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# Diagnosis and management of chronic viral hepatitis: antigens, antibodies and viral genomes

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Approximately 500 million people, i.e. one fifth of the world population, are chronically infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) 1, 2. Each year, over 1,5 million people die from HBV- and/or HCV-related chronic liver diseases, such as end-stage cirrhosis and hepatocellular carcinoma (HCC). HCC is one of the most common cancers worldwide and in 2002, HBV and HCV have been responsible for at least half a million of these cancers 3.

HBV belongs to the Hepadnaviridae family, genus Orthohepadnavirus. HBV is the smallest human enveloped DNA virus, with a genome of approximately 3,200 base pairs. The partially double-stranded circular DNA genome encodes at least four overlapping open reading frames, including the surface (preS/S), precore/core (preC/C), polymerase (P) and X genes 4. HBV strains are classified into 8 genotypes (A to H) based on an 8% or more DNA sequence difference over the full genome 5, 6. The different HBV genotypes have distinct geographical distributions and appear to bear different biological properties that may affect the clinical outcome of HBV-related disease 7–14.

HCV belongs to the Flaviviridae family, genus Hepacivirus. HCV is an enveloped RNA virus, with a genome of approximately 9,500 nucleotides. The single positive-strand RNA genome contains a major open reading frame that encodes a large polyprotein of approximately 3,000 amino acids. The polyprotein co- and post-translational processing yields a number of structural and non-structural proteins 15. HCV strains are classified into 6 major genotypes, and a seventh one has been recently identified 16, 17. The HCV genotypes also have distinct geographical distributions and the genotype is an essential prognosis marker of the likelihood of HCV eradication during antiviral treatment 18.

Virological tools are needed to diagnose chronic HBV and HCV infections, they may be useful to establish their prognosis, but they have found their principal application in guiding treatment decisions and assessing the virological responses to therapy.

## VIROLOGICAL TOOLS

The virological diagnosis and monitoring of HBV and HCV infections is based on the use of a variety of virological markers (Table 1). Six HBV markers are used in clinical practice, including HBs antigen (HBsAg), anti-HBs antibodies, HBe antigen (HBeAg), anti-HBe antibodies, anti-HBc antibodies (including total anti-HBc antibodies and anti-HBc IgM) and HBV DNA. Three HCV markers are useful in clinical practice, including total anti-HCV antibodies, HCV genotype and HCV RNA.

### Viral antigens and antibodies

#### *Principles of viral antigen and antibody detection and quantification*

The detection (and eventually the quantification) of viral antigens and of specific antibodies in body fluids is based on the use of sandwich enzyme immunoassays (EIAs). Recombinant antigens or antibodies are used to capture circulating antibodies or antigens, respectively, onto the wells of microtiter plates, microbeads or specific holders adapted to close automated devices. The presence of antigens or antibodies is revealed by antibodies (in the case of antigen) or anti-antibodies (in the case of antibody) labeled with an enzyme that catalyzes the transformation of a substrate into a colored compound. The optical density (OD) ratio of the reaction (sample OD/internal control OD) is proportional to the amount of antigens or antibodies in the sample. EIAs are cheap, easy to use, can be fully automated and are well adapted to large volume testing.

### *HBV serological markers*

#### *Hepatitis B surface antigen (HBsAg)*

Hepatitis B surface antigen is bore by three distinct viral proteins encoded by the pre-S/S gene: the major (or small) surface protein encoded by the S gene, the most abundantly produced surface protein; the middle surface protein, encoded by the preS2 + S gene; and the large surface protein, encoded by the preS1 + preS2 + S gene. HBsAg is present in great excess in the blood of HBV-infected patients,

essentially associated with noninfectious particles including spherical particles and tubular ones (approximately  $10^{13}$  particles per milliliter) 19 .

HBsAg is detected early during acute infection, on average 6 to 10 weeks after exposure and several weeks before the onset of clinical and biological symptoms (Figure 1A ). The analytical sensitivity of the available commercial HBsAg EIAs has been improved recently. Indeed, current HBsAg detection assays detect at least 0.15 nanograms (ng) per milliliter (mL) of circulating HBsAg, i.e. approximately 350 international units (IU) per mL 20 . This improvement has reduced the “window period”, i.e. the post-exposure period during which HBsAg is undetectable, to up to 9 days 20 . In addition, the ability of HBsAg assays to detect HBV variants bearing nucleotide substitutions in the S gene leading to modifications of the tri-dimensional structure of HBsAg has been improved compared to the previous generations of tests 21 –23 . The specificity of current HBsAg detection assays is of more than 99.5%. False-positive results can be exceptionally observed in pregnant women, autoimmune diseases, and chronic liver diseases of other causes 23 –25 . They can also sometimes be observed in heparinized, hemolyzed (hemoglobin above 1.4 g/dL) or icteric (bilirubin above 52.8  $\mu$ mol) blood specimens 24 . In practice, it is recommended that the first detection of HBsAg be confirmed by neutralization in order to eliminate a false-positive result.

Evolution of acute hepatitis B towards chronic HBV carriage is defined by HBsAg persistence for more than 6 months 26 . Under certain circumstances, HBsAg may not be detectable during chronic hepatitis B: (i) in low-replication asymptomatic HBV carriers; (ii) in the case of HBV variants bearing nucleotide substitutions in the S gene leading to the synthesis of an HBsAg that is not recognized by commercial assays 27 , 28 ; (iii) when infection resolves spontaneously or after successful antiviral therapy in chronic HBV-infected patients who may subsequently achieve an HBs seroconversion; (iv) in hepatitis delta HBV-co-infected patients, where hepatitis delta virus most often inhibits HBV replication and expression 27 , 29 .

HBsAg quantification can now be performed by means of a fully automated chemiluminescent microparticle immunoassay. HBsAg quantification is easy, cheap and may provide a mean to establish the prognosis of antiviral therapy in the future. HBsAg quantification indeed appears to be a surrogate marker of the amount of covalently closed circular DNA (cccDNA), the persistent intrahepatic form of HBV DNA, in the hepatocytes and a predictor of a sustained virological response to antiviral treatment off therapy. During antiviral therapy, HBsAg decrease is a predictor of subsequent HBsAg clearance, the principal goal of antiviral treatment, which may be subsequently followed by an HBs seroconversion characterized by the appearance of anti-HBs antibodies 30 –32 .

The estimated annual incidence of spontaneous HBsAg clearance from serum in chronically infected patients is 1% to 2% in Caucasians 33 . It may be lower (0.1% to 0.8%) in high endemicity areas where infection is usually acquired perinatally or in the early childhood 34 –36 .

#### ***Anti-hepatitis B surface (anti-HBs) antibodies***

The presence of anti-HBs antibodies (in the presence of anti-HBc antibodies) witnesses the recovery of chronic HBV infection and life-long immunity against HBV. The anti-HBs titer often varies over time, and anti-HBs antibodies may become undetectable several years after the acute episode. The assessment of anti-HBs antibody levels by different assays is not accurate and consistent, yielding incomparable quantitative results in spite of the calibration with international standards 37 .

After vaccination against HBV, isolated anti-HBs antibodies must be present at a titer of more than 10 mIU/mL to confer efficient protection.

#### ***Anti-hepatitis B core (anti-HBc) antibodies***

Anti-hepatitis B core antibodies are detected early during HBV infection (Figure 1A ). Two isotypes of anti-HBc antibodies can be detected, including: anti-HBc IgM, a marker of acute infection that can be also detected at low levels during the immune elimination phase of chronic hepatitis B and during exacerbations in inactive carriers; anti-HBc IgG which rise in parallel to HBsAg at the acute phase of infection and persist for life-long whatever the outcome of HBV infection (Table 1 ).

In contrast to anti-HBs antibodies, anti-HBc IgG are not neutralizing in vivo . False-negative detection of anti-HBc antibodies may rarely occur in immunosuppressed patients. A rare HBV variant harboring an in-frame deletion of the core gene defective for HBc antigen synthesis, named HBV-2, has been reported not to elicit a detectable immune response to HBc antigen in immunocompetent patients. We recently reported the case of an organ donor with low-level HBV DNA (3.0  $\text{Log}_{10}$  IU/mL) lacking both HBsAg and anti-HBc antibodies; several substitutions in both the HBsAg and HBc antigen sequences were found in this donor 38 .

Anti-HBc antibodies may be the only marker present in chronically infected patients, for instance in low-replication asymptomatic HBV carriers with low HBsAg production. Attempts to further characterize anti-HBc antibodies in a prognostic purpose, such as determining anti-HBc IgG subclass profiles or anti-HBc antibody affinity, have failed so far.

## ***Hepatitis B e antigen (HBeAg) and anti-HBe antibodies***

The HBe protein, which bears the HBe antigen, is a nonstructural HBV protein encoded by the preC/C gene. It is secreted by infected cells under various soluble forms that vary in size between 16 and 20 kDa. The HBe protein contains two epitopes: e1, conformational, and e2, linear. The e1 epitope sequence overlaps that of the conformational HBe epitope, so that e1 is presented at the surface of HBV nucleocapsids and expressed, together with HBe, at the surface of infected cells. Although HBe protein secretion does not appear to be essential for HBV lifecycle, its presence is associated with immune tolerance, high-level HBV replication and high transmissibility.

During acute infection, HBeAg can be detected on average 6 to 12 weeks after exposure (Figure 1A ). HBeAg clearance is generally associated with a decrease of viremia and an aminotransferase flare. It is followed by the appearance of anti-HBe antibodies during the HBe seroconversion phase. Persistence of HBeAg is generally observed in the patients who develop chronic infection.

In chronic HBV infection, two phenotypes can be observed, including HBeAg-positive and HBeAg-negative chronic hepatitis B. In HBeAg-positive chronic hepatitis B, the preC/C gene has a wild-type sequence, no anti-HBe antibodies are found, and the presence of HBeAg in peripheral blood is generally associated with a high HBV DNA level. In HBeAg-negative chronic hepatitis B, the patients are infected with a variant virus, bearing nucleotide substitutions in the precore region and/or in the basal core promoter region of the preC/C gene. The most frequent nucleotide substitution occurs at position 1896 (G1896A) in the precore region of the preC/C gene. It inserts a stop codon in the precore sequence that prevents the HBe protein from being synthesized. The second group of nucleotide polymorphisms is located within the core promoter region. The most frequent changes are at positions 1762 (A1762T) and 1764 (G1764A). They down-regulate HBe protein production up to 70%. Other mutations susceptible to alter HBe protein production have also been described 33, 39. Different variants bearing precore and/or basal core promoter mutations can be observed in the same patient in variable proportions. Although a few cases of de novo infection with HBeAg-negative viruses have been described in the literature, HBeAg-negative variants are generally selected during the immune clearance of chronic HBV infection. An important outcome of the immunoeelimination phase is indeed seroconversion from HBeAg to anti-HBe, characterized by the clearance of HBeAg followed by the appearance of anti-HBe antibodies. The seroconversion phase may result in an HBeAg-negative chronic hepatitis B or in an inactive carrier state characterized by normal aminotransferase levels and undetectable or low-level (< 2 000 IU/mL) HBV DNA. The estimated annual incidence of spontaneous HBeAg seroclearance in chronically infected patients is 5% to 15% 40, 41.

## ***HCV serological markers***

### ***Total anti-HCV antibodies***

The serological window, characterized by detectable HCV RNA and core antigen in the absence of anti-HCV antibodies, has been estimated to be of approximately 60 days on average 42. Anti-HCV antibodies appear on average 2 to 8 weeks after the acute phase of infection and persist for life in patients who develop chronic HCV infection (Figure 1B ). The presence of both anti-HCV antibodies and HCV RNA does not allow to distinguish acute hepatitis C from an acute exacerbation of chronic hepatitis C or an acute hepatitis of another cause in a patient with chronic hepatitis C. The anti-HCV IgG avidity index within the first 8 days following the onset of clinical symptoms may be useful in identifying actual acute HCV infection cases 43.

The significance of the presence of anti-HCV IgM during HCV infection is unclear. Anti-HCV IgM have been reported in 50% to 93% of patients with acute hepatitis C and 50% to 70% of patients with chronic hepatitis C 44–46. Therefore, anti-HCV IgM cannot be used as a reliable marker of acute HCV infection, and IgM assays have not be used in clinical practice. However, the serial measurements of the anti-HCV IgM titers based on at least three determinations from the fifth to the fifteenth day from the onset of the symptoms may identify patients with acute hepatitis C 47.

### ***Serological determination of HCV genotype***

The HCV genotype can be determined by means of a competitive ELISA assay using genotype-specific antigens 48. This assay allows to identify the 6 HCV genotypes (1 to 6) but not the subtype.

## ***Viral genome***

### ***Genome detection and quantification***

#### ***Principles of genome detection and quantification***

Viral genome detection and quantification can be achieved by using two categories of molecular biology-based techniques, including target amplification (such as polymerase chain reaction (PCR)) and signal amplification (such as hybrid capture or the branched DNA assay). Whatever technique used, HBV DNA and HCV RNA international units per milliliter must be preferred to any other quantitative unit and should now be implemented in all commercial quantitative assays. Conversion factors can be used to establish a relationship between the IUs and the non standardized copies 49, 50. The classical techniques for viral genome detection and quantification are now progressively being replaced by real-time PCR assays in most virology laboratories (Table 2).

Real-time PCR techniques have a broad dynamic range of quantification, well suited to the clinical needs (upper range of quantification: 7 to 8 Log<sub>10</sub> IU/mL). Real-time PCR is more sensitive than classical PCR, with lower limits of detection of the order of 10 to 15 IU/mL for both HBV DNA and HCV RNA. Real-time PCR assays do not yield false-positive results due to carryover contaminations, and they can be fully automated. Real-time PCR has become the technique of choice to detect and quantify viral genomes in clinical practice. Several real-time PCR assays are now commercially available for the detection and quantification of both HBV DNA and HCV RNA (Table 2).

### ***HBV DNA detection and quantification***

HBV DNA detection and quantification is useful in clinical practice to: (i) to diagnose chronic hepatitis B with viral replication; (ii) establish the prognosis of liver disease and assess the risk of progression towards cirrhosis and hepatocellular carcinoma 51 ; (iii) identify patients who need antiviral therapy and offer them the most adapted treatment; (iv) monitor the virological response to therapy and identify amino acid substitutions responsible for resistance to nucleos(t)ide analogues.

The presence of HBV DNA in peripheral blood is a reliable marker of HBV replication. HBV DNA is detectable within a few days after infection. It generally increases to reach a peak at the time of acute hepatitis, and then progressively decreases and disappears when the infection resolves spontaneously 52 . In the patients progressing towards chronic HBsAg carriage, chronic infection evolves through successive phases. HBV DNA levels are not stable over time and depend on the infection phase: the immunotolerance phase is characterized by high levels of viral replication; the immuno-elimination phase is characterized by generally lower, often fluctuating HBV DNA levels; the inactive carrier stage is characterized by very low or undetectable levels of viral replication, depending on the sensitivity of the assay; reactivation phases, that are facilitated by immunosuppressive therapies, are generally associated with high levels of replication.

HBV DNA assays based on real-time PCR technology are now replacing the classical techniques in most virology laboratories. Three real-time PCR platforms are currently available for detection and quantification of HBV DNA: the Cobas Taqman<sup>®</sup> platform, which can be used together with automated sample preparation with the Cobas AmpliPrep<sup>®</sup> system (CAP-CTM, Roche Molecular System, Pleasanton, California), the Real-Art<sup>®</sup> HBV PCR Assay (Artus-Biotech, Qiagen, Hamburg, Germany), and the Abbott platform (Abbott Diagnostic, Chicago Illinois), which uses the m 2000<sub>RT</sub> amplification platform together with the m 2000<sub>SP</sub> device for sample preparation ( Table 2 ). These assays have been shown to accurately quantify HBV DNA levels in clinical practice 53 , 54 .

### ***HCV RNA detection and quantification***

HCV RNA detection and quantification is useful in clinical practice to: (i) diagnose chronic HCV infection; (ii) identify patients who need antiviral therapy; (iii) monitor the virological responses to antiviral therapy, and (iv) in the future, identify amino acid substitutions responsible for resistance to specific inhibitors of HCV viral proteins 50 .

The presence of HCV RNA in peripheral blood is a reliable marker of HCV replication. HCV RNA can be detected 1 to 3 weeks after infection, approximately 1 month before the appearance of total anti-HCV antibodies. The presence of HCV RNA after 6 months signs chronic HCV infection. HCV RNA levels remain relatively stable over time in chronically infected patients.

HCV RNA assays based on real time PCR are now used in clinical virology laboratories for RNA detection and quantification. Two real-time PCR platforms are currently available for detection and quantification of HCV RNA: the Cobas Taqman<sup>®</sup> platform, which can be used together with automated sample preparation with the Cobas AmpliPrep<sup>®</sup> system (CAP-CTM, Roche Molecular System), and the Abbott platform (Abbott Diagnostic), which uses the m 2000<sub>RT</sub> amplification platform together with the m 2000<sub>SP</sub> device for sample preparation. Another assay, developed by Siemens Medical Solutions Diagnostics (Tarrytown, New-York) will be available soon (Table 2 ). The intrinsic performances of available tests differ. Indeed, approximately 15% of HCV genotype 2 and 30% of HCV genotype 4 samples are substantially underestimated in the CAP-CTM, most likely because of nucleotide mismatches, whereas this problem has not been found with the Abbott assay 55 , 56 .

## ***Analysis of the nucleotide sequence of the viral genome***

### ***Principles of nucleotide sequence analysis***

Viral genome sequence analysis is generally based on direct sequencing (so-called population sequencing) that provides the full sequence of the analyzed fragment, or reverse hybridization that identifies specific nucleotides or motifs at given positions. In practice, signature sequences are used: (i) to classify viral strains into phylogenetic groups of clinical interest, such as the 8 HBV genotype (A to H) or the 6 HCV genotypes (1 to 6); (ii) to identify nucleotide substitutions of the precore and basal core promoter regions of the preC/C gene; (iii) to identify amino acid substitutions known to confer viral resistance to specific antiviral inhibitors.

### ***Genotype determination***

The reference method for viral genotype determination is phylogenetic analysis of sequences generated after PCR amplification of a portion of the viral genome relative to reference sequences. Commercial assays have been developed. The Trugene<sup>®</sup> HBV Genotyping Kit (Siemens Medical Solutions Diagnostics) can determine the HBV genotype after amplification of a region overlapping the open reading frame encoding the S and P genes. The Trugene<sup>®</sup> 5'NC HCV Genotyping kit (Siemens Medical Solutions Diagnostics) has been developed for HCV genotype determination by direct sequencing of a portion of the 5' non-coding region (NCR) of the viral genome. Direct sequence analysis is the gold standard for genomic sequence analysis. However, it only identifies viral variants representing at least 20 to 25% of the circulating viral populations.

Reverse hybridization of PCR amplicons to membrane-bound probes is more sensitive than direct sequence analysis to detect minor variants representing as few as 5% the whole viral population 57 . Line probe assays (INNO-LiPA, Innogenetics, Gent, Belgium) use a series of short immobilized oligonucleotide probes to discriminate among different PCR fragments. A commercial assay has been developed for HBV genotype determination (INNO-LiPA HBV Genotyping) 58 . The most recent version of the line probe assay for HCV genotype determination (VERSANT<sup>®</sup> HCV Genotype 2.0 Assay, Siemens Medical Solutions Diagnostics) bears consistently improved accuracy for HCV genotype 1 subtype and HCV genotype 6 determination compared to the previous assays 59 –62 .

The utility of HBV genotype determination in clinical practice is debated. The positive and negative predictive values of the HBV genotype on disease progression and treatment outcomes have not been determined at the individual patient level, and further large clinical studies are warranted before implementing HBV genotype determination in practice 63 . In contrast, HCV genotype determination is mandated before the initiation of therapy with pegylated interferon alpha and ribavirin because it is the best predictor of treatment response and it determines the dose of ribavirin and treatment duration 18 .

#### ***Identification of amino acid substitutions associated with viral resistance***

Direct sequence analysis and reverse hybridization methods are used to identify amino acid substitutions known to confer resistance to antiviral drugs before the viral level increases. At present, early detection of HBV substitutions conferring resistance to nucleos(t)ide analogues is used to alter therapy in order to avoid rebound and hepatitis flare. Commercial assays are available. The Trugene<sup>®</sup> HBV Genotyping Kit (Siemens Medical Solutions Diagnostics) is based on direct sequence analysis of a portion of the reverse transcriptase domain of the HBV polymerase gene 64 . A new generation of reverse hybridization assay, INNO-LiPA HBV DR v3, has been developed in 2008 to detect amino acid substitutions associated with lamivudine, adefovir and entecavir resistance. Reverse hybridization methods have been shown to detect changes in viral quasispecies composition early, on average 6 months before direct sequencing analysis 65 , 66 .

## **PRACTICAL USE OF VIROLOGICAL TOOLS IN THE MANAGEMENT OF CHRONIC HEPATITIS B**

Serological and molecular HBV markers are used in clinical practice to diagnose chronic HBV infection, assess the prognosis of the disease, guide therapy and monitor treatment responses.

### **Diagnosis of chronic HBV infection**

The persistence of HBsAg for more than 6 months defines chronic HBV carriage. In a chronic HBV carrier, chronic hepatitis B is defined by an elevated serum HBV DNA (generally  $> 2 \times 10^3$  to  $2 \times 10^4$  IU/mL), with persistent or intermittent elevation of aminotransferase levels and signs of chronic hepatitis B on liver biopsy 67 –70 . In a chronic HBV carrier, the HBV DNA level should be systematically measured by means of a sensitive and accurate method. Real-time PCR assays should be preferred and the results must be expressed in international units per milliliter 26 . The HBV DNA level is indeed a key determinant of liver disease progression towards cirrhosis or HCC 51 , 71 . HBV DNA level measurement is also crucial for therapeutic decision-making.

Figure 1A shows the kinetics of HBV markers during chronic HBV infection. HBsAg and total anti-HBc antibodies are always present. The presence of HBeAg (in the absence of anti-HBe antibodies) is generally associated with high-level viral replication and high transmissibility. HBV replication levels are substantially lower on average in HBeAg-negative patients. Inactive HBV carriers have a low level of viral replication ( $< 2 \times 10^3$  IU/mL), normal aminotransferase levels, no HBeAg and positive anti-HBe antibodies.

### **Assessment of disease severity and prognosis**

The HBV DNA level and aminotransferase activity provide valuable prognostic information. The REVEAL study, which included more than 3,000 Taiwanese patients with chronic HBV infection, showed that an HBV DNA level higher than  $4.3 \text{ Log}_{10}$  (i.e.  $2 \times 10^4$  IU/mL) was associated with a significant risk of progression of chronic hepatitis B to cirrhosis and HCC, independently of the HBeAg serostatus and level of aminotransferase activity 51 , 71 . In addition, the risk of HCC is significantly related to the level of HBV replication and starts raising above  $2 \times 10^3$  IU/mL 72 . The risk of HCC is low in the absence of detectable HBV DNA, except in patients with cirrhosis.

## **Treatment of HBV infection**

The goal of chronic hepatitis B therapy is to prevent progression of liver disease to its life-threatening complications, cirrhosis and HCC. This can be achieved if HBV replication is durably abolished or significantly reduced. In HBeAg-positive patients, HBeAg clearance followed by the HBe seroconversion phase can be achieved in some cases with short-term therapy and ensures long-term control of viral replication. In HBeAg-negative patients, long-term antiviral suppression of viral replication is needed in most cases. The loss of HBsAg, eventually associated with an HBs seroconversion, is the most desirable endpoint of therapy but is rarely achieved.

### ***Decision to treat***

The decision to treat chronic hepatitis B is based on the assessment of multiple parameters including clinical, biological and histologic parameters. Antiviral treatment is currently recommended in patients with an HBV DNA titer above 2,000 IU/mL, elevated serum alanine aminotransferase activity (above the upper limit of normal), and/or evidence of chronic hepatitis with or without cirrhosis. Antiviral treatment should be considered in patients with a low level of viral replication who show mild to moderate necro-inflammatory activity and/or fibrosis. In HBV-infected patients with normal aminotransferase activity and an HBV DNA level below 2,000 IU/mL, repeated HBV DNA and ALT determinations are recommended every 3 to 6 months.

### ***Selection of optimal therapy***

The current treatment of chronic hepatitis B is based on the use of two categories of antiviral compounds: pegylated interferon alpha and nucleos(t)ide analogues that inhibit the reverse transcriptase domain of viral polymerase. Five nucleos(t)ide analogues are approved in Europe or in US for the treatment of chronic hepatitis B, including lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir disoproxil fumarate.

In HBeAg-positive patients, first-line treatment with pegylated interferon alpha is recommended if aminotransferase levels are elevated and the HBV DNA level is moderate (below  $2 \times 10^6$  IU/mL). Although HBV genotypes A and B globally appear to better respond to interferon-based therapy than genotypes C and D, HBV genotype determination is not yet recommended to guide the therapeutic decision in the absence of strong individual predictive value.

Most of the other patients, whatever the HBe serostatus, and in HBeAg-positive patients who did not achieve an HBe seroconversion during or after interferon alpha therapy, the use of nucleos(t)ide analogues is recommended. Entecavir or tenofovir must be preferred as first-line treatment because they both potently inhibit HBV replication and they have a high genetic barrier to resistance. Combinations of nucleos(t)ide analogues with no cross-resistance increases the genetic barrier to resistance and thus better prevents resistance on the long-term than mono- or sequential therapy. De novo combinations can be used as first-line treatment in patients with high HBV DNA levels and are unlikely to reach undetectable HBV DNA on monotherapy.

### ***Treatment monitoring***

HBV treatment monitoring is based on HBV DNA quantifications and ALT determinations every 3 to 6 months, whatever the HBe serostatus and antiviral treatment 63 .

In HBeAg-positive patients, treatment efficacy is witness by the loss of HBeAg with may be followed by a seroconversion to anti-HBe antibodies. It is generally associated with a profound reduction of serum HBV DNA levels and normalization of aminotransferase activity. Ideally in HBe seroconverters, HBV DNA should be undetectable with a sensitive real-time PCR assay (lower limit of detection of the order 10 to 15 IU/mL) and aminotransferase activity should be normal 73 . If this is not the case, the patient may have switched from HBeAg-positive to HBeAg-negative therapy and may need sustained therapeutic suppression of viral replication.

In HBeAg-positive patients with no HBe seroconversion and in HBeAg-negative patients, the goal of antiviral treatment is to achieve a profound and durable HBV DNA suppression. The HBV DNA level should be undetectable on treatment with a sensitive real-time PCR assay (lower limit of detection: 10 to 15 IU/mL) 63 . If the HBV DNA level remains detectable after 48 weeks, a second antiviral compound with no cross-resistance with the first one must be added in order to prevent subsequent resistance.

In the patients who have responded and have been compliant, resistance should be suspected if the HBV DNA level rises by more than  $1 \text{ Log}_{10}$  IU/mL above nadir in two consecutive samples taken one month part 63 , 74 . The identification of selected amino acid substitutions known to be associated with resistance to the administered drug(s) by means of molecular testing can help guide treatment adaptation. Consensus decisional algorithms will need to be established before systematic genotypic resistance testing can be recommended to adapt the treatment strategy to the resistance profile of the infecting viral strain.

### **Monitoring of untreated chronic HBV infections**

Antiviral treatment is not recommended in patients with an HBV DNA level below 2,000 IU/mL and normal aminotransferase activity 67 . However, a regular monitoring should be performed. Aminotransferase activity should be assessed every month for the three first months, and then every 6–12 months. In untreated patients, the severity of liver inflammation and fibrosis must be evaluated by means of a liver biopsy or non-invasive serological or ultrasound-based testing when persistent or intermittent elevation of aminotransferase levels has been observed. HBV DNA quantification every 6 months is useful to detect an increase in viral replication and eventually reconsider the treatment indication.

## **PRACTICAL USE OF VIROLOGICAL TOOLS IN THE MANAGEMENT OF CHRONIC HEPATITIS C**

Serological and molecular markers are used in clinical practice to diagnose chronic hepatitis C, guide treatment decisions and monitor the antiviral efficacy of treatment.

### **Diagnosis of chronic HCV infection**

The persistence of HCV RNA for more than 6 months defines chronic HCV infection. In patients with clinical and/or biological signs of chronic liver disease, chronic hepatitis C is diagnosed by the simultaneous presence of anti-HCV antibodies and HCV RNA. Detectable HCV replication in the absence of anti-HCV antibodies is exceptional with current anti-HCV enzyme immunoassays. It is almost exclusively observed in profoundly immunodepressed, hemodialysis or agamaglobulinemic subjects 75 , 76 .

### **Treatment of HCV infection**

The current standard treatment for chronic hepatitis C is the combination of pegylated interferon alpha-2a or -2b and ribavirin 77 . The efficacy endpoint of chronic hepatitis C treatment is the sustained virological response (SVR), defined by an undetectable HCV RNA in serum with a sensitive assay (lower limit of detection of 50 IU/mL or less) 24 weeks after the end of treatment.

### ***Decision to treat and indication of treatment***

The decision to treat chronic hepatitis C depends on multiple parameters including a precise assessment of the severity of liver disease, the presence of absolute or relative contra-indications to therapy, and the patient's willingness to be treated.

HCV genotype determination should be systematically determined before treatment, as it determines the indication, the duration of treatment, the dose of ribavirin, and the virological monitoring procedure (Figure 2 ) 18 . Genotypes 2- and 3-infected patients require 24 weeks of treatment and a low dose of ribavirin, i.e. 800 mg daily. In contrast, genotype 1-, 4-, 5- and 6-infected patients require 48 weeks of treatment and a high, body-weight based dose of ribavirin, i.e. 1000 to 1400 mg daily.

### ***Treatment monitoring***

Monitoring of HCV RNA levels is recommended to tailor treatment to the actual virological response. A sensitive assay with a broad range of quantification should be used. A real-time PCR assay should ideally be used. In HCV genotype 1-infected patients, the HCV RNA level should be measured before therapy and 12 weeks after its initiation (Figure 2 ). The lack of a 12-week virological response (i.e. no change or an HCV RNA decrease of less than 2 Log<sub>10</sub> at week 12) indicates that the patient has virtually no chance to achieve an SVR and should stop treatment. In contrast, treatment must be continued when a 2 Log<sub>10</sub> drop in HCV RNA level has been observed at week 12, until week 48 if HCV RNA is undetectable, until week 72 if HCV RNA is still detectable at week 12 28 , 78 .

Recent studies have suggested that the patients who achieve a rapid virological response, defined by an undetectable HCV RNA (< 50 IU/mL) at week 4 of therapy, could benefit from shorter treatment duration, i.e. 24 weeks in patients infected with HCV genotypes 1, 4, 5 or 6 and 12 to 16 weeks in those infected with HCV genotypes 2 or 3 79 –83 .84 . These results however need confirmation and new algorithms should be drawn to tailor treatment duration to the virological response at week 4 without losing a chance of viral eradication.

The sustained virological response corresponds to a cure of infection in more than 99% of cases. In the mid-term future, triple combination therapy with pegylated interferon alpha, ribavirin and a specific HCV inhibitor will likely become the standard treatment of chronic hepatitis C. The SVR will remain the endpoint of therapy. On-treatment monitoring and the corresponding decision algorithms will need to be established.

### **Monitoring of untreated chronic HCV infections**

In patients with no indication or with contra-indications to therapy, the HCV RNA level has no prognostic value. The level of HCV replication does not correlate with the severity of liver disease, nor with the risk of liver disease progression to cirrhosis or HCC. Repeated aminotransferase level assessments are recommended. Assessment of liver inflammation and fibrosis by means of liver biopsy or

non-invasive serological or ultrasound-based testing is needed in the case of persistent or intermittent elevation of aminotransferase levels  
77 .

## References:

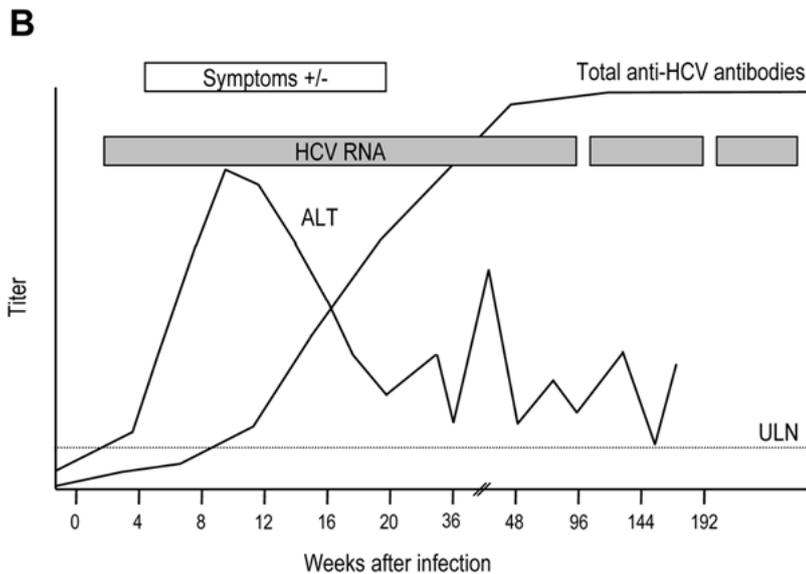
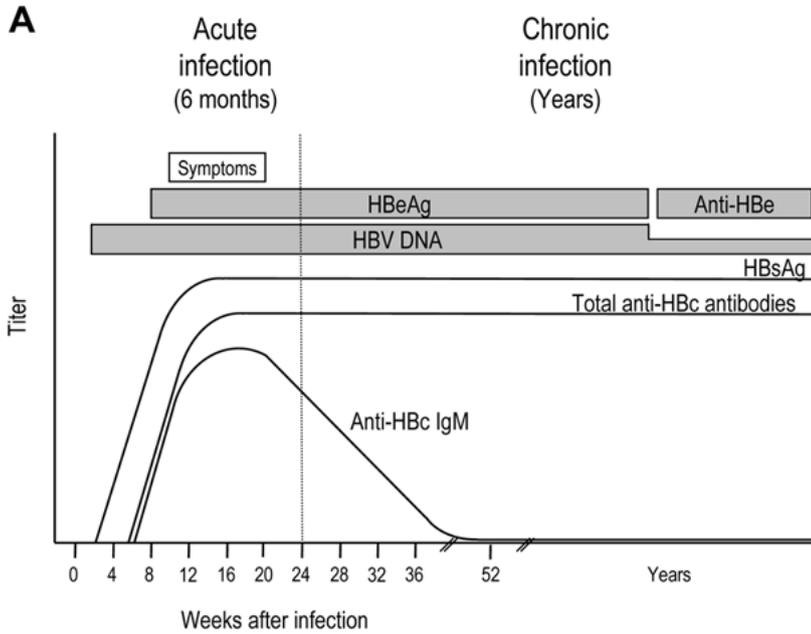
- 1 . Lavanchy D . Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures . *J Viral Hepat* . 2004 ; 11 : 97 - 107
- 2 . Shepard CW , Finelli L , Alter MJ . Global epidemiology of hepatitis C virus infection . *Lancet Infect Dis* . 2005 ; 5 : 558 - 67
- 3 . Parkin DM . The global health burden of infection-associated cancers in the year 2002 . *Int J Cancer* . 2006 ; 118 : 3030 - 44
- 4 . Robinson WS , Clayton DA , Greenman RL . DNA of a human hepatitis B virus candidate . *J Virol* . 1974 ; 14 : 384 - 91
- 5 . Kidd-Ljunggren K , Miyakawa Y , Kidd AH . Genetic variability in hepatitis B viruses . *J Gen Virol* . 2002 ; 83 : 1267 - 80
- 6 . Schaefer S . Hepatitis B virus taxonomy and hepatitis B virus genotypes . *World J Gastroenterol* . 2007 ; 13 : 14 - 21
- 7 . Chu CM , Liaw YF . Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline . *J Hepatol* . 2005 ; 43 : 411 - 7
- 8 . Chu CM , Liaw YF . Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B . *Gastroenterology* . 2007 ; 133 : 1458 - 65
- 9 . Flink HJ , van Zonneveld M , Hansen BE , de Man RA , Schalm SW , Janssen HL . Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype . *Am J Gastroenterol* . 2006 ; 101 : 297 - 303
- 10 . Funk ML , Rosenberg DM , Lok AS . Worldwide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants . *J Viral Hepat* . 2002 ; 9 : 52 - 61
- 11 . Grandjacques C , Pradat P , Stuyver L , Chevallier M , Chevallier P , Pichoud C , Maisonnas M , Trepo C , Zoulim F . Rapid detection of genotypes and mutations in the pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity . *J Hepatol* . 2000 ; 33 : 430 - 9
- 12 . Janssen HL , van Zonneveld M , Senturk H , Zeuzem S , Akarca US , Cakaloglu Y , Simon C , So TM , Gerken G , de Man RA , Niesters HG , Zondervan P , Hansen B , Schalm SW . Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial . *Lancet* . 2005 ; 365 : 123 - 9
- 13 . Livingston SE , Simonetti JP , McMahon BJ , Bulkow LR , Hurlburt KJ , Homan CE , Snowball MM , Cagle HH , Williams JL , Chulanov VP . Hepatitis B virus genotypes in Alaska Native people with hepatocellular carcinoma: preponderance of genotype F . *J Infect Dis* . 2007 ; 195 : 5 - 11
- 14 . Orito E , Ichida T , Sakugawa H , Sata M , Horiike N , Hino K , Okita K , Okanoue T , Iino S , Tanaka E , Suzuki K , Watanabe H , Hige S , Mizokami M . Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan . *Hepatology* . 2001 ; 34 : 590 - 4
- 15 . Chevaliez S , Pawlotsky JM . Editor: Tan S-L . HCV genome and life cycle . *Hepatitis C viruses: genome and molecular biology: Horizon Bioscience* . 2006 ; 5 - 47
- 16 . Simmonds P , Bukh J , Combet C , Deleage G , Enomoto N , Feinstone S , Halfon P , Inchauspe G , Kuiken C , Maertens G , Mizokami M , Murphy DG , Okamoto H , Pawlotsky JM , Penin F , Sablon E , Shin IT , Stuyver LJ , Thiel HJ , Viazov S , Weiner AJ , Widell A . Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes . *Hepatology* . 2005 ; 42 : 962 - 73
- 17 . Murphy DG , Chamberland J , Dandavino R , Sablon E . A new genotype of hepatitis C virus originating from central Africa . *Hepatology* . 2007 ; 46 : (Suppl 1) 623A -
- 18 . Hadziyannis SJ , Sette H Jr , Morgan TR , Balan V , Diago M , Marcellin P , Ramadori G , Bodenheimer H Jr , Bernstein D , Rizzetto M , Zeuzem S , Pockros PJ , Lin A , Ackrill AM . Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose . *Ann Intern Med* . 2004 ; 140 : 346 - 55
- 19 . Kan M , Gerlich W . Structure and molecular virology . Blackwell ; 2005 ;
- 20 . Biswas R , Tabor E , Hsia CC , Wright DJ , Laycock ME , Fiebig EW , Peddada L , Smith R , Schreiber GB , Epstein JS , Nemo GJ , Busch MP . Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection . *Transfusion* . 2003 ; 43 : 788 - 98
- 21 . Gibb R , Nimmo GR , O'Loughlin P , Lowe P , Drummond D . Detection of HBsAg mutants in a population with a low prevalence of hepatitis B virus infection . *J Med Virol* . 2007 ; 79 : 351 - 5
- 22 . Muhlbacher A , Weber B , Burgisser P , Eiras A , Cabrera J , Louisirirochanakul S , Tiller FW , Kim HS , Helden JV , Bossi V , Echevarria JM . Multicenter study of a new fully automated HBsAg screening assay with enhanced sensitivity for the detection of HBV mutants . *Med Microbiol Immunol* . 2008 ; 197 : 55 - 64
- 23 . Weber B , Van der Taelen-Brule N , Berger A , Simon F , Geudin M , Ritter J . Evaluation of a new automated assay for hepatitis B surface antigen (HBsAg) detection VIDAS HBsAg Ultra . *J Virol Methods* . 2006 ; 135 : 109 - 17
- 24 . Weber B , Bayer A , Kirch P , Schluter V , Schlieper D , Melchior W . Improved detection of hepatitis B virus surface antigen by a new rapid automated assay . *J Clin Microbiol* . 1999 ; 37 : 2639 - 47
- 25 . Weber B , Dengler T , Berger A , Doerr HW , Rabenau H . Evaluation of two new automated assays for hepatitis B virus surface antigen (HBsAg) detection: IMMULITE HBsAg and IMMULITE 2000 HBsAg . *J Clin Microbiol* . 2003 ; 41 : 135 - 43
- 26 . Lok AS , McMahon BJ . Chronic hepatitis B . *Hepatology* . 2001 ; 34 : 1225 - 41
- 27 . Shukla NB , Poles MA . Hepatitis B virus infection: co-infection with hepatitis C virus, hepatitis D virus, and human immunodeficiency virus . *Clin Liver Dis* . 2004 ; 8 : 445 - 60
- 28 . Berg T , von Wagner M , Nasser S , Sarrazin C , Heintges T , Gerlach T , Buggisch P , Goeser T , Rasenack J , Pape GR , Schmidt WE , Kallinowski B , Klinker H , Spengler U , Martus P , Alshuth U , Zeuzem S . Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin . *Gastroenterology* . 2006 ; 130 : 1086 - 97
- 29 . Su CW , Huang YH , Huo TI , Shih HH , Sheen IJ , Chen SW , Lee PC , Lee SD , Wu JC . Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients . *Gastroenterology* . 2006 ; 130 : 1625 - 35
- 30 . Akhan SC , Yulugkural Z , Vahaboglu H . Response to interferon-alpha in chronic hepatitis B patients with and without precore mutant strain and effects on HBsAg titers . *Chemotherapy* . 2007 ; 53 : 402 - 6
- 31 . Chan HL , Wong VW , Tse AM , Tse CH , Chim AM , Chan HY , Wong GL , Sung JJ . Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response . *Clin Gastroenterol Hepatol* . 2007 ; 5 : 1462 - 8
- 32 . Ozaras R , Tabak F , Tahan V , Ozturk R , Akin H , Mert A , Senturk H . Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment . *Dig Dis Sci* . 2008 ;
- 33 . Fattovich G , Bortolotti F , Donato F . Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors . *J Hepatol* . 2008 ; 48 : 335 - 52
- 34 . Liaw YF , Sheen IS , Chen TJ , Chu CM , Pao CC . Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study . *Hepatology* . 1991 ; 13 : 627 - 31
- 35 . Nam SW , Jung JJ , Bae SH , Choi JY , Yoon SK , Cho SH , Han JY , Han NI , Yang JM , Lee YS . Clinical outcomes of delayed clearance of serum HBsAg in patients with chronic HBV infection . *Korean J Intern Med* . 2007 ; 22 : 73 - 6
- 36 . Wu TT , Hsu HC , Chen DS , Sheu JC , Su IJ , Chen SL , Chuang SM . Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma . *J Hepatol* . 1987 ; 4 : 45 - 51
- 37 . Huzly D , Schenk T , Jilg W , Neumann-Haefelin D . Comparison of nine commercially available assays for quantification of antibody response to hepatitis B virus surface antigen . *J Clin Microbiol* . 2008 ; 46 : 1298 - 306
- 38 . Challine D , Chevaliez S , Pawlotsky JM . Hepatitis B virus (HBV) replication in organ, tissue and cell donors with and without HBV serological markers . *Gastroenterology* . 2008 ; in press
- 39 . Ingman M , Lindqvist B , Kidd-Ljunggren K . Novel mutation in hepatitis B virus preventing HBeAg production and resembling primate strains . *J Gen Virol* . 2006 ; 87 : 307 - 10

- 40. Brown SD, Barbara AJ, Lambert T, Wilson DV. Spontaneous loss of HBeAg and development of anti-HBe during long-term follow-up of blood donors found to be HBsAg-positive. *Br J Biomed Sci*. 1995; 52: 106 - 9
- 41. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000-summary of a workshop. *Gastroenterology*. 2001; 120: 1828 - 53
- 42. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. *Transfusion*. 2005; 45: 254 - 64
- 43. Coppola N, Pisapia R, Marrocco C, Martini S, Vatiro LM, Messina V, Tonziello G, Sagnelli C, Filippini P, Piccinino F, Sagnelli E. Anti-HCV IgG avidity index in acute hepatitis C. *J Clin Virol*. 2007; 40: 110 - 5
- 44. Hellstrom UB, Sylvan SP, Decker RH, Sonnerborg A. Immunoglobulin M reactivity towards the immunologically active region sp75 of the core protein of hepatitis C virus (HCV) in chronic HCV infection. *J Med Virol*. 1993; 39: 325 - 32
- 45. Negro F, Troonen H, Michel G, Giostra E, Albrecht M, Perrin L, Hadengue A. Lack of monomeric IgM anti-hepatitis C virus (HCV) core antibodies in patients with chronic HCV infection. *J Virol Methods*. 1996; 60: 179 - 82
- 46. Sagnelli E, Coppola N, Marrocco C, Coviello G, Rossi G, Battaglia M, Sagnelli C, Messina V, Tonziello A, Scolastico C, Filippini P. Diagnosis of HCV related acute hepatitis by serial determination of IgM to HCV: a preliminary observation. *J Biol Regul Homeost Agents*. 2003; 17: 207 - 10
- 47. Sagnelli E, Coppola N, Marrocco C, Coviello G, Battaglia M, Messina V, Rossi G, Sagnelli C, Scolastico C, Filippini P. Diagnosis of hepatitis C virus related acute hepatitis by serial determination of IgM anti-HCV titres. *J Hepatol*. 2005; 42: 646 - 51
- 48. Pawlotsky JM, Prescott L, Simmonds P, Pellet C, Laurent-Puig P, Labonne C, Darthuy F, Remire J, Duval J, Buffet C, Etienne JP, Dhumeaux D, Dussaix E. Serological determination of hepatitis C virus genotype: comparison with a standardized genotyping assay. *J Clin Microbiol*. 1997; 35: 1734 - 9
- 49. Gish RG, Locarnini SA. Chronic hepatitis B: current testing strategies. *Clin Gastroenterol Hepatol*. 2006; 4: 666 - 76
- 50. Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. *Hepatology*. 2002; 36: S65 - 73
- 51. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006; 295: 65 - 73
- 52. Whalley SA, Murray JM, Brown D, Webster GJ, Emery VC, Dusheiko GM, Perelson AS. Kinetics of acute hepatitis B virus infection in humans. *J Exp Med*. 2001; 193: 847 - 54
- 53. Chevaliez S, Bouvier-Alias M, Laperche S, Pawlotsky JM. Performance of the Cobas AmpliPrep/Cobas TaqMan real-time PCR assay for hepatitis B virus DNA quantification. *J Clin Microbiol*. 2008; 46: 1716 - 23
- 54. Thibault V, Pichoud C, Mullen C, Rhoads J, Smith JB, Bitbol A, Thamm S, Zoulim F. Characterization of a new sensitive PCR assay for quantification of viral DNA isolated from patients with hepatitis B virus infections. *J Clin Microbiol*. 2007; 45: 3948 - 53
- 55. Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Overestimation and underestimation of hepatitis C virus RNA levels in a widely used real-time polymerase chain reaction-based method. *Hepatology*. 2007; 46: 22 - 31
- 56. Chevaliez S, Bouvier-Alias M, Pawlotsky JM. Performance of the Abbott m2000SP/m2000RT real-time polymerase chain reaction assay for hepatitis C virus RNA quantification. submitted
- 57. Sertoz RY, Erensoy S, Pas S, Akarca US, Ozgenc F, Yamazhan T, Ozacar T, Niesters HG. Comparison of sequence analysis and INNO-LiPA HBV DR line probe assay in patients with chronic hepatitis B. *J Chemother*. 2005; 17: 514 - 20
- 58. Hussain M, Chu CJ, Sablon E, Lok AS. Rapid and sensitive assays for determination of hepatitis B virus (HBV) genotypes and detection of HBV precore and core promoter variants. *J Clin Microbiol*. 2003; 41: 3699 - 705
- 59. Bouchardeau F, Cantaloube JF, Chevaliez S, Portal C, Razer A, Lefrere JJ, Pawlotsky JM, De Micco P, Laperche S. Improvement of hepatitis C virus (HCV) genotype determination with the new version of the INNO-LiPA HCV assay. *J Clin Microbiol*. 2007; 45: 1140 - 5
- 60. Chevaliez S, Bouvier-Alias M, Vandervet C, Pawlotsky JM. HCV genotype determination in clinical practice: weaknesses of assays based on the 5' noncoding region and improvement with the core-coding region. *Hepatology*. 2007; 46: (Suppl 1) 839A -
- 61. Stelzl E, van der Meer C, Gouw R, Beld M, Grahovac M, Marth E, Kessler HH. Determination of the hepatitis C virus subtype: comparison of sequencing and reverse hybridization assays. *Clin Chem Lab Med*. 2007; 45: 167 - 70
- 62. Verbeeck J, Stanley MJ, Shieh J, Celis L, Huyck E, Wollants E, Morimoto J, Fariro A, Sablon E, Jankowski-Hennig M, Schaper C, Johnson P, Van Ranst M, Van Brussel M. Evaluation of Versant hepatitis C virus genotype assay (LiPA) 2.0. *J Clin Microbiol*. 2008; 46: 1901 - 6
- 63. Pawlotsky JM, Dusheiko G, Hatzakis A, Lau D, Lau G, Liang TJ, Locarnini S, Martin P, Richman DD, Zoulim F. Virologic monitoring of hepatitis B virus therapy in clinical trials and practice: recommendations for a standardized approach. *Gastroenterology*. 2008; 134: 405 - 15
- 64. Gintowt AA, Germer JJ, Mitchell PS, Yao JD. Evaluation of the MagNA Pure LC used with the Trugene HBV Genotyping Kit. *J Clin Virol*. 2005; 34: 155 - 7
- 65. Libbrecht E, Doutreloigne J, Van De Velde H, Yuen MF, Lai CL, Shapiro F, Sablon E. Evolution of primary and compensatory lamivudine resistance mutations in chronic hepatitis B virus-infected patients during long-term lamivudine treatment, assessed by a line probe assay. *J Clin Microbiol*. 2007; 45: 3935 - 41
- 66. Osioy C, Villeneuve JP, Heathcote EJ, Giles E, Borlang J. Detection of rtN236T and rtA181V/T mutations associated with resistance to adefovir dipivoxil in samples from patients with chronic hepatitis B virus infection by the INNO-LiPA HBV DR line probe assay (version 2). *J Clin Microbiol*. 2006; 44: 1994 - 7
- 67. Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H, Wright TL. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. *Clin Gastroenterol Hepatol*. 2006; 4: 936 - 62
- 68. Lok AS. Chronic hepatitis B. *N Engl J Med*. 2002; 346: 1682 - 3
- 69. Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. *Hepatology*. 2004; 39: 857 - 61
- 70. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med*. 2002; 137: 1 - 10
- 71. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology*. 2006; 130: 678 - 86
- 72. Chen CJ, Yang HI, Su J, Jen CL, You SL, Chen CF, Iloeje UH. Serial monitoring of viral load and serum alanine transferase level and the risk of hepatocellular carcinoma (HCC): R.E.V.E.A.L. -HBV study update. *J Hepatol*. 2008; 48: (Suppl 2) S61 -
- 73. Lai CL, Yuen MF. The natural history and treatment of chronic hepatitis B: a critical evaluation of standard treatment criteria and end points. *Ann Intern Med*. 2007; 147: 58 - 61
- 74. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology*. 2007; 45: 507 - 39
- 75. Lok AS, Chien D, Choo QL, Chan TM, Chiu EK, Cheng IK, Houghton M, Kuo G. Antibody response to core, envelope and nonstructural hepatitis C virus antigens: comparison of immunocompetent and immunosuppressed patients. *Hepatology*. 1993; 18: 497 - 502
- 76. Thio CL, Nolt KR, Astemborski J, Vlahov D, Nelson KE, Thomas DL. Screening for hepatitis C virus in human immunodeficiency virus-infected individuals. *J Clin Microbiol*. 2000; 38: 575 - 7
- 77. NIH Consensus Statement on Management of Hepatitis C. NIH Consens State Sci Statements. 2002; 19: 1 - 46
- 78. Sanchez-Tapias JM, Diago M, Escartin P, Enriquez J, Romero-Gomez M, Barcena R, Crespo J, Andrade R, Martinez-Bauer E, Perez R, Testillano M, Planas R, Sola R, Garcia-Bengoechea M, Garcia-Samaniego J, Munoz-Sanchez M, Moreno-Otero R. Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology*. 2006; 131: 451 - 60
- 79. Dalgard O, Bjoro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H. Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology*. 2004; 40: 1260 - 5
- 80. Ferenci P, Bergholz U, Laferl H, Scherzer TM, Maieron A, Gschwantler M, Brunner H, Hubmann RR, Datz C, Bishof M, Stauber R, Steindl-Munda P. 24 weeks treatment regimen with peginterferon alpha-2a (40 kDa) (Pegasys) plus ribavirin (Copegus) in HCV genotype 1 or 4 "super-responders". *J Hepatol*. 2006; 44: S6 -

- 81 . Mangia A , Santoro R , Minerva N , Ricci GL , Carretta V , Persico M , Vinelli F , Scotto G , Bacca D , Annese M , Romano M , Zechini F , Sogari F , Spirito F , Andriulli A . Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3 . *N Engl J Med* . 2005 ; 352 : 2609 - 17
- 82 . von Wagner M , Huber M , Berg T , Hinrichsen H , Rasenack J , Heintges T , Bergk A , Bernsmeier C , Haussinger D , Herrmann E , Zeuzem S . Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C . *Gastroenterology* . 2005 ; 129 : 522 - 7
- 83 . Zeuzem S , Buti M , Ferenci P , Sperl J , Horsmans Y , Cianciara J , Ibranyi E , Weiland O , Noviello S , Brass C , Albrecht J . Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia . *J Hepatol* . 2006 ; 44 : 97 - 103
- 84 . Fried MW , Hadziyannis SJ , Shiffman M , Messinger D , Zeuzem S . Rapid virological response is a more important predictor of sustained virological response (SVR) than genotype in patients with chronic hepatitis C virus infection . *J Hepatol* . 2008 ; 48 : (Suppl 2 ) S5 -

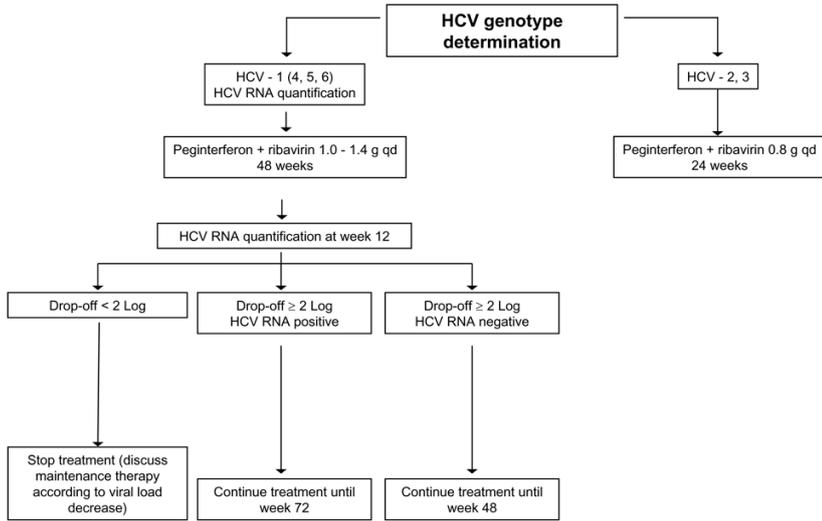
**Figure 1**

Kinetics of virological markers during chronic viral hepatitis: (A) chronic HBV infection; (C) chronic HCV infection.



**Figure 2**

Algorithms for the use of HCV virological tools in the treatment of chronic hepatitis C, according to the HCV genotype.



**Table 1**

Virological markers for the diagnosis and management of chronic HBV and HCV infections

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**Hepatitis B virus**

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**Direct markers**

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Hepatitis B surface antigen (HBsAg)

Hepatitis B e antigen (HBeAg)

HBV DNA

HBV genotype \*

HBV resistance substitutions \*

ccDNA \*

**Indirect markers**

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Anti-HBs antibodies

Anti-HBc antibodies (IgM & IgG)

Anti-HBe antibodies

---

**Hepatitis C virus**

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**Direct markers**

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Capsid antigen \*

HCV RNA

HCV genotype

**Indirect markers**

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Total anti-HCV antibodies

\* not routinely available

**Table 2**

Commercially available real-time PCR assays for HBV DNA and HCV RNA detection and quantification

Assay	Manufacturer	Method	Lower limit of detection	Dynamic range
<b>Hepatitis B virus</b>				
Cobas Taqman HBV	Roche Molecular Systems, Pleasanton, California	Real-time PCR on Cobas Taqman after automated extraction (Cobas Ampliprep)	12 IU/mL	54 IU/mL -110 000 000 IU/mL
Real-Art HBV PCR assay	Qiagen, Hamburg, Germany	Real-time PCR after manual extraction	4 IU/mL	4 to 9 IU/mL -100 000 000 IU/mL
Abbott Real-time HBV	Abbott Diagnostic, Chicago, Illinois	Real-time PCR on m 2000 <sub>RT</sub> after automated extraction (m 2000 <sub>SP</sub> )	10 IU/mL	10 UI/mL - 1 000 000 000 IU/mL
<b>Hepatitis C virus</b>				
Cobas Taqman HCV	Roche Molecular Systems, Pleasanton, California	Real-time PCR on Cobas Taqman after automated extraction (Cobas Ampliprep)	15 IU/mL	46 IU/mL - 69 000 000 IU/mL
Abbott Real-time HCV	Abbott Diagnostic, Chicago, Illinois	Real-time PCR m 2000 <sub>RT</sub> after automated extraction(m 2000 <sub>SP</sub> )	12 to 30 IU/mL	12 IU/mL -100 000 000 UI/mL