

pressing the development of HCC, branched chain amino acids (BCAA)¹¹ are implemented. Also, nutrient supplements are utilized for stabilizing liver function.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HBV and HCV ranging from chronic hepatitis to cirrhosis of various severities annually, since the fiscal year 2002. The principal aim of these guidelines is to decrease the incidence of HCC due to hepatitis virus infections in Japan. In accordance with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic viral liver disease every fiscal year. This article summarizes guidelines for the treatment of liver disease due to HBV. Guidelines for the treatment of liver disease due to HCV for the fiscal year 2008 are reported in the accompanying paper. They are formulated on evidence-based data that have been accumulated by members and cooperators of the study group. It will be necessary to improve these guidelines in the next fiscal year and henceforth, in accordance with many pieces of new evidence that are expected to evolve through enduring efforts and keen insights of members and cooperators of the study group.

In the treatment of chronic hepatitis B, novel therapeutic strategies have continued to evolve in previous guidelines. In guidelines of the fiscal year 2008, diverse new treatment arms are introduced for gaining the eventual goal of the "drug-free state".

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has been drafted and displayed on the web site (www.jsh.or.jp/medical/index.html [in Japanese]) as well, guidelines for the treatment of a spectrum of liver diseases due to HBV, ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. In view of the eventual goal of decreasing the incidence of HCC due to HBV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been introduced to the treatment of cirrhosis due to HBV infection. As a general rule, antiviral treatments are the mainstay in guidelines for the treatment of chronic hepatitis B. In addition to them, it is necessary to always keep in mind the fundamental concepts of these guidelines. It is our sincere hope that, for the treatment of each patient, readers will conduct their

clinical practice on the basis of these concepts, and then refer to appropriate individual guidelines, when they make decisions regarding treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in making treatment choices.

We can foretell that there is no end to the treatment of patients with chronic hepatitis and cirrhosis due to HBV, as it will keep evolving and improving in future guidelines. The enduring efforts of doctors and scientists, in pursuit of this goal, will fill in wide social and economic gaps in medical practices being served to the nation, and produce substantial and efficient interest in the medical economy on a national basis. In conducting treatment of patients with liver disease due to HBV infection, according to these guidelines, many new and unforeseen facets may surface that will require further improvements. Hence, it will be necessary to evaluate the therapeutic efficacy of these guidelines, and revise or add necessary supplements to them as required in the future.

REFERENCES

- 1 Serfaty L, Thabut D, Zoulim F *et al.* Sequential treatment with lamivudine and interferon monotherapies in patients with chronic hepatitis B not responding to interferon alone: results of a pilot study. *Hepatology* 2001; 34: 573–7.
- 2 Shindo M, Chayama K, Mochida S. Antiviral activity, dose-response relationship, and safety of entecavir following 24 week oral dosing in nucleoside-naïve Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial. *Hepatol Int* 2009.
- 3 Kobayashi H, Fujioka S, Kawaguchi MK. Two cases of development of entecavir resistance during entecavir treatment for nucleoside-naïve chronic hepatitis B. *Hepatol Int* 2009; 3: 403–10.
- 4 Suzuki F, Toyoda J, Katano Y *et al.* Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 2008; 23: 1320–6.
- 5 Shakado S, Watanabe H, Tanaka T. Combination therapy of lamivudine and adefovir in Japanese patients with chronic hepatitis B. *Hepatol Int* 2009; 2: 361–9.
- 6 Akuta N, Suzuki F, Kawamura Y *et al.* Virological response and hepatocarcinogenesis in lamivudine-resistant hepatitis B virus genotype C patients treated with lamivudine plus adefovir dipivoxil. *Intervirology* 2008; 51: 385–93.
- 7 Hosaka T, Suzuki F, Suzuki Y *et al.* Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine. *J Gastroenterol* 2007; 42: 368–74.

- 8 Yatsuji H, Suzuki F, Sezaki H *et al.* Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. *J Hepatol* 2008; 48: 923-31.
- 9 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494-500.
- 10 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multi-centre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; 56: 1747-53.
- 11 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; 35: 204-14.

Review Article

Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan

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In the 2008 guidelines for the treatment of patients with chronic hepatitis C, pegylated interferon (Peg-IFN) combined with ribavirin for 48 weeks are indicated for treatment-naïve patients infected with hepatitis C virus (HCV) of genotype 1. Treatment is continued for an additional 24 weeks (72 weeks total) in the patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA during 13–36 weeks on treatment. Re-treatment is aimed to either eradicate HCV or normalize transaminase levels for preventing the development of hepatocellular carcinoma (HCC). For patients with compensated cirrhosis, the clearance of HCV RNA is aimed toward improving histological damages and decreasing the development of HCC. The recommended therapeutic regimen is the initial daily dose of 6 million international units (MIU) IFN continued for 2–8 weeks

that is extended to longer than 48 weeks, if possible. IFN dose is reduced to 3 MIU daily in patients who fail to clear HCV RNA by 12 weeks for preventing the development of HCC. Splenectomy or embolization of the splenic artery is recommended to patients with platelet counts of less than $50 \times 10^3/\text{mm}^3$ prior to the commencement of IFN treatment. When the prevention of HCC is at issue, not only IFN, but also liver supportive therapy such as stronger neo-minophagen C, ursodeoxycholic acid, phlebotomy, branched chain amino acids (BCAA), either alone or in combination, are given. In patients with decompensated cirrhosis, by contrast, reversal to compensation is attempted.

Key words: chronic hepatitis, cirrhosis, hepatocellular carcinoma, hepatitis C virus, interferon, liver supportive therapy, pegylated interferon, ribavirin

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INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health Labor and Welfare of Japan, recruiting many specialists from all over the nation. They have been improved every year with many supplementary issues that have evolved, as our understanding of various aspects of viral hepatitis deepens and treatment options widen with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to infection with hepatitis C virus (HCV) in Japan. It is hoped that these guidelines will be accepted widely and implemented for helping as many patients as possible who suffer from sequelae of persistent HCV infection.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with HCV-induced liver disease covering a wide range from those with normal aminotransferase levels to those with decompensated cirrhosis.

GUIDELINES FOR THE PRIMARY TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

TABLE 1 SUMMARIZES the antiviral therapy of treatment-naïve patients with chronic hepatitis C. In comparison with previous guidelines, the duration of combined treatment with pegylated interferon (Peg-IFN) and ribavirin is extended to 48–72 weeks for patients infected with HCV of genotype 1 in high viral loads (HVL: ≥ 5 log IU/mL by the Japanese criteria).^{1,2} For patients infected with HCV of genotype 2 in HVL, Peg-IFN- $\alpha 2b$ and ribavirin for 24 weeks are indicated.

To patients with HCV-1 in low viral loads (LVL: < 5 log IU/mL), either the standard IFN (not conjugated with polyethylene glycol) for 24 weeks, or the weekly monotherapy with Peg-IFN- $\alpha 2a$ for 24–48 weeks, is given.³ Patients with HCV-2 in LVL receive either the standard IFN for 8–24 weeks, or the weekly monotherapy with Peg-IFN- $\alpha 2a$ for 24–48 weeks.

GUIDELINES FOR THE RE-TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

FOR PATIENTS WHO receive re-treatment, first, it is imperatively prerequisite to: (i) identify factors for non-response to previous treatments; and (ii) decide whether to aim for clearance of HCV or to prevent the progression of hepatitis that can accelerate the development of hepatocellular carcinoma (HCC), and this can be monitored by alanine aminotransferase (ALT) and α -fetoprotein (AFP) levels toward normalizing or stabilizing their levels (Table 2).⁴ Second, IFN combined with ribavirin is the mainstay of re-treatment of patients with chronic hepatitis C. Third, long-term IFN monotherapy is recommended to patients who are not indicated to IFN/ribavirin or who have failed to respond to the combination therapy. However, some patients do not tolerate IFN due to side-effects or their complicating morbidities. In addition, IFN monotherapy does not always improve ALT levels. Such patients need to receive liver supportive therapy including stronger neominophagen C (SNMC)⁵ and ursodeoxycholic acid (UDCA),⁶ as well as phlebotomy, either alone or in combination. Therapeutic target ALT levels are: (i) within $\times 1.5$ the upper limit of normal (ULN) for patients in fibrosis stage 1 (F1); and (ii) less than 30 IU/L in those in fibrosis stages 2 or 3 (F2/F3), as far as possible.

Table 1 Guidelines for the primary treatment of patients with chronic hepatitis C

| Genotypes | Genotype 1 | Genotype 2 |
|---|---|---|
| Viral loads | | |
| High viral load ≥ 5.0 log IU/mL ≥ 300 fmol/L ≥ 1 Meq/mL | <ul style="list-style-type: none"> • Peg-IFN-$\alpha 2b$ (Peg-Intron) + ribavirin (Rebetol) for 48–72 weeks • Peg-IFN-$\alpha 2a$ (Pegasys) + ribavirin (Copegus) for 48–72 weeks | <ul style="list-style-type: none"> • Peg-IFN-$\alpha 2b$ (Peg-Intron) + ribavirin (Rebetol) for 24 weeks |
| Low viral load < 5.0 log IU/mL < 300 fmol/L < 1 Meq/mL | <ul style="list-style-type: none"> • Standard IFN for 24 weeks • Peg-IFN-$\alpha 2a$ (Pegasys) for 24–48 weeks | <ul style="list-style-type: none"> • Standard IFN for 8–24 weeks • Peg-IFN-$\alpha 2a$ (Pegasys) for 24–48 weeks |

Peg-IFN, pegylated interferon.

Table 2 Guidelines for re-treatment of chronic hepatitis C**Principles**

Selection has to be made between termination of HCV infection and normalization/stabilization of ALT as well as AFP levels (toward preventing aggravation of liver disease and development of HCC), after evaluating factors for non-response in the primary IFN treatment.

- 1 "IFN plus ribavirin" is the mainstay of re-treatment of patients who have failed to respond to the primary IFN therapy.
- 2 Long-term IFN is recommended to patients in whom ribavirin is not indicated or who have failed to respond to IFN/ribavirin; self-injection at home is approved for IFN- α (not for Peg-IFN).
- 3 Patients who are not indicated to IFN or have failed to improve ALT and AFP levels, in response to IFN, receive liver supportive therapy (SNMC, UDCA) and phlebotomy, either alone or in combination.
- 4 For preventing aggravation of liver disease (and development of HCC), ALT levels need to be controlled within $1.5 \times \text{ULN}$ in patients in stage 1 fibrosis (F1), and as far as possible, 30 IU/L or lower in those in fibrosis stages 2–3 (F2/F3).
- 5 In treatment combined with ribavirin, dose and mode need to be selected, taking into consideration factors contributing to the response, such as age, sex, progression of liver disease, mutations in the HCV genome (amino acid substitutions in the core protein [aa70/aa91] and ISDR) and HCV RNA titers determined by the real-time PCR.

AFP, α -fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS C

FOR THE FISCAL year 2008, the following items were supplemented to the treatment of chronic hepatitis C (Table 3).

- 1 The treatment of patients infected with HCV-1 in HVL with Peg-IFN/ribavirin for 72 weeks is modified by the early virological response (EVR) within 12 weeks after the start. Patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.^{1,2}
- 2 Patients with HCV-1 in HVL who fail to clear HCV RNA detectable by real-time PCR but in whom

ALT levels normalize are continued on Peg-IFN/ribavirin until 48 weeks, so that normalized ALT levels endure longer after the completion of therapy.⁷

- 3 Patients who are not indicated to Peg-IFN/ribavirin, or who have failed to respond to previous treatments, receive long-term IFN monotherapy. During the first 2 weeks, IFN in the conventional dose is given daily or three times a week. Patients who do not clear HCV RNA during the maximal treatment period of 8 weeks receive half the conventional dose of IFN indefinitely.⁸

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C IN NORMAL ALT LEVELS

AS IN PREVIOUS guidelines, patients with chronic hepatitis C having normal ALT levels are stratified into four groups by ALT levels and platelet counts (Table 4). Patients with chronic hepatitis C who have normal ALT levels are reported to gain the sustained virological response (SVR) to antiviral treatments comparably frequently as those having elevated ALT levels. Taking this into consideration, patients with ALT levels of 30 IU/L or less and platelet counts of $150 \times 10^3/\text{mm}^3$ or more are followed for ALT every

Table 3 Supplements to guidelines for chronic hepatitis C

- 1 Criteria for extending the duration of Peg-IFN/ribavirin (to 72 weeks) in patients infected with HCV-1b in HVL: patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.^{1,2}
- 2 Patients with HCV-1b in HVL who fail to lose HCV RNA detectable by real-time PCR, but in whom ALT levels normalize by 36 weeks, Peg-IFN/ribavirin is given till 48 weeks for maintaining normalized ALT levels long after the completion of treatment.
- 3 Long-term IFN monotherapy in patients who are not indicated to Peg-IFN/ribavirin, or have failed to respond to it: the usual dose of IFN daily or three times in week is given for the first 2 weeks, and when HCV RNA does not disappear within the maximal duration of 8 weeks, long-term treatment with half the usual dose of IFN is continued indefinitely.

ALT, alanine aminotransferase; HCV, hepatitis C virus; HVL, high viral loads; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon.

Table 4 Guidelines for the treatment of patients with normal ALT levels toward preventing the development of HCC

| Platelets | $\geq 150 \times 10^3/\text{mm}^3$ | $< 150 \times 10^3/\text{mm}^3$ |
|----------------|--|---|
| ALT | | |
| ≤ 30 IU/L | <ul style="list-style-type: none"> Follow for ALT every 2–4 months. If ALT levels elevate, start antiviral treatments taking into consideration the possibility of SVR and risk for HCC. | <ul style="list-style-type: none"> Liver biopsy, if possible, and consider antiviral treatments for patients in A2/F2. Follow for ALT every 2–4 months, and consider antiviral treatments when ALT levels elevate, for patients without biopsy. |
| 31–40 IU/L | <ul style="list-style-type: none"> Consider antiviral treatments for patients younger than 65 years. | <ul style="list-style-type: none"> Start treatments for chronic hepatitis C. Select treatments according to genotypes, viral load, age of patients, etc. |

ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

2–4 months. If ALT levels increase in them, antiviral treatments are considered based on the possibility of resolving HCV infection and the risk for developing HCC. In view of significant fibrosis present in patients with platelet counts of less than $150 \times 10^3/\text{mm}^3$, they are recommended to receive liver biopsy, if this is possible. Patients in fibrosis stage F2 or higher are evaluated for the indication to antiviral treatments. Patients with ALT levels between 31 and 40 IU/L are classified by platelet counts. Antiviral treatments are considered in those aged younger than 65 years who have platelet counts of $150 \times 10^3/\text{mm}^3$ or more, while guidelines for patients with chronic hepatitis are applied to those with platelet counts of less than $150 \times 10^3/\text{mm}^3$.^{9,10}

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HCV

PATIENTS WITH COMPENSATED cirrhosis who are not infected with HCV-1 in HVL receive either IFN- β or IFN- α (Table 5). Since the fiscal year 2008, IFN- α has been approved for the treatment of patients infected with HCV-1 in HVL, with the aim of resolving infection and normalizing ALT as well as AFP levels by long-term therapy. Treatment duration was set at 1 year or longer, and because the longer the treatment duration the higher the SVR rate, 36 weeks has been recommended as the optimal treatment duration. Because the normalization of ALT/AST is important, even in patients who fail to clear HCV infection by these therapeutic regimens, treatment is better conducted for maintaining normal ALT/AST levels. Guidelines for maintaining liver function for preventing the development of HCC include liver supportive therapy with glycyrrhizin⁵ and UDCA,⁶ either alone or in combination. For treatment toward suppressing the

development of HCC, branched chain amino acids (BCAA)¹¹ or phlebotomy are adopted. Also, nutrient supplements are applied for stabilizing liver function.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CIRRHOSIS DUE TO HCV

THE FOLLOWING ITEMS have been appended to supplement guidelines for the treatment of type C cirrhosis (Table 6).

Table 5 Guidelines for treatment of type C cirrhosis

| | |
|------------|---|
| Principles | Compensated: termination of HCV infection Decompensated: reversal to compensation and prevention of HCC |
| Methods | <ol style="list-style-type: none"> Eradication of HCV and normalization of ALT/AST (for patients with compensated cirrhosis). <ol style="list-style-type: none"> HCV-1b in HVL (≥ 5 log IU/mL) <ul style="list-style-type: none"> IFN-α (Sumiferon) Others <ul style="list-style-type: none"> IFN-α (Sumiferon) IFN-β (Feron) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC. <ol style="list-style-type: none"> Liver supportive therapy <ul style="list-style-type: none"> Stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA), etc. Branched chain amino acids (BCAA [Livact]) Phlebotomy Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis). |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HVL, high viral loads; IFN, interferon.

Table 6 Supplements to guidelines for type C cirrhosis

- 1 To start with, IFN for compensated cirrhosis is desired at 6 MIU daily for 2–8 weeks, as far as possible, and to continue for 48 weeks or longer, as for chronic hepatitis C.
- 2 In patients with compensated cirrhosis who fail to clear HCV RNA within 12 weeks on IFN, long-term therapy at 3 MIU should be considered for preventing HCC.
- 3 In patients with platelet counts $<50 \times 10^3/\text{mm}^3$, splenectomy or embolization of splenic artery is recommended before re-treatment, and after thorough evaluation has been made on the response to IFN to be expected.
- 4 For the prevention of HCC, not only IFN, but also liver supportive therapy (SNMC, UDCA, etc.), phlebotomy and branched chain amino acids, either alone or in combination, are recommended for improving ALT/AST and AFP levels.

AFP, α -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MIU, million international units; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

- 1 For treatment of type C cirrhosis with IFN, the initial dose of 6 million international units (MIU) daily is continued as long as possible (2–8 weeks). Thereafter, long-term IFN for 48 weeks or longer is desired as in the treatment of chronic hepatitis C.
- 2 In the treatment of type C cirrhosis, patients who fail to achieve EVR with the clearance of HCV RNA from serum within 12 weeks should receive long-term IFN at a dose of 3 MIU.
- 3 For patients with type C cirrhosis who have platelet counts of less than $50 \times 10^3/\text{mm}^3$, splenectomy or embolization of the splenic artery is desirable before commencing IFN therapy, after the efficacy of IFN has been evaluated thoroughly.¹²
- 4 For preventing the development of HCC, improvement in ALT, AST and AFP levels are aimed. Toward this end, not only IFN, but also liver supportive therapy (SNMC and UDCA), phlebotomy and BCAA are used, either alone or in combination.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HCV ranging from chronic hepatitis to cirrhosis of various severities for the fiscal

year 2008. The principal aim of these guidelines is to decrease the incidence of HCC due to HCV infection in Japan. In accord with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic hepatitis C. They are prepared on evidence-based data that have been accumulated by members and cooperators of the study group. It is necessary to improve these guidelines in the next fiscal year and thereafter, in accordance with many pieces of new evidence that are expected to emerge through enduring efforts of members and cooperators of the study group.

In the treatment of chronic hepatitis C, the duration of antiviral treatments is extended to 72 weeks, which has been approved as of the fiscal year 2008, and criteria for the eligibility of extended treatment duration are clearly defined. Long-term antiviral treatments, extended up to 72 weeks, are hoped to increase the SVR even further. In addition, comprehensive guidelines for the treatment of cirrhosis have been improved with substantial additions, and their criteria for the indication made explicit.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has drafted, and also displayed online (www.jsh.or.jp/medical/index.html [in Japanese]), guidelines for a spectrum of liver diseases due to HCV, from chronic hepatitis to cirrhosis of various severities. In view of the eventual goal of decreasing the incidence of HCC due to HCV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been constructed for the treatment of cirrhosis due to HCV infection. As a general rule, antiviral treatments constitute the main body of guidelines for the treatment of chronic hepatitis C. Furthermore, the fundamental concept of these guidelines would need to be kept in mind always. It is our sincere hope that, for the treatment of each patient, readers will base their clinical practice on these guidelines, and refer to appropriate individual guidelines, when they make a decision on the treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in treatment choice.

It is our sincere desire that treatment of patients with chronic hepatitis and cirrhosis due to HCV will proceed following these guidelines. Efforts along these lines will rectify a wide gap in medical treatment served to the nation and raise substantial and efficient interest in the medical economy on the national basis. In practicing treatment according to these guidelines, it will be nec-

essary to evaluate their therapeutic efficacy, and revise or add necessary supplements to them as required in the future.

REFERENCES

- 1 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; 131: 451–60.
- 2 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; 81: 452–8.
- 3 Iwasaki Y, Shiratori Y, Hige S. A randomized trial of 24 versus 48 weeks of interferon alpha-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatology Int* 2009; 3: 468–79.
- 4 Nomura H, Kashiwagi Y, Hirano R *et al.* Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: a pilot study. *Hepatology Res* 2007; 37: 490–7.
- 5 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494–500.
- 6 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multi-centre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; 56: 1747–53.
- 7 Sezaki H, Suzuki F, Kawamura Y *et al.* Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatology Res* 2007; 37: 787–92.
- 8 Akuta N, Suzuki F, Kawamura Y *et al.* Efficacy of low-dose intermittent interferon-alpha monotherapy in patients infected with hepatitis C virus genotype 1b who were predicted or failed to respond to pegylated interferon plus ribavirin combination therapy. *J Med Virol* 2008; 80: 1363–9.
- 9 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatology* 2005; 43: 599–605.
- 10 Okanoue T, Itoh Y, Minami M *et al.* Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts. *Hepatology Res* 2008; 38: 27–36.
- 11 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatology Res* 2006; 35: 204–14.
- 12 Morihara D, Kobayashi M, Ikeda K *et al.* Effectiveness of combination therapy of splenectomy and long-term interferon in patients with hepatitis C virus-related cirrhosis and thrombocytopenia. *Hepatology Res* 2009; 39: 439–47.

Comparison of Hepatitis B Virus DNA, RNA, and Core Related Antigen as
Predictors of Lamivudine Resistance in Patients with Chronic Hepatitis B

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Comparison of Hepatitis B Virus DNA, RNA, and Core Related Antigen as Predictors of Lamivudine Resistance in Patients with Chronic Hepatitis B

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The clinical usefulness of hepatitis B virus (HBV) DNA, RNA, and core related antigen (HBcrAg) assays for predicting the appearance of HBV DNA breakthrough was evaluated and compared in patients with chronic hepatitis B undergoing lamivudine therapy. Methods: Thirty six patients with chronic hepatitis B who received lamivudine therapy for more than 1 year were enrolled. HBV RNA was measured simultaneously with HBV DNA (HBV RNA/DNA) using a real-time detection polymerase chain reaction assay with a preceding step of reverse-transcription. HBV DNA was measured by an HBV AMPLICOR monitor kit. HBcrAg was measured using a chemiluminescence enzyme immunoassay. Results: Sixteen patients (44 %) developed HBV DNA breakthrough during the median observation period of 48.4 months (range 7.4–87.8 months). Afterwards, HBV DNA breakthrough was prospected using the three parameters taken 6 months after starting lamivudine therapy. The cut-off levels for predictions were determined by receiver operating characteristic curves, and were 2.6 log copies/ml for HBV DNA, 3.8 log U/ml for HBV RNA/DNA, and 4.0 log U/ml for HBcrAg. Sensitivity, specificity, and accuracy for predicting HBV DNA breakthrough were 25 %, 100 %, and 67 % respectively for HBV DNA. Similarly, they were 50 %, 90 %, and 72 % for HBV RNA/DNA, and 100 %, 40 %, and 67 % for HBcrAg. Conclusion: Our findings confirm that HBV DNA is useful for identifying patients who are at high risk for HBV breakthrough. HBcrAg is useful for isolating those who are at low risk, and HBV RNA/DNA showed predictive characteristics similar to HBV DNA with higher sensitivity and the highest accuracy. *Shinshu Med J 58 : 153–162, 2010*

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Key words: hepatitis B virus, HBV core related antigen, HBV RNA, lamivudine, breakthrough

I Introduction

Lamivudine (LAM), a nucleoside analogue, inhibits the replication of hepatitis B virus (HBV), reduces hepatitis, and improves histological findings of the liver during long-term therapy. Lamivudine treatment has also been reported to reduce the risk of complicating hepatocellular carcinoma¹⁾²⁾. However, relapse of hepatitis due to the appearance of

resistant viruses is a major drawback of lamivudine therapy^{3)–5)}. Recently, new nucleoside and nucleotide analogues have been developed, such as adefovir dipiboxil and entecavir, which develop resistant viruses far less frequently than

Abbreviations: HBV, hepatitis B virus; HBcrAg, hepatitis B virus core related antigen; HBV RNA/DNA, hepatitis B virus RNA and DNA; LAM, lamivudine; YMDD, tyrosine-methionine-aspartic acid-aspartic acid; HBsAg, hepatitis B virus surface antigen; HBeAg, hepatitis B virus e antigen; HBeAb, anti-hepatitis B antibody; RTD-PCR, real-time detection polymerase chain reaction assays; ROC, receiver operating characteristic; cccDNA, covalently closed circular DNA.

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lamivudine, but these analogues still face the problem of resistant viruses⁶⁾⁻⁸⁾.

The concentration of HBV DNA in serum decreases and usually becomes undetectable during lamivudine administration⁹⁾⁻¹²⁾, but can rapidly increase when tyrosine-methionine-aspartic acid-aspartic acid (YMDD) mutations induce lamivudine resistant strains¹³⁾⁻¹⁶⁾. Thus, the measurement of HBV DNA is useful for monitoring the anti-viral effects of lamivudine, but monitoring these effects by HBV DNA level alone is not satisfactory because lamivudine resistance occurs even in patients who show undetectable levels of serum HBV DNA¹³⁾¹⁷⁾¹⁸⁾.

We previously described a HBV core-related antigen (HBcrAg) assay¹⁹⁾ which measures the total amount of protein encoded by the pre-core and core regions of the HBV genome, including core, e, and p22cr²⁰⁾ antigens. In those experiments, the serum level of HBcrAg was shown to be useful for predicting the occurrence of lamivudine resistance in a manner different from serum levels of HBV DNA²¹⁾. We also reported that serum HBV RNA is detectable in a form incorporated into virus particles in patients with hepatitis B undergoing lamivudine therapy, which could possibly represent a new viral marker of different significance than that of HBV DNA in lamivudine therapy²²⁾.

Thus, in the present study, we sought to compare the clinical usefulness of using HBV DNA, HBV RNA, and HBcrAg to predict the occurrence of lamivudine resistance, as detected by HBV DNA breakthrough in patients with chronic hepatitis B receiving lamivudine therapy.

II Patients and Methods

A Patients

A total of 36 patients with chronic hepatitis B who started LAM therapy at Shinshu University Hospital between July 2002 and February 2004 were enrolled in this study. They consisted of 28 men and 8 women and had a median age of 55 years (range 29-80 years) at the commencement of LAM therapy. Chronic hepatitis B was defined as positive for HBV

surface antigen (HBsAg) for more than 6 months with liver histological findings consistent with chronic hepatitis. All patients had elevations in serum alanine aminotransferase (ALT) levels, as well as detectable HBV DNA for at least 6 months. Immediately prior to LAM administration, 23 of the patients were positive for HBV e antigen (HBeAg) and 13 were positive for anti-HBV e antibody (HBeAb) and negative for HBeAg. The HBV genotype was C in all patients except three (two were genotype B and one was F). Patients received 100 mg doses of LAM daily for at least 12 months. No patient underwent treatment with any other anti-viral agent, such as interferon, before or during the present study, and all patients were negative for hepatitis C and human immunodeficiency virus antibodies. Written informed consent was obtained from each patient. This study was approved by the Ethics Committee of Shinshu University.

Serum samples were collected from the start of LAM therapy on a monthly basis and were stored frozen at -20 °C or below until assayed. The occurrence of LAM resistance was defined as a rapid increase in serum HBV DNA with the appearance of the YMDD mutations. Using these criteria, resistance appeared in 16 (44 %) of the 36 patients. The median period from the start of LAM therapy to the occurrence of resistance was 18.2 months, with a range of 7.4 to 57.7 months.

B Routine laboratory tests

Serological markers for HBV, including HBsAg, HBeAg, and HBeAb, were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd., Tokyo, Japan). Six HBV genotypes (A-F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami et al²³⁾. The YMDD motif, a LAM-resistant mutation in the active site of HBV polymerase, was detected using an enzyme-linked mini-sequence assay kit (HBV YMDD Mutation Detection Kit, Genome Science Laboratories Co., Ltd., Tokyo, Japan)²⁴⁾. Serum concentration of HBV DNA was determined using an AMPLICOR HBV monitor kit (Roche, Tokyo, Japan), which had a

quantitative range of 2.6 to 7.6 log copies/ml.

C HBV core-related antigen assay

Serum HBcrAg was measured using a chemiluminescence enzyme immunoassay (CLEIA) as reported previously²⁶. In brief, 100 μ l aliquots of serum were mixed with pretreatment solution containing 15 % sodium dodecylsulfate. After incubation at 70 °C for 30 min, 50 μ l pretreated serum was added to wells coated with monoclonal antibodies against denatured HBV core and e antigens (HB44, HB61, and HB114) and filled with 100 μ l assay buffer. The mixture was then incubated for 2 hrs at room temperature. After washing with buffer, either alkaline phosphatase-labeled HB50 monoclonal antibody (specific for denatured HBV core antigen) or a mixture of HB91 and HB110 monoclonal antibodies (specific for denatured HBV core and e antigens) were added to wells and incubated for 1 hr at room temperature. After washing again, CDP-Star with Emerald II (Applied Biosystems, Bedford, MA) was added and plates were incubated for 20 min more at room temperature. The relative chemiluminescence intensity was measured, and HBcrAg concentrations were read by comparison to a standard curve generated with recombinant pro-hepatitis B e antigen (amino acids -10 to 183 of the precore/core gene product). The concentration of HBcrAg was expressed as units/ml and the immunoreactivity of recombinant pro-hepatitis B e antigen at 10 fg/ml was defined as 1 unit/ml. The lower detection limit of this assay was set at 2 log units/ml. Sera containing over 7 log units/ml of antigen were diluted 10 or 100 fold in normal human serum and measured again to obtain the end titer.

D HBV RNA/DNA

The High Pure Viral Nucleic Acid Kit (Roche Diagnostics) was used for isolation of HBV DNA and RNA from serum. Briefly, 200 μ l of serum was added to 250 μ l of freshly prepared working solution (6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl [pH 4.4] and 20 % [vol/vol] Triton X-100) supplemented with 20 μ g of poly (A) carrier RNA and 900 μ g of Proteinase K. After incubation for 10 min at 72 °C, 100 μ l of isopropanol was added and

the mixture was transferred into a High Pure filter tube fitted with a collection tube. The filter tube was centrifuged for 1 min at 8,000 rpm in a standard tabletop centrifuge at room temperature, then attached to a new collection tube. An inhibitor removal buffer (5 M guanidine-HCl, 20 mM Tris-HCl [pH 6.6] in ethanol) was added to the upper reservoir and the tube was centrifuged again for 1 min at 8,000 rpm. After being washed with 250 μ l of wash buffer (20 mM NaCl, 2 mM Tris-HCl [pH 7.5] in ethanol), the filter was placed in a new collection tube and 50 μ l of RNase- and DNase-free water was added to elute the DNA and RNA. After centrifugation for 1 min at 8,000 rpm, the eluted DNA and RNA was stored at -80 °C. Synthesis of cDNA was performed at 42 °C for 30 min in a 20 μ l reaction mixture containing 10 μ l of the extracted DNA and RNA, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 1 mM dNTP (1 mM each dATP, dGTP, dCTP and dTTP), 1 mM DTT, 100 nM reverse primer for the HBV surface gene (5'-GGTTGGTGAGTGATTGGAGGTT-3'; nt. 345 to 324), 40 units of RNasin (TaKaRa, Kyoto, Japan), and 200 units of SuperScript II RNase H- Reverse Transcriptase (Invitrogen, Carlsbad, CA). The reaction mixture was inactivated by heating to 70 °C for 15 min, then cooled to -80 °C until real-time detection polymerase chain reaction (RTD-PCR) assays. A 4 μ l aliquot of DNA and cDNA solution was used for RTD-PCR, which was performed with the Light Cycler System (Roche Diagnostics) as reported previously²⁶. The two primers and TaqMan probe used were designed from a region of the HBV surface gene: forward primer; 5'-ACAACATCAGGATTCCTAGGAC-3' (nt. 166 to 187), reverse primer as stated above (nt. 345 to 324), and TaqMan probe; 5'-FAM-CAGAGTCTAGACTCGTGGTG-GACTTC-TAMRA-3' (nt. 244 to 269). An HBV genome (nt. 20 to 1805) subcloned into a pUC vector was used as an internal standard. The lower detection limit for the HBV RNA/DNA assay was set at 2.6 log copy/ml.

E Statistical analysis

The proportion of each clinical factor was

compared between the groups with or without HBV DNA breakthrough using the χ^2 and Fisher's exact probability tests, and group medians were compared using the Mann-Whitney's U test. Correlations between HBV RNA/DNA and HBV DNA or HBcrAg were tested using Spearman's analysis. The rates of HBV DNA breakthrough during LAM treatment between higher and lower level groups of HBV DNA, HBcrAg and HBV direct RT-PCR were analyzed using the Kaplan-Meier method, and the difference in incidences was assessed with the log-rank test. Receiver operating characteristic (ROC) curves were used to decide each cut-off point for predicting HBV DNA breakthrough. All tests were performed using the SPSS 10.0 J statistical software package (SPSS Inc., Chicago, IL). P values less than 0.05 were considered significant.

III Results

A Comparison of characteristics between patients with and without HBV DNA breakthrough

HBV DNA breakthrough occurred in 16 (44.4 %) of 36 patients during the follow-up period. The cumulative HBV DNA breakthrough incidence in all patients was 8.3 % at 12 months, 27.8 % at 24 months, 33.5 % at 36 months, 40.2 % at 48 months, and 48.2 % at 60 months. The clinical characteristics at baseline in the 16 patients with HBV DNA breakthrough and 20 patients without are shown in Table 1. The median follow-up period did not differ,

and no significant differences were observed in any other characteristics, including ALT, HBV DNA, HBcrAg, and HBV RNA/DNA.

B Changes in serum HBV DNA, HBcrAg and HBV RNA/DNA

Changes in serum levels of HBV DNA, HBcrAg, and HBV RNA/DNA from baseline to 6 months of lamivudine therapy are shown in Fig. 1. HBV DNA decreased rapidly and became undetectable within 6 months in all except 4 patients with HBV DNA breakthrough. HBcrAg decreased more slowly than HBV DNA, and became undetectable only in 2 (6 %) of the 36 patients at 6 months. HBV RNA/DNA decreased faster than HBcrAg, but slower than HBV DNA, and became undetectable at 6 months in 15 (42 %) of the 36 patients.

Although HBV RNA/DNA was significantly correlated with both HBV DNA (Fig. 2A) and HBcrAg (Fig. 2B) at the start of lamivudine therapy, this association was lost at 6 months because over 90 % of patients became undetectable for HBV DNA (Fig. 2C). On the other hand, HBV RNA/DNA retained its correlation with HBcrAg at 6 months (Fig. 2D). Although significant, the HBcrAg to HBV RNA/DNA ratio tended to be lower when compared to baseline.

C Prediction of occurrence of lamivudine resistance

The occurrence of lamivudine resistance was next prospectively using the levels of HBV DNA, HBcrAg, and HBV RNA/DNA measured at 6

Table 1 Baseline characteristics of patients with and without HBV DNA breakthrough during lamivudine therapy

| | with breakthrough | without breakthrough | p |
|--|-------------------|----------------------|-------|
| Number | 16 | 20 | |
| Age (y.o.) ^a | 56.0 (29.5–64.0) | 53.6 (41.0–79.5) | 0.660 |
| Gender (male/female) | 13/3 | 15/5 | 1.000 |
| Follow-up period (months) ^a | 53.2 (21.5–84.0) | 67.5 (45.8–89.5) | 0.421 |
| HBeAg (+/–) | 10/6 | 9/11 | 0.508 |
| HBeAb (+/–) | 6/10 | 11/9 | 0.508 |
| ALT (IU/l) ^a | 60 (22–499) | 119 (20–1816) | 0.156 |
| HBV DNA (log copy/ml) ^a | 6.7 (4.8–>7.6) | 6.1 (3.9–>7.6) | 0.338 |
| HBcrAg (log U/ml) ^a | 5.9 (4.3–8.2) | 5.8 (2.9–8.7) | 0.683 |
| HBV RNA/DNA (log U/ml) ^a | 6.2 (4.8–8.3) | 6.3 (4.4–8.8) | 0.916 |

^a Data are expressed as median (range)

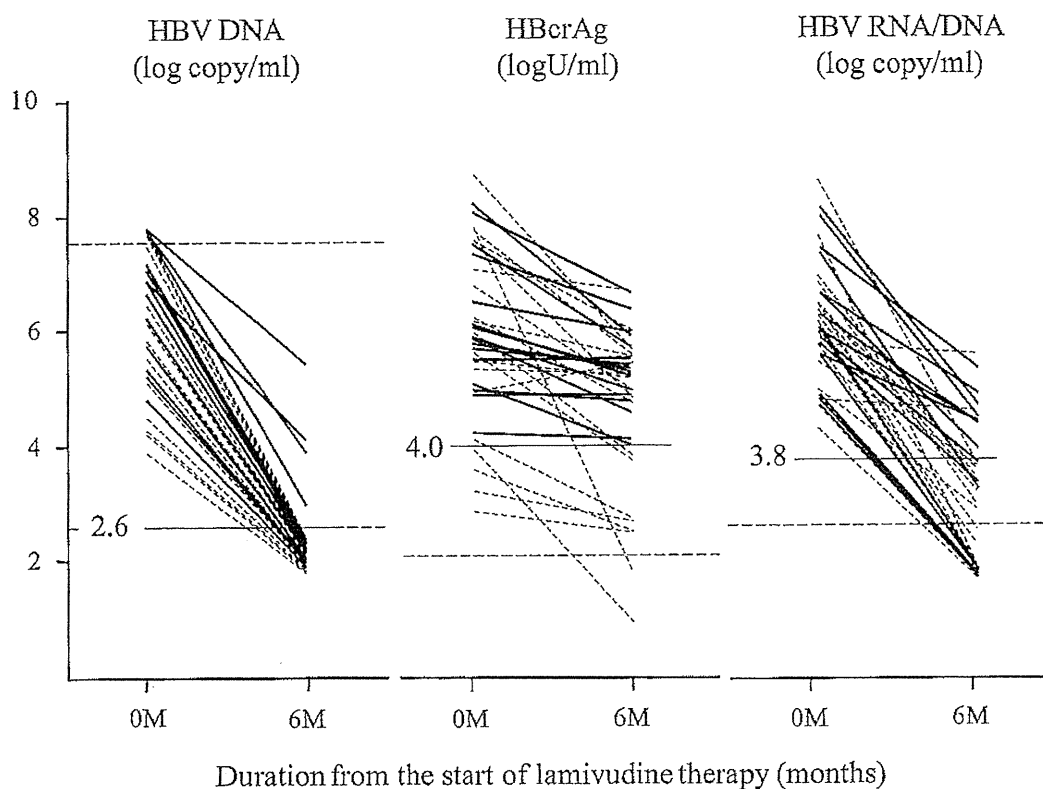


Fig. 1 Changes in serum levels of HBV DNA, HBcrAg, and HBV RNA/DNA from baseline to month 6. The solid line indicates patients with HBV DNA breakthrough during the observation period, and the broken line indicates patients without. The horizontal broken line indicates the lower detection limit of each assay. Cut-off values for predicting lamivudine resistance were 2.6 log copies/ml in HBV DNA (the same as the lower detection limit), 4.0 log U/ml in HBcrAg (solid line) and 3.8 log copies/ml in HBV RNA/DNA (solid line). The upper detection limit of HBV DNA is also shown by a horizontal broken line at 7.6 log copy/ml because 4 patients showed levels higher than the upper detection limit at the baseline.

months after starting lamivudine therapy. The cut-off values of HBV DNA (2.6 log copies/ml), HBcrAg (4.0 log U/ml), and HBV RNA/DNA (3.8 log copies/ml) for prediction of resistance was determined by ROC analysis. The sensitivity, specificity, and accuracy for predicting breakthrough were 25 %, 100 %, and 67 % respectively for HBV DNA. Similarly, they were 100 %, 40 %, and 67 % for HBcrAg, and 50 %, 90 %, and 72 % for HBV RNA/DNA. The positive predictive values for predicting HBV DNA breakthrough by HBV DNA, HBcrAg, and HBV RNA/DNA combined was 100 %, 57.1 %, and 80.0 % respectively. Similarly, the negative predictive values were 63.6 %, 100 %, and 62.5 %.

The cumulative occurrence of HBV DNA break-

through was compared using a log-rank test between two groups of patients divided by the cut-off values of HBV DNA ($P < 0.0003$), HBcrAg ($P = 0.0088$), and HBV RNA/DNA ($P = 0.0011$) (Fig. 3). All 4 patients whose HBV DNA levels were higher than the cut-off showed lamivudine resistance within 24 months of the start of therapy. None of the 9 patients whose HBcrAg levels were less than the cut-off showed lamivudine resistance during the follow-up period. HBV RNA/DNA showed an intermediate character between HBV DNA and HBcrAg in predicting the occurrence of HBV DNA breakthrough.

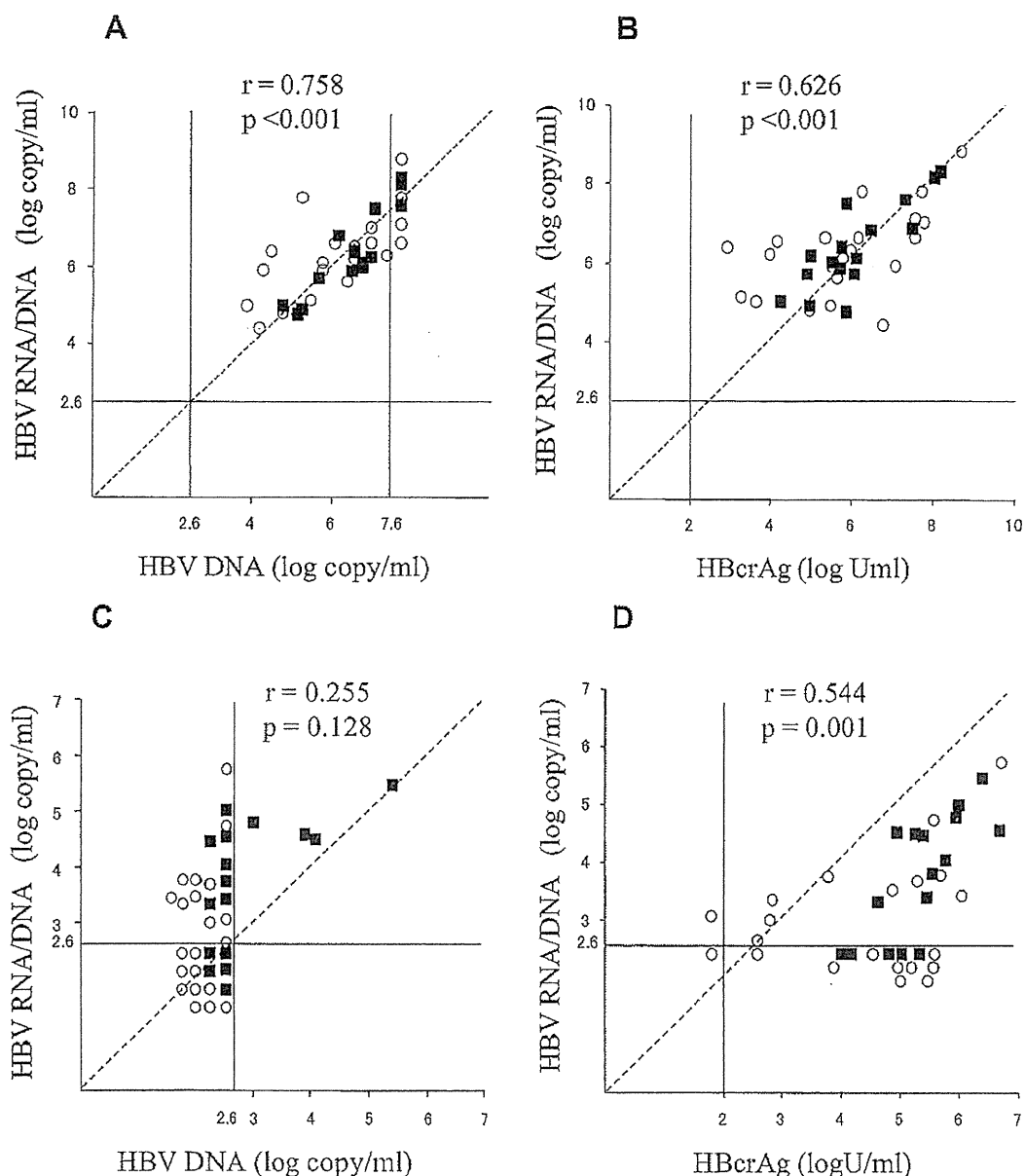


Fig. 2 Correlation between the levels of the three parameters at baseline and at 6 months after starting lamivudine administration: (A) between HBV DNA and HBV RNA/DNA at baseline, (B) between HBcrAg and HBV RNA/DNA at baseline, (C) between HBV DNA and HBV RNA/DNA at 6 months, and (D) between HBcrAg and HBV RNA/DNA at 6 months. Closed squares indicate patients with breakthrough during the observation period, and open circles indicate patients without.

IV Discussion

HBV is an enveloped DNA virus containing a relaxed circular DNA genome that is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells which serves as a transcriptional template for the production of viral RNA. Reverse transcription of pregenomic

RNA and second-strand DNA synthesis then occurs in the cytoplasm within viral capsids formed by the HBV core protein. Because lamivudine inhibits reverse transcription of pregenomic RNA, it directly suppresses production of HBV virions, and serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. On the other hand, the amount of cccDNA decreases quite slowly

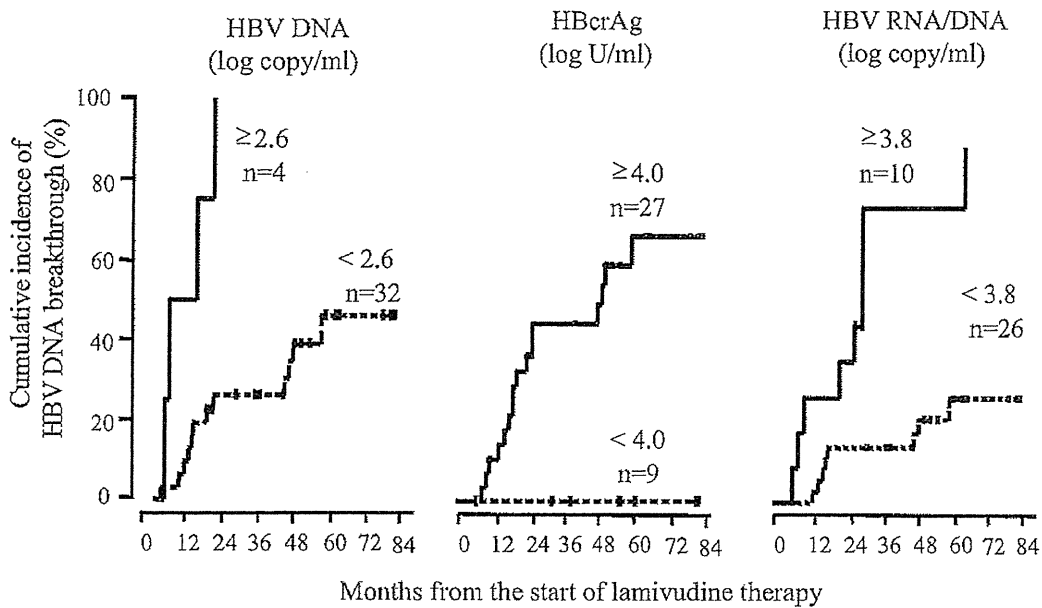


Fig. 3 Cumulative occurrence of HBV DNA breakthrough between two groups of patients classified by the selected cut-off values of HBV DNA (2.6 log copy/ml), HBcrAg (4.0 log U/ml), and HBV RNA/DNA (3.8 copy/ml).

after commencement of nucleoside analogues. Intrahepatic HBV cccDNA has been reported to be superior to serum HBV DNA in predicting virologic response to nucleoside/nucleotide analogue therapies, such as lamivudine²⁹⁻³⁰. However, the measurement of cccDNA seems ill-suited for clinical use because it requires a liver biopsy and complicated measurements. The HBcrAg assay developed by our research group has been shown to be useful for identifying patients who are at low risk of developing lamivudine resistance during therapy²¹, as well as those who are at low risk of hepatitis reactivation after cessation of lamivudine administration^{31,32}. The serum HBcrAg levels were well correlated with intrahepatic cccDNA level after HBV DNA became undetectable during anti-viral therapy³³. Here, serum HBcrAg may reflect the intrahepatic cccDNA better than serum HBV DNA under lamivudine therapy since lamivudine inhibits the synthesis of HBV DNA from pregenomic RNA transcribed from cccDNA, but does not inhibit synthesis of viral proteins which are translated from viral mRNA directly.

Maturation of the HBV genome occurs in nucleocapsids. Viral polymerase initiates encapsidation by

binding to the encapsidation signal, epsilon, and a secondary structure on the pregenomic RNA, which is then complexed with core proteins to form nucleocapsids. The polymerase-epsilon interaction is also the first step in initiating reverse transcription of pregenomic RNA to yield the negative DNA strand of the viral genome^{29,32}. Therefore, we can hypothesize that HBV particles containing pregenomic HBV RNA are produced rather than those containing HBV DNA during lamivudine therapy. Lamivudine inhibits reverse transcription of pregenomic RNA, suggesting that HBV particles containing HBV RNA are produced and may account for the majority of HBV particles²². Accordingly, it is also possible that serum HBV RNA reflects intrahepatic cccDNA and is useful for predicting the occurrence of lamivudine resistance. Recently, Hatakeyama et al. reported that serum HBV RNA was a predictor of early emergence of the YMDD mutant in patients treated with lamivudine³⁴. In a previous study, we demonstrated a method to measure serum HBV RNA only by eliminating HBV DNA. However, this method is prohibitively complicated for testing many samples because it includes a digestion step with DNAase.

As such, HBV DNA and RNA were measured simultaneously in the present study by eliminating the digestion step. Because the proportion of HBV DNA to HBV RNA at 6 months was quite low, we believe that the possibility of HBV RNA as a predictor for lamivudine resistance could be tested.

This study compared the abilities of HBV DNA, HBV RNA/DNA, and HBcrAg for predicting occurrence of lamivudine resistance. Lamivudine resistance was monitored by HBV DNA breakthrough because it is more sensitive than hepatitis breakthrough in detecting resistance. The specificity of HBV DNA breakthrough was confirmed by the existence of YMDD mutations. HBV DNA appears useful for detecting patients who are at high risk of developing lamivudine resistance, but not for selecting patients who are at low risk because the positive predictive value was as high as 100 % and sensitivity as low as 25 %. On the contrary, HBcrAg was useful for identifying patients who are at low risk of lamivudine resistance, but not for patients who are at high risk since the negative predictive value was as high as 100 % and specificity as low as 40 %. HBV DNA breakthrough did not occur until 60 months from the start of lamivudine therapy in 9 patients whose HBcrAg was less than 4.0 log U/ml at 6 months of therapy.

The ability of HBV RNA/DNA for predicting the occurrence of lamivudine resistance landed between those of HBV DNA and HBcrAg; both the positive (80 %) and negative (63 %) predictive values were intermediate. The accuracy (72 %) of HBV RNA/DNA was highest among the three parameters. Thus, HBV RNA/DNA is presumed to have the predictive characteristics of both HBV DNA and HBcrAg, with the additional feature of including a

wider range of patients.

Lamivudine has already been eliminated from first line therapy in naive chronic hepatitis B patients due to a higher incidence of developing resistant mutations than new antiviral agents, such as adefovir dipivoxil and entecavir. However, a considerable number of patients who began lamivudine administration in the past still take this treatment, so the present study may be valuable to such patients when they consider changing therapies in the future. For example, lamivudine patients who show low levels of HBV RNA/DNA or HBcrAg do not necessarily need to change their therapy to a new antiviral regimen. Additionally, since the main mechanisms of suppressing HBV replication are similar among lamivudine, entecavir, and adefovir dipivoxil, it is possible that HBV DNA, HBV RNA/DNA, and HBcrAg are useful for monitoring the antiviral effects of these drugs as well. However, further studies are required to determine whether these three assays are indeed applicable to antiviral agents other than lamivudine.

In conclusion, monitoring HBV DNA is useful for identifying patients with chronic hepatitis B under lamivudine therapy who are at high risk of lamivudine resistance, and measurement of HBcrAg is useful for isolating those who are at low risk of HBV DNA breakthrough. The predictive characteristics of HBV RNA/DNA are similar to that of HBV DNA with higher sensitivity, and show the highest accuracy.

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References

- 1) Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J: Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 351: 1521-1531, 2004
- 2) Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, Okita K, Hayashi N, Okanoue T, Iino S, Tanikawa K: Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2,795 patients. *Hepatology Research* 32: 173-184, 2005

- 3) Leung NW, Lai CL, Chang TT, Gran R, Lee CM, Ng KY, Lim SG, Wu PC, Dent JC, Edmundson S, Condreay LD, Chien RN ; Asia Hepatitis Lamivudine Study Group : Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates : results after 3 years of therapy. *Hepatology* 33 : 1527-1532, 2001
- 4) Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM : Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 30 : 567-572, 1999
- 5) Kim JW, Lee HS, Woo GH, Yoon JH, Jang JJ, Chi JG, Kim CY : Fatal sub massive hepatic necrosis associated with tyrosine-methionine-aspartate-aspartate- motif mutation of hepatitis B virus after long-term lamivudine therapy. *Clin Infect Dis* 33 : 403-405, 2001
- 6) Villet S, Pichoud C, Villeneuve JP, Trepo C, Zoulim F : Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient. *Gastroenterology* 131 : 1253-1261, 2006
- 7) Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS : Evolution of multi-drug resistant hepatitis B virus during sequential therapy. *Hepatology* 44 : 703-712, 2006
- 8) Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, Chung YH, Yoo W, Kim SO : Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 43 : 1385-1391, 2006
- 9) Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER : Histological outcome during long-term lamivudine therapy. *Gastroenterology* 124 : 105-117, 2003
- 10) Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M : A Preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 333 : 1657-1661, 1995
- 11) Doong SL, Tsai CH, Schinazi RF, Liotta DC, Cheng YC : Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues. *Proc Natl Acad Sci U S A* 88 : 8495-8499, 1991
- 12) Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF : A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 339 : 61-68, 1998
- 13) Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM : Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 30 : 567-572, 1999
- 14) Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, Merle P, Abidi H, Trepo C, Zoulim F : Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. *Hepatology* 32 : 1078-1088, 2000
- 15) Yuen MF, Kato T, Mizokami M, Chan AO, Yuen JC, Yuan HJ, Wong DK, Sum SM, Ng IO, Fan ST, Lai CL : Clinical outcome and virologic profiles of severe hepatitis B exacerbation due to YMDD mutations. *J Hepatol* 39 : 850-855, 2003
- 16) Kumashiro R, Kuwahara R, Ide T, Koga Y, Arinaga T, Hisamochi A, Ogata K, Tanaka K, Sata M : Subclones of drug-resistant hepatitis B virus mutants and the outcome of breakthrough hepatitis in patients treated with lamivudine. *Intervirology* 46 : 350-354, 2003
- 17) Suzuki F, Tsubota A, Arase Y, Suzuki Y, Akuta N, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kobayashi M, Matsuda M, Satoh J, Takagi K, Kumada H : Efficacy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. *Intervirology* 46 : 182-189, 2003
- 18) Zollner B, Schafer P, Feucht HH, Schroter M, Petersen J, Laufs R : Correlation of hepatitis B virus load with loss of e antigen and emerging drug-resistant variants during lamivudine therapy. *J Med Virol* 65 : 659-663, 2001
- 19) Rokuhara A, Tanaka E, Matsumoto A, Kimura T, Yamaura T, Orii K, Sun X, Yagi S, Maki N, Kiyosawa K : Clinical evaluation of a new enzyme immunoassay for hepatitis B virus core-related antigen ; a marker distinct from viral DNA for monitoring lamivudine treatment. *J Viral Hepat* 10 : 324-330, 2003
- 20) Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, Tanaka E, Kiyosawa K, Ohno S, Maki N : Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. *J Biol Chem* 280 : 21713-21719, 2005
- 21) Tanaka E, Matsumoto A, Suzuki F, Kobayashi M, Mizokami M, Tanaka Y, Okanoue T, Minami M, Chayama K,

- Imamura M, Yatsuhashi H, Nagaoka S, Yotsuyanagi H, Kawata S, Kimura T, Maki N, Iino S, Kiyosawa K: Measurement of hepatitis B virus core-related antigen is valuable for identifying patients who are at low risk of lamivudine resistance. *Liver Int* 26 : 90-96, 2006
- 22) Rokuhara A, Matsumoto A, Tanaka E, Umemura T, Yoshizawa K, Kimura T, Maki N, Kiyosawa K: Hepatitis B Virus RNA is Measurable in Serum and can be a New Marker for Monitoring Lamivudine Therapy. *J Gastroenterol* 41 : 785-790, 2006
- 23) Mizokami M, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, Robertson BH: Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 450 : 66-71, 1999
- 24) Kobayashi S, Shimada K, Suzuki H, Tanikawa K, Sata M: Development of a new method for detecting a mutation in the gene encoding hepatitis B virus reverse transcriptase active site (YMDD motif). *Hepatol Res* 17 : 31-42, 2000
- 25) Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, Maki N: Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 40 : 439-445, 2002
- 26) Lee WM: Hepatitis B virus infection. *N Engl J Med* 337 : 1733-1745, 1997
- 27) Mason WS, Halpern MS, England JM, Seal G, Egan J, Coates L, Aldrich C, Summers J: Experimental transmission of duck hepatitis B virus. *Virology* 131 : 375-384, 1983
- 28) Summers J, Mason WS: Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. *Cell* 29 : 403-415, 1982
- 29) Tuttleman JS, Pourcel C, Summers J: Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell* 47 : 451-460, 1986
- 30) Sung JJ, Wong ML, Bowden S, Liew CT, Hui AY, Wong VW, Leung NW, Locarnini S, Chan HL: Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. *Gastroenterology* 128 : 1890-1897, 2005
- 31) Shinkai N, Tanaka Y, Orito E, Ito K, Ohno T, Hirashima N, Hasegawa I, Sugauchi F, Ueda R, Mizokami M: Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 36 : 272-276, 2006
- 32) Matsumoto A, Tanaka E, Minami M, Okanoue T, Yatsuhashi H, Nagaoka S, Suzuki F, Kobayashi M, Chayama K, Imamura M, Yotsuyanagi H, Nagaoka S, Maki N, Kawata S, Kumada H, Iino S, Kiyosawa K: Low serum level of hepatitis B virus core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol Res* 37 : 661-666, 2007
- 33) Suzuki F, Miyakoshi H, Kobayashi M, Kumada H: Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. *J Med Virol* 81 : 27-33, 2009
- 34) Hatakeyama T, Noguchi C, Hiraga N, Mori N, Tsuge M, Imamura M, Takahashi S, Kawakami Y, Fujimoto Y, Ochi H, Abe H, Maekawa T, Kawakami H, Yatsuji H, Aisaka Y, Kohno H, Aimitsu S, Chayama K: Serum HBV RNA is a predictor of early emergence of the YMDD mutant in patients treated with lamivudine. *Hepatology* 45 : 1179-1186, 2007

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<特別寄稿>

日本肝臓学会コンセンサス神戸 2009 : B 型肝炎の診断と治療

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索引用語: B型肝炎 自然経過 遺伝子型 ウイルス変異 抗ウイルス剤

はじめに

B型肝炎はアジア・アフリカ諸国に特に多い疾患であるが、その病態はC型肝炎に比べて、やや複雑であり、治療においても迷うことの多い疾患である。従って、B型肝炎の病態・診断・治療に関するコンセンサスを得ることは肝疾患の臨床に携わる者にとって重要である。2009年の第45回日本肝臓学会総会(工藤正俊会長)のコンセンサス パネルディスカッションにおいて、B型肝炎の病態・診断・治療に関するコンセンサス パネルディスカッションが開催された。B型肝炎をテーマとしたコンセンサス パネルディスカッションはこれまで第5回、第7回、第10回の日本肝臓学会大会に於いて行なわれており、今回が4回目となるが、近年、B型肝炎の病態がこれまで以上に明らかにされ、また各種診断法や抗ウイルス剤の発展は目覚しく、この意味で、時機を得たものと考えられる。本稿は、日本肝臓学会における、B型肝炎のコンセンサス パネルディスカッションの発表内容を纏め、アンサーパッド方式で、会場の肝臓学会会員からコンセンサスの得られた事項について記載した。アンサーパッドの参加者は200人であり、アンサーパッドにて3分の2以上の賛同が得

られた事項を Consensus statement とし、うち臨床的に推奨すべきものを Recommendation とした。

コンセンサスの目安となる、エビデンスレベルなどに関しては、各種ガイドラインの標準的な報告システム¹⁾に基づいて記載した。

1. B型肝炎ウイルス(HBV)感染症の自然経過

自然経過の研究ではランダム化試験などの比較検討がないので、通常のエビデンスレベル分類をこれに当てはめるのは不適切である。2009年にHepatologyでB型肝炎が特集されており、ここに自然経過に適合するエビデンスレベル分類が提唱されていたので²⁾、本項目ではこれを用いた。この分類の要約は以下の如くである。Level 1は住民を対象とした経時的なコホート研究で、1aが非キャリアの対照があるもの、1bはこれが無いものに分けられる。Level 2aは医療機関でのコホート研究、2bはコホート研究の中でのケース・コントロール研究、2cはケース・コントロール研究である。Level 3は症例報告や観察研究となる。

キャリアの経過は、ALT値、HBe抗原、HBV DNA量、予測される免疫状態から、これを病期に分けることが可能である^{3)~7)}。Table 1にこれらの病期を示す。また、これらの病期は能動的に変化することが知られている。免疫寛容期では、HBV増殖は活発であるがALT値は正常で、組織学的にも正常か軽度の炎症に留まる。周産期に感染した場合、免疫寛容期は思春期~若年成人まで続く^{8)~10)}。

HBe抗原陽性の慢性肝炎では、HBV排除に働く宿主の免疫反応が起こり肝炎が惹起される。HBe抗原陽性の慢性肝炎が長期に続くと肝硬変へ進行するが、多くの患者ではHBe抗体へセロコンバージョンし非活動性キャリアとなる^{11)~14)}。比較的エビデンスレベルの高い

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