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Review Article

The use of molecular assays in the management of viral hepatitis

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Abstract

Molecular assays are instrumental in the clinical management of viral hepatitis. During the past years, a wide variety of molecular assays have been developed and implemented. This considerably improved the understanding of the natural history and pathogenesis of Hepatitis B virus (HBV), Hepatitis C virus (HCV) or Hepatitis delta virus (HDV) hepatitis, but also caused uncertainties in the selection of the most appropriate assays for clinical requirements. Indeed, a rational choice and application of these assays requires adequate knowledge of the performance of the single test. Moreover, the choice of the most accurate assay for patients' needs and physicians' objectives, needs to be oriented to specific contexts, such as diagnosis, management or treatment. In the past, a hurdle in the routine use of assays for hepatitis viruses nucleic acid quantification was represented by the availability of only "home brew" methods which lacked standardization. Major improvement in addressing the use of molecular assays for viral hepatitis has been derived from recent standardization procedures that allowed a comparison between different tests after results were given as International Units. In addition, it should be reminded that, before getting into the market, molecular assays should be approved by European regulation authorities and validated using internationally recognized standards. A subsequent clinical validation should address the diagnostic accuracy of the assay. These proceedings have the aim of identifying which molecular tests, among those currently available, meet clinical requirements for each specific application.

Keywords: Genotypes; HDV RNA; Qualitative HCV RNA; Quantitative HBV DNA; Quantitative HCV RNA; Viral hepatitis

1. Introduction

Despite the increasing number of molecular assays currently available for hepatitis viruses, their performances are

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Table 1
Class of recommendation based on the quality of evidence (SIGN 50)

Class		
A	Meta-analysis, systematic review, RCT	1 ++ (very low risk of bias) 1 + (low risk of bias)
B	Systematic review of case–control or cohort studies Case–control or cohort studies (very low risk of bias)	2 ++
C	Well conducted case–control or cohort studies (low risk of bias)	2 +
D	Non-analytic studies Expert opinion; formal consensus	3 4

not always comparable. In addition, among hepatologists, virologists and clinical pathologists, no clear consensus exists on the application of different assays in clinical practice. Recently, standardization procedures allowed the comparison of data obtained with different assays or in different laboratories by means of the expression of results in International Units (IU). Although standard units do not represent the exact number of virions in a serum sample, they allow both HBV and HCV for the conversion of viral copies/ml into IU/ml and permit comparison of different quantitative assays. This premises encouraged, in Italy, the organization of a Consensus Conference defined as "II AISF Single Topic" and entitled "Use of molecular biology assays in viral hepatitis" which was held in San Giovanni Rotondo on the 2nd–3rd December 2005. The Conference had the aim to review current knowledge of molecular assays for viral hepatitis with the objective of developing consensus statements on their appropriate clinical use. The Conference essentially followed the problem oriented process used for preparing the National Institute for Clinical Excellence (NICE) guidelines and technology assessment. Statements and recommendations were graded for their strength and quality using a grading system based on the Scottish Intercollegiate Guideline Network (Table 1).

Indications reported below are the conclusions merged during and after the meeting, from the systematic review of the literature and from a multi-disciplinary debate. This short version of the consensus summarises the main conclusions and recommendations from the conference. A more detailed version of these recommendations with additional information on technologies, background and supporting data is available online at the AISF website (www.webasif.it).

Definitions adopted in the document are the following as shown in (Table 2).

2. HCV infection

Diagnosis of HCV infection in the presence of HCVAb or in their absence when a primary infection is suspected requires that HCV RNA be evaluated by molecular assays with a sensitivity of ≤50 IU/ml (A) (Table 3a).

2.1. Diagnosis of active or inactive infection

Up to 40% of HCV infected patients undergo spontaneous HCV RNA clearance [1]. As a consequence, a positive HCVAb ELISA test does not always mean an active infection to be diagnosed [2]. To distinguish between past or current infection a molecular assay of sensitivity ≤50 IU/ml is required (A). A negative HCV RNA test may suggest resolved HCV infection (B), but in order to exclude intermittent viremia, HCV RNA should be tested repeatedly. For negative results repeat evaluation, at least 6 months apart are required (B).

In patients on chronic dialysis or in patients with immunodeficiency, when chronic HCV infection is suspected, HCV RNA should be evaluated independently of a negative HCVAb test.

The availability of a real time nucleic acid amplification assay which offers, at the same time, high sensitivity (<50 IU/ml) to detect low HCV RNA levels and a wide dynamic range to quantify very high viral loads before and during treatment, is desirable (D).

2.2. Use of molecular testing for disease diagnosis

Data from the literature have shown, in the majority of the studies, lack of a correlation between viral load and liver damage or disease outcome [3]. Quantitative evaluation of HCV RNA does not correlate with severity of histologic damage and cannot be used as a marker of disease progression (C), although in patients with advanced disease a decrease in HCV RNA levels has been demonstrated over time (C).

In chronic HCV infection, fluctuations in HCV RNA levels are of limited entity (maximum 1 $\log_{10}$) and do not justify repeat testing out of treatment (C).

2.3. Use of molecular testing as a guide for initiating and monitoring treatment and in prediction of response

All HCVAb positive subjects with detectable HCV RNA, aged between 18 and 70 years are potential candidates for treatment. Current guidelines recommend as standard antiviral treatment of HCV infection the combination of