



## 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.

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**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](http://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  $\leq 50$  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  $\leq 50$  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<sub>10</sub>) 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

Table 2

Virus	Test	Applications
		<b>Diagnosis of infection</b> <i>Active:</i> HCVAb positive subject, with HCV RNA detectable independently of levels. <i>Past:</i> HCVAb positive subject, with undetectable HCV RNA.
HCV	Qualitative or quantitative HCV RNA	<b>Treatment response evaluation</b> <i>RVR:</i> HCV RNA undetectable after 4 weeks of treatment. <i>EVR:</i> HCV RNA undetectable or $\leq 2$ log reduction in HCV RNA levels after 12 weeks from the start of treatment. <i>ETR:</i> HCV RNA undetectable at the end of treatment. <i>SVR:</i> HCV RNA undetectable 24 weeks after the end of treatment. <i>Relapse:</i> HCV RNA previously undetectable, detectable at FU. <i>Non-response:</i> HCV RNA still detectable at 24 weeks of treatment.
		<b>Diagnosis</b> <i>HBV carrier:</i> HBsAg positive subject either HBeAg or HBeAb positive with or without hepatic damage. <i>Occult HBV carrier:</i> subject with detectable HBV DNA in serum or in liver in the absence of HBsAg. <i>Active infection:</i> HBV DNA $>10^5$ copies/ml.
HBV	Qualitative HBV DNA	HBV DNA $<10^5$ copies/ml with 3–4 log fluctuations in antiHBe positive subjects.
	Quantitative HBV DNA	<i>Inactive infection:</i> HBV DNA $<10^4$ copies/ml, in more than one occasion in the absence of hepatic damage. <b>Treatment response<sup>a</sup></b> <i>Suppression of viral replication:</i> HBV DNA $<10^3$ copies/ml or reduction in HBV DNA levels in comparison with baseline, during treatment, at the end of treatment or at follow up. <b>Assessment of treatment resistance<sup>b,c,d</sup></b> <i>Genotypic resistance:</i> detection of mutations responsible of reduced response to treatment. <i>Primary resistance:</i> lack of 1 log reduction in HBV DNA levels at month 3 from the start of treatment with nucleot(s)ide analogs. <i>Secondary resistance:</i> loss of previous treatment response due to the selection of resistant mutants. <i>Virologic breakthrough:</i> 1 log increase in HBV DNA levels in comparison with the nadir at 2 different evaluations in patients compliant to treatment. <i>Cross resistance:</i> mutations conferring resistance to more than 1 drug.
	Specific mutations research	
	Quantitative HBV DNA	
	Quantitative HBV DNA	
	Quantitative HBV DNA	
	Specific mutations research	
HDV	Qualitative HDV RNA	<i>Active phase infection:</i> high IgG titers with antiHDV IgM $\pm$ HDV RNA. <i>Latent phase infection:</i> intranuclear HDAg in hepatocytes and negative HBsAg in serum. <i>Virologic response:</i> HDV RNA undetectable (in house PCR)
HBV HCV	Qualitative HCV RNA	<i>Active HCV infection:</i> HBsAg, HCVAb positive with HCV RNA detectable in serum.
HBVHIV		AntiHIV and HBsAg positive
HCVHIV	Qualitative HCV RNA	<i>Active HCV:</i> antiHIV positive with HCV RNA detectable in serum.

RVR = rapid virologic response, EVR = early virologic response, ETR = end of treatment response, SVR = sustained virologic response, FU = follow up.

<sup>a</sup> HBsAg/antiHBs seroconversion: defines complete response in patients on IFN treatment.

<sup>b</sup> Biochemical breakthrough: on treatment alanine aminotransferase (ALT) increase.

<sup>c</sup> Clinical resistance: virologic and biochemical breakthrough  $\pm$  liver disease progression.

<sup>d</sup> Phenotypic resistance: capability of the virus of replicating in vitro in the presence of the antiviral drug as a consequence of mutations in the polymerase gene.

pegylated interferon and ribavirin. Two different pegylated interferon alpha 2b and alpha 2a are available [4–6]. Randomized controlled trials demonstrated that this combination achieves 54–56% of SVR. A number of baseline and on-treatment factors predict SVR after combination therapy [7].

Table 3a

HCV RNA conversion factors from copies to IU/ml

Roche Amplicor 2.0	1 IU/ml = 2.4 copies/ml
Bayer bDNA 3.0	1 IU/ml = 5.2 copies/ml
NGI SuperQuant	1 IU/ml = 3.4 copies/ml
LCx HCV RNA	1 IU/ml = 3.8 copies/ml

Six different genotypes of HCV are known. RCT showed with the current standard therapy SVR in 42–52% of patients infected with genotypes 1–4 and in 84% of those infected with genotype 2 or 3. As demonstrated by several studies, the HCV genotype represents the main predictive factor of response and should be evaluated before the start of treatment (A). In HCV infection, there is no indication of the level of HCV RNA on which when to treat does not exist (A).

Due to the different assays and cut offs used, viremia levels do not demonstrate an equally relevant impact in all the studies. Assessment of HCV RNA levels before treatment might be considered a predictive criterion of response in patients infected with genotypes 1 and 3 (B).

In genotype 1 infection, monitoring of viral response at week 12 during treatment with peginterferon and ribavirin has become a rule on which to decide discontinuation of ineffective treatment. In fact, in genotype 1 infected patients with yet detectable HCV RNA or with less than 2 log drop in HCV RNA levels, the likelihood of achieving SVR has been shown to decline to less than 2% [5]. On the contrary, HCV RNA undetectable at week 4 and persistently negative by a sensitive assay, has been recently shown to be predictive of the highest SVR rate. It has been suggested that the early the viral clearance the higher the rate of SVR [8].

Very low levels of circulating HCV RNA at the end of treatment are potentially useful in identifying patients who will relapse after discontinuation of therapy [9]. However, the clinical relevance of a more sensitive assay to be used at the end of treatment to prospectively decide whether or not to stop treatment has not yet been proved. The use of extremely sensitive assays at the end of treatment has also been proposed with the aim of shortening follow up duration from 24 to 12 weeks. Current guidelines recommend discontinuing treatment in patients with genotype 1 at week 12 in the absence of a 2 log drop in HCV RNA levels (B). Regardless of genotype, recent evidence suggests that HCV RNA undetectable from week 4 and throughout the whole treatment course is highly predictive of SVR (B). RVR should be established by an assay of sensitivity of 50 IU/ml (B).

The experts panel agreed on the need to monitor treatment response at the baseline and at week 12 using the same quantitative assay. The use of the same qualitative assay is recommended at 4, 12 and 24 weeks for treatments lasting 48 weeks, and at 4 and 12 weeks, for treatment durations of 24 weeks (A). Evidence suggesting the use of a test more sensitive than 50 IU/ml at the ETR to decide whether or not to extend treatment duration or reduce length of follow up are yet limited. Therefore, to evaluate ETR response, a sensitivity of 50 IU/ml is required (C).

#### 2.4. Use of molecular tests in the assessment of treatment response

The primary aim of HCV treatment is the achievement of SVR. The importance of an accurate assay in demonstrating undetectable HCV RNA at the end of follow up is widely recognized. The performance characteristic of the assay used in this context is most notably sensitivity. However, the occurrence of delayed relapse among patients in whom low levels of serum HCV RNA have been revealed by extremely sensitive experimental techniques at the end of 24 week standard follow up period is very unusual [10,11]. SVR should be assessed by a molecular assay with a sensitivity of  $\leq 50$  IU/ml (A). At present, no evidence supports the use of molecular assays with a lower detection limit, or assessment of HCV RNA in liver or PBMC (B). HCV RNA in patients with SVR should be evaluated after 18 months from ETR and every year to ascertain a permanent long-term response.

#### 2.5. Use of molecular tests to establish treatment duration

In genotype 2 and 3 infected patients and in patients with genotype 1 and low viremia levels, the rapidity of virologic response has been shown to be highly predictive of SVR even after treatments of duration shorter than that currently recommended [12–14]. Thus, HCV RNA at week 4 may be evaluated in order to determine duration of therapy. Shorter treatment duration can be adopted in patients with genotype 2 and 3 when HCV RNA at week 4 is below 50 IU/ml [15,16] (B). In patients with genotype 1 and low viremia levels at baseline further studies are required to confirm whether RVR established with a molecular assay of sensitivity  $\leq 50$  IU/ml allows shortening treatment duration (C).

#### 2.6. HCV/HIV: diagnosis of co-infection and of disease

All the HIVAb positive and HCVAb positive subjects should undergo HCV RNA testing by a high sensitive molecular assay (B). The diagnostic approach is similar to that of HCV mono-infected patients [17].

In HIV patients with acute hepatitis or with low CD4 levels, when HCV infection is suspected, even after a negative serologic test, HCV RNA should be measured repeatedly (B).

As several diagnostic instruments provide information on the presence and severity of HCV/HIV related liver damage, molecular assays concur with liver biopsy and ALT evaluation with the assessment of liver disease [18]. In case of HIV co-infection, HCV RNA should be assessed by the most sensitive available molecular assay (D).

#### 2.7. Decision to treat, choice of treatment and prediction of response

Antiviral therapy allows HCV RNA eradication in a well established period of time, therefore all HIV positive patients are candidates to treatment of chronic HCV infection with a combination of Peg IFN and ribavirin of chronic HCV infection [17]. Decision to treat should be based on HCV RNA evaluation by quantitative assay with low detection limit of 50 IU/ml (A) and large dynamic quantification range should be used in monitoring viremia at baseline and at week 12 (B).

### 3. HBV infection

#### 3.1. Use of molecular tests to define the presence of infection

To define the presence of HBV infection, serological rather than molecular assays are needed. In the absence of HBsAg, the diagnosis of HBV infection relies on the presence of HBV

Table 3b  
HBVDNA conversion factors from copies to IU/ml

Platform	Version	Company	Conversion factor
Real-time	CAP-CTM HBV	ROCHE	1 IU = 5.82 copies
bDNA	Versant 3.0	BAYER	1 IU = 5.6 copies

DNA only in two conditions [19,20]: when a primary infection is suspected (A) or in patients with isolated antiHBc undergoing immune-suppression with the aim of evaluating the risk of viral reactivation (C). In the first case a high sensitive molecular assay is required (A).

### 3.2. To define active or inactive carrier condition or acute or chronic disease

As internationally established, in patients with positive HBeAg, to define active infection HBV DNA assays with detection limit of  $1.8 \times 10^2$  and dynamic range adequate to discriminate HBV DNA levels  $\geq 1.8 \times 10^4$  IU/ml are required (B) (Table 3b). As chronic liver disease is usually associated with HBV DNA of these levels, this evaluation also suggests the presence of disease [21–23]. In HBeAg negative/antiHBe positive patients, levels persistently below this cut off identify the inactive carriers [24–26]. HBV DNA and ALT monitoring for at least 12 months, in association with a marker of hepatic damage as IgM antiHBc when available is required to exclude an active infection in HBeAg negative/antiHBeAg positive patients with normal ALT and with HBV DNA  $< 1.8 \times 10^4$  IU/ml at the first evaluation (D).

### 3.3. Use of molecular tests for therapeutic decision-making

In patients with chronic hepatitis B in active phase, antiviral treatment is indicated. High levels of HBV DNA and immunomediated damage of hepatocytes containing HBV, characterize active HBV infection, demonstrated by elevated serum ALT levels, inflammatory activity and variable degree of liver fibrosis. There are two main forms of HBsAg positive hepatitis, the HBeAg positive form associated with wild type infection, HBV DNA levels usually higher than  $10^6$  copies/ml and elevated ALT, and the HBeAg negative form associated with core promoter and/or pre-core mutant virus [24–26]. In this form, either HBV DNA or ALT levels are fluctuating over time. For treatment decision, HBsAg positive patients should be evaluated in at least two occasions 1 to 3 months apart by the same molecular assay with a large dynamic range (A). HBV DNA levels  $> 1.8 \times 10^4$  IU/ml, in HBeAg positive, or  $> 1.8 \times 10^3$  IU/ml, in HBeAg negative antiHBe positive patients, indicate the need of treatment (A). In patients with normal ALT and viremia  $< 1.8 \times 10^4$  IU/ml, to exclude an active infection, monitoring of HBV DNA, ALT and antiHBc IgM for at least 12 months is recommended (D).

Antiviral treatment of chronic hepatitis B relies currently on two categories of medications: immune modulators such as interferon alpha and peginterferon and viral polymerase inhibitors that belong to the nucleoside and nucleotide analog family [27–35]. With Interferons the course of therapy has a finite duration, whereas with nucleoside analog therapy is long-term. The main goal of antiviral therapy is to control HBV replication and to induce remission of liver disease activity; the secondary end point is to eventually obtain sustained antiviral response and finally antiHBs loss and seroconversion [36,37]. A predictive role of levels of HBV DNA before treatment has been reported only in studies with pegylated interferon or in lamivudine resistant patients for decision to start add-on adefovir (B). Lack of viremia decrease, by week 12 on antiviral treatment predicts unfavourable response to lamivudine or adefovir. Levels of HBV DNA at the end of a course of treatment with pegylated interferon is not predictive of response (A). Patients should always be monitored with the same assay at least twice before starting treatment and then with the same highly sensitive assay with a wide dynamic range at baseline and during treatment (B).

### 3.4. Response evaluation

Definition of virologic response depends on whether the treatment is interferon or nucleos(t)ide analog based [24,26,38]. Response is marked by the clearance of serum HBV DNA. A very sensitive molecular assay, which requires a lower detection limit of  $1.8 \times 10^2$  IU/ml, should be used to demonstrate HBV DNA suppression (B).

### 3.5. Application of molecular tests to clinical variability of HBV

- Clinical relevance of HBV genotype. Increasing evidence suggests that HBV genotype influences the natural history of HBV infection and genotype A (versus D) and B (versus C) are associated with a better interferon treatment outcome [32,34,39–42]. However, in Italy, the role of genotype is limited by the high prevalence of genotype D. At the present time, available evidence does not support the evaluation of genotypes in clinical practice (C).
- How and when to test for viral resistance to nucleos(t)ide analogs. The risk of the emergence of drug-resistance is now defined in patients on lamivudine (66% after 4 years), adefovir (29% after 5 years) and entecavir (10% after 2 years) but not in patients treated with tenofovir, emtricitabine or telbivudine [43–45]. In compliant patients, viral resistance should be suspected when HBV DNA levels increase by 1 log<sub>10</sub> or more, thus diagnosis of primary resistance requires monitoring HBV DNA levels at baseline and after 3 months of treatment. The same molecular assay, with analytical sensitivity adequate to detect 1 log<sub>10</sub> increase in HBV DNA levels in compari-

son with the lowest value attained on treatment, should be used (B).

For an early evaluation of secondary resistance, the assay should be repeated every 6 months for adefovir and every 3 months for lamivudine [24–27,35]. After the first year the interval should be shortened to 3 months in non-cirrhotic patients and to 2 months in patients with cirrhosis (A). Evidence to support different management in patients treated with other antivirals of more recent introduction is lacking [32–34,39,42,46]. As the number of nucleos(t)ide analogues is increasing, the characterization of mutations associated with resistance may be advisable.

### 3.5.1. Role of molecular tests in the diagnosis of HDV infection

HDV screening should be performed by HDVAg assessment in all HBV infected subjects (A). In patients with chronic infection, detectable HDV RNA may confirm the diagnosis (B).

### 3.5.2. Role of molecular tests in disease diagnosis

HDV RNA detectable by PCR (low detection limit  $<10^4$ ) or nested-PCR with low detection limit of 10 copies/ml suggests the presence of an active delta infection (D).

High HDV RNA levels can be considered a marker of liver damage and disease progression (C).

Levels of HBV DNA should be evaluated at the same time and suggest simultaneous active HBV infection when higher than  $1.8 \times 10^3$  IU/ml ( $10^4$ – $10^5$  copies/ml) (B).

In patients with HDV RNA  $<10^4$  copies/ml or positive only by nested PCR, evaluation should be repeated with the same assay every 4–6 months (D).

### 3.5.3. Role of molecular test for treatment decision and to assess virologic response

Patients with detectable HDV RNA, raised ALT and liver damage require treatment (C). HDV RNA levels determined by semi-quantitative or qualitative nested PCR (B) (performable only in a limited number of centres) is useful in monitoring treatment response [47,49].

In repeated controls the same molecular test is recommended (D).

**3.5.3.1. HIV/HBV co-infection.** All HIV positive patients should be screened for HBV infection by serological evaluations (A).

HBV DNA evaluation of HIV positive patients with isolated antiHBe should be performed yearly or in the presence on ALT increase (B) and always before immunosuppressive therapies to predict the risk of HBV reactivation (D).

In all HIV positive and HBsAg positive subjects (B), quantitative evaluation of HBV DNA should be performed. Molecular assays should be used as in HBV mono-

infection [17,48]. In case of severe immunosuppression or when HDV infection is suspected, HDV RNA or HDVAg should be searched for, even in subjects non-reactive for HDVAb (C).

**3.5.3.2. Diagnosis of disease.** An HBV DNA assay should have a lower detection limit of  $1.8 \times 10^2$  IU/ml ( $1 \times 10^3$  copies/ml) and a wide dynamic range is to be used for diagnosis and monitoring of untreated patients (C).

The use of the same assay is recommended to monitor HBV DNA levels in a single patient (D) over time.

**3.5.3.3. Role of molecular tests in treatment decision-making.** In co-infected patients, HBV DNA levels are usually very high, thus quantitative HBV DNA assay for treatment decision should have the lowest detection limit and an extended range of linearity to allow accurate quantification (C) [50]. While some studies have highlighted that the magnitude of viral load decline may be associated with subsequent immune mediated ALT flare and HBe seroconversion, evidence is insufficient [51–53]. At a variance with mono-infected, ALT cannot be considered predictors of treatment response (C). In HIV/HBV co-infected patients, evaluation of HBV genotype or quantification of HBV DNA cannot be considered useful in predicting treatment response (C).

Monitoring HBV DNA levels should be performed in a single patient with the same assay over time (D). As in mono-infected patients, also in HIV co-infection but only when patients are compliant to the HAART, a  $1 \log_{10}$  HBV DNA increase demonstrates antiviral resistance [38]. HBV DNA evaluation should be performed at closer intervals than in mono-infected patients independently of any severity of liver damage (B).

**3.5.3.3.1. HBV/HCV co-infection.** In patients positive for antiHCV and HBsAg, HBV DNA levels should be evaluated by a quantitative assay with a wide dynamic range (D) and HCV RNA by an assay with a sensitivity of 50 IU/ml (D). As levels of viremia may fluctuate [54], HBsAg/antiHCV positive subjects should be re-evaluated every 3–6 months to establish which infection requires antiviral treatment (D).

**3.5.3.3.2. Disease diagnosis.** HBV/HCV co-infection is associated with more severe hepatic damage. Assays and monitoring timing are similar to those employed in HBV mono-infected patients (D).

In HBV/HCV co-infected subjects, HDV infection should be searched for [55]. The search should be repeated over time as HDV RNA may fluctuate widely (D).

**3.5.3.3.3. Treatment decision.** HBsAg and HCVAb positive patients are considered to be certainly HBV- and probably HCV-infected. HBV DNA search in this setting should be done by a quantitative test with a wide dynamic range (D) while HCV RNA should be determined by the most sensitive assay available (D).

In patients in whom, at the first evaluation, only one viral nucleic acid is detectable, quantitative assessment of HBV

DNA and qualitative assessment of HCV RNA should be repeated at least every 3–6 months to detect possible variations of the two nucleic acid levels (D).

In co-infected patients no evidence supports the decision to treat HBV or HCV first (B).

At present, clinical management of patients with HBV/HCV co-infection should not be different from that adopted in mono-infected patients (D).

When treating a single infection, the possible reactivation of the untreated infection should be periodically excluded by re-testing every 3–6 months (D).

## 4. Orthotopic liver transplant

### 4.1. HCV positive patients

A positive molecular assay with low detection limit of  $\leq 50$  IU/ml defines the presence of re-infection (A); levels of HCV RNA at week 4 represent one among several factors, predictive of disease progression (C). To diagnose disease recurrence [56] virologic data should be integrated with histological evaluation of liver damage and hepatic enzymes evaluation (B). HCV RNA levels represent one among several factors associated with progressive disease (B).

### 4.2. Treatment decision

With the therapeutic instruments currently available [57–59], the decision to treat relies on a combination of virologic, biochemical and histological data (A). With HCV RNA detectable at week 4 by a molecular assay with sensitivity  $< 50$  IU/ml it is recommended to start treatment as soon as the general condition of the transplanted patient allows to achieve the best likelihood of success (D).

Treatment response should be evaluated as in immunocompetent patients (A). No evidence supports the use of treatment or monitoring criteria different from those adopted out of the transplantation setting (C). Treatment discontinuation after 12 weeks can be recommended when an HCV RNA decrease of at least 2log is not attained (D). Assessment of the presence of HCV RNA in the liver is a useful indicator of SVR (C).

### 4.3. OLT in HBV infection

#### 4.3.1. 4.2.1 Application of HBV DNA assessment

HBV DNA levels before OLT are the main predictors of post-OLT successful prophylaxis [60–65]. As a consequence, HBV DNA should be evaluated by molecular tests with detection limit of at least  $1.8 \times 10^2$  IU/ml ( $10^3$  copies/ml) and a wide dynamic range (A).

To exclude the risk of possible recurrence, viremia levels should be monitored as soon as the patients enter the transplantation list and every two months until OLT (A).

HBV DNA levels  $> 10^3$  IU ( $10^5$  copies/ml) at the moment of transplant are associated with a significant risk of recurrence (A) independently of the type of prophylaxis (lamivudine, HBIG only or combined prophylaxis) (B). HBV DNA levels between  $1.8 \times 10^4$  and  $10^2$  IU/ml ( $10^{5-3}$  copies/ml), even when attained with antiviral treatment, are associated with a maximum 10% risk of recurrence in case of combined prophylaxis (B).

In patients with phenotypic resistance to lamivudine, HBV DNA levels should be lowered to  $10^4$  IU/ml ( $10^5$  copies/ml) before OLT by adding adefovir with the objective of adopting prophylaxis with two antiviral drugs or of suspending HBIG one year after OLT (C).

#### 4.3.2. HBV DNA and post-OLT recurrence

HBV recurrence is defined by a positive HBsAg test, whereas detectable HBV DNA, in a patient previously negative, indicates re-infection (A).

We recommend the use of a molecular test with lower detection limit of ( $10^3$  copies/ml)  $2 \times 10^2$  IU/ml (A). In case of a positive test, a combined prophylaxis is required. Monitoring HBV DNA after transplantation in an HBsAg positive subject on HBIG prophylaxis or on HBIG plus LAM prophylaxis is of low clinical relevance (B).

In absence of immune-depression, to evaluate treatment response, ALT levels should be considered (A). On treatment, molecular evaluation should be performed every 3 months (A).

In case of undetectable HBV DNA or  $< 1.8 \times 10^2$  IU/ml ( $10^3$  copies/ml) (A) HBIG withdrawal (B) or prophylaxis with only one antiviral drug may be suggested (C).

#### 4.3.3. “de novo” HBV infection in HBsAg negative recipients

“de novo” HBV infection occurs in case of antiHBc positive antiHBs negative donor.

Screening of patients with “de novo” infection requires HBsAg rather than HBV DNA evaluation by molecular assays (B). The risk of “de novo” infection depends on the replicative status of the donor but a correlation between serum HBV DNA levels and risk of “de novo” infection has not been demonstrated (A). The risk correlates with the anti-corporeal status of recipients, the highest being observed in patients negative for both antiHBs and antiHBc [66–68]. Patients with isolate antiHBc have a 10–15% risk. Vaccinated recipients with adequate antibody response do not have any risk (A).

Assessment of intra-hepatic HBV DNA may be considered the gold standard in the diagnosis of occult infection (A). Molecular test for HBV DNA assessment in the liver should be standardized to demonstrate occult infection (B) with the aim of restricting the use of prophylaxis only to recipients of livers from donor antiHBc-positive donors and ascertained presence of HBV DNA in the liver (A).

### Practice points

- Appropriate use of molecular assays for HCV infected patients requires a sensitivity  $\leq 50$  IU/ml to diagnose active infection or establish SVR response after treatment, a sensitivity of 50 IU/ml to establish RVR and ETR response and a large dynamic range of quantitative assays to establish EVR response.

- To characterize the status of an HBV carrier as active or inactive, a molecular assay should have a lower detection limit of  $1.8 \times 10^2$  IU/ml and an upper detection limit of HBV DNA levels  $\geq 1.8 \times 10^4$  IU/ml.

HBV DNA levels  $>1.8 \times 10^4$  IU/ml in HBeAg+ve or  $1.8 \times 10^3$  IU/ml in HBeAg–ve/HBeAb+ve patients are recommended before starting treatment. Viremia levels  $<1.8 \times 10^2$  IU/ml commonly indicate adequate HBV DNA suppression during treatment.

In patients on antiviral treatment, resistance is usually heralded by 1 log<sub>10</sub> HBV DNA increase after 3 months from the start of treatment. For an early evaluation of secondary resistance, timing of repeat HBV DNA quantitation needs to be differentiated in accordance with the drug used and the severity of the underlying liver disease.

- In the transplant setting, recurrence of HBV infection in the liver graft should be assessed by means of a molecular assay with detection limit of  $1.8 \times 10^2$  IU/ml and a wide dynamic range.

### Research agenda

- Studies of proficiency to assess accuracy of quantitative HCV RNA assays in different laboratories.
- Clinical relevance of evaluation of cccDNA to establish response to HBV treatment.
- Relevance of collection of large database as those based on virtual phenotype with the aim to correlate HBV mutations and clinical meaning.

### Conflict of interest statement

None declared.

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