.

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have undetectable HCV RNA if tested by the bDNA assay.[8,10-14] Although the detection of HCV RNA by PCR assays is considered to be the gold standard for the diagnosis of HCV infection, the lack of uniformity in current PCR assays has tarnished this standard. In a recent international collaborative study, 86 laboratories submitted 136 data forms on a panel of coded sera.[15] Of these data sets, 99 were tested using a PCR assay developed in-house, 28 using a commercially available PCR assay (AMPLICOR; Roche Molecular Diagnostics Systems, Branchburg, NJ), and 9 using other amplification methods. Only 16% of the data forms had faultless results, 29% missed the weak positive sample only, and 55% had false-positive and/or false-negative results. These data highlight the urgent need to standardize PCR assays for the detection of HCV RNA. Standardization is especially important for quantitative HCV RNA assays where up to 1,000-fold differences in HCV RNA levels have been reported.[15]

HCV Genotyping. There are at least six genotypes of HCV and more than 30 subtypes. HCV genotyping provides important information in epidemiological studies but does not help in confirming the diagnosis of HCV infection.

Liver Biopsy. Characteristic histological features that are more frequently found in patients with chronic hepatitis C than in patients with chronic hepatitis B or autoimmune hepatitis have been reported.[16-18] However, none of these features is pathognomonic for chronic hepatitis C. Liver biopsies are not necessary for the diagnosis of HCV infection.

DIAGNOSTIC ALGORITHMS

The need for and the choice of supplementary and confirmatory tests depend on the clinical setting and the likelihood of a true-positive EIA result.[9,19] Different diagnostic algorithms may be considered for patients presenting with chronic liver disease or elevated alanine aminotransferase (ALT) levels as opposed to asymptomatic blood donors or individuals with normal ALT levels. It can also be argued that individuals with or without risk factors for HCV infection should be evaluated differently. However, identification of risk factors is more subjective and dependent on the experience and skills of the clinician. In view of the possibility of a false-negative EIA-2 result, modified diagnostic algorithms are recommended for immunocompromised patients and patients with acute hepatitis C.

Anti-HCV–Positive (EIA-2) Patients Presenting With Chronic Liver Disease or Elevated ALT Level. The majority (78%-98%) of patients who present with chronic liver disease and are anti-HCV positive by EIA-2 have chronic HCV infection as defined by the detection of HCV RNA in serum using PCR assays (Fig. 1).[10,20-23] Most (80%-90%) EIA-2–positive patients with chronic liver disease are RIBA positive, 90% of whom will be HCV RNA positive; approximately 10% are RIBA indeterminate, 60% to 70% of whom will be HCV RNA positive.[22,23] Failure to detect HCV RNA in all patients may be related to insensitivity of the PCR assays, degradation of HCV RNA during sample collection and storage, intermittent viremia, resolved HCV infection, or false-positive EIA result. These patients should be retested for HCV RNA using the most reliable PCR assay available, and attention should be paid to optimize the conditions for sample collection and storage to prevent RNA degradation. Investigations into other causes of chronic liver disease should be performed in patients who are repeatedly HCV RNA negative and when clinically indicated. Studies performed on recently collected sera in experienced laboratories have yielded HCV RNA detection rates of greater than 90%.[20] Thus, it can be argued that supplementary and confirmatory tests for the diagnosis of HCV infection are in general unnecessary in anti-HCV (EIA-2)–positive patients who present with chronic liver disease, especially those who have risk factors for HCV infection. When confirmatory tests are performed, PCR assays for HCV RNA are more appropriate than RIBA. Although qualitative PCR assay for HCV RNA will suffice to confirm the diagnosis, quantitative tests may be considered if treatment is contemplated (vide infra) because many studies have found that pretreatment serum HCV RNA level is the most important independent predictor of response to treatment.

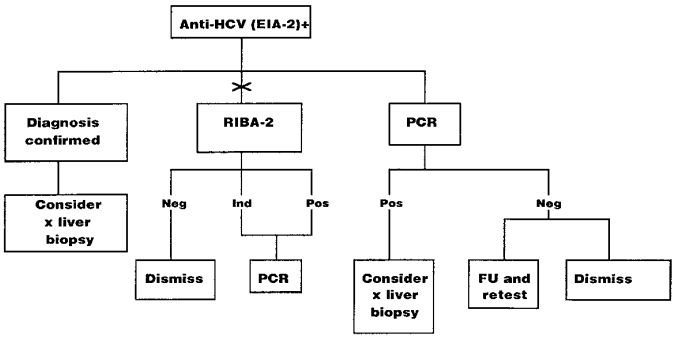

FIG. 1. Algorithm for the diagnostic evaluation of anti-HCV (EIA-2)–positive patients with chronic liver disease. Most (>90%) of these patients have ongoing HCV infection. Thus, confirmatory tests may not be necessary. When indicated, PCR assay for HCV RNA is more appropriate than RIBA. Pos, positive; ind, indeterminate; neg, negative; FU, follow-up.

Anti-HCV–Positive (EIA-2) Blood Donors and Individuals With Normal ALT Levels. Contrary to patients with chronic liver disease, only 30% to 40% anti-HCV (EIA-2)–positive blood donors are RIBA positive and 20% to 40% are RIBA indeterminate (Fig. 2). The percentage of EIA-2–positive blood donors who have detectable HCV RNA in serum when tested by PCR assay varies from 70% to 90% for those who are RIBA positive to 2% to 40% for those who are RIBA indeterminate, to none among those who are RIBA negative, giving an overall detection rate of 35% to 45% (Table 1).[24-33] Thus, supplementary or confirmatory tests for HCV infection should always be performed in EIA-2–positive blood donors. Two algorithms can be considered for the evaluation of EIA-2–positive blood donors (Fig. 2).

The first algorithm is to retest for anti-HCV using RIBA, and then test for HCV RNA using PCR assay in those who are RIBA positive or indeterminate. RIBA-negative donors can be dismissed. PCR-positive donors should be further evaluated to assess the severity of liver disease. The disposition of RIBA-positive or -indeterminate donors who are PCR negative is less clear. Several studies have found that individuals with isolated reactivity to C-100 or 5-1-1 are invariably PCR negative.[27,28,33] However, HCV RNA can be detected in 0% to 80% of individuals with isolated reactivity to C22 or C33.[27,29,34] Histological evidence of chronic hepatitis has been reported in 7.5% to 18% of RIBA-indeterminate donors,[29,35] but liver disease appeared to be confined to those who were PCR positive. Among RIBA-positive donors, three studies reported that 6 of 29 (21%) PCR-negative donors had chronic

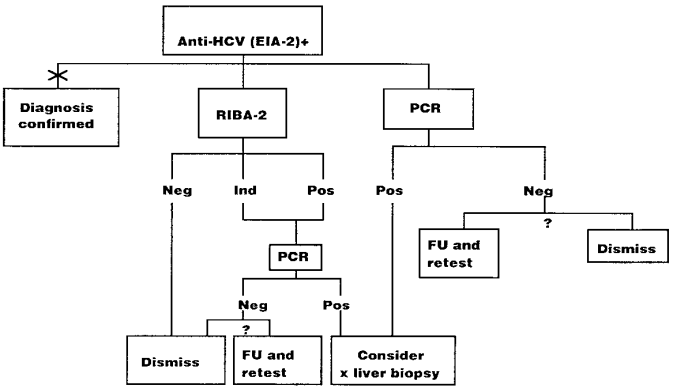

FIG. 2. Algorithm for the diagnostic evaluation of anti-HCV (EIA-2)–positive blood donors and individuals with normal aminotransferase levels. Confirmatory tests should always be performed in these individuals. The most appropriate approach is to retest for anti-HCV using RIBA and then test for HCV RNA using PCR assay in those who are RIBA positive or indeterminate. Direct confirmation using PCR assay is not recommended because of the poor standardization of current assays. Pos, positive; ind, indeterminate; neg, negative; FU, follow-up.

persistent hepatitis, but none had chronic active hepatitis or cirrhosis.[32,36,37] However, one study found that 17 of 34 (50%) RIBA-positive, PCR-negative donors had chronic hepatitis, 4 of whom had chronic active hepatitis.[30] The high rate of significant liver disease in PCR-negative donors in the latter study may be related to insensitivity of the PCR assay because HCV RNA was detected in only 65% (64 of 98) of RIBA-positive donors in contrast to the 89% detection rate in other studies.[27,31] These data suggest that RIBA-indeterminate donors who are PCR negative can be dismissed if the PCR assay is reliable. One might even argue that HCV RNA testing is unnecessary in donors who have isolated reactivity to C100 or 5-1-1. In view of the finding of chronic hepatitis in 21% to 50% of RIBA-positive, PCR-negative donors, retesting for HCV RNA is recommended for these individuals to exclude the possibility of intermittent viremia or false-negative result because of suboptimal assay.

The second algorithm is to test all EIA-2–positive blood donors for HCV RNA by PCR assay because 60% to 70% will eventually be tested for HCV RNA. This is potentially a one-step approach. However, it is not clear if all the PCR-negative donors can be safely dismissed because significant liver disease can be found in PCR-negative donors, predominantly those who are RIBA positive. Follow-up and retesting of all the PCR-negative donors (55%-65%) will be more tedious and expensive than the first algorithm. Clearly, the choice between the two diagnostic algorithms depends on the availability of reliable PCR assays that can serve as the gold standard for the diagnosis of HCV infection. Early studies on stored sera reported that patients with chronic hepatitis C may be intermittently PCR negative.[38,39] However, more recent studies found that the vast majority of patients with chronic hepatitis C who are not receiving interferon therapy are persistently viremic.[40,41] Further studies using standardized PCR assays are needed to clarify the frequency of intermittent viremia in untreated patients with chronic HCV infection and to determine the prevalence of significant liver disease among RIBA-positive, PCR-negative individuals. While these data are collected and standardized PCR assays are developed, the first diagnostic algorithm is preferred for the evaluation of EIA-positive blood donors.

Other individuals who are found to be anti-HCV (EIA-2)–positive with normal ALT levels should be evaluated using the same algorithm for blood donors. Although it is possible that these individuals may have a false-positive EIA test result or resolved HCV infection, many (especially those who are RIBA positive) are viremic, and some may develop elevated ALT levels on follow-up evaluation. Studies in EIA-2–positive blood donors found that 40% to 60% had normal ALT levels at the time of blood donation, but 30% to 50% of these donors developed intermittent or persistent elevation in ALT levels during a 6- to 12-month follow-up period.[35,42] Among the RIBA-positive blood donors, HCV RNA was detected in 55% to 65%[30,32] and chronic hepatitis in 30% to 70%[30,35,42-44] of those with persistently normal ALT levels.

Immunocompromised Patients. Immunocompromised patients such as hemodialysis patients and transplant recipients have impaired antibody response to HCV antigens, especially C-100 and 5-1-1.[45,46] False-negative results in EIA-2 for anti-HCV have been reported in 2.5% to 10.5% of patients who were PCR positive.[46-49] Thus, tests for HCV RNA should be performed in immunocompromised patients who are anti-HCV negative when there is clinical suspicion of HCV infec-

TABLE 1. RIBA and PCR Testing in Anti-HCV (EIA-2)–Positive Blood Donors

			RIBA-2		PCR Positive	
Reference	Country	No. of Donors	% Positive	% Indeterminate	No.	%
Prati[26]	Italy	483	36	26	170/259	66*
Kleinman[27]	USA	70	39	30	28/48	58*
Serfaty[42]	France	483	36	23	NT	
Rossini[28]	Italy	477	36	23	10/40	25†
Prieto[30]	Spain	975	33	39	64/98	65‡
Salmeron[31]	Spain	228	63	20	80/126	63*
Mean			40	27	67*	
95% Confidence interval			31-49	21-32	60-75*	

Abbreviation: NT, not tested.

* RIBA-positive and -indeterminate donors.

† RIBA-indeterminate donors.

‡ RIBA-positive donors.

tion. In view of the complexity and high costs of PCR assays, it is impractical to recommend HCV RNA testing as the initial diagnostic test for these patients. Recent studies suggest that EIA-3 is more sensitive and can detect anti-HCV seroconversion earlier than EIA-2 in hemodialysis patients.[50,51] These data need to be confirmed.

Patients With Acute Hepatitis. Anti-HCV, as detected by EIA-2, is positive in approximately 50% of patients with acute hepatitis C at the time of presentation, and in 90% of patients at some point during the acute illness.[52] Tests for HCV RNA, preferably by qualitative PCR assays, permit earlier diagnosis and institution of treatment since several randomized controlled trials have shown that interferon therapy can reduce the rate of chronic infection.[53-57]

ASSESSMENT OF THE SEVERITY OF LIVER DISEASE

Most patients with acute or chronic hepatitis C have no or nonspecific symptoms. Thus, history and physical examination are unreliable in assessing the severity of liver disease, except in patients with decompensated cirrhosis.

Liver Biopsy. Liver histology is the gold standard for assessing the severity of liver disease. Liver biopsy is the only means to diagnose well-compensated cirrhosis. It is useful in determining not only inflammatory activity but also the extent of fibrosis. Histological grading of inflammatory activity and staging of fibrosis have also been shown to correlate with the risk of subsequent progression to cirrhosis.[58] In addition, fibrosis score or cirrhosis has been identified to be one of the most important independent predictive factors for response to interferon treatment.[59-63]

Serum Aminotransferase Levels. Although anti-HCV–positive patients who have elevated ALT levels are more likely to have significant liver disease on liver biopsy than those who have persistently normal ALT levels,[25] histological evidence of chronic hepatitis can be found in 30% to 70% of RIBA-2– and PCR–positive individuals despite persistently normal ALT levels. Among patients with elevated ALT levels, there is a weak correlation between the ALT level and histological diagnosis or histology activity index.[64]

Serum HCV RNA Level. Regardless of the assay used to quantify serum HCV RNA levels, all published studies found no correlation between serum HCV RNA and ALT levels.[8,41,43,65-68] Several studies have reported that blood donors with normal ALT levels tended to have lower serum HCV RNA levels than patients with chronic hepatitis C.[69-71] However, other investigators failed to confirm these observations.[43] Data on the correlation between serum HCV RNA level and liver histology are conflicting. Some studies found no correlation,[67,72] one study reported lower serum HCV RNA levels in patients with more advanced liver disease,[12] whereas other studies demonstrated progressive increase in serum HCV RNA levels in patients with more advanced liver disease.[20,65,73] In view of the overlap in serum HCV RNA levels among patients with different stages of liver disease, quantifying HCV RNA levels in serum will not help in assessing the severity of liver disease.

HCV Genotyping. Many investigators have examined the relation between HCV genotype and severity of liver disease. Most of the studies have focused on comparisons between genotype 1b and other genotypes, predominantly types 2 and 3. The vast majority of these studies reported a higher prevalence of genotype 1b among patients with cirrhosis and hepatocellular carcinoma than in patients with chronic hepatitis.[24,25,72,74-78] In addition, several studies found a higher prevalence of genotype 1b among patients with chronic liver disease versus asymptomatic blood donors and individuals with normal ALT levels.[25,26,79] Nevertheless, some of these studies also noted that patients with genotype 1b were older.[24,25,72,79,80] Thus, it is possible that longer duration of infection rather than genotype 1b per se accounted for the more advanced liver disease. Other studies have not confirmed an association between genotype 1b and the presence of severe or advanced liver disease.[67,81-83] Several reasons may account for the conflicting data: imbalance in the number of patients with each genotype (many studies had very few patients with non-1b genotypes), failure to include patients with the full spectrum of liver disease from asymptomatic blood donors with normal ALT levels to hepatocellular carcinoma (some studies included patients with mild to severe chronic hepatitis only), and different typing techniques (with the potential for misclassification). All the studies cited above were cross-sectional studies.

Two recent studies examined the relation between HCV genotype and development of progressive liver disease with opposite conclusions. In one study, 136 Japanese patients (96 type 1b, 36 type 2) were followed-up for a mean of 9.6 years (range, 5-26 years). The initial inflammatory and fibrosis scores were comparable between patients with types 1 and 2. However, patients with genotype 1 had significantly higher inflammatory as well as fibrosis scores on the follow-up biopsies compared with patients with genotype 2.[84] In addition, a significantly higher percentage of patients with genotype 1 developed hepatocellular carcinoma during follow-up (29% vs. 5.6%; $P < .001$). These findings differed from those in a second study in which 85 Italian patients (47 type 1, 30 type 2) were followed-up for a mean of 66.9 months (range, 12-119 months).[21] There was no correlation between HCV genotypes and progression of liver disease, death, or development of hepatocellular carcinoma. Studies in liver transplant recipients with recurrent hepatitis C also reached opposite conclusions. Two studies reported more severe liver disease in patients with genotype 1b,[85,86] but these findings were not confirmed in another study.[87]

It is unclear if genotype 1b is more pathogenic. Because a wide spectrum of liver disease has been found in association with each genotype, genotyping does not help in assessing the severity of liver disease, and until further data become available, genotyping has no role in predicting prognosis.

EVALUATION FOR TREATMENT

Once the diagnosis of HCV infection is confirmed and the severity of liver disease has been assessed, patients should be evaluated for treatment. The decision to treat or not to treat depends on many factors, including age of the patient, presence of symptoms, severity of liver disease, likelihood of response to treatment, concomitant medical problems, and contraindications to the use of interferon therapy. In general, the decision should be made jointly by the physician and patient after the latter has been informed of the pros and cons of treatment.

The most important factors that have been identified to be associated with a favorable response to interferon treatment are low pretreatment serum HCV RNA level, HCV genotype non-1, and low fibrosis score or lack of cirrhosis.

TABLE 2. Correlation Between Pretreatment Serum HCV RNA Levels and Response to Interferon Therapy

Reference	Country	No. of Patients	Sustained Biochemical Response (%)	
			bDNA$^+$	bDNA$^-$
Yuki[11]	Japan	93	33	76
Yamada[12]	Japan	60	25	63
Martinot-Peignoux[13]	France	141	12	39
Rumi[59]	Italy	234	11	44
Magrin[14]	Italy	100	10	33
Tsubota[61]	Japan	185	47	75
Total		813	23	55

NOTE. Odds ratio, 4.37 (95% confidence interval, 3.12-6.14).

Serum HCV RNA Level. Every study that has examined the relation between pretreatment serum HCV RNA level and response to interferon therapy has concluded that low pretreatment serum HCV RNA level is associated with a higher rate of response.[11-14,59,61,88-96] The difference is more striking when sustained response was compared against transient and no response. Response in most of these studies was defined as normalization in serum ALT level. In several studies, low pretreatment serum HCV RNA level was found to be associated with favorable response regardless of the genotype.[88,90,92] These findings suggest that all patients considered for treatment should have quantification of serum HCV RNA level. The results can be used to counsel patients on the likelihood of response and may influence the patient's decision on treatment. However, there is insufficient data at this stage to define inclusion or exclusion criteria for treatment based on serum HCV RNA level. It is also unclear which assay should be used for quantification. The bDNA assay is technically easy, highly reproducible, has good linearity and similar efficiencies for various genotypes, but it is less sensitive; therefore, patients who have undetectable HCV RNA in the bDNA assay would need to be tested by the PCR assay to ascertain that they are viremic before treatment. Quantitative PCR assays are more sensitive but tedious to perform, poorly standardized, inconsistent, have variable efficiencies for different genotypes, and have limited range of linearity.[97]

Many published studies used in-house quantitative PCR assays that are poorly standardized, and the results were expressed in different units. Studies that used bDNA assays for quantification of pretreatment serum HCV RNA level found that the odds ratio for sustained biochemical response in patients who were PCR positive but bDNA negative was 4.4 (95% confidence interval, 3.1-6.1) compared with those who were bDNA positive (Table 2).[11-14,59,61] Nevertheless, sustained biochemical response was achieved in 23% (95% confidence interval, 11%-44%) of patients who were bDNA positive before treatment. In addition, all except one study[12] failed to identify a cut-off value in serum HCV RNA level above which response was nil, underscoring the difficulty in using serum HCV RNA level to select patients for treatment.

HCV Genotype. Many studies found that patients with genotype 1b have lower response rates compared with genotypes 2 and 3.[59,61,88,90-99] However, in most countries, genotype 1b is the most prevalent genotype. In addition, several studies found that satisfactory response can be obtained in patients with genotype 1b and low HCV RNA level.[12,88,90,92] Thus, it is impractical and unnecessary to exclude patients from treatment based on genotyping result, although the results can help in advising patients on the likelihood of response.

Liver Histology. Liver biopsy is frequently performed to assess severity of liver disease before consideration for treatment. Several studies have found that patients with high fibrosis score or cirrhosis have lower response rates to interferon therapy. The results of liver biopsy can help predict prognosis and likelihood of response to treatment.[58-63]

MONITORING RESPONSE TO TREATMENT

The goals of treatment are sustained biochemical (normalization in ALT level) and virological (undetectable HCV RNA in serum) response. It is now recognized that in some patients there is a discrepancy between biochemical and virological response[100] and that patients who normalize their ALT level but remain HCV RNA positive are more likely to relapse than those who have normal ALT and undetectable HCV RNA levels.[60,101] Because the aim is to determine viral clearance, the most sensitive test (qualitative PCR assay) should be used. bDNA assay has no role in defining virological response. The most appropriate time for testing is just before completion of treatment. However, there is no data to suggest that prolonging treatment in patients who have achieved biochemical but not virological response after 6 to 12 months of therapy will achieve viral clearance if treatment is prolonged.

Several studies have shown that early normalization of ALT level (week 12) is associated with biochemical response at the end of treatment.[102] Continuing treatment at the same or higher doses in patients who failed to normalize their ALT levels after 12 weeks of interferon treatment at 3-million-unit doses is associated with very low rate of sustained response.[103,104] These data suggest that treatment should be withdrawn in patients who failed to normalize their ALT levels after 12 weeks of treatment. However, one study reported histological improvement in patients who completed a long course (18 months) of interferon therapy despite their failure to normalize ALT levels.[105] This observation needs to be confirmed before changing our current recommendation to withdraw treatment based on lack of initial ALT response.

Recent studies reported an association between rapid viral clearance and sustained response to interferon therapy.[106,107] In one study, clearance of HCV RNA at week 4 was the best predictor of sustained biochemical response; HCV RNA became undetectable by week 4 in 73%, 26%, and 0% of the

TABLE 3. Diagnostic Evaluation of Hepatitis C

	RIBA	HCV RNA Assays			HCV Genotype
		Qual PCR	Quant PCR	bDNA	
Confirmation of diagnosis	+	+	±	−	−
Assessment of severity of liver disease	−	−	−	−	−
Evaluation for treatment	−	−	+	+	−
Determination of response to treatment	−	+	−	−	−
Monitoring progress of liver disease	−	−	−	−	−

Abbreviations: qual, qualitative; quant, quantitative.

patients who had, respectively, sustained, transient, and no response ($P = .02$).[106] These data suggest that the decision to discontinue treatment based on failure of initial response may be made earlier by testing for viral clearance. However, these data were based on small numbers of patients. Further studies using more standardized PCR assays are needed to define the optimal time for assessment of initial response that will identify all sustained responders without including a large percentage of transient and nonresponders.

Based on the above discussions, patients undergoing treatment should be confirmed to be HCV RNA positive before initiation of treatment, preferably using a quantitative assay. PCR assay for HCV RNA should be repeated after 12 weeks of therapy to determine initial response. Patients who continue to have elevated ALT and detectable HCV RNA are unlikely to respond and should have their treatment discontinued. Those who have normalized ALT and undetectable HCV RNA should continue treatment for a total period of 12 months. PCR assay should be repeated at the end of treatment to document virological response. Patients with sustained biochemical response should be retested for HCV RNA by PCR assay 6 to 12 months after completion of therapy to document sustained virological response. More frequent testing for HCV RNA is in general unnecessary. There is no role for retesting anti-HCV or HCV genotyping during or after treatment.

MONITORING PROGRESS IN UNTREATED PATIENTS

Progress of liver disease in untreated patients is monitored clinically and biochemically by serial blood tests of ALT level as well as blood counts, liver profile, and prothrombin time to detect evidence of hypersplenism and impaired hepatic synthetic function. Repeated tests for HCV RNA levels and HCV genotyping are unnecessary and do not help in determining disease progression. Serial liver biopsies are most reliable in monitoring progress but least acceptable to patients.

CONCLUDING PERSPECTIVE

Currently, EIA for anti-HCV is the most practical screening test for the diagnosis of HCV infection. The need for and the choice of confirmatory tests depend on the clinical setting (Table 3). In general, qualitative PCR assay for serum HCV RNA is the best confirmatory test. However, EIA-positive blood donors and individuals with normal ALT levels may be evaluated by RIBA first, PCR assay for HCV RNA being performed only in those who are RIBA positive or indeterminate. Quantifying serum HCV RNA level by bDNA or quantitative PCR assays can help to predict response to interferon treatment, but it is unclear if these values should be used to select patients for treatment. Response to treatment should include documentation of viral clearance by qualitative PCR assay (Table 3). Liver biopsy is the most reliable means to assess severity of liver disease and to predict prognosis. Tests for serum HCV RNA level and HCV genotyping do not help in assessing severity or progress of liver disease.

Clearly, the most important task at hand is to standardize tests for the detection and quantification of HCV RNA in serum. Further studies using standardized assays for serum HCV RNA should be performed to determine the frequency of intermittent viremia in patients with chronic hepatitis C who are not receiving treatment, the prevalence of significant liver disease in individuals who are RIBA positive but PCR negative, the predictive value of pretreatment serum HCV RNA level for sustained (biochemical and virological) response to interferon treatment, and the criteria for withdrawal of treatment based on lack of initial response.

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