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Diagnosis of patients with suspected chronic hepatitis C infection

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ABSTRACT

The current optimal approach to detecting hepatitis C virus (HCV) infection involves screening people for risk factors and only testing selected individuals at risk. Blood transfusion from infectious donors, unsafe therapeutic injection practices, and illegal intravenous drug use have been the predominant modes of transmissiom of HCV infection. Virological markers that are currently used for the clinical management of patients with hepatitis C include serologic assays (ELISA or immunoblot assays), which detect specific antibodies (IgG) to HCV, and virological assays, which detect serum HCV RNA, by highly sensitive qualitative and quantitative techniques. The applicability of these tests is for the diagnoses and monitoring of the treatment but they have no role in the assessment of disease severity or prognosis. Patients diagnosed with HCV infection must be educated in order to avoid the spread of the disease to other people.

Key words. Hepatitis C. Diagnosis. Risk factors. Serologic tests.

INTRODUCTION

There is an important population of patients who are currently infected with hepatitis C virus (HCV) but who are undiagnosed and asymptomatic. The optimal approach to detecting HCV infection involves screening for risk factors of exposure to the virus and only testing selected individuals at a risk.

Throughout the world, blood transfusions from infectious donors, unsafe therapeutic injection practices, and illegal intravenous drug use have been the predominant modes of transmission of HCV infection. In most developed countries, blood donor testing has virtually eliminated transfusion-related transmission of HCV, and nosocomial and iatrogenic transmission is uncommon. In these countries, illegal intravenous drug use has been identified as the major risk factor during the past 40 years and the highest prevalence of infection is found in young to middle-aged adults. In many developing countries, unsafe therapeutic injection practices appears to be responsible for the geographic clustering of high rates of infection because of inadequate or nonexistent supplies of sterile syringes, administration of injections by nonprofessionals outside the medical setting, and administration of medications by injection. In some countries, the risk from unsafe therapeutic injections has decreased, as indicated by high prevalence rates of HCV infection only in older people. In other countries, this transmission mode continues to be a major source of HCV infection, with high prevalence rates of HCV infection found among people in all age groups.

Characterizing the epidemiology of hepatitis C in individual countries is crucial to developing and implementing effective preventive measures. For some countries, ensuring safe blood supplies and healthcare-related procedures is the highest priority. For others, the priorities are the prevention of illegal injection drug use, harm reduction counseling, and testing to identify HCV-infected people for medical evaluation and management.[1, 2]

Because symptoms are generally absent in individuals with chronic HCV infection, recognition of infection requires risk factor screening, which should be performed, when indicated, in combination with appropriate HCV testing and counseling.[3] Table 1

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Table 1. People for whom HCV screening is recommended.

- People who have injected illicit drugs in the recent or remote past, including those who have injected only once and do not consider themselves to be drug users.
- People with conditions associated with a high prevalence of HCV infection including those:
  - With HIV infection.
  - With hemophilia who received clotting factor concentrates prior to 1987.
  - Who have ever received hemodialysis.
  - With unexplained abnormal liver function tests.
- Recipients of transfusions or organ transplants prior to July 1992 including persons who:
  - Were notified that they had received blood from a donor who later tested positive for HCV infection.
  - Received a transfusion of blood or blood products.
  - Received an organ transplant.
- Children born to HCV-infected mothers.
- Healthcare, emergency medical, and public safety workers after a needle stick injury or mucosal exposure to HCV-positive blood.
- People who are or have been in prison.
- Current sexual partners of HCV-infected people.

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lists people who should be routinely screened for HCV infection.[4]

DIAGNOSIS OF CHRONIC HEPATITIS C

Laboratory testing

The repertoire of virological markers currently used for the clinical management of patients with hepatitis C includes serologic assays (ELISA or immunoblot assays), which detect specific antibodies (IgG) to HCV, and virological assays, which detect serum, HCV RNA by highly sensitive qualitative and quantitative techniques. These assays play no role in the assessment of disease severity or prognosis.

The use of serologic and virological tests has become essential in the management of HCV infection in order to:

- Diagnose infection.
- Assess the virological response to therapy and guide treatment decisions.

Serologic assays

Tests that detect anti-HCV are used both to screen for and to diagnose HCV infection. Such tests detect antibodies directed against various HCV epitopes in plasma or in serum. Three enzyme immunoassays (EIAs) that have been approved by the US Food and Drug Administration (FDA) for clinical use are the Abbott HCV EIA 2.0 (Abbott Laboratories, Abbott Park, IL), ORTHO HCV Version 3.0 ELISA (Ortho-Clinical Diagnostics, Raritan, NJ), and the enhanced chemiluminescence immunoassay (CIA) VITROS Anti-HCV assay (Ortho-Clinical Diagnostics, Raritan, NJ). The specificity of current EIAs for anti-HCV is greater than 99%;[5] however, sensitivity is more difficult to evaluate.

These tests can occasionally provide false positive results, which is why confirmation using virological assays to determine HCV RNA may be necessary. Undertaking confirmation tests is essential in patients with normal alanino aminotransferase (ALT) levels and in patients with elevated levels of gamma globulin. Outside these circumstances, as for the great majority of patients who are anti-HCV positive with elevated ALT levels, it is not essential to carry out confirmation tests.[6,7]

The recombinant immunoblot assay Chiron RIBA HCV 3.0 SIA is also FDA approved. However, the widespread availability of nucleic acid testing has rendered RIBA testing for HCV clinically obsolete.[8,9]

Virological assays

Historically, the qualitative assays have been more sensitive. With the recent availability of real-time polymerase chain reaction (PCR)-based assays and transcription-mediated amplification (TMA) assays, with a sensitivity of 10-50 IU/mL, qualitative assays are being replaced in most settings by highly sensitive quantitative real-time PCR assays.[10,11]

Another virological assay is the HCV core anti-

gen EIA assay, which can detect total HCV core antigen in serum. The HCV core antigen levels are expressed in pg/mL and closely correlate with HCV RNA levels. One picogram of HCV core antigen is equivalent to approximately 8,000 IU of HCV RNA. Although the HCV core antigen assay can be used as a surrogate marker of HCV replication to monitor the early virological response during antiviral therapy, its clinical use is limited by the low sensitivity as the current version of the assay does not detect HCV core antigen below an HCV RNA level of 20,000 IU/mL.[12]

All currently available assays have excellent specificity, in the range of 98 to 99%. Since the IUs have been refined and standardized across laboratories throughout the world, they are now the preferred unit for reporting test results, rather than viral copy number.[13,14]

These assays are mainly used in the early monitoring of the virological response to antiviral therapy and in the prediction of treatment outcome. For monitoring purposes, it is important to use the same laboratory test before and during therapy.

The commercial assays available for the detection (qualitative assays) or quantification (quantitative assays) of HCV RNA are listed in Tables 2 and 3.

Diagnosis of chronic HCV infection and interpretation of assays

In patients with clinical or biological signs of chronic liver disease, screening for chronic HCV infection generally requires testing for antibodies against both HCV (anti-HCV) and HCV RNA. A sensitive quantitative HCV RNA assay is recommended for diagnosis because it also provides information on the viremia. Although here we recommend the use of quantitative assays, their use in Latin American countries will depend on the availability of tests.

The differentiation between acute and chronic HCV infection depends on the clinical presentation, namely the presence of symptoms, such as jaundice,

Table 2. FDA-approved qualitative assays for detection of HCV RNA.

Assay and manufacturer	Method of detection	Lower limit IU/mL	Setting
Amplicor HCV v2.0 (Roche Molecular Systems)	Manual RT-PCR	50	Diagnosis and monitoring
Cobas Amplicor HCV v2.0 (Roche Molecular Systems)	Semiautomated RT-PCR	50	Diagnosis and monitoring
Ampliscreen (Roche Molecular Systems)	Semiautomated RT-PCR	< 50	Blood screening
Versant HCV RNA Qualitative Assay (Siemens)	Semiautomated TMA	10	Diagnosis and monitoring
Procleix HIV-1/HCV Assay (Chiron Corporation)	Manual TMA	< 50	Blood screening

RT-PCR: Reverse transcription polymerase chain reaction. TMA: Transcription-mediated amplification. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, Management and treatment of Hepatitis C: an update. Hepatology 2009;49(4):1335–1374.

Table 3. Available assays for quantification of HCV in serum/plasma.

Assay	Method	IU/mL Conversion factor	Dynamic range (IU/mL)	FDA approved
• Amplicor HCV Monitor (Roche Molecular Systems)	Manual RT-PCR	0.9 copies/mL	600-500,000	Yes
• Cobas Amplicor HCV Monitor V2.0 (Roche Molecular Systems)	Semiautomated RT-PCR	2.7 copies/mL	600-500,000	Yes
• Versant HCV RNA 3.0 Assay (bDNA) (Siemens Diagnostics)	Semiautomated bDNA	5.2 copies/mL	615-7,700,000	Yes
• LCx HCV RNA-Quantitative Assay (Abbott Diagnostics)	Semiautomated RT-PCR	3.8 copies/mL	25-2,630,000	No
• SuperQuant (National Genetics Institute)	Semiautomated RT-PCR	3.4 copies/mL	30-1,470,000	No
• Cobas Taqman HCV Test (Roche Molecular Systems)	Semiautomated real-time PCR		43-69,000,000	Yes
• Abbott RealTime (Abbott Diagnostics)	Semiautomated RT-PCR		12-100,000,000	No

RT-PCR: Reverse transcription polymerase chain reaction. TMA: Transcription-mediated amplification. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, Management and treatment of Hepatitis C: an update. Hepatology 2009;49(4):1335–1374.

Table 4. Interpretation of HCV assays: anti-HCV *vs* HCV RNA.

Interpretation
Positive-Positive: acute or chronic HCV depending on the clinical context.
Positive-Negative: resolution of HCV; acute HCV during period of low-level viremia. False Anti-HCV positive.
Negative-Positive: early acute HCV infection; chronic HCV in immunosuppressed patients; false positive HCV RNA test.
Negative-Negative: absence of HCV infection.

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and whether or not there is a prior history of prolonged elevated ALT levels. After acute exposure, detection of HCV RNA usually precedes the detection of antibody reactivity in serum; HCV RNA can be identified as early as two weeks following exposure whereas anti-HCV antibodies are generally not detected before 8-12 weeks. The positive response may vary between these two markers of HCV infection, requiring careful analysis for interpretation (Table 4).

It is possible for both anti-HCV and HCV RNA to be positively identified in a patient with a recent elevation of ALT levels. This scenario is consistent with either acute HCV infection when there is a recent risk of exposure, with an exacerbation of a chronic HCV infection, or with an acute hepatitis of other etiology in a patient with chronic HCV infection. Another possibility is the detection of anti-HCV but not HCV RNA. This scenario most commonly represent a recovery from HCV infection. Less likely it may represent an acute HCV infection during a period of transient clearance of HCV RNA, an anti-HCV false positive or an HCV RNA false negative result. The reverse scenario, a negative anti-HCV with a positive HCV RNA, can be seen in the early stage of an acute infection prior to the development of antibodies and in cases of chronic infection in immunosuppressed patients. Alternatively, it may represent a false positive the HCV RNA result. For all cases, retesting for anti-HCV and HCV RNA 4-6 months later should resolve the issue. Finally, if the patient has elevated ALT levels but the tests for anti-HCV and HCV RNA are negative, both acute and chronic hepatitis C may be excluded and another diagnosis should be considered.

Approximately 30% of the patients with chronic HCV infection have normal ALT levels at diagnosis, regardless of the age and gender, and many of them maintain normal liver function tests during prolonged follow-up. Reactivation of liver disease with ALT flares may be seen in a significant number of these cases over time. Some of these HCV patients with normal ALT levels have significant fibrosis or cirrhosis, as shown by liver biopsy. Virological or biochemical testing cannot predict the presence of significant liver disease or the risk of future ALT flares.[15]

RECOMMENDATIONS

1. Patients suspected of having acute or chronic HCV infection should first be tested for anti-HCV (**Class I, Level B**).
2. HCV RNA testing should be performed in:
   a) Patients who test positive for anti-HCV (**Class I, Level B**).
   b) Patients for whom antiviral treatment is being considered, using a sensitive quantitative assay (**Class I, Level A**).
   c) Patients with unexplained liver disease whose anti-HCV test is negative and who are immunosuppresed or suspected of having acute HCV infection (**Class I, Level B**).

3. HCV genotyping should be performed in all HCV-infected patients prior to interferon-based treatment in order to determine the dose and duration of therapy and to estimate the likelihood of response (**Class I, Level A**).

Indiscriminate screening is not recommended for:
   a) The general population.
   b) Non household contacts of HCV infected patients.
   c) Pregnant women.
   d) Health care workers who are not at significant risk.

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