Hepatitis C: new perspectives in diagnosis
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by Ron Gasbarro, PharmD, MS Journ

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Abstract
This article reviews and discusses the state-of-the-art tests and assays used in the diagnosis and management of hepatitis C virus (HCV) infection. Screening tests for the presence of HCV and confirmatory tests are discussed and put into clinical perspective. More recent generations of the enzyme-linked immunosorbent assays (ELISA) and the recombinant immunoblot assays (RIBA) are compared with their predecessors in terms of sensitivity and specificity. HCV molecular assays, such as detection of HCV RNA by reverse transcription and polymerase chain reaction, are considered in terms of their use in following therapeutic activity. Quasispecies detection, genotyping and serotyping, used to unravel the genetic idiosyncrasies of the virus so that more accurate therapeutic strategies can be planned, are covered. Finally, liver biopsy is discussed in terms of patient prognosis and with it, non-invasive markers of histological status, such as hyaluronic acid and serum procollagenase-III peptide are assigned their optimal time and place in the treatment of patients with HCV infection.

Introduction
The discovery of the hepatitis C virus (HCV) genome was accomplished just a decade ago. Today, applied biotechnology strengthens our means to manage and prevent chronic hepatitis C and its complications. Many techniques, such as the enzyme-linked immunosorbent assay (ELISA) and the recombinant immunoblot assay (RIBA), are in their third-generations with increased sensitivity and more specificity. [1-3] Others, such as the branched-chain DNA and polymerase chain reaction (PCR), are considered inadequate in range and variability, and in sensitivity. [4]

Additionally, while the detection of antibodies to HCV (anti-HCV) has become the most practical means of diagnosing both past and present infection, even these new generation tests can fall short. Detection of anti-HCV following infection averages 3 months with some cases occurring six months later, weeks after serum aminotransferase has peaked. Patients who are immunosuppressed following transplantation, or immunocompromised secondary to human immunodeficiency virus (HIV), can have HCV infection without detectable antibodies. Here, HCV RNA viral assays are needed. Finally, although third-generation ELISAs are more specific than their predecessors, false-positive results are prevalent among low-risk blood donors. [2] What are the additional tests that can give a more accurate picture of the patient’s virological status? Are they practical? Are they necessary? This paper will describe and discuss the state-of-the-art techniques in the diagnosis of HCV and their clinical relevance.

Screening tests for hepatitis C infection
Since the identification and molecular characterization of the non-A, non-B hepatitis virus in 1989 by Choo, [5] a variety of diagnostic tests based on the detection of anti-HCV antibodies in serum samples have been developed and refined. The first-generation anti-HCV test that was commercially available and widely used was an ELISA that incorporated the c100-3 epitope from the nonstructural NS4 region. [See Figure 1] Three generations of serodiagnostic anti-HCV antigen tests have been developed, with each new generation providing incremental improvements in the sensitivity to anti-
HCV antibodies. [See Table 1] Many of the screening tests employ enzyme-linked immunosorbent assays (ELISA), such as the ORTHO HCV 3.0 ELISA Test System (ELISA 3.0 HCV; Ortho Clinical Systems, Raritan, NJ), MONOLISA HCV (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France), and the Abbott HCV 3.0 (HCV EIA 3.0; Abbott Laboratories, North Chicago, Il). The third-generation ELISAs, recently approved by the Food and Drug Administration for blood donor screening, are designed to detect antibodies to four recombinant HCV proteins [See Table 2]. The third-generation assay differs from the second-generation ELISA by substitution of the NS5 protein for the 5-1-1 antigen. However, this substitution does not appear to account for the increased sensitivity of the newer assay. [4] The average period for HCV seroconversion after blood transfusion has been shortened with each generation as well: 7 to 8 weeks with ELISA-3 as compared with 10 weeks with ELISA-2 and 16 weeks with ELISA-1. [4]

ELISA assays have many advantages in the diagnostic setting including ease of automation, ease of use, relative cost-effectiveness low variability and high sensitivity in screening before liver transplantation. Some of the major disadvantages include suboptimal sensitivity and specificity, an abundance of false-positives in low-prevalence populations and poor sensitivity in post-liver transplant patients because of immunosuppression [6].

Although false-positive ELISA results are a problem in low-prevalence settings, the accuracy of the third-generation test is very good in high-prevalence populations, and therefore, supplemental anti-HCV tests may not be necessary in high-risk patients with a positive anti-HCV screen. A study by Pawlotsky et al [1] determined whether a double ELISA determination and confirmation of positive ELISA results with immunoblot assays were still useful in clinical laboratories performing routine HCV diagnosis. The study showed that one single ELISA 3.0 determination was sufficient for diagnosis of HCV infection in clinical laboratories eliminating confirmation of positive or weakly positive ELISAs with immunoblot assays.

Automated systems can improve workflow in the clinical laboratory such as the Abbott AxSYM, an automated system that provides random- and continuous-access testing for immunoassays, 20 onboard reagents, primary tube sampling, and a throughput of 80 to 120 tests per hour. The AxSYM incorporates three separate analytical technologies for processing immunoassays: microparticle immunoassay, fluorescence polarization immunoassay, and ion-capture immunoassay. The system incorporates both common and technology-specific subsystems controlled by a real-time software scheduling processor. Tests can be processed in one- or two-step sandwich or competitive formats, with variable pipetting steps, incubation periods, optical read formats, and wash sequences. Abbott expects to launch an updated version called AxSYM 2. This new system will use existing AxSYM tests, but will incorporate additional labor-saving features to simplify and improve the testing process for mid-volume laboratories. The AxSYM line is not available in the United States pending FDA approval. [7]

The PRISM (Abbott) is another automated, high-volume, donor screening immunoassay analyzer in which a sample is split between disposable reaction trays in a group of linear tracks. The system's pipettor uses noninvasive sensing of the sample volume and disposable pipet tips. Each assay track has (a) a conveyor belt for moving reaction trays to predetermined functional stations, (b) temperature-controlled tunnels, (c) noncontact transfer of the reaction mixture between incubation and detection wells, and (d) single-photon counting to detect a chemiluminescence (CL) signal from the captured immunochemical product. Multiple channels on the PRISM accommodate hepatitis and retrovirus assays and use either serum or plasma. [8] The assay methodology, a combination of microparticle capture and direct detection of a CL signal on a porous matrix, offers excellent sensitivity, specificity,
Supplemental or confirmatory tests for HCV
An accurate diagnosis of hepatitis C virus (HCV) is necessary before treatment and counseling of patients begins. As stated above, false-positive results following ELISA testing continue to be noted among low-risk blood donors. While elevated aminotransferases and high-risk factors for infection are indicative of active infection and hepatitis, additional testing for antibody specificity can further document HCV infection. A number of confirmatory and/or supplemental serodiagnostic tests are available to cross-check seropositive results obtained with ELISA screening tests. An HCV diagnosis can be confirmed by the recombinant immunoblot assay (RIBA). RIBA identifies antibodies to individual HCV antigens and has a higher specificity than ELISA. [3] Confirmation can involve the use of either a four-antigen RIBA (RIBA HCV 2.0; Chiron Corporation, Emeryville, CA) or strip immunoblot assay (SIA). The second-generation RIBA, or RIBA-2, uses the same recombinant antigens as the ELISA-2. [See Table 2] A more sensitive third generation RIBA is in the process of obtaining FDA licensing and has recently been introduced in Europe.

How do RIBAs compare with other diagnostic tests? RIBAs are technically more demanding than ELISA. Also, RIBA positivity is not always a true indicator of active infection by HCV because recovered patients may stay anti-HCV positive for years. Conversely, RIBAs are simpler, more standardized, and more reproducible than tests for HCV RNA, such as the branched chain DNA assay. The third-generation RIBA has resolved many of the RIBA-2 interdeterminate samples. However, only 50% of the RIBA-3 positive blood donors are HCV RNA positive by polymerase chain reaction (PCR) assay. [3, 9]

Supplemental tests can also indicate whether or not a patient will respond to a particular therapy, in this case, interferon (INF). INNO-LIA HCV Ab III is a confirmatory assay, like RIBA-3, and exposes several specific HCV peptides from two non-overlapping core regions (C1 and C2), E2, NS4 and NS5A regions, and recombinant NS3 of the HCV polyprotein. The HCV nonstructural 5A (NS5A) protein may contribute to the interferon-resistant phenotype of HCV. In the mechanism of HCV resistance to interferon therapy, the NS5A protein represses the action of PKR, a protein activated by interferon that shuts down viral protein synthesis thereby inhibiting replication of the HCV. A study by Frangeul et al [10] used both RIBA-3 and INNO-LIA HCV Ab III in determining which patients with chronic hepatitis C would respond better to interferon (INF). An association was found between a primary response to INF therapy and reactivity towards the NS5A antigen in the INNO-LIA HCV Ab III as well as the NS5 in the RIBA-3 with no differences between the two tests. As further studies become available, both RIBA-3 and INNO-LIA HCV Ab III could become simple predictive markers of INF response.

Molecular assays: detection and quantitation
While the diagnosis of HCV is currently based on the detection of antibodies via ELISA, the technique is less sensitive in the early phases of HCV infection and cannot differentiate between active infection and disease resolution. Also, immunocompromised patients such as those who are infected with HIV, or hemodialysis patients, produce fewer antibodies. [11] The direct molecular qualitative detection of HCV RNA by reverse transcription (RT) and PCR are considered the gold standard for the diagnosis of HCV infection and for assessing the antiviral response to INF therapy. Quantitative assessment of HCV RNA levels, via signal amplification and quantitative PCR (Q-PCR), are valuable tools in the clinical management of patients before, during and after therapy. PCR-based assays are able to ascertain minute amounts of HCV RNA in serum or plasma. HCV RNA detection by PCR helps to resolve weakly positive or negative ELISA results when clinical signs and/or risk factors are
compatible with HCV infection.

A variety of home-brew or in-house PCR assays to test for the seropresence of HCV RNA are available. However, numerous factors add to the variability of such PCR testing including the handling and storage of samples, DNA product contamination, correct design of amplification parameters, and efficacy of postamplification detection. To that end, only 5 of 31 (16%) laboratories scored perfectly on a standardized test. Of these 5, a 100-fold difference in sensitivity was reported for the dilution series. Almost one-third of the laboratories produced false negative and/or false positive results. The investigators concluded that contamination was a major problem. Contamination can come from two areas: carry-over results in the amplification of PCR products that were synthesized during previous PCR reactions, and cross-contamination whereby one sample is contaminated by a positive sample at any given step in the procedure.

A reliable, standardized assay for HCV RNA can convey 1) whether a patient will likely respond to INF therapy, 2) if a virologic response has occurred and 3) promote a better comprehension of the relationship between viral load and the natural history of chronic HCV infection. In studies by Davis et al [14] and McHutchison et al [15], HCV RNA levels correlated with rates of response to INF and/or ribavirin therapy. Late clearance of HCV RNA from serum during combination therapy was associated with a sustained response, a phenomenon not typically seen in patients treated with INF alone. [McHutchison] The genotype and the pretreatment serum HCV RNA level were related; response rate was as high as 100% in patients with low HCV RNA at baseline and a genotype other than type 1 [14].

The only standardized Q-PCR is the Roche Monitor assay but experience with this assay has been limited. The main strength of Q-PCR assays, however, is their high analytic sensitivity with reports as low as 1,000 RNA copies per milliliter. [4] The biggest disadvantages include high assay variability and limited linear range above 1 million RNA copies per milliliter. [4]

In contrast, the branched chain DNA assay (bDNA), a signal amplification technique, is highly standardized. The second generation bDNA provides a modest increase in sensitivity compared to the previous generation and minimal bias in measuring HCV RNA levels for the major HCV genotypes. Direct detection of as few as 1,000 hepatitis viral genomes is possible. [16] In a study [17] using the Quantiplex HCV RNA 2.0 assay (bDNA-2), the Quantiplex RNA assay (bDNA-1) and the Roche monitor assay, highly reproducible results were observed upon repeat testing of samples by both the bDNA-1 and the bDNA-2. A greater variability was observed in the Roche Monitor assay (correlation coefficient of 0.537, compared with 0.942 and 0.964 for the bDNA-1 and bDNA-2 assays, respectively). Significant differences in the efficiency of detection of genotypes 1, 2, and 3 were observed for the bDNA-1 and Roche Monitor assays, whereas the bDNA-2 assay and nested PCR at limiting dilution were able to quantify genotypes with equal sensitivity. These results suggest that many of the previous studies evaluating the effect of genotype and virus load on the response to INF using methods such as the Roche Monitor assay and other competitive PCR methods require reinterpretation. Differences in efficiency of quantitation should be considered in future trials that investigate the relationship between genotype and virus load.

The clinical value of bDNA assay has been the object of several performance studies. Jacob et al [13] compared the relative sensitivities of first-and-second generation branched nucleotide assays (Quantiplex HCV RNA 1.0 and 2.0, respectively, Chiron, Emeryville, CA) for the detection of HCV RNA to that of a RT-PCR method (Monitor, Roche Molecular Systems, Nutley, NJ) in 53 patients with chronic hepatitis C. They concluded that both methods can be used to detect HCV RNA in patients.
who are infected with the genotypes that are most commonly encountered in the United States. The HCV RNA 2.0 bDNA assay may offer advantages when attempting to quantify high-level viremia. Lu et al [11] compared the Roche Amplicor Monitor to the Chiron bDNA assay in quantitative measurement of serum HCV in patients with chronic hepatitis C. The serum of the patients was qualitatively positive by RT-PCR. They concluded that the Roche Amplicor HCV Monitor test kits and the Chiron bDNA are equally sensitive in the quantitative measurement of serum HCV RNA in patients with chronic hepatitis C and can be reliably used in measuring HCV viremia clinically. Roth et al [18] observed that RT-PCR and bDNA in clinically significant agreement in measurement of HCV RNA concentrations, despite subtype-specific differences. However, they stressed that when monitoring an individual patient, kits and methods should not be interchanged. Comparing the bDNA with a semi-quantitative cDNA-polymerase chain reaction (cDNA-PCR) in monitoring HCV RNA levels, the bDNA assay was not as sensitive as cDNA-PCR, given its user friendliness and quantitative results, but it is considered a useful test for monitoring HCV RNA levels in patients treated with INF. However, patients who are non-reactive in the bDNA assay have to be re-tested by cDNA-PCR because low viral titers are not detected by the bDNA assay. [19]

Actual sensitivities of the tests cannot be deduced from the manufacturers’ stated HCV RNA cut-offs. Analytic sensitivity is estimated according to the smallest amount of HCV RNA detected and reliably quantified. Manufacturers of the Roche Monitor assign a cut-off of 1,000 copies per milliliter. Manufacturers of the Chiron bDNA 2.0 assay state the cut-off is 200,000 equivalent genomes per milliliter. This suggests that the Roche product is the more sensitive of the two assays. In reality, the Roche copy and the Chiron genome do not represent the same amount of HVC RNA in a clinical sample. [20] A global standardized system is needed that utilizes quantified standards, such as nucleic acid transcripts of the same nature, length and sequence.

Quasispecies
The RNA-dependent polymerase of RNA viruses is highly error prone and lacks proofreading capabilities. Consequently, within any given individual, HCV exists as a heterogeneous mixture of closely related viruses called quasispecies. In contrast to HCV genotypes, which vary by 31% to 35% of bases over the entire length of the genome, quasispecies vary from each other by 1% to 9% of bases. [21] The quasispecies nature of HCV has several potentially significant biological consequences. They are likely an important factor in the inability of acutely infected individuals to clear infection. Additionally, mutations in the viral populations likely contribute to drug "resistance" during INF treatment and to the ineffectiveness of isolate-specific vaccines.

Both direct and indirect methods of detecting and quantifying quasispecies within an individual exist. Early studies utilized cloned PCR products, a procedure that is relatively easy to perform and reliable, but labor-intensive. Indirect methods of measuring the number of different viral populations within an individual include single-strand conformation polymorphism (SSCP). In SSCP analysis, products of PCR are subjected to electrophoretic analysis under denaturing conditions, such that single-stranded RNA is obtained. Single nucleotide polymorphisms result in different mobilities of the single-stranded fragments. Depending on the quasispecies diversity, as well as the sensitivity of the RNA staining technique, a range of the most prevalent variants can be observed. Using SSCP, investigations of large patient cohorts with chronic HCV infection can be undertaken. [22]

In direct sequencing, PCR products obtained are not cloned from PCR fragments, but all RNA strands with varying sequences are directly submitted en masse to sequence analysis. The presumed sequence represents the master sequence and can show degeneration at certain positions, that is, certain positions
may, for example, show both an adenine and guanine residue. Degeneration can only be observed when the minor sequence is observed in 20 percent or more of the RNA strands. [23]

Quasispecies measurement can be used to predict INF responsiveness in patients with mutations in HCV genotype. Polya et al [24] used nucleotide sequencing to show that INF therapy frequently exerts pressure on the second envelope glycoprotein gene hypervariable region 1 (HVR1) in HCV-infected individuals. This pressure results in quasispecies distribution in such persons with genetic complexity significantly higher in transfusion recipients than in intravenous drug users. Laskus et al [25] used SSCP and sequencing to determine that, in the presence of HIV-1 infection, viral sequence differences existed in the same tissue samples, arguing in favor of extrahepatic HCV replication that can interfere with HCV therapy.

Sequence determination and phylogenetic analysis
Seqencing of the E2 HVR1, followed by phylogenetic analysis is recommended for studying patient-to-patient transmission (i.e., hematolgy ward), analysis of interspousal transmission, noscomial infections in a hemodialysis unit, and in geographical regions with a high endemicity of only one subtype (e.g., subtype 1b in Belgium or Sicily). In a multivariate analysis of pretreatment parameters with a sustained virological response to treatment, three parameters appear to be independent predictors of a treatment response: a low viral load (P<.04), a low anti-HCV core IgM titer (P=.03) and a low genetic complexity of HVR1 major variants (P<.04). [24] However, the E2 HVR is too heterogeneous to be of value for classification of HCV genotypes. Instead, the 5’ NCR, core, E1 and NS5B regions are frequently amplified for the purpose of genotypic classification.

Phylogenetically analyzing a subtype needs to factor in, beyond simple sequence differences, the possibility of mutation and reversions. Nucleotide sequence analysis of hepatitis C virus (HCV) strains showed substantial variability leading to a classification into several genotypes and subtypes. The data correlating HCV genotypes and subtypes with hepatitis C viremia levels, demographic characteristics of patients (age, mode of transmission, duration of infection), and severity of liver disease conflict. The interpretation of clinical studies is further complicated because the molecular methods used lacked specificity for genotyping/subtyping and underestimated viremia levels, especially in patients infected with HCV genotypes 2 and 3.

Zeuzem et al [26] sought a connection between phylogenetic analysis of HCV isolates and viremia, liver function tests, and histology. HCV subtyping was performed by sequence and phylogenetic analysis of the nonstructural (NS)-5 region and assessed serum HCV-RNA concentration by a validated genotype-independent quantitative reverse-transcription-polymerase chain reaction assay using an internal RNA standard. There were no significant differences between median serum HCV-RNA concentrations in patients infected with different genotypes/subtypes. Although patients infected with HCV-1b were older, no biochemical or histological evidence was obtained that this subtype is associated with more severe liver disease. Furthermore, this study showed a lack of correlation between the serum HCV-RNA concentration, biochemical parameters, and liver histology. The median serum HCV-RNA levels in patients with chronic persistent hepatitis, chronic active hepatitis, and liver were not significantly different and no correlation was shown between HCV genotypes/subtypes, viremia, liver function test results, and histology.

Genotyping/Subtyping: Divergence within the human population
Not only do HCV quasispecies sequences express variability in different regions of the genomes, but isolates also differ among themselves. All isolates separate into phylogenetically related clusters called
subtypes. One or several subtypes can be classified into several major types that show similarities over 65 percent to 75 percent over the total genome. The term genotype is used generically to refer to subtypes, types or both. Use of the term genotype to describe quasispecies variants is not appropriate. Eleven HCV genomes are known to exist [23] as well as more than 90 subtypes [27], with more subtypes being discovered at a continuous rate. [23]

Genetic variation can determine the success of therapy in a patient with HCV. For example, INF-alpha therapy is more effective in genotypes 1a, 2, 3 and 5 than in 1b and 4 infections. Post-transplantation, recurrent subtype 1b and type 4 infections proceed much faster to chronic hepatitis in the new graft, while subtypes 1a and 2a show very similar, more benign recurrences. [23] Most importantly, HCV genotypes are distributed differently, depending on geography and etiology. For these reasons, genotyping is important.

Several screening tests have been developed to identify HCV genotype, and include reverse hybridization line probe assay (LiPA, Innogenetics, Zwijnaarde, Belgium), restriction fragment length polymorphism (RFLP) of the PCR amplicons, and nested PCR with genotype-specific parameters to the core region.

The optimal genotyping region is reported to be the 5’ untranslated region (UR) because of high conservation within, but considerable variation between, genotypes. LiPA technology is based on the reverse hybridization principle in that biotinylated PCR fragments are hybridized to a selection of highly specific immobilized probes. In a second step, the biotin group in the hybridization complex is exposed by incubation with a streptavidin-alkaline phosphatase complex and the appropriate chromogen compounds. Previously, an LiPA was developed that allowed discrimination of HCV types and subtypes and was capable of detecting single nucleotide differences in the 5’ UR. In a more recent study, 21 probes dispersed over seven variable 5’ UR areas were applied to an LiPA and used to analyze 506 HCV-infected sera from different geographical regions resulting in an abundance of subtypes. The investigators concluded that the selected probes detected the corresponding signature motifs in the seven variable regions with 100% reliability. [27] In addition, these motifs allowed correct type interpretation of samples collected worldwide, with the exclusion of Vietnam, Thailand, or Vietnamese patients residing in European hospitals. Finally, subtyping specificities vary according to geographical region, with 11 prototype subtyping patterns identifying the majority of samples from Europe and the Americas. These results indicate that the LiPA is a reliable assay applicable to routine typing and subtyping of HCV specimens. [28]

A study that used LiPA to determine HCV genotypes in tertiary referral centers through the United States found that the proportion of patients with HCV had types 1 (71.5%), 2 (13.5%) and 3 (5.5%). Patients with HCV type 1 had a longer estimated duration of infection compared to patients with HCV type 3 (P=.004) and type 4 (P<.05). Disease activity and viremia levels did not differ among patients with HCV types 1, 2 or 3 but patients with type 4 had a lower level of viremia than patients with type 1 (P<.05). [29]

In RFLP analysis, a single PCR fragment is amplified from a certain region of the HCV genome with universal primers. Restriction enzyme recognition sites present in the DNA fragment usually show subtype- or type-specific distribution. Thus, restriction fragments with varying lengths are created after cutting the PCR fragment with one or several restriction endonucleases. The electrophoretic separation of these fragments lets the observer infer the approximate lengths of the restricted fragments and, in turn, identify the genotype.
A Spanish study used RFLP analysis to identify the HCV genotypes in their country and to show a relationship between the genotype and disease severity. The results showed that genotype 1b was associated with advanced liver disease, including hepatocellular carcinoma and cirrhosis in Spain. However, the investigators conceded that the results may be related to a cohort-effect caused by overrepresentation of genotype 1b in older patients with more advanced disease. [30]

Genotypes of HCV differ in their biologic effects. Variations in HCV genotype have major implications in the design of HCV vaccines and biotherapeutic agents. Antibody elicited by one genotype may not protect from reinfection with other variants, as has been observed with other enveloped viruses. Some HCV-infected individuals infected experience multiple episodes of acute hepatitis. It is unclear whether these episodes are due to reinfection with HCV or to reactivation of the original virus infection. Genotype-related differences may also include viral replication rates, mutation rates, histologic inflammatory activity, disease severity and INF response. The development and clinical application of HCV genotyping assays are the object of much research.

Serotyping
Determination of hepatitis C virus (HCV) genotype could be routinely run in the future to tailor treatment schedules for patients with chronic hepatitis C. The suitabilities of two versions of a serological, so-called serotyping assay (Murex HCV Serotyping Assay version 1-3 [SA1-3] and Murex HCV Serotyping Assay version 1-6 [SA1-6]; Murex Diagnostics Ltd.), based on the detection of genotype-specific antibodies directed to epitopes encoded by the NS4 region of the genome, for the routine determination of HCV genotypes were studied by Pawlotsky. [31] The NS4, E1 and a small variable region in the core yield type-specific antigenic determinants. Type-specific B-cell epitopes have also been reported in the NS4A and NS4B regions, thus, single or branched peptides obtained from the NS4A and NS4B regions can be used for serotyping. For serological determination of HCV genotype in the study, SA1-3 and SA1-6 were compared to reverse hybridization line probe assay (LiPA). The results showed that SA1-6 had increased sensitivity relative to SA1-3 but remained less sensitive than the genotyping assay on the basis of PCR amplification of HCV RNA. Cross-reactivities between different HCV genotypes could be responsible for the mistyping of 8 percent (SA1-3) and 6 percent (SA1-6) of the samples. Subtyping of 1a and 1b is still not possible with the existing peptides, but discriminating between subtypes may not be necessary for routine use.

Liver biopsy
Grading and staging liver biopsy lesions is important in HCV, particularly for patients with necroinflammation, septal fibrosis and regions of modularity on initial biopsy who are at high risk for developing advanced necrosis in the ensuing decade. [32] Liver biopsy, when combined with periodic serum alanine aminotransferase (ALT) measurements, can be useful in determining the severity or activity of liver disease and the stage or degree of fibrosis. Rate of progression to cirrhosis has been seen to accelerate in patients whose initial biopsies showed high-grade and high-stage lesions. Liver biopsy is recommended prior to treatment to obtain a baseline disease stage and to exclude other forms of liver disease, such as concurrent alcoholic liver disease and iron overload. However, liver biopsy is costly and carries its own risk. Thus, monitoring patients who are being treated should involve serial ALT measurements and qualitative HCV RNA testing.

RT-PCR has been used to obtain molecular evidence for intrahepatic HCV replication occurring shortly after liver transplantation. The level of replication does not correlate with the development of recurrent hepatitis, which suggests that HCV can replicate without inducing morphological evidence of liver damage as seen by biopsy. [33] RT-PCR can also be used to clarify the relationship between anti-HCV to HCV infection of the liver by detecting HCV sequences in liver tissue. These findings were
found feasible in the study reported by Shieh, et al. [34] However, repeatedly negative RT-PCR tests for HCV RNA in serum does not indicate the absence of HCV from the liver. [35] The majority of long-term responders who have been on INF therapy do test negative for HCV RNA in the liver, suggesting definite eradication of HCV RNA infection. [36] RT-PCR with unlabeled primers followed by in situ hybridization (RT-PCR-ISH) and in situ RT-PCR with FITC-labeled probes (RT-PCRd) showed HCV signal in all liver biopsies of patient who had been treated with INF one year after treatment stopped. [37]

The TORDJI-22 MoAb (BioGenex, San Ramon, Calif) is specific for the C-100 protein of the hepatitis C virus, and was compared with RT-PCR of tissue for viral RNA. Immunohistochemistry with the TORDJI-22 monoclonal antibody was found to be a very specific, fairly sensitive diagnostic test for hepatitis C virus in fixed liver tissues. [38] The identification of hepatitis C virus antigen (HCVAg) in liver tissue indicates that the presence of viral antigens in hepatocytes of patients with transplants and recurrent HCV infection is a consistent finding one month or longer after transplantation. However, the relationship between the antigen and the development of pathologic changes remains to be investigated. [39]

Non-invasive markers of fibrosis or cirrhosis
The synthesis and deposition of hyaluronic acid (HA) increases during fibrogenesis. Serum HA is a useful marker in chronic CV patients in that it can be used to monitor patients at risk of progressive fibrosis, in controlled clinical trials, as a measure of antifibrogenic response and in those in whom liver biopsy is difficult or contraindicated. [40] Cirrhosis can be correctly diagnosed in over 90% of patients with chronic liver disease by observing serum HA concentration. [41] Serum procollagen-III peptide has been shown to be valuable in predicting the development of chronic active fibrogenic liver disease. In chronic viral C hepatitis, the levels of HCV-RNA correlate directly with the severity of hepatic histology and inversely with response to INF therapy. Serum procollagen-III peptide provides a relatively noninvasive means of following disease progression. [42] Laminin is an extracellular matrix component that, when measured in the serum, correlates with severe complications of liver cirrhosis. Patients who present with elevated serum laminin have a high risk of developing severe complications. Both HA and laminin can be used as prognostic markers in addition to the Child criteria in liver cirrhosis. [43]

Cryoglobulin
Among the many conditions that are spawned by HCV infection, mixed cryoglobulinemia is by far the most closely linked to HCV infection. A high frequency of mixed cryoglobulinemia (types II and III) is seen in patients with chronic HCV infection. In HCV-positive patients, stage of liver disease correlate with the prevalence of cryoglobulinaemia. Patients with type II cryoglobulins showed a significantly higher risk of cirrhosis and of extrahepatic manifestations while patients with type III cryoglobulins had a significantly higher prevalence of hepatocellular carcinoma. In one study by Donada et al [44], type II cryoglobulin patients had an odds ratio of 11.9 of death from extrahepatic complications during follow-up while type III patients had an odds ratio of 3.4 of dying from hepatic disease.

Conclusions
Tests used in the diagnosis and management of HCV infection each have their time and place as Table 3 and the algorithm in Figure 2 illustrate. ELISAs have many advantages in the diagnostic setting including ease of automation, ease of use, relative cost-effectiveness low variability and high sensitivity in screening before liver transplantation. But the false-positives that they can produce can be cleared through the use of RIBAs. INNO-LIAs can determine whether a patient will respond to INF
therapy. Taken one step further, measurement of HCV RNA by reverse transcription or PCR can actually plot virological response to INF. Detecting and quantifying quasispecies can account for why a particular patient is unable to clear an HCV infection or has become INF-resistant as well as explain why an isolate-specific vaccine is not effective. The genetic, geographical and etiological variations in the hepatitis C virus are observable via genotyping, allowing for more exact diagnoses. Genotyping also has important implications in the design of hepatitis-related vaccines and biotherapeutic agents. Liver biopsy can be useful in determining the severity or activity of liver disease and the stage or degree of fibrosis. Non-invasive markers, such as hyaluronic acid and serum procollagen-III peptide can deliver histological information on patients for whom biopsy is not tolerable or is contraindicated.

References


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