Virological tools for optimal management of chronic hepatitis C

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Abstract

Serological and virological tests are useful in management of HCV patients, and include anti-HCV antibody assays, measurement of HCV RNA and HCV genotyping. They are used to diagnose infection, initiate treatment and assess the virological response to antiviral therapy. Monitoring of viral kinetics during the early phases of antiviral treatment is crucial in making treatment decisions concerning arrest of treatment and optimization of its duration. A 2-log drop in viral load at week 12 (early virological response) has good negative predictive value when assessing the sustained virological response (SVR), as most patients without a 2-log drop in viral load at week 12 will not attain a SVR. In contrast, undetectable HCV RNA at week 4 (rapid virological response) has good positive predictive value, as patients with undetectable HCV RNA at week 4 have high probability of reaching a SVR. Recent data suggest that some rapid responders can be treated for shorter periods than usually recommended without compromising their chance of a sustained response. On the other hand, slow virological responders infected with genotype 1 should be treated longer to increase the probability of viral eradication. Future studies should focus on identification of the earliest criterion which is both highly sensitive and highly specific in order to predict early SVR and non-response, as well as to avoid useless treatment prolongation. (Acta gastroenterol. belg., **2009**, 72, **421-424**).

Key words : hepatitis C, viral kinetics, antiviral therapy, sustained virological response.

Abbreviations : HCV, hepatitis C virus ; ALT, Alanine aminotransferase ; IFN, interferon ; SVR, sustained virological response ; EVR, early virological response ; RVR, rapid virological response.

Introduction

Current treatment of patients infected with hepatitis C virus (HCV) involves a combination of pegylated interferon (IFN) α-2a or -2b and ribavirin. The primary objective of such treatment is a sustained virological response (SVR) defined by undetectable HCV RNA 24 weeks after the end of treatment, since patients with viral eradication generally do not experience fibrosis progression (1). Moreover, SVR is associated with less HCV-related complications and better survival in cirrhotic patients (2, 3). The use of serological and virological tests has become essential in management of HCV infection. They are helpful in diagnosing infection, guiding treatment decisions and assessing the virological response to antiviral therapy. The present review will describe virological tools useful in HCV patients, and techniques for successfully using them.

Virological tools in chronic hepatitis C

Virological assays useful in management of HCV patients include anti-HCV antibody assays, measurement of HCV RNA and HCV genotyping.

The most common anti-HCV antibody tests used for HCV detection are enzyme-linked immunosorbent assay (ELISA) tests for detecting antibodies directed against various HCV epitopes. Third-generation assays currently in use yield very few false-positive and false-negative results in immunocompetent patients (4).

The HCV genotype can be determined by several methods. The reference method is direct sequencing of NS5B or E1 regions of the viral genome (5). This assay can identify the six HCV genotypes (1 to 6) and subtypes. Mistyping is rare but mis-subtyping may occur in 10% to 25% of cases. In addition, the HCV genotype can be determined by competitive ELISA assay using genotype-specific antigens (6). This assay provides interpretable results in 90% of cases and enables identification of the six HCV genotypes, but not the subtypes.

HCV RNA can be detected and quantified using two types of molecular-biology-based techniques : target amplification ("classic" or "real-time" polymerase chain reaction - PCR - or transcription-mediated amplification) and signal amplification (branched DNA assay). Real-time PCR assays are now widely used by most laboratories. In "real-time" PCR, each round of amplification leads to emission of a fluorescent signal, and the number of signals per cycle is proportional to the amount of HCV RNA in the starting sample (7,8). As a result, real-time PCR techniques have a broad dynamic range of quantification (upper range of quantification : 7–8 log10 IU/mL). Moreover, they are more sensitive than classical PCR (lower limits of detection on the order of 10–15 IU/mL), do not yield false-positive results due to carryover contamination and can be fully automated. Therefore, real-time PCR has become the technique of choice for detecting and quantifying HCV RNA in clinical practice.

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Use of virological tools in clinical practice

Serological and virological tests are useful to : 1/ diagnose HCV infection ; 2/ identify patients who need antiviral therapy ; 3/ monitor the virological response during antiviral therapy ; and 4/ define SVR.

Detection of antibodies to HCV is used to diagnose acute and chronic HCV infection. Anti-HCV antibodies usually appear 2–8 weeks after the acute phase of infection and persist for life. Diagnosis of acute hepatitis C also requires use of an HCV RNA assay with a lower limit of detection of 50 IU/mL or less (9). The onset of HCV RNA precedes that of HCV antibodies by a few days to several weeks. Patients who recover from the infection will become HCV RNA-negative, whereas those who develop chronic infection will remain HCV RNA-positive. The persistence of HCV RNA for more than 6 months defines chronic HCV infection. Patients who are severely immunodepressed, hemodialyzed or with agammaglobulinemia may have chronic infection without detectable anti-HCV antibodies (10,11), although this situation has become rare with use of current third-generation assays.

In the setting of chronic infection, pre-treatment determination of genotype and HCV RNA will affect decisions concerning initiation of treatment, determination of treatment duration, ribavirin dosage and the virological monitoring procedure. It should be noted that the level of HCV RNA does not correlate with the severity of liver disease, nor with risk of complications. Therefore, these tests have no prognostic value in untreated patients.

Only patients with detectable HCV RNA should be considered for pegylated IFN and ribavirin combination therapy. The decision to treat depends on multiple factors, including genotype, which offers prognostic information, as patients infected with genotype 2 or 3 respond more favourably to treatment than those infected with genotype 1. Naive patients infected with genotype 1 have a 40-50% likelihood of reaching SVR with pegylated IFN and ribavirin combination therapy, whereas those infected with genotype 2 or 3 have 80% probability of SVR (12,13). The HCV genotype also determines the length of pegylated IFN and ribavirin combination therapy, as HCV-1, 4, 5 and 6 patients should be treated for 48 weeks, whereas a 24-week course of treatment is sufficient in HCV-2 and 3 patients. SVR is defined as undetectable HCV RNA (with a lower limit of detection of 50 IU/mL or less) 6 months after the end of treatment, and corresponds to a cure of infection in more than 99% of cases (14).

During the past few years, efforts have been made to optimize the chances of SVR. One of the most promising strategies consists of tailoring treatment duration to virological response during therapy. For this purpose, HCV RNA quantification must be performed at baseline and at different time points during treatment using the same technique, in order to ensure comparability of the results at different time points. When envisaging tailoring therapy, patients must be considered individually according to prior treatment and to genotype.

In naive patients infected with genotype 1, the week 12 stopping rule recommends that patients without a 2-log drop in viral load (the so-called “early virological response”, or EVR) should discontinue treatment (15,16). The negative predictive value of this criterion for predicting SVR is almost 100%, indicating that prolongation of treatment in these patients is useless and should therefore be avoided. In contrast, patients with at least a 2-log drop in viral load at week 12 have around 60% probability of SVR (15,16) and should therefore continue treatment. However, it is important to note that not all patients with a 2-log drop in viral load at week 12 will have the same probability of attaining SVR. Patients with a rapid virological response (RVR), defined as undetectable HCV RNA at week 4 (with a lower limit of detection of 50 IU/mL or less), are those who have the best chances of reaching SVR (positive predictive value around 80-90%) (17-23). It has been suggested that, in HCV-1 patients with RVR and low baseline viral load (< 400,000 IU/mL), a 24-week course of pegylated IFN and ribavirin combination therapy might be sufficient without losing the chance to attain a SVR (20-22). However, patients with cirrhosis or other factors which might negatively influence the virological response should not be considered for shortened therapy. On the other hand, HCV-1 patients with a 2-log drop in viral load, but who are still HCV-RNA-positive at week 12 and who become HCV-RNA-undetectable at week 24 for the first time (the co-called “slow virological response”), may be considered for prolonged therapy because their probability of reaching SVR is approximately only 25-30%. Recent studies have shown that extending the treatment to 72 weeks in these patients led to an improvement in SVR rates. Indeed, in 3 recent studies, it was shown that the probability of SVR increased by 15 to 30% with such extended treatment. It went from 16-44% for a classic 48-week treatment schedule to 44-69% for a 72-week course of treatment (17,24-26).

When considering the time point at which a virological response can be assessed to predict SVR during antiviral treatment, the RVR criterion has a clear advantage as it is available 2 months before the EVR criterion. However, RVR, though highly specific, is not sensitive enough as it identifies only 20% of the HCV-1 patients who will attain SVR. Thus, this criterion cannot be used in clinical practice to decide treatment discontinuation. When considering the safety profile of antiviral treatment, it is mandatory to find a criterion that is both highly sensitive and highly specific so as to predict early on a SVR or non-response, as well as to avoid useless treatment prolongation. One recent study compared the area under the receiver operating characteristic curves of reduction in viral load at different time points during antiviral therapy in HCV-1 patients with normal ALT (27). This statistical approach was specifically designed to find the earliest and most optimal criterion

predictive of SVR so as to prevent arbitrary identification. In that study, day 28 was the earliest and most optimal time point at which reduction of viral load was predictive of SVR, and a 2-log drop in viral load was the best cut-off for predicting SVR. Interestingly, the 28-day 2-log drop criterion had a predictive value similar to that of the EVR available 2 months later. If these results are confirmed in larger studies, this new criterion might be used early on to decide treatment discontinuation in patients with low probability of viral clearance.

In naive patients infected with genotype 2 or 3, preliminary data suggest that treatment can be shortened in RVR patients with low baseline viral load (< 400,000 or 800,000 IU/mL depending on the study) (28-30). However, the ACCELERATE study, which included nearly 1000 HCV-2 and -3 patients with RVR, did not confirm these results and revealed a slight but significantly lower SVR rate in the shortened treatment group due to higher relapse rates (31). A recent meta-analysis suggested that 16 weeks of treatment may be sufficient in RVR patients treated with adapted ribavirin doses (32). As in the case of HCV-1 patients, patients with extensive fibrosis and other factors which negatively influence the virological response should not be considered for shortened therapy (33,34). It is still unknown as to whether prolonged therapy is beneficial in HCV-2 or -3 patients without RVR.

In naive patients infected with genotype 4, one study suggested that 36-week therapy may be sufficient in those with low baseline viral load (< 600,000 IU/mL in that study) (35), but current data are not robust enough to recommend shortened treatment in HCV-4 patients.

In previously treated patients, EPIC and REPEAT studies recommended stopping treatment in patients who remain HCV RNA positive at week 12 (36,37). Patients who become HCV RNA-negative (with a lower limit of detection of 50 IU/mL in the REPEAT study and 125 IU/mL in the EPIC study) after 12 weeks of treatment had 49 to 56% probability of SVR with a 48-week course of pegylated IFN and ribavirin combination therapy. This criterion, more restrictive than classical EVR, is now used as the stopping point in previously treated patients.

Conclusions

Virological assays are indispensable in the diagnosis and management of individuals infected with HCV. Monitoring of viral kinetics during antiviral treatment is crucial in making treatment decisions, such as defining when to stop and optimizing the length of treatment. Well-validated algorithms are needed to help clinicians tailor treatment duration according to the individual patient without hindering the possibility of a SVR. Future studies should use a statistical approach specifically aimed at finding the earliest and most optimal time point at which the decline in viral load becomes the best predictor of SVR. This optimal criterion should help clinicians early on to predict response and non-response, as well as to avoid useless treatment prolongation.

In the near future, triple therapy combining pegylated IFN, ribavirin and anti-protease or anti-polymerase will likely become the standard treatment for chronic hepatitis C. A SVR will remain the endpoint of therapy. New algorithms will need to be established so as to more efficiently monitor the virological response during triple therapy.

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