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 On the other hand, it requires a lengthy administration period, due to the propensity for flare-up if treatment is withdrawn, increasing the likelihood of drug-resistant mutations and raising safety issues.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

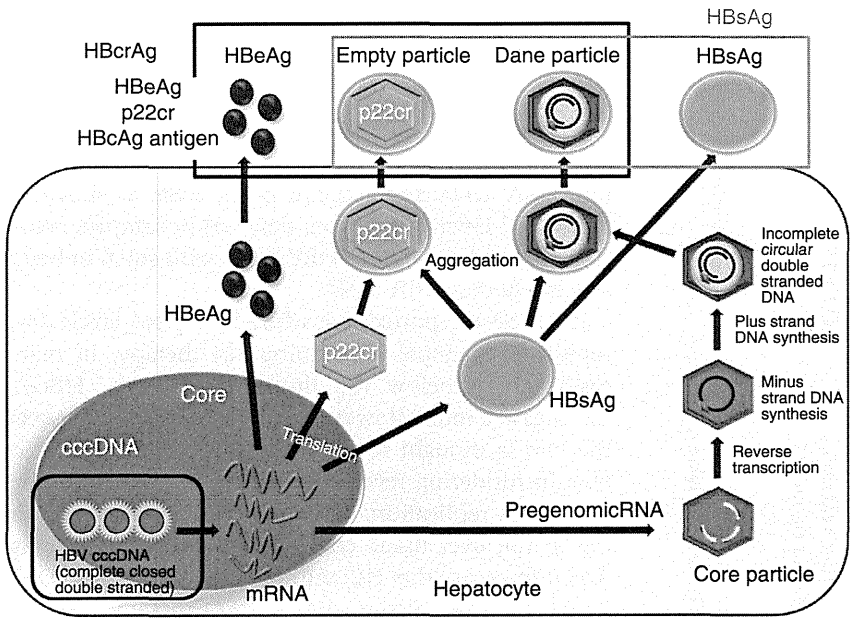

Figure 2 HBV related markers.

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

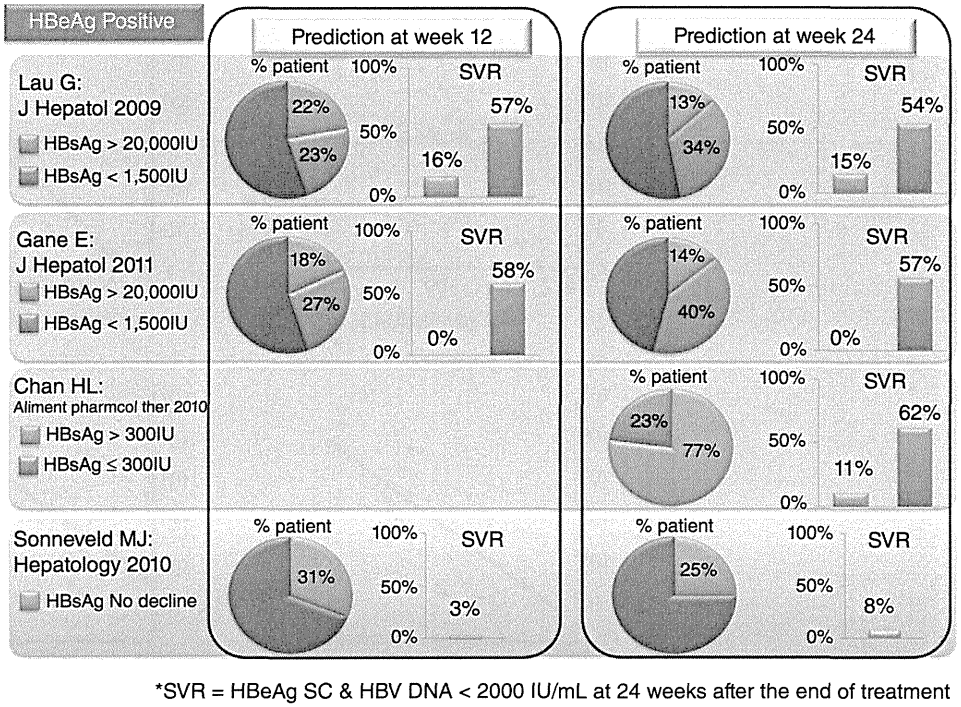

Figure 3 HBsAg measurement is a useful predictor of outcomes in HBeAg positive chronic HBV patients undergoing a 48 week Peg-IFN α therapy regimen.

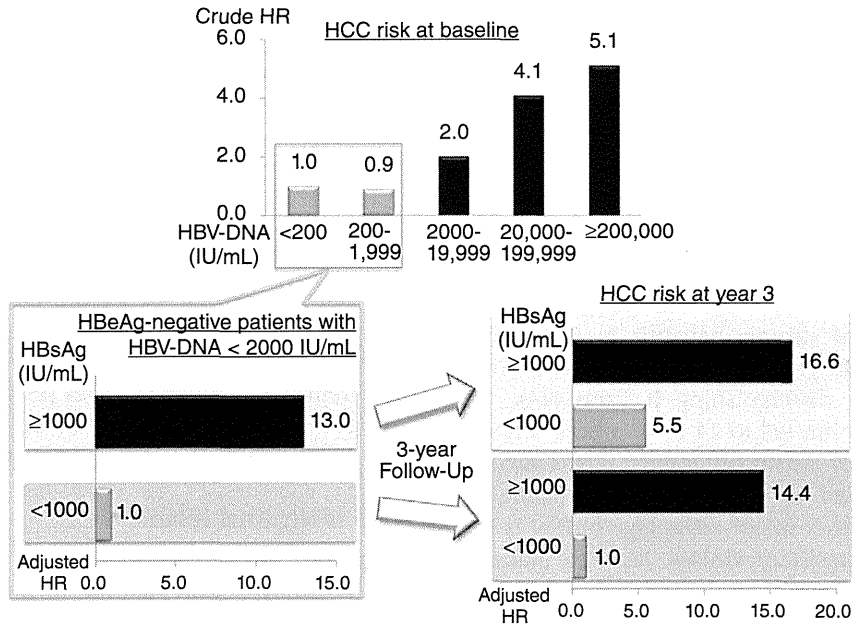

Figure 4 Correlation between HBsAg levels and HCC development in HBeAg negative patients with low viral load.

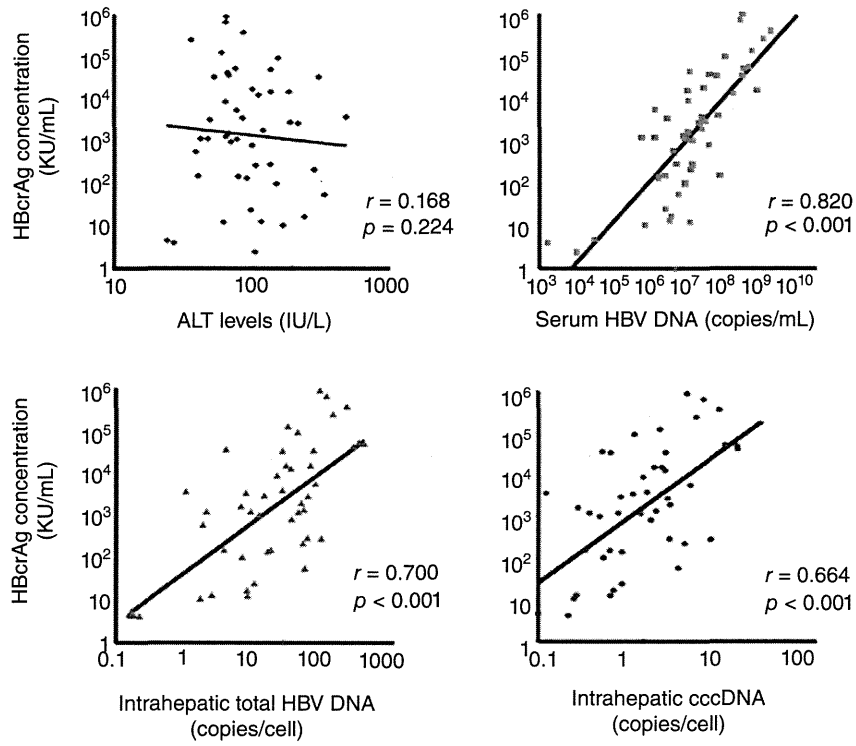

Figure 5 Correlation between HBcrAg, serum HBV DNA levels, and total hepatic HBV DNA and cccDNA.

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.