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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*,**

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.

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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.[1] 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.[2–4]

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.

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.[5]

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

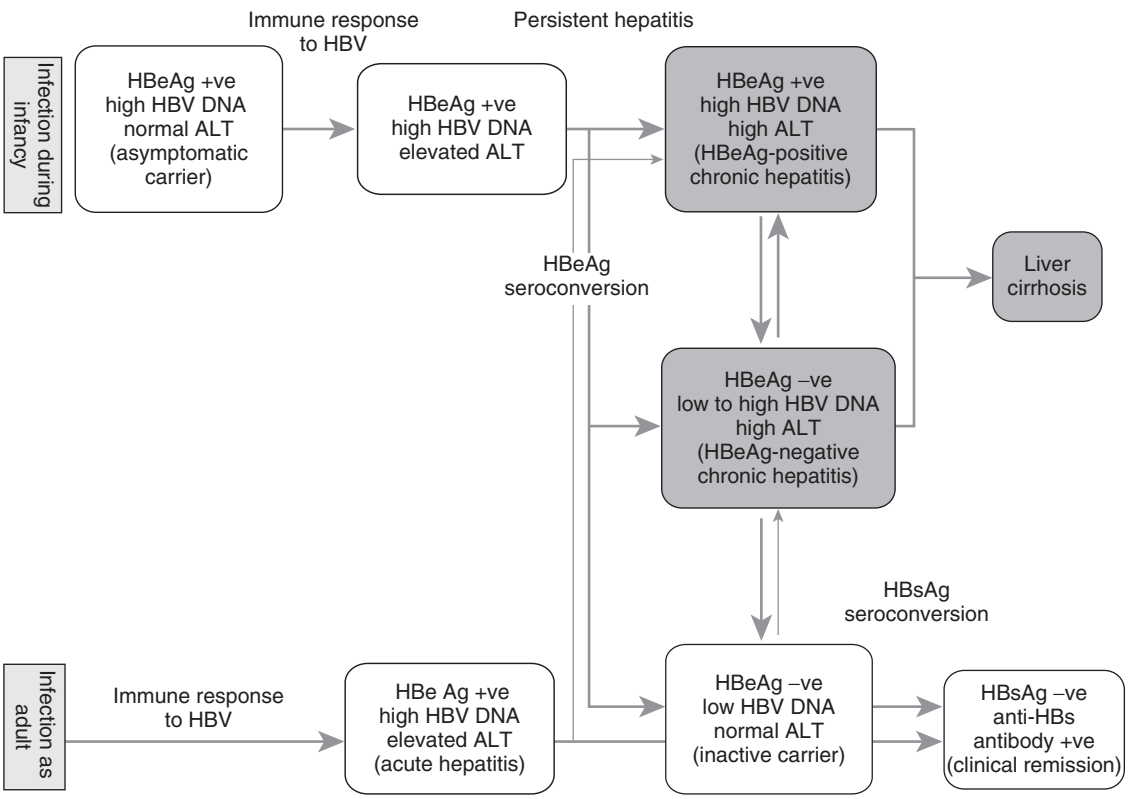


Figure 1 Natural course of persistent HBV infection.

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.[5]

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 (≤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[*1]	Persistent normal[*1]
HBeAg	Negative[*2]	Negative[*2]
HBV DNA[*3]		
On-treatment (Ongoing NA therapy)	Negative	Negative
Off-treatment (IFN completed/NA therapy ceased[*4])	< 4 log copies/ml	Negative[*5]

Notes
[*1]. Normal range of ALT is defined as ≤30 U/L.
[*2]. Conversion to HBeAg-negative in HBeAg-positive cases, and maintain HBeAg-negative in HBeAg-negative cases.
[*3]. As measured using high-sensitivity PCR (real-time PCR).
[*4]. At 24–48 weeks following completion of antiviral therapy.
[*5]. 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, immunopotentiation	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)[6] and the Asia-Pacific Association for the Study of the Liver (APASL)[7] 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[8–21] and negative cases,[15,22–26] 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%[8]	67~75%[14,15]
Long term	13%[11–13]	93~94%[15,16]
HBeAg seroconversion		
Short term	24~36%[8–10]	16~21%[14,15]
Long term	37~60%[11–13]	34~44%[17–19]
ALT normalization		
Short term	37~52%[8–10]	68~81%[14,15]
Long term	47%[11–13]	87~95%[15,20]
Long term goals		
HBsAg elimination		
Short term	2.3~3.0%[8–10]	1.7%[14]
Long term (overall)	11%[11]	0.6~5.1%[16,17,21]
Long term (responders*)	30%[11]	

Peg-IFN (Peg-IFNα-2a[8–10,12] and Peg-IFNα-2b[11,13]):
Short term: 24 weeks after ending treatment.[8–10]
Long term: Three years after ending treatment.[11]
*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.[14]
Long term: Two years[20,21], three years,[17–19] four years,[15] and five years[16] after starting treatment.

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

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

Peg-IFN (Peg-IFNα-2a:[22–24])
Short term: 24 weeks after ending treatment.[22]
Long term: Three years[23] and five years[24] after ending treatment.
*Responders: HBV DNA negative three years after ending treatment (15% of total).
Entecavir
Short term: One year after starting treatment.[25]
Long term: Four years after starting treatment.[15]

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),[27] the European Association for the Study of the Liver (EASL),[6] the Asia Pacific Association for the Study of the Liver (APASL),[7] and the Japanese Ministry of Health, Labour and Welfare (MHLW) research group[28] 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)[6]	EASL (2012)[7]	APASL (2008)[27]	MHLW (2013)[28]
HBeAg-positive chronic hepatitis				
HBV DNA (log copies/mL)	≥5	≥4	≥5	≥4
ALT	1) >2 × ULN 2) 1–2 × ULN >40 years Family history of HCC → liver biopsy	1) >1 × ULN 2) <1 × ULN → liver biopsy	1) >2 × ULN 2) ≤2 × ULN >40 years → liver biopsy	≥31 U/l
HBeAg-negative chronic hepatitis				
HBV DNA (log copies/mL)	≥4	≥4	≥4	≥4
ALT	1) >2 × ULN 2) 1–2 × ULN >40 years Family history of HCC → liver biopsy	1) >1 × ULN 2) <1 × ULN → liver biopsy	1) >2 × ULN 2) ≤2 × ULN >40 years → liver biopsy	≥31 U/L
Cirrhosis				
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 cancinogenesis.[29] 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.[4,30–32] 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,[33] 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 ≥31 U/L since 2008,[28] and thus the current Guidelines propose a normal ALT range for chronic hepatitis of ≤30 U/L, with ≥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.[34] 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.[35]

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 (≤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 ≤ 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 ≥40 years with high HBV DNA levels,[2,36,37] or platelet counts <150 000 /μl, or a family history of HCC,[38,39] 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.[40–44] 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 γ globulin levels, and serum α macroglobulin levels, but none of these should be used as the sole marker.[45]

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 ≥31 U/L and HBV DNA levels ≥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 ≥40 with a high HBV DNA levels, platelet count <150 000 /μl 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†‡§	≥31 U/L	≥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 ≤ 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 ≥40 with high HBV DNA levels, platelet count <150 000 /μl 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 cirrhosis 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 ≥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.[46–52] It is important to be aware of the ongoing risk of HCC even where cccDNA has been eliminated, due to HBV genome recombination.[53–55]

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

HBV 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.[56] 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.[57] 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.[58]

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.[59] 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.[5] 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 requires, as HBV genotype Bj and the 1896 mutation have been identified as independent risk factors for fulminant hepatitis.[60] 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)	Often becomes chronic (5%–10%)
	Asian/African strains (HBV/A1/Aa)	Increasing prevalence, particularly in younger age groups
B	Asian strains (HBV/Ba)	Often becomes fulminant
	Japanese strains (HBV/B1/Bj)	10%–20% of total
C	Southeast Asia (HBV/Cs)	High rate HCC
	East Asia (HBV/Ce)	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.[61] 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.[62] Reports from Europe suggest that HBV genotype D is more resistant to IFN treatment than HBV genotype A, with a poor overall prognosis.[63]

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.[34] 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	log copies/mL	
TaqMan (Roche)	Serum/blood plasma	20~1.7×10^8	⇒ (×5.82)	116~ 9.9×10^8	2.1~9.0	4.07 log copies/mL
AccuGene (Abbott)	Serum/blood plasma	10~1.0×10^9	⇒ (×3.41)	34~ 3.4×10^9	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).

2.3 HBsAg quantification

HBsAg is an antigen within the HBV envelope that is present within the blood as the Dane particle as well as empty particles, small spherical particles and tubular particles, all of which are generated from covalently closed circular DNA (cccDNA) in the hepatocytes, as shown in Figure 2.

Qualitative reagents have traditionally been used for measuring HBsAg and for the diagnosis of hepatitis B. But recent years have seen the development of a number of new quantitative reagents with considerable potential for prognosis and evaluation of therapeutic effects.[64,65] Table 11 lists reagents used for measuring HBsAg.

Observations generated by qualitative reagents are expressed in terms of a cut-off index (COI), where a value of 1.0 or higher is deemed positive and higher measurements are semiquantitative, used for reference purposes. Common quantitative reagents include Architect (Abbott) and HISCL (Sysmex). Table 11 shows the threshold criteria and measurement ranges in IU/mL. Quantification covers a wide range through dilution. A newly developed quantitative reagent for HBsAg called Lumipulse HBsAg-HQ claims ten times the sensitivity of conventional reagents, and shows considerable potential for clinical settings.

HBsAg levels vary in accordance with factors such as age, HBV DNA levels and HBV genotype.[66] HBV DNA is considered unsuitable for evaluating therapeutic effects because the HBV DNA levels often falls below the limit of detection shortly after the commencement of antiviral treatment. Several reports therefore recommend monitoring the HBsAg levels over time instead. There have been overseas studies of HBeAg positive patients with chronic hepatitis B stating that the HBsAg levels at 24 weeks after commencing administration of Peg-IFN α-2a, either in isolation or in combination with lamivudine, can be used to predict HBeAg seroconversion, HBV DNA levels and HBsAg elimination rate at 24 weeks after the end of treatment.[67] Similarly, it has been reported that the HBsAg levels at 12 and 24 weeks in a 48 week Peg-IFN therapy regimen can be used to predict HBeAg seroconversion and HBV DNA negative status (sustained viral response or SVR) six months after the end of treatment, as shown in Figure 3.[68–71]

On the other hand, it has been reported that by monitoring the rate of decline in HBsAg levels during treatment of HBeAg negative chronic hepatitis B patients – specifically at 12, 24 and 48 weeks – it is possible to predict the HBV DNA levels one year after the end of treatment as well as disappearance of HBsAg five years later.[72,73]

Some researchers argue that HBsAg monitoring is necessary not only for predicting antiviral therapeutic effects, but throughout the natural course of HBV. A prospective study in Taiwan of the natural course of HBV infection in patients with no history of antiviral

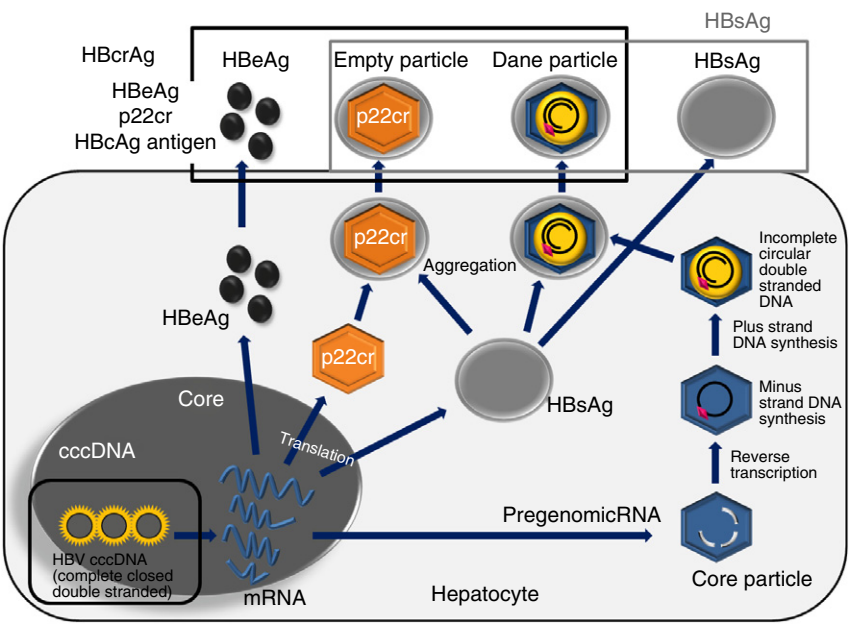


Figure 2 HBV related markers.

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	Sysmex	Fujirebio
Principle of operation		CLEIA	ECLIA	CLIA	CLIA	CLEIA	CLEIA
Unit		COI (qualitative)	COI (qualitative)	COI (qualitative)	IU/mL (quantitative)	IU/mL (quantitative)	IU/mL (quantitative)
Antibodies	Capture	Poly	Mono (two types)	Mono	Mono (two types)	Mono (various)	Mono (two types)
	Conjugate	Mono (two types)	Poly/mono	Mono	Poly	Mono (various)	Mono (two types)
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.[29]

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.[51] 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.

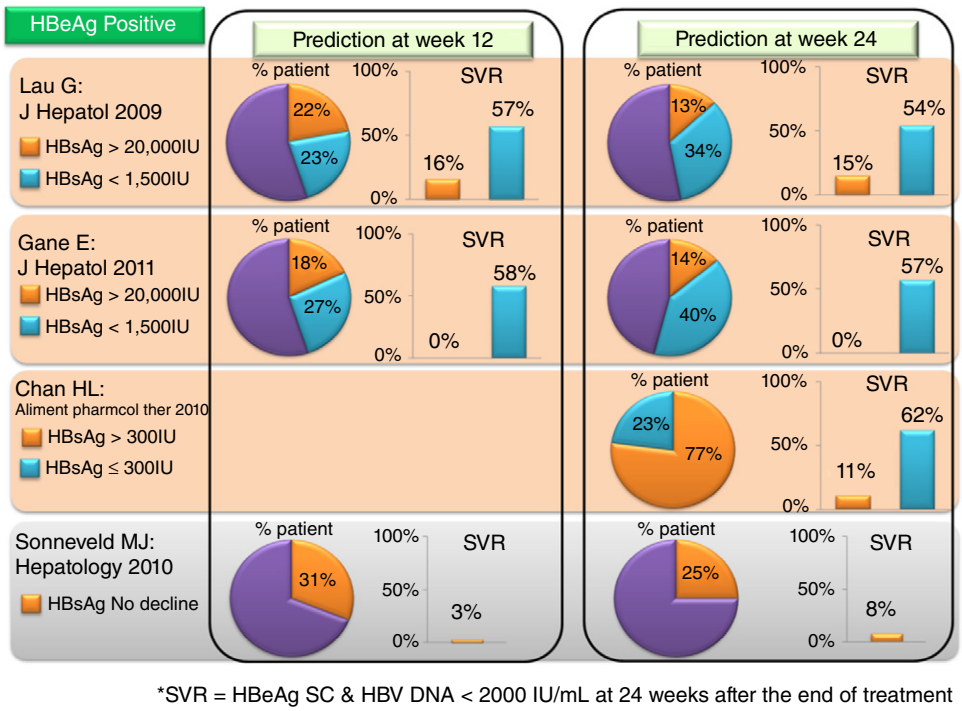
Recommendation

- ***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 precore 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.[74] A positive correlation was also observed between total HBV DNA and cccDNA in the liver, as shown in Figure 5.[75] 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.[76] 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 α therapy regimen.

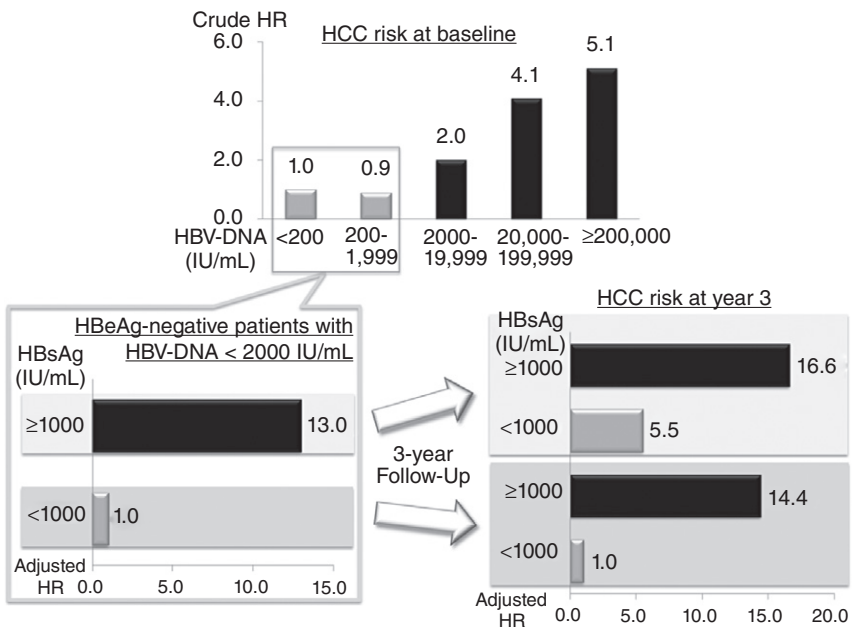


Figure 4 Correlation between HBsAg levels and HCC development in HBeAg negative patients with low viral load.

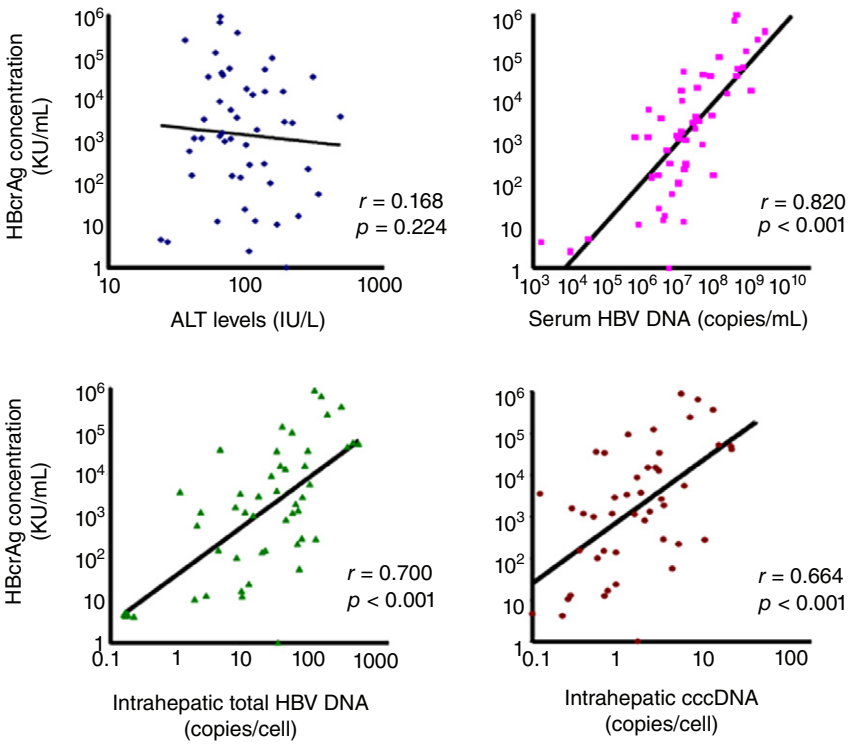


Figure 5 Correlation between HBcrAg, serum HBV DNA levels, and total hepatic HBV DNA and cccDNA.

providing a useful serum marker for predicting flare-ups during therapy[77] and determining when to conclude treatment.[78]

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 immunopotentiation 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 IFNα and IFNβ for HBeAg positive chronic active HBV treatment. In 2011, coverage was extended to the pegylated agent Peg-IFNα-2a for chronic active HBV, irrespective of HBeAg status.

3.1 Antiviral effects of IFN[79–81]

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 IFNα and IFNβ. When IFNα or IFNβ 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 IFNα and IFNβ

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.[82] 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 IFNα 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.[83–85]

IFNβ is a natural non-pegylated agent that is administered three or more times per week either by intravenous injection or infusion. IFNβ binds to the same type I IFN receptors as IFNα 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 IFNα.

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.[86] 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.[87–89] 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.[90] Reports from Asia however suggest that the effect is not sustained in the long term, with negative conversion of HBsAg being relatively rare.[87,90] 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[91] 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%.[91]

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.[92,93] 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.[94] An even longer therapy regimen of 24 months achieved sustained quiescence of hepatitis in 30% of cases and 18% HBsAg elimination after six years.[95] 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.[96]

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-IFNα-2a

Pegylated IFN is available as Peg-IFNα-2a (40kD branched strand PEG covalently bonded to IFNα-2a) and Peg-IFNα-2b (12kD single strand PEG urethane bonded to IFNα-2a). In Japan, only Peg-IFNα-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-IFNα-2a in the blood remains within the therapeutic range for approximately 168 hours after administration, reaching the peak concentration (Cmax) 72 to 96 hours after administration.[97] A study in Asia comparing the therapeutic effects of Peg-IFNα-2a and conventional IFNα-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-IFNα-2a compared to 12% of patients treated with conventional IFNα-2a, a statistically significant difference ($P = 0.036$). The HBeAg seroconversion rate was also higher for Peg-IFNα-2a (33% versus 25%), indicating the superiority of the pegylated agent.[98]

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-IFNα-2a for 48 weeks, the second Peg-IFNα-2a together with lamivudine for 48 weeks, and the third lamivudine only for 48 weeks.[8] While all three groups returned similar HBeAg seroconversion rates at the end of the treatment period (27%, 24% and 20% respectively), the Peg-IFNα-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-IFNα-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.[12] The NEPTUNE study of four arms of Peg-IFNα-2a dosage (90 μg *vs* 180 μg) and treatment period (24 weeks *vs* 48 weeks) found that the group administered 180 μg for 48 weeks had the highest HBeAg seroconversion rate (36.2%), followed by 180 μg for 24 weeks (25.8%), 90 μg for 48 weeks (22.9%) and 90 μg for 24 weeks (14.1%).[10]

One study in Japan used a non-inferiority test on natural IFNα to evaluate the therapeutic effects of Peg-IFNα-2a therapy for HBeAg positive chronic active hepatitis B [9]. A sample of 207 HBeAg positive chronic active hepatitis B patients was grouped as follows: Peg-IFNα-2a 90 μg for 24 weeks = 41 patients, Peg-IFNα-2a 180 μg for 24 weeks = 41 patients, Peg-IFNα-2a 90 μg for 48 weeks = 41 patients, Peg-IFNα-2a 180 μg for 48 weeks = 41 patients, and natural IFNα 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 ≤40 U/L) at 24 weeks after the end of treatment was 4.9% for Peg-IFNα-2a 90 μg for 24 weeks, 17.1% for Peg-IFNα-2a 90 μg for 48 weeks, 9.8% for Peg-IFNα-2a 180 μg for 24 weeks, 19.5% for Peg-IFNα-2a 180 μg for 48 weeks, and 7.0% for natural IFNα for 24 weeks. These results indicate a greater therapeutic benefit for patients receiving Peg-IFNα-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-IFNα-2a at either 90 or 180 μg for 48 weeks for chronic active HBV patients.[99] 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.[100]

Several studies are looking into the potential long-term benefits of Peg-IFNα-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.[12] 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-IFNα-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.[11] 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.[100]

According to a long-term follow-up study in China of 85 patients administered Peg-IFNα-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

seroconversion. Overall, HBeAg seroconversion at five years after the end of treatment was seen in an impressive 60% of the total sample.[13]

Recommendation

- ***Clinical studies in Japan have found that 17% – 20% of patients with HBeAg positive chronic hepatitis B administered Peg-IFNα-2a at either 90 or 180 μg dosage for 48 weeks experience the target therapeutic benefits of HBeAg seroconversion, HBV-DNA <5.0 log copies/mL and ALT ≤40 U/L.***

3.3.2 Therapeutic effect in cases of HBeAg negative chronic hepatitis

An overseas comparative study of three treatment regimens for HBeAg negative patients (Peg-IFNα-2a for 48 weeks, Peg-IFNα-2a plus lamivudine for 48 weeks, and lamivudine only for 48 weeks) reported ALT normalization rates of 59%, 60% and 44% respectively, and HBV DNA negative conversion rates of 43%, 44% and 29% respectively at 24 weeks after finishing treatment.[22] Thus, the Peg-IFNα-2a groups demonstrated better results on both parameters. The long term benefits (negative HBV DNA and normal ALT levels at 72 weeks) were likewise stronger in the two Peg-IFNα-2a groups (15% and 16% compared to 6% for lamivudine only), although the effect tended to be less sustained overall compared to HBeAg positive patients. The HBV DNA levels <400 copies/mL were found in 19% of patients, and HBsAg elimination was observed in 3%.[22]

Meanwhile, a study of 61 patients with HBeAg negative chronic active hepatitis B in Japan compared the therapeutic effects from Peg-IFNα-2a dosages of 90 μg (32 patients) and 180 μg (29 patients). In terms of virological benefits, the target HBV DNA levels at finishing treatment (<4.3 log copies/mL) was achieved in 78.1% of the 90 μg group and 93.1% of the 180 μg group. After 24 weeks, these figures had fallen to 37.5% and 37.9% respectively, whereas the biochemical target (ALT ≤40 U/L) was achieved in 68.8% and 65.5% of patients respectively.[9] It should be noted that, as with the HBeAg positive study, the overwhelming majority of the patients in this study (58/61; 95%) were <50 years of age.

A long term follow-up study of 230 HBeAg negative patients treated with Peg-IFNα-2b (with or without lamivudine) reported HBV DNA negative conversion (DNA <4.0 log copies/m) in 21% of patients after five years, and HBsAg elimination in 5% after one year and 12% after five years.[23] Meanwhile, an Italian study of 128 genotype D HBeAg negative patients administered Peg-IFNα-2a over an extended period of 96 weeks (180 μg for 48 weeks then 135 μg for 48 weeks) reported 29% of cases reaching the virological target HBV DNA levels of <2000 IU/mL. It can be seen that this is considerably higher than the corresponding figure of 12% for the 48 week treatment regimen. HBsAg elimination rates were also better after 96 weeks (6%) compared to 48 weeks (0%).[24] Thus, the efficacy of Peg-IFNα-2a therapy on patients with HBeAg negative chronic hepatitis B can be considerably improved by extending the therapy period. In Japan however there is no national medical insurance approval for treatment regimens longer than 48 weeks.

Recommendation

- ***A clinical study in Japan reported that 38% of patients with HBeAg negative chronic hepatitis B administered Peg-IFNα-2a at either 90 or 180 μg dosage for 48 weeks achieved the virological target of a HBV DNA levels <4.3 log copies/mL 24 weeks after the end of treatment.***

3.4 IFN therapy for HBV-associated cirrhosis

It was demonstrated that IFN treatment of compensated HBV cirrhosis produced much the same outcomes and adverse effects to IFN therapy as in non-cirrhotic patients, and in Asian patients in whom HBeAg had been successfully eliminated the HBsAg elimination rate was boosted by a factor of 6.63 times, effectively suppressing progression of liver fibrosis and hepatocarcinogenesis.[101] A study of 24 patients with HBeAg positive compensated cirrhosis administered Peg-IFNα-2b (with or without lamivudine) for 52 weeks reported 30% efficacy (defined as HBeAg seroconversion and HBV DNA <4.0 log copies/mL) at 26 weeks after finishing treatment. This figure is significantly higher than the corresponding 14% for non-cirrhotic cases. Histological improvement was observed in 66% of cases, also significantly higher than the 22% for non-cirrhotic cases, with similar adverse reactions.[102] It should be noted however that IFN, unlike NAs, has an immunopotentiation effect that can increase the risk of acute exacerbation of hepatitis through immunological destruction of HBV infected cells. IFN therapy is contraindicated for HBV-associated decompensated cirrhosis patients in particular, who are at risk of potentially fatal adverse reactions such as deterioration of liver function.[103] In Japan there is insufficient evidence regarding the efficacy and safety of IFN therapy for HBV associated cirrhosis, and consequently this is not approved by

national medical insurance. Hence HBV-associated cirrhosis should be treated with NAs.

Recommendation

- ***There is insufficient evidence in Japan on the efficacy and safety of IFN therapy for HBV-associated compensated cirrhosis, and NA therapy is recommended instead. IFN treatment is contraindicated for patients with HBV decompensated cirrhosis.***

3.5 Should NAs be administered at the same time?

IFN administered in combination with lamivudine produces improved HBV DNA negative conversion and ALT normalization outcomes compared to lamivudine alone, for both HBeAg positive and negative patients. Meanwhile, studies comparing IFN plus lamivudine combination therapy with IFN monotherapy found similar therapeutic effects[8,22,104] and similar persistent benefits.[96,105,106] IFN in combination with adefovir was likewise found to have roughly the same therapeutic effect six months after treatment as IFN alone.[107] It has been reported that Peg-IFN in combination with entecavir or adefovir produces better negative conversion of HBsAg and reduction in cccDNA levels.[108,109] However in the absence of a broad consensus on this at the present point in time, there cannot be said to be sufficient evidence for improved therapeutic effects of IFN administered in combination with NAs.

Recommendation

- ***There is insufficient evidence for improved therapeutic effects of IFN administered in combination with NAs.***

3.6 Factors that determine therapeutic effect

Factors reported to determine the therapeutic effect of conventional IFN include HBV genotype,[104,110,111] age,[112] and the degree of fibrosis.[113] However, as shown below, Peg-IFN has a high therapeutic effect compared to conventional IFN, and has high efficacy against HBV genotype A, but its therapeutic effect is not influenced by other HBV genotypes or patient age. Currently, regardless of whether a patient is HBeAg positive or negative, there is no established method for predicting the treatment response prior to Peg-IFN treatment, with the exception of HBV genotype A (Tables 12,13).

3.6.1 HBV genotype

Concerning correlations between genotype and therapeutic effect, for conventional IFN therapeutic effect is

Table 12 Reports on favourable factors affecting Peg-IFN therapeutic effect for HBeAg positive cases

	Liaw[10]	Lau[8]	Buster[114]	Janssen[115]	Sonneveld[116]	Hayashi[9]
Dosage	α-2a 90/180 μg	α-2a 180 μg ± LAM 100 mg	α-2a 180 μg α-2b 100 μg	α-2b 100 μg ± LAM100 mg	α-2a/α-2b ± LAM100 mg	α-2a 90/180 μg
Administration period	24/48 weeks	48 weeks	α-2a: 48 weeks α-2b: 52 weeks	52 weeks	32~104 weeks	24/48 weeks
Cases	548	542	788	307	205	164
Race	NS			NS	NS	
Age	NS		Elderly	NS	Elderly	Young†
Gender	NS		Female	NS	NS	Female†
ALT	High†	NS	High	High	NS	NS
HBV DNA levels	Low	Low	Low	Low	Low	NS
HBsAg levels	Low					
Genotype	NS	NS	A (*vs* D)	A (*vs* D)	A (*vs* D)	
IL28B					Major	

†Tendency but not statistically significant.
LAM, lamivudine; NS, Not significant.

Table 13 Reports on favourable factors affecting Peg-IFN therapeutic effect for HBeAg negative cases

	Bonino[117]	Rijckborst[118]	Moucari[119]	Marcellin[23]	Hayashi[9]
Dosage	α-2a 180 μg ± LAM 100 mg	α-2a 180 μg ± RIB 1000/1200 mg	α-2a 180 μg	α-2a 180 μg ± LAM 100 mg	α-2a 90/180 μg
Administration period	48 weeks	48 weeks	48 weeks	48 weeks	24/48 weeks
Cases	518	107	48	230	61
Race	NS	NS		NS	
Age	Young	NS	NS	NS	NS
Gender	Female	NS	NS	NS	NS
ALT	High	NS	High	High	NS
HBV DNA levels	Low	NS	NS	NS	NS
HBsAg levels		NS	NS		
Genotype	B, C (vs. D)	NS	NS	NS	

LAM, lamivudine; NS, not significant; RIB, ribavirin.

reported to be high for genotypes A and B compared to genotypes C and D.[104,110,111] For treatment using the minimum dosage (90 μg) of Peg-IFNα-2a or short period (24 weeks), poorer therapeutic response has also been reported for genotypes C compared to genotype B.[98] However, the recent NEPTUNE study evaluated the therapeutic effect of Peg-IFNα-2a 180 μg/48 weeks, finding the response rate of antiviral therapy was the same for genotypes B and C, and genotype was not a predictive factor for therapeutic effect.[10] Possible reasons for this are that due to increased therapeutic effect from administration of Peg-IFNα-2a 180 μg for 48 weeks, any influence on the therapeutic effect from genotype C was lost. The results of other large scale clinical trials for HBeAg positive cases indicated strong Peg-IFN therapeutic effect for genotype A compared to genotype D,[114,115] but no difference in therapeutic effect between genotype B and genotype C was seen[8] (Table 12). In HBeAg negative cases also, no significant difference in response rate was found between genotype B and genotype C[23,117–119] (Table 13).

3.6.2 HBsAg levels

In recent years highly sensitive measurement of HBsAg levels has become possible, and it has been noted that HBsAg levels are useful in predicting IFN therapeutic effect. Although it is difficult to predict the therapeutic effect from the pretreatment HBsAg levels, the amount and rate of reduction in HBsAg levels during treatment are useful in predicting therapeutic effect.

A European study of 202 HBeAg positive patients administered Peg-IFNα±lamivudine for 52 weeks found that in cases where elimination of HBeAg and HBV DNA <10 000 copies/mL were achieved, the reduction of HBsAg levels at 12 weeks since treatment start correlated significantly with HBsAg elimination an average of 3 years after treatment completion.[71] In other reports, in patients administered Peg-IFNα, the HBsAg levels at 12 weeks after commencement of treatment is important for predicting therapeutic effect, and in cases where the HBsAg levels declined to 1500 IU/mL or less, the rate of elimination of HBeAg is high,[120,121] and subsequent elimination of HBsAg can be expected. In a Hong Kong study of 92 cases administered Peg-IFNα±lamivudine for 32–48 weeks, in cases where the HBsAg levels at 12 weeks after commencement of treatment was <1500 IU/mL, and declined to <300 IU/mL at 24 weeks, the therapeutic effect was high 1 year after treatment, and therapeutic effect was high particularly at 24 weeks in cases where the HBsAg levels declined ≥1 log IU/mL to ≤300 IU/mL.[70]

Even in HBeAg negative patients, when HBV DNA non-detection is defined as effective at 24 weeks after completion of 48 weeks administration of Peg-IFNα, the HBsAg levels at treatment completion is reduced to 2.1 ± 1.2 log IU/mL in effective cases, and if the HBsAg levels reduction at 12 weeks and 24 weeks treatment is ≥0.5 log IU/mL or ≥1.0 log IU/mL respectively, it has been reported as a highly effective response.[119] Furthermore, in a study by Brunetto *et al.*, in cases where the reduction in HBsAg during treatment is ≥1.1 log IU/mL, and the HBsAg at 48 weeks is ≤1.0 log IU/mL, the rate of decrease in the HBsAg levels at 3 years after completion of treatment was markedly high.[122] Furthermore, it has been reported that a decline of 10% or more in the HBsAg levels at the 12 week mark correlated with therapeutic effect 1 year after treatment, and HBsAg elimination after 5 years.[123] On the other hand, there is no way

to use the rate of decrease in HBV DNA levels to distinguish between responders and non-responders. From these results, HBsAg levels are more useful than HBV DNA levels in predicting the therapeutic effect of IFN treatment. However, these reports are all from overseas, and no Japanese evidence is yet available concerning IFN therapy and HBsAg levels.

3.6.3 Age and fibrosis

A Japanese study reported that with conventional IFN, therapeutic effect declines in patients aged ≥35 years,[112] but in a European study analyzing the therapeutic effect of conventional IFN in 496 HBeAg positive patients, based on 10 control trials, no correlation was seen between age and therapeutic effect.[124] A Japanese clinical trial of a 48 week course of Peg-IFNα-2a 180 μg found the combined efficacy rates (ALT ≤40 U/L, HBeAg seroconversion, HBV DNA <5.0 log copies/mL at 24 weeks after completion of treatment) were 15.0% and 23.8% respectively for ≥35 years and <35 years, with a tendency to greater efficacy in the younger group, but some effective cases also seen in the older age group.[9] In overseas trials, no correlation has been found between Peg-IFN therapeutic effect and patient age,[10,115] although there have been reports that in HBeAg positive cases, the therapeutic effect is better in older patients.[114,116] Regardless of whether HBeAg status, there is no clear consensus concerning the relationship between Peg-IFN therapeutic effect and patient age (Tables 12,13). Furthermore, for conventional IFN in patients with advanced fibrosis, the therapeutic effect declined,[113] but for Peg-IFN no correlation was seen between therapeutic effect and fibrosis.[102]

Taken together, due to the improved therapeutic effect seen with Peg-IFN, as with genotype C, factors such as age and advanced fibrosis which impair the therapeutic effect of conventional IFN are no longer significant prognostic factors for Peg-IFN therapy (Tables 12,13).

3.6.4 IL28B gene

In recent years it has been reported that for chronic hepatitis C, single nucleotide polymorphisms (SNPs) in proximity to the IL28B gene correlate extremely strongly with the therapeutic effect of Peg-IFNα+ribavirin combination therapy against genotype 1. A recent study of 205 HBeAg positive patients reported that, even in chronic hepatitis B, high HBeAg seroconversion and HBsAg elimination rates were seen in IL28B major homozygotes.[116] However, no conclusion has yet been reached about the correlation between IL28B genotype and IFN therapeutic effect in chronic hepatitis B, and further investigation and evaluation are required about the effect of host genome factors, including IL28B polymorphisms.

Recommendations

- ***HBV genotype, patient age and degree of fibrosis are factors reported to influence therapeutic effect of conventional IFN treatment. However, Peg-IFN has a greater therapeutic effect than conventional IFN, and high efficacy against HBV genotype A, but its therapeutic effect is not influenced by HBV genotypes B/C or patient age.***
- ***Currently, there is no established method for predicting the treatment response prior to Peg-IFN treatment, regardless of whether a patient is HBeAg positive or negative.***
- ***The amount and rate of reduction of HBsAg levels at 12 weeks and 24 weeks during Peg-IFNα therapy are useful for predicting therapeutic effect. However, no Japanese evidence is yet available concerning IFN therapy and HBsAg levels.***

3.7 Adverse reactions

Adverse reactions associated to IFN treatment are seen in almost all patients. The most common adverse reactions are influenza-like symptoms such as general malaise, fever, headache and joint pain, seen in 60–95% of patients. These influenza-like symptoms can be controlled in most cases by administering an antipyretic analgesic. Hematological testing often shows leukopenia, with white cell counts <1000/mm^3 in approximately 60% of cases. Leukopenia, neutropenia and thrombocytopenia often progress until the fourth week of administration, and then stabilize. However, with the exception of immunocompromised patients and those with cirrhosis, there is no increased risk of infection or hemorrhage associated with neutropenia or thrombocytopenia.[125]

ALT elevation is seen more frequently during IFN treatment for chronic hepatitis B than for chronic hepatitis C. This is considered to be due to the immunostimulatory action of IFN, and normally treatment can be continued, but caution is required in patients with decreased hepatic reserve to avoid liver failure. Neuropsychiatric symptoms such as depression and insomnia occur in 5–10% of patients, and are more common in those with pre-existent neuropsychiatric symptoms or a history of depression. Neuropsychiatric symptoms are classified into depression-specific symptoms and depression-related autonomic nervous

symptoms,[126–128] with selective serotonin reuptake inhibitors (SSRIs) reported to be useful in treating the former. IFN can also trigger or aggravate autoimmune conditions such as chronic thyroiditis, so the utmost caution is required when administering IFN to patients with autoimmune diseases. Interstitial pneumonitis, another reported adverse reaction to IFN therapy, can be serious and even life threatening. It usually occurs after two months of therapy, or in the latter stages of treatment. A rapid and appropriate response is required following the onset of respiratory symptoms such as a dry cough or dyspnea, including an immediate chest CT scan. Determination of serum KL-6 levels is also useful in the diagnosis of interstitial pneumonitis. Other reported adverse reactions to IFN therapy include cardiomyopathy, fundal hemorrhage, and cerebral hemorrhage.

The adverse reaction profile of Peg-IFN differs somewhat to that of non-pegylated IFN. In a Japanese clinical trial of Peg-IFNα-2a monotherapy, the adverse reactions with a higher reported frequency than non-pegylated Peg-IFNα-2a were skin reactions such as erythema at the injection site and hematological reactions such as decreases in the white cell or platelet counts. On the other hand, mild to moderate adverse reactions such as influenza-like symptoms, including fever and joint pains, or malaise and loss of appetite, were milder than with standard non-pegylated IFNα-2a.[129] The cessation rate due to adverse reactions to Peg-IFNα treatment is 2–8%.

Recommendations

- ***Reported adverse reactions to IFN therapy include influenza-like symptoms, reduction in blood cell counts, neuropsychiatric symptoms, autoimmune phenomena, interstitial pneumonitis, cardiomyopathy, fundal hemorrhage, and cerebral hemorrhage.***
- ***Pegylation stabilizes serum IFN levels, ameliorating influenza-like symptoms such as fever and joint pains.***
- ***Patients self-injecting at night minimizes influenza-like symptoms associated with natural IFN-α.***
- ***IFN-β should be considered in patients unable to tolerate IFN-α due to depression or other causes.***

4. PHARMACOTHERAPY (2) – NAs

NAS DIRECTLY SUPPRESS the HBV replication process. In particular, they specifically inhibit reverse transcriptase coded by the HBV itself, and powerfully inhibit negative and positive strand DNA synthesis in the HBV living environment (Fig. 2). As a result, HBV DNA levels in the blood quickly decline and ALT levels also improve. Effectiveness is achieved through continued administration, but if treatment stops the proliferation of virus reoccurs at high frequency causing recurrence of hepatitis.[130] The effect of eliminating HBV-infected hepatocytes is weak.

NAs currently approved by medical insurance system in Japan comprise 3 agents: lamivudine, adefovir and entecavir. In Japan, lamivudine, the first of the NAs, were approved by medical insurance in 2000, followed by adefovir in 2004 and entecavir in 2006 (Table 2).

If administration of the NAs is ceased, in many cases the HBV DNA levels rise again, returning to pre-treatment levels.[131–134] Even in cases where HBeAg seroconversion occurred during administration of a NA (lamivudine), it was found similarly that HBV DNA quantity rose again and HBeAg reappeared.[135,136] Furthermore, after treatment ceases, cases have been reported where ALT levels rose to ≥500 U/L, and total bilirubin rose to ≥2.0 mg/dL.[137] Accordingly, in order to achieve the aim of improved long term outcomes, in general it is necessary not to stop administration of the NAs, and provide continuous maintenance treatment to inhibit HBV reproduction.

4.1 Lamivudine

Lamivudine is a reverse transcriptase inhibitor, originally developed for treatment of human immunodeficiency virus (HIV). Like HIV, HBV passes through a transcriptase process in its lifecycle, so a reverse transcriptase inhibitor has therapeutic effect. Lamivudine has a structure (3TC-TP) similar to deoxycytidine triphosphate (dCTP), which is used as a foundation substance when reverse transcriptase synthesizes DNA using RNA as a template. For this reason lamivudine binds to reverse transcriptase during DNA synthesis and inhibits further DNA synthesis. This mechanism inhibits reproduction of the HBV virus and reduces HBV DNA levels. The dosage of lamivudine is 100 mg per day. Lamivudine has almost no adverse reactions and is very safe. Reported therapeutic results for lamivudine in HBeAg positive patients in Asian and other overseas countries are ALT normalization rates of 40–87% 1 year after commencement of treatment, 85% after 2 years, and HBV DNA negative conversion rates (solution-hybridization or branched chain DNA assays) of 44–87% after 1 year, and 74% after 2 years.[131,138,139] Reported HBeAg seroconversion rate are 17–28% after 1 year, 25–29% after 2 years, 40% after 3 years, and 50% after 5 years.[138–141] Furthermore, histological

improvement is also reported 1 year after commencement of treatment.[142]

The short term effects of lamivudine are also favorable in HBeAg negative patients.[134,143,144] In a Japanese study,[139] the HBV DNA negative conversion rate (HBV DNA <0.5 Meq/mL) was 94% after 1 year of treatment and 92% after 2 years, and the ALT normalization rate was 89% after 1 year, and 82% after 2 years. However, the HBV DNA negative conversion rate decreases over the long term.[96]

A major problem with lamivudine is the occurrence of drug resistance (YMDD motif mutation). In lamivudine-resistant viruses, mutation occurs in the amino acid sequence called the YMDD motif inside the RNA dependent DNA polymerase region. In other words, M (methionine) inside the YMDD motif mutates into V (valine) or I (isoleucine). As a result, changes occur in the polymerase structure, lamivudine bonding is reduced and its effectiveness declines. It has also been shown in *in vitro* tests that lamivudine resistance occurs due to YMDD motif mutation.[145,146]

In general, lamivudine-resistant viruses appear 6–9 months after treatment starts, and increase as treatment continues.[139,147–154] In Japanese studies, the incidence of lamivudine-resistant viruses was 13–15% at 1 year, 25–32% at 2 years, 29–45% at 3 years, 51–60% at 4 years, 63–65% at 5 years, and 70% at 6 years.[139,149–154] Past studies have identified HBeAg positive status at baseline, high HBV DNA load at baseline, cases where the HBV DNA load fails to fall below 3–4 log copies/mL after 3–6 months of treatment, persistent HBeAg positive status, cirrhosis, and genotype A as risk factors for the emergence of lamivudine-resistant viruses.[139,147,149–151,154]

Usually, no abnormalities are seen in blood tests immediately after the emergence of lamivudine-resistant viruses, but rising HBV DNA levels (breakthrough) and rising ALT levels (breakthrough hepatitis) are seen within 3–4 months of emergence of resistance in at least 70–80% or more of cases.[149,152,155] Great caution is required in these cases because breakthrough hepatitis can sometimes be more serious than hepatitis prior to lamivudine therapy.[156,157] Due to the high risk of emergence of lamivudine-resistant virus, currently lamivudine is not regarded as the first choice NA.

Recommendation

- ***Long-term lamivudine administration is associated with a high risk of emergence of resistant virus. Accordingly, lamivudine is not the first choice NA.***

4.2 Adefovir

Adefovir (adefovir dipivoxil) is an analog of adenine (dATP). Adefovir inhibits HBV reproduction not only through antagonistic competition with dATP, but by also acting as a chain terminator to stop the DNA extension process and inhibit HBV replication. *In vitro*, adefovir not only exhibits a similar antiviral effect to lamivudine against natural strains of HBV, but it has also been shown to be effective against lamivudine-resistant strains.[145] Its effectiveness against cases of exacerbated hepatitis due to lamivudine-resistant virus has been confirmed in actual clinical practice.[158–168] Adefovir therapy is officially approved by Japanese medical insurance system at a dosage of 10 mg daily.

Following 48 weeks of adefovir monotherapy in HBeAg positive patients, the HBV DNA negative conversion rate was 21%, and the HBeAg seroconversion rate 12%, with no resistant virus detected.[169] Following long term administration for 5 years, the HBV DNA levels declined an average of 4.05 log copies/mL, ALT levels declined by ≥50 U/L in 63% of cases, the DNA negative conversion rate was 39%, the HBeAg negative conversion rate was 58%, and seroconversion was reported in 48%. The incidence of adefovir-resistant virus was 21%.[170] In HBeAg negative patients, after 48 weeks of administration the HBV DNA negative conversion rate was 51% as expected, the ALT normalization rate was 72%, and resistant virus was not detected.[171] In another study, after 5 years of adefovir therapy, the HBV DNA negative conversion rate was 67%, the ALT normalization rate 69%, the histological improvement rate (Ishak fibrosis scores) 71%, whereas the incidence of resistant virus (rtA181T/V, rtN236T) was 0% at 1 year, 3% at 2 years, 11% at 3 years, 18% at 4 years and 29% at 5 years, and re-elevation of ALT was 11%.[172] Reported factors associated with adefovir-resistant virus are where treatment switched from lamivudine to adefovir monotherapy, advanced age, genotype D, and lamivudine-resistant virus.[173,174]

Important adverse reactions to adefovir are renal dysfunction and hypophosphatemia. After 4–5 years administration, creatinine levels increased to ≥0.5 mg/dL in 3–9% of patients,[170,172] and eGFR declined ≥20% in 2.6% at 1 year, 14.8% at 3 years, and 34.7% at 5 years.[175] Furthermore, treatment discontinuation due to renal dysfunction and decline in eGFR <50 mL/min was significantly more common in the group administered adefovir than in the non-treatment group (relative risk = 3.68). Renal dysfunction was more likely to occur in patients aged ≥50 years, patients

with mildly reduced eGFR at commencement of treatment (50–80 mL/min), and patients with hypertension or diabetes.[176] In a Japanese study, administration of adefovir for an average of 38 months caused elevated creatinine levels in 38% of cases, exceeding 1.4 mg/dL in 11% of cases. Factors associated with elevated creatinine levels were advanced age and long term therapy.[165] Elevated creatinine levels can be managed by reducing the dose of adefovir (such as alternate day administration). Hypophosphatemia (<2.0 or <2.5 mg/mL) was seen in 3–16% of cases,[165,170] and elevation of serum creatinine level was also observed in most of these cases.[165] Cases of Fanconi syndrome have also been reported,[165,177,178] indicating the need for careful monitoring.

Recommendations

- ***Adefovir long term monotherapy is moderately effective. However, resistant HBV may emerge with long term administration.***
- ***Care should be taken with long term administration of adefovir for the possible onset of renal dysfunction and hypophosphatemia (including Fanconi syndrome).***

4.3 Entecavir

Entecavir is a NA with a structure resembling that of guanosine (a guanine nucleoside), with a powerful and selective inhibitor effect against HBV DNA polymerase. The mechanism of its activity involves intracellular phosphorylation of entecavir and conversion into activated entecavir-triphosphate (ETV-TP). Through competition with the natural substrate deoxyguanosine triphosphate (dGTP), ETV-TP inhibits all 3 types of HBV polymerase activity during HBV DNA replication: (1) priming, (2) reverse transcription when the minus strand DNA is synthesized from mRNA, and (3) synthesis of plus strand DNA. *In vitro* experiments have demonstrated not only that entecavir has stronger antiviral activity than lamivudine or adefovir against HBV wild strains, but it is also effective against lamivudine-resistant strains.[179] Entecavir has had health insurance approval in Japan since 2006, for administration of 0.5 mg per day in treatment-naïve cases.

In Europe studies of entecavir therapy in patients naïve to NAs, in both HBeAg positive cases and negative patients, HBV DNA negative conversion rates and ALT normalization rates were higher for entecavir than for lamivudine.[14,25,180] The greatest characteristic of entecavir is that it has a lower incidence of viral resistance than lamivudine. For this reason entecavir is currently the treatment of first choice when using NAs. Resistance to entecavir is exhibited by amino acid mutation of either rtT184, rtS202 or rtM250, in addition to the lamivudine resistant amino acid mutations at rtM204V and rtL180M.[181] In the abovementioned study, increased HBV DNA levels were seen in 22 out of 679 patients until the 96th week of therapy. Only 1 case of entecavir-resistant HBV was confirmed at 1 year, and 1 more case at 96 weeks, in one of which lamivudine-resistant HBV had already been detected at the commencement of entecavir therapy.[180]

Long term results have been reported for entecavir administration for 5 years.[16,182] The HBV DNA negative conversion rate was 55–81% at 1 year, 83% at 2 years, 89% at 3 years, 91% at 4 years and 94% at 5 years, and the ALT normalization rate was 65% at 1 year, 78% at 2 years, 77% at 3 years, 86% at 4 years and 80% at 5 years, while the incidence of resistant HBV was 0.2% at 1 year, 0.5% at 2 years, and 1.2% at 3–5 years. However, in these studies, entecavir 0.5 mg daily was not continuously administered in all cases. On the other hand, in a report from Hong Kong of continuous entecavir therapy for 3 years, the HBV DNA negative conversion rate was 81% at 1 year, 90% at 2 years and 92% at 3 years; the ALT normalization rate was 84% at 1 year, 88% at 2 years and 90% at 3 years; and the HBeAg seroconversion rate was 22% at 1 year, 41% at 2 years and 44% at 3 years.[19] From of these cases, 1 case of resistant HBV was confirmed at 3 years.

In results from Japan concerning NAs naïve cases,[15,18,183] the HBV DNA negative conversion rate was 77–88% at year 1, 83–93% at year 2, 95% at year 3, and 96% at year 4. The ALT normalization rate was 83–87% at year 1, 88–89% at year 2, 92% at year 3, and 93% at year 4. The HBeAg seroconversion rate was 12–20% at year 1, 18–20% at year 2, 29% at year 3, and 38% at year 4. Histological evaluation also confirmed improvement in the Knodell necroinflammatory score and fibrosis score at 1 year and 3 years.[18] The incidence of entecavir-resistant HBV was 3.3% at 3 years.[18]

In consideration of the high risk of resistant HBV associated with long term administration of lamivudine, some studies have examined the results of a change from lamivudine to entecavir.[184–186] In cases where the HBV DNA levels during lamivudine therapy remained <2.6 log copies/mL, HBV DNA continued negative after switching to entecavir, and entecavir-resistant virus was not detected. On the other hand, when the HBV DNA levels is ≥2.6 log copies/mL at the time of switching, entecavir-resistant HBV may appear irrespective of whether lamivudine-resistant virus was already present.

Concerning problems with safety, almost no adverse reactions of clinical importance were reported. Points to keep in mind are that entecavir is not suitable for long term continuous therapy for women desiring to bear children due to the risk of teratogenesis, and the safety of long term administration has not been established.

Recommendations

- ***Favourable results are obtained with entecavir in patients naïve to NAs, with a low incidence of resistant virus, currently making entecavir the first-choice NA.***
- ***Switching to entecavir is recommended in patients in whom the HBV DNA negative conversion occurs with lamivudine therapy.***

4.4 Treatment of NA-resistant HBV

4.4.1 Lamivudine-resistant HBV

It has been reported that if lamivudine-resistant HBV appears and the viral load increases, onset of hepatitis is likely; furthermore, in some cases the hepatitis may become severe.[157,187] Accordingly, treatment with an antiviral agent is required if lamivudine-resistant HBV appears. IFN, adefovir and entecavir have been confirmed effective against lamivudine-resistant HBV, and are currently approved for Japanese medical insurance.

Although IFN can be used to a certain extent to treat hepatitis associated with lamivudine-resistant HBV, there are problems with adverse reactions and a limited treatment duration.[188,189] On the other hand, adefovir has good long term efficacy against lamivudine-resistant HBV, with mild adverse reactions and suitable for long term therapy, so currently adefovir is recommended. Rather than switch from lamivudine to adefovir, lamivudine and adefovir in combination provides a stronger antiviral effect.[190] The long term effect of lamivudine+adefovir combination therapy against lamivudine-resistant HBV has been reported as an HBV DNA negative conversion rate (<2.6 log copies/mL) using the Amplicor testing of 56–82% at 1 year, 74–84% at 2 years, 81–86% at 3 years, 80–92% at 4 years, and 85–86% at 5 years.[158,159,161,164,165,167] Reported factors relating to the antiviral effect of lamivudine+adefovir combination therapy include DNA load (low value), albumin level (low), ALT level (high), HBeAg (negative), and HBV DNA negative conversion during lamivudine therapy.[159,165,166,168] Reported ALT normalization rates were 67–81% at 1 year, 75–83% at 2 years, 80–92% at 3 years, 82–90% at 4 years, and 85% at 5 years.[158,159,161,164,165,167] HBeAg negative conversion rates for HBeAg positive cases at the time of commencement of combination therapy were 20–23% at 1 year, 17–25% at 2 years, 14–61% at 3 years; seroconversion rates were 5% at 1 year, 11% at 2 years, and 14% at 3 years.[159,161,166] Reported factors related to HBeAg negative conversion were ALT level (high), and the history of IFN therapy in the past.[159,166] If hepatitis associated with lamivudine-resistant HBV occurs, adefovir resistance develops if therapy is changed from lamivudine to adfovir, but if lamivudine+adefovir combination therapy is administered, the reported incidence of HBV resistant to both agents is low.[191]

Entecavir therapy is also administered to patients with lamivudine-resistant HBV (including cases unresponsive to lamivudine). The short-term results for entecavir therapy are good, and in some USA studies reported an HBV DNA negative conversion rate of 21% at 1 year, and 34–40% at 2 years, and an ALT normalization rate of 65% at 1 year, and 81% at 2 years.[192,193] However, the appearance of entecavir-resistant HBV associated with long term administration of entecavir has been confirmed. The incidence of entecavir-resistant HBV was 6% at 1 year and 8–13% at 2 years, and rebound of the HBV DNA load due to entecavir-resistant HBV was 1% at 1 year and 9% at 2 years. A Japanese study reported favorable results with a HBV DNA negative conversion rate of 16% at 6 months and 33% at 1 year, and ALT normalization rate of 78% at 6 months and 81% at 1 year,[194–196] although entecavir-resistant HBV was detected in 26% of cases up to year 3, in whom hepatitis rebounded in 40%.[196] In this way, entecavir therapy for lamivudine-resistant (or unresponsive) HBV may also produce viral strains resistant to entecavir.

Recommendations

- ***Lamivudine+adefovir combination therapy is recommended for treatment of lamivudine-resistant HBV.***
- ***Entecavir therapy of lamivudine-resistant HBV may also produce viral strains resistant to entecavir.***

4.4.2 Adefovir-resistant HBV

Reported adefovir-resistant mutations include rtA181V/T, rtI233V and rtN236T in the HBV polymerase reverse transcriptase (rt) region. Of these mutations, *in vitro* and *in vivo* testing has demonstrated sensitivity to both lamivudine and entecavir for the rtN236T mutation, but lamivudine resistance for the rtA181V mutation.[7,197] In 132 patients with lamivudine-resistant HBV treated with lamivudine+adefovir combination therapy, multiple resistant strains were seen in 3 cases before the commencement of adefovir therapy, and in 2 further

cases after therapy commenced (overall incidence 4%).[168]

Entecavir+adefovir combination therapy is administered to patients with HBV resistant to both lamivudine and adefovir, with undetermined results. On the other hand, in reports from Europe, in cases with resistance to lamivudine or adefovir monotherapy, or resistant/unresponsive to lamivudine+adefovir combination therapy, administration of the new agent tenofovir (median treatment period 23 months) yielded HBV DNA negative conversion in 79% of cases, HBeAg negative conversion in 24%, and HBsAg negative conversion in 3%.[198] In cases where lamivudine was ineffective and there was no response after at least 24 weeks of adefovir therapy, 12 weeks of tenofovir monotherapy or tenofovir+ lamivudine combination therapy reduced the HBV DNA load by a mean 2.19 log IU/mL, with HBV DNA negative conversion rates after 48 weeks and 96 weeks of 46% and 64% respectively.[199] Tenofovir is effective against multiresistant HBV strains, and it is hoped that it will be approved for use in clinical practice in Japan.

Recommendation

- ***Entecavir+adefovir combination therapy is administered to patients with HBV resistant to both lamivudine and adefovir, with undetermined results.***

4.4.3 Entecavir-resistant HBV

Entecavir-resistance involves one of the amino acid mutations, rtT184, rtS202 or rtM250 in addition to the amino acid mutations rtM204V and rtL180M that confer lamivudine resistance.[181] Efficacy has been reported for lamivudine+adefovir and for entecavir+adefovir combination therapy against entecavir-resistant HBV.[200,201] On the other hand, another study found that HBV DNA negative conversion was not achieved with lamivudine+adefovir combination therapy, but lamivudine+tenofovir combination therapy was effective.[202] At present the long term results for these combined therapy methods are unclear, and further studies including therapeutic results for tenofovir will be required.[7,203]

Recommendations

- ***Lamivudine+adefovir or entecavir+adefovir combination therapy is recommended for the treatment of entecavir-resistant HBV infection.***
- ***Tenofovir can be expected to be effective against multiagent resistant HBV strains.***

4.5 Towards a drug-free state

NA therapy for chronic hepatitis B produces a strong antiviral effect compared to IFN therapy, irrespective of HBV genotype, and has the added benefit of a low level of adverse reactions. On the other hand, with NA therapy, resistant mutations can appear with long term administration, the safety of long term administration has not been confirmed, and medical costs are high. Accordingly, when good therapeutic efficacy is achieved, cessation of NA therapy may be considered. However, there is a high likelihood of hepatitis recurrence following treatment cessation,[78] so it is important to identify cases unlikely to relapse and to cease NA therapy only in patients in whom treatment cessation is considered feasible. Sequential therapy is also being trialed, whereby the NAs are ceased after switching over to IFN, with the aim of continued therapeutic effect, or even achieving HBsAg negative conversion, after stopping NA therapy.

4.5.1 Cessation of NAs

NAs exert antiviral effects through inhibition of HBV DNA reverse transcriptase, but are unable to eliminate cccDNA present in hepatocyte nuclei. Accordingly, after cessation of NA therapy, even if HBV DNA negative conversion has occurred, this cccDNA becomes a template for HBV replication to resume, leading to recurrence of hepatitis.[204] Accordingly, HBV DNA negative conversion cannot be used as the sole criterion for cessation of NA therapy.

In such cases, HBcrAg and HBsAg become useful markers. A significant positive correlation has been reported between HBcrAg and cccDNA, even during NA therapy.[205,206] In fact, evaluation of cases of exacerbated hepatitis following cessation of NA therapy revealed significantly lower levels of HBcrAg (3.2 *vs* 4.9, $P = 0.009$) in the non-recurrence group compared to the recurrence group,[207] indicating that HBcrAg is a potential marker for cessation of NA therapy. Similarly to HBcrAg, HBsAg is thought to be little affected by NA transcriptase inhibition, and the retreatment rate after cessation of NA therapy was significantly lower for the group with low HBsAg levels (<1000 IU/mL) at the time of cessation (18% *vs* 63%, $P = 0.049$).[208]

Based on the above results, the MHLW research group produced a report titled "Studies concerning efficacy of IFN therapy aimed at creation of treatment discontinuation standards and treatment discontinuation in NAs therapy for hepatitis B", setting out policy regarding cessation of NA therapy.[209,210] A summary is shown in

Table 14 Conditions required for cessation of NA therapy

Patient criteria
• Both the treating physician and the patient fully understand that after cessation of NA therapy, there is a high incidence of recurrence of hepatitis, possibly severe
• Follow-up is possible after treatment cessation, and appropriate treatment is possible even if hepatitis recurs
• Even if recurrence of hepatitis occurs, it is unlikely to be severe if the degree of fibrosis is mild and the hepatic reserve is good
Laboratory criteria
• At least 2 years of administration of NAs
• Undetectable serum HBV DNA levels (using real time PCR) at the time of treatment cessation
• Negative serum HBeAg at the time of treatment cessation.

Table 14. To determine the criteria for therapy cessation, as shown below in Table 15, HBsAg and HBcrAg levels at therapy cessation were scored, the final score allocated to the following 3 categories of risk of relapse, and the success rate was predicted. Successful cessation was defined as "finally resulting in inactive carrier status, i.e. ALT ≤30 U/L and HBV DNA <4.0 log copies/mL". Studies have shown that if this inactive carrier status is achieved, there is no progression of liver disease, and risk of HCC also declines.[34,211]

Recommendations

- ***The following 3 patient criteria must be met for cessation of NA therapy: (1) Both the treating physician and the patient fully understand that after cessation of NA therapy, there is a high incidence of recurrence of hepatitis, possibly severe; (2) Follow-up is possible after treatment cessation, and appropriate treatment is possible even if hepatitis recurs, (3) Even if recurrence of hepatitis occurs, it is unlikely to be severe if the degree of fibrosis is mild and the hepatic reserve is good.***
- ***The 3 laboratory criteria for cessation of NA therapy are: (1) At least 2 years of administration of NAs; (2) undetectable serum HBV DNA levels (using real time PCR); (3) negative serum HBeAg at the time of treatment cessation.***
- ***When the above criteria are met, it is possible to predict the risk of relapse from HBsAg and HBcrAg levels at the time of cessation of therapy. NA therapy should be continued in the high risk group.***

4.5.2 Sequential therapy

As described earlier, although NAs inhibit replication of HBV DNA, they have no effect on cccDNA, whereas IFN has a weak effect on HBV reproduction inhibition, but

Table 15 Risk of relapse following cessation of NA therapy

HBsAg load at cessation (IU/mL)	Score	HBcrAg load at cessation (U/mL)	Score
<1.9 log (80)	0	<3.0 log	0
≥1.9 log (80), <2.9 log (800)	1	≥3.0 log, <4.0 log	1
≥2.9 log (800) IU/mL	2	≥4.0 log	2

Relapse risk	Total score	Predicted success rate	Evaluation
Low risk group	0	80~90%	Group for which cessation may be considered. However, even in the low risk group, recurrence of hepatitis can occur, so vigilance is required.
Moderate risk group	1~2	Approx. 50%	Group for which cessation may be considered depending on circumstances. This group requires further evaluation concerning cessation criteria and methods.
High risk group	3~4	10~20%	Continued treatment is recommended for this group. However, for patients aged <35, the cessation success rate is relatively high at 30~40%.

has immunomodulatory effects including increasing viral antigen presentation to host cells, with antiviral effects persisting after completion of administration. Accordingly, a number of clinical trials have been conducted using IFN in combination with NAs. Combination therapy regimens are either synchronous combination therapy or sequential combination therapy, where a NA is administered synchronously with IFN for a fixed period, then switched over to IFN monotherapy (or the switchover is from NA monotherapy to IFN monotherapy, with no synchronous administration period). Synchronous combined therapy was aimed to enhance therapeutic efficacy. However, the antiviral effects of synchronous Peg-IFN+lamivudine combination therapy may be higher than lamivudine monotherapy during treatment, but its therapeutic effect has been reported to be almost the same as Peg-IFN monotherapy.[8,22,115] Accordingly, at this time there is insufficient evidence that therapeutic effect improves with synchronous administration of IFN and NAs.

As with synchronous therapy, sequential therapy can be used with the aim of "enhanced therapeutic efficacy", or for "suppression of recurrence of hepatitis after cessation of NAs". Initially, Serfaty *et al*. conducted a sequential therapy study with 14 patients with HBeAg positive chronic hepatitis B in whom IFN treatment was ineffective. Lamivudine monotherapy was administered for 20 weeks, then IFN+lamivudine combination therapy for 4 weeks, followed by IFN monotherapy for 24 weeks, producing favorable therapeutic results with an HBeAg seroconversion rate of 45%, and HBV DNA negative conversion rate of 57%.[212] However, subsequent studies of sequential therapies following a variety of protocols have failed to demonstrate a significant enhancement of therapeutic efficacy.[213–215] A Japanese multicenter collaborative trial of sequential therapy following a similar method to Saferty *et al*. also found no significant enhancement of therapeutic efficacy in comparison to IFN monotherapy as a historical control.[216] However, this study did show that in almost all responders, HBeAg negative conversion occurred during initial lamivudine monotherapy. It has also been reported that in sequential entecavir+IFN combination therapy, a high rate of efficacy was demonstrated in patients where HBeAg negative conversion was seen during entecavir monotherapy.[215] Accordingly, in Japan the aim of sequential therapy is not to enhance therapeutic efficacy through addition of NAs, but rather as a method for safely discontinuing NAs, and currently is indicated in "patients who have undergone HBeAg negative conversion during NA therapy, or are HBeAg negative". Currently the MHLW research group is conducting prospective trials with the aim of evaluating the efficacy and safety of sequential therapy using Peg-IFN, with the following as the main entry criteria: (1) at least 2 years of NA therapy; and (2) HBeAg negative and HBV DNA load <3.0 log copies/mL (preferably undetectable HBV DNA using real time PCR). As evidence is accumulated, the indications for sequential therapy should become clearer.

Comprehensive studies are lacking concerning sequential therapy in cases where a favorable therapeutic response is maintained by NA therapy. Ning *et al*. conducted a randomized controlled study with 102 HBeAg positive patients without cirrhosis who were administered entecavir for 4 years, resulting in HBV DNA <3.0 log copies/mL and HBeAg <100 PEIU/mL. The sequential therapy group was administered entecavir+Peg-IFNα-2a synchronous combination therapy for 8 weeks, then Peg-IFN monotherapy for 40 weeks, and the entecavir monotherapy group was treated with entecavir alone. They reported that no difference between groups in the HBV DNA load, but a higher rate of HBsAg negative conversion during treatment for the sequential therapy group (27%, 4/15). As described above, in Japan sequential therapy is conducted with the aim of safely ceasing NAs, and there is no data concerning HBsAg negative conversion.

4.5.3 Retreatment following cessation of NAs or completion of sequential therapy

Recurrence of hepatitis following cessation of NA therapy (including sequential therapy) has the potential to become severe, and retreatment may be necessary. The abovementioned MHLW research group proposed criteria for retreatment after cessation of NA therapy. A retrospective analysis of patients who became inactive carriers found that approximately 2/3 experienced transient elevation of HBV DNA or ALT levels after cessation of NA therapy, clarifying that retreatment was not necessary for all cases of HBV DNA or ALT rebound.[208] However, a return to inactive carrier status is unlikely in cases with elevation of ALT ≥80 U/L or HBV DNA ≥5.8 log copies/mL, and retreatment should be considered.

Recommendations

- ***The aim of sequential therapy is not enhancement of the therapeutic efficacy of NAs, but as a method of safe cessation of NA therapy, and is currently indicated in "patients who have undergone HBeAg negative conversion during NA therapy, or are HBeAg negative".***

- ***Following cessation of NA therapy or completion of sequential therapy, a return to inactive carrier status is unlikely in cases with elevation of ALT ≥80 U/L or HBV DNA ≥5.8 log copies/mL, and retreatment should be considered.***

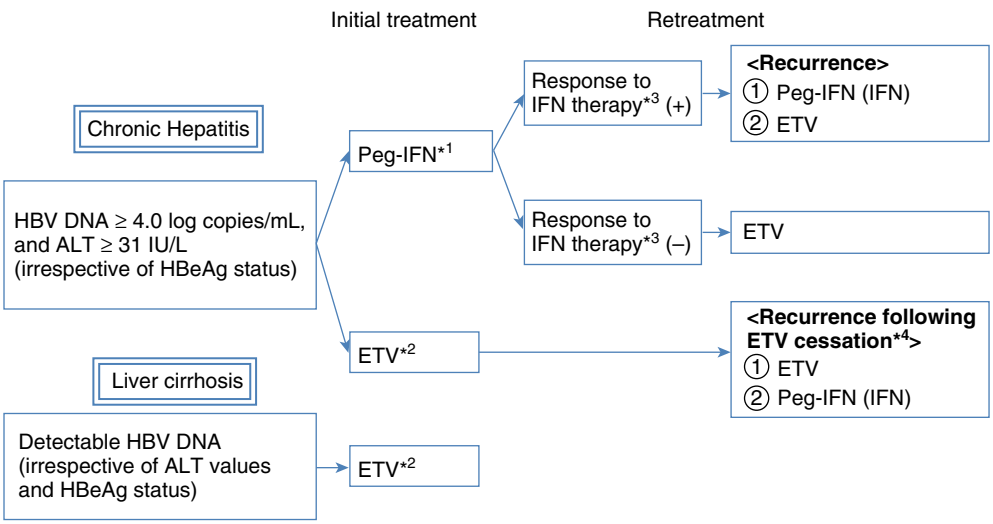
5. TREATMENT OF CHRONIC HEPATITIS AND LIVER CIRRHOSIS

5.1 Basic principles of antiviral therapy (Fig. 6)

5.1.1 Chronic hepatitis (initial treatment)

PEG-IFN THERAPY FOR a finite duration may provide drug-free, long-lasting HBeAg seroconversion, and also HBsAg negative conversion, with no development of drug resistance. For conventional IFN treatment, therapeutic efficacy fell for patients 35 years or older and for genotype C,[112] but in Peg-IFN clinical trials in Japan as well as overseas, there was no significant correlation between therapeutic efficacy and genotype or age.[8–10,115,124] Taking these characteristics into consideration, Peg-IFN monotherapy should be generally considered the first choice for initial treatment of chronic hepatitis, regardless of HBeAg status or HBV genotype. In cases where avoidance of long-term administration of NAs is preferable, particularly for young patients and women desiring to bear children, Peg-IFN is the treatment of first choice. It should be noted that, in Japanese clinical trials, ≥95% of subjects are aged <50 years, in both HBeAg positive and negative groups, and the efficacy of Peg-IFN therapy has not been adequately assessed in patients aged ≥50 years.[100] A full explanation may be warranted that the HBeAg seroconversion rate and HBV DNA negative conversion rate are not necessarily high, that it is difficult to efficacy in individual cases prior to treatment, and possible adverse reactions.

On the other hand, in cases where Peg-IFN is contraindicated for tolerability, or in cases with cirrhosis, entecavir therapy is administered initially with the aim of maintaining long term remission. However,



*1 Full explanation required that HBeAg seroconversion rate and HBV DNA negative conversion rate are not necessarily high, that effectiveness prediction for each case prior to treatment is difficult, and explanation of expected adverse reactions.
*2 After confirming no intention to produce children, explain fully the need for long-term continuous administration, and explain the risk of resistant mutations.
*3 Use ALT normalization, HBV DNA load decline (HBsAg load decline), and in HBeAg positive cases, use HBeAg negative conversion for reference, then make the judgment at 24-48 weeks after treatment completion.
*4 Retreatment standard for relapse after ETV cessation: HBV DNA ≥ 5.8 log copies/ml, or ALT ≥ 80 IU/L.

Figure 6 Basic protocol for antiviral treatment.

lamivudine therapy is recommended in cases of acute exacerbation of hepatitis associated with jaundice, because transaminases can rise in these patients following entecavir administration. When a prolonged treatment period is likely, a switch should be made to entecavir. Before commencing entecavir therapy, it is necessary to fully explain the need for long term continuous treatment, possible safety problem during pregnancy and the risk of resistant mutations, before obtaining informed consent.

5.1.2 Chronic hepatitis (retreatment)

In cases where the HBV DNA and ALT levels declined and hepatitis became quiescent following treatment with conventional IFN or Peg-IFN treatment, retreatment with Peg-IFN therapy should be considered if hepatitis recurs. Even in patients where quiescence of hepatitis was not obtained by conventional IFN therapy, retreatment with Peg-IFN is an option. However, in cases where tolerability of conventional IFN therapy is poor, and in cases where quiescence of the hepatitis is not obtained by the preceding Peg-IFN therapy, entecavir therapy is administered with the aim of maintaining long term remission. Even in cases of recurrence of hepatitis following cessation of entecavir therapy, retreatment with entecavir should be considered. The criteria for recurrence of hepatitis are HBV DNA levels ≥5.8 log copies/mL, or ALT levels ≥80 U/L.[209]

5.1.3 Liver cirrhosis

In Japan, there is insufficient evidence for the efficacy and safety of IFN treatment for HBV cirrhosis, and it is not officially approved. The initial treatment for liver cirrhosis is long term continuous entecavir therapy.

Recommendations

- ***In general, Peg-IFN monotherapy should be considered the first choice treatment for chronic hepatitis, irrespective of HBeAg status or HBV genotype.***
- ***Retreatment using Peg-IFN should be considered in patients with chronic hepatitis when recurrence of hepatitis occurs following treatment with conventional IFN or Peg-IFN. Entecavir therapy should be administered to IFN non-responders, with no efficacy from earlier IFN therapy. Even in cases of recurrence of hepatitis following cessation of entecavir therapy, retreatment with entecavir should be considered.***
- ***The initial treatment for liver cirrhosis is long term continuous entecavir therapy.***

5.2 HBeAg positive chronic hepatitis

5.2.1 Timing of commencement of treatment

Even if they are HBeAg positive, asymptomatic carriers in the immune tolerance phase with ALTs consistently within the normal range present few abnormal histological findings. Furthermore, irrespective of the NAs or IFN, seroconversion rates from antiviral therapy are low at <10%.[217–222] For these reasons, treatment is not indicated in asymptomatic carriers.[223] HBV DNA, HBeAg and ALT levels should be monitored at 3–6 month intervals, and treatment considered if ALT levels rise.[32,224–227]

Treatment is indicated in patients with HBeAg positive chronic hepatitis B with HBV DNA levels ≥4.0 log copies/mL and ALT ≥31 U/L.[4,30–32] If there is no evidence of advanced fibrosis, and the patient is not considered at risk of fulminant hepatitis, it may be advisable to withhold treatment for another year while monitoring ALT, HBeAg and HBV DNA levels, anticipating natural HBeAg seroconversion, since the annual likelihood of natural HBeAg seroconversion is 7–16% per annum.[4,30–32] However, if HBeAg seroconversion does not occur, persistent hepatitis may cause progression of hepatic fibrosis,[2,4,228] necessitating treatment to prevent this. HBeAg positivity and elevated HBV DNA levels are independent risk factors for hepatocellular carcinogenesis and progression to liver cirrhosis,[2,34,37,211,229–231] and patient age (≥40 years) is also a risk factor for progression of liver cirrhosis and HCC.[2,36,37] The risk of HCC is also higher in patients with platelet counts <150 000, reflecting progression of hepatic fibrosis, or a family history of HCC.[38,39] Accordingly, treatment should be positively considered in patients with any of the abovementioned risk factors, even if they do not meet the criteria for commencement of treatment. Liver biopsy (or noninvasive alternative) should be performed as an optional investigation to determine the extent of fibrosis, and treatment is indicated if hepatic fibrosis is diagnosed.

Treatment should be commenced immediately, without a monitoring period, in patients with acute exacerbations of hepatitis associated with jaundice, or if there are concerns about liver failure.

Recommendations

- ***Treatment is not indicated in HBeAg positive asymptomatic carriers.***
- ***Treatment is indicated in patients with HBeAg positive chronic hepatitis cases with HBV DNA levels ≥4.0 log copies/mL and ALT ≥31 U/L.***
- ***When ALT levels increase in patients with HBeAg positive chronic hepatitis, if there is no evidence of***

advanced fibrosis, and the patient is not considered at risk of fulminant hepatitis, one option is to defer treatment for approximately one year. However, if HBeAg seroconversion does not occur naturally, treatment is indicated to prevent progression of hepatic fibrosis due to persistent hepatitis.

- ***For patients who do not meet the criteria for commencement of treatment, in but have a high risk of HCC, liver biopsy (or noninvasive alternative) should be performed as an optional investigation to determine the extent of fibrosis, and treatment is indicated if hepatic fibrosis is diagnosed.***
- ***Treatment should be commenced immediately, without a monitoring period, in patients with acute exacerbations of hepatitis associated with jaundice, or if there are concerns about liver failure.***

5.2.2 Selection of therapeutic agent

In patients with HBeAg positive chronic hepatitis, the risk of liver failure is reduced by negative conversion of HBeAg, and life expectancy increased,[2,34,211,228–232] so the short term target of antiviral therapy is HBeAg seroconversion, and the ultimate long term target is negative conversion of HBsAg.

In general Peg-IFN monotherapy is considered the treatment of first choice for initial antiviral therapy, taking into consideration the absence of drug resistance, and relatively high probability that a prolonged HBeAg seroconversion, in a drug free state, can be achieved with treatment for a finite duration.

HBeAg seroconversion rates are no more than 24%–36% at 24 weeks after completion of 48 weeks of Peg-IFN therapy,[8–10] but in responders that achieved HBeAg seroconversion, HBeAg negative status was maintained in 77%–86% of patients in drug free status.[11–13] Even in cases who failed to achieve HBe seroconversion at the conclusion of treatment, delayed seroconversion occurs in 14% of cases 1 year later,[12] in 27% 3 years later,[11] and in 69% 5 years later.[13] The HBsAg negative conversion rate was low at 2.3%–3.0% of all patients 24 weeks after the conclusion of treatment,[8–10] but in responders who achieved HBeAg seroconversion, the HBsAg negative conversion rate was at an extremely high rate, 30% 3 years after treatment completion,[11] and 64% (with conventional IFN) 14 years after treatment completion.[233]

Entecavir is the first choice in patients at high risk of progression of hepatic fibrosis to liver cirrhosis. Furthermore, in cases where Peg-IFN is ineffective or contraindicated, entecavir therapy is administered with the aim of maintaining long term remission.

Higher rates of HBV DNA negative conversion and ALT normalization are achieved after 1 year of entecavir therapy than with Peg-IFN therapy.[14,25,183] Furthermore, after 4–5 years of long term continuous treatment, even higher levels of therapeutic efficacy are achieved, with HBV DNA negative conversion rates of 94%–96%, and ALT normalization rates of 80%–93%.[15,16] The HBeAg seroconversion rate was no better than 12%–22% after 1 year,[14,15,18,19,183] lower than for Peg-IFN, but the seroconversion rate increases with long term continuous treatment, and even if HBeAg seroconversion does not occur at the 2 year mark, after 5 years the seroconversion rate was 23%,[16] and a report from Japan indicated that the seroconversion rate was 38% after 4 years.[15] On the other hand, the HBsAg negative conversion rate is lower than for Peg-IFN, only 1.7% 48 weeks after commencement of treatment,[14] and 0.6%–5.1% after 3–5 years of treatment.[16,17,21]

In patients administered NA therapy that achieve HBeAg seroconversion and maintain HBV DNA negative status long term, cessation of NA therapy can be considered. The criteria established by the MHLW research group mentioned earlier should be referred to when considering stopping cessation of NA therapy, with less than 10% of patients meeting these criteria.[208] Sequential therapy with Peg-IFN, aiming at drug free status, can also be considered, although at present there is a lack of evidence supporting this method. HBeAg reappeared in 50% or more of cases where lamivudine therapy was ceased after seroconversion,[130] whereas seroconversion was maintained in 73%–77% of cases treated with entecavir.[20] There is little data available concerning HBeAg following cessation of entecavir, and more data needs to be gathered regarding this subject.

Low HBV DNA levels and high ALT levels are factors related to therapeutic efficacy that are common to both IFN and NA therapy, although both factors change along with natural course. These factors should be considered, in addition to the degree of necessity of treatment, in choosing the appropriate timing for commencement of treatment.

Recommendations

- ***In general, Peg-IFN monotherapy, with the aim of HBeAg seroconversion, is considered the treatment of first choice for initial antiviral therapy in patients with HBeAg positive chronic hepatitis.***
- ***Retreatment with Peg-IFN can be considered when required in responders to initial treatment with conventional IFN.***

- ***In patients with cirrhosis, and in cases where Peg-IFN is ineffective or contraindicated, entecavir is the first choice therapy with the aim of maintaining long term remission.***
- ***Lamivudine therapy is recommended in cases of acute exacerbation of hepatitis associated with jaundice.***

5.3 HBeAg negative chronic hepatitis

5.3.1 Timing of commencement of treatment

If HBeAg seroconversion occurs naturally or through treatment, in approximately 80% of cases HBV DNA levels remain low value, and ALT levels within the normal range, the patient becoming an HBeAg negative inactive carrier. HBeAg negative inactive carriers have a low risk of liver cirrhosis and HCC, with a good long-term prognosis,[4,30,32,50,234–239] and if HBV DNA negative conversion occurs, HBsAg also undergoes negative conversion in 1%–3% of patients per year.[240]

However, over the long term hepatitis recurrence is seen in 10%–20% of patients first diagnosed as HBeAg negative inactive carrier,[32,50,227,238,241] so accurate differentiation between the true inactive carrier state and HBeAg negative chronic hepatitis is difficult. In the current Guidelines, inactive carriers are defined as "patients in a drug free status (no antiviral therapy), and where three or more blood tests taken over the course of at least one year satisfy all the following conditions: (1) Persistently negative HBeAg; (2) Persistently normal ALT levels (≤30 U/L); and (3) HBV DNA <4.0 log copies/mL". Where advanced fibrosis is suspected on the basis of imaging studies or platelet counts, a liver biopsy should be conducted to assess the need for treatment.

Even after the diagnosis of inactive carrier status has been made, patients should be monitored every 6–12 months, and treatment is indicated if ALT levels increase. The incidence of hepatitic activity of at least moderate grade on liver biopsy in patients with ALT <40 U/L measured at least 3 times in 1 year is 7% if HBV DNA is 4–5 log copies/mL, 1.4% if HBV DNA is <4 log copies, and the incidence of hepatic fibrosis of at least moderate grade is 10% and 0.7%, respectively.[35] Accordingly, even if ALT levels remain within the normal range, liver biopsy is an option if HBV DNA is ≥4 log copies/mL, and treatment should also be considered.

It is common for patients with HBeAg negative chronic hepatitis to exhibit repeated transient increases in ALT and HBV DNA levels, and the likelihood of natural remission is low.[228,242–244] Progression of fibrosis at an advanced age is common compared to patients with HBeAg positive chronic hepatitis, so HBeAg negative chronic hepatitis should be considered a more advanced disease stage.[228,243,245] Even in patients with HBeAg negative chronic hepatitis, a high HBV DNA load, age ≥40 years, and a family history of HCC are independent risk factors for progression to liver cirrhosis and HCC,[2,34,36,37,211,229–231] so treatment should be actively considered if any of these factors are present. If hepatic fibrosis is confirmed by liver biopsy (or noninvasive alternative) as an optional investigation, treatment is indicated.

Recommendations

- ***In patients with HBeAg negative chronic hepatitis, progression of fibrosis at an advanced age is common compared to patients with HBeAg positive chronic hepatitis, so HBeAg negative chronic hepatitis should be considered a more advanced disease stage.***
- ***As for HBeAg positive chronic hepatitis, treatment is indicated in patients with HBeAg negative chronic hepatitis cases with HBV DNA ≥4.0 log copies/mL and ALT ≥31 U/L.***
- ***Even for cases fitting the criteria for inactive carrier status, if advanced fibrosis is suspected on the basis of imaging studies or platelet counts, a liver biopsy should be conducted. If hepatic fibrosis is confirmed, treatment is indicated.***
- ***Even after the diagnosis of inactive carrier status has been made, patients should be monitored every 6–12 months, and treatment is indicated if ALT levels increase.***

5.3.2 Selection of treatment

The initial aim of treatment of patients with HBeAg negative chronic hepatitis is to lead to inactive carrier status, with the additional aim of continued HBV DNA negative conversion in patients with advanced fibrosis. The ultimate aim is HBsAg negative conversion.

As for HBeAg positive patients, Peg-IFN is the therapy of first choice. Peg-IFN treatment of HBeAg negative patients decreases HBV DNA levels in 43%–44% of cases, with maintenance of HBV DNA levels <4.0 log copies/mL in 25%–28% of cases.[23] However, the HBV DNA negative conversion rate was 19% 24 weeks after the conclusion of treatment,[22] and long term was only 18%–21%,[23,24] with a lower probability of maintaining HBV DNA negative conversion compared to entecavir. On the other hand, the HBsAg negative conversion rate was 2.8%–4.0% 24 weeks after conclusion of treatment,[107] and 8.7%–12% 3 years after.[23,24] In responders

who achieved HBV DNA negative conversion, the HBsAg negative conversion rate is 44% at 3 years,[23] and in patients with HBsAg levels <10 IU/mL at conclusion of treatment, the rate is extremely high at 52%,[122] characteristics not seen with entecavir therapy. In this way, Peg-IFN monotherapy of HBeAg negative patients does not yield high overall rates of HBV DNA continuous negative conversion, but Peg-IFN is the treatment of first choice because in responders a drug free state and HBsAg negative conversion can be achieved with a finite duration of treatment. However, all these results are from overseas, and there is no Japanese data concerning elimination of HBsAg by Peg-IFN therapy.

On the other hand, as for HBeAg positive chronic hepatitis, patients at high risk of progression of hepatic fibrosis to liver cirrhosis, and in cases where Peg-IFN is ineffective or contraindicated, entecavir is the treatment of first choice.

With entecavir treatment, the HBV DNA negative conversion rate is 90% after 48 weeks of treatment,[25] and long term it is extremely high at 100%,[15] enabling certain achievement of HBV DNA negative conversion irrespective of pretreatment factors. However, the relapse rate after treatment cessation is high at 97%, so long term continuous treatment is the norm. The HBsAg negative conversion rate at 48 weeks after treatment commencement is reported as 0%.[25] Even with long term continuous treatment, HBsAg negative conversion is considered rare, but there have been reports of NA therapy with lamivudine yielding a HBsAg negative conversion rate of 6.9% at 9 years,[246] and for adefovir 5% at 3.8 years.[172] There are very few reports of the long term therapeutic results with entecavir, and further studies will be required to elucidate the HBsAg negative conversion rate with long term treatment.

Recommendations

- ***In patients with HBeAg negative chronic hepatitis, the overall rate of HBV DNA continuous negative conversion is not high with Peg-IFN therapy, but in responders we can expect high rates of drug free state and HBsAg negative conversion. Peg-IFN should also be considered the treatment of first choice for patients with HBeAg negative chronic hepatitis.***
- ***In patients at high risk of progression of hepatic fibrosis to liver cirrhosis, and in cases where Peg-IFN is ineffective or contraindicated, entecavir is the treatment of first choice with the aim of maintaining long term remission.***
- ***Lamivudine therapy is recommended in cases of acute exacerbation of hepatitis associated with jaundice.***

5.4 Liver cirrhosis

Compared to non-cirrhotic chronic hepatitis, patients with liver cirrhosis are at greater risk of chronic liver failure and HCC, necessitating more aggressive intervention, and the short term goal of treatment is not reduction in the HBV DNA load, but to keep HBV DNA persistently undetectable. IFN can cause acute exacerbation of hepatitis during treatment; particularly in patients with decompensated cirrhosis there is a risk of liver failure and serious infection, so IFN is contraindicated.[247,248] There are reports of efficacy for IFN and Peg-IFN therapy of compensated cirrhosis similar to that for chronic hepatitis,[102,221,249] but consideration of maintenance of continuous HBV DNA negative conversion, and safety issues, makes entecavir the first choice treatment.

5.4.1 Compensated cirrhosis

By suppressing HBV replication, NAs inhibit progression of fibrosis and progression of compensated cirrhosis to decompensated cirrhosis. In a randomized controlled clinical trial that randomly allocated lamivudine and a placebo to 651 patients with liver cirrhosis or advanced fibrosis, the proportion of patients with increased Child Pugh scores declined with lamivudine therapy (3.4% *vs* 8.8%), and the proportion of patients whose disease stage progressed also declined (7.8% *vs* 17.7%).[250] Long term continuous entecavir therapy ameliorates hepatic fibrosis, in 57% of all patients after 3 years of treatment, and in 85% of patients with advanced fibrosis, including liver cirrhosis.[18] With continuous treatment for an average of 6 years, hepatic fibrosis improved in 88% of all patients, and in 100% of cases of patients with advanced fibrosis, including liver cirrhosis.[251] In other words, liver cirrhosis is not an irreversible condition, and with long term continuous entecavir therapy it is possible to ameliorate fibrosis.

Relapse after cessation of NA therapy presents a risk of liver failure, so in general treatment continues for the rest of the patient's life. Cessation of treatment can be considered in cases of HBsAg negative conversion, but no results are available concerning long term outcomes following cessation of NA therapy. Even in patients exhibiting histological improvement of fibrosis, or patients meeting the criteria for cessation of treatment in chronic hepatitis, the lack of clear data regarding the pros and cons of treatment cessation means it cannot be recommended.

Recommendations

- ***Entecavir is the treatment of first choice for compensated cirrhosis.***
- ***Long term continuous entecavir therapy ameliorates hepatic fibrosis, including liver cirrhosis.***
- ***Relapse after cessation of NA therapy presents a risk of liver failure, so in general treatment continues for the rest of the patient's life.***

5.4.2 Decompensated cirrhosis

The aim of treatment for decompensated cirrhosis is reversal of liver failure through improving hepatic function. Although several studies have reported improved hepatic function with lamivudine therapy,[249,252–254] fewer studies have evaluated the therapeutic efficacy in patients with decompensated cirrhosis of entecavir, which is currently the treatment of first choice.

In a report on 70 patients with decompensated cirrhosis administered entecavir, the therapeutic results after 1 year were 89% for undetectable HBV DNA, 22% for HBeAg seroconversion, and 76% for ALT normalization, similar to results for compensated cirrhosis. Albumin levels rose from 2.8 g/dL to 3.2 g/dL, total bilirubin fell from 3.0 mg/dL to 1.9 mg/dL, and the prothrombin time (PT) improved from 16.3 sec to 13.9 s. As a result, after treatment for 1 year in 49% of cases the Child-Turcotte-Pugh score improved by ≥2 points, declining from the pretreatment average 8.1 ± 1.7 to 6.6 ± 2.4, and 66% of cases improved to Child class A. Similarly, the MELD score decreased from 11.1 ± 3.8 to 8.8 ± 2.3.[255] In a trial where 191 cases of decompensated cirrhosis were allocated randomly to entecavir or adefovir for 96 weeks in a comparison of therapeutic efficacy, a higher rate of HBV DNA negative conversion was seen with entecavir (57% *vs* 20%), and in both groups the Child-Turcotte-Pugh score improved or was maintained in 2/3 of patients.[256] Although entecavir improves hepatic function in patients with decompensated cirrhosis in this way, in order to avoid relapse after cessation of treatment, lifelong continuation of treatment is recommended. On the other hand, the 1 year survival rate was 87% in the first study,[255] and the 6 month survival rate in the latter study was 88%,[256] indicating deaths from failure usually occur in the 3–6 months before the onset of therapeutic effect of NAs. We must recognize that a liver transplant is required to save such cases.[252] Also, for decompensated cirrhosis with a MELD score of ≥20, 5 cases were reported of entecavir therapy causing lactic acidosis, of whom one patient died.[257] Accordingly, careful monitoring is required during treatment of decompensated cirrhosis.

Recommendations

- ***Entecavir is the treatment of first choice for decompensated cirrhosis. Although improvement of hepatic function can be expected, in order to avoid relapse after cessation of treatment, lifelong continuation of treatment is the norm.***
- ***There is a report of lactic acidosis associated with entecavir therapy for decompensated cirrhosis, necessitating careful monitoring.***
- ***IFN is contraindicated for decompensated cirrhosis, because of the risk of liver failure and serious infection.***

5.5 Suppression of HCC by antiviral therapy

5.5.1 IFN

Studies into the effects of IFN on carcinogenesis have all involved conventional IFN, and none Peg-IFN. Randomized controlled clinical trials evaluating the effects of IFN therapy on carcinogenesis comprise one study of 121 patients with HBeAg positive chronic hepatitis (liver cirrhosis; 10.3% of treated cases and 14.7% of controls),[258] and one small study evaluating 64 patients with HBeAg positive chronic hepatitis.[259] The results of the two trials differed; the former found a reduction in carcinogenesis (1.5% *vs* 11.8%, $P = 0.043$), whereas the latter trial found no carcinogenesis suppression effect (3.0% *vs* 6.4%). Even two comparatively large-scale case-controlled studies that matched the clinical backgrounds yielded contradictory results. One study observed HBeAg positive patients, 233 treated with IFN and 233 untreated for 6.8 years, with cancers detected in 2% of treated patients and 7% of untreated controls, showing carcinogenesis significantly reduced in the IFN therapy group ($P < 0.025$).[90] On the other hand, the other study of HBeAg positive patients, 208 treated with IFN and 203 untreated, found no significant difference in the rate of carcinogenesis (2.9% *vs* 0%).[260] Although many other studies have evaluated the relationship between IFN therapy and carcinogenesis,[261–266] they have all been cohort studies and their results do not consistently demonstrate a carcinogenesis suppressor effect for IFN. In these cohort studies, the carcinogenesis rate in the control group (untreated patients) varies greatly from 0% to 30.8%, and the rate including patients with cirrhosis also varies from 0% to 100%, with considerable differences in subject clinical backgrounds. These differences in the clinical background of applicable cases may be related to the variations in the reported carcinogenesis suppression effect of IFN.

A number of meta-analyses have examined the relationship between IFN therapy and carcinogenesis. One

analysis of 11 studies comprising 1006 patients treated with IFN and 1076 untreated controls found IFN therapy significantly reduced the carcinogenesis risk ratio to 0.59.[267] Another meta-analysis of 8 studies found that, although carcinogenesis was suppressed in IFN treated patients compared to untreated controls (risk difference 5.0%), the carcinogenesis suppression effect was found in a subgroup of ethnic Asians, where the carcinogenesis rate in the untreated controls was ≥10%, and ≥70% of subjects were HBeAg positive.[268] A third meta-analysis of 7 studies evaluated the therapeutic effect of IFN in patients with cirrhosis, 122 cases of HCC developed in 1505 patients with liver cirrhosis, and a carcinogenesis risk difference of 6.4% in IFN treated patients compared to untreated controls.[269] The authors discussed that, although all 7 studies indicated a tendency for IFN therapy to suppress carcinogenesis, only 3 studies showed a significant difference, of which 2 studies were results from Asia. Then they concluded that the overall significant difference disappeared with elimination of the last 2 Asian studies, and no firm conclusion was made concerning carcinogenesis suppression by IFN therapy. Another meta-analysis of 12 studies examining 1292 IFN treated patients and 1450 untreated controls, IFN therapy significantly reduced the carcinogenesis risk ratio to 0.66.[270] A sub-analysis indicated that carcinogenesis was suppressed by IFN therapy in liver cirrhosis patients (11.6% *vs* 21.5%, risk ratio 0.53, 95% CI: 0.36–0.78), whereas for non-cirrhosis patients the cancer rate was low, 0.9% in treated patients and 1.1% in untreated controls, showing no significant difference.

In this way, the carcinogenesis suppression effect of IFN therapy differs according to the patient's clinical background. For patients with liver cirrhosis and a high risk of carcinogenesis, a carcinogenesis suppression effect is obtained, but for patients with chronic hepatitis and a low risk of carcinogenesis, the results concerning carcinogenesis suppression effect are not consistent. Further large-scale studies will be required to draw any definite conclusions. In addition, there have been no studies that provide a detailed evaluation of the antiviral effects of IFN treatment, i.e. whether the carcinogenesis suppression effect differs according to HBV DNA suppression, HBeAg seroconversion or ALT normalization; this issue requires further evaluation.

Recommendations

- ***Suppression of carcinogenesis by IFN therapy has been confirmed by meta-analyses.***
- ***However, studies of carcinogenesis suppression by IFN have comprised a variety of clinical backgrounds, such as carcinogenesis rate and proportion of patients with liver cirrhosis, and the carcinogenesis suppression effect stratified for antiviral effect has not been evaluated, leading to contradictory results.***

5.5.2 NAs

Only one randomized controlled trial examining the effect of lamivudine therapy on carcinogenesis has evaluated patients with liver cirrhosis and advanced fibrosis, with a carcinogenesis rate of 3.9% for the lamivudine treated group, significantly lower than that of 7.4% for the untreated group.[250] In a Japanese case-controlled multicenter collaborative study, matching factors such as age, gender, liver fibrosis, family history, albumin levels and platelet counts, the carcinogenesis rate for the 377 lamivudine treated patients was 0.4% per year, and 2.5% for controls with matched clinical backgrounds, indicating that lamivudine therapy suppresses carcinogenesis.[271] In a comparison of 142 patients with HBeAg positive chronic hepatitis treated with lamivudine and 124 untreated controls, carcinogenesis was significantly suppressed (0.7% *vs* 2.4%).[272] In a cohort study comparing 872 lamivudine treated patients with 699 historical controls, the annual carcinogenesis rate was 0.95% in patients with liver cirrhosis where HBV replication was continuously suppressed by lamivudine therapy, compared to 4.10% in patients with liver cirrhosis not administered lamivudine, 2.18% where lamivudine resistance occurred, and 5.26% for the group in whom lamivudine could not adequately suppress HBV replication. These results indicated that the carcinogenesis rate declines in patients with liver cirrhosis if HBV replication is continuously suppressed by lamivudine treatment.[273]

The above results are from before introduction of adefovir against lamivudine resistant strains. In a cohort study where lamivudine therapy was administered to patients with HBeAg negative chronic hepatitis B, followed by adefovir therapy in lamivudine-resistant cases, the carcinogenesis rate was 7.7% in 195 patients not administered lamivudine, compared with 1.1% in 92 patients in whom remission was maintained out of a total 201 lamivudine treated patients, and 1.8% in the remaining 109 patients in whom lamivudine was ineffective or resistance developed. Furthermore, among patients with appearance of lamivudine resistance, the carcinogenesis rate was 0% in 79 patients administered adefovir, and 6.7% in patients not administered adefovir, indicating that even in lamivudine-resistant cases, if HBV replication was suppressed continuously by adefovir combination therapy, carcinogenesis was

suppressed.[96] In a meta-analysis of 5 studies, including the one above, of a total 2289 patients, carcinogenesis occurred in 32/1267 patients (2.5%) in the lamivudine treated group, and 120/1022 (11.7%) in the untreated group. Lamivudine therapy reduced the carcinogenesis risk ratio to 0.22 by; furthermore, in a sub-analysis of 753 patients with liver cirrhosis the carcinogenesis risk ratio was 0.17 with lamivudine therapy, and in a sub-analysis of patients without liver cirrhosis the carcinogenesis risk was 0.21, both sub-analyses indicating a significant suppression effect.[270]

The efficacy of entecavir therapy in suppressing carcinogenesis was evaluated in a cohort study that matched clinical backgrounds using propensity scores. The results showed a 5 year carcinogenesis rate of 3.7% for the entecavir treated group, significantly less than that of 13.7% for the untreated control group. Entecavir therapy reduced the carcinogenesis risk ratio to 0.37, and also suppressed carcinogenesis in patients with liver cirrhosis.[274] Furthermore, in a recent cohort study with patients with liver cirrhosis, the 5 year carcinogenesis rate was reduced to a risk ratio of 0.55 for the entecavir treated group compared to the historical control group.[275]

Recommendation

- ***Lamivudine and entecavir therapy suppress carcinogenesis.***

6. TREATMENT OF OTHER CONDITIONS ASSOCIATED WITH HBV

6.1 Acute hepatitis

ACUTE HEPATITIS B is a disease with a strong tendency to natural resolution, with more than 90% of sufferers becoming HBsAg negative, then anti-HBs antibody positive, without treatment. In essence, no treatment is necessary for these patients. Administration of corticosteroids or glycyrrhizin formulations, with the aim of ameliorating hepatic inflammation, may instead cause hepatitis to be prolonged or become chronic, and should be avoided.[276]

Lamivudine is effective in cases of severe (prothrombin time <40%) or fulminant (prothrombin time <40%, and grade 2 or worse hepatic encephalopathy) hepatitis. According to Tillman *et al*., following administration of lamivudine to 20 patients with severe hepatitis, prothrombin time < 36%, 18 survived (of whom 3 received liver transplants).[277] Liu *et al*. investigated the efficacy of lamivudine therapy for fulminant hepatitis, reporting an improvement in the survival rate from 15.4% to 36.8%.[278] At present, administration of lamivudine is recommended to commence before the prothrombin time reaches 40%. Lamivudine therapy should be ceased when HBsAg negative conversion occurs.

There is insufficient evidence concerning entecavir therapy for severe acute hepatitis. A study comparing entecavir and lamivudine in the treatment of exacerbations of chronic hepatitis B found that entecavir was superior in antiviral effect to lamivudine, but a tendency to prolongation of jaundice was identified.[279] Caution is required in administering entecavir to acute hepatic dysfunction associated with jaundice.

At present, more than half of Japanese patients with acute hepatitis B are infected with HBV genotype A. Acute hepatitis B has been shown to be more likely to be prolonged or become chronic in patients with HBV genotype A.[280–282] The usefulness of NA therapy with the aim of preventing chronic disease has yet to be established, and is not recommended overseas either.

Acute hepatitis B, with sexual transmission as the main route of infection, can be a coinfection with HIV. To avoid drug resistance, treatment of HIV infection requires the use of at least 3 antiviral agents. Of the NAs approved for the treatment of hepatitis B in Japan, lamivudine has a strong anti-HIV effect, and adefovir and entecavir have weak anti-HIV effects.[283,284] It is therefore necessary to confirm whether coinfection with HIV is present before commencing NA therapy for acute hepatitis B, and take care to avoid HIV monotherapy. There has been some indication that entecavir monotherapy in patients with HBV/HIV coinfection, who are not receiving fully suppressive antiretroviral regimens, may lead to the emergence of drug resistant HIV strains.[283]

Recommendations

- ***Lamivudine therapy is recommended for patients with severe acute hepatitis B, commencing before the prothrombin time goes below 40%. Lamivudine should be ceased when HBsAg testing becomes negative.***
- ***Presence of coinfection with HIV should be determined before commencing lamivudine therapy.***

6.2 Fulminant hepatitis

6.2.1 Diagnosis and pathology

Approximately 40% of cases of fulminant hepatitis in Japan are caused by HBV.[285] The etiology of fulminant hepatitis B can be broadly divided into rapid progressive acute infection (transient infection) and acute exacerbation in an HBV carrier. A recently devised etiological

classification of acute liver failure further divides acute exacerbation in an HBV carrier into 3 categories: (1) asymptomatic or inactive carrier without drug exposure, (2) reactivation in asymptomatic or inactive carrier receiving immunosuppressive and/or anti-cancer drugs, and (3) reactivation by immunosuppressive and/or anti-cancer drugs in patients with resolved HBV infection (*de novo* hepatitis B).[286,287]

Both the pathological state and prognosis differ between patients with a rapidly progressive acute infection and those with acute exacerbation of the carrier state. The former is hepatitis in the process of clearing HBV, in which amelioration of the hepatitis can be expected as the viral load decreases. The latter, however, is hepatitis caused by HBV reactivation in a carrier with a persistent infection, and hepatitis will persist as long as viral proliferation continues. The survival rate is relatively favorable at 53% with medical therapy of acute infections, but only 16% in cases of acute exacerbation of the carrier state.[285] The prognosis is particularly poor in cases of fulminant hepatitis B occurring in patients with HBV reactivation.[288]

Differentiation between acute infection and acute on chronic infection can be difficult, even using HBV markers from before and after the onset of infection. For the etiological diagnosis of fulminant hepatitis B, we measure HBsAg, anti-HBs antibody, anti-IgM-HBc antibody, anti-HBc antibody, and HBV DNA levels. We can differentiate between acute infection and acute exacerbation of the carrier state through the presence of HBsAg prior to disease onset, and positive conversion of anti-HBs antibody during the disease course. If these markers are indeterminate, the anti-IgM-HBc antibody and anti-HBc antibody titers at the time of disease onset may be considered. In general, in acute infections anti-IgM-HBc antibody are positive with a high titer, whereas HBc antibody have a low titer. In carriers, the anti-IgM-HBc antibody titer is low, and the anti-HBc antibody titer is high. At present, anti-IgM-HBc antibody titers are usually measured using the CLIA (chemiluminescent immunoassay) method, with a cut-off titer of 10.0 for differentiation between acute infection and acute on chronic infection.[289] Determination of anti-HBc antibody titers using the CLIA method is becoming more common, although this has actually made differentiation between acute infection and acute on chronic infection more difficult in comparison with the earlier RIA (radioimmunoassay) and EIA (enzyme immunoassay) 1:200 dilution methods. HBV reactivation should be suspected in patients on immunosuppressive therapy or chemotherapy before or at the time of disease onset.

A variety of HBV variants have been reported in association with fulminant hepatitis B, and preferably the HBV genotype, and the presence of precore and core promoter mutations should be determined. The B1/Bj genotype is common in fulminant hepatitis associated with acute infections,[5] and high incidences of core promoter (A1762T/G1764A) and precore (G1896A/G1899A) mutations have also been reported.[5,60,290–293] An association has also been reported between preS2 variants, S antigen variants, and fulminant hepatitis B.[294–296] On the other hand, no specific variants have been identified in HBV carriers developing acute exacerbation.

Recommendation

- ***HBsAg, anti-HBs antibody, anti-IgM-HBc antibody, anti-HBc antibody, and HBV DNA levels should be determined in patients with fulminant hepatitis B to make the etiological diagnosis. Determination of HBV genotype and the presence of precore and core promoter mutations is also desirable.***

6.2.2 Principles of treatment

In general, acute hepatitis B is a condition that resolves naturally, with no need for treatment. NAs are indicated in cases where there is concern about possible rapid progression or severe hepatitis, although there are no clear indications for their use. The AASLD Guidelines state that treatment is indicated in prolonged hepatitis (>4 weeks of prolonged INR and hyperbilirubinemia).[297] It is important to commence antiviral therapy using NAs as soon as fulminant hepatitis B is suspected, whether it is a rapidly progressive acute infection or acute exacerbation of the carrier state. Even after commencement of NA therapy once fulminant hepatitis has been diagnosed, it takes some time for the antiviral effect to appear, and improved outcomes are not always achieved, so antiviral therapy should be commenced before the onset of fulminant hepatic failure. The treatment of fulminant hepatitis is not directed solely at the etiological cause, but is a multidisciplinary treatment encompassing protective therapy, artificial liver support, general care, and prevention of complications. Outcomes are generally poor for medical treatment of fulminant hepatitis B, so liver transplantation should be considered as soon as possible.

6.2.3 NAs

A randomized controlled clinical trial of lamivudine in the treatment of severe hepatitis B (bilirubin ≥10 mg/

dL, PT-INR 1.4–1.6) found that early administration of lamivudine significantly reduced the incidence of hepatic failure and mortality.[278] A retrospective study of lamivudine therapy for fulminant or severe hepatitis B with PT-INR ≥2.0 found that 82.4% (14/17) of patients in the treated group survived and cleared HBsAg within 6 months, whereas the survival rate in the historical control group not administered lamivudine was only 20% (4/20), with a significant difference seen between groups ($P < 0.001$).[277] Other studies have demonstrated the efficacy of lamivudine in the treatment of fulminant hepatitis B, with no reports of problems with safety, such as adverse reactions.[298,299] Although there are no clear guidelines for when to stop NA therapy, negative conversion of HBsAg is usually the indicator for treatment cessation.

Administration of NAs is the mainstay of treatment of acute exacerbation of the carrier state. The viral load is already high at the time of onset of fulminant hepatitis, by which stage a therapeutic response to NAs is unlikely, necessitating commencement of NA therapy before the onset of severe or fulminant hepatitis B. Although subject numbers were low, the "Prospective study of the efficacy of lamivudine" in patients with acute exacerbation of the carrier state, conducted by an MHLW study group, found that 71% (5/7) patients administered lamivudine when a prothrombin time declined to ≤40% died, but all patients administered lamivudine when a prothrombin time was ≥60% survived. They therefore recommended that lamivudine should be administered to patients with acute exacerbation of the carrier state without delay, before the prothrombin time goes below 60%.[300] On the other hand, in patients with acute exacerbation of chronic hepatitis B, lamivudine should be administered before the total bilirubin level exceeds 5 mg/dL.[300] The cessation criteria for NA therapy in patients with acute exacerbation of the carrier state are the same as for chronic hepatitis B.

Even when liver transplantation is indicated, early NA therapy is effective in preventing recurrent HBV infection following transplantation. Post-transplant HBsAg positive conversion is considered less common after transplantation for HBV-associated acute hepatic failure than for chronic liver disease, although it is difficult to predict post-transplant recurrence. At present, the standard prophylactic regimen in HBsAg positive recipients is to commence NA therapy prior to transplantation, then introduce high titer hepatitis B immunoglobulin (HBIG) intraoperatively, and continue NA + HBIG dual therapy postoperatively.[301,302]

Of the NAs, a number of studies have demonstrated that lamivudine ameliorates acute liver failure.[277,278,298,303] Although evidence is scarce, amelioration of acute liver failure has also been suggested for entecavir and tenofovir.[304–306] Caution is required when administering entecavir to jaundiced patients with acute hepatic dysfunction, as a post-administration rise in transaminases may occur. Adefovir therapy is not recommended, as it has only weak antiviral activity, and is nephrotoxic. Caution is also required with the use of tenofovir, as latent nephrotoxicity has been reported.

6.2.4 IFN

IFN is occasionally administered in combination with a NA when treating fulminant hepatitis B in Japanese patients, because it often occurs in HBV carriers.[307] There is, however, a dearth of evidence clearly demonstrating the usefulness of IFN in the treatment of fulminant hepatitis.[308,309] Caution for adverse effects including worsening liver function and bone marrow suppression is required in administering IFN to these patients, either using a low dosage or using IFN-β in an intravenous formulation to avoid hemorrhagic complications. When fulminant hepatitis occurs in an HBV carrier, it is important to suppress persistent hepatic inflammation as quickly as possible, for which corticosteroids are administered in combination with antiviral therapy. A clinical trial of the usefulness of corticosteroid pulse therapy in combination with NA therapy in the treatment of fulminant hepatitis B is currently being conducted by an MHLW study group.

Recommendations

- ***Antiviral therapy for fulminant hepatitis B should be commenced as soon as possible using NAs, whether it is a rapidly progressive acute infection or acute exacerbation of the carrier state.***
- ***NAs should be administered immediately to patients with severe acute hepatitis B, aiming to commence therapy before the prothrombin time goes below 40% in patients with severe acute hepatitis B, and before the prothrombin time goes below 60% in patients with acute exacerbation of the carrier state.***
- ***IFN may be administered in combination with NAs. However, careful attention should be paid to possible exacerbation of hepatic dysfunction or the development of decline of blood cell counts during treatment.***

6.3 HBV reactivation

Reactivation of HBV refers to a rise in the hepatitis B viral load caused by immunosuppression or chemo-

therapy in a patient with HBV infection. Reactivation of HBV is classified into reactivation from the carrier state and reactivation in a patient with resolved HBV infection (HBsAg negative, and anti-HBc antibody or anti-HBs antibody positive). Hepatitis associated with reactivation in a patient with resolved HBV infection is called "*de novo* hepatitis B". Not only is severe disease common in cases of hepatitis associated with reactivation of HBV, but also treatment of concurrent conditions is made difficult by the onset of hepatitis, so it is extremely important to prevent the onset of hepatitis itself. The basic strategy for prevention and treatment of HBV reactivation associated with powerful immunosuppressant or chemotherapy regimens should follow the guidelines summarized below, based on the "Guidelines for the prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy (Revised version)"[310,311] produced by an MHLW study group (Fig. 7). An MHLW study group currently conducting a multicenter nationwide prospective clinical trial of preemptive antiviral therapy to prevent HBV reactivation during treatment of malignant lymphoma with rituximab has published the results of interim analyses.[312] As for HBV reactivation caused by immunosuppressive and anti-cancer therapies rather than rituximab, the MHLW "HBV Reactivation through Immunosuppressive and/or Anti-cancer Therapies" research group has also reported its results.[313] Furthermore, the Japan College of Rheumatology has published "A proposal for management of rheumatic disease patients with hepatitis B virus infection receiving immunosuppressive therapy".[314]

6.3.1 Risk of reactivation

The risk of reactivation of HBV is mainly governed by the HBV infection status and the degree of immunosuppression. The HBV infection status is classified into chronic active hepatitis, inactive carrier, and resolved infection. This corresponds to the risk of reactivation in descending order. There is no evidence available concerning asymptomatic carriers in the immune tolerance phase, the incidence of further activation of HBV, or whether NA therapy can prevent activation. The risks of HBV reactivation and the onset of hepatitis or fulminant hepatitis vary with the exact immunosuppressant or chemotherapy agents used, and the incidences of these events are unclear. When immunosuppressive therapy or chemotherapy including powerful agents such as rituximab is administered, careful attention should be paid to the possibility of reactivation in HBsAg positive patients including inactive carriers, and patients with resolved infection. When standard immunosuppressive therapy or chemotherapy is administered, reactivation in HBsAg positive patients including inactive carriers is the main problem, but caution is also required with in patients with resolved HBV infection, as there have been reports of HBV reactivation in such patients with HBV DNA levels <2.1 log copies/mL, either administered corticosteroid monotherapy, or administered standard chemotherapy for the treatment of solid malignancies.[313] Risk factors for HBV reactivation in HBsAg positive patients are HBeAg positive status and high HBV DNA levels. Although most patients with resolved HBV infection are positive for both anti-HBc and anti-HBs antibody, some are either anti-HBc antibody positive or anti-HBs antibody positive alone. Although anti-HBs antibody act to suppress HBV reactivation, reactivation is still possible in patients positive for anti-HBs antibody alone.[315–317]

HBV reactivation is commonly associated with hepatitis, which can vary from mild and transient hepatitis to severe and fatal. The onset of hepatitis associated with HBV reactivation is not always during immunosuppressive therapy or chemotherapy, but may occur after its interruption or cessation. In particular, severe hepatitis associated with HBV reactivation has been reported after cessation of corticosteroid and methotrexate therapy.[318–321] Moreover, conditions such as fibrosing cholestatic hepatitis (FCH) may present when viral replication is increased in the immunosuppressed state.[322,323]

6.3.2 Screening (Fig. 7)

Screening for HBV infection should be performed in all patients undergoing immunosuppressive therapy or chemotherapy, irrespective of whether abnormalities of hepatic function are evident or not. HBsAg levels should be measured in all patients prior to commencement of treatment. In HBsAg positive patients, HBeAg, anti-HBe antibody, and HBV DNA levels should also be measured. A real-time PCR should be used for measurement of HBV DNA levels. In HBsAg negative patients, anti-HBc antibody and anti-HBs antibody should also be measured. Patients positive for anti-HBc or anti-HBs antibody are diagnosed as patients with resolved HBV infection. However, this excludes those positive for anti-HBs antibody alone due to prior hepatitis B vaccination. The next step for patients with resolved HBV infection is measurement of HBV DNA levels. For measurement of HBsAg, anti-HBc antibody and anti-HBs antibody, a highly sensitive test such as the CLIA or CLEIA method should be used. If HBV infection is diagnosed, the past history of hepatitis should be elicited, and screening for

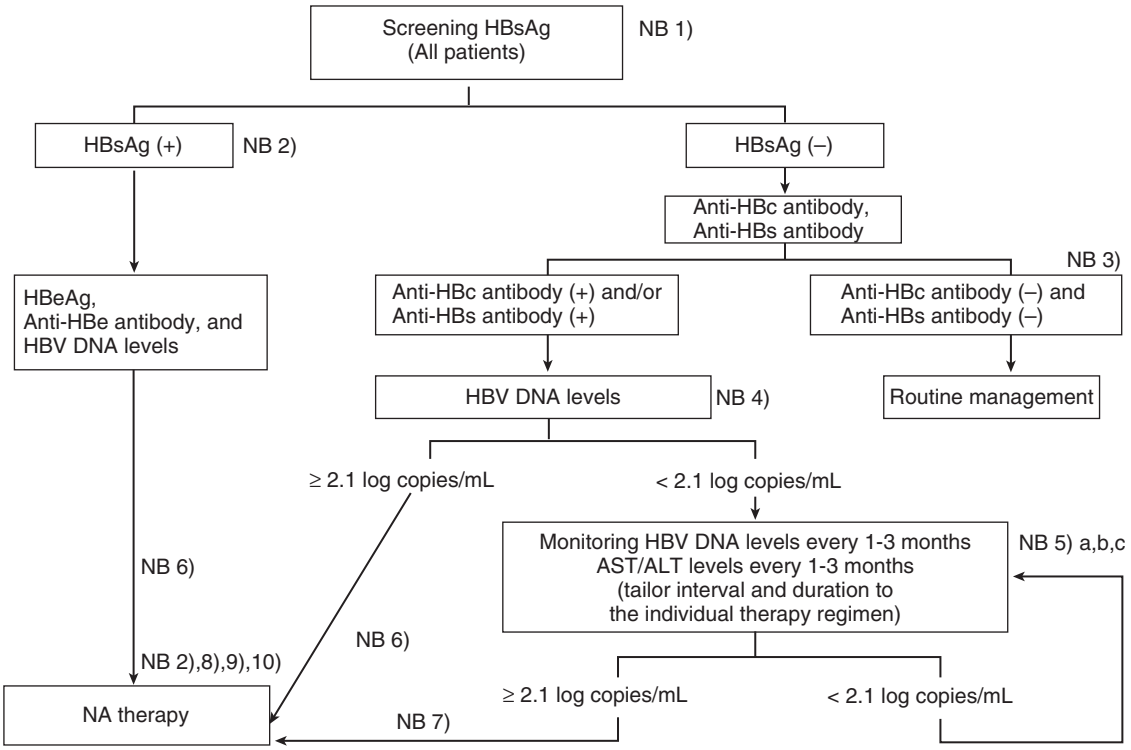


Figure 7 Guidelines for the prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy.

Addendum: Caution is required when administering powerful chemotherapeutic agents for hematological malignancies, as during or following completion of treatment some HBsAg positive or negative patients will develop hepatitis B due to reactivation of HBV, and some of these will go on to suffer fulminant hepatitis. Consideration should also be given to the possibility of HBV reactivation in association with standard chemotherapy for hematological malignancies or solid cancers, and immunosuppressive therapy for autoimmune diseases, such as rheumatic and collagen diseases. The incidences of HBV reactivation, hepatitis and fulminant hepatitis associated with standard chemotherapy and immunosuppressive therapy are not known, and there is a lack of evidence on which to base guidelines. Furthermore, prevention of fulminant hepatitis is not guaranteed with NA therapy.

NB 1) HBV carriers and patients with resolved hepatitis B should be screened prior to immunosuppressive therapy or chemotherapy. First HBsAg testing should be performed to determine whether they are an HBV carrier. HBsAg negative patients should be tested for anti-HBc antibody and anti-HBs antibody, to confirm past infection. Highly sensitive testing methods should be used for measurements of HBsAg, anti-HBc antibody and anti-HBs antibody.

NB 2) A hepatologist should be consulted concerning HBsAg positive patients. A hepatologist should preferably be consulted for all patients administered NAs.

NB 3) In some patients undergoing retreatment who did not undergo testing for anti-HBc or HBs antibody at the time of their initial chemotherapy, and in patients who have already commenced immunosuppressive therapy, antibody titers may be low, in which case measurement of HBV DNA levels is preferable.

NB 4) Patients with resolved HBV infection should be screened using real-time PCR measurement of HBV DNA levels.

NB 5)

a. Caution is required when treating patients with resolved HBV infection with rituximab + corticosteroid or fludarabine chemotherapy, or when they undergo hematopoietic stem cell transplantation, as these patients are at high risk of HBV reactivation. HBV DNA levels should be monitored on a monthly basis during treatment, and for at least 12 months afterward. Long-term monitoring is required for hematopoietic stem cell transplant recipients.

b. Although the incidence is low, there is a risk of HBV reactivation with standard chemotherapy regimens. HBV DNA levels should be measured every 1–3 months, with the interval and duration tailored to the individual therapy regimen. It is best to err on the side of caution with patients undergoing treatment for hematological malignancies.

Figure 7 *Continued*

c. There is also a risk of HBV reactivation associated with immunosuppressive therapy using corticosteroids, immunosuppressant agents, or molecular targeted therapy with immunosuppressant or immunomodulator activity. HBV DNA levels should be monitored on a monthly basis in patients on immunosuppressive therapy for at least 6 months after commencement or alteration (including cessation) of treatment. After 6 months, the interval and duration should be tailored to the individual therapy regimen.

NB 6) Administration should be commenced as soon as possible, before commencement of immunosuppressive therapy or chemotherapy.

NB 7) Administration should be commenced as soon as the HBV DNA levels exceed 2.1 log copies/mL, during or after immunosuppressive therapy or chemotherapy. If this occurs during treatment, it is preferable to consult with a hepatologist, and not immediately cease the immunosuppressant or antineoplastic agent with immunosuppressive activity.

NB 8) Entecavir is the recommended NA.

NB 9) Cessation of NA therapy can be considered if the following criteria are met.

In patients who were HBsAg positive at the time of screening, when the criteria for cessation of NA therapy in cases with chronic hepatitis B are met.

In patients who were anti-HBc antibody and/or anti-HBs antibody positive at the time of screening:

1 NA therapy has been continued for at least 12 months after completion of immunosuppressive therapy or chemotherapy.
2 ALT (GPT) levels have been normalized during this period (excluding causes of elevated ALT levels other than HBV).
3 negative conversion of HBV DNA has occurred during this period.

NB 10) Patients should be carefully monitored, including measurement of HBV DNA levels, for at least 12 months following completion of NA therapy. Monitoring methods depend on package inserts of each NA. NA therapy should be immediately resumed if HBV-DNA levels exceed 2.1 log copies/mL during monitoring period.

chronic liver disease performed, including abdominal ultrasonography. In HBV DNA positive patients, testing for HBV genotype, precore mutations and core promoter mutations is desirable.

Recommendations

- ***Screening for HBV infection should be performed in all patients undergoing immunosuppressive therapy or chemotherapy, who are at risk of HBV reactivation.***
- ***Screening for HBV infection should be performed in a systematic fashion, using a highly sensitive test, and include measurement of levels of HBsAg, anti-HBc and anti-HBs antibodies, and HBV DNA.***

6.3.3 Basic strategy for prevention and treatment of reactivation

When immunosuppressive therapy or chemotherapy, with the associated risk of HBV reactivation, is administered to patients with chronic active hepatitis, NA therapy should be commenced beforehand as possible. Immunosuppressive therapy is considered safe in patients with chronic hepatitis under cover of antiviral therapy.[324] When immunosuppressive therapy or chemotherapy, with the associated risk of HBV reactivation, is administered to HBsAg positive inactive carriers, prophylactic NA therapy should be commenced without delay beforehand. Patients with resolved HBV infection and HBV DNA levels ≥2.1 log copies/mL on pretreatment screening should be administered prophylactic NA therapy beforehand, as for inactive carriers. Patients with resolved HBV infection and HBV DNA levels <2.1 log copies/mL on pretreatment testing should undergo regular monitoring of HBV DNA levels during and after their immunosuppressive therapy or chemotherapy. If HBV DNA levels exceed 2.1 log copies/mL during monitoring, preemptive NA therapy should be commenced immediately. The interval between tests should be of the order of 1–3 months, although the monitoring duration and intervals can be adjusted in accordance with the nature of the immunosuppressive therapy or chemotherapy.

A survey conducted by an MHLW study group found that increased HBV DNA levels were not necessarily detected in patients with resolved HBV infection, after HBV DNA levels (real-time PCR) were <2.1 log copies/mL and amplification reaction signals were detected in pretreatment monitoring, or HBV DNA levels were <2.1 log copies/mL and amplification reaction signals were detected in monitoring during treatment. They concluded that HBV reactivation can be diagnosed when HBV DNA levels exceed 2.1 log copies/mL, and it is reasonable to commence NA therapy at that point.[313]

The usefulness of prophylactic lamivudine therapy prior to chemotherapy in HBV carriers has been demonstrated in prospective studies.[325–328] Although few in number, some studies have shown prophylactic entecavir and tenofovir therapy to be useful.[329–331] The

genetic barrier to resistance to lamivudine is low, so resistant strains are likely to appear if the virus has a high capacity to proliferate, or the period of administration is long, and at present entecavir therapy is recommended.

The criteria for cessation of NA therapy are the same as for cessation of NA therapy in HBsAg positive patients. For anti-HBc or anti-HBs antibody positive patients, NA therapy should be continued for at least 12 months after completion of immunosuppressive therapy or chemotherapy, although NAs may be ceased during this period if continued ALT normalization and HBV DNA negative conversion are seen. However, close follow-up including HBV DNA monitoring is necessary for at least 12 months after cessation of NA therapy.

Recommendations

- ***When immunosuppressive therapy or chemotherapy, with the associated risk of HBV reactivation, is administered to HBsAg positive inactive carriers, or patients with resolved HBV infection and HBV DNA levels ≥2.1 log copies/mL on pretreatment screening tests, NA therapy should be commenced without delay.***
- ***Patients with resolved HBV infection and HBV DNA levels <2.1 log copies/mL on pretreatment screening tests should undergo regular monitoring of HBV DNA levels during and after their immunosuppressive therapy or chemotherapy. If HBV DNA levels exceed 2.1 log copies/mL during monitoring, preemptive NA therapy should be commenced.***
- ***Entecavir is the recommended NA.***
- ***The criteria for cessation of NA therapy are the same as for cessation of NA therapy in HBsAg positive patients. For patients with resolved HBV infection, NA therapy should be continued for at least 12 months after completion of immunosuppressive therapy or chemotherapy, although cessation of NAs may be considered during this period if continued ALT normalization and HBV DNA negative conversion are seen.***
- ***Close follow-up including HBV DNA monitoring is necessary for at least 12 months after cessation of NA therapy. If HBV DNA levels exceed 2.1 log copies/mL during the follow-up period, NA therapy should be recommenced immediately.***

6.3.4 Liver transplantation

HBV reactivation is a potential problem in recipients of a liver transplant from an HBsAg negative and anti-HBc antibody positive donor. In a report from a time before prophylactic HBIG administration became standard, HBV reactivation occurred in 15 out of 16 recipients of liver transplants from anti-HBc antibody positive donors, one of whom died from FCH.[332] It is preferable to exclude anti-HBc antibody positive donors, but a strategy is needed when transplantation of a liver from such a donor cannot be avoided. One such strategy is to administer HBIG during the transplantation procedure, and maintain anti-HBs antibody levels postoperatively. Postoperative administration of NA therapy, or NA+HBIG combination therapy, is also considered useful.[333,334] Early commencement of NA therapy following HBV reactivation has also been reported to be effective.[335]

6.3.5 Transplantation of other organs

HBV reactivation is seen in a high proportion (50–94%) of HBsAg positive patients undergoing transplantation of kidneys and other organs.[336–339] Following HBV reactivation, rapid progression is seen from chronic hepatitis B to liver cirrhosis, which becomes the cause of death. Prophylactic NA therapy is recommended for HBsAg positive and/or anti-HBc antibody positive patients, commencing prior to the transplantation procedure.

6.3.6 Hematopoietic stem cell transplantation

HBV reactivation is seen in a high proportion (≥50%) of HBsAg positive patients undergoing of hematopoietic stem cell transplantation.[340] The rate of HBV reactivation is 14–20% in patients with resolved HBV infection.[341,342] The risk of HBV reactivation is higher with allogeneic bone marrow transplantation than with autologous bone marrow transplantation. This is thought to be due to the need for long term corticosteroid and immunosuppressant therapy for graft-versus-host disease (GVHD) with allogeneic transplantation. Characteristic of reactivation in patients with resolved HBV infection undergoing hematopoietic stem cell transplantation is the delayed onset of HBV reactivation, influenced by immunosuppressant therapy and delayed immune reconstitution.[343,344] The median interval between transplantation and HBsAg positive conversion is long at 19 months (range 6–52 months),[345] necessitating long term HBV DNA monitoring after transplantation.

6.3.7 Chemotherapy including rituximab

The risk of HBV reactivation is high with chemotherapy using rituximab or fludarabine for hematological malignancies, reported to be 20–50% in carriers and 12–23% in patients with resolved HBV infection.[316,346] Prospective HBV DNA monitoring studies conducted in Japan and Taiwan found the risk of HBV reactivation to be

approximately 10% in patients with resolved HBV infection.[312,347] For HBV reactivation associated with rituximab+corticosteroid combination therapy, the rate of fulminant hepatitis was high, and mortality also high in cases of fulminant hepatitis.[288,348]

The Taiwanese group conducted a multicenter collaborative prospective clinical trial of monthly HBV DNA monitoring in patients with malignant lymphoma who underwent chemotherapy including rituximab.[347] Using an HBV DNA cutoff value of 3.0 log copies/mL, they defined HBV reactivation as an increase in the HBV DNA levels at least 10 times greater than baseline. As a result, HBV reactivation was seen in 9.3% (14) of patients, in 5 of whom hepatic dysfunction was seen. Of these, serious hepatic dysfunction (ALT increase ≥10 times upper limit of normal) associated with HBV reactivation was seen in 2 patients, but it did not develop into fulminant hepatitis, and no deaths were reported.

In Japan, an MHLW study group is conducting a multicenter collaborative clinical trial with patients with malignant lymphoma who underwent rituximab+corticosteroid combination therapy with the aim of determining the usefulness of HBV DNA monitoring during treatment. They have published their interim analysis results.[312] Using an HBV DNA cutoff value of 1.8 log copies/mL, they defined HBV reactivation as a HBV DNA levels above the cutoff value (greater than the signal detection sensitivity), and commenced NA therapy. HBV reactivation was seen in 16/187 patients, but there were no cases of hepatitis associated with HBV reactivation.

These results strongly suggest the necessity for highly sensitive HBV DNA monitoring and the immediate commencement of NA therapy as soon as HBV DNA becomes detectable. This supports the validity of the present MHLW guidelines for the management of HBV reactivation.

6.3.8 Standard chemotherapy

For standard chemotherapy regimens, the incidence of HBV reactivation is relatively high in inactive carriers, but only 1–3% in patients with resolved HBV infection.[325,349,350] The incidence of HBV reactivation is higher for chemotherapy regimens that include corticosteroids or anthracycline anti-cancer agents.[345,351,352] A prospective study conducted by an MHLW study group found that standard chemotherapy for solid cancers in patients with resolved HBV infection induced HBV reactivation (HBV DNA ≥2.1 log copies/mL) in 1 out of 36 patients. The HBV DNA levels in that patient was 2.4 log copies/mL, and entecavir therapy was commenced immediately, with no evidence of the onset of hepatitis. Chemotherapy for hematological malignancies, not including rituximab, induced 1 case of hepatitis over the 3 month monitoring period.[313]

In general, monitoring of HBV DNA levels in patients undergoing standard chemotherapy for solid cancers should be performed at intervals of 1–3 months, although the monitoring duration and intervals can be adjusted in accordance with the nature of the chemotherapy. More intensive surveillance is required for hematological malignancies. If reactivation occurs during chemotherapy, it is preferable to consult with a hepatologist, and not immediately cease the antineoplastic agent with immunosuppressive activity.

6.3.9 Immunosuppressive therapy for rheumatic and connective tissue diseases

It is characteristic of immunosuppressive therapy for autoimmune diseases, such as rheumatic and connective tissue diseases, that multiple immunosuppressant agents including methotrexate and corticosteroids are administered for long periods. Immunosuppressant agents known to be associated HBV reactivation include corticosteroids, immunosuppressant agents (azathioprine, cyclophosphamide, cyclosporine and mycophenolate mofetil), anti-rheumatic agents with immunosuppressive activity (methotrexate, tacrolimus, leflunomide and mizoribine), and biological agents such as anti-TNF-α agents.[353,354] A prospective study conducted by an MHLW study group found that immunosuppressive therapy for rheumatic and connective tissue diseases in patients with resolved HBV infection induced HBV reactivation (HBV DNA ≥2.1 log copies/mL) in 6 out of 121 patients (2 patients with pretreatment HBV DNA <2.1 log copies/mL, signal detected, 4 patients with pretreatment HBV DNA <2.1 log copies/mL, signal not detected). The timing of reactivation was within 6 months after commencement of treatment in all cases.[313] Accordingly, HBV DNA monitoring at monthly intervals is desirable for at least 6 months after commencement or alteration of immunosuppressive therapy. There is insufficient evidence concerning monitoring more than 6 months after commencement or alteration of immunosuppressive therapy, so the monitoring duration and intervals can be adjusted in accordance with the nature of the treatment. If HBV reactivation occurs during immunosuppressive therapy, it is preferable to consult with hepatologist, and not immediately cease the immunosuppressant agent.

6.3.10 Novel molecular targeted therapies

Although evidence is lacking concerning the risk of HBV reactivation with novel molecular targeted therapies, there have been reports of hepatitis associated with several molecular targeted therapeutic agents.[355–357] In particular, caution is required with molecular targeted therapeutic agents with immunosuppressive or immunomodulating activity, necessitating more intensive surveillance.

Recommendations

- ***Monthly HBV DNA monitoring should be performed for patients undergoing hematopoietic stem cell transplantation or chemotherapy including rituximab, corticosteroids or fludarabine, during treatment and for at least 12 months after its completion.***
- ***HBV DNA monitoring should be performed every 1–3 months for patients undergoing chemotherapy for hematological malignancies, not including rituximab, and standard chemotherapy for solid malignancies, although the monitoring duration and intervals can be adjusted in accordance with the nature of the treatment.***
- ***Monthly HBV DNA monitoring should be performed at monthly intervals for patients undergoing immunosuppressive therapy for rheumatic or connective tissue diseases, for at least 6 months after commencement or alteration of treatment. After 6 months, the monitoring duration and intervals should be decided in accordance with the nature of the treatment.***
- ***If HBV reactivation occurs during chemotherapy or immunosuppressive therapy, it is preferable to consult with a hepatologist, and not immediately cease the anti-neoplastic agent with immunosuppressive activity or immunosuppressant agent.***

6.4 Coinfection with HIV

6.4.1 Epidemiology

As we saw above in the section on acute HBV, coinfection with HBV and HIV infection may occur. HIV patients exhibit an HBsAg positive rate of 6.3%[358] and anti-HBs antibody positive rate of around 60%.[359] It has been reported that immunopathy associated with HIV can increase the likelihood of HBV infection becoming chronic by as much as 23%.[360] Over 80% of HBsAg positive Japanese HIV-infected patients have HBV genotype A[361], which contributes to the higher HBsAg positive rates among HIV sufferers. Thus, coinfection with HIV can occur in patients with chronic hepatitis B as well as those with acute hepatitis B.

6.4.2 Basic principles

NAs are the mainstay of HBV therapy in patients coinfected with HIV. Antiretroviral therapy (ART) for HIV infection involves a combination of three or more anti-HIV agents. Table 16 shows anti-HIV agents that are also active against HBV. Nucleoside analog reverse transcriptase inhibitors (NRTI) are generally used as two of the anti-HIV agents. They will normally have anti-HBV activity as well, to discourage the development of drug-resistant HBV.

Table 16 Anti-HIV drugs also active against HBV*

Common name	Product name	Code	Dosage	Remarks
Lamivudine	Epivir	3TC	300 mg once or twice daily	Reduce dosage for renal failure Different dosage to Zefix
Emtricitabine	Emtriva	FTC	200 mg	Reduce dosage for renal failure
Tenofovir disoproxil fumarate	Viread	TDF	300 mg	Reduce dosage for renal failure
Emtricitabine + tenofivir disoproxil fumarate	Truvada	TDF+FTC	One tablet	Reduce dosage for renal failure
Zidovudine + lamivudine	Combivir	AZT+3TC	Two tablets twice daily	Reduce dosage for renal failure Contraindicated if hemoglobin <7.5 g/dL Contraindicated in combination with ibuprofen
Abacavir + lamivudine	Epzicom	ABC+3TC	One tablet	Reduced dosage for renal failure Contraindicated in severe hepatic dysfunction

*All these of the above are classed as nucleoside analog reverse transcriptase inhibitors (NRTI). Other options include anti-HIV agents such as non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), integrase inhibitors and CCR-5 inhibitors.

In patients with very low CD4 counts (well below the normal range of 800–1200/μL), ART may cause exacerbation of hepatitis due to recovery of cellular immunity, in a phenomenon known as Immune Reconstitution Inflammatory Syndrome (IRIS). In the majority of cases, IRIS is observed within 16 weeks of starting ART. It can be difficult to distinguish between IRIS and drug-induced liver injury.

An issue with ART is the potential for drug-induced liver injury associated with the use of anti-HIV agents, particularly protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). The risk of liver injury generally decreases during ongoing ART;[362] it is however more likely in patients with advanced liver fibrosis, and particularly cirrhosis. Cessation of ART or a change in the agents used should be considered if liver injury is detected or hepatic function deteriorates.

Prolonged administration of tenofovir and/or adefovir can lead to renal damage.[363] In the case of tenofovir, this may be irreversible.[364] For this reason, changes in the drug regimen should be considered before the estimated glomerular filtration rate (eGFR) falls below 60% or phosphorus reabsorption falls below 70%.

6.4.3 Problems with treatment and responses

Before commencing ART including anti-HBV agents, it is important to check for a history of treatment with anti-HBV agents such as lamivudine, adefovir, entecavir or any of the anti-HIV drugs listed in Table 16. If any of these agents have been administered in the past, an infectious diseases specialist should be consulted regarding the choice of ART agents.

Functional hepatic reserve should also be evaluated prior to commencing ART including anti-HBV agents, given that IRIS can potentially exacerbate hepatitis in patients with a low hepatic reserve. Protease inhibitors and NNRTIs known to cause hepatic dysfunction should be avoided with these patients.

Entecavir is not recommended for patients coinfected with HIV and HBV not being administered anti-HIV agents, as it can lead to the emergence of drug-resistant HIV.

All the abovementioned factors should be considered in selecting the ART regimen. The ART regimen should consist of a backbone of either tenofovir (TDF) with emtricitabine (FTC), or tenofovir (TDF) with lamivudine (3TC), together with a key drug (integrase inhibitor, NNRTI or PI).

Where IRIS occurs during ART including anti-HBV agents, it is usually only transient in nature. Although it is generally held that cessation of ART should be considered when transaminase levels reach more than five to ten times the baseline level, it is preferable to address the problem without interrupting ART.

If it proves necessary to cease administration of an anti-HIV drug with anti-HBV activity (such as lamivudine, emtricitabine, tenofovir or Truvada (emtricitabine+tenofovir)) due to adverse reactions associated with ART, there is a danger of recurrence or aggravation of hepatitis. Where possible, two anti-HBV agents should be administered instead. Consideration should be given to entecavir+adefovir combination therapy.

It is rare for treatment to be indicated for HBV alone, and "treatment of HIV infection not indicated or not wanted". If this situation does arise, Peg-IFNα-2a therapy should be considered.

Specific directions regarding coinfections with HBV and HIV are set out in the HIV Guidelines.[365,366]

Recommendations

- ***In patients with very low CD4 counts (well below the normal range of 800–1200/μL), ART may exacerbate hepatitis due to recovery of cellular immunity.***
- ***When administering ART, we should take into consideration the potential for anti-HIV agents to cause drug-induced liver injury.***
- ***Before commencing ART involving anti-HBV agents, it is important to check for a history of treatment with anti-HBV agents.***
- ***Before commencing ART involving anti-HBV agents, it is important to evaluate functional hepatic reserve.***
- ***The ART regimen should consist of a backbone of either tenofovir (TDF) with emtricitabine (FTC), or tenofovir (TDF) with lamivudine (3TC), together with a key drug (integrase inhibitor, non-nucleoside reverse transcriptase inhibitor or protease inhibitor).***
- ***If it is necessary to cease administration of an anti-HIV drug with anti-HBV activity due to adverse reactions associated with ART, there is a danger of recurrence or aggravation of hepatitis. Where possible, two anti-HBV agents should be administered instead. Consideration should be given to entecavir+adefovir combination therapy.***

CONFLICTS OF INTEREST

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