



Improvement and New Aspects of HCV Testing for Clinical Management Kliniğin Yönetiminde Hepatit C Testlerinde Gelişmeler ve Yeni Yaklaşımlar

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ABSTRACT

Recent increase in approvals of orally delivered pharmaceuticals for the treatment of patients with infection by Hepatitis C virus and of a point-of-care assay for community screening of HCV infection have generated impetus to widen the identification of HCV-infected individuals. Diagnosis of HCV infection is, however, still based on the detection of anti-HCV antibody, or core antigen test and nucleic acid testing as supplementary test. As treatment is intended for individuals with current infection, testing for evidence of infection would need to centre on the detection of HCV viremia. Minimizing the complexity and costs associated with HCV RNA testing and speeding the development and validation of HCV Core antigen assays expedite the identification of HCV-viremic individuals. Validation and cost-effectiveness studies are ongoing to inform the best practices for detecting HCV viremia and for distinguishing between resolved HCV infection and false positivity for HCV antibody in persons in whom HCV RNA is not detected. Diagnosing recent HCV infection and engagement in surveillance of drug-resistant HCV are two important points in management of HCV infection. Successful implementation of these measures brightens prospects for the eventual elimination of HCV. (*Viral Hepatitis Journal* 2013; 19(3): 93-8)

Key words: Hepatitis C virus, HCV RNA, HCV core antigen, new method, diagnostic algorithm

ÖZET

Son zamanlarda Hepatit C enfeksiyonu olan hastaların tedavisi için oral yolla verilen onaylı ilaçların artması ve bu enfeksiyon için hasta başı testlerin toplum taramasında kullanılması, Hepatit C enfekte bireylerin tanı konmasında genişletici bir etki yaratmıştır. Buna rağmen HCV enfeksiyonunun yaygın tanısı, anti-HCV antikor veya çekirdek (kor) antijen testine ve teyit edici test olarak nükleik asit saptanmasına dayanmaktadır. Tedavi mevcut enfeksiyonu olan hastaları hedeflemekte iken, enfeksiyonun kanıtı için testler, HCV viremisini tespiti odaklanmaktadır. HCV RNA nükleik asit testi ile ilgili karmaşıklığı ve maliyetleri en aza indirmek ve HCV kor antijen testlerinin geliştirilmesi ve validasyonunun gerçekleşmesi, HCV-viremis olan bireylerin tanımlanmasını hızlandıracaktır. Validasyon ve maliyet-etkinlik çalışmaları, HCV viremisini en iyi şekilde tespit etmek için ve HCV RNA tespit edilmeyen kişilerdeki yalancı antikor pozitifliği ile geçirilmiş HCV arasındaki ayrımı yapmak amacıyla sürekli yürütülen uygulamalardır. HCV enfeksiyonu yönetimindeki önemli nokta; yeni enfeksiyonu tanımlamak ve ilaca dirençli HCV sürveyansını sürdürmektir. Bu iki noktanın çözümlenmesi sürecindeki olumlu gelişmeler, HCV'nin ortadan kaldırılması için umutları güçlendirecektir. (*Viral Hepatit Dergisi* 2013; 19(3): 93-8)

Anahtar Kelimeler: Hepatit C virus, HCV RNA, HCV kor antijen, yeni metod, tanı algoritması

Introduction

Hepatitis C virus (HCV) belongs to the Flaviviridae family, Hepacivirus genus. This virus has a 9600-nucleotide genome, encoding structural and nonstructural (NS) proteins. The structural proteins include the core protein and the envelope glycoproteins, which form the viral particle; and the NS proteins include the p7, NS2, NS3, NS4, and NS5 proteins, which are essential for the viral replication (1). The phylogenetic analysis of the whole genome or the representative subgenomic regions revealed that HCV is divided into six major genotypes and many subtypes (2,3). It is estimated that 2.35% of the world population, are chronically infected with HCV, i.e. approximately 160 million people worldwide (4). The persistence of HCV infection is a major cause of liver cirrhosis and hepatocellular carcinoma. HCV infection is often asymptomatic, thus diagnosis relies heavily on clinical laboratory assays, including the serological immunoassays for antibodies to HCV (anti-HCV) or core antigen, and nucleic acid testing (NAT) for HCV RNA (5,6). Diagnostic procedures used in laboratories to confirm HCV infection are principally based on the detection of antibodies against recombinant HCV polypeptides (7).

Centers for Disease Control and Prevention (CDC) has recommendation of HCV testing for persons with risks for HCV infection (8-13). In 2003, CDC published guidelines for the laboratory testing and result reporting of antibody to HCV (11). In 2012, CDC revised testing recommendations to include one-time HCV testing for all persons born during 1945-1965 regardless of other risk factors (8).

Changes in HCV Testing Technologies

Since 2003, there have been developments with important implications for HCV testing:

1. Rapid test for HCV antibody: The OraQuick HCV Rapid Antibody Test (OraSure Technologies) is a rapid assay for the presumptive detection of HCV antibody in finger-stick capillary blood and venipuncture from whole blood. Its sensitivity and specificity are similar to those of FDA-approved, laboratory-conducted HCV antibody assays (13,14).

2. HCV salivary tests: Point-of-care (POC) HCV salivary tests can be used in the field, in emergency departments, in medical and dental clinics and, eventually, at home. Clinicians can use a number of oral samples to diagnose viruses, including whole saliva, gingival crevicular fluid, oral swabs of mucosal tissue, dental plaque, oral biopsy specimens. Saliva, consists primarily of secretory IgA (sIgA), whereas oral mucosal transudate contains a mixture of sIgA, IgG and IgM. OraSure Technologies markets an FDA approved test for HCV that uses finger-stick blood; in addition, the company has a salivary test that is used widely in Europe (15).

3. Detection and Quantification of HCV: Levels of Hepatitis C core antigen correlate with levels of HCV RNA in patients with chronic HCV infection. Assays that quantify HCV core antigen can therefore be used as alternatives to those that measure levels of HCV RNA, as shown in various populations of patients. It is less expensive and easier to use than current HCV RNA tests for diagnosis of chronic hepatitis C and monitoring of antiviral therapy. However, the HCV Ag assay's lower limit of detection corresponds to HCV RNA levels of 500-3000 IU/mL, depending on the HCV

genotype. Assays for HCV core antigen are therefore not suitable for response-guided therapy, according to current guidelines (16).

4. Discontinuation of RIBA HCV. The Chiron RIBA HCV 3.0 Strip Immunoblot Assay that was recommended for supplemental testing of blood samples after initial HCV antibody testing is no longer available (11-13). As a result, the only other FDA-approved supplemental tests for HCV infection are those that detect HCV viremia. There are some new products at the market for screening and confirmatory test for HCV. MP Diagnostics MultiSure HCV is a multi-parameter qualitative immunochromatographic assay for the in-vitro detection of Ab to HCV in human whole blood, plasma or serum (17).

5. Predictive markers for HCV:

IL28B: Recent genome-wide association studies have identified genetic variations near the IL28B gene which are strongly associated with spontaneous and treatment-induced clearance of HCV infection. Protective IL28B variations are strongly associated with on-treatment viral kinetics and approximately 2-fold increased sustained virologic response (SVR) rates in HCV genotype 1 and 4 patients. In HCV genotype 1 patients, IL28B variations were shown to be the strongest pre-treatment predictor of virologic response. In the treatment of HCV genotype 2 and 3 infected patients, IL28B variations play only a minor role. Preliminary data indicate that IL28B variations are also associated with treatment outcome of regimens, including directly acting antiviral (DAA) agents, though their impact seems to be attenuated compared to standard treatment (18,19).

IP 10: A series of reports have demonstrated that chemokine Interferon- γ inducible protein 10 (IP-10, CXCL10) is a promising single marker correlate for liver fibrosis, and an IL-28b independent negative predictor of treatment outcome in HCV infected patients. Ruhwald et al. established that IP-10 levels in plasma can be detected after the plasma has been dried on filter paper, but more studies are needed to substantiate IP-10 as a valid biomarker for liver fibrosis (20).

Identifying Current HCV Infections

In 2011, FDA approved boceprevir and telaprevir for treatment of chronic hepatitis C genotype 1 infection, in combination with pegylated interferon and ribavirin, in adult patients with compensated liver disease. Boceprevir and telaprevir inhibit HCV replication. Persons who complete treatment using either of these drugs combined with pegylated interferon and ribavirin are more likely to clear virus, compared to those given standard therapy based on pegylated interferon and ribavirin (13,15). Because antiviral treatment is only useful for persons with current HCV infection, these persons need to be distinguished from other persons. HCV RNA in blood can be detected by nucleic acid testing (NAT) only in persons who are currently infected. This is a marker for HCV viremia so persons with reactive results after HCV antibody testing should be evaluated for the presence of HCV RNA in their blood (13).

Benefits of Testing for Current HCV Infection

Accurate testing to identify current infection is important because:

1) To help clinicians and other providers correctly identify persons infected with HCV, as a result that preventive services, care and treatment can be provided.

2) To notify tested persons of their infection status, enabling them to make informed decisions about medical care and options for HCV treatment, take measures to limit HCV-associated disease progression (e.g., avoidance of alcohol intake, and vaccination against hepatitis A and B), and minimize risk for transmitting HCV to others.

3) To inform persons who are not currently infected of their status and the fact that they are not infectious.

Recommended Testing Sequence of CDC

The testing sequence in CDC guidance is intended for use by primary care and public health providers seeking to implement CDC recommendations for HCV testing. In most cases, persons identified with HCV viremia have chronic HCV infection. This testing sequence is not intended for diagnosis of acute hepatitis C or clinical evaluation of persons receiving specialist medical care, for which specific guidance is available (13,18).

Testing for HCV infection begins with either a rapid or a laboratory-conducted assay for HCV antibody in blood. A nonreactive HCV antibody result indicates no HCV antibody detected. A reactive result indicates one of the following: 1) current HCV infection, 2) past HCV infection that has resolved, or 3) false positivity (13) (Table 1). A reactive result should be followed by NAT for HCV RNA. If HCV RNA is detected, that indicates current HCV infection. If HCV RNA is not detected, that indicates either past, resolved HCV infection, or false HCV antibody positivity (8-13).

Initial Testing for HCV Antibody: An FDA-approved test for HCV antibody should be used. If the OraQuick HCV Rapid Antibody test is used, the outcome is reported as reactive or nonreactive. If a laboratory-based assay is used, the outcome is reported as reactive or nonreactive without specifying cutoff ratios.

Testing for HCV RNA. An FDA-approved NAT assay intended for detection of HCV RNA in serum or plasma of at-risk patients who test reactive for HCV antibody should be used. There are several possible operational steps toward NAT after initial testing for HCV antibody (20,21).

1. If the blood sample collected is reactive for HCV antibody during initial testing, blood from a subsequent venipuncture is submitted for HCV NAT.

2. If the HCV antibody test is reactive, two specimens are collected in separate tubes at the same time: one tube for initial HCV antibody testing; and a second tube for HCV NAT.

3. The same sample of venipuncture blood used for initial HCV antibody testing, if reactive, is directed to HCV NAT without another blood draw for NAT.

4. A separate venipuncture blood sample is submitted for HCV NAT if the OraQuick HCV Rapid Antibody Test for initial testing of HCV antibody has used finger-stick blood.

Supplemental Testing for HCV Antibody

If testing is desired to distinguish between true positivity and biologic false positivity for HCV antibody, testing may be done with a second different HCV antibody assay approved by FDA for diagnosis of HCV infection. HCV antibody assays vary according to their antigens, test platforms, and performance characteristics, so biologic false positivity is unlikely to be exhibited by more than one test when multiple tests are used on a single specimen (13,23,24). Interpretation of results of tests for HCV infection is given in Table 1 (13).

Clinical Use of Virologic Tools for HCV

Diagnosis and decision to treat: Patients are diagnosed with chronic HCV infection based on the detection of antibodies to HCV by enzyme immunoassays and HCV RNA by a sensitive molecular biology-based technique that has a detection limit of about 10 to 15 IU/mL. All treatment-naïve patients with compensated liver disease and detectable HCV RNA should be considered for therapy. Assessment of liver disease severity is important for decision making. Patients infected with HCV genotype 1, in whom pegylated IFN- α and ribavirin combination therapy failed to eradicate the virus, should be considered for retreatment because they might benefit from the triple combination of pegylated IFN- α , ribavirin, and a protease inhibitor (25,26).

Table 1. Interpretation of results of tests for hepatitis C virus (HCV) infection and further actions (13)

Test outcome	Interpretation	Further action
HCV antibody nonreactive	No HCV antibody detected	*Sample can be reported as nonreactive for HCV antibody. No further action required. If recent HCV exposure in person tested is suspected, test for HCV RNA.
HCV antibody reactive	Presumptive HCV infection	A repeatedly reactive result is consistent with current HCV infection, or past HCV infection that has resolved, or biologic false positivity for HCV antibody. Test for HCV RNA to identify current infection.
HCV antibody reactive, HCV RNA detected	Current HCV infection	[†] Provide person tested with appropriate counseling and link person tested to medical care and treatment.
HCV antibody reactive, HCV RNA not detected	No current HCV infection	No further action required in most cases. If distinction between true positivity and biologic false positivity for HCV antibody is desired, and if sample is repeatedly reactive in the initial test, test with another HCV antibody assay. In certain situations [§] follow up with HCV RNA testing and appropriate counseling.

* If HCV RNA testing is not feasible and person tested is not immunocompromised, do follow-up testing for HCV antibody to demonstrate seroconversion. If the person tested is immunocompromised, consider testing for HCV RNA. [†]It is recommended before initiating antiviral therapy to retest for HCV RNA in a subsequent blood sample to confirm HCV RNA positivity. [§] If the person tested is suspected of having HCV exposure within the past 6 months, or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Table 2. Commercially Available Real-Time Target Amplification Assays for HBV DNA and HCV RNA Detection and Quantification (33)							
Assay	Manufacturer	Method	Automated extraction Device	Amplification Device	Volume required (μ L)	LLOD (IU/mL)	Dynamic range of quantification (IU/mL)
HCV RNA							
COBAS TaqMan HCV Test, v2.0	Roche Molecular Pleasanton, CA	Real-time PCR	Manual with high pure system viral nucleic acid kit	COBAS TagMan	650	20 IU/mL	25 (1.4 log ₁₀)-3.0 X 10 ⁸ to 3.9 X 10 ⁸ (8.5-8.6 log ₁₀) IU/mL
COBAS Ampliprep-COBAS TaqMan (CAP/CTM) HCV Test, v2.0	Roche Molecular Pleasanton, CA	Real-time PCR	COBAS Ampliprep	COBAS TagMan	650	15 IU/mL	15 (1.2 log ₁₀)-1.0 X 10 ⁸ (8.0 log ₁₀) IU/mL
Real Time HCV	Abbott Molecular, Des	Real-time PCR	m2000SP	m2000RT	200 or 500	12 IU/mL (for 500 μ L) 30 IU/mL (for 200 μ L)	12 (1.1 log ₁₀) 1.0 10 ⁸ (8.0 log ₁₀) IU/mL
Artus HCV QS-RGQ Assay ^a	Qiagen, Hilden, Germany	Real-time PCR	QIAasymphony RGQ	Rotor-Gene Q	1000	36.2 IU/mL	67.6 (1.8 log ₁₀) 17.7 10 ⁸ (7.2 log ₁₀) IU/mL
VERSANT HCV RNA 1.0 Assay (kPCR)	Siemens Medical Solutions Diagnostics, Tarrytown, NY	Real-time PCR	Sample preparation (SP) module	Amplification and Detection (AD) Module	500	15 IU/mL	15 (1.2 log ₁₀) 1.0 10 ⁸ (8.0 log ₁₀) IU/mL
Fluorion HCV QNP 3.0	Iontek, Turkey	Real-time PCR	Hamilton Nimbus	Fluorion Detection System - BioRad CFX96	600	32 IU/ml	32 (1.49 log ₁₀)-1.0 X 10 ⁹ (8.5 log ₁₀) IU/mL
RTA Voltran VLDS HCV Real Time PCR	RTA Laboratories, Turkey	Real-time PCR STAR ^{LET}	Hamilton MICROLAB	Bio-Rad CFX96	200	15 IU/ml	14 (1.15 log ₁₀)-1.0 x 10 ⁹ (9.0 log ₁₀) IU/ml

LLOD, lower limit of detection.
^aAvailable only for EDTA-plasma specimens.

Selecting therapy: The HCV genotype should be determined before treatment is started. Patients infected with HCV genotypes other than type 1 should be treated with pegylated IFN- α and ribavirin alone. For patients infected with genotype 2 or 3, the dose of ribavirin is 0.8 g/day, and the treatment duration is 24 weeks; patients with genotypes 2 and 3 with baseline factors that indicate low responsiveness should receive weight-based ribavirin. For patients infected with genotypes 5 and 6, the dose of ribavirin is based on body weight, and treatment should last up to 72 weeks, depending on the virologic response during treatment (27).

Patients infected with HCV genotype 1 who qualify for therapy should receive the triple combination of pegylated IFN- α , ribavirin, and either telaprevir or boceprevir. The subtype (1a or 1b) does not need to be determined for patients who receive the combination of pegylated IFN- α and ribavirin, or triple drug combinations that include a protease inhibitor, because genotype 1 subtypes do not affect treatment decisions with these therapeutic strategies. Further trials are needed to improve the selection of patients infected with HCV genotype 1 who might not need

protease inhibitors, such as young patients with mild fibrosis, a low baseline level of HCV RNA, or the CC IL28B genotype (28).

Monitoring treatment: The end point for HCV therapy is an sustained virologic response (SVR), characterized by undetectable HCV RNA (<10-15 IU/mL) 24 weeks after the end of treatment: this corresponds with viral eradication in more than 99% of cases. Monitoring of HCV RNA levels during treatment is key in determining virologic response, in guiding duration of treatment, and in deciding futility of treatment. This is particularly important when direct-acting antivirals (DAAs) are used, as reviewed by Barritt and Fried (29). Failure of triple combination therapy to clear HCV is associated with outgrowth of variant populations that are resistant to the protease inhibitor used. However, resistance testing based on HCV sequence analysis of the protease region has no utility in clinical practice (30). Resistant variants are present in almost all infected patients before therapy begins, but technologies available in clinical virology laboratories are not sensitive enough to detect them; negative results are therefore uninterpretable. In addition, the profile of HCV variants at the start of therapy does not appear to affect the outcome of

treatment when the protease inhibitor is combined with pegylated IFN- α and ribavirin. At the time of treatment failure (virologic breakthrough or relapse), the resistant population is always enriched, and phase 2 and 3 trials have reported that it is dominant in 50% to 70% of patients (31,32). Resistance testing is still not indicated at this time because the results will have no impact on subsequent treatment decisions (30).

Clinical research: In cohort studies, surveillance studies, and clinical trials of HCV DAA or host-targeting agents, given alone or in combination, serial HCV RNA testing is needed to characterize the virologic response and detect virologic breakthrough and relapse (Table 2). Resistance variants should always be analyzed in patients who fail to respond to therapy to characterize the nature and dynamics of the selected viral populations and identify amino acid substitutions selected by the DAAs. When several antiviral drugs with different target sites and mechanisms of action are combined, all the target sites should be studied. Sensitive sequencing methods such as next-generation sequencing (NGS) are particularly useful for characterizing the dynamics of sensitive and resistant virus populations over time (33,34).

Conclusion

In conclusion, new virological methods that detect and quantify HCV RNA or HCV core antigen are now available. Real time target amplification (PCR or TMA) methods are well standardized and widely used in clinical practice to diagnose and monitor HCV infection. POC tests offer substantial benefits for the management of hepatitis C infection, mainly by shortening the time of results and/or by making the test available at the bedside or in remote care centers. New matrix specimens, such as oral fluids and finger-stick capillary whole blood represent promising alternatives to venous puncture. However, further prospective studies are needed to establish diagnostic and monitoring algorithms, as well as to guide appropriate interventions such as treatment or referral.

Conflict of interest: None declared.

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