

New markers for diagnosis and management of chronic hepatitis C virus infection

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Over 130 million individuals are chronically infected with hepatitis C virus (HCV) worldwide [1]. In spite of the existence of antiviral treatments based on dual or triple therapies with substantial cure rates that vary according to the viral genotype, chronic HCV infection remains the first cause of chronic hepatitis and cirrhosis in Europe and has become the first indication for liver transplantation in industrialized countries [2]. The complications of HCV infection represent the 10th most frequent cause of death of infectious origin worldwide, with approximately 350,000 deaths per year and more than 7,500 in the United States in 2004 [3]. Chronic hepatitis C remains a global health problem, with considerable geographic variation of its prevalence. A high prevalence of chronic HCV infection has been reported in African and Eastern Mediterranean countries, with a prevalence of 14.7% in the general population of Egypt, whereas the HCV prevalence varies from 0.4-0.8% in Sweden, Germany, the Netherlands and France to over 1.9% in the United States, and over 5% in some communities in Italy [4-6]. Because chronic hepatitis C is often asymptomatic until advanced liver disease stages develop, up to approximately 60% of infected patients are unaware of their infection and related liver disease [7]. A late detection and diagnosis of HCV infection has important clinical implications, because advanced fibrosis and cirrhosis have been identified as independent pretreatment predictors of failure of treatments based on interferon- α , as well as key factors of morbidity and mortality [8]. Early diagnosis and treatment of HCV infection can reduce the risk of development of long-term complications and prevent further transmission.

Virology tools developed over the past 20 years are routinely used to diagnose and monitor chronic HCV infection. Classical assays include molecular tools used to detect and quantify HCV RNA, and techniques that determine the HCV sequence in order to determine the genotype and the subtype and eventually identify clinically relevant amino acid substitutions associated with resistance to direct acting antivirals (DAA). New assays capable of detecting and quantifying HCV core antigen are now approved in Europe and are available for research use only in the

United States. Moreover, recent advances include alternatives approaches that require whole blood or oral fluid samples and will be used to extend HCV screening and improve access to care in regions without molecular biology laboratories.

A variety of biological markers are useful for the virological diagnosis and monitoring of chronic HCV infection, as well as screening of hepatitis C infection. Virological markers [total anti-HCV antibodies (Ab), core antigen titer, HCV RNA level and viral genotype], biochemical markers (ALT activity) and histological markers (hepatic fibrosis stage and necroinflammatory activity grade) are currently used in clinical practice to diagnose and monitor chronic hepatitis C. Among them, HCV RNA quantification before treatment is important for decision making. All treatment-naïve patients with compensated liver disease and detectable HCV RNA are candidates for therapy. New molecular methods for quantifying HCV RNA in blood are based on real-time PCR (polymerase chain reaction) or TMA (transcription-mediated amplification) assays. These assays are fully or partly automated. With a broader range of linear quantification, a lower limit of detection (LLOD) in the order of 10-15 IU/mL, and an identical LLOD and lower limit of quantification (LLOQ) for the most recent assays, these techniques are well suited to the clinical needs. They are recommended to quantify HCV RNA in international liver society guidelines [9-11]. The HCV genotype should be determined before treatment is started. Indeed, the HCV genotype drives the treatment indication. Patients infected with HCV genotypes other than 1 should be treated with pegylated interferon (pegIFN) α -2a or-2b and ribavirin only. Patients infected with HCV genotype 1 should receive the triple combination of pegIFN, weight-based ribavirin and an NS3/4A protease inhibitor (either telaprevir or boceprevir). Direct sequence analysis (population sequencing) is the gold standard for HCV genotype determination. The viral region sequenced should be carefully chosen, because not all the regions provide accurate typing and subtyping [12]. Alternative methods to sequencing techniques have been developed for routine clinical use. The most widely used assay is based on reverse hybridization (line probe assay). New technologies, so-called next generation sequencing (NGS), are now available for analyzing viral genome sequences, particularly amino acid substitutions associated with resistance to DAAs. NGS methods offer the capacity to generate a large amount of data, but the results are often difficult to interpret due to the lack of bioinformatic tools to analyze them [13]. In addition, resistance testing based on HCV sequence analysis of the NS3/4A region has no utilities in clinical practice with the currently available drug regimens [14].

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HCV core antigen quantification has been proposed as a surrogate marker of HCV RNA levels in patients with chronic HCV infection [15-17]. In this issue, Hadziyannis *et al* [18] report an interesting evaluation of the new Abbott automated assay for HCV core antigen in a large number of chronically HCV infected patients. The study showed a significant positive correlation between HCV RNA and core levels. The LOD was 1,200 IU/mL of HCV RNA. These assays represent a credible alternative to those measuring HCV RNA levels, because they are less expensive (one-third the cost of an HCV RNA level measurement) and easier to use than current HCV RNA tests for the diagnosis of chronic hepatitis C and the monitoring of antiviral therapy. The only test approved in Europe, but not by the US Food and Drug Administration, however has a lower limit of detection corresponding to HCV RNA levels of the order of 500 to 3,000 IU/mL, depending on the HCV genotype. Thus, HCV core antigen quantification is not suitable to response-guided therapy, according to current guidelines, which use "undetectable" at 10-20 IU/mL as decision criterion.

In the last 20 years, the availability and use of point-of-care (POC) tests have greatly increased and expanded to all fields of medicine. In the setting of infectious diseases, most existing POC tests consist of immunoassays. Some non-immunological POC tests, based on nucleic acid detection and quantification, are currently in development for HCV. POC tests are alternative methods to whole blood samples collected by venous puncture because they can use original specimen matrices such as oral fluid or finger-stick capillary whole blood. Several rapid diagnostic tests are already European Conformity (CE)-marked and are currently in evaluation in clinical settings. Blood can be collected on filter paper, known as the dried blood spot method, allowing the storage of desiccated blood for transport at room temperature via regular mail or courier services. Theoretically, DBS can be used for detection and eventually quantification of all virological parameters used to diagnose and monitor HCV infection. However, standardization and automation are urgently needed to improve the accuracy of these methods.

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.

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