

Table 1 Clinical characteristics of patients

| | |
|---------------------------------------|-------------|
| Male, n (%) | 643 (50%) |
| Age (years) | 54.2 ± 11.9 |
| BMI (kg/m ²) | 23.4 ± 3.1 |
| Alcohol consumption ≥20 g/day, n (%) | 44 (3%) |
| Diabetes Mellitus, n (%) | 197 (15%) |
| AST level (IU/L) | 68.9 ± 45.3 |
| ALT level (IU/L) | 92.9 ± 75.9 |
| GGT level (IU/L) | 41.2 ± 38.2 |
| Platelet count (×10 ¹⁰ /L) | 16.4 ± 5.2 |
| HCV genotype, n (%) | |
| 1b | 873 (68.2%) |
| 2a | 236 (18.4%) |
| 2b | 139 (10.9%) |
| 3 | 2 (0.2%) |
| Not determined | 29 (2.3%) |
| Histological findings | |
| Grade of activity, n (%) | |
| A0 | 154 (12%) |
| A1 | 574 (45%) |
| A2 | 441 (34%) |
| A3 | 110 (9%) |
| Stage of fibrosis, n (%) | |
| F0 | 24 (2%) |
| F1 | 591 (46%) |
| F2 | 378 (30%) |
| F3 | 242 (19%) |
| F4 | 44 (3%) |
| Grade of steatosis, n (%) | |
| 0% | 384 (30%) |
| 1-9% | 543 (42%) |
| 10-29% | 215 (17%) |
| ≥30% | 137 (11%) |
| SVR to interferon therapy, n (%) | 393 (31%) |
| Development of HCC, n (%) | 68 (5%) |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ -glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

angiography or tumor biopsy to confirm the diagnosis. Diagnostic criteria of HCC on radiological findings were hyper-vascularity at angiography or hyper-attenuation at triphasic contrast-enhanced computerized tomography or magnetic resonance imaging during the hepatic arterial phase.

Statistical analysis

The SPSS software package ver. 15.0 was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. The time for the development of HCC was defined as the time from the completion of IFN therapy to the time of diagnosis. Annual incidence of

HCC was calculated using the person-years method. Effect of hepatic steatosis on time to development of HCC was analyzed by the Kaplan-Meier method and log-rank test, after stratification by age, sex, BMI, degree of fibrosis and response to IFN therapy, as well as multivariate analysis using Cox proportional hazards regression analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

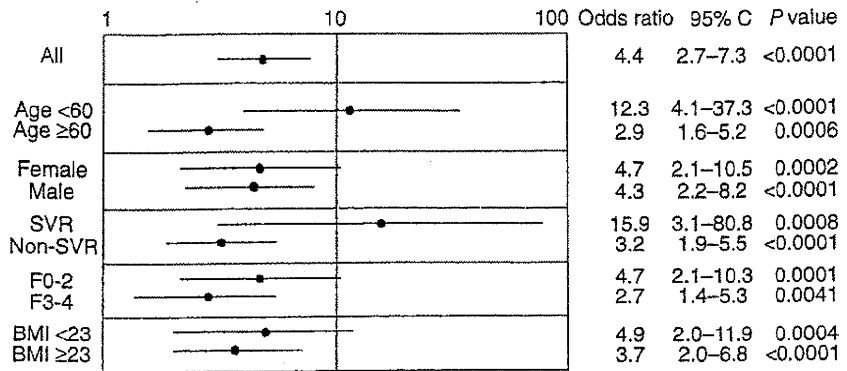
Background factors for steatosis

PATIENTS WITH A steatosis grade of 10% or greater were older (53.6 ± 12.6 vs 56.0 ± 9.8, *P* = 0.001), had a higher BMI (23.0 ± 3.0 vs 24.6 ± 3.3, *P* < 0.0001), higher frequency of diabetes (12% vs 24%, *P* < 0.0001), higher serum levels of aspartate aminotransferase (AST) (66 ± 46 vs 75 ± 43, *P* = 0.002), γ -glutamyltransferase (GGT) (37 ± 52 vs 52 ± 33, *P* < 0.0001), total cholesterol (173 ± 32 vs 179 ± 33, *P* = 0.005), triglycerides (123 ± 56 vs 145 ± 68, *P* < 0.0001), and a lower serum level of albumin (4.2 ± 0.3 vs 4.1 ± 0.3, *P* = 0.005) and lower platelet counts (16.6 ± 5.2 vs 15.7 ± 5.1, *P* = 0.007). Histological grade of activity (A2-3: 39% vs 54%, *P* < 0.0001), and stage of fibrosis (F3-4: 18% vs 34%, *P* < 0.0001) were higher. The proportion of non-sustained virological response (SVR) to IFN also was higher (35% vs 19%, *P* < 0.0001). These results indicate that hepatic steatosis in hepatitis C is related to metabolic factors and associated with other risk factors for the development of HCC such as older age, advanced stage of fibrosis, and non-SVR to IFN therapy.

Factors associated with the development of HCC

Hepatocellular carcinoma developed in 68 patients during follow up. An overall annual incidence of HCC development was 1.19% by person-years. The annual incidence of HCC development by person-years was higher in patients with higher grade of steatosis: 0.45% for patients without steatosis, 0.78% for patients with 1-9% of steatosis, 2.30% for patients with 10-29% of steatosis, and 3.56% for patients with 30% of steatosis. The relative risk of hepatic steatosis (grade of ≥10%) for HCC development was 4.39 (95% confidence interval 2.66-7.26, *P* < 0.0001). The difference remained significant, even after stratification for other risk factors such as IFN therapy, stage of fibrosis, age, sex and BMI (Fig. 1). When analyzed by the multivariate Cox proportional hazards regression method, a higher grade of steatosis,

Figure 1 Relative risk differences of hepatocellular carcinoma (HCC) among patients with and without steatosis. The relative risk of hepatic steatosis (grade $\geq 10\%$) for HCC development was analyzed, after stratification for other risk factors such as interferon (IFN) therapy, stage of fibrosis, age, sex and body mass index (BMI). SVR, sustained virological response.



older age, male sex, higher BMI, an advanced stage of fibrosis and non-SVR to IFN therapy were independent risk factors associated with the development of HCC (Table 2). The adjusted risk ratio of hepatic steatosis was 3.04 (95% confidence interval 1.82-5.06, $P < 0.0001$). The presence of diabetes and consumption of ethanol were not significant. Figure 2(a) shows the Kaplan-Meier curve of the time to development of HCC in the entire cohort. The cumulative incidence of HCC was significantly higher with hepatic steatosis of 10% or greater. To adjust for other risk factors, patients were stratified according to response to IFN therapy, stage of fibrosis, age, sex and BMI. The difference remained significant, even after stratification for these confounding factors (Fig. 2b-f). Three patients died after the development of HCC. All were over 60 years old, and had significant steatosis. The impact of hepatic steatosis on the survival rate could not be analyzed due to the small number of death.

DISCUSSION

IN THIS STUDY, we have shown that the presence of significant steatosis is an independent risk factor for

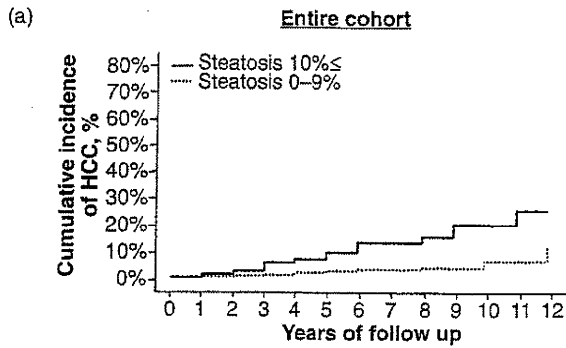
the development of HCC in chronic hepatitis C. Our study involved the largest number of patients, compared to previous reports, and this enabled us to adjust for other known risk factors for HCC. The impact of steatosis on HCC development remained significant even after adjusting for other risk factors such as older age, male sex, higher BMI, advanced fibrosis and non-SVR to IFN therapy. These findings indicate the need of intensive surveillance for HCC in patients with significant steatosis and provide an argument for therapeutic interventions aimed at reducing steatosis, in order to reduce the risk of HCC.

The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed and the possible mechanism has been discussed.¹⁶ There are several cohort studies on this topic but their results are conflicting. The first report included 20 patients with SVR to IFN, 51 patients with non-SVR to IFN and 90 patients who did not receive IFN therapy.¹⁷ In this cohort of 161 patients, older age, absence of IFN therapy, cirrhosis and steatosis were associated with HCC development. Another study involved 25 patients with HCC and an equal number of patients who did not develop HCC, matched for

Table 2 Multivariate analysis of risk factors for hepatocellular carcinoma

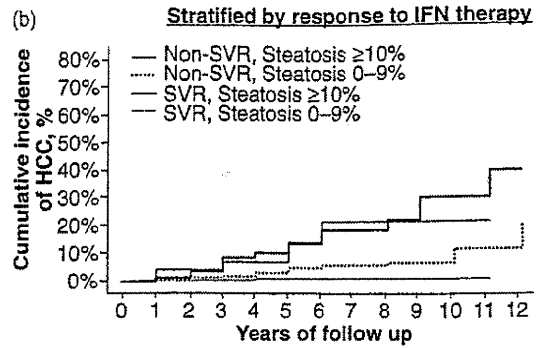
| Predictor | | Odds ratio (95% CI) | P-value |
|-----------------------------|-------------------------|---------------------|---------|
| Age | By every 10 years | 1.09 (1.05-1.13) | <0.0001 |
| Sex | Male vs female | 2.12 (1.28-3.51) | 0.004 |
| Stage of fibrosis | F3-4 vs F0-2 | 4.30 (2.59-7.14) | <0.0001 |
| Grade of steatosis | $\geq 10\%$ vs $< 10\%$ | 3.04 (1.82-5.06) | <0.0001 |
| Response to IFN | Non-SVR vs SVR | 2.43 (1.13-5.23) | 0.023 |
| Diabetes | Present vs absent | 0.75 (0.42-1.33) | 0.319 |
| Ethanol consumption (g/day) | ≥ 20 vs < 20 | 0.50 (0.07-3.60) | 0.478 |
| BMI (kg/m ²) | ≥ 23 vs < 23 | 1.69 (1.02-2.86) | 0.043 |

BMI, body mass index; CI, confidence interval; IFN, interferon; SVR, sustained virological response.



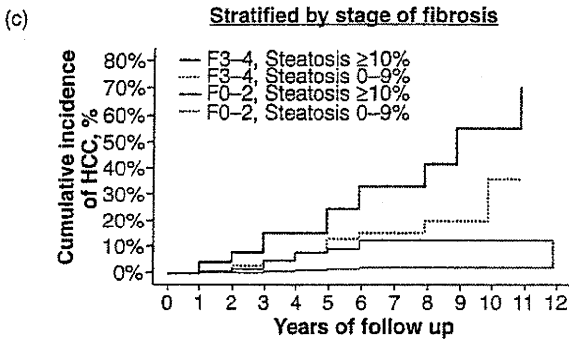
Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|
| Steatosis 0-9% | 927 | 824 | 620 | 503 | 320 | 227 | 161 | 117 | 77 | 49 | 27 | 10 |
| Steatosis ≥10% | 352 | 271 | 207 | 157 | 113 | 83 | 54 | 48 | 32 | 17 | 9 | 1 |



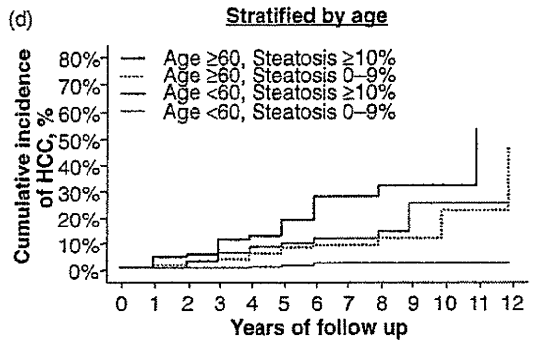
Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|
| SVR | | | | | | | | | | | | |
| Steatosis 0-9% | 326 | 254 | 204 | 153 | 81 | 55 | 33 | 21 | 15 | 10 | 5 | 0 |
| Steatosis ≥10% | 67 | 50 | 34 | 22 | 14 | 10 | 4 | 4 | 4 | 2 | 2 | 0 |
| Non-SVR | | | | | | | | | | | | |
| Steatosis 0-9% | 601 | 507 | 416 | 350 | 239 | 172 | 128 | 96 | 62 | 39 | 22 | 10 |
| Steatosis ≥10% | 285 | 221 | 173 | 135 | 99 | 73 | 50 | 44 | 28 | 15 | 7 | 1 |



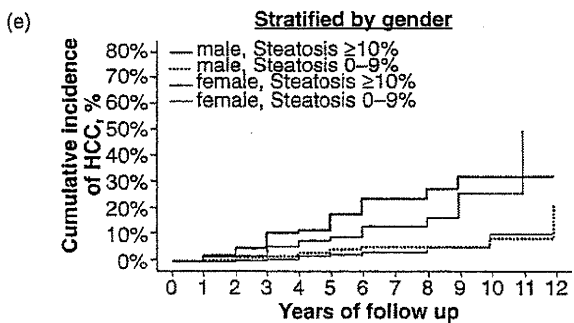
Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|
| F0-2 | | | | | | | | | | | | |
| Steatosis 0-9% | 759 | 623 | 509 | 415 | 266 | 188 | 137 | 99 | 64 | 39 | 25 | 10 |
| Steatosis ≥10% | 234 | 190 | 146 | 107 | 77 | 55 | 37 | 32 | 19 | 11 | 6 | 1 |
| F3-4 | | | | | | | | | | | | |
| Steatosis 0-9% | 118 | 81 | 61 | 50 | 36 | 28 | 17 | 16 | 13 | 6 | 3 | 0 |
| Steatosis ≥10% | 168 | 138 | 111 | 88 | 54 | 39 | 23 | 18 | 13 | 10 | 2 | 0 |



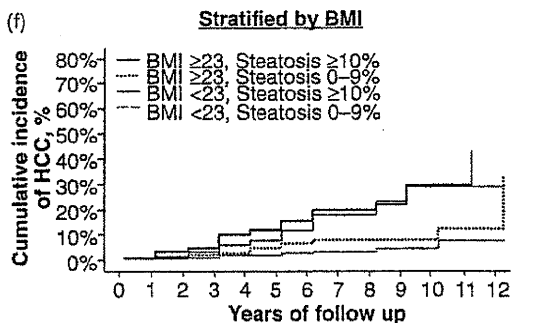
Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|---|
| Age <60 | | | | | | | | | | | | |
| Steatosis 0-9% | 549 | 457 | 367 | 298 | 188 | 148 | 111 | 83 | 53 | 33 | 19 | 7 |
| Steatosis ≥10% | 193 | 154 | 111 | 83 | 61 | 48 | 34 | 31 | 23 | 12 | 6 | 1 |
| Age ≥60 | | | | | | | | | | | | |
| Steatosis 0-9% | 378 | 304 | 253 | 205 | 132 | 79 | 50 | 34 | 24 | 16 | 8 | 3 |
| Steatosis ≥10% | 159 | 117 | 96 | 74 | 52 | 35 | 20 | 17 | 9 | 5 | 3 | 0 |



Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|----|----|----|----|----|---|
| Male | | | | | | | | | | | | |
| Steatosis 0-9% | 470 | 389 | 319 | 265 | 169 | 126 | 90 | 65 | 46 | 30 | 17 | 7 |
| Steatosis ≥10% | 173 | 134 | 98 | 73 | 54 | 40 | 21 | 21 | 15 | 8 | 6 | 1 |
| Female | | | | | | | | | | | | |
| Steatosis 0-9% | 457 | 372 | 301 | 238 | 151 | 101 | 71 | 52 | 31 | 19 | 10 | 3 |
| Steatosis ≥10% | 179 | 137 | 109 | 84 | 59 | 43 | 33 | 27 | 17 | 9 | 3 | 0 |



Number of patients at risk

| | | | | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|----|----|----|----|----|---|
| BMI ≥23 | | | | | | | | | | | | |
| Steatosis 0-9% | 417 | 346 | 269 | 213 | 129 | 94 | 66 | 49 | 31 | 19 | 8 | 4 |
| Steatosis ≥10% | 226 | 176 | 137 | 101 | 71 | 55 | 34 | 33 | 20 | 10 | 5 | 0 |
| BMI <23 | | | | | | | | | | | | |
| Steatosis 0-9% | 510 | 415 | 351 | 290 | 191 | 133 | 95 | 68 | 46 | 30 | 19 | 6 |
| Steatosis ≥10% | 126 | 95 | 70 | 56 | 42 | 28 | 20 | 15 | 12 | 7 | 4 | 1 |

Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) among patients with steatosis (solid line) and without steatosis (dotted line), stratified by other risk factors. The cumulative incidence of HCC was (a) significantly higher in patients with a steatosis grade of 10% or greater ($P < 0.0001$ by the log-rank test), even after (b) stratification by the response to interferon therapy ($P < 0.0001$ for sustained virological response [SVR] and non-SVR by the log-rank test), (c) stratification by the stage of fibrosis ($P < 0.0001$ for F0-2 and $P = 0.0036$ for F3-4 by the log-rank test), (d) stratification by age ($P = 0.0001$ for age ≥ 60 and $P < 0.0001$ for age < 60 by the log-rank test), (e) stratification by sex ($P < 0.0001$ for men and women by the log-rank test), and (f) stratification by body mass index (BMI) ($P < 0.0001$ for BMI ≥ 23 kg/m² and < 23 kg/m² by the log-rank test). The number of patients at risk is shown below each graph.

age, sex, HCV genotype and stage of fibrosis.¹⁹ In this study, only ALT and albumin were identified as predictors of HCC and steatosis was not. The authors acknowledged the small size of the cohort as a limitation and emphasized the need for larger cohort studies. The third study analyzed explanted liver from cirrhotic patients who underwent liver transplantation and included 32 patients with HCC and 62 patients without HCC.¹⁸ The authors found that older age, higher α -fetoprotein levels and steatosis were significantly associated with HCC. The major advantage of this study was the standardization of fibrosis stage to cirrhosis. On the other hand, a limitation was the retrospective nature of the study; steatosis was evaluated after the diagnosis of HCC, when cirrhosis already was present (fibrosis stage F4). Because steatosis has been reported to decrease once cirrhosis has developed, this study may have underestimated the grade of steatosis present prior to the development of HCC. Thus, we cannot simply apply their findings to a clinical setting where biopsies are usually obtained before the development of cirrhosis and years before the development of HCC. Based on that background, the principal aim of this study was to analyze the association between hepatic steatosis and the development of HCC in chronic hepatitis C patients, adjusting for known risk factors. We found that steatosis was an independent risk factor by the multivariate Cox proportional hazards regression analysis and by the Kaplan-Meier method and log-rank test after stratification by other risk factors. To our surprise, the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI.

How steatosis contributes to the development of HCC remains unclear. Several studies including ours,¹⁰ indicated that hepatic steatosis promotes the progression of hepatic fibrosis,¹¹⁻¹⁵ which potentiates the risk of HCC indirectly. On the other hand, the ob/ob mouse model of NAFLD showed that hepatic neoplasia developed in the absence of advanced fibrosis, supporting the concept that metabolic abnormalities related to obesity initiate

the neoplastic process.⁸ Leptin, an adipocytokine related to steatosis in chronic hepatitis C,²¹ was shown recently to be mitogenic in human liver²² and thus may be a link between steatosis and HCC development. Otherwise, steatosis may be responsible for increased lipid peroxidation and reactive oxygen species which induce genetic damage.²³⁻²⁵ Another study showed that mice transgenic for the HCV core gene developed hepatic steatosis early in life and thereafter HCC which indicates that the HCV core protein has a chief role in the development of both steatosis and HCC development.²⁶ The precise mechanism of the association between steatosis and carcinogenesis needs further investigation.

The higher incidence of HCC in patients with significant steatosis has important clinical implications. The most important question is whether therapeutic interventions aimed at reducing steatosis could reduce the risk of HCC in chronic hepatitis C. Because the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI, we hypothesize that modification of lifestyle and the amelioration of hepatic steatosis may efficiently prevent hepatocarcinogenesis in patients having concomitant risk factors. Apparently, further prospective studies focusing on this point are necessary. Weight reduction may provide an important treatment strategy because one study indicated that weight reduction in chronic hepatitis C leads to a reduction in steatosis and an improvement in fibrosis despite the persistence of HCV infection.²⁷ Alternatively, insulin resistance may be another target of therapy because a study showed that the administration of pioglitazone led to metabolic and histological improvement in subjects with non-alcoholic steatohepatitis.²⁸ A limitation of the present study was that data for the plasma insulin concentration was not available and thus insulin resistance could not be assessed. Whether insulin resistance plays a role in hepatocarcinogenesis or its amelioration could improve steatosis and ultimately prevent development of HCC in chronic hepatitis C awaits future investigation.

Another important finding of the present study was that steatosis was a significant risk factor for the development of HCC in patients with SVR to IFN therapy. Thus, steatosis may play a role in carcinogenesis in patients who have cleared HCV. Several studies have shown that the incidence of HCC is reduced but not eliminated in those with SVR to IFN.^{29–31} Because the predictors of HCC development in SVR patients have not been established to date, steatosis may be used to identify patients who need intensive surveillance and long-term follow up, even after the clearance of HCV. In conclusion, we showed that hepatic steatosis is significantly associated with the development of HCC in chronic hepatitis C independent of age, sex, BMI, degree of fibrosis and response to previous IFN therapy. Steatosis may be a useful marker for identifying patients at higher risk for HCC. Further studies are needed to evaluate the hypothesis that therapeutic interventions aimed at reducing steatosis may prevent hepatocarcinogenesis.

ACKNOWLEDGMENTS

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Review Article

Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009

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Recently, much progress has been made in the field of hepatitis B, such as natural history of the disease in relation to the amount of hepatitis B virus (HBV) DNA, genotypes of HBV influencing the natural course and treatment effects, mutations of HBV influencing the severity of the disease and development of hepatocellular carcinoma, and antiviral treatment such as nucleos(t)ide analogues and pegylated interferon. To make the consensus for the diagnosis, management and treatment of hepatitis B, a meeting was held during 45th annual meeting of Japan Society of Hepatology (JSH) in June 2009. In the meeting, recommendations and informative statements were discussed on the following subjects: (i) natural history of HBV infection; (ii) clinical implication of HBV genotypes; (iii) HBV mutations and their potential impact on

pathogenesis of HBV infection; (iv) indications for antiviral treatment of chronic hepatitis B; (v) nucleos(t)ide analogues for chronic hepatitis B; and (vi) interferon therapy for chronic hepatitis B. The presenters reviewed the data on these subjects and proposed the consensus statements and recommendations. These statements were discussed among the organizers and presenters, and were approved by the participants of the meeting. In the current report, the relevant data were reviewed and the 12 consensus statements and nine recommendations on chronic hepatitis B were described.

Key words: genotype, hepatitis B virus, interferon, mutation, natural history, nucleotide analogue

Hepatitis B virus (HBV) is one of the most distributed viruses which infect humankind. More than 3 billion people, one half of the world's population, have been exposed to HBV during their life.¹ Acute infection in adults is self-limited in general whereas infection during early childhood will develop into persistent chronic infection in most individuals.² More than 400 million people worldwide are chronically infected with HBV and are at risk of developing life-threatening complications

including liver cirrhosis and hepatocellular carcinoma (HCC).¹ HBV is a major public health problem worldwide especially in East Asia and Africa. In Japan, approximately 1.5 million people are infected with HBV and it is one of the major causes of HCC and chronic hepatic failure. Other complications of HBV infection include fulminant hepatitis and acute liver failure.

The consensus meeting for diagnosis, management and treatment for hepatitis B was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M Kudo), where the recommendations and informative statements were discussed. Although the JSH consensus meeting of hepatitis B had been held four times so far, recommendations were hitherto published only in Japanese and this is the first report in English. Established

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information for pathogenesis and contributing factors for disease progression which was agreed by the organizers and presenters are shown as “consensus statements”, and clinically useful consensus are shown as “recommendations”. The quality of recommendations or informative statements are required to show a “level” (assessing strength or certainty) of evidence and “grading” of recommendations or assessment according to a standard reporting system of clinical guidelines.³

NATURAL HISTORY OF HBV INFECTION

AN EVALUATION OF studies on the natural history of HBV infection was done using the scoring system proposed by MacMahon *et al.*⁴ in the present analysis because scoring systems for treatment studies cannot always be applied directly to those using natural history. The proposed scoring system consists of levels 1 (1a, 1b), 2 (2a, 2b, 2c), and 3. Level 1a is defined as a population-based longitudinal cohort study with a hepatitis B surface antigen (HBsAg) negative comparison group. Level 1b is identical to level 1a, but with no comparison group. Level 2a is defined as a clinic-based longitudinal cohort study, level 2b is a population-based or clinic-based cohort nested case–control study, and level 2c is a cross-sectional clinic-based study. Level 3 is defined as an observation study case series.

The natural history of chronic HBV infection can be classified into several phases based on levels of alanine aminotransferase (ALT), hepatitis B e-antigen (HBeAg) status, amounts of HBV DNA, and estimated immunological states.^{4–9} A representative classification of these phases is shown in Table 1. In the immune tolerance phase, HBeAg is positive, serum levels of ALT are normal, histological activities of hepatitis are absent or minimal, and levels of HBV DNA are elevated. The

immune tolerance phase is thought to occur most frequently in individuals who are infected through perinatal transmission, and this phase usually lasts until adolescence or young adulthood.^{10–12}

The chronic hepatitis B phase is characterized by elevated ALT and HBV DNA levels. In this phase, the host’s immune system recognizes HBV as being foreign and initiates an immune response that results in hepatitis. In cases who are HBeAg positive, active hepatitis can be prolonged and may result in cirrhosis. However, chronic hepatitis B eventually transitions into an inactive phase with a loss of HBeAg positivity in the majority of patients. Seroconversion to anti-HBe and the fall of serum HBV DNA to low levels result in the disappearance of disease activity, despite persisting HBsAg and low levels of HBV DNA.^{13–16} Seroconversion rates range 7–16% per year according to reports with higher evidence levels (levels 1b, 2a).^{16–19} Factors associated with seroconversion are age (level 1b),²⁰ ALT levels (level 1b), occurrence of acute exacerbation of hepatitis (level 1b),^{19,21} and genotype (level 2c).^{22,23}

The seroconversion of HBeAg results in the transition from hepatitis phase to inactive carrier phase, which is generally thought to be a benign course for HBV carrier, but sometimes hepatitis can be reactivated spontaneously.²⁴ Patients experiencing reactivation undergo another transition, with increases in HBV DNA and ALT levels and disease activity without reappearance of HBeAg.²⁴ This phase is referred to as HBeAg negative chronic hepatitis B. Occasional severe hepatitis B flare-ups with middle range HBV DNA levels (3–8 log copies/mL) occur in this phase.^{8,25} HBeAg negative chronic hepatitis B is caused by mutant strains of HBV unable to produce HBeAg,^{25,26} and tends to develop into cirrhosis and complicate HCC more than HBeAg positive chronic hepatitis B.^{27–30}

Table 1 Phases in the natural history of HBV carriers (modified from ⁴)

| Phase | Hepatitis | Blood | | | Liver |
|-------------------|-------------|-------|-------|-------|--------|
| | | DNA | HBeAg | HBsAg | cccDNA |
| Immune tolerance | – | 8–11 | + | + | + |
| HBeAg positive | Usually | 6–10 | + | + | + |
| Chronic hepatitis | Persistent | | | | |
| HBeAg negative | Often | 3–8 | – | + | + |
| Chronic hepatitis | Fluctuating | | | | |
| Inactive carrier | – | <4 | – | + | + |
| Recovery | – | – | – | – | + |

HBV DNA: log copies/mL. cccDNA, covalently close circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Many factors that are associated with the development of HCC have been reported so far. Higher age (level 1a), male sex (level 1a), presence of cirrhosis (level 2a) and familial cluster of carriers (level 2c) are reported as host factors.^{31,32} Viral factors include high viral load (level 1b),^{33–36} existence of pre-core and core promoter mutations (level 2a), genotype C and high ALT levels (level 1b). High viral load should be considered as a factor in patients over 35–40 years of age. Co-infection with hepatitis C virus, hepatitis D virus or HIV (level 2a), drinking habit (level 2c) and exposure to aflatoxin (level 2c) are reported as social and environmental factors.^{37–39} Other lifestyle-related factors, such as smoking habit, obesity and complications from diabetes mellitus, have been documented as well.

Consensus 1

In patients with chronic hepatitis B, seroconversion of HBeAg usually results in the transition from hepatitis phase to inactive carrier phase, which generally has low HBV replication and normal ALT levels. However, reactivation of chronic hepatitis can spontaneously occur without the reappearance of HBeAg. At this point, active hepatitis continues and the risk of complicating cirrhosis and HCC is high in patients with HBeAg negative chronic hepatitis B. (Level 1b.)

In the inactive carrier phase, HBV replication is continuously suppressed as a result of predominantly host immunological pressure against HBV. Patients in the inactive carrier phase generally have a benign course because active hepatitis subsides and the risk of HCC decreases.^{19,20,24,40} However, regular follow up is required because reactivation of HBV sometimes occurs spontaneously or as a result of immunosuppressive therapy.^{19,24}

Hepatitis B surface antigen is known to fall to undetectable levels in some inactive carriers. This HBsAg negative phase, referred to as the recovery phase, has no hepatitis and a low risk of HCC. Still, caregivers must be aware that patients who are old or cirrhotic have a relatively higher risk of HCC.^{41,42} Disappearance of HBsAg in the recovery phase does not indicate complete eradication of HBV because the HBV genome remains as covalently close circular DNA (cccDNA) in the nucleus of hepatocytes.

Consensus 2

2-1 HBV can not be completely eradicated using any currently existing treatment measures. (Level 2a.)

2-2 Patients in the inactive carrier or recovery phase have a benign clinical course. However, regular follow up of such patients is required because reactivation of hepatitis B and ensuing HCC can occur. (Level 1b, 2a.)

Clinicians have to consider two types of hepatitis B reactivation: one during the inactive carrier phase and the other in the recovery phase.⁴ Both types of reactivation have been attributed with increasing incidence to strong immunosuppressive therapies. De novo hepatitis B, a reactivation of hepatitis B in the recovery phase, tends to develop into fulminant hepatitis, which has a very high mortality rate.^{43–46} Thus, establishment of effective measures to prevent reactivation of hepatitis B is necessary.

Consensus 3

- 3-1 Reactivation of hepatitis B can occur during the inactive carrier or recovery phases and stems mainly from strong immunosuppressive treatment courses. (Level 2a.)
- 3-2 Recent advances in medical care have increased the use of immunosuppressive agents and thus the incidence of hepatitis B reactivation. (Level 2a.)
- 3-3 Reactivation of hepatitis B tends to develop into fulminant hepatitis. (Level 2a.)

Recommendation 1

In addition to the loss or seroconversion of HBeAg, a substantial decrease in HBV viral load and subsequent disappearance of hepatitis are the primary targets in the treatment of patients with chronic hepatitis B. (Level 1b.)

Recommendation 2

The main goals of HBV carrier treatment are patients in the inactive carrier and recovery phases. However, caregivers should be aware that reactivation of hepatitis B and complication of HCC can occur even in these benign phases. (Level 1b.)

Recommendation 3

Reactivation of hepatitis B due to immunosuppressive therapy tends to develop into severe hepatitis, thus requiring the establishment of effective preventative measures. (Level 2a.)

CLINICAL IMPLICATION OF HBV GENOTYPES

DISTINCT CLINICAL AND/OR virological characteristics of the HBV infection have been reported in different geographical parts of the world and are increasingly associated with host factors, environmental factors and the genetic diversity of the infecting virus.⁴⁷ HBV is classified into at least eight genotypes (A–H) based on an intergroup divergence of 8% or more in the complete nucleotide sequence and a number of subgenotypes (Aa/A1, Ae/A2, Bj/B1, Ba/B2, Cs/C1, Ce/C2, D1, D2, and so forth) that are currently known to have distinctive association with ethnic and/or geographical distribution.⁴⁸

Association between HBV genotype and clinical manifestation

Acute hepatitis

The universal vaccination program against HBV has significantly reduced the number of new infection cases in most countries with levels of endemicity estimated from intermediate to high.⁴⁹ However, efficiency of universal vaccination in countries with a low level of endemicity still remains controversial. Japan is one of the countries with a low level of endemicity and mainly vertical (mother to baby) transmission route.⁵⁰ In Japan, HBV vaccination in combination with HBV immunoglobulin treatment is the only recommended measure for infants born to HBsAg positive mothers. Studies in Japan indicated genotype C (subgenotype Ce/C2) to be the major type in the country and genotype B (subgenotype Bj/B1) is the second distributed. Surveillance studies have shown a recent trend toward increase in number of acute hepatitis B infection among young adults mainly through sexual contacts.^{51,52} Although most cases are associated with genotype C infection, there is a continuous trend toward increase in prevalence of genotype A among acute hepatitis cases.^{51,53–56} Patients infected with genotype C have been known to be rarely associated with development of chronic persistence after acute infection in immune competent adults in Japan (1%) in contrast to the higher rates of those infected with genotype A (6–23%).^{53,54} A recent multicenter study in Japan indicated a trend among chronic hepatitis B patients toward increase in prevalence of genotype A (from 1.7% in 2002 to 3.5% in 2006), whereas other genotypes remained stable at their prevalence during the same period.⁵⁷ The shift in genotype prevalence with the increase of genotype A among chronically infected carriers can be explained by higher risk of genotype A to develop persistence. This is consistent with higher rates

of chronic persistence after acute infection in adults in European countries where genotype A is prevalent (10%).^{48,58} This is also consistent with results of *in vitro* and *in vivo* comparisons of different genotype strains showing different dynamics of replication: slow for genotype A and rapid by genotype C.^{59,60} The surveillance study indicated that all patients treated with lamivudine (LVD) recovered from acute hepatitis, whereas none of the three patients who developed a chronic outcome had received antiviral treatment during their acute phase of infection, indicating that LVD might be able to prevent the chronic outcome.⁵⁴ Cumulatively, these data indicate the clinical importance of routine genotyping for acute hepatitis B patients.

Fulminant hepatitis

One of the most serious complications of acute HBV infection is fulminant hepatitis. In Japan, the annual number of fulminant hepatitis reported was approximately 400 cases, with approximately half of these caused by HBV infection. Despite its rather low incidence, fulminant hepatitis is a national problem because the mortality rate is extremely high.⁶¹ It is important to understand factors predisposing for development of fulminant hepatitis. Viral factors associated with the development of fulminant hepatitis are mutations in the core promoter (T1762/A1764)⁶² and the pre-core region (A1896).^{54,63,64} However, these findings were not consistent with studies in Europe and the USA.^{65–67} A large-scale cross-sectional study in Japan revealed association between genotype B (subgenotype Bj/B1) infection and development of fulminant hepatitis; on the other hand, no cases of fulminant hepatitis were registered among those infected with genotype A (subgenotype Ae/A2).⁵⁴ Differences in genotypes circulating in Asia and Europe/USA may indicate that distinct viral factors are playing roles in manifestation of infection by different genotype.

Chronic hepatitis

Chronic HBV infection is the most common cause of HCC in Asia.⁶⁸ Efficient surveillance and early diagnosis of development of this life complication requires risk stratification of chronic hepatitis B patients. Older age, male sex and liver cirrhosis are well recognized factors associated with increased risk of HCC.^{69,70} In addition, recent large-scale population-based and clinical case-control studies carried out in Asia, have shown that infecting virus factors associated with a high risk of HCC, include HBV DNA levels,^{71,72} HBV basal core promoter mutations,³⁵ genotype C (vs B),^{22,36,73,74} and sub-

genotype Ce/C2.^{71,75} There are data indicating that genotype C infection associated with a higher viral load than genotype B.⁷⁶ Association of genotype F with HCC was found to be higher than that of genotype C in Alaskan natives.^{77,78} Unfortunately, there are few prospective studies examining other HBV genotypes for association with adverse outcomes. Genotype A (subgenotype Aa/A1) was found in association with development of HCC in young adults in South Africa.^{79,80} However, very high rates of detection of subgenotype Aa/A1 among asymptomatic carriers suggest contribution of environmental factors (aflatoxin contained in food) for the development of HCC. In comparison with Aa/A1, HCC associated with Ae/A2 is found primarily in older individuals. In addition, the rate of complications, including HCC, for those infected with subgenotype Ae/A2 appears to be less than that found in those infected with genotype D, C or F1.^{77,81} A prospective study in Spain showed that genotype A (presumably Ae/A2) infection was associated with a significantly higher cumulative rate of sustained biochemical remission, HBV DNA and HBsAg clearance in patients with chronic HBV infection than genotype D infection.⁸¹

Consensus 4

- 4-1 Recently, there is an increase of HBV genotype A proportion among acute hepatitis B infection cases in Japan. (Level 3.)
- 4-2 HBV genotype A acute infection has a tendency to evolve in chronic hepatitis compared to genotype B/C. (Level 3.)
- 4-3 Antiviral therapy of acute infection might be efficient in prevention of chronic carrier stage. (Level 3.)
- 4-4 Genotype C compared with genotype B is associated with higher risk of outcome in HCC in chronic carriers. (Level 2a, grade B.)
- 4-5 Genotype A compared with genotype D and F in chronic carriers is associated with better prognosis in terms of spontaneous ALT normalization and DNA clearance. (Level 2a, grade B.)

HBV MUTATIONS AND THEIR POTENTIAL IMPACT ON PATHOGENESIS OF HBV INFECTION

THE HBV GENOME consists of double-stranded DNA, 3200 bp in length. HBV replicates through reverse transcription of a RNA intermediate, the prege-

nome RNA, different from all known mammalian DNA viruses. HBV infection is characterized by high levels of virus production, however, the HBV reverse transcriptase is an error-prone enzyme lacking proof-reading capacity, resulting in a large number of nucleotide substitutions during replication. The misincorporation rate has been estimated to be of the order of 10^{10} incorrect nucleotide incorporations per day. As a result, HBV has a quasispecies distribution in infected patients.

Naturally occurring mutations identified in the HBV genome are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers. Among them, several specific mutations have been shown to be associated with the pathogenesis of HBV infection.

HBeAg seroconversion

A HBV strain harboring stop codon mutation in the precore region was first reported in anti-HBe positive patients with chronic hepatitis.²⁵ The precore region located upstream of the core region is involved in the production and secretion of HBeAg protein. HBeAg is secreted into blood after removal of N-terminal 19 amino acids (a.a.) and C-terminal 34 a.a. from HBeAg precursor protein composed of precore and core regions. Nucleotide substitution of G to A at nt 1896 confers stop codon (TAG) mutation from tryptophan (TGG) at codon 28 in the precore region, resulting in a failure to produce HBeAg protein.⁸²⁻⁸⁴ Although controversial, 10 genotypes have been identified tentatively so far⁸⁵ and genotypes affect the occurrence of stop codon mutation in the precore region. The stop codon mutation in the precore region (G1896A) is rarely encountered in HBV genomes of genotype A, some of genotype C and F, because they possess C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.⁸⁶

The HBV core promoter regions located upstream of core region are involved in the transcription of precore mRNA and pregenomic RNA. Nucleotide substitution of A to T at nt 1762 combined with substitution of G to A at nt 1764 in the core promoter region give rise to a reduced transcription of precore mRNA and increased level of viral DNA, resulting in a decreased production of HBeAg protein and enhanced viral replication.⁸⁷⁻⁸⁹

Consensus 5

Nucleotide substitution G1896A confers stop codon mutation in the precore region. Nucleotide substitution A1762T combined with substitution G1764A in

the core promoter region give rise to a reduced transcription of precore mRNA. These nucleotide changes in combination with a reduction of HBeAg caused by suppressed replication of HBV are closely associated with HBeAg seroconversion. (Level 2b, grade B.)

Association between HBV mutations and clinical manifestation

Fulminant hepatitis

Precore and core promoter mutations are very frequent in patients with fulminant hepatitis from Asia^{62,63,90} and the Middle East.⁶⁴ However, these mutations were not detected in those from Western countries.^{65,67,91,92} This difference could be attributable to the difference of genotype prevalence, frequent genotype Ae and rare Bj in Western countries.⁸⁶ The patients infected with the former genotype rarely have precore mutant virus, while the latter frequently have the mutant virus. Stop codon mutation in the precore region is inhibited in genotype A because of C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.⁹³

Ozasa *et al.* analyzed the difference of host and viral factors between 40 patients with fulminant hepatitis B and 256 with acute self-limited hepatitis B in a multi-center cross-sectional study,⁵⁴ and showed that precore stop codon mutation of G1896A and genotype Bj are associated with fulminant hepatitis in Japan. They also reported the marked enhancement of viral replication by introducing either G1896A or A1762T/G1764A mutation into the Bj clone in *in vitro* transfection study. Because this type of HBV mutant is found not only in patients with fulminant hepatitis but also in asymptomatic HBV carriers,⁹⁴ the interaction between the virus and the host's immune response might influence the outcome of HBV infection.

In addition to the mutants mentioned above, pre-S2 defective virus or HBV defective in secretion because of surface gene mutations are reported in patients with fulminant hepatitis. These mutant viruses showed a characteristic feature of virus retention in hepatocytes and misassembly with high replication capacity.^{95–97}

HCC development

Evidence has been accumulating over the past decade that the risk of developing cirrhosis and HCC is influenced by the patient's viral status, such as genotype, viral load and genomic mutations. Naturally occurring

mutations have been identified in the structural and non-structural genes as well as the regulatory elements of the virus, and these mutations are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers.⁹⁸

A double mutation, A1762T/G1764A in the basal core promoter region has been found in patients with advanced liver disease and HCC. Several case-control studies,^{30,35,99–102} retrospective cohort studies^{103,104} and one prospective cohort study¹⁰⁵ confirmed this finding, while some conflicting results were also reported in the case-control studies^{106,107} and one prospective study.¹⁰⁸

The role of deletions in the pre-S region of the HBV genome has been shown to be associated with the development of progressive liver diseases including HCC. Several case-control studies confirmed this finding.^{27,107–110} A further mapping study of the pre-S region showed that all the deletion regions encompassed T- and B-cell epitopes and most of them lost one or more functional sites including the polymerized human serum albumin-binding site.¹⁰⁹ Deletion of these functional sites may cause intracellular retention of HBV envelope proteins and viral particles and contribute to more progressive liver damage and HCC development.

In addition to these common mutations, several other mutations, C1653T in the enhancer II region, T1753C/A/G in the core promoter region, and G1317A/T1341C/A/G in enhancer I region, have been reported to be associated with the development of HCC in some case-control studies.^{30,107,111}

Consensus 6

There is some evidence that emergence of HBV genomic mutations arising during the course of chronic infection influence the outcome of chronic liver disease. Among them, core promoter mutations A1762T/G1764A might have a potential for developing progressive liver disease and HCC. (Level 2a, grade B.)

HBsAg escape mutant

The HBsAg mutant was first described in a child born to a HBsAg positive mother who developed acute hepatitis B in spite of vaccination and passive immunization against HBV.¹¹² This viral strain contained a substitution of glycine to arginine at position 145 (sG145R) and was able to escape the immune surveillance, resulting in an infection despite the presence of anti-HBs antibodies, vaccine escape mutant. Similar mutants have been detected all over the world.^{113–115}

Patients after liver transplantation for HBV-related chronic liver disease who had received anti-HBs antibodies to prevent re-infection of the graft showed an "immune escape mutant".¹¹⁶⁻¹¹⁸ Furthermore, "diagnosis escape mutants" have also been described because HBsAg detection assays are based on anti-HBs antibodies.¹¹⁹ The emergence of these variants may contribute to occult HBsAg negative HBV infection.¹²⁰

The HBV genome is organized in such a way that the envelope gene is overlapped by the polymerase gene; therefore, HBV with changes in the polymerase gene associated with resistance to the nucleos(t)ide analog which are described in detail in section 5 may have consequent changes in the envelope gene. A triple mutant causing LVD resistance (rtV173L + rtL180M + rtM204V), which have an enhanced replication capacity compared with rtL180M + rtM204V alone, causes two amino acid changes in the overlapping surface gene (sE164D + sI195M). This mutant reduces anti-HBs binding to levels seen only with the vaccine escape mutant sG145R.¹²¹ Some patients treated with LVD showed seroclearance of HBsAg with detectable circulating HBV DNA. An sP120A mutation was associated with HBsAg seroconversion in these patients and this mutation produces a reduced anti-HBs binding which causes the failure to detect HBsAg.¹²²

Consensus 7

Amino acid substitutions, deletions or insertions across the "a" determinant of HBsAg, such as a substitution sG145R, give rise to vaccine and immunoglobulin escape mutant. (Level 4, grade C.)

INDICATIONS FOR ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS B

ONCE THE LIVER is persistently infected with HBV, it is difficult to eradicate the virus. It is reported that the natural clearance rate of HBsAg in asymptomatic HBsAg carriers is approximately 1-2% per year.¹²³ Therefore, the first goal in treating chronic hepatitis B is to prevent patients from progression to cirrhosis and occurrence of HCC.

When the initiation of antiviral therapy for chronic hepatitis B is considered, it is very important to estimate the fibrosis stage of each patient. If possible, a liver biopsy should be performed in order to obtain sufficient information to determine the extent of hepatic fibrosis. When the fibrosis stage of patients with chronic hepatitis B is moderate to severe, or when the patients

have cirrhotic liver, the administration of antiviral therapy should be considered. When inflammatory activity is high and the fibrosis seems to be progressive, the introduction of antiviral therapy should also be considered.

In order to prevent the occurrence of hepatic fibrosis and HCC, virological factors as well as biochemical factors are important. A long-term follow-up study of untreated HBsAg positive individuals in Taiwan in which the cumulative incidence of HCC and cirrhosis were studied for 13 years revealed that high baseline HBV DNA was associated with increased risk of HCC and cirrhosis. Incidence rate of HCC in patients whose viral load of HBV DNA was less than 300 copies/mL was 1.3%, whereas in patients whose viral load was more than 1 000 000 copies/mL the incidence rate was 14.9%.³³ Moreover, incidence of cirrhosis in patients whose viral load was less than 300 copies/mL was 4.5%, whereas it was 36.2% in patients whose viral load was more than 1 000 000 copies/mL.¹²⁴ Therefore, the introduction of antiviral therapy should be considered based on biochemical and virological findings.

As mentioned above, although high viral load of HBV DNA is one of the strong risk factors in predicting poor prognosis of HBV carriers, low HBV DNA level does not rule out risk in Asian patients. Among HBeAg positive patients, HBV DNA levels of less than 10⁵ copies/mL predicted better histological outcome; however, 14.3% of patients still had established fibrosis.¹²⁵ The liver biopsy is also very useful for such cases.

Recommendation 4

- 4-1 Introduction of antiviral therapy should be considered on the biochemical and virological findings. (Level 2a, grade B.)
- 4-2 Antiviral therapy should be considered for patients with low virus load but progressed hepatic fibrosis. (Level 2a, grade B.)
- 4-3 Liver biopsy finding (if available) should be useful to determine the introduction of antiviral therapy. (Level 2a, grade B.)

On the other hand, when patients with HBV have obscure or mild fibrosis, a close observation without any medication could be considered for them. Once antiviral therapy with a nucleos(t)ide analogue is started, it is very difficult to stop. Therefore, for patients who are in an inactive carrier state and whose fibrosis stage is relatively mild, a coarse observation without any treatment could be a useful choice to treat the patients.

Young patients with chronic hepatitis B, especially those who are HBeAg positive, often face the flare-up of hepatitis. Because such patients are likely to achieve spontaneous HBe seroconversion and go into an inactive carrier state, unnecessary antiviral therapy should be avoided for them. A close observation without any medications should be considered for young patients or those with mild fibrosis.

Recommendation 5

Indication of antiviral therapy for chronic hepatitis B: Observation without therapy should be considered for young patients or those with mild fibrosis. (Level 3, grade B.)

NUCLEOS(T)IDE ANALOGUES FOR CHRONIC HEPATITIS B

AS STATED ABOVE, the goal of antiviral therapy in patients with chronic hepatitis B is to prevent cirrhosis and HCC. Maintaining persistent suppression of HBV replication reduces the development of cirrhosis and HCC. In the last decade, there has been a major advance in the treatment of chronic hepatitis B with nucleos(t)ide analogues such as LVD, adefovir (ADV), entecavir (ETV), telbivudine and tenofovir.^{126–132} In treatment by nucleos(t)ide analogues for chronic hepatitis B in Japan, LVD, ADV and ETV are mainly used at present. Nucleos(t)ide analogues are potent inhibitors of the polymerase/reverse transcriptase and are easy to administer p.o. to chronic hepatitis B patients because of low adverse effects and strong efficacy to suppress HBV replication. Thus, nucleotide analogue therapy could rescue liver decompensation, reduce fibrosis progression and prevent the development of HCC.^{133–136} On the other hand, there are major disadvantages including requirement of prolonged or even indefinite therapy for most patients and the high incidence of antiviral resistance. Disadvantages of nucleos(t)ide analogues include the development of antiviral resistance.^{137–140} Drug-resistant viruses emerge during the treatment and could be associated with flare-up of hepatitis. Due to no proof of reading activity of HBV polymerase, the spontaneous substitution rate of HBV genome is high in the natural course of the disease. Through the selection of pre-existing resistant variants and gradual accumulation of new a.a. substitutions, the mutations exhibiting the best replication capacity in the presence of the drug are selected under the circumstance of antiviral pressure.

The level of intrinsic resistance and the replicative fitness determine the mutant spread and hence the annual incidence of drug resistance.

LVD

Lamivudine was the first nucleoside analogue licensed for the treatment of chronic HBV infection in Japan in 1999. LVD was given at a dose of 100 mg daily and has excellent safety and tolerability.^{141–143}

Liaw *et al.* reported that continuous treatment with LVD delays the clinical progression of chronic hepatitis B with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and risk of HCC (level 1b).¹³⁴ Matsumoto *et al.* also showed that LVD therapy effectively reduces the incidence of HCC in Japanese patients with chronic hepatitis B.¹⁴⁴ Thus, it is generally considered that control of viral load using nucleos(t)ide analogues is effective to prevent complicating HCC in patients with active chronic hepatitis B.

Consensus 8

The control of viral load using nucleos(t)ide analogues reduces the risk of complicating HCC in patients with chronic hepatitis B. (Level 1b, grade B.)

Lamivudine resistance is characterized by the mutation of the highly conserved tyrosine, methionine, aspartate, aspartate (YMDD) nucleotide-binding motif in the catalytic domain of the enzyme. YMDD to YIDD (rtM204I) or YVDD (rtM204V) mutations are associated with LVD resistance.^{142,145,146} These resistant mutants appear to replicate less efficiently than the wild-type virus *in vitro*, however, additional mutations such as rtV173L and rtL180M can restore partially the replication capacity *in vitro*.^{147,148} LVD resistance occurred in approximately 20% of patients after 1 year, which increased to approximately 70% after 5 years (Fig. 1).

A meta-analysis, which included Asian patients and North American/European patients, indicated that HBV subtype ayw (genotype D) appears to respond significantly better to LVD treatment than does HBV subtype adw (genotype A). Insufficient suppression of the adw subtype during the early phase of treatment may lead to the high incidence of LVD resistance in HBV subtype adw.¹⁴⁹ In a study comparing the virological outcome among infections with HBV genotypes A, B and C, patients infected with genotype A had the lowest rate of HBV DNA clearance than those with genotype B or C, and had the highest incidence of resistant mutations.¹⁵⁰

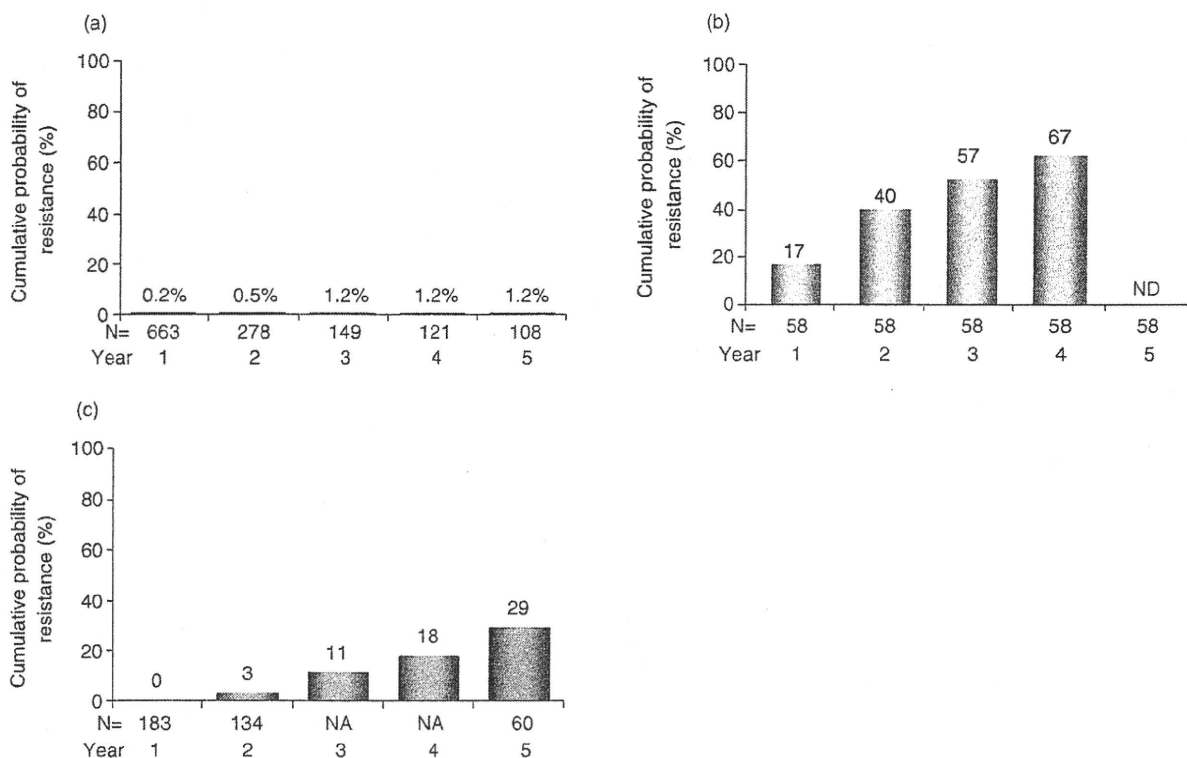


Figure 1 Cumulative probability of resistance after the initiation of entecavir (ETV), lamivudine (LVD) and adefovir (ADV) for patients with hepatitis B e-antigen. (a) Cumulative probability of resistance after the initiation of ETV.¹⁵⁹ (b) Cumulative probability of resistance after the initiation of LVD.¹³⁸ (c) Cumulative probability of resistance after the initiation of ADV.¹⁵³

Lamivudine or hepatitis B immunoglobulin (HBIg) treatment induced vaccine/HBIg-escape mutations sP120T and sG145R in combination with LVD-resistance mutations. These mutations are associated with rtT128N and rtW153Q in the polymerase protein and have been found to partially restore the *in vitro* replicative capacity of LVD-resistant HBV.¹²¹

Another LVD resistant mutation, rtA181T, concomitantly generates a stop codon in the surface antigen (sW172stop), resulting in impaired secretion of HBsAg.¹⁵¹ Neither the adefovir associated resistance mutation rtN236T nor the tenofovir associated resistance mutation rtA194T causes changes in the envelop protein.

ADV

Adefovir dipivoxil is a prodrug of ADV and has structural similarity to the natural substrate, dATP. Several studies have also been conducted using ADV.^{128,152-154} In HBeAg positive patients, treatment with ADV for 1 year resulted in HBeAg seroconversion in 12%, serum HBV DNA in less than 10³ copies/mL in 21% and normaliza-

tion of ALT in approximately 48% of patients.¹²⁷ The rate of HBeAg seroconversion increased to 29% after 2 years and 43% after 3 years of treatment. In HBeAg negative patients, serum HBV DNA of less than 10³ copies/mL and normalization of ALT were observed in 51% and 72%, respectively, after 1 year of ADV.¹⁵⁴ After 5 years of therapy, the serum HBV DNA were less than 10³ copies/mL in 67% of patients, and ALT level normalized in 69%. The reported incidence of ADV resistance is 0% after 1 year, 3% after 2 years and 29% after 5 years of antiviral therapy (Fig. 1).¹⁵⁴ The primary mutations associated with ADV resistance are rtN236T and rtI233V in the D domain and rtA181V in the B domain of HBV polymerase. In comparison with more than 100-fold decrease in sensitivity to LVD associated with the two primary mutations, the rtN236T mutation confers only a 5-10-fold decrease in sensitivity to ADV *in vitro*,¹⁵⁵ which may explain the delayed emergence of this mutant.

In LVD-resistant patients treated with ADV monotherapy, the rate of antiviral resistance was 6-18% after

1 year and 21–38% after 2 years.^{156,157} Switching therapy from LVD to ADV may enhance the acquisition of another mutation and induce replication of HBV DNA.^{158–160} On the other hand, combination therapy of LVD and ADV effectively suppressed viral replication and maintained high efficacy in LVD-resistant patients with chronic HBV infection.

ETV

Entecavir is a guanine analogue and Chang *et al.* have reported that ETV is effective in reducing the serum level of HBV DNA compared with LVD in HBeAg positive patients (Table 2).¹⁵⁹ The cumulative proportion of patients with undetectable HBV DNA (<300 copies/mL) increased to 81% after 1 year of therapy and 93% after 5 years of therapy.¹⁶⁰ After 1 year of treatment with ETV, the serum ALT level was normalized in approximately 70% of patients, and increased to 90% of patients after 5 years. Lai *et al.* have reported that ETV is more efficacious in HBeAg negative patients compared with LVD (Table 2).¹⁶¹ ETV is the most potent of the currently available anti-HBV drugs because it affects multiple functions of the polymerase, including priming, reverse transcription and DNA elongation.¹⁶²

Entecavir was licensed for the treatment of chronic hepatitis B in Japan in 2006. In nucleos(t)ide-naïve patients, ETV is given at dose of 0.5 mg/day.

The rate of ETV resistance was extremely low in nucleoside-naïve patients.^{160,163,164} The incidence of ETV resistance in nucleos(t)ide analogue-naïve patients was reported to be 1.2% at 3 years (Fig. 1).^{160,163,164} HBeAg loss was observed in 8% of these patients. The response to ETV was lower in LVD-resistant patients than in nucleos(t)ide analogue-naïve patients. In LVD-resistant patients, 20% of patients had undetectable HBV DNA levels after 48 weeks of ETV therapy, and the resistance rate to ETV was 26% at 3 years. Patients with HBeAg at the initiation of ETV had a resistance rate to ETV of 36% at 3 years. On the other hand, patients without HBeAg at the initiation of ETV did not have resistance to ETV at 3 years (Fig. 2).^{160,165} In LVD-resistant patients, the risk of the development of resistance to ETV is much higher than those without LVD resistance.^{160,165}

The resistance to ETV is principally associated with the mutations rtM250V, rtI169T or rtS202I in addition to the primary LVD resistance mutations rtM204V + rtL180M. The need for multiple mutations to induce ETV resistance suggests a higher genetic barrier to resistance and explains the low rate of resistance to ETV in nucleos(t)ide analogue-naïve patients.

Table 2 Efficacy of nucleoside analogues for chronic hepatitis B

| | n | Change of HBV DNA (log copies/mL) | Subject: HBeAg positive patients ¹⁵⁹ | | P | SC |
|---|-----|-----------------------------------|---|---|----------------------|----------|
| | | | Change of HBV DNA (log copies/mL) | Negativity of HBV DNA of <300 copies/mL | | |
| ETV 0.5 mg | 354 | -6.9 | P < 0.001 | 67% | P < 0.001 | 21% |
| LVD 100 mg | 355 | -5.4 | | 36% | | 18% |
| | | | | | | P = 0.33 |
| Subject: HBeAg negative patients ¹⁶¹ | | | | | | |
| | n | Change of HBV DNA (log copies/mL) | Negativity of HBV DNA of <300 copies/mL | | Normalization of ALT | |
| ETV 0.5 mg | 325 | -5.0 | 90% | P = 0.001 | 78% | P < 0.05 |
| LVD 100 mg | 323 | -4.5 | 72% | | 71% | |

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; LVD, lamivudine; SC, seroconversion; VR, virological response.

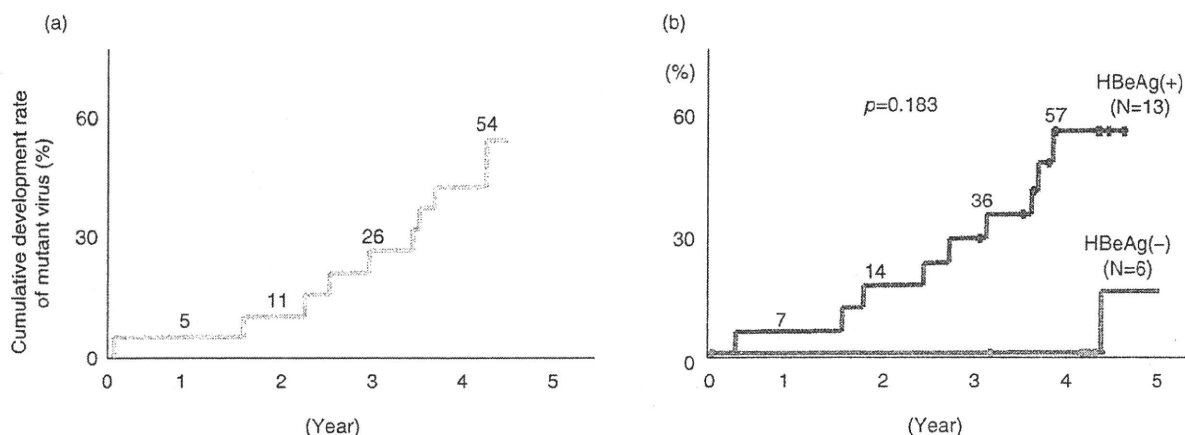


Figure 2 Cumulative development rate of mutant virus after the initiation of entecavir monotherapy in hepatitis B patients with resistance after the administration of lamivudine monotherapy.¹⁶⁴ (a) Cumulative development rate of mutant virus in all patients. (b) Cumulative development rate of mutant virus based on the difference of hepatitis B patients with positive hepatitis B e-antigen (HBeAg) and hepatitis B patients with negative HBeAg.

Consensus 9

Drug-resistant virus with specific mutations in the polymerase/reverse transcriptase gene emerges during nucleos(t)ide analogue therapy in chronic hepatitis B patients. The rtM204V/I and rtL180M mutations are associated with LVD resistance, the rtN236T and rtI233V or rtA181V with ADV resistance, and the rtM250V or rtT184G or rtS202I combined with rtM204V + rtL180M with ETV resistance. (Level 4, grade C.)

Recommendation 6

When patients with chronic hepatitis B are treated with nucleos(t)ide analogues, ETV should be given as the first-line drug because of its high efficacy and low emergence of viral resistant mutant. (Level 1b, grade A.)

Recommendation 7

The combination therapy of LVD and ADV is an effective treatment for LVD-resistant patients. (Level 1b, grade B.)

INTERFERON THERAPY FOR CHRONIC HEPATITIS B

INTERFERON (IFN) WAS the first antiviral treatment approved for chronic HBV infection. IFN- α and - β

have a predominantly antiviral effect but also have an immunomodulatory effect and antiproliferative effect which is in contrast to direct antiviral agents such as nucleos(t)ide analogues. The duration of treatment is defined (usually 24–48 weeks) in IFN therapy. This finite duration of therapy is an advantage over direct antiviral agents which are usually given indefinitely. The long-term outcome of therapy is more precisely described in IFN compared to LVD due to its longer history of clinical usage.

Selection of patients

Factors associated with favorable response to IFN therapy are vigorously studied (Table 3). For HBeAg positive patients, high pretreatment ALT levels,¹⁶⁶ high grade of necroinflammation on liver histology and low serum HBV DNA level have consistently been shown to be predictive of favorable response.¹⁶⁷ Other predictive factors include female sex,¹⁶⁶ younger age,^{168,169} and HBV genotype A versus D or B versus C.^{169,170} Patients fulfilling these predictors are the best candidates for IFN treatment. For HBeAg negative patients, there is no consistent predictor of response. Adverse events such as severe infection or exacerbations of liver disease were common when IFN was given for decompensated cirrhosis. Thus, patients with decompensated cirrhosis should not be treated with IFN due to a risk of precipitating hepatic failure and fatal complications.^{171,172}

Table 3 Predictive factors for response to interferon therapy

| Predictive factors | HBeAg positive | HBeAg negative |
|--------------------|---------------------------|--------------------------------|
| Race | No correlation | No correlation |
| Age | No correlation or Younger | No correlation or Younger |
| Sex | No correlation or Female | No correlation or Female |
| ALT | Higher level | No correlation or Higher level |
| Activity | Higher grade | No correlation |
| Fibrosis | Conflicting | No correlation |
| HBV DNA titer | Lower titer | No correlation or lower titer |
| Genotype | A > D, B > C | A > D, B > C |
| Precore | Conflicting | No correlation |
| Core promoter | mutant | |

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

Recommendation 8

Younger age, high ALT levels, low HBV load, genotype A or B and high inflammatory activity in liver biopsy are predictive of good response to IFN. IFN therapy should be considered in patients fulfilling these predictors. (Level 2a, 2b, grade B.)

Recommendation 9

Interferon should be avoided for patients with decompensated cirrhosis. (Level 4, grade D.)

Standard IFN therapy in HBeAg positive chronic hepatitis B

A meta-analysis of 16 randomized controlled studies have shown that treatment with IFN- α for 16–24 weeks versus an untreated control is associated with higher rate of HBeAg loss (33% vs 12%), HBeAg seroconversion (difference of 18%), undetectable HBV DNA by hybridization or branched chain assay (37% vs 17%), HBsAg loss (7.8% vs 1.8%) and ALT normalization (difference of 23%) (Table 4).¹⁷³ A controlled trial has shown that extending therapy for up to 32 weeks in patients who remained HBeAg positive at the end of 16 weeks of

therapy improved the rate of HBeAg seroconversion.¹⁷⁴ The durability of HBeAg seroconversion is more than 80%, and even delayed seroconversion could occur in 10–15% of patients 1–2 years after completion of therapy.^{175–177} The loss of HBsAg is reported to occur in 12–65% of patients who cleared HBeAg.^{175,178} However, this is a rare event in Asian patients.^{176,177}

Consensus statement 10

10-1 In HBeAg positive patients, treatment with IFN versus untreated control is associated with higher rate of HBeAg loss, HBeAg seroconversion, undetectable HBV DNA, HBsAg loss and ALT normalization. Extension of therapy improves the rate of HBeAg seroconversion. (Level 1a,1b.)

10-2 Durability of HBeAg seroconversion is more than 80%. The loss of HBsAg is rare in Asian patients. (Level 1b.)

Standard IFN therapy in HBeAg negative chronic hepatitis B

Although the rate of response at the end of therapy is 60–90%, the durability of long-term response is less

Table 4 Standard interferon therapy for HBeAg positive chronic hepatitis B. Meta-analysis of 16 randomized controlled trials

| | Interferon | Control | P-value |
|-------------------|------------|-------------------|---------|
| Loss of HBV DNA | 37% | 17% | 0.0001 |
| Loss of HBeAg | 33% | 12% | 0.0001 |
| Loss of HBsAg | 7.8% | 1.8% | 0.001 |
| Seroconversion | | Difference of 18% | 0.002 |
| ALT normalization | | Difference of 23% | 0.0001 |

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

than 50%.^{179,180} Longer duration of therapy is associated with improved durability of response: 10-15% with 4-6 months of therapy, 22-30% with 6-12 months of therapy and 30% with 24 months of therapy.¹⁸¹⁻¹⁸⁴

Consensus statement 11
 11-1 Durability of response is less than 50% in HBeAg negative patients. (Level 1b.)
 11-2 Longer duration of therapy (>48 weeks) is associated with improved durability of response. (Level 2b.)

Pegylated IFN (PEG IFN)

Twenty four weeks of PEG IFN- α -2a monotherapy had higher rate of combined response (loss of HBeAg, suppression of HBV DNA <500 000 copies/mL and ALT normalization) compared to standard IFN- α -2a.¹⁸⁵ Another study with 24 weeks of PEG IFN- α -2b monotherapy also showed a higher rate of HBeAg loss and HBV DNA suppression compared to standard IFN- α -2b.¹⁶⁹

Controlled studies comparing the 48 weeks of PEG IFN- α -2a and LVD in HBeAg positive and negative patients revealed that PEG IFN had a higher rate of sustained response.^{170,171} Seroconversion of HBeAg (32% vs 19%), ALT normalization (41% vs 28% in HBeAg positives and 59% vs 44% in HBeAg negatives), HBV DNA suppression (HBV DNA <10 000 copies/mL, 32% vs 22% in HBeAg positives; HBV DNA <20 000 copies/mL, 43% vs 29% in HBeAg negatives) and negative HBV DNA (14% vs 5% in HBeAg positives and 19% vs 7% in HBeAg negatives) were more frequent in PEG IFN treated patients.

Differences were reported in outcome of the antiviral treatment of patients infected with different genotypes; genotype B is associated with a higher rate of antiviral response to IFN treatment than HBV genotype C among Asian patients with HBeAg positive chronic hepatitis B.^{169,186,187} In multicenter trials comparing combination therapy of PEG IFN- α -2b and LVD versus PEG IFN- α -2b alone, it was shown that treatment with PEG IFN- α -2b is the best therapy to achieve HBsAg clearance in patients with genotype A compared with D.^{188,189}

Combination or sequential therapy

Combination of two antiviral agents with different mechanisms of action seems a logical approach to improve efficacy. In fact, simultaneous combination of LVD and PEG IFN has a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone. However, there is no difference in treatment response between the simultaneous combination of LVD and IFN or PEG IFN compared to IFN or PEG IFN alone (Table 5).^{132,133,170}

There are several clinical trials of sequential therapy with LVD followed by IFN.¹⁹⁰⁻¹⁹⁴ Common to all studies is that the sequential therapy had no advantage over IFN alone. Some studies have shown the suggestive evidence that sequential therapy had a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone (Table 5).¹⁹⁰⁻¹⁹⁴ However, because the study protocols and their results are variable, a conclusive result could not be drawn.

Table 5 Sequential therapy of lamivudine and interferon

| | | BR | SC | VR | LVD-R |
|---|------------|-----|-----|-----|-------|
| Manesis <i>et al.</i> 2006 (n = 36) ¹⁹⁰ | Sequential | 39% | NA | 28% | |
| | IFN | 22% | NA | 19% | |
| Shi <i>et al.</i> 2006 (n = 162) ¹⁹¹ | Sequential | 53% | NA | 14% | 0% |
| | LVD | 36% | NA | 18% | 23% |
| Yurdaydin <i>et al.</i> 2005 (n = 78) ¹⁹³ | Sequential | 51% | NA | 54% | 24% |
| | LVD | 41% | NA | 59% | 53% |
| Sarin <i>et al.</i> 2005 (n = 75) ¹⁹⁴ | Sequential | 40% | 40% | 40% | 15% |
| | LVD | 14% | 11% | 16% | 8% |
| Schalm <i>et al.</i> 2000 (n = 226) ¹⁹² | Sequential | 50% | 36% | 55% | 0% |
| | IFN | 50% | 22% | 49% | 0% |
| | LVD | 63% | 19% | 63% | 31% |

BR, biochemical response; IFN, interferon; LVD, lamivudine; LVD-R, lamivudine resistant mutation; NA, not applicable because hepatitis B e-antigen patients are studied; SC, seroconversion; VR, virological response.

Long-term outcome

The end-point of antiviral therapy is to prevent liver cirrhosis and HCC. Meta-analysis of five studies including 935 patients revealed that IFN treatment significantly decreased the incidence of cirrhosis with the combined risk ratio of 0.65 (95% confidence interval [CI] = 0.47–0.91).¹⁹⁵ Meta-analysis of 11 studies including 2082 patients revealed that IFN treatment significantly decreased the incidence of HCC with the combined risk ratio of 0.59 (95% CI = 0.43–0.81).¹⁹⁵ These results suggest that IFN prevents progression of liver disease to liver cirrhosis or delays the development of HCC, as long as it is within 4–7 years of follow up which is the length of follow up in these studies. Sustained response to IFN therapy was associated with increased survival.^{175,181,196,197} To further elucidate the impact of IFN on the natural course of chronic hepatitis B, studies with larger populations followed for longer periods may be needed.

Consensus statement 12

- 12-1 IFN therapy prevents progression to cirrhosis or the development of HCC. (Level 1a.)
12-2 IFN therapy is associated with improved survival. (Level 1b.)

Adverse effects

The most frequent adverse effects are flu-like symptoms, fatigue, myelosuppression and dermal reaction at the injection site. Others include alopecia, depression and thyroid dysfunction. Less frequent but severe adverse events include interstitial pneumonitis, exacerbation of underlying autoimmune disorders, cerebral vascular events and flare of hepatitis.

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