

degree of hepatic fibrosis in relapsers. However, the classification F4 is not included in Japanese clinical trials, and there have been no reports of therapeutic results stratified for the degree of hepatic fibrosis.

In this way, the response to previous therapy is at present the most important predictive factor for SVR rates achieved by SMV + Peg-IFN + RBV triple therapy.

There is presently no evidence available concerning the therapeutic efficacy of SMV + Peg-IFN + RBV triple therapy in non-responders to previous TVR + Peg-IFN + RBV triple therapy. However, retreatment with SMV-based therapy, with particular caution regarding adverse reactions, is an option in patients previously administered TVR-based therapy who were unable to tolerate adequate dosages of one or more agents due to adverse reactions.

When previously treated patients undergo retreatment with a combination including RBV, if RBV was not included in the previous IFN or Peg-IFN monotherapy regimen, the response to the earlier therapy is not a strong predictive factor for the efficacy of further treatment, so in general follow the treatment protocol for treatment-naïve patients. If the HCV RNA decrease at week 12 of the previous treatment is unknown, but it is clear that HCV RNA did not become negative, follow the retreatment protocol for null responders.

#### Recommendations

- The response to previous therapy is the best indicator for the response to retreatment in patients who were non-responders to previous IFN/Peg-IFN + RBV combination therapy. The relationship between IL28B SNPs and therapeutic efficacy is unclear at present.
- Retreatment with RBV combination therapy in patients previously administered IFN or Peg-IFN monotherapy should in general follow the treatment protocol for treatment-naïve cases. If the HCV RNA decrease at week 12 of the previous treatment is unknown, but it is clear that HCV RNA did not become negative, follow the null response retreatment protocol.
- There is presently no evidence available concerning the therapeutic efficacy of SMV + Peg-IFN + RBV triple therapy in non-responders to previous TVR + Peg-IFN + RBV triple therapy.

## 4.2 Selection of antiviral therapy in previously-treated patients (retreatment) (Fig. 7A, 7B)

### 4.2.1 Elderly patients

SMV + Peg-IFN + RBV triple therapy should be commenced promptly if treatment is likely to be tolerated.

In particular, relapsers and partial responders are favorable indications. As for null responders, in the overseas clinical trial (ASPIRE), SVR rates of approximately 50% were achieved when SMV + Peg-IFN + RBV combination therapy administered to null responders to previous treatment. Introduction of this regimen is therefore recommended to null responders, although it may be an option to await the advent of newer agents with fewer adverse reactions if problems with tolerability are anticipated. TVR + Peg-IFN + RBV triple therapy is another option, although it is recommended that TVR therapy should be commenced at a reduced dosage of 1500 mg/day as in treatment-naïve cases, and great caution is still required in its use.

The risk of hepatocellular carcinogenesis is high in elderly patients, and when viral eradication cannot be achieved protective therapies (SNMC, UDCA) should be administered with the aims of biochemical improvement and inhibiting hepatocellular carcinogenesis.<sup>1</sup> Long-term low dose Peg-IFN (IFN) therapy is another option.<sup>1</sup>

#### Recommendations

- In retreatment of elderly patients, if treatment is likely to be tolerated, SMV + Peg-IFN + RBV triple therapy should be administered to relapsers and partial responders.
- SVR rates of approximately 50% are achieved with SMV + Peg-IFN + RBV combination therapy in null responders to previous treatment, and introduction of this regimen is therefore recommended to null responders as well. If problems with tolerability are anticipated, it may be an option to await the advent of newer agents with fewer adverse reactions.
- When viral therapy is not administered, protective therapies should be administered to patients with abnormal ALT levels. Long-term low dose Peg-IFN (IFN) therapy is another option.

### 4.2.2 Non-elderly patients

As with elderly patients, as a general rule non-elderly patients with advanced fibrosis and associated high risk of hepatocellular carcinogenesis should be administered SMV + Peg-IFN + RBV combination therapy. Even in patients with mild fibrosis and a lower risk of carcinogenesis, a high SVR rate of approximately 90% is achieved with SMV + Peg-IFN + RBV combination therapy in relapsers and partial responders. Therefore, if treatment is likely to be tolerated, SMV-based triple therapy should be administered to this patient group.

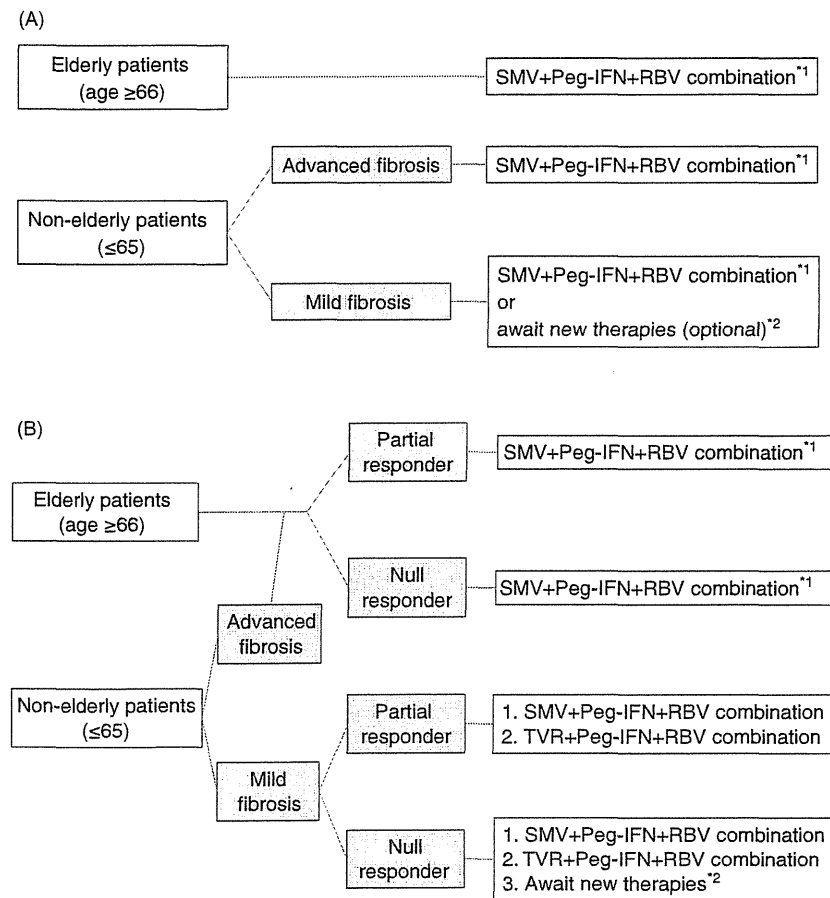


Figure 7 Treatment flow chart for previously-treated patients (retreatment). A. Relapsers. B. Non-responders. “Previously-treated” refers to previous treatment with Peg-IFN(IFN)/RBV combination therapy. Consider IFNβ + RBV combination if depressive symptoms present. Follow the null response retreatment protocol in non-responders if the quantitative decrease in HCV RNA at week 12 of the previous treatment is unknown. \*1 TVR + Peg-IFN + RBV triple therapy is another option (TVR should be commenced at a reduced dosage of 1500 mg/day in the elderly). \*2 Protective therapy or low dose Peg-IFN/IFN therapy if abnormal ALT levels.

On the other hand, for non-elderly null responders with mild fibrosis, if adverse reactions are a concern, it may be reasonable to await the advent of newer agents with fewer adverse reactions. When there are no problems with tolerability, SMV + Peg-IFN + RBV combination therapy can be commenced in patients who meet the therapeutic indications for antiviral therapy (ALT > 30 U/L or platelet count < 150 000/μL).

TVR + Peg-IFN + RBV triple therapy is an alternative option in cases with mild fibrosis, where safety is relatively guaranteed.

**Recommendations**

- In general, SMV + Peg-IFN + RBV triple therapy should be administered for retreatment of non-elderly

patients with advanced fibrosis, as for elderly patients.

- Even in patients with mild fibrosis, a high SVR rate of approximately 90% is achieved with SMV + Peg-IFN + RBV combination therapy in relapsers and partial responders. If treatment is likely to be tolerated, SMV-based triple therapy should be therefore administered to this patient group.
- On the other hand, for non-elderly null responders with mild fibrosis, if adverse reactions are a concern, it may be reasonable to await the advent of newer agents with fewer adverse reactions. When there are no problems with tolerability, SMV + Peg-IFN + RBV combination therapy can be commenced in patients who meet the therapeutic indications for antiviral

therapy (ALT > 30 U/L or platelet count < 150 000/ $\mu$ L).

- In non-responders (partial and null responders), TVR + Peg-IFN + RBV triple therapy is an alternative option in cases with mild fibrosis, if treatment is likely to be tolerated.

## CONFLICTS OF INTEREST

THE MEMBERS OF Drafting Committee for Hepatitis Management Guidelines have received royalty from SRL, lecture fees from Ajinomoto Pharmaceuticals, MSD, Daiichi-Sankyo, Dainippon-Sumitomo Pharma, Mitsubishi Tanabe Pharma, Chugai Pharmaceutical, Bristol-Myers-Squibb, Janssen Pharmaceutical Companies, and research support from Eisai, MSD, Kan Research Institute, Chugai Pharmaceutical, Mitsubishi Tanabe Pharma, Dainippon-Sumitomo Pharma, Toray, Minophagen Pharmaceutical.

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## Special Report

## JSH Guidelines for the Management of Hepatitis B Virus Infection

Drafting Committee for Hepatitis Management Guidelines and the Japan Society of Hepatology<sup>\*,\*\*</sup>

## PREFACE

THE JAPAN SOCIETY of Hepatology established the Drafting Committee for Hepatitis Management Guidelines in November 2011, and published the Guidelines for the Management of Hepatitis C in May 2012 (English version, Jan 2013). Thence the Committee decided our next task of high priority is to produce the practical guidelines for hepatitis B, also a significant burden to the health care system. Here the Committee has launched the Guidelines for the Management of

Hepatitis B Virus Infection. As with hepatitis C virus, this is a field that changes rapidly with the accumulation of new evidence, accompanied by changes in the level of evidence, so we have elected not to show evidence levels. We plan to update these guidelines at appropriate intervals, as new evidence comes to hand.

## 1. INTRODUCTION

## 1.1 Hepatitis B virus

IT IS ESTIMATED that there are 400 million patients of persistent hepatitis B virus (HBV) infection in the world.<sup>1</sup> In Japan, the HBV infection rate is around 1%. HBV infection at birth or during infancy leads to persistent infection in over 90% of cases. Approximately 90% of these undergo seroconversion from HBe antigen (HBeAg) positive at the initial stage to anti-HBe antibody positive and become inactive carriers, and in virtually all cases the condition effectively stabilizes. But in the remaining 10% the virus remains active, leading to chronic hepatitis, and in around 2% of cases annually, there is further progression to liver cirrhosis, with potential for hepatocellular carcinoma (HCC) and liver failure.<sup>2–4</sup>

Clinical research on HBV dates back to the discovery of the Australia antigen (later renamed HBs antigen; HBsAg) by Blumberg *et al.* in 1964. Prince *et al.* and Okouchi *et al.* subsequently reported a link between the Australia antigen and hepatitis. And there have been various other discoveries demonstrating that the existence of an asymptomatic carrier, who does not develop hepatitis following HBV infection and indicating HBV as a cause of chronic liver diseases. The base form of HBV, known as the Dane particle, was discovered in 1970, followed by the identification of HBeAg in 1972. In 1979, the whole HBV genome was successfully cloned from virus particles, enabling measurement of the virus gene (HBV DNA) for the first time.

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In Japan, screening for the HBsAg was introduced at blood centers in 1972. 1986 was the year of the introduction of an anti-HBV vaccine and immunoglobulin for newborns designed to prevent vertical (mother-to-child) infection. This was highly effective in arresting the development of new HBV carriers through vertical infection, causing a marked decline in HBsAg positive rates among juveniles. The incidence of acute hepatitis caused by HBV infection, however, has not declined, mainly as a result of horizontal transmission associated with sexual activity. In recent years, there has been an increase in infection rates for the HBV genotype A, which frequently causes persistent infection.<sup>5</sup>

**1.2 Natural history of patients with persistent HBV infection**

HBV in itself is considered to have little or no cytotoxicity. Hepatocellular damages are generally caused by cellular immunity associated with cytotoxic T cells, which represent the host’s immune response attacking HBV infected cells. Other immunity-associated cells such as antigen-specific helper T cells, macrophages,

natural killer cells and natural killer T cells also contribute to inflammation and illness. Patients suffering from persistent HBV infection generally are categorized into four phases defined by the host immune response and the replication of HBV DNA, as shown in Figure 1.

(1) Immune tolerance phase

In infants, when the host immune response is immature, HBV infection inevitably leads to persistent infection. This is followed by a state of immune tolerance, with high levels of HBeAg and HBV DNA replication activity. The host in this phase is termed as an asymptomatic carrier, with ALT levels within the normal range and negligible activity of hepatitis. Infectivity is high. In most cases, infection during infancy is followed by a prolonged immune tolerance period lasting from a few to more than 20 years.

(2) Immune clearance phase

By adulthood, the immune response to HBV becomes an active one, which develops active hepatitis in the immune clearance phase. During the process of HBeAg seroconversion, with disappearance of HBeAg and appearance of anti-HBe antibody, the replication of

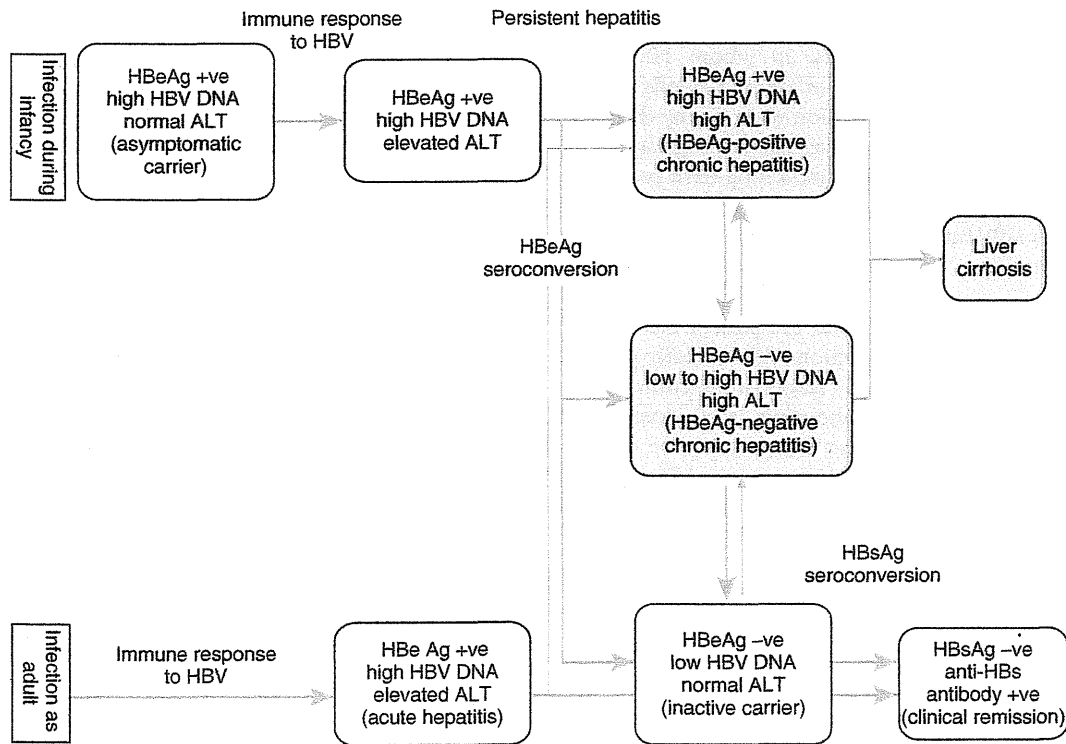


Figure 1 Natural course of persistent HBV infection.

HBV DNA is inhibited, thereby encouraging quiescence of hepatitis. However liver disease can progress in cases of persistent hepatitis that remain HBeAg positive for extended periods (HBeAg-positive hepatitis).

### (3) Low replicative phase (inactive phase)

HBeAg seroconversion usually results in quiescence of hepatitis, with HBV DNA levels dropping below 4 log copies/mL (inactive carrier). In 10–20% of cases, however, HBeAg seroconversion is followed by increased HBV replication in the HBeAg negative state, causing the exacerbation of hepatitis (HBeAg-negative hepatitis). In a further 4–20% of cases, the HBeAg actually reappears and anti-HBe antibody disappears, a phenomenon known as reverse seroconversion.

### (4) Remission phase

In some cases, HBeAg seroconversion causes appearance of anti-HBs antibody and disappearance of HBsAg. In the remission phase, improvement is seen in both blood tests and liver biopsy findings. The natural rate of disappearance of HBsAg in patients with persistent HBV infection is thought to be around 1%.

The natural course of persistent HBV infection can be therefore a progression from HBeAg-positive asymptomatic carrier, through HBeAg-positive (or negative) chronic hepatitis, to cirrhosis. HCC occurs at an annual rate of 5–8% in patients with cirrhosis. At the same time, however, in inactive carriers, in whom HBV DNA declines and serum ALT values are persistently normal following HBeAg seroconversion without any therapeutic intervention, there is a lower risk of progression and hepatocarcinogenesis with a good long-term prognosis. Thus it is important that treatment of patients with persistent HBV infection should be based on a thorough understanding of the natural course as described above.

Where infection occurs after the patient has reached adulthood, an immune reaction will normally develop against HBV during the early stages of infection. After a period of acute hepatitis, the virus is eliminated and quiescence occurs. With the rising incidence of HBV genotype A in recent years, however, we have seen an increasing number of adult infection cases progressing to chronic hepatitis.<sup>5</sup>

## 1.3 Treatment goals – what should we aim for?

The treatment goal of antiviral therapy for persistent HBV infection is to improve the life expectancy and quality of life (QOL) of the patient with HBV infection.

HBV infection is directly associated with the life expectancy in three ways, due to acute liver failure,

chronic liver failure, and HCC. Of these three, acute liver failure usually presents the most difficult challenge in terms of prediction and prevention. Management usually centers on preventing HBV reactivation associated with immunosuppressant agents. Meanwhile, the risk factors for chronic liver failure and HCC associated with persistent HBV infection are known, and can be successfully eliminated via antiviral therapy in order to reduce the risk of disease. In other words, we can say that the treatment goal of antiviral therapy in patients with persistent HBV infection should be to inhibit activity of hepatitis and progression of hepatic fibrosis in order to prevent chronic liver failure and reduce the risk of HCC, thereby improving the life expectancy and QOL of the patient with HBV infection. HBsAg is considered the most effective surrogate marker for achieving this ultimate goal, and HBsAg elimination should be defined as the long-term goal of antiviral therapy in patients with persistent HBV infection (Table 1).

Antiviral therapy has three short term goals leading to the elimination of HBsAg: persistent normalization of ALT ( $\leq 30$  U/L), HBeAg negative and anti-HBe antibody positive (HBeAg seroconversion in HBeAg-positive cases and maintain HBeAg negative status in HBeAg-negative cases), and suppression of HBV DNA replication.

Target serum HBV DNA levels differ between chronic hepatitis and cirrhosis, and also depending on the therapeutic agents. Nucleos(t)ide analogue (NA) therapy is highly effective at producing negative HBV DNA, and at maintaining a negative status through treatment. Thus the on-treatment goal should be to attain an HBV DNA negative status, as determined using high-sensitivity real-time PCR, for both chronic hepatitis and cirrhosis alike. For interferon (IFN) therapy, since HBeAg seroconversion and HBsAg reduction or elimination are expected outcomes following completion of therapy, there is no need for an on-treatment goal of reduced HBV DNA. It should be recommended to complete the full course of therapy over 24 to 48 weeks.

The off-treatment goal (i.e., after IFN therapy has concluded and NAs are no longer administered) is the absence of active hepatitis with no risk of further progression on no medication. Accordingly, the target at 24 to 48 weeks after the end of treatment is set as  $< 4.0$  log copies/mL for chronic hepatitis, and negative HBV DNA for cirrhosis.

### Recommendations

- *The treatment goal for antiviral therapy in patients with persistent HBV infection is to prevent liver failure and inhibit HCC by suppressing activity of hepatitis*

Table 1. Treatment goals for antiviral therapy

	Chronic hepatitis	Liver cirrhosis
Long-term goal	HBsAg elimination	HBsAg elimination
Short-term goals		
ALT	Persistent normal <sup>1</sup>	Persistent normal <sup>1</sup>
HBeAg	Negative <sup>2</sup>	Negative <sup>2</sup>
HBV DNA <sup>3</sup>		
On-treatment (Ongoing NA therapy)	Negative	Negative
Off-treatment (IFN completed/NA therapy ceased <sup>4</sup> )	< 4 log copies/ml	Negative <sup>5</sup>

## Notes

<sup>1</sup>. Normal range of ALT is defined as ≤30 U/L.

<sup>2</sup>. Conversion to HBeAg-negative in HBeAg-positive cases, and maintain HBeAg-negative in HBeAg-negative cases.

<sup>3</sup>. As measured using high-sensitivity PCR (real-time PCR).

<sup>4</sup>. At 24–48 weeks following completion of antiviral therapy.

<sup>5</sup>. NA therapy should not to be ceased in patients with cirrhosis.

*and progression of liver fibrosis, thereby improving the patient's life expectancy and overall QOL.*

- *HBsAg is considered the most effective surrogate marker for attaining this treatment goal. The long-term goal of antiviral therapy is to eliminate HBsAg.*
- *The three short-term goals of antiviral treatment prior to elimination of HBsAg are persistent normalization of ALT, HBeAg negative and positive anti-HBe antibody, and suppression of HBV DNA replication.*
- *The on-treatment goal is negative HBV DNA; this applies to both chronic hepatitis and cirrhosis.*
- *Since HBeAg seroconversion and reduction (or elimination) of HBsAg are expected outcomes following completion of therapy, on-treatment HBV DNA target levels are not applied, and it should be recommended to complete a full course of treatment of 24 to 48 weeks.*
- *The off-treatment goals (following IFN therapy and cessation of NAs) are <4.0 log copies/mL HBV DNA (chronic hepatitis), and negative HBV DNA (cirrhosis).*

#### 1.4 Pharmacotherapy – which agents should we use?

Currently IFN and NAs are employed in antiviral therapy for persistent HBV infection. Table 2 lists the approval process of main antiviral therapy agents used in Japan by national medical insurance.

IFN therapy is intended to achieve lasting benefits from a limited treatment period. IFN therapy was first introduced to Japan in 1987. Initially, it was limited to a 28-day course of treatment, although this was extended to 6 months in 2002. In 2011, Peg-IFN (pegylated interferon) was approved for treatment of

chronic hepatitis B in clinical settings. In addition to inhibiting the replication of HBV DNA, IFN has both antiviral and immunomodulatory effects. Therapeutic effects of IFN further improved with the advent of Peg-IFN.

IFN therapy offers some key advantages. Treatment is for a fixed period, and if an adequate therapeutic response is achieved, no further treatment is required. IFN therapy can therefore produce lasting therapeutic benefits in the drug-free state. Furthermore, overseas studies have reported that IFN therapy is also highly effective at eliminating HBsAg over the long term. However, disadvantages include the fact that only 20–30% of HBeAg positive cases and 20–40% of HBeAg negative cases respond well to Peg-IFN treatment; patients are required to attend hospital weekly; there are several possible adverse reactions associated with treatment; and finally, Peg-IFN treatment for cirrhosis is not currently approved by Japanese national medical insurance.

Meanwhile, NAs are a form of antiviral agent originally developed as a pharmacological therapy for

Table 2 Approval process of antiviral therapy in Japan

1987	Conventional interferon (28-day course, HBeAg positive only)
2002	Conventional interferon (six-month course, HBeAg positive only)
2000	Lamivudine
2004	Adefovir
2006	Entecavir
2011	Peg-IFN

**Table 3** Peg-IFN versus entecavir – key characteristics

	Peg-IFN	Entecavir
Mechanism	Induces antiviral proteins, immunopotentialiation	Directly inhibits virus replication
Route of administration	Subcutaneous injection	Oral
Therapy period	Limited to 24–48 weeks	Generally unrestricted (long-term)
Drug resistance	None	Around 1% after 3 years
Adverse effects	Frequent and varied	Rare
Teratogenicity/carcinogenicity	None	Teratogenic; possibly carcinogenic when administered for long periods
Use during pregnancy	Generally contraindicated during pregnancy*	Generally contraindicated during pregnancy
Decompensated liver cirrhosis	Contraindicated	Allowed
Therapeutic response rate	20–30% in HBeAg positive, 20–40% in HBeAg negative (difficult to estimate)	Very high
Ongoing benefits post therapy	Very high where seroconversion occurs	Low

\*Guidelines for the treatment of chronic hepatitis B from the European Association for the Study of the Liver (EASL)<sup>6</sup> and the Asia-Pacific Association for the Study of the Liver (APASL)<sup>7</sup> prohibit administration of Peg-IFN to pregnant women.

human immunodeficiency virus (HIV). Once it was established that NAs also hinder the reverse transcription mechanism in HBV proliferation, the use of lamivudine, adefovir and entecavir for hepatitis B was approved over the period 2000 to 2006. NAs have a powerful inhibiting effect on HBV DNA proliferation, regardless of genotype, and act as antiviral agents and promote quiescence of hepatitis in nearly all patient types, including those of more advanced age with little prospect of spontaneous remission.

In particular entecavir, currently the first-choice drug, has a very low incidence of resistant mutations compared to lamivudine, and is highly effective at HBV DNA negative conversion and ALT normalization, irrespective of baseline factors. It has virtually no adverse reactions in the short term. On the other hand, it requires a lengthy administration period, due to the propensity for flare-up if treatment is withdrawn, increasing the likelihood of drug-resistant mutations and raising safety issues. Entecavir is also said to be less successful than IFN treatment in reducing the HBsAg load.

Thus, Peg-IFN and entecavir have quite different pharmacological properties and cannot be compared directly, as shown in Table 3. In both HBeAg positive<sup>8–21</sup> and negative cases,<sup>15,22–26</sup> Peg-IFN has been shown to be more effective in terms of the long term goal of HBsAg elimination, while entecavir is more effective in terms of the short-term goals of normalizing ALT and suppressing HBV DNA proliferation (see Tables 4,5). Peg-IFN

**Table 4** Peg-IFN versus entecavir – outcomes for HBeAg positive patients

	Peg-IFN	Entecavir
Short term goals		
HBV DNA negative		
Short term	14% <sup>8</sup>	67–75% <sup>14,15</sup>
Long term	13% <sup>11–13</sup>	93–94% <sup>15,16</sup>
HBeAg seroconversion		
Short term	24–36% <sup>8–10</sup>	16–21% <sup>14,15</sup>
Long term	37–60% <sup>11–13</sup>	34–44% <sup>17–19</sup>
ALT normalization		
Short term	37–52% <sup>8–10</sup>	68–81% <sup>14,15</sup>
Long term	47% <sup>11–13</sup>	87–95% <sup>15,20</sup>
Long term goals		
HBsAg elimination		
Short term	2.3–3.0% <sup>8–10</sup>	1.7% <sup>14</sup>
Long term (overall)	11% <sup>11</sup>	0.6–5.1% <sup>16,17,21</sup>
Long term	30% <sup>11</sup>	
	(responders*)	

Peg-IFN (Peg-IFN $\alpha$ -2a<sup>8–10,12</sup> and Peg-IFN $\alpha$ -2b<sup>11,13</sup>):

Short term: 24 weeks after ending treatment.<sup>8–10</sup>

Long term: Three years after ending treatment.<sup>11</sup>

\* Responders: HBe negative at 26 weeks after the end of treatment (37% of total, though 21% received additional lamivudine treatment).

Entecavir

Short term: One year after starting treatment.<sup>14</sup>

Long term: Two years<sup>20,21</sup>, three years,<sup>17–19</sup> four years,<sup>15</sup> and five years<sup>16</sup> after starting treatment.



**Table 5** Peg-IFN versus entecavir – outcomes for HBeAg negative patients

	Peg-IFN	Entecavir
<b>Short term goals</b>		
HBV DNA negative		
Short term	19–20% <sup>22</sup>	90–99% <sup>15,25</sup>
Long term	18–21% <sup>23,24</sup>	100% <sup>15</sup>
Reduced HBV DNA levels		
Short term	43–44% <sup>22</sup>	
(<20,000 copies/mL)		
Long term	25–28% <sup>23</sup>	
(<10,000 copies/mL)		
ALT normalization		
Short term	59–60% <sup>22</sup>	78–85% <sup>15,25</sup>
Long term	31% <sup>23</sup>	91% <sup>15</sup>
<b>Long term goals</b>		
HBsAg elimination		
Short term	2.8–4.0% <sup>22</sup>	0.3% <sup>25</sup>
Long term (overall)	8.7–12% <sup>23,24</sup>	0% <sup>15</sup>
Long term (responders*)	44% <sup>23</sup>	

Peg-IFN (Peg-IFN $\alpha$ -2a:<sup>22–24</sup>)

Short term: 24 weeks after ending treatment.<sup>22</sup>

Long term: Three years<sup>23</sup> and five years<sup>24</sup> after ending treatment.

\* Responders: HBV DNA negative three years after ending treatment (15% of total).

Entecavir

Short term: One year after starting treatment.<sup>25</sup>

Long term: Four years after starting treatment.<sup>15</sup>

and entecavir also differ in terms of predictive factors for therapeutic efficacy, as shown in Table 6. It is therefore important that treatment of HBV should be tailored to the individual patient, based on a thorough understanding of the natural course of the disease and of the key differences between Peg-IFN and entecavir.

### Recommendations

- Peg-IFN and entecavir are substantially different pharmacotherapeutic agents that do not bear direct comparison.
- HBV treatment regimens should be tailored to the individual patient, based on a thorough understanding of the natural course of the disease and of the key differences between Peg-IFN and entecavir.

### 1.5 Indications for treatment – who should we treat?

Indications for antiviral therapies for persistent HBV infection are based on the need for treatment, related to a range of factors such as age, disease stage, degree of liver disease (inflammation and fibrosis), and risk of further progression to liver cirrhosis and/or HCC. The three key criteria that are currently used in determining whether to treat are histological progression, ALT levels and HBV DNA levels. In numerous reports on factors linked to antiviral therapeutic effects, ALT and HBV DNA levels have been shown to influence the progression of the disease, and are also noted as common factors associated with therapeutic effects for both IFN and NAs. Guidelines from the American Association for the Study of Liver Diseases (AASLD),<sup>27</sup> the European Association for the Study of the Liver (EASL),<sup>6</sup> the Asia Pacific Association for the Study of the Liver (APASL),<sup>7</sup> and the Japanese Ministry of Health, Labour and Welfare (MHLW) research group<sup>28</sup> all nominate these factors as patient selection criteria, as shown in Table 7. ALT and HBV DNA levels change over the natural course of the disease, and this must be taken into account when deciding when to initiate treatment.

Recently a link has been posited between HBsAg levels and carcinogenesis, with some reports claiming that patients with high HBsAg levels (even when the HBV

**Table 6** Peg-IFN versus entecavir – predictive factors for therapeutic efficacy

	HBeAg positive		HBeAg negative	
	Peg-IFN	Entecavir	Peg-IFN	Entecavir
Race	None	None	None	None
Age	Inconsistent	None	None or young	None
Gender	None or female	None	None or female	None
ALT	High	High	None or high	None or high
HBV DNA levels	Low	Low	None or low	Low
HBsAg levels	Low		None	
Genotype	None or A (vs D)	None	None or B, C (vs D)	None
IL28B	Major			

**Table 7** Treatment target selection criteria in leading guidelines

	AASLD (2009) <sup>6</sup>	EASL (2012) <sup>7</sup>	APASL (2008) <sup>27</sup>	MHLW (2013) <sup>28</sup>
<b>HBeAg-positive chronic hepatitis</b>				
HBV DNA (log copies/mL)	≥5	≥4	≥5	≥4
ALT	1) >2 × ULN 2) 1–2 × ULN	1) >1 × ULN 2) <1 × ULN	1) >2 × ULN 2) ≤2 × ULN	≥31 U/l
	>40 years Family history of HCC → liver biopsy	→ liver biopsy	>40 years → liver biopsy	
<b>HBeAg-negative chronic hepatitis</b>				
HBV DNA (log copies/mL)	≥4	≥4	≥4	≥4
ALT	1) >2 × ULN 2) 1–2 × ULN	1) >1 × ULN 2) <1 × ULN	1) >2 × ULN 2) ≤2 × ULN	≥31 U/l
	>40 years Family history of HCC → liver biopsy	→ liver biopsy	>40 years → liver biopsy	
<b>Cirrhosis</b>				
HBV DNA (log copies/mL)	≥4 (<4†)	detectable	≥4	≥2.1
ALT	>1 × ULN (>2 × ULN†)	normal	normal	normal

†If ALT >2 × ULN, treatment may be indicated even when HBV DNA is <4 log copies/mL.

DNA level is less than 4 log copies/mL following HBeAg seroconversion) have higher rates of further progression and carcinogenesis.<sup>29</sup> However there is still insufficient evidence on the link between HBsAg levels and long term outcomes, and further studies are required before HBsAg levels can be incorporated into the patient selection criteria.

#### Recommendations

- The three key criteria currently used to determine whether to treat persistent HBV infection are histological progression, ALT levels and HBV DNA levels.
- The question of whether HBsAg levels should be added to these criteria requires further studies.

#### 1.5.1 Chronic hepatitis – who are not indicated for treatment?

Indications for treatment for chronic hepatitis include abnormal ALT levels, high HBV DNA levels, and presence of histological liver disease. Treatment is therefore not indicated when ALT levels are within the normal range and histological disease is mild or absent altogether – in other words, for HBeAg positive asymptomatic carriers during the immune tolerance phase and

inactive carriers following HBeAg seroconversion. Note that in cases of HBeAg-positive chronic hepatitis with elevated ALT levels, there is a 7–16% probability (in annual terms) of the HBeAg seroconversion over the natural course of the disease.<sup>4,30–32</sup> Therefore, it may be advisable in such cases to wait a year before commencing treatment, in the anticipation of HBeAg seroconversion, where there is no evidence of advanced fibrosis and the patient is considered not at risk of fulminant hepatitis.

#### Recommendations

- Treatment is not indicated in HBeAg-positive asymptomatic carriers and HBeAg-negative inactive carriers.
- In patients with HBeAg-positive chronic hepatitis with elevated ALT levels with no evidence of advanced fibrosis and not considered at risk of acute liver failure, it may be advisable to wait for 12 months before commencing treatment.

#### 1.5.2 Definition of inactive carriers

The diagnosis of inactive carrier status requires considerable caution.

The first issue concerns the definition of the threshold for abnormal ALT levels. There is no broad consensus in the medical profession on what constitutes the upper limit of normal (ULN) for ALT levels. In nearly all clinical studies conducted in Japan and elsewhere, the normal value is defined as the standard or control value for the institution conducting the study. Some researchers have proposed an ULN of 30 U/L for males and 19 U/L for females,<sup>33</sup> although these figures have not been validated for hepatitis B. The threshold ALT value as treatment indication seems to be slowly lowered, encouraging more aggressive therapeutic intervention. In Japan, an MHLW research group has defined the indication for treatment at an ALT levels  $\geq 31$  U/L since 2008,<sup>28</sup> and thus the current Guidelines propose a normal ALT range for chronic hepatitis of  $\leq 30$  U/L, with  $\geq 31$  U/L defined as abnormal and therefore the trigger for treatment. When elevated ALT levels are associated with factors unrelated to HBV, such as fatty liver, or consumption of drugs and/or alcohol, antiviral therapy is not indicated.

Similarly, consensus is lacking on the definition of a normal HBV DNA level. As Table 7 shows, the latest AASLD, EASL and APASL guidelines employ differing treatment indications, although in all these guidelines levels have been progressively lowered in line with advances in treatment regimes. In cases of persistent HBV infection, studies have demonstrated that HCC occurs even in patients with normal ALT levels and cancer rates increase in line with the HBV DNA levels, with a statistically significant increase in the rate of carcinogenesis when the HBV DNA levels are over 4 log copies/mL.<sup>34</sup> Liver biopsies in HBeAg negative patients with ALT levels consistently lower than 40 U/L (measured at least three times in a year) indicate negligible active hepatitis and fibrosis when the HBV DNA levels is less than 4 log copies/mL, with a good long term prognosis.<sup>35</sup>

Therefore, in the current Guidelines, inactive carriers after HBeAg seroconversion in whom treatment is not indicated is defined as subjects in a drug free status (no antiviral therapy) satisfy all the following conditions in three or more blood tests taken over the course of at least one year:

- 1 Persistently negative HBeAg;
- 2 Persistently normal ALT levels ( $\leq 30$  U/L); and
- 3 HBV DNA  $< 4.0$  log copies/mL.

Note that patients who satisfy the above conditions but exhibit fibrosis are considered to have a high risk of hepatocarcinogenesis. Therefore, if fibrosis is suspected on the basis of imaging studies or platelet counts, a

liver biopsy should be conducted to assess the need for treatment.

In the current Guidelines, the abovementioned off-treatment goals for chronic hepatitis are consistent with the definition of an HBeAg negative inactive carrier, namely an HBV DNA level of less than 4.0 log copies/mL. Accordingly, when the off-treatment goal is achieved the patient becomes an HBeAg negative inactive carrier and treatment is no longer required.

#### Recommendation

- An HBeAg negative inactive carrier is defined as a patient who satisfies three key requirements in three or more blood tests taken over the course of a year or more: HBeAg negative, ALT  $\leq 30$  U/L, and HBV DNA  $< 4$  log copies/mL.

#### 1.5.3 Indications for liver biopsy

A liver biopsy provides valuable information for determining whether antiviral therapy is indicated. In cases where ALT levels are normal or show a gradual or intermittent increase, a liver biopsy is optionally considered, irrespective of whether the treatment indication thresholds given below are met. Treatment is indicated when findings of liver biopsy demonstrate moderate or greater liver fibrosis (Metavir 2 or more) or active hepatitis. A liver biopsy is particularly important in patients  $\geq 40$  years with high HBV DNA levels,<sup>2,36,37</sup> or platelet counts  $< 150\,000$  / $\mu$ l, or a family history of HCC,<sup>38,39</sup> due to the increased risk of carcinogenesis. Since it is often difficult to distinguish whether fibrosis is advanced or not in HBeAg negative inactive carriers, a liver biopsy is required in order to ensure an accurate diagnosis. Conversely, a liver biopsy solely for the purpose of assessing treatment indication is not considered necessary for clinically demonstrable cases of cirrhosis or chronic hepatitis where the ALT levels is persistently greater than twice the upper limit of normal.

Hepatic fibrosis can be evaluated via noninvasive alternatives to biopsy, such as serum fibrosis markers, imaging studies including CT and ultrasound, and liver stiffness measurement.<sup>40–44</sup> Confirmation of hepatic fibrosis using any of these techniques is considered a treatment indication. Note that the use of serum fibrosis markers alone is not sufficiently accurate for assessment of the degree of fibrosis. There are several useful serum fibrosis markers, including platelet count, serum  $\gamma$  globulin levels, and serum  $\alpha$  macroglobulin levels, but none of these should be used as the sole marker.<sup>45</sup>

#### 1.5.4 Chronic hepatitis – who are indicated for treatment?

Chronic hepatitis cases that qualify as neither asymptomatic carriers nor inactive carriers are indicated for antiviral therapy. As Table 8 shows, cases of chronic hepatitis with ALT of 31 U/l or more and HBV DNA levels of 4.0 log copies/mL or more should be indicated for treatment, irrespective of HBeAg status and age. Patients who meet the definition of an inactive carrier but exhibit positive HBV DNA and progression of fibrosis are considered to have a high risk of hepatocarcinogenesis and should be indicated for treatment.

##### Recommendations

- Treatment is indicated in patients with chronic hepatitis with ALT levels  $\geq 31$  U/L and HBV DNA levels  $\geq 4$  log copies/mL, regardless of HBeAg status.
- Even in those cases not meeting the above criteria, if ALT levels rise slowly or intermittently, or the patient is aged  $\geq 40$  with a high HBV DNA levels, platelet count  $< 150\,000$  / $\mu$ l and/or family history of HCC, or if advanced fibrosis is suspected by imaging studies, the risk of hepatocarcinogenesis is high and liver biopsy (or noninvasive alternative) should be performed as an optional investigation to determine the extent of fibrosis.

Table 8 Treatment indications for persistent HBV infection

	ALT	HBV DNA levels
Chronic hepatitis†‡§	$\geq 31$ U/L	$\geq 4.0$ log copies/mL
Cirrhosis	–	Detectable

##### Notes

†The chronic hepatitis criteria apply to both HBeAg positive and negative patients.

‡Treatment is not indicated in asymptomatic and inactive carriers (defined as HBeAg negative, ALT  $\leq 30$  U/L, and HBV DNA  $< 4$  log copies/mL measured at least three times over a period of one year or more). In patients with HBeAg positive hepatitis with rising ALT levels, no evidence of advanced fibrosis and not considered at risk of acute liver failure, it may be advisable to withhold treatment for a year while monitoring ALT, HBeAg and HBV DNA levels. Note that treatment is indicated in inactive carriers with both positive HBV DNA and advanced fibrosis.

§In cases where ALT is rising slowly or intermittently, or the patient is aged  $\geq 40$  with high HBV DNA levels, platelet count  $< 150\,000$  / $\mu$ l and/or family history of HCC, or if advanced fibrosis is suspected by imaging studies, liver biopsy (or noninvasive alternative) should be performed to determine the extent of fibrosis.

- Even in patients meeting the definition of an inactive carrier, the combination of positive HBV DNA and advanced fibrosis suggests a high risk of hepatocarcinogenesis, and treatment is indicated.

#### 1.5.5 Liver cirrhosis

The criteria for treatment of chronic hepatitis – ALT and HBV DNA levels – are also considered in patients with cirrhosis. However, more aggressive therapeutic intervention is normally required and the treatment indications are different, since the risk of progression to hepatic failure and HCC is increased in cirrhotic patients. As Table 8 shows, treatment is indicated in cirrhotic patients with detectable HBV DNA irrespective of HBeAg status, ALT levels or HBV DNA levels, whereas if HBV DNA is below the detectable threshold antiviral treatment is not indicated.

##### Recommendation

- Treatment is indicated in patients with liver cirrhosis with detectable HBV DNA, regardless of HBeAg status and ALT or HBV DNA levels.

#### 1.5.6 Follow-up taking into consideration risk of hepatocarcinogenesis

Certain patients on a monitoring regimen with no treatment may yet be at high risk of hepatocarcinogenesis and should be placed under HCC surveillance with regular imaging, particularly those with contributing factors such as age  $\geq 40$ , male, alcohol consumption, high HBV load, family history of HCC, simultaneous infection with HCV/HDV/HIV, advanced liver fibrosis, low platelet count associated with advanced fibrosis, genotype C, and core promoter mutation. In patients with chronic hepatitis who become HBsAg negative and anti-HBs antibody positive, if cirrhosis was already present prior to elimination of HBsAg there is a high risk of hepatocarcinogenesis.<sup>46–52</sup> It is important to be aware of the ongoing risk of HCC even where cccDNA has been eliminated, due to HBV genome recombination.<sup>53–55</sup>

##### Recommendations

- Patients under a monitoring regimen who are at a high risk of hepatocarcinogenesis should be placed under HCC surveillance with regular imaging.
- It is important to be aware of the risk of HCC in cases of chronic hepatitis in whom HBsAg has disappeared.

## 2. CLINICAL SIGNIFICANCE OF HBV MARKERS

**H**BV MARKERS ARE an indispensable tool for the evaluation of acute hepatitis, chronic hepatitis and cirrhosis caused by HBV. Of the many different HBV markers used in clinical settings, in this section we will discuss HBV genotype, HBV DNA, HBsAg and HB core related antigens (HBcrAg), which are central to predicting disease course and therapeutic effects.

### 2.1 HBV genotype

Generally speaking, DNA viruses have fewer genetic mutations than RNA viruses; yet HBV, a DNA virus, is characterized by a viral proliferation mechanism including reverse transcription, and high rates of mutation.<sup>56</sup> HBV genotypes are classifications used to denote differences in the nucleic acid sequence associated with these genetic mutations. At present, nine genotypes have been identified, from A through J (with genotype I being a subtype of C). Types A, B, C and D account for nearly all genotypes extant in Japan. HBV genotype detection techniques include RFLP (restriction fragment length polymorphism), EIA (enzyme immunoassay), and nucleic acid sequence phylogenetic analysis. Of these only EIA, the technique developed by Usuda *et al.*, is approved by Japanese national medical insurance. EIA uses a combination of monoclonal antibodies capable of recognizing genotype-specific amino acids in the PreS2 domain.<sup>57</sup> Many differences have been reported in the clinical picture of HBV genotypes, which are useful for predicting outcomes and therapeutic effects, as shown in Table 9.<sup>58</sup>

HBV genotype A has been linked to horizontal infection among young people in Japan, with a steady

increase seen in the relative incidence of HBV genotype A, most notably in urban areas.<sup>59</sup> Recent studies have demonstrated a marked increase in infection rates for HBV genotype Ae, a genotype traditionally more prevalent in Western countries. This trend is particularly noticeable among young people in Japan, and has been attributed to sexual transmission and illicit drug usage. The normal pattern for a person who becomes infected with HBV during adulthood is a period of acute hepatitis after which the virus is eliminated, leading to quiescence of hepatitis. But with HBV genotype A, the virus tends to remain in the body after the acute phase, making the patient more likely to become a HBV carrier.<sup>5</sup> Nevertheless, outcomes are generally favorable for infections with HBV genotype A.

HBV genotype B is divided into two subtypes: HBV genotype Bj, found in Japan, and HBV genotype Ba, found in the rest of Asia. The Japanese strain (HBV genotype Bj) is distributed widely throughout Japan, from the Tohoku region and parts of Hokkaido in the north to Okinawa in the south. It generally causes very mild disease; most cases remain indefinitely as asymptomatic carriers with a negligible incidence of HCC. However, the Bj subtype has a mutation that can enter site 1896 in the pre-core region. Infection with the pre-core mutation strain causes the virus to proliferate rapidly through the body, potentially leading to fulminant hepatitis. Caution is required, as HBV genotype Bj and the 1896 mutation have been identified as independent risk factors for fulminant hepatitis.<sup>60</sup> HBV genotype Ba is a recombinant gene arrangement resembling in part HBV genotype C from the core promoter through to the core. HBV genotype Ba reportedly has a relatively high HCC risk, though the characteristics differ significantly between subtypes.

Table 9 Characteristics of HBV genotypes

Genotype	Regional specificity	Clinical characteristics in Japan
A	Western strains (HBV/A2/Ae) Asian/African strains (HBV/A1/Aa)	Often becomes chronic (5%–10%) Increasing prevalence, particularly in younger age groups
B	Asian strains (HBV/Ba) Japanese strains (HBV/B1/Bj)	Often becomes fulminant 10%–20% of total
C	Southeast Asia (HBV/Cs) East Asia (HBV/Ce)	High rate HCC Around 85% of total
D	Southern Europe, Egypt, India, etc.	Rare in Japan, resistant to treatment
E	Distributed through Western Africa	Extremely rare in Japan
F	Primarily central and southern America	Extremely rare in Japan
G	Reported in France, Germany, North America, etc.	Extremely rare in Japan
H	Primarily in central and southern America	Extremely rare in Japan
J	Borneo?	Extremely rare in Japan

HBV genotype C has a high HCC risk (higher even than HBV genotype Ba) and poor prognosis.<sup>61</sup> HBV genotype C is resistant to conventional IFN treatment.

HBV genotype D is normally found in Western countries. There are several localized pockets of infection and a number of subtypes in existence. The most common form is HBV genotype D1, which has been studied extensively and found to include a specific genetic mutation linked to disease phenotype.<sup>62</sup> Reports from Europe suggest that HBV genotype D is more resistant to IFN treatment than HBV genotype A, with a poor overall prognosis.<sup>63</sup>

#### Recommendations

- *HBV genotype A has been linked to horizontal infection among young people in Japan, who often become carriers following the acute hepatitis phase.*
- *Among HBV genotype B, subtype Bj is found only in Japan. Most cases remain asymptomatic carriers indefinitely, with negligible risk of HCC. However infection with pre-core mutations can lead to fulminant hepatitis.*
- *HBV genotype C has a high HCC risk and is resistant to conventional IFN treatment. The prognosis is poor.*

## 2.2 HBV DNA quantification

HBV DNA quantification is for assessment of liver disease, evaluation of therapeutic effects, and diagnosis of breakthrough hepatitis via HBV mutation. It is also linked to prognosis, since high HBV DNA levels indicates a high risk of cancer.<sup>34</sup> Conventional techniques for measuring HBV DNA levels in the past included the Amplicor HBV Monitor test (Roche Diagnostics Systems, Branchburg, NJ, USA) and the HBV DNA TMA-HPA test (transcription-mediated amplification-hybridization protection assay, Chugai Diagnostics Science, Tokyo). Real-time detection PCR testing has

become more popular in recent years, as it offers greater sensitivity and a wider measurement range. Real-time detection PCR installs primers and a probe on the well conserved S domain sequences on the HBV genome. The HBV probe is a short oligonucleotide for 5'-end fluorescence labeling and 3'-end quencher labeling. Real-time PCR HBV DNA quantification offers both high sensitivity and a broad dynamic range for detecting the quantity of PCR products based on PCR cycles once the fluorescence intensity reaches a given level. In addition to evaluation of antiviral therapeutic effects, improved sensitivity allows detection of viral breakthroughs, detection of HBV in HBeAg negative cases and latent HBV infections, as well as early prediction of exacerbation of hepatitis and HBV reactivation. Given that results correlate well with those of TMA methods, the real-time PCR method is now recommended for HBV DNA quantification in clinical settings.

Note the difference in units for HBV DNA levels. In the current Guidelines and in Japan in general, HBV DNA is expressed as copies/mL, but elsewhere the unit IU/mL is used (IU stands for international units). The AASLD, EASL and APASL guidelines all use IU/mL. Table 10 shows conversion rates between IU/mL and copies/mL. For example, the general treatment cutoff of 2000 IU/mL is equivalent to 4.07 log copies/mL (conversion rate 5.82) using the TaqMan method (Roche). Note that conversion rates may differ between real-time PCR methods; for example, the same treatment standard would be 3.83 log copies/mL (conversion rate 3.41) using the AccuGene method (Abbott). Further research is required into these discrepancies.

#### Recommendation

- *Real-time PCR is recommended for HBV DNA quantification in the clinical setting.*

Table 10 HBV DNA quantification using real-time PCR TaqMan versus AccuGene – measurement ranges and conversion rate

Method	Sample	Measurement range			Equivalent to 2,000 IU/mL	
		IU/mL	Conversion rate	copies/mL		
TaqMan (Roche)	Serum/blood plasma	20–1.7×10 <sup>8</sup>	⇒ (×5.82)	116– 9.9×10 <sup>8</sup>	2.1–9.0	4.07 log copies/mL
AccuGene (Abbott)	Serum/blood plasma	10–1.0×10 <sup>9</sup>	⇒ (×3.41)	34– 3.4×10 <sup>9</sup>	1.53–9.5	3.83 log copies/mL

Due to different conversion rates for TaqMan and AccuGene (IU to copies), reported values expressed as copies/mL cannot be compared directly (1:1).



Table 11 Reagents for HBsAg measurement

Device Trade Name	LUMIPULSE HBsAg	cobas ECLusys HBsAg II	ADVIA Centaur HBsAg	ARCHITECT HBsAg QT	HISCL HBsAg	LUMIPULSEHBsAg-HQ
Manufacturer	Fujirebio	Roche Diagnostics	Siemens Healthcare Diagnostics	Abbott Japan	Symex	Fujirebio
Principle of operation	CLEIA COI (qualitative) Poly	ECLIA COI (qualitative) Mono (two types) Poly/mono	CLIA COI (qualitative) Mono	CLIA IU/mL (quantitative) Mono (two types) Poly	CLEIA IU/mL (quantitative) Mono (various) Mono (various)	CLEIA IU/mL (quantitative) Mono (two types) Mono (two types)
Antibodies	Capture					
Conjugate	Mono		Mono			
Reaction time (min)	30	18	30	30	17	30
Sample volume (μL)	100	50	100	75	20	100
Positive criterion	C.O.I ≥ 1.0	C.O.I ≥ 1.0	C.O.I ≥ 1.0	≥ 0.05 IU/mL	≥ 0.03 IU/mL	≥ 0.005 IU/mL
Measuring range†	0.1-2000 C.O.I.	0.001-C.O.I.	0.1-1000 Index	0.05-250 IU/mL (manual/auto dilution)	0.03-2500 IU/mL (auto dilution)	0.005-150 IU/mL (auto dilution)

†Theoretical value range.

therapy (see Fig. 4) found that the rate of HCC development increases with the baseline HBV DNA levels (>2000 IU/mL), while the actual incidence of HCC in HBeAg negative patients with a low virus load (below 2000 IU/mL) correlated with the HBsAg levels.<sup>29</sup>

Thus, patients with HBV-DNA <2000 IU/mL (=4 log copies/mL), but HBsAg ≥1000 IU/mL, are still at high risk of developing HCC. The risk is greater still if the HBsAg levels remain ≥1000 IU/mL for three years. A prospective study in Alaska reported the incidence of HCC at 0.0368/year following elimination of HBsAg. This is significantly lower in statistical terms than the reported 0.1957/year for patients with persistently positive HBsAg.<sup>51</sup> We may conclude that the elimination of HBsAg effectively reduces cccDNA in the liver, in turn inhibiting carcinogenesis.

Thus, monitoring of the HBV DNA levels during antiviral treatment of chronic HBV should be augmented by regular observation of HBsAg levels in line with a long term treatment goal of elimination of HBsAg.

**Recommendation**

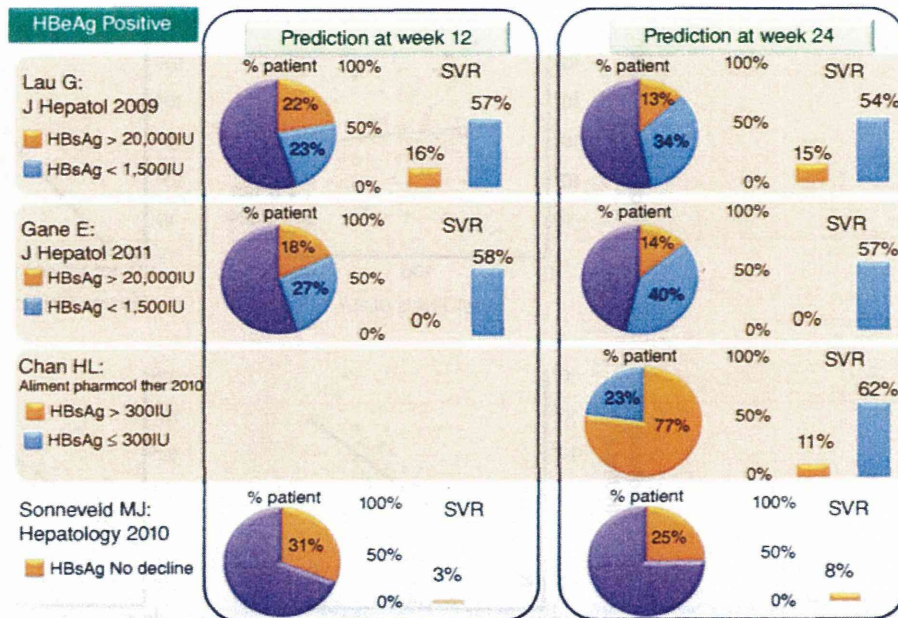
- In antiviral treatment of chronic hepatitis B, both HBV DNA and HBsAg levels should be monitored in line with a long term treatment goal of eliminating HBsAg.

**2.4 HBcrAg**

As Figure 2 shows, HBcrAg is the generic term for three types of antigen structural protein: HBcAg translated from pregenomic mRNA, HBeAg translated from pre-core mRNA and p22cr antigen. This provides a simple measurement framework, developed in Japan, that can be used to generate automated results in a relatively short time frame. In patients not on antiviral therapy, HBcrAg correlated positively with serum HBV DNA levels, in both HBeAg positive and negative patients alike.<sup>74</sup> A positive correlation was also observed between total HBV DNA and cccDNA in the liver, as shown in Figure 5.<sup>75</sup> HBcrAg has been detected in samples below the limit of detection for HBV DNA, with equal or better sensitivity than HBV DNA.

It has been reported that while HBV DNA levels drop rapidly in patients undergoing NA therapy, in many cases falling below the limit of detection, HBcrAg declines at a much slower rate.<sup>76</sup> The divergence between the two is thought to be attributable to the action of NAs in hindering reverse transcription and preventing HBV DNA replication, while the HBV cccDNA remaining in the liver tissue continues to discharge HBcrAg. And it turns out that HBcrAg correlates with the cccDNA levels in liver tissue during NA therapy, thereby





\*SVR = HBeAg SC & HBV DNA < 2000 IU/mL at 24 weeks after the end of treatment

Figure 3 HBsAg measurement is a useful predictor of outcomes in HBeAg positive chronic HBV patients undergoing a 48 week Peg-IFN  $\alpha$  therapy regimen.

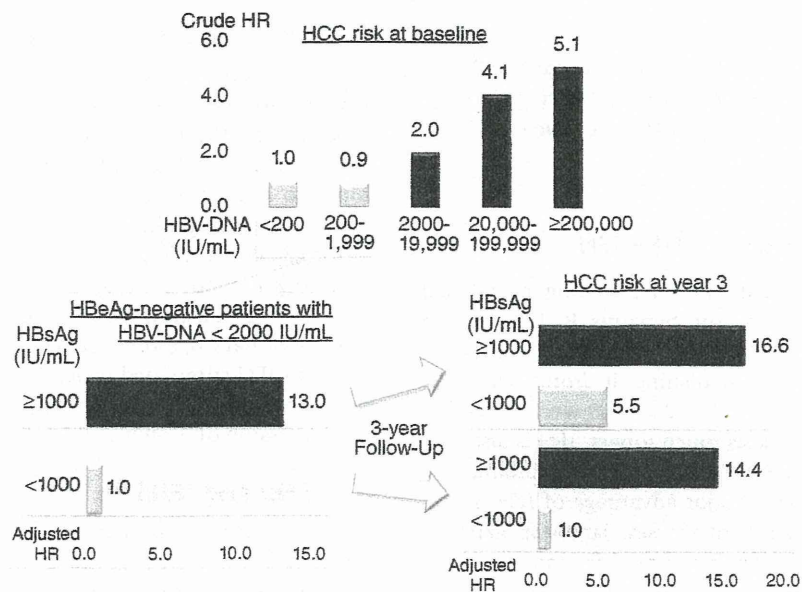


Figure 4 Correlation between HBsAg levels and HCC development in HBeAg negative patients with low viral load.

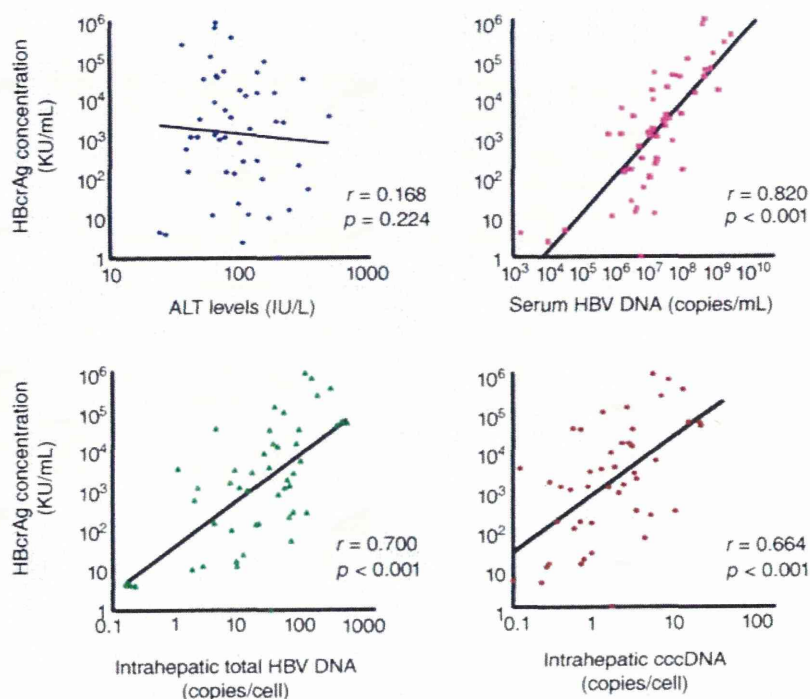


Figure 5 Correlation between HBcrAg, serum HBV DNA levels, and total hepatic HBV DNA and cccDNA.

providing a useful serum marker for predicting flare-ups during therapy<sup>77</sup> and determining when to conclude treatment.<sup>78</sup>

#### Recommendation

- HBcrAg levels correlate with liver tissue cccDNA levels, and serves as a useful marker for predicting flare-ups during NA therapy and determining when to finish treatment.

### 3. PHARMACOTHERAPY (1) – IFN

THE ANTIVIRAL AGENT IFN has long been used for treatment of chronic hepatitis B. IFN has an immunopotentiating effect in addition to its antiviral proliferative effect, distinguishing it from NAs. IFN therapy is generally limited to 24 to 48 weeks, whereas NA therapy normally lasts much longer. IFN is also free of teratogenicity and is therefore more suitable for young people. Another major advantage of IFN is that it does not create resistant viruses. Japanese national medical insurance schemes have for many years approved the non-pegylated agents IFN $\alpha$  and IFN $\beta$  for HBeAg positive chronic active HBV treatment. In 2011,

coverage was extended to the pegylated agent Peg-IFN $\alpha$ -2a for chronic active HBV, irrespective of HBeAg status.

#### 3.1 Antiviral effects of IFN<sup>79–81</sup>

The mechanism behind the antiviral effect of IFN is thought to work as follows. IFN binds to type I IFN receptors on the target cell membrane, which are the same for both IFN $\alpha$  and IFN $\beta$ . When IFN $\alpha$  or IFN $\beta$  binds to a receptor the tyrosine-protein kinase JAK1 is activated, causing phosphorylation of tyrosine residue in the cell domain of the IFN receptor. This in turn leads to phosphorylation of STAT1 and the formation of dimers that transmit information to the cell nucleus. This information induces and stimulates a variety of different IFN-stimulated genes (ISGs), including antiviral genes and immunomodulator genes that promote the expression of proteins which have an antiviral effect.

#### 3.2 IFN $\alpha$ and IFN $\beta$

Non-pegylated conventional IFN is unstable in the body. It has a short half-life in blood of just three to eight hours and by 24 hours is below the limit of detection.<sup>82</sup> For this reason, it must be administered at least

three times per week during treatment for chronic hepatitis B. In conventional IFN treatment there is an ongoing cycle of the serum IFN level rising and falling, which can cause adverse effects such as fevers, chills and headaches. Natural IFN $\alpha$  among the conventional IFN is approved for self-medication via injection together with fortnightly hospital visits. Patients can self-inject just before going to sleep at night to align the blood IFN concentration more closely with the cycle of cortisone levels in the body, thereby mitigating adverse effects such as fever.<sup>83–85</sup>

IFN $\beta$  is a natural non-pegylated agent that is administered three or more times per week either by intravenous injection or infusion. IFN $\beta$  binds to the same type I IFN receptors as IFN $\alpha$  and exhibits the same antiviral effect, but with a different adverse reaction profile. It is recommended for patients affected by depression who are considered unsuitable for IFN $\alpha$ .

### 3.2.1 Therapeutic effect in patients with HBeAg positive chronic hepatitis

In a meta-analysis (n = 837) of randomized clinical controlled trials conducted overseas in 1993, the IFN therapy group had an HBeAg negative conversion rate of 33% and an HBV DNA negative conversion rate of 37%. The corresponding rates for the untreated group were 12% and 17% respectively. These findings demonstrate the benefit of IFN therapy.<sup>86</sup> Negative conversion for HBsAg was also higher at 7.8% for the IFN group compared to 1.8% for the untreated group. Sustained ongoing HBeAg seroconversion was observed in almost 90% of cases, as well as delayed seroconversion (occurring one or two years after the conclusion of therapy) in 10%–15% of cases.<sup>87–89</sup> Thus, in cases where IFN therapy in HBeAg positive patients successfully bring about HBeAg seroconversion, there is an ongoing effect that acts to hinder progression to cirrhosis and HCC, and the prognosis is therefore much improved.<sup>90</sup> Reports from Asia however suggest that the effect is not sustained in the long term, with negative conversion of HBsAg being relatively rare.<sup>87,90</sup> This may be attributable to host-specific factors such as race as well as genotype, infection period, and route of infection.

Collation of 24 studies of therapeutic outcomes in HBeAg positive patients with chronic hepatitis B in Japan<sup>91</sup> yielded HBeAg negative conversion rates of 29% after one year of IFN therapy and 55% after two years, and HBeAg seroconversion rates of 12% after one year and 29% after two years. These figures are higher than the corresponding natural conversion rates of 10% and 5% respectively, indicating the efficacy of IFN therapy.

However, there have also been reports of cases that revert to HBeAg positive status after completion of treatment, and hepatitis fails to subside. It should be noted that at the time these studies were conducted, most IFN therapy regimens in Japan lasted only four weeks. With a longer IFN treatment regimen, the HBeAg negative conversion rate six months after the completion of the therapy is considerably higher at 29%.<sup>91</sup>

### 3.2.2 Therapeutic effect in patients with HBeAg negative chronic hepatitis

Japanese national medical insurance does not cover conventional IFN therapeutic agents for the treatment of HBeAg negative chronic hepatitis B.

Overseas studies, mainly from Europe, report impressive biochemical and virological therapeutic benefit rates of 60%–90% in HBeAg negative patients following IFN therapy. At the same time, however, subsequent increases in HBV DNA levels and recurrence of hepatitis are also common, with sustained effects in only 10%–15% of patients for four to six months of IFN therapy, and 22% for 12 months of therapy.<sup>92,93</sup> An Asian study of IFN therapy regimens lasting six to ten months identified therapeutic benefits six months after the end of therapy in 30% of cases, compared to just 7% in the control group.<sup>94</sup> An even longer therapy regimen of 24 months achieved sustained quiescence of hepatitis in 30% of cases and 18% HBsAg elimination after six years.<sup>95</sup> In light of these findings, continued administration of IFN is recommended overseas for patients with HBeAg negative chronic hepatitis B. IFN therapy has also been shown to suppress carcinogenesis and deliver improved life expectancies in HBeAg negative patients with chronic hepatitis B, as with HBeAg positive patients.<sup>96</sup>

#### Recommendation

- IFN therapy has been shown to produce significant improvements in HBeAg positive chronic HBV patients with respect to HBeAg negative conversion, HBeAg seroconversion, HBV DNA negative conversion and ALT normalization, compared to an untreated control group.

### 3.3 Peg-IFN $\alpha$ -2a

Pegylated IFN is available as Peg-IFN $\alpha$ -2a (40kD branched strand PEG covalently bonded to IFN $\alpha$ -2a) and Peg-IFN $\alpha$ -2b (12kD single strand PEG urethane bonded to IFN $\alpha$ -2a). In Japan, only Peg-IFN $\alpha$ -2a is approved by medical insurance for the treatment of

chronic active hepatitis B. PEG is a neutral, water-soluble molecule with no inherent toxicity. The molecular weight is governed by the number of ethylene oxide subunits. Pegylation of IFN has two objectives: to alter the pharmacokinetics in the body, and to prevent IFN from being recognized and rejected by the host's immune system.

The concentration of Peg-IFN $\alpha$ -2a in the blood remains within the therapeutic range for approximately 168 hours after administration, reaching the peak concentration (C<sub>max</sub>) 72 to 96 hours after administration.<sup>97</sup> A study in Asia comparing the therapeutic effects of Peg-IFN $\alpha$ -2a and conventional IFN $\alpha$ -2a reported a complete response (i.e. elimination of HBeAg, suppression of HBV DNA and normalization of ALT) in 28% of patients treated with Peg-IFN $\alpha$ -2a compared to 12% of patients treated with conventional IFN $\alpha$ -2a, a statistically significant difference ( $P = 0.036$ ). The HBeAg seroconversion rate was also higher for Peg-IFN $\alpha$ -2a (33% versus 25%), indicating the superiority of the pegylated agent.<sup>98</sup>

### 3.3.1 Therapeutic effect in cases of HBeAg positive chronic hepatitis

In an overseas comparative study, 814 HBeAg positive patients were divided into three groups: the first was administered Peg-IFN $\alpha$ -2a for 48 weeks, the second Peg-IFN $\alpha$ -2a together with lamivudine for 48 weeks, and the third lamivudine only for 48 weeks.<sup>8</sup> While all three groups returned similar HBeAg seroconversion rates at the end of the treatment period (27%, 24% and 20% respectively), the Peg-IFN $\alpha$ -2a groups showed significantly better HBeAg seroconversion rates 24 weeks after the end of treatment (32%, 27% and 19%). Virological outcomes 24 weeks after treatment were also better in the Peg-IFN $\alpha$ -2a groups, with 32% of patients <5 log copies/mL HBV DNA, 14% <400 copies/mL, and HBsAg seroconversion in 3%. A sub-analysis looking specifically at Asian patients yielded 31% HBeAg seroconversion, consistent to seroconversion rates for the overall sample.<sup>12</sup> The NEPTUNE study of four arms of Peg-IFN $\alpha$ -2a dosage (90  $\mu$ g vs 180  $\mu$ g) and treatment period (24 weeks vs 48 weeks) found that the group administered 180  $\mu$ g for 48 weeks had the highest HBeAg seroconversion rate (36.2%), followed by 180  $\mu$ g for 24 weeks (25.8%), 90  $\mu$ g for 48 weeks (22.9%) and 90  $\mu$ g for 24 weeks (14.1%).<sup>10</sup>

One study in Japan used a non-inferiority test on natural IFN $\alpha$  to evaluate the therapeutic effects of Peg-IFN $\alpha$ -2a therapy for HBeAg positive chronic active hepatitis B.<sup>9</sup> A sample of 207 HBeAg positive chronic active

hepatitis B patients was grouped as follows: Peg-IFN $\alpha$ -2a 90  $\mu$ g for 24 weeks = 41 patients, Peg-IFN $\alpha$ -2a 180  $\mu$ g for 24 weeks = 41 patients, Peg-IFN $\alpha$ -2a 90  $\mu$ g for 48 weeks = 41 patients, Peg-IFN $\alpha$ -2a 180  $\mu$ g for 48 weeks = 41 patients, and natural IFN $\alpha$  for 24 weeks = 43 patients. The proportion in each group achieving the combined outcome (HBeAg seroconversion, HBV-DNA <5.0 log copies/mL and ALT  $\leq$ 40 U/L) at 24 weeks after the end of treatment was 4.9% for Peg-IFN $\alpha$ -2a 90  $\mu$ g for 24 weeks, 17.1% for Peg-IFN $\alpha$ -2a 90  $\mu$ g for 48 weeks, 9.8% for Peg-IFN $\alpha$ -2a 180  $\mu$ g for 24 weeks, 19.5% for Peg-IFN $\alpha$ -2a 180  $\mu$ g for 48 weeks, and 7.0% for natural IFN $\alpha$  for 24 weeks. These results indicate a greater therapeutic benefit for patients receiving Peg-IFN $\alpha$ -2a, depending on dosage and treatment period. Based on the results of these clinical trials, national medical insurance approval was extended in September 2011 to a treatment regimen of Peg-IFN $\alpha$ -2a at either 90 or 180  $\mu$ g for 48 weeks for chronic active HBV patients.<sup>99</sup> It should be noted however that 97% (157 of 164) of the HBeAg positive patients in the Japanese clinical study were under 50 years of age, with very few over 50 years of age.<sup>100</sup>

Several studies are looking into the potential long-term benefits of Peg-IFN $\alpha$ -2a therapy. One study found that 14% of patients who did not respond at the end of therapy displayed HBeAg seroconversion one year after treatment, with this effect being sustained in 86% of cases.<sup>12</sup> Similarly, a long term follow-up study (average follow-up period three years) of 172 patients with HBeAg positive chronic hepatitis B treated with Peg-IFN $\alpha$ -2b confirmed that HBeAg negative remained in 81% of patients where HBeAg negative conversion had been observed at 26 weeks after treatment. Delayed HBeAg negative conversion was seen in a further 27% of cases where conversion had not occurred at that point. Elimination of HBsAg occurred in 30% of patients who were HBeAg negative at 26 weeks after treatment and in 11% of the total sample.<sup>11</sup> It is important, however, to note the context of this study: 31% of the long term cases were genotype A, known to respond well to IFN, and 47% of the total and 21% of the HBeAg negative group were administered additional NA therapy.<sup>100</sup>

According to a long-term follow-up study in China of 85 patients administered Peg-IFN $\alpha$ -2a and lamivudine (average follow-up period six years), 77% of those who well responded at the end of treatment subsequently demonstrated HBeAg seroconversion after five years while 57% recorded HBV DNA levels <10 000 copies/mL. Even 69% of those who did not respond at the end of treatment subsequently demonstrated HBeAg