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*JAMA*. 2007;297(7):724-732 (doi:10.1001/jama.297.7.724)

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# Molecular Diagnostics of Hepatitis C Virus Infection

## A Systematic Review

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**C**HRONIC HEPATITIS C VIRUS (HCV) infection occurs frequently in the United States and worldwide. The Centers for Disease Control and Prevention estimates that at least 3.2 million persons in the United States are chronically infected.<sup>1</sup> In the 1990s, at least 10 000 deaths annually were directly attributable to hepatitis C, with a projection of a tripling of the hepatitis C–related deaths by 2020.<sup>1,2</sup> Chronic hepatitis C is an important and emerging factor in hepatocellular carcinoma and is now the leading indication for liver transplantation.<sup>3</sup> Unfortunately, HCV infection is often underdiagnosed. More than 50% of people at highest risk for HCV are infected yet are unaware of their disease, leading to spread of the infection and lost treatment opportunities.<sup>4</sup>

Molecular virological techniques play a key role in diagnosis and monitoring of treatment. Because it is difficult to culture the virus, molecular techniques were instrumental in first identifying HCV, making it one of the first pathogens to be identified purely by molecular diagnostics.<sup>5</sup> Hepatitis C virus infection is confirmed by the de-

**Context** Hepatitis C virus (HCV) is a common blood-borne pathogen that relies heavily on nucleic acid testing for confirmation of infection. Nucleic acid tests are invaluable for the diagnosis of HCV infection and provide critical prognostic information for guiding treatment and measuring the response to antiviral therapy.

**Objective** To review the currently available molecular diagnostic tests for HCV, their clinical applications, and how these tests shed light on the natural history of HCV.

**Evidence Acquisition** Search of MEDLINE (1966 to July 2006), article reference lists, and national meeting abstracts for the diagnosis and applications of molecular diagnostic tests for HCV. Studies were selected on the basis of clinical relevance.

**Evidence Synthesis** Qualitative nucleic acid tests have low limits of detection (<50 IU HCV RNA/mL) and are used for confirmation of HCV infection and for screening blood donations. Hepatitis C virus genotype test results provide important prognostic information related to therapeutic response and are routinely used for selecting treatment regimens. Quantitative HCV RNA testing provides prognostic information regarding likelihood of treatment response and plays an important role in monitoring the antiviral response to treatment. *Sustained virological response* is defined as testing negative for HCV RNA 6 months after cessation of therapy. Recent studies suggest that the rate of response to therapy is also important. For example, conversion to an HCV RNA negative test result after 4 weeks of therapy constitutes a *rapid virological response* and is a strong predictor of treatment success. Patients who have not had an *early virological response*, defined as at least a 2-log decline in HCV RNA after 12 weeks of therapy, are unlikely to respond with an additional 36 weeks of therapy, and should stop therapy.

**Conclusions** A sensitive nucleic acid test should be used to confirm all cases of acute or chronic HCV infection. A genotype test and quantitative HCV RNA test should be performed on all patients prior to therapy to best assess probability of response and to aid in selection of appropriate therapeutic regimen. Monitoring HCV RNA during treatment provides important information on likelihood of sustained virological response. The same type of quantitative HCV RNA test should be used throughout a patient's treatment course.

JAMA. 2007;297:724-732

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tection of viral RNA through nucleic acid tests (NATs), and these tests are used to monitor the response to antiviral therapy. We review currently available molecular diagnostic tests for HCV, their clinical applications, and how these tests shed light on the natural history and optimal management of hepa-

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**Clinical Review Section Editor:** Michael S. Lauer, MD. We encourage authors to submit papers for consideration as a Clinical Review. Please contact Michael S. Lauer, MD, at lauerm@ccf.org.

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titis C. Readers are referred to a prior review in this series for a more general overview of the clinical management of hepatitis C.<sup>6</sup>

## EVIDENCE ACQUISITION

We searched the MEDLINE database from 1966 to July 2006 for English-language articles using the following search terms: *HCV*, *hepatitis C/diagnosis*, *hepatitis C/virology*, *hepacivirus/physiology*, *hepatitis C/treatment*, *polymerase chain reaction/methods*, *polymerase chain reaction/standards*, *polymerase chain reaction*, *sensitivity and specificity*, *accuracy*, *genotype*, *virus replication*. We further reviewed meeting abstracts from the 2006 American Association for the Study of Liver Disease and the European Association for the Study of the Liver for relevant articles. We based our recommendations on laboratory diagnosis and evaluation on the 2002 National Institutes of Health Consensus Guidelines, the 2004 American Association for the Study of Liver Disease Practice Guidelines, and the 2003 Centers for Disease Control and Prevention Screening and Testing Guidelines.<sup>7-9</sup>

## EVIDENCE SYNTHESIS

### Natural History of HCV Infection

Among patients exposed to HCV, 15% to 40% will clear the infection within 6 months<sup>10,11</sup> (FIGURE 1). The remaining 60% to 85% of patients who still have detectable HCV RNA for 6 months are considered to be chronically infected.<sup>8</sup> A minority of chronically infected patients will have persistently normal alanine aminotransferase (ALT) levels.<sup>13</sup> As a result, ALT levels and a positive HCV serology result are not adequate for the diagnosis of chronic HCV; instead, detection of HCV RNA is required to establish the diagnosis. Results from longitudinal viremia studies have indicated that spontaneous resolution of chronic HCV infections occurs at a rate of 0.50% to 0.74% per person-year annually.<sup>14,15</sup> Unfortunately, up to 20% of individuals with chronic hepatitis C eventually develop liver cirrhosis, which may be

complicated by hepatocellular carcinoma, hepatic decompensation, or death.<sup>16</sup>

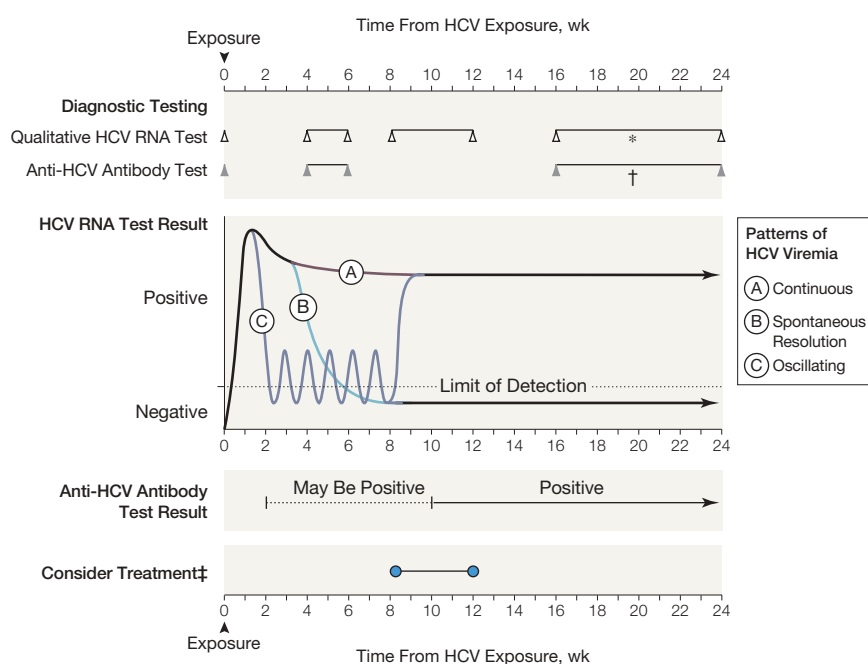
### Nucleic Acid Testing for HCV

Nucleic acid tests directly detect the presence of HCV RNA using a combination of amplification and detection techniques. Except for certain uncommon clinical situations, NATs have supplanted the recombinant immunoblot assay as the preferred test to confirm HCV infection. Nucleic acid tests are classified into qualitative tests (qualitative polymerase chain reaction [PCR], transcription-mediated amplification [TMA]), and quantitative tests (branched-chain DNA [bDNA]) am-

plification, quantitative PCR, and real-time PCR). Guidelines covering the indications, interpretation, and recommended tests are listed in TABLE 1<sup>9,17-19</sup> and FIGURE 2.

In general, NATs are quite sensitive and specific (TABLE 2). A negative NAT result following a positive serological test result is usually indicative of a resolved infection. However, intermittent or low-level viremia may occur during chronic infection,<sup>15</sup> and for this reason clinicians should perform a second NAT 6 to 12 months later. In addition, those patients with ongoing exposure to HCV can be reinfected. A positive HCV NAT result indicates active infection regardless of antibody test

**Figure 1.** Use of Molecular Tests for Diagnosis of Acute Hepatitis C Virus Infection Following Known Exposure



Most patients develop asymptomatic infection but will often have high-level viremia and elevated alanine aminotransferase (ALT) levels in the acute infection period. Hepatitis C virus (HCV) RNA can be detected 1 to 3 weeks after infection, approximately 1 month before the appearance of antibodies. If HCV RNA is still detectable after 6 months, the patient is considered to be chronically infected and the HCV RNA level will remain more or less stable (within 0.5 log).<sup>12</sup> The plot shows a schematic of 3 major scenarios to explain when to initiate treatment within 8 to 12 weeks after exposure for those who do not have spontaneous resolution. In cases when the date of exposure is unknown, we recommend HCV testing at initial evaluation and again 3 to 4 months later. Suggested time frames for testing are indicated based on known time of exposure, results of prior diagnostic testing, and goal of determining if or when to start treatment.

\*Repeat HCV RNA testing at 16 to 24 weeks if the patient had previous negative qualitative test results.

†Repeat anti-HCV antibody testing at 16 to 24 weeks if patient had a negative anti-HCV test result at 4 to 6 weeks after exposure.

‡Consider treatment if a patient tests positive for HCV RNA 8 to 12 weeks after infection (optimal time to initiate treatment; see Table 3).

results. In acute infections, as in occupational exposures, the NAT result will become positive within 1 to 3 weeks, several weeks earlier than serological tests (Figure 1).<sup>9</sup>

### Qualitative Tests

Alberti and colleagues<sup>20</sup> demonstrated that detection of HCV RNA in patient serum is the definitive marker of hepatitis C liver disease regardless of serum ALT levels. Thus, documentation of HCV viremia is the hallmark of HCV diagnostics in antibody-positive and in HCV antibody-negative patients with unexplained ALT elevations or liver disease documented by liver biopsy.<sup>21</sup> The most commonly used qualitative NATs use reverse transcription PCR to detect viral RNA as reviewed elsewhere.<sup>22,23</sup> There are 3 widely used tests for qualitative detection of HCV RNA, including 2 commercially available kits (AMPLICOR 2.0 and Ampliscreen 2.0, both by Roche Diagnostics, Indianapolis, Ind) and a reference laboratory test known as UltraQual (National Genetics Institute, Los Angeles, Calif; Table 2). The sensitivity is more than 96% and their specificities are more than 99%, using antibody status and elevated ALT as the gold standard.<sup>24-26</sup> These tests have a very low limit of detection, less than 50 IU/mL.

The TMA test is a newer qualitative NAT, which appears to be more sensitive than reverse transcription-PCR tests. The VERSANT HCV RNA Qualitative Assay (Bayer Diagnostics, Emeryville, Calif) has a lower detectable limit of 5 IU/mL and sensitivity of more than 98%.<sup>27</sup> In 1 tube, HCV RNA is captured using sequence-specific hybridization and is amplified via T7 RNA polymerase; RNA amplicons are then detected using chemiluminescent probes.

### Clinical Applications of Qualitative NATs

Qualitative NATs are used to confirm viremia (especially low-level viremia)<sup>22,28</sup> and to screen blood donations. Nucleic acid testing of blood donations for HCV RNA dramatically reduced the incidence of posttransfusion hepatitis C, with the risk of HCV acquisition dropping from 1 per 276 000 donations to 1 per 2 million donations.<sup>29</sup> In current practice, the VERSANT and Procleix HIV-1/HCV assays (Gen-Probe, San Diego, Calif) are used for screening blood and organ donations. Increased sensitivity of these tests may well prevent 56 additional HCV transmissions annually compared with antibody screening.<sup>29</sup>

Nucleic acid tests also have clinically important applications in predicting patients at risk for virological relapse once therapy stops and in diagnosing acute HCV.<sup>19,30-32</sup> In a recent study, patients who were prior non-responders to interferon had reverse transcription-PCR and TMA testing of sera samples after 20 and 24 weeks of peginterferon and ribavirin therapy.<sup>33</sup> Importantly, 45 participants who tested negative for HCV RNA by reverse-transcription PCR tested positive for HCV RNA by TMA at both the 20- and 24-week visits. None of these 45 participants achieved sustained virological response. Overall, TMA was superior to PCR in predicting sustained virological response (positive predictive value, 66% vs 52%). However, further studies are needed to validate the use of TMA in this clinical setting.

### Quantitative Tests

There are 3 types of tests to quantify HCV RNA: quantitative reverse transcription-PCR, real-time PCR, and bDNA (Table 2).<sup>22</sup> Quantitative PCR tests include MONITOR 2.0 (Roche Diagnostics) and SuperQuant (National Genetics Institute); they provide comparable results.<sup>34</sup> The bDNA method differs from reverse transcription-PCR tests

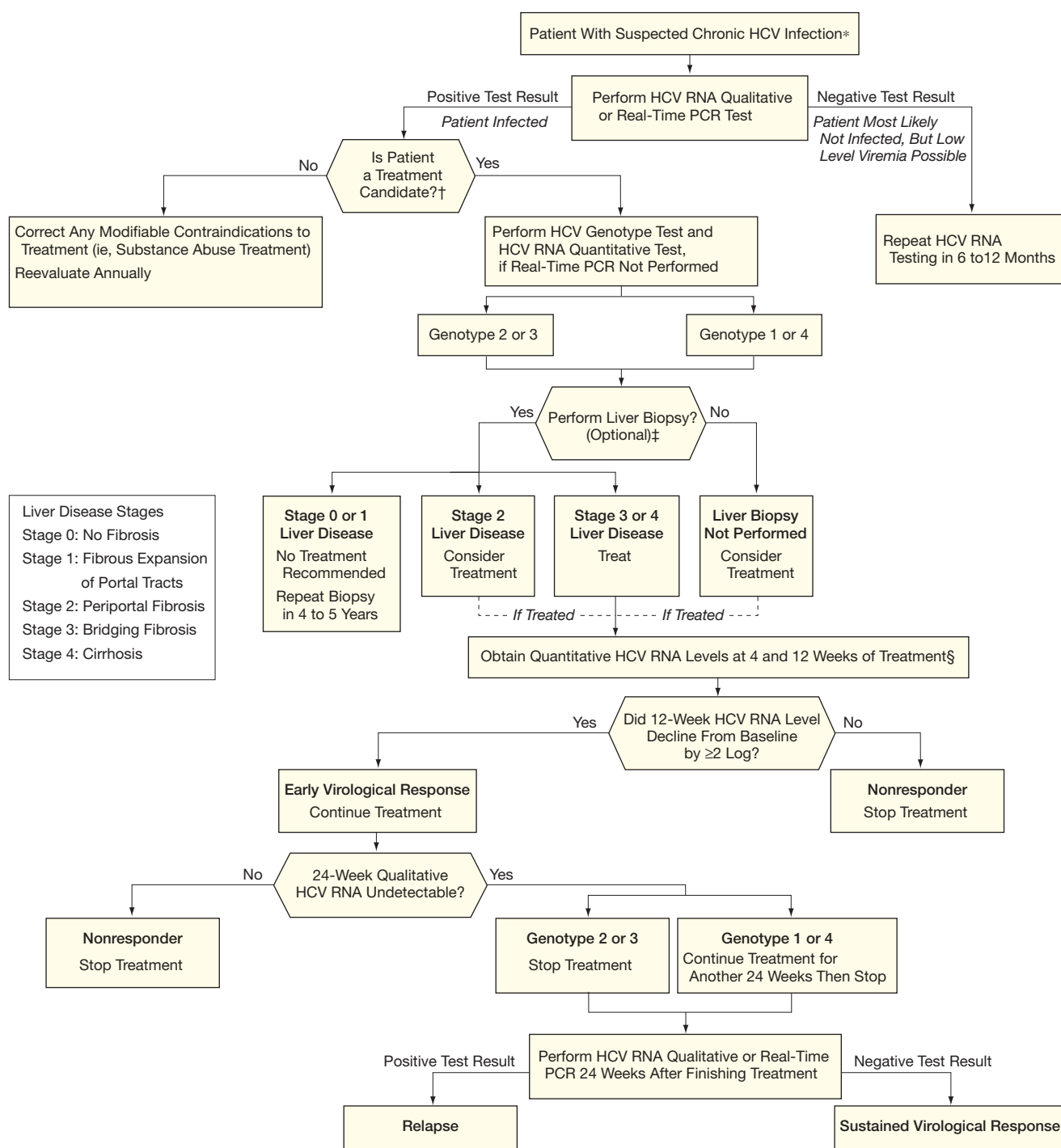
**Table 1.** Guidelines for Hepatitis C Virus RNA Testing\*

Clinical Situation	Test to Use	Interpretation and Comments
Acute infection suspected	Qualitative PCR or real-time PCR	Check HCV RNA and HCV antibody 4-6 wk after exposure Check HCV RNA at 8-12 wk; if positive, consider therapy Check HCV RNA and HCV antibody 4-6 mo after exposure
Chronic infection suspected† HCV antibody positive	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo
HCV antibody negative but unexplained liver disease or immunocompromised	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected, unless acute HCV infection is supported by clinical situation. HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo
HCV antibody and RNA positive, eligible for treatment	Quantitative tests such as quantitative PCR, bDNA, or real-time PCR	>800 000 IU/mL is considered high, more difficult to treat Use same quantitative assay before treatment and measure 4- and 12-wk responses
Infant born to HCV positive mother; infant still antibody positive at 18 mos	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo

Abbreviations: bDNA, branched-chain DNA; HCV, hepatitis C virus; PCR, polymerase chain reaction

\*Guidelines adapted from Strader, Polywka and Centers for Disease Control and Prevention.<sup>9,9,17,18</sup>

†Most recent exposure to HCV more than 6 months prior.

**Figure 2.** Algorithm for Testing and Treatment of Chronic Hepatitis C Virus Infection

HCV indicates hepatitis C virus; PCR, polymerase chain reaction.

\*Chronic infection is suspected if a patient's most recent HCV exposure was more than 6 months before testing or if the patient does not have features of acute hepatitis C (recent seroconversion, alanine aminotransferase greater than 5 times the upper limit of normal, with or without features of hepatitis [ie, jaundice]).

†Treatment candidates include those without any absolute contraindications to treatment<sup>6</sup> or those without relative contraindications (thyroid disease, depression) that cannot be safely managed.

‡Liver biopsy is the most accurate method of determining the severity of liver disease.

§If HCV RNA levels are negative at 4 weeks, there is a high probability of sustained virological response.

in that the detection signal is amplified rather than target RNA. The third generation assay (VERSANT bDNA 3.0, Bayer Corp, Tarrytown, NY) has a lower limit of detection of 615 IU/mL and an upper range of 7.7 million IU/mL.<sup>35</sup> It is highly reproducible and the specificity ranges from 96% to 98.8%.

A critically important advance in molecular diagnostics has been the adaptation of real-time PCR methods to quantify HCV RNA. Using TaqMan technology, real-time PCR yields quantitative results with comparable sensitivity to qualitative tests. In addition, real-time PCR can accurately quantify HCV RNA levels over a linear range exceeding 6 logs (ie, 10 IU/mL to 100 million IU/mL) for purposes of therapeutic monitoring (Table 2).<sup>36</sup> Therefore, a single test result serves the purpose of both quantitative and qualitative HCV NATs. The assay is faster and more cost-effective than the other techniques and has already replaced other NAT testing platforms at many institutions. However, real-time PCR assays are presently available only as in-house tests.

Because the initial HCV RNA quantification techniques reported results in different units, direct comparisons were often difficult. With the adoption of a World Health Organization international standard, units from different assays are now interconvertible. However, because there is still variability between the various assays, it is recommended that clinicians use the same assay throughout the treatment course of any given patient. Given the reduced sensitivity of quantitative NAT for HCV RNA detection, it is prudent to retest all negative specimen results by the more sensitive qualitative NATs (ie, TMA or reverse transcription-PCR).

### Clinical Applications of HCV RNA Quantification Techniques

Early clinical trials showed that patients with a baseline HCV RNA level of more than 2 million copies/mL had a 9% lower sustained virological response rate than those with less than 2 million copies/mL.<sup>37</sup> Using the World Health Organization international standard, it was determined that 800 000 IU/mL corresponds to 2 million copies/mL. By

extrapolation of these findings, a *high viral load* is considered greater than 800 000 IU/mL and a *low viral load* is defined as less than 800 000 IU/mL.<sup>38</sup> Further studies have found that patients with low HCV RNA levels had a 15% to 39% better response rate than those with high HCV RNA levels, a finding that is consistent across trials using different formulations and dosages of interferon.<sup>39-43</sup> However, many of these early studies used quantification tests that had not been approved by the US Food and Drug Administration; thus, these prediction cutoffs may not hold for the more commonly used bDNA and real-time PCR assays.

The rate of virological response has become an important parameter to monitor during treatment of chronic hepatitis C (Figure 2 and FIGURE 3). *Sustained virological response* is defined as testing negative for HCV RNA 6 months after cessation of therapy and is the gold standard for treatment response. Monitoring changes in HCV viral load after 4 and 12 weeks of therapy predicts the likelihood of sustained virological response.<sup>44,45</sup> Patients who test negative

**Table 2.** Characteristics of Available Nucleic Acid Tests for Hepatitis C Virus

Assay	Method	Lower Limit of Detection or Range, IU/mL	Used to Confirm Viremia	Role in HCV Therapeutic Monitoring*
<b>Qualitative tests</b>				
Ultra-Qual	RT-PCR	40	Yes (blood donation screening)	NA
AMPLICOR (v2.0)	RT-PCR	50	Yes	Determination of rapid and sustained virological response
Ampliscreen (v2.0)	RT-PCR	50	Yes (blood donation screening)	NA
Procleix HIV-1/HCV assay	TMA†	5	Yes (blood and organ donation screening)	NA
Versant	TMA	5	Yes	Determination of rapid and sustained virological response
<b>Quantitative tests</b>				
SuperQuant	RT-PCR	40-2 million	No	Determination of pretreatment levels and early virological response
Monitor (v2.0)	RT-PCR	600-500 000	No	Determination of pretreatment levels and early virological response
Quantiplex bDNA (v3.0)	Branched-chain amplification	615-7.7 million	No	Determination of pretreatment levels and early virological response
TaqMan real-time PCR	RT-PCR	10-100 million	Yes	Determination of pretreatment levels, rapid, early, and sustained virological response

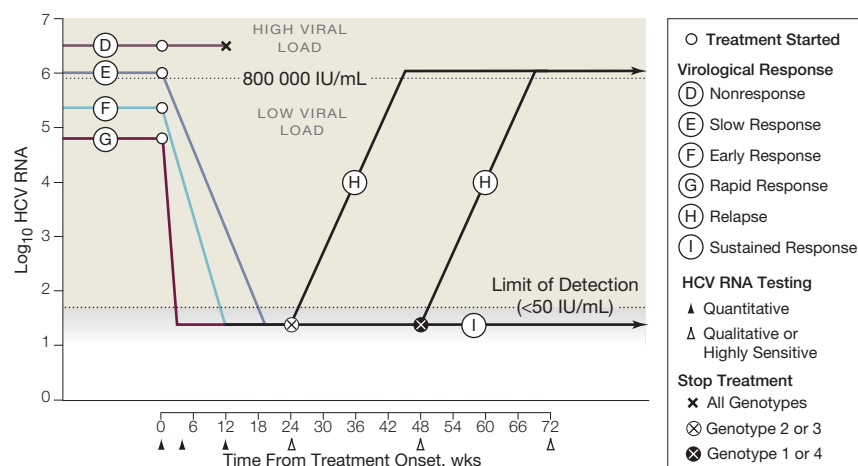
Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; NA, not applicable; PCR, polymerase chain reaction; RT, reverse transcription; TMA, transcription mediated amplification.

\*Pretreatment viral load is predictive of successful therapy; rapid virological response refers to undetectable HCV RNA after 4 weeks of treatment; early virological response, HCV RNA levels have declined by more than 2 log<sub>10</sub> after 12 weeks of treatment; sustained virological response, an undetectable HCV RNA level 24 weeks after treatment cessation.

for HCV RNA at 4 weeks are defined as having a *rapid virological response*,<sup>41,46</sup> and those who test negative at 12 weeks are defined as having an *early virological response*. Compared with week-12 testing, week-4 viral load testing provides slightly better positive predictive value (75% vs 67%).<sup>47</sup> However, week-12 results form the basis of so-called “stopping rules” during hepatitis C therapy. For patients infected with genotype 1 HCV whose HCV RNA levels have not declined by at least 2 logs after 12 weeks of therapy, the chance of sustained virological response is 0% to 3% and cessation of therapy should be considered.<sup>9,46</sup> One should cautiously interpret viral load declines that are close to the 2-log cutoff because of poorer predictive value at this border.<sup>48</sup>

Other clinical scenarios in which quantitative HCV RNA testing has been studied include spontaneous remission, sexual transmission, and determining severity of liver disease. Rapidly declining HCV RNA levels in the acute phase may predict spontaneous clearance,<sup>32</sup> whereas levels lower than 800 000 IU/mL in the chronic phase were associated with spontaneous negativity.<sup>15</sup> Although high-serum HCV RNA levels predict both oral and genital shedding of HCV,<sup>49-51</sup> the relationship between serum HCV RNA levels and sexual transmission has not been proven to date. The relationship between HCV RNA levels and liver disease severity remain controversial. Several studies have reported significant associations between HCV RNA levels and either liver inflammation or liver fibrosis.<sup>53-56</sup> However, other studies failed to find such associations, perhaps due to different methods for HCV RNA quantification and/or differences in patient cohort characteristics between positive and negative studies.<sup>57-59</sup> Although the prevailing opinion is that HCV RNA levels remain fairly constant over time and are not predictive of disease outcome, the question is not fully resolved and the issue deserves more careful study from both clinical and laboratory perspectives.<sup>28</sup> Therefore, a liver biopsy is important to determine the severity of liver disease.

**Figure 3.** Monitoring Treatment Response With Molecular Testing of Patients With Chronic Hepatitis C Virus Infection



The plot shows a schematic of 4 possible responses during treatment of chronic hepatitis C virus (HCV) infection and stopping rules. Suggested times for HCV RNA testing are shown along the same scale.

### Genotyping Tests

Hepatitis C virus is classified into 6 major genotypes, numbered 1 through 6, which vary in nucleotide sequence by as much as 30%. These genotypes occupy unique geographical niches. In the United States, genotype 1 accounts for 71.5% of the total cases, genotype 2 for 13.5%, genotype 3 for 5.5%, and genotype 4 for 1.1%.<sup>60</sup> Several tests are available for assigning HCV genotype. Most assays target the highly conserved 5' non-coding region (5'NCR) of the HCV genome, but spurious mutations within the 5'NCR result in misclassification of HCV genotypes in 5% to 8% of cases. The commonly used line probe assay (INNO-LiPA HCV II, Bayer) misclassified 4 (8%) of 50 genotype 1a isolates as genotype 2a, a difference that has significant implications on treatment decisions and outcomes.<sup>61</sup>

### Clinical Applications of Genotyping Tests

Genotype tests are important clinically because they predict most accurately the chance of antiviral response, dictate the duration of therapy, and determine the dosage of ribavirin. Genotype is the strongest predictor of response to interferon and ribavirin; patients who had genotype 2 or 3 were 3 to 6 times more

likely to achieve sustained virological response in the 2 large registration trials of peginterferon.<sup>40,44</sup> Furthermore, for genotypes 2 and 3 (but not genotype 1), rates of sustained virological response were equivalent with 6 months vs 12 months of therapy.<sup>62</sup> Therefore, any patient deemed to be a candidate for peginterferon and ribavirin should undergo the genotype test before initiating therapy.

### Controversies

Molecular diagnostic testing for HCV has provided a crucial tool for addressing significant controversies in HCV management. For example, 1 meta-analysis of published articles up to 2002 reported that sustained virological response rates varied from 37% to 100% in the treatment of acute HCV compared with 12% of untreated patients.<sup>63</sup> Recent clinical trials incorporating NAT has helped to optimize the timing and duration of treatment, resulting in sustained virological response rates exceeding 80% in some situations (TABLE 3). In a similar fashion, HCV NATs have been used to optimize treatment of chronic HCV infection. In the research setting, HCV RNA testing of nonserum reservoirs has raised the controversial issue of occult hepatitis C.

**Table 3.** Optimization of Acute Hepatitis C Virus Therapy\*

Source	Study Design	Total No. of Patients	Clinical Parameter Optimized	Percentage of Patients With Sustained Virological Response†
Kamal et al, <sup>64</sup> 2006	Randomized controlled trial	129	Timing of treatment initiation‡	95 (Initiation at 8 weeks) 92 (Initiation at 12 weeks) 75 (Initiation at 20 weeks)
Kamal et al, <sup>65</sup> 2006	Randomized controlled trial	102	Duration of therapy§	68 (8-week duration) 82 (12-week duration) 91 (24-week duration)
Kamal et al, <sup>66</sup> 2004	Open-label, controlled trial receiving peginterferon with or without ribavirin§	54	Adjunctive ribavirin	85 (With ribavirin) 80 (Without ribavirin)

Abbreviations: NA, not applicable.

\*Response parameters were defined using sensitive nucleic acid tests.

†Information in the parentheses indicates treatment variable under study.

‡Time treatment initiated after exposure.

§Treatment initiated at 12 weeks after hepatitis C virus exposure for a maximum course of 24 weeks.

**Treatment of Acute HCV Infection**

Recent randomized trials have used molecular diagnostics to clarify controversies surrounding the timing of treatment initiation, the duration of therapy and the use of adjunctive ribavirin for acute HCV infection. Therapy of patients with acute genotype 1 infection is especially important because therapy is shortened and much more efficacious compared with therapy during the chronic phase (sustained virological response, 42%-52%).<sup>40,44,64</sup> In a series of well-defined studies, Kamal and colleagues<sup>65-67</sup> performed frequent ultrasensitive NATs (reverse transcription-PCR and TMA) to define the effect of various therapeutic regimens on the HCV persistence or clearance during acute infection. The molecular tests were critical for defining treatment responses. Several controversial issues were resolved, including (1) optimal time after infection to initiate therapy (8-12 weeks); (2) optimal treatment duration (24 weeks); and (3) the important point that ribavirin is apparently not required for optimal responses during acute infection, thus reducing the risk of major adverse effects (anemia). These studies illustrate well the critical role of molecular diagnostics in shaping clinical management of acute HCV infections.

**Treatment Durations for Chronic Hepatitis C**

Hepatitis C virus NATs also played a critical role in optimization of therapeutic decision making during treatment of chronic

HCV infections. Expert guidelines recommend 48 weeks of peginterferon plus 1000 to 1200 mg of ribavirin (combination therapy) for patients with genotype 1 infection and 24 weeks of peginterferon plus 800 mg of ribavirin for genotypes 2 or 3 infection.<sup>8,9</sup> Since these guidelines were published, several studies have explored shortened treatment courses to reduce major neuropsychiatric and hematological adverse effects. One series of studies evaluated the efficacy of shortening treatment duration in patients infected with genotype 1 HCV who develop a rapid virological response. Researchers found that 24% of the patients receiving combination therapy achieved a rapid virological response. Among these patients with a rapid virological rate, sustained virological response rates were equivalent for 24 vs 48 weeks of therapy using weight-based ribavirin (88%-91%).<sup>45</sup> Preliminary results of an intention-to-treat analysis of a prospective randomized trial indicate that 77% of participants with genotype 1 and a rapid virological response achieved sustained virological response after a 24-week treatment course.<sup>47</sup> This rate is much higher than the 42% to 52% rate reported in randomized trials using 48 weeks of treatment for participants with genotype 1.<sup>40,44,45,64</sup> Thus, defining rapid virological response (undetectable RNA by week 4) may be very useful for shortening treatment durations in individuals infected with genotype 1. Randomized trials have also studied the impact of shortening the treatment course for patients with genotype 2 or 3 infections from 24 weeks to 12 to 16 weeks, with mixed results.<sup>68-70</sup> Although

early results suggested that rapid virological response may identify patients requiring only 12 to 16 weeks of therapy, preliminary results from a large prospective study have not confirmed this association. Thus, results defined by molecular diagnostic testing do not support shortening treatment for patients with genotype 2 or 3 infections, and we recommend 24 weeks of therapy in this circumstance until more definitive data are available.

Other studies have examined the converse issue of extending treatment for patients with a slower virological response defined by lack of an early virological response (HCV RNA levels decreased by 2 logs after 12 weeks of therapy). In an early randomized trial, patients with genotype 1 who were naïve to interferon were given 48 vs 72 weeks of combination therapy, and there was no difference in sustained virological response.<sup>71</sup> However, patients with early virological response showed significant improvement in sustained virological response from an additional 24 weeks of therapy (29% vs 17%). This result emphasizes the potential utility of intratreatment HCV RNA quantification for tailoring duration of therapy. When considering the option of extended treatment, it is important to consider the significant adverse effects of HCV therapy, including major depression and anemia. Furthermore, the treatment paradigms that rely on rapid virological response and early virological response for tailoring therapy may not apply to emerging therapies and will need to be studied. Nevertheless, molecular diagnostic testing for HCV has played and will continue to play a criti-

cal role in optimization of therapy for chronic HCV infection.

### Occult HCV Infection

If a patient develops an undetectable HCV RNA level 24 weeks after completion of therapy, it is generally believed that HCV infection has been eradicated. This assumption is based on several long-term surveillance studies, including one that found 72 of 75 patients had undetectable HCV RNA levels in serum samples for a mean of 4 years after achieving a sustained virological response.<sup>72</sup> This study also found that none of 27 patients had detectable HCV RNA in liver biopsy specimens performed 1 to 5 years after treatment.

Several recent studies have challenged the conclusion that HCV is truly eradicated after a documented sustained virological response. In one study, researchers detected HCV RNA in 15 of 17 serum samples and in 9 of 12 samples of peripheral blood mononuclear cells taken from patients previously reported as having tested negative for HCV RNA after either spontaneous or treatment-induced resolution.<sup>70</sup> A second research group reported detection of HCV RNA frequently in liver biopsies and peripheral blood mononuclear cells from patients with abnormal liver function tests whose serum sample tested negative for HCV antibody and RNA.<sup>73</sup> Overall, 48 of 100 patients with undiagnosed hepatitis had HCV RNA in liver biopsy specimens and 40 of 100 had HCV RNA in their peripheral blood mononuclear cells, leading to speculation of a new clinical entity designated as *occult hepatitis C*. A study of patients who tested positive for HCV antibodies but negative for HCV RNA with normal serum ALT levels found that 10 of 12 patients had HCV RNA in liver biopsies.<sup>74</sup> Finally, Lee et al<sup>75</sup> reported that a patient with hypogammaglobulinemia had HCV recrudescence at the time of steroid treatment after an acute infection, despite 8 years of having negative HCV RNA test results. Because of the technical difficulties in evaluating HCV replication *ex vivo*, the possibility of reinfection

and the controversies in the literature, additional rigorous studies are needed to confirm these reports of occult HCV infection, both during natural infection and after therapy.

### CONCLUSIONS AND PERSONAL PERSPECTIVE

The diagnosis, monitoring, and treatment of HCV infection represent a new paradigm in the field of virology. Hepatitis C virus was the first pathogenic human virus identified purely by molecular methods. Active disease is defined by detection of viral RNA in serum regardless of antibody or ALT levels. The transition of acute to chronic HCV infection is defined by HCV RNA detection 6 months after exposure. Monitoring of the antiviral response is largely by measuring the presence and levels of virus using molecular diagnostics. Without question, the diagnosis and management of hepatitis C rely heavily on accurate molecular diagnostic tests. Important emerging issues include defining the optimal use of molecular testing across all drug regimens, including several new antiviral agents presently under evaluation in clinical trials and resolution of the controversial claim that occult HCV infection may occur.

**Author Contributions:** Dr Gretch had full access to all of the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. *Study concept and design:* Scott, Gretch. *Acquisition of data:* Scott, Gretch.

*Analysis and interpretation of data:* Scott, Gretch. *Drafting of the manuscript:* Scott, Gretch. *Critical revision of the manuscript for important intellectual content:* Gretch. *Study supervision:* Gretch.

**Financial Disclosure:** None reported.

**Funding/Support:** No funding was received for this review. Dr Scott reports that he is the recipient of a Mentored Patient-Oriented Research Career Development Award (K23) from the National Center for Research Resources. Dr Gretch reports that he is supported by National Institutes of Health grants AI 049168, AI 066209, and DK 92318.

**Role of the Sponsor:** Organizations providing salary support for the authors played no role in the design and conduct of the study, including collection, management, analysis, and interpretation of the data, and the preparation, review, and approval of the manuscript.

**Acknowledgment:** We thank Richard Wilson, MD, Chia Wang, MD, MSc, and Larry Corey, MD, for helpful editorial comments, all from the University of Washington and Harborview Medical Center, Seattle. None received compensation for their assistance. We also thank Georgia St. Aubyn, Harborview Medical Center, for her invaluable support in the preparation of the manuscript, whose assistance is part of her regular duties.

### REFERENCES

- Centers for Disease Control and Prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR Recomm Rep*. 1998;47(RR-19):1-39.
- Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl*. 2003;9:331-338.
- Shuhart MC, Bronner MP, Gretch DR, et al. Histological and clinical outcome after liver transplantation for hepatitis C. *Hepatology*. 1997;26:1646-1652.
- Kwiatkowski CF, Fortuin Corsi K, Booth RE. The association between knowledge of hepatitis C virus status and risk behaviors in injection drug users. *Addiction*. 2002;97:1289-1294.
- Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*. 1989;244:359-362.
- Flamm SL. Chronic hepatitis C virus infection. *JAMA*. 2003;289:2413-2417.
- Alter MJ, Kuhnert WL, Finelli L; Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. *MMWR Recomm Rep*. 2003;52(RR-3):1-13, 15.
- National Institutes of Health. National Institutes of Health Consensus Development Conference Statement: management of hepatitis C: 2002-June 10-12, 2002. *Hepatology*. 2002;36(suppl 1):S3-S20.
- Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology*. 2004;39:1147-1171.
- Alter MJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States: the Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *N Engl J Med*. 1992;327:1899-1905.
- Jauncey M, Micallef JM, Gilmour S, et al. Clearance of hepatitis C virus after newly acquired infection in injection drug users. *J Infect Dis*. 2004;190:1270-1274.
- Nguyen TT, Sedghi-Vaziri A, Wilkes LB, et al. Fluctuations in viral load (HCV RNA) are relatively insignificant in untreated patients with chronic HCV infection. *J Viral Hepat*. 1996;3:75-78.
- Bruce MG, Bruden D, McMahon BJ, et al. Hepatitis C infection in Alaska Natives with persistently normal, persistently elevated, or fluctuating alanine aminotransferase levels. *Liver Int*. 2006;26:643-649.
- Watanabe H, Saito T, Shinzawa H, et al. Spontaneous elimination of serum hepatitis C virus (HCV) RNA in chronic HCV carriers: a population-based cohort study. *J Med Virol*. 2003;71:56-61.
- Scott JD, McMahon BJ, Bruden D, et al. High rate of spontaneous negativity for hepatitis C virus RNA after establishment of chronic infection in Alaska Natives. *Clin Infect Dis*. 2006;42:945-952.
- Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med*. 2001;345:41-52.
- Polywka S, Pembrey L, Tovo PA, Newell ML. Accuracy of HCV-RNA PCR tests for diagnosis or exclusion of vertically acquired HCV infection. *J Med Virol*. 2006;78:305-310.
- US Public Health Service. Updated US Public Health Service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. *MMWR Recomm Rep*. 2001;50(RR-11):1-52.
- Carithers RL Jr, Marquardt A, Gretch DR. Diagnostic testing for hepatitis C. *Semin Liver Dis*. 2000;20:159-171.
- Alberti A, Morsica G, Chemello L, et al. Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. *Lancet*. 1992;340:697-698.
- Gretch DR. Diagnostic tests for hepatitis C. *Hepatology*. 1997;26(3 suppl 1):435-475.
- Ferreira-Gonzalez A, Shiffman ML. Use of diagnostic testing for managing hepatitis C virus infection. *Semin Liver Dis*. 2004;24(suppl 2):9-18.

23. Morishima C, Chung M, Ng KW, Brambilla DJ, Gretch DR. Strengths and limitations of commercial tests for hepatitis C virus RNA quantification. *J Clin Microbiol.* 2004;42:421-425.
24. Albadalejo J, Alonso R, Antinozzi R, et al. Multi-center evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol.* 1998;36:862-865.
25. Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. *J Clin Microbiol.* 2002;40:4407-4412.
26. Nolte FS, Fried MW, Shiffman ML, et al. Prospective multicenter clinical evaluation of AMPLICOR and COBAS AMPLICOR hepatitis C virus tests. *J Clin Microbiol.* 2001;39:4005-4012.
27. Ross RS, Viazov SO, Hoffmann S, Roggendorf M. Performance characteristics of a transcription-mediated nucleic acid amplification assay for qualitative detection of hepatitis C virus RNA. *J Clin Lab Anal.* 2001;15:308-313.
28. Morishima C, Gretch DR. Clinical use of hepatitis C virus tests for diagnosis and monitoring during therapy. *Clin Liver Dis.* 1999;3:717-740.
29. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. *N Engl J Med.* 2004;351:760-768.
30. Desombere I, Van Vlierberghe H, Couvent S, Clinckspoor F, Leroux-Roels G. Comparison of qualitative (COBAS AMPLICOR HCV 2.0 versus VERSANT HCV RNA) and quantitative (COBAS AMPLICOR HCV monitor 2.0 versus VERSANT HCV RNA 3.0) assays for hepatitis C virus (HCV) RNA detection and quantification: impact on diagnosis and treatment of HCV infections. *J Clin Microbiol.* 2005;43:2590-2597.
31. Sarrazin C, Hendricks DA, Sedarati F, Zeuzem S. Assessment, by transcription-mediated amplification, of virologic response in patients with chronic hepatitis C virus treated with peginterferon alpha-2a. *J Clin Microbiol.* 2001;39:2850-2855.
32. Hofer H, Watkins-Riedel T, Janata O, et al. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. *Hepatology.* 2003;37:60-64.
33. Morishima C, Morgan TR, Everhart JE, et al. HCV RNA detection by TMA during the hepatitis C antiviral long-term treatment against cirrhosis (Halt-C) trial. *Hepatology.* 2006;44:360-367.
34. Konnick EQ, Erali M, Ashwood ER, Hillyard DR. Performance characteristics of the COBAS Amplicor Hepatitis C Virus (HCV) Monitor, Version 2.0, International Unit assay and the National Genetics Institute HCV Superquant assay. *J Clin Microbiol.* 2002;40:768-773.
35. Elbeik T, Surtihadi J, Destree M, et al. Multi-center evaluation of the performance characteristics of the bayer VERSANT HCV RNA 3.0 assay (bdNA). *J Clin Microbiol.* 2004;42:563-569.
36. Barbeau JM, Goforth J, Caliendo AM, Nolte FS. Performance characteristics of a quantitative TaqMan hepatitis C virus RNA analyte-specific reagent. *J Clin Microbiol.* 2004;42:3739-3746.
37. McHutchison JG, Gordon SC, Schiff ER, et al. Hepatitis Interventional Therapy Group. Interferon alpha-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med.* 1998;339:1485-1492.
38. Pawlotsky JM, Bouvier-Alias M, Hezode C, Darthuy F, Remire J, Dhumeaux D. Standardization of hepatitis C virus RNA quantification. *Hepatology.* 2000;32:654-659.
39. Hayashi J, Kawakami Y, Nabeshima A, et al. Comparison of HCV RNA levels by branched DNA probe assay and by competitive polymerase chain reaction to predict effectiveness of interferon treatment for patients with chronic hepatitis C virus. *Dig Dis Sci.* 1998;43:384-391.
40. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet.* 2001;358:958-965.
41. Trabaud MA, Bailly F, Si-Ahmed SN, et al. Comparison of HCV RNA assays for the detection and quantification of hepatitis C virus RNA levels in serum of patients with chronic hepatitis C treated with interferon. *J Med Virol.* 1997;52:105-112.
42. Weiland O, Braconier JH, Fryden A, Norkrans G, Reichard O, Uhnoo I. Influence of pre-treatment factors on outcome of interferon-alpha treatment of patients with chronic hepatitis C. *Scand J Infect Dis.* 1999;31:115-118.
43. Yamada G, Takatani M, Kishi F, et al. Efficacy of interferon alpha therapy in chronic hepatitis C patients depends primarily on hepatitis C virus RNA level. *Hepatology.* 1995;22:1351-1354.
44. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med.* 2002;347:975-982.
45. Jensen DM, Morgan TR, Marcellin P, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology.* 2006;43:954-960.
46. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alpha-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology.* 2003;38:645-652.
47. Ferenci P, Fried MW, Shiffman ML, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alpha-2a (40 KD)/ribavirin. *J Hepatol.* 2005;43:425-433.
48. Terrault NA, Pawlotsky JM, McHutchison J, et al. Clinical utility of viral load measurements in individuals with chronic hepatitis C infection on antiviral therapy. *J Viral Hepat.* 2005;12:465-472.
49. Wang CC, Morishima C, Chung M, et al. High serum hepatitis C virus (HCV) RNA load predicts the presence of HCV RNA in saliva from individuals with chronic and acute HCV infection. *J Infect Dis.* 2006;193:672-676.
50. Nowicki MJ, Laskus T, Nikolopoulou G, et al. Presence of hepatitis C virus (HCV) RNA in the genital tracts of HCV/HIV-1-coinfected women. *J Infect Dis.* 2005;192:1557-1565.
51. Bourlet T, Levy R, Maertens A, et al. Detection and characterization of hepatitis C virus RNA in seminal plasma and spermatozoon fractions of semen from patients attempting medically assisted conception. *J Clin Microbiol.* 2002;40:3252-3255.
52. Hisada M, O'Brien TR, Rosenberg PS, Goedert JJ. Virus load and risk of heterosexual transmission of human immunodeficiency virus and hepatitis C virus by men with hemophilia: the Multicenter Hemophilia Cohort Study. *J Infect Dis.* 2000;181:1475-1478.
53. Hagiwara H, Hayashi N, Fusamoto H, Kamada T. Quantitative analysis of hepatitis C virus RNA: relationship between the replicative level and the various stages of the carrier states or the response to interferon therapy. *Gastroenterol Jpn.* 1993;28(suppl 5):48-51.
54. Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M, Omata M. Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: increase of the virus in advanced liver disease. *Hepatology.* 1993;18:16-20.
55. Gordon SC, Kodali VP, Silverman AL, et al. Levels of hepatitis C virus RNA and liver histology in chronic type C hepatitis. *Am J Gastroenterol.* 1994;89:1458-1461.
56. Fanning L, Kenny E, Sheehan M, et al. Viral load and clinicopathological features of chronic hepatitis C (1b) in a homogeneous patient population. *Hepatology.* 1999;29:904-907.
57. Lau JY, Davis GL, Kniffen J, et al. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet.* 1993;341:1501-1504.
58. Martinot-Peignoux M, Marcellin P, Pouteau M, et al. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alpha therapy in chronic hepatitis C. *Hepatology.* 1995;22:1050-1056.
59. Yun ZB, Reichard O, Chen M, et al. Serum hepatitis C virus RNA levels in chronic hepatitis C—importance for outcome of interferon alpha-2b treatment. *Scand J Infect Dis.* 1994;26:263-270.
60. Lau JY, Mizokami M, Kolberg JA, et al. Application of six hepatitis C virus genotyping systems to sera from chronic hepatitis C patients in the United States. *J Infect Dis.* 1995;171:281-289.
61. Anderson JC, Simonetti J, Fisher DG, et al. Comparison of different HCV viral load and genotyping assays. *J Clin Virol.* 2003;28:27-37.
62. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med.* 2004;140:346-355.
63. Alberti A, Boccato S, Vario A, Benvenuto L. Therapy of acute hepatitis C. *Hepatology.* 2002;36(suppl 1):S195-S200.
64. Kamal SM, Fouly AE, Kamel RR, et al. Peginterferon alpha-2b therapy in acute hepatitis C: impact of onset of therapy on sustained virologic response. *Gastroenterology.* 2006;130:632-638.
65. Kamal SM, Moustafa KN, Chen J, et al. Duration of peginterferon therapy in acute hepatitis C: a randomized trial. *Hepatology.* 2006;43:923-931.
66. Kamal SM, Ismail A, Graham CS, et al. Pegylated interferon alpha therapy in acute hepatitis C: relation to hepatitis C virus-specific T cell response kinetics. *Hepatology.* 2004;39:1721-1731.
67. Mangia A, Santoro R, Minerva N, et al. Peginterferon alpha-2b and ribavirin for 12 vs 24 weeks in HCV genotype 2 or 3. *N Engl J Med.* 2005;352:2609-2617.
68. von Wagner M, Huber M, Berg T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology.* 2005;129:522-527.
69. Shiffman M, Pappas S, Bacon B, et al. Utility of virological response at weeks 4 and 12 in the prediction of SVR rates in genotype 2/3 patients treated with peginterferon alpha-2a (40KD) plus ribavirin: findings from ACCELERATE [abstract]. *Hepatology.* 2006;44(suppl 1):316A.
70. Berg T, von Wagner M, Nasser S, et al. Extended treatment duration for hepatitis C virus type 1: comparing 48 vs 72 weeks of peginterferon-alpha-2a plus ribavirin. *Gastroenterology.* 2006;130:1086-1097.
71. Marcellin P, Boyer N, Gervais A, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med.* 1997;127:875-881.
72. Radkowski M, Gallegos-Orozco JF, Jablonska J, et al. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology.* 2005;41:106-114.
73. Castillo I, Pardo M, Bartolome J, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis.* 2004;189:7-14.
74. Carreno V, Pardo M, Lopez-Alcorcho JM, Rodriguez-Inigo E, Bartolome J, Castillo I. Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. *J Infect Dis.* 2006;194:53-60.
75. Lee WM, Polson JE, Carney DS, Sahin B, Gale M Jr. Reemergence of hepatitis C virus after 8.5 years in a patient with hypogammaglobulinemia: evidence for an occult viral reservoir. *J Infect Dis.* 2005;192:1088-1092.