Review

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Diagnostics in hepatitis C: The end of response-guided therapy?

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Summary

On-treatment hepatitis C virus (HCV) RNA has been used to predict response to interferon (IFN)-based therapy. The concept of response-guided treatment (RGT) was established to determine optimal treatment duration and to early identify patients not responding to futile therapies. RGT helped to improve sustained virologic response (SVR) rates and lower the rates of adverse effects. RGT was of particular importance for telaprevir- and boceprevir-based triple therapies. RGT strategies are dependent on highly sensitive and reproducible HCV RNA quantification. However, different HCV RNA assays are used in routine clinical practice and these differ significantly in their performance characteristics. The development of IFN-free therapies has fundamentally changed the role of on-treatment HCV RNA for SVR prediction. Given the high efficacy and excellent tolerability of IFN-free regimens, the interest in treatment individualization has decreased. However, shorter treatment durations may still be desirable, particularly with respect to the high costs of current IFN-free direct-acting antiviral agents (DAAs). Moreover, some difficult-to-treat patients remain, e.g., those infected with HCV genotype 3 in whom the current standard of care may not always be sufficient to achieve SVR, especially in treatment-experienced patients with cirrhosis. Here, a RGT extension may be feasible. However, current data on the predictive value of on-treatment HCV RNA are limited and have shown conflicting results. As more potent DAAs become available, the role of response prediction may diminish further. Currently, shorter treatment duration is only based on baseline HCV RNA whereas no RGT strategy is recommended for any of the approved DAA regimens available.

Keywords: Response-guided therapy; Pegylated interferon; Direct-acting; Antivirals; On-treatment HCV RNA; HCV RNA assay.

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Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that can progress to cirrhosis and hepatocellular carcinoma [1]. Chronic HCV infection accounts for approximately 500,000 deaths each year [2]. Following the discovery of HCV in the late 1980s, development of molecular methods for the detection of nucleic acids was a milestone towards successful treatment of HCV infection [3]. Until today, direct detection and quantification of viral nucleic acid (HCV RNA) is generally regarded as the definite diagnostic criterion to document active HCV infection, regardless of the presence of anti-HCV antibodies and/or elevated liver enzymes [4,5]. Moreover, the primary goal of HCV therapy is the achievement of a sustained virologic response (SVR), defined as undetectable HCV RNA by a sensitive assay 12 or 24 weeks after treatment completion [5].

Qualitative assays were the first nucleic acid assays available. However, with these assays only the presence or absence of active HCV infection can be confirmed. Development of quantitative assays also allowed for on-treatment response monitoring and SVR prediction. This became an integral part of pegylated interferon (PegIFN)-based treatment, which was the mainstay of antiviral therapy until 2013 [6]. On-treatment HCV RNA levels were used to determine optimal treatment duration and to early identify patients not responding to antiviral therapy who were advised to stop treatment due to futility [7]. The paradigm of response-guided treatment (RGT) also became a key concept of HCV therapy following the approval of first generation HCV protease inhibitors (PIs) in 2011. With PI-based triple therapies, higher SVR rates could be achieved using shorter treatment durations compared with PegIFN/RBV alone [8–11]. RGT was further facilitated by the increase in sensitivity and accuracy of real-time PCR-based assays. Today, highly sensitive assays with a limit of detection (LOD) ⩽15

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Key point

-Highly sensitive real-time PCR-based assays are recommended to be used to monitor HCV RNA during and after therapy.
-Several commercially available assays are approved for treatment monitoring.
-Differences in assays sensitivities and quantification must be addressed when comparing and interpreting HCV RNA results.

International Units (IU)/ml are universally recommended for monitoring treatment response [5]. However, several quantitative nucleic acid tests with different sensitivities and different ranges of quantification are commercially available [12]. These differences in performance characteristics must be addressed when comparing assay results in a given clinical setting.

PegIFN-based dual and triple therapies are associated with severe side effects that can sometimes even be fatal [12–15]. The concept of RGT was implemented not only to increase SVR but also to shorten treatment, which may be associated with better tolerability and patient compliance. In 2014, the first IFN-free regimens became available. Currently, IFN-free treatment with direct-acting antiviral agents (DAAs) is the standard of care in many countries. These treatments are generally safe and well tolerated, and viral eradication can be achieved in the vast majority of patients [16]. DAA treatment durations are mostly short, ranging from 8–24 weeks only, with most patients achieving undetectable HCV RNA relatively early during antiviral therapy. This has fundamentally changed the role of on-treatment HCV RNA monitoring. Indeed, the original concept of RGT is now very much in question as on-treatment HCV RNA may no longer be used to increase SVR significantly. Instead, RGT may still be useful for other reasons, in particular for its cost-saving potential.

In this review we discuss the role of on-treatment HCV RNA quantification to guide treatment duration in light of the therapeutic advances that have evolved over the past years.

Virologic tools for HCV RNA quantification

Molecular assays for HCV RNA detection and quantification are routinely used to diagnose and monitor treatment of patients with chronic HCV infection. These assays have evolved over the past 20 years in parallel with the tremendous advances in the therapeutic field. Currently, several HCV RNA assays are commercially available that use different combinations of amplification and detection methods (Table 1). These include signal amplification techniques, such as branched DNA amplification, and target amplification techniques, such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) [12,17].

Real-time PCR technology

The classic PCR technique involves thermal cycling and a thermostable DNA polymerase that has both reverse transcriptase and polymerase activity. During the process, HCV RNA is transcribed into complimentary DNA, which serves as a template in the PCR reaction. The number of DNA copies is doubled with each PCR cycle. However, the number of amplicons can only be analysed at the end of the PCR reaction. Quantification is based on competitive amplification of the HCV target sequence and a known amount of a quantification standard that is added to each reaction tube.

The limitations of classic end-point PCR assays, such as relatively low sensitivity, narrow dynamic range and lack of automation have been largely overcome with the advent of real-time PCR technology [18–20]. Real-time PCR relies on the detection and quantification of a fluorescent reporter that is linked to a quencher and annealed to the target sequence. The reporter signal is released during each PCR cycle and increases in direct proportion to the amount of the PCR product. By measuring the amount of fluorescence emission at each cycle, it is possible to collect the PCR data during the exponential growth phase in real-time as opposed to end-point detection.

Commercially available real-time HCV RNA assays

Several real-time PCR assays are commercially available. The two most widely used assays the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM; Roche Diagnostics, Rotkreuz, Switzerland) and the Abbott RealTime HCV assay (ART; Abbott Molecular, Des Plaines, IL, USA) as well as the COBAS TaqMan for use with the High Pure System (HPS/CTM; Roche Diagnostics), which is used in most clinical trials, are discussed herein. All three assays have received Conformité Européenne (CE) marking and United States Food and Drug Administration (FDA) approval for monitoring HCV RNA during antiviral therapy.

For assay standardization purposes, a WHO international standard for HCV was established. The standard comprises genotype 1a HCV RNA positive plasma and has been calibrated in IU/ml [21]. All assays are calibrated against this standard, which is currently available in its 5th version (https://www.nibsc.org/documents/ifu/14-150.pdf). However, despite calibration to the standard material, significant differences between assays have been reported, i.e., lower quantification by ART and higher quantification by CAP/CTM [22,23]. This may result from different reasons, including inter-individual and genotype-specific sequence variability, even within the highly conserved 5'-UTR which commonly serves as the primer binding region, and differences in assay properties, such as the internal control [23,24].

The COBAS TaqMan HCV assay was the first real-time PCR assay to be approved for the guidance of antiviral therapy. The assay is highly sensitive and linear over a broad dynamic range [25,26]. A common misconception is that there is only one COBAS assay available. However, for HCV RNA extraction either the manual HPS viral nucleic acid kit that uses glass fibre columns or the fully automated COBAS AmpliPrep (CAP) instrument that uses magnetic