Table 5 Supplements to guidelines for the treatment of patients with chronic hepatitis B (part II)

- Self-injection of IFN at home is recommended to patients, who are eligible to do it, for improving their quality of life.
- Treatment with nucleos(t)ide analogs should be continued in patients in whom cirrhosis or HCC has been cured.
- Antiviral treatment is considered in patients with ALT levels of ≥31 IU/L. To patients aged 35 years or older in whom viral replication persists, even to those with normal ALT levels, antiviral treatments are indicated. It is possible, however, to follow for outcomes in patients who are elderly or HBeAg-negative and in whom antiviral treatments are difficult, while they receive liver supportive therapy (e.g. SNMC, UDCA).
- In patients co-infected with HBV and HIV, entecavir cannot be used due to the possibility for emergence of HIV variants resistant to antiretroviral therapies.
- Immunosuppressive and anticancer drugs should be used with utmost caution, even in patients with low HBV DNA titers and normal ALT levels, because they can induce severe liver damage along with elevation in HBV DNA titers.

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS B (PART II)

FURTHER, THE FOLLOWING five supplements have been added to the 2008 guidelines (Table 5).

To patients who are eligible, self-injection of IFN at home is recommended, taking into consideration their QOL. Because IFN-based therapies are not recommended for patients in whom HBV has been transmitted by perinatal infection, sequential treatment with IFN plus entecavir serves as another option in their antiviral treatment.

Treatment with nucleos(t)ide analogs should be extended to patients in whom cirrhosis or hepatocellular carcinoma (HCC) has been cured after successful therapies.

Antiviral treatment has to be considered in patients with ALT levels of 31 IU/L or more. Patients aged 35 years or older with normal ALT levels but in whom HBV replication persists, need to be considered for antiviral treatments. Elderly and HBeAg-negative patients, as well as those to whom the administration of antiviral drugs is difficult, can be followed regularly while they receive liver supportive therapy (e.g. stronger neo-minophagen C,[9] ursodeoxycholic acid [UDCA][10]).

Patients co-infected with HBV and HIV type 1 cannot receive entecavir due to the possibility of emergence of HIV mutants resistant to antiretroviral drugs.

Even in patients with low HBV DNA titers and normal ALT levels, HBV DNA loads can increase massively to induce severe liver damages in them, while they receive immunosuppressive or anticancer drugs. Hence, utmost caution should be exercised if they are to undergo antiviral treatments.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HBV

TABLE 6 SUMMARIZES guidelines for the treatment of patients with type B cirrhosis. Patients with compensated or decompensated cirrhosis, who are infected with HBV, receive entecavir for persistent clearance of HBV DNA detectable by the real-time polymerase chain reaction and normalization of aspartate aminotransferase as well as ALT levels. Combined lamivudine plus adefovir therapy are indicated for patients in whom HBV mutants resistant to lamivudine or entecavir have developed. Guidelines for maintaining liver function, for preventing the development of HCC, include liver supportive therapy with glycyrrhizin and UDCA, either alone or in combination. For treatment toward sup-

Table 6 Guidelines for treatment of type B cirrhosis

Principles
- Compensated: termination of HBV infection by antiviral treatment with entecavir as the mainstay.
- Decompensated: reversal to compensation and prevention of HCC.

Methods
(1) Eradication of HBV and normalization of ALT/AST (compensated and decompensated cirrhosis).
  a) Entecavir.
  b) Combined lamivudine and adefovir (for patients with HBV mutants resistant to lamivudine or entecavir).
(2) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC.
  a) Liver supportive therapy such as SNMC or UDCA.
  b) Branched chain amino acids (Livact).
(3) Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

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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-fee 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.

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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-naive 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 $50 \times 10^3/mm^3$ 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-naive 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-α2b 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-α2a 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-α2a 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 ×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	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 48–72 weeks • Peg-IFN-α2a (Pegasys) + ribavirin (Copegus) for 48–72 weeks	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 24 weeks
Low viral load <5.0 log IU/mL <300 fmol/L <1 Meq/mL	• Standard IFN for 24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks	• Standard IFN for 8–24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks

Peg-IFN, pegylated interferon.

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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 × 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 $150 \times 10^3/mm^3$ 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.

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

Platelets	$\geq 150 \times 10^3/mm^3$	$<150 \times 10^3/mm^3$
ALT		
≤30 IU/L	• 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.	• 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	• Consider antiviral treatments for patients younger than 65 years.	• 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 $150 \times 10^3/mm^3$, 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 $150 \times 10^3/mm^3$ or more, while guidelines for patients with chronic hepatitis are applied to those with platelet counts of less than $150 \times 10^3/mm$.[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 glycyrrhizin[5] 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
(1) Eradication of HCV and normalization of ALT/AST (for patients with compensated cirrhosis).
   a) HCV-1b in HVL (≥5 log IU/mL)
      IFN-α (Sumiferon)
   b) Others
      IFN-α (Sumiferon)
      IFN-β (Feron)
(2) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC.
   a) Liver supportive therapy
      Stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA), etc.
   b) Branched chain amino acids (BCAA [Livact])
   c) Phlebotomy
(3) 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.

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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 $<50 \times 10^3/mm^3$, 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 $50 \times 10^3/mm^3$, 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-

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

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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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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 carcinoma[1)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

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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.

lamivudine, but these analogues still face the problem of resistant viruses[6)-8)].

The concentration of HBV DNA in serum decreases and usually becomes undetectable during lamivudine administration[9)-12)], but can rapidly increase when tyrosine-methionine-aspartic acid-aspartic acid (YMDD) mutations induce lamivudine resistant strains[13)-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 DNA[13)17)18)].

We previously described a HBV core-related antigen (HBcrAg) assay[19)] which measures the total amount of protein encoded by the pre-core and core regions of the HBV genome, including core, e, and p22cr[20)] 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 DNA[21)]. 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 therapy[22)].

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 al[23)]. 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 previously[25]. In brief, 100 μl aliquots of serum were mixed with pretreatment solution containing 15 % sodium dodecylsulfate. After incubation at 70 °C for 30 min, 50 μl 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 μl 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 immunereactivity 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 μl of serum was added to 250 μL of freshly prepared working solu tion (6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl [pH 4.4] and 20 % [vol/vol] Triton X-100) supplemented with 20 μg of poly (A) carrier RNA and 900 μg of Proteinase K. After incubation for 10 min at 72 °C, 100 μl 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 μl 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 μl 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 μl reaction mixture containing 10 μL of the extracted DNA and RNA, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 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 μl aliquot of DNA and cDNA solution was used for RTD-PCR, which was performed with the Light Cycler System (Roche Diagnostics) as reported previously[25]. The two primers and TaqMan probe used were designed from a region of the HBV surface gene : forward primer ; 5'-ACAACATCAG-GATTCCTAGGAC-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 prospected 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)

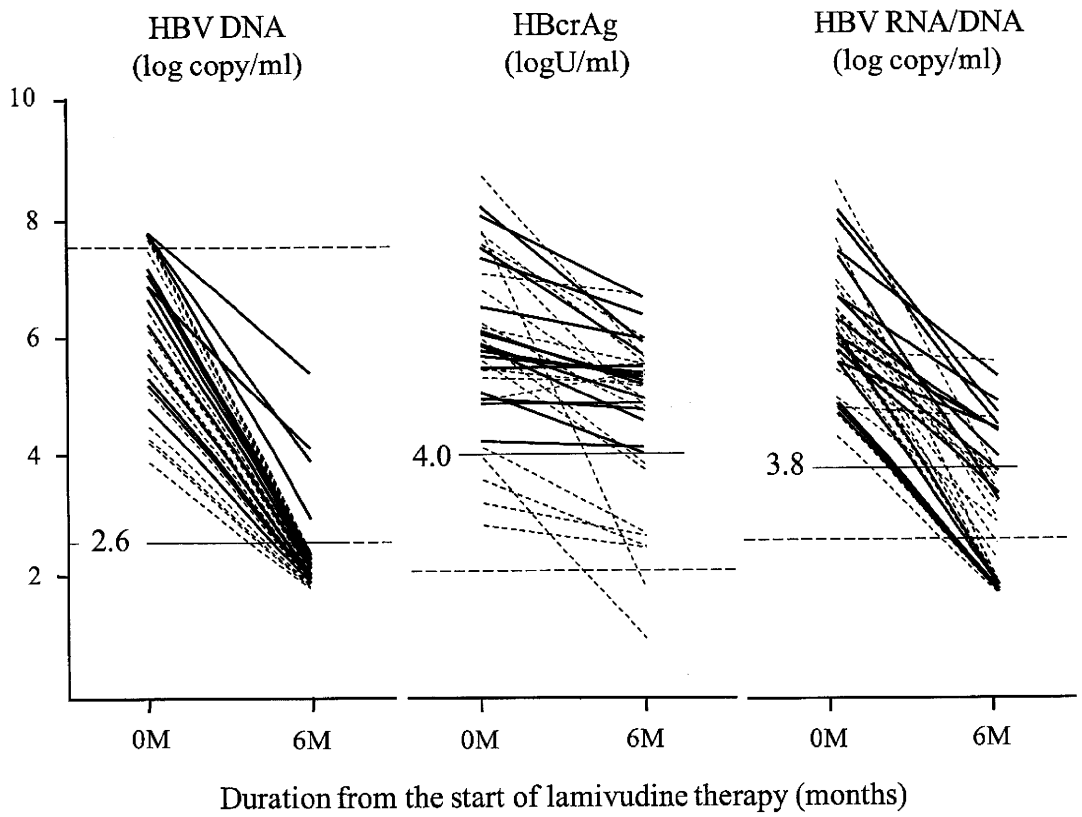

Fig. 1 Changes in serum levels of HBV DNA, HBcrAg, and HBV RNA/DNA from baseline to month 6. The solid line indicates patients with HBV DNA breakthrough during the observation period, and the broken line indicates patients without. The horizontal broken line indicates the lower detection limit of each 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 breakthrough 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.

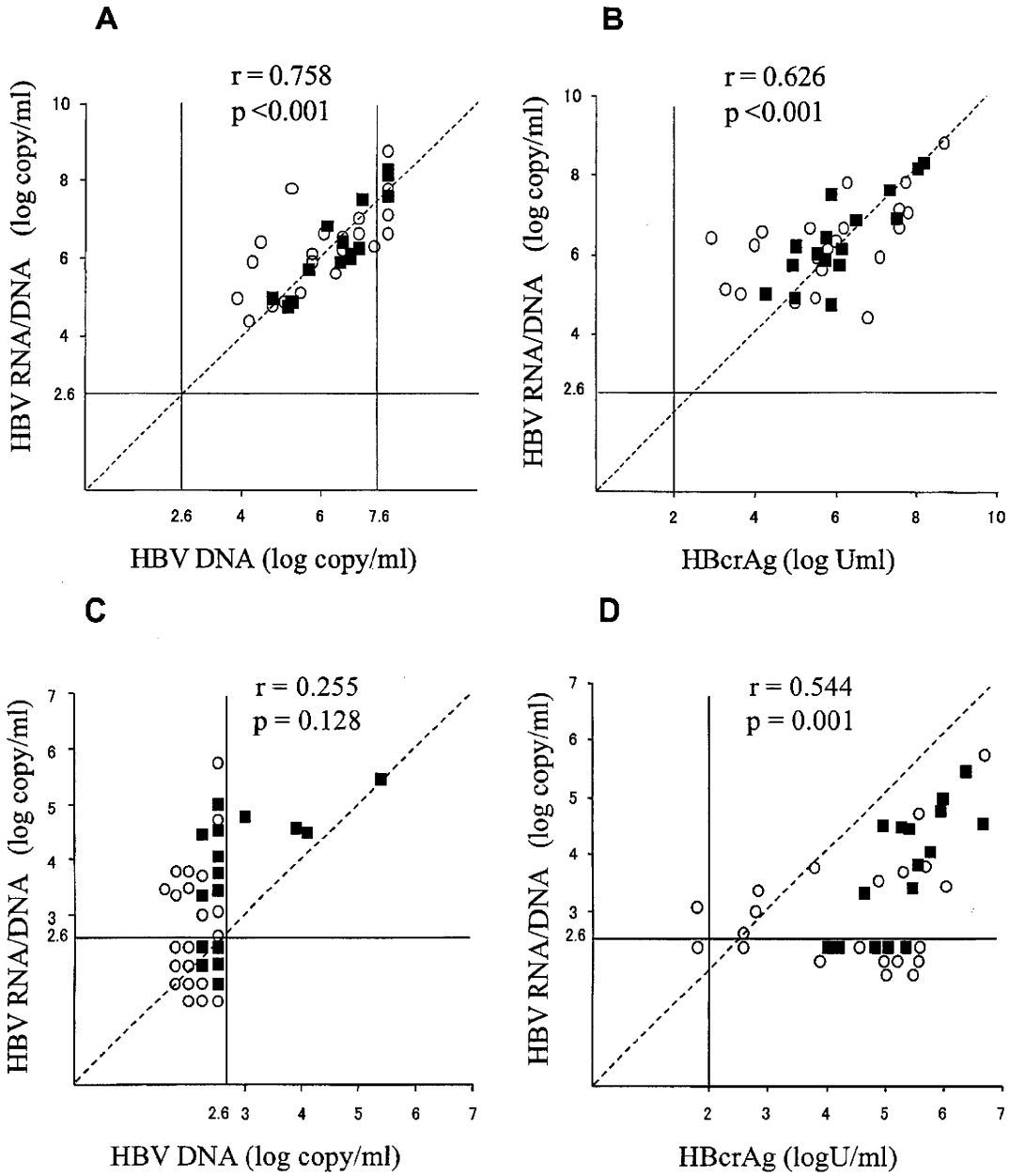

Fig. 2 Correlation between the levels of the three parameters at baseline and at 6 months after starting lamivudine administration: (A) between HBV DNA and HBV RNA/DNA at baseline, (B) between HBcrAg and HBV RNA/DNA at baseline, (C) between HBV DNA and HBV RNA/DNA at 6 months, and (D) between HBcrAg 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

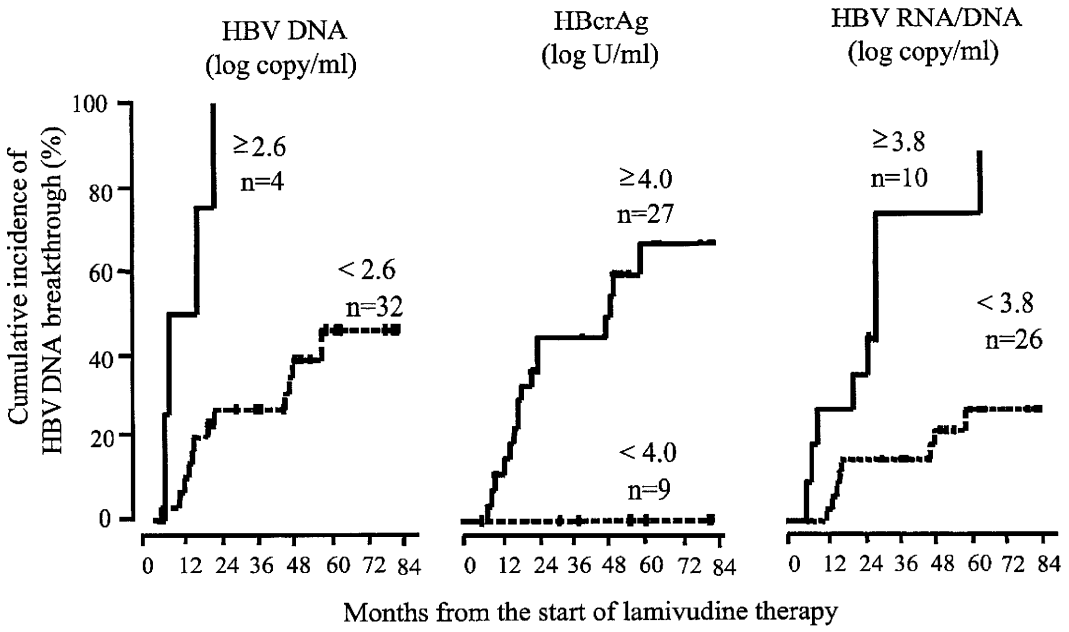

Fig. 3 Cumulative occurrence of HBV DNA breakthrough between two groups of patients classified by the selected cut-off values of HBV DNA (2.6 log copy/ml), HBcrAg (4.0 log U/ml), and HBV RNA/DNA (3.8 copy/ml).

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 lamivudine[26)-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 therapy[21)], 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 therapy[33)]. 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 particles[22)]. 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 lamivudine[34)]. 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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