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Diagnosis and Management of Chronic Hepatitis B virus Infection

1. Introduction

About 2 billion people worldwide have been infected with the hepatitis B virus (HBV). Of those, at least 350 million are suffering from a chronic infection.
HBV infection is detected mainly on the basis of serological parameters. A chronic (persistent) HBV infection is present if the HBs-Ag is detectable in the serum more than 6 months after the time of infection. The chronicity rate is on average 5-10 %, but varies greatly according to the age of the person at infection (90 % with perinatal infection, 20 % in adolescents). If the infection, which is often asymptomatic, manifests itself as an inflammatory liver disease, it is called "hepatitis B". The complications of hepatitis B are inflammatory liver cell damage, cirrhosis and hepatocellular carcinoma (HCC). Early, effective antiviral therapy reduces morbidity and mortality. For this reason timely diagnosis is extremely important. The monitoring and treatment of chronic HBV infection calls for the broad use of molecular biological diagnostic techniques.

2. Course of the chronic infection

For a long time only two phases were identified in the chronic course of the infection: HBe antigen-positive patients with active liver disease and HBe antigen-negative patients who were regarded as asymptomatic carriers.
More sensitive molecular biological detection techniques and a better understanding of the pathogen-

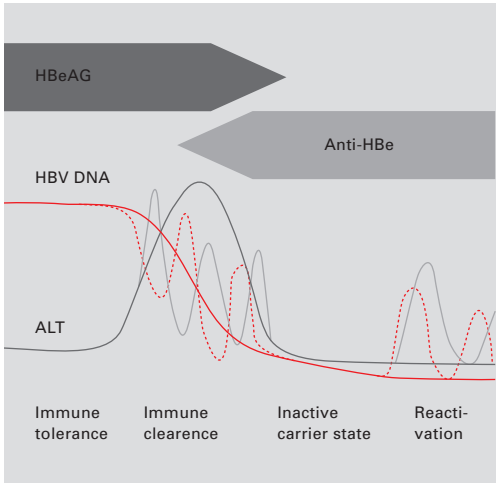

Fig. 1
Natural course of chronic HBV infection
(modified according to 6)

specific immune response in the meantime paint a more complex picture of the chronic form. It is now known to consist of **4 characteristic phases** (Fig. 1).
The immune tolerance phase, which can last for a very long time, especially with perinatally acquired hepatitis B, is in particular notable for very high virus DNA titres. Despite this, no or only very moderate liver cell damage is observed ("high viraemic carrier state").
The inflammatory processes of hepatitis B are caused only by the start of the immune defence, in particular by cytotoxic T-lymphocytes and the associated lysis of hepatocytes.

	I. Immune tolerance	II. Immune clearance	III. Inactive carrier state	IV. Reactivation
HBe-Ag	+	+/-	-	-
Transaminases	normal	increased/fluct.	normal	increased/fluct.
HBV-DNA	very high	high/fluct.	low/negative	high/fluct.
Histology	normal	inflammatory	mild/normal	inflammatory
Cirrhosis/HCC risk	low	correlates with duration and intensity of the phase	low	increased
Comments	Typical of perinatally acquired infection. May last several decades. Brief or absent with later age of infection.	Transaminase flares characteristic. Usually HBe seroconversion.	May last indefinitely.	Occurs in some carriers (25% in 9 years in an Asian population).

Table 1
Characteristics of the 4 phases of chronic hepatitis B virus infection

The characteristic transaminase flares (fluctuating levels) in the immune clearance phase must be particularly emphasized. These are often preceded by a peak in the fluctuating viraemia (DNA titre).
HBe seroconversion and a reduction in the viraemia may subsequently occur. The HBe antigen often remains positive, however. The duration of the immune clearance and the frequency and intensity of the flares correlates with the risk of cirrhosis and HCC.
A considerable proportion of inactive carriers experience reactivation of hepatitis B. Transaminase flares and fluctuating DNA titres are characteristic of this phase too. Against this background, serial testing of the transaminases and the DNA titres is superior to individual testing.

3. Importance of the virus load

Testing of the virus load, in addition to the serum transaminases, is now an essential part of the evaluation of any patient with chronic hepatitis B (see point 4) and of treatment monitoring (see point 6). Modern methods for the quantitative detection of HBV-DNA (virus load determination) have a very high sensitivity, with a lower detection limit of approx. 10 IU/ml (approx. 50 genome copies/ml).
The clinical threshold value for distinguishing an active from a latent infection and for a decision on treatment has until now been set at a fairly arbitrary level of 10^5 copies/ml (approx. 2×10^4 IU/ml).
Recent studies in large (Asian) populations have now shown that the level of virus load is an absolutely independent risk factor and predictor for the progression of the disease.
Beginning with a value of 10^4 copies/ml the risk of cirrhosis and HCC is significantly increasing proportionately with increasing virus load (see Figure 2). This close correlation is clearly still present even after adjustment for all other risk factors.
For this reason a new clinical threshold level of 10^4 copies/ml (corresponding to approx. 2×10^3 IU/ml) has been included in the German and European guidelines as a decision-making criteria (2, 8). It is also used in the US guidelines (4).

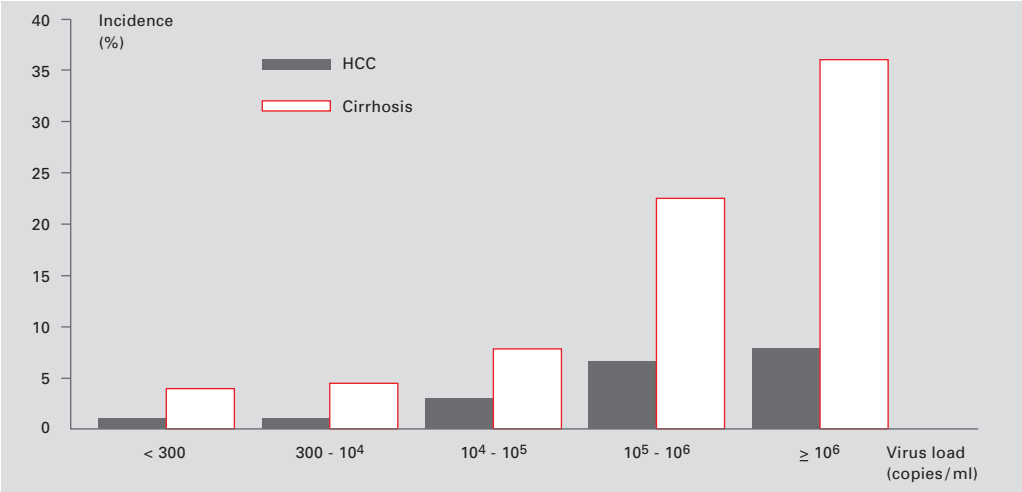

Fig. 2
Cirrhosis and HCC risk (percent of patients) as a function of the initial virus load after 11 years' follow-up (source: 1, 3)

4. Monitoring of the chronic infection

If treatment is not indicated (<10^4 copies/ml HBV-DNA and normal transaminase levels), a persistent HBV infection necessitates regular follow-up, depending on the clinical course (Table 2).
Particular attention is paid today to asymptomatic HBs-Ag carriers, since approx. 30 % of this group have a virus load of >10^4 copies/ml. They have an increased HCC risk. Reactivation of hepatitis B with an inflammatory course and progression of the disease occurs in a considerable proportion of the patients over the years.
The liver biopsy is generally considered useful to determine inflammatory activity, the degree of fibrosis and other possible causes of liver disease. The decision to perform a biopsy always depends on whether the histological result influences the therapeutic approach. There is no need for a biopsy if treatment is indicated irrespective of the histological result or clinically confirmed cirrhosis is present (2, 8).

Chronic hepatitis B	**HBs-Ag carrier status**
▪ Liver inflammation parameters ▪ Liver function parameters ▪ CBC ▪ Quick value/INR ▪ HBe-Ag (if initially positive) ▪ HBs-Ag (if HBe-Ag negative) ▪ Quantitative HBV-DNA ▪ Increased HCC risk: epigastric ultrasonography, AFP	▪ Transaminase activity ▪ Quantitative HBV-DNA ▪ HBe-Ag/anti HBe (every 12 months) ▪ With a rise in transaminases see left-hand column ▪ Increased HCC risk: epigastric ultrasonography, AFP every 12 months
Depending on clinical activity, initially every 3, later every 12 months (with cirrhosis every 6 months).	First year: 3x Second year: at least 2x Then every 12 months

Table 2
Monitoring of chronic HBV infection (2)

5. Indication for treatment

In addition to (pegylated) interferon alfa (IFN), the nucleoside/nucleotide analogues lamivudine, adefovir, entecavir, telbivudine and tenofovir are antiviral drugs approved for the treatment of hepatitis B.

Acute infection
An acute infection resolves spontaneously in 95-99 % of adults. It is not yet known whether antiviral therapy of the acute infection has a positive effect on the duration and progression of the disease. For this reason antiviral treatment is indicated only in special, exceptional cases, e.g. to avoid a fulminant course where liver function is already impaired.

Chronic infection
The decision to treat a chronic infection is made on the basis of the virus load in serum (the current threshold value is 10^4 copies or 2×10^3 IU/ml) and the serum transaminase level or the degree of inflammation/fibrosis in the biopsy (the HBe-Ag/HBe Ab status has no bearing on the decision).

There is a clear need for treatment in the following situations (Table 2):

HBV-DNA: > 2×10^3 IU/ml and

AST/ALT: > 2 x ULN* or

Histology: inflammatory activity

HBV-DNA: Detectable and
clear fibrosis or (decompensated) cirrhosis

*ULN: Upper Limit of Normal

There is no need for treatment in inactive HBs-Ag carriers who repeatedly have normal transaminases and whose HBV-DNA is negative or very low (< 2×10^3 IU/ml).
In other cases careful consideration is needed. In particular, patients with high viraemia, despite normal transaminase levels, have an increased risk of cirrhosis and HCC. It is now known that an inflammatory hepatic process or fibrosis can be present even with normal transaminase levels. For this reason the virus load plays an increasingly crucial role in diagnosing the need for treatment and needs to be checked regularly.

6. Treatment monitoring

The aim of treatment is to prevent cirrhosis, hepatocellular carcinoma and transplantation by suppressing virus replication and reducing inflammation of the liver tissue as efficiently as possible. Permanent elimination of the virus by HBs Ab seroconversion is achieved in just 5-10 % of cases. In the other cases a response to treatment is defined according to various criteria.

These are checked regularly (every 3-6 months) using the appropriate surrogate markers during and after treatment (2):

- Virological response:
  HBV-DNA < 2×10^3 IU/ml (ideal: < 60 IU/ml)
- Immunological response:
  Permanent HBe seroconversion
- Biochemical:
  Permanent AST/ALT normalization
- Histological:
  Reduction in inflammatory activity/
  degree of fibrosis

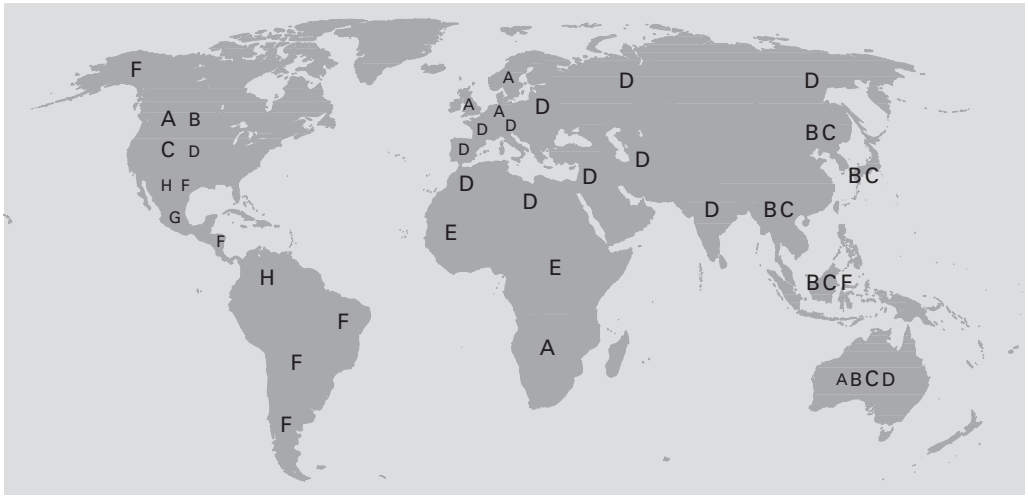

Fig. 3
Global distribution of HBV genotypes

7. Importance of the genetic variants of the hepatitis B virus

7.1 HBV genotyping

On the basis of genome-wide sequence differences of more than 8 %, eight genotypes are currently identified. These genotypes (A-H) show a specific geographical distribution (see Figure 3).
More and more study results describe significant clinical differences between the individual genotypes as regards the natural course of infection, the progression rate of the disease and the response to treatment.
Identification of the patient's genotype is currently of specific diagnostic relevance when choosing the suitable antiviral treatment regimen.
Genotypes A and B show a higher response rate with interferon treatment than genotypes C and D. For this reason genotyping is increasingly being used in decisions on treatment with IFN or nucleoside/nucleotide analogues.

7.2 Drug resistance

The clinically necessary long-term therapy of HBV infection with all the nucleoside/nucleotide analogues registered to date leads to the development of substance-specific resistance mutations. These are located on the HBV polymerase gene (see Figure 4). The cumulative resistance rate is very high, especially with lamivudine therapy, at approx. 20 % per year of treatment (approx. 70 % after 5 years). The lowest resistance rate in the limited period covered by the studies to date has been shown by the substance entecavir with a rate of < 1 % after a 3-year period of treatment.
Resistance development is associated with virological breakthrough and jeopardizes the outcome of treatment. It leads to a worsening of the clinical condition. Early detection is therefore very important.
Resistance testing should be considered if there is a rebound in viraemia during therapy or if there is no initial response to treatment and if patient compliance has been confirmed. This allows the treatment to be changed or stepped up at short notice (2).

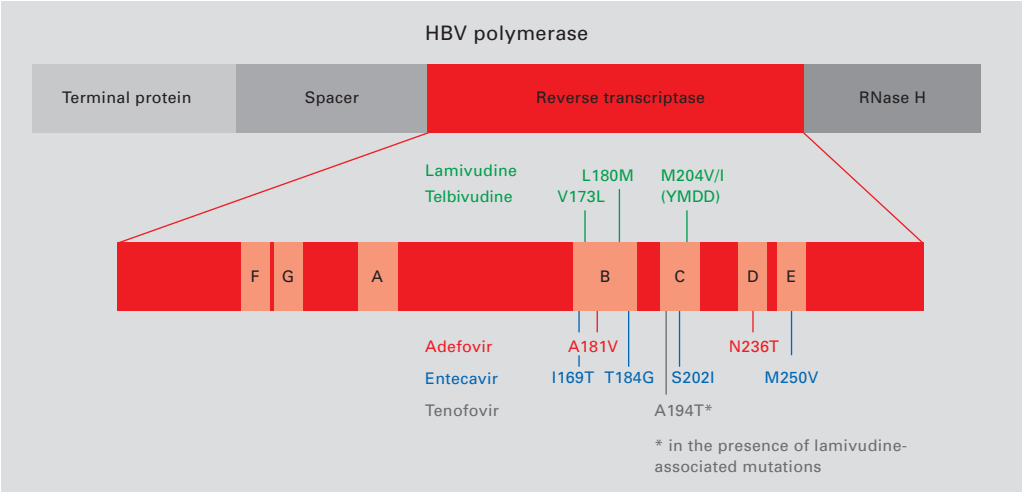

Fig. 4
Location of resistance-associated mutations in the HBV polymerase region which lead to the specified amino acid substitutions during the corresponding nucleoside/nucleotide treatment.

7.3 "Immune escape" mutants

These variants are based on several mutations of the antigenic "a" determinant of the HBs antigen. A conformational change of this peptide has such a strong effect on the binding of neutralizing anti HBs Abs that the virus eludes detection by commercial HBs-Ag tests. This variant may also, despite passive or active immunization, have high replication activity and, in the presence of HBs antibodies, develop chronic hepatitis B ("immune escape").

Potential mutations of the "a" determinant can be considered in the following situations:

- Isolated HBc reactivity
- Discordant HBs-Ag results from various assays
- HBe-Ag positive patients with negative HBsAg
- Simultaneous presence of HBs-Ag and HBs Abs (except in seroconversion)

The prevalence of the variant in Europe is estimated to be very low, except in the Mediterranean region. Testing of the "a" determinant is still of no particular importance in routine diagnostic investigations.

7.4 HBV e antigen (HBe) variants

The HBe antigen largely corresponds to the core protein with, at the N terminus, a short peptide which is coded by the precore region. The HBe antigen is not a constituent of the virus particle, but is secreted into the bloodstream. Its function is not fully understood. HBe antibodies are formed during HBe seroconversion. As a result the HBe antigen is eliminated and the serum HBV-DNA drops by several log stages.
The virus often escapes this immunological selection pressure by developing anti HBe escape variants which halt production of the HBe antigen.
Clinically, infections manifest themselves with precore/core variants in severe forms. Fulminant hepatitis B is also associated with these HBe variants, which are also found more often in chronic active hepatitis and hepatocellular carcinoma than in asymptomatic carriers.
There is also a connection between the lack of response to treatment with IFN-alfa and these variants. Testing for the HBe variants is not routinely performed.

Relevant laboratory analyses

- HBV DNA qantitative
- HBV genotyping
- HBV drug resistance
- AST, ALT, AFP, HBV serology

(see our test list for individual specimen requirements)

- HBV immune escape and precore/ core variants can also be requested (contact us for further information)

8. References

1. Chen CJ et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295:65-73.

2. Cornberg M et al. Prophylaxe, Diagnostik und Therapie der Hepatitis-B-Virus-(HBV) Infektion. Z. Gastroenterol 2007; 45: 1-50.

3. Iloeje UH et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006; 130:678-686.

4. Lok, AS, McMahon, BJ. Chronic hepatitis B. Hepatology 2007; 45:507-539.

5. Valsamakis, A. Molecular testing in the diagnosis and management of chronic hepatitis B. Clin Microbiol Rev 2007; 20; 426-439.

6. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: What we knew in 1981 and what we know in 2005. Hepatology 2006; 43 Suppl.1:S173-S181.

7. Gößner, N. Diagnostik viraler Hepatitiden. Bioscientia Bericht 78, 2008

8. European Association fo the Study of the Liver. EASL. Clinical Practice Guidelines: Management of chronic hepatitis B. J Hepatol 2009; 50: in press.

9. http://www.who.int/mediacentre/factsheets/fs204/en/index.html

10. Robert Koch Institut: Epidemiologisches Bulletin 49; Dezember 2007

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Bioscientia
Institut für Medizinische Diagnostik GmbH
Konrad-Adenauer-Strasse 17
55218 Ingelheim
Germany

phone: +49 6132 7 81-203
+49 6132 7 81-224
+49 6132 7 81-165
fax: +49 6132 7 81-236

int.support@bioscientia.com
www.bioscientia.com

Author:
Dr. rer. nat. Peter Gohl
Molecular Biologist

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