ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

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Original Article

Long-term outcomes of add-on adefovir dipivoxil therapy to ongoing lamivudine in patients with lamivudine-resistant chronic hepatitis B

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Aim: Add-on adefovir dipivoxil (ADV) therapy has been a standard rescue treatment for patients with lamivudine (LAM)-resistant chronic hepatitis B, but the overall benefits of long-term add-on ADV therapy are still limited. The aim of this study was to evaluate the long-term efficiency of add-on ADV treatment and to explore predictive factors associated with it.

Methods: A total of 158 patients with LAM-resistant chronic hepatitis B were included in this retrospective, multicenter, nationwide study in Japan. After confirming LAM resistance, ADV was added to LAM treatment. Three types of events were considered as outcomes: virological response, hepatitis B e antigen (HBeAg) clearance and alanine aminotransferase (ALT) normalization. Virological response was defined as serum hepatitis B virus (HBV) DNA levels of less than 3 log copies/mL. Baseline factors contributing to these outcomes were examined by univariate and multivariate analyses.

Results: The median total duration of ADV treatment was 41 months (range, 6–84). The rate of virological response was 90.8% at 4 years of treatment; HBeAg clearance and ALT normalization were achieved by 34.0% and 82.7%, respectively, at the end of follow up. Each outcome had different predictive factors: baseline HBV DNA and albumin level were predictive factors for virological response, history of interferon therapy and ALT level for HBeAg clearance, and sex and baseline albumin level for ALT normalization.

Conclusion: Long-term add-on ADV treatment was highly effective in LAM-resistant chronic hepatitis B patients in terms of virological and biochemical responses. Lower HBV replication and lower albumin level at baseline led to better outcomes.

Key words: adefovir dipivoxil, alanine aminotransferase normalization, chronic hepatitis B, hepatitis B e antigen clearance, lamivudine resistance, virological response

INTRODUCTION

CHRONIC HEPATITIS B (CHB) is an important cause of morbidity and mortality worldwide.[1–3] The main goals of therapy in CHB patients are to prevent the development of liver failure, due to subsequent liver

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cirrhosis, and the emergence of hepatocellular carcinoma (HCC). All of these are likely be achieved by suppressing hepatitis B virus (HBV) replication, which thereby leads to remission of liver disease.[4]

Lamivudine (LAM) treatment has been used to prevent the progression of CHB and the development of HCC.[5] LAM is an effective and well-tolerated treatment for patients with CHB, but it has the major limitation of drug-resistant mutants arising at a rate of 16–32% during the first year of treatment and increasing by 15% with each additional year of treatment.[6–8] The widespread use of LAM monotherapy in CHB patients before introduction of entecavir, which is more potent, has progressively increased the numbers of patients with LAM-resistant HBV mutant strains.

Adefovir dipivoxil (ADV) has been reported to be effective in suppressing HBV replication and approved as a standard therapy in LAM-resistant patients.[9,10] However, data concerning the long-term efficacy of ADV treatment in LAM-resistant CHB patients are still limited. The aims of this study were to evaluate the long-term efficiency of ADV add-on treatment based on virological response (VR), hepatitis B e antigen (HBeAg) clearance and alanine aminotransferase (ALT) normalization, and to explore the predictive factors associated with ADV add-on treatment.

METHODS

Patients

A TOTAL OF 158 patients (109 males and 49 females) were included in this retrospective study from 21 medical centers of the National Hospital Organization (NHO) in Japan. Both HBeAg positive and negative CHB patients were considered eligible if they had documented LAM resistance confirmed by detection of mutations in the YMDD motif of the reverse transcriptase gene of the virus (genotypic resistance), elevated serum HBV DNA levels (≥4 log copies/mL and/or >1 log copies/mL elevation from the LAM on-treatment nadir) and/or elevated serum ALT levels (>40 IU/L). Patients were excluded if they had decompensated liver cirrhosis, HCC at the initiation of ADV, or if they had co-infections (human immunodeficiency virus, hepatitis C virus) or other concomitant liver diseases such as autoimmune liver disease. Patients with no available clinical, biochemical, serological or virological data at baseline as well as every 6 months during treatment were also excluded.

Patient records were extracted from each institutional database. All data were labeled with their respective institution and pooled. In total, 20 variables were examined to evaluate the long-term responses. The following variables were used as baseline factors: sex, HBeAg status, liver disease, age, body mass index, duration of LAM monotherapy, history of interferon (IFN) therapy, serum HBV DNA level, aspartate aminotransferase (AST), ALT, γ-glutamyl transpeptidase (γ-GTP), platelet (PLT) counts, and total bilirubin (T-Bil), albumin (Alb), prothrombin time (PT) and α-fetoprotein (AFP) levels. All were measured at the initiation of ADV therapy. For each variable, it was not used in the stepwise analysis if missing data accounted for more than 10% of the cases.

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all patients and approval of this study was obtained from the NHO.

Statistical analysis

Three types of events were considered as outcomes: (i) VR; (ii) HBeAg clearance; and (iii) ALT normalization. VR was defined as serum HBV DNA levels of less than 3 log copies/mL by a quantitative real-time polymerase chain reaction assay, and ALT normalization was defined as a decrease in ALT levels to less than 31 IU/L during the on-treatment follow-up period. Baseline factors that could have an impact in the prediction of VR, HBeAg clearance as well as ALT normalization were investigated. The predictive value of several baseline parameters for VR was evaluated using time-to-event methods, because of the varying length of follow up. Time-to-event analysis was carried out using Kaplan–Meier estimates to draw cumulative incidence curves, compared by log–rank tests, as well as using univariate and multivariate Cox's proportional hazards models in combination with stepwise regression analysis. Factors contributing to HBeAg clearance and ALT normalization during ADV add-on therapy were estimated using multivariate multiple logistic regression analysis in combination with stepwise regression analysis. A stepwise variable selection procedure was used for variables that were at least marginally associated with the outcomes.

Covariates included in these analyses were binomial or continuous variables. Quartile analysis was initially performed separately for each continuous variable to make the decision regarding cut-off points. At first, we divided each continuous data into quarters to convert numerical values into four categorical values. Then, we estimated whether there was a regular trend among these four ordinal categorical data with outcome and selected a cut-off point among the 25th, 50th and 75th percentiles so that these variables could be appropriately

Table 1 Baseline characteristics at the initiation of add-on ADV therapy in LAM-resistant CHB patients based on HBeAg status

Baseline characteristics	HBeAg positive $n = 99$	HBeAg negative $n = 59$
Age (years)	51.6 (25.5–80.4)	59.3 (33.3–76.9)
Sex (male/female)	73/26	36/23
Liver disease (CH/cirrhosis)	79/20	38/21
Duration of LAM therapy (months)	29.8 (6.0–82.4)	39.3 (8.4–91.2)
History of IFN therapy (months)	39	15
HBV DNA (log copies/mL)	7.5 (2.1–7.6)	5.9 (2.1–7.6)
≤6	15	31
6–7.5	38	21
>7.5	46	7
Total bilirubin (mg/dL)	0.8 (0.3–5.2)	0.9 (0.41–3.7)
AST (IU/L)	60 (18–959)	60 (17–464)
ALT (IU/L)	80 (11–697)	86 (17–724)
γ-GTP (IU/L)	38 (12–325)	53 (10–740)
Albumin (g/dL)	4.3 (2.6–5.4)	4.3 (2.7–5.2)
Platelet count ($\times 10^4/mm^3$)	15.5 (3.7–50.0)	12.3 (1.7–33.2)

Continuous variables are expressed in median (range) and categorized variables in number.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; γ-GTP, γ-glutamyl transpeptidase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LAM, lamivudine.

dichotomized. The hazards ratio (HR) and the odds ratio (OR) are presented with 95% confidence intervals (CI) and *P*-values, with less than 0.05 being considered statistically significant. All data analyses were processed using the R statistical software ver. 2.13.

RESULTS

IN THIS RETROSPECTIVE nationwide analysis of add-on ADV therapy in Japan, a total of 158 patients were enrolled from 2003–2010, consisting of 99 HBeAg positive and 59 HBeAg negative patients. Table 1 summarizes the baseline characteristics of the study population; most were HBV genotype C. At the time of this analysis, the median total duration of ADV treatment was 41 months (range, 6–84), and the median time of LAM monotherapy, prior to initiation of ADV, was 34 months (range, 6–91).

VR

Figure 1 shows a Kaplan–Meier curve displaying the cumulative probability of VR based on HBV DNA levels among HBeAg positive and negative patients. Patients with a lower HBV DNA level displayed earlier VR than those with a higher HBV DNA level among both HBeAg positive and negative patients ($P < 0.001$, $P = 0.002$,

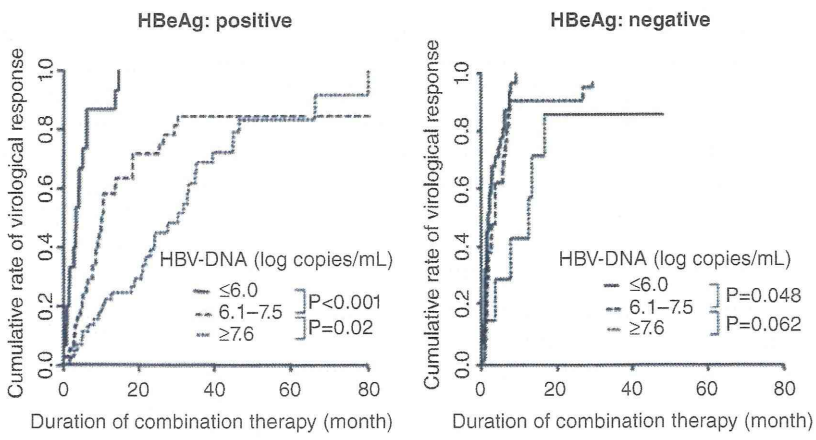

Figure 1 Cumulative rate of virological response on treatment with lamivudine plus adefovir dipivoxil depending on hepatitis B virus (HBV) DNA load in HBeAg positive and negative patients. hepatitis B e antigen (HBeAg) negativity and low HBV replication had a higher probability of virological response compared with HBeAg positivity or higher HBV replication. —, ≤6.0; ---, 6.1–7.5; ·····, ≥7.6.

Table 2 Univariate and multivariate Cox's regression analysis of predictors of virological response

Variable	HBeAg positive $n = 99$				HBeAg negative $n = 59$	
	Univariate		Multivariate		Univariate	
	HR	*P*-value	HR	*P*-value	HR	*P*-value
Age (years) (<45/45≤)	0.91	0.69			0.66	0.34
Sex (male/female)	1.07	0.86			0.71	0.21
Liver disease (CH/cirrhosis)	0.61	0.069			1	0.99
Duration of LAM therapy (months) (<34/34≤)	0.92	0.76			1.72	0.076
History of IFN therapy (-/+)	0.83	0.43			0.89	0.73
HBV DNA (log copies/mL) (<7.0/7.0≤)	0.28	<0.001	<0.001	<0.001	0.44	0.012
Total bilirubin (mg/dL) (<1.0/1.0≤)	1.66	0.067	1.73	0.06	1.54	0.13
AST (IU/L) (<100/100≤)	1.57	0.061			1.11	0.71
ALT (IU/L) (<130/130≤)	1.51	0.085			1.05	0.87
γ-GTP (IU/L) (<70/70≤)	1.53	0.113			1.33	0.3
Albumin (g/dL) (<4.1/4.1≤)	0.51	0.011	0.48	0.0065	1.41	0.32
Platelet count ($\times 10^4/mm^3$) (<15/15≤)	0.93	0.77			1.1	0.74

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HR, hazard ratio; IFN, interferon; γ-GTP, γ-glutamyl transpeptidase; LAM, lamivudine.

respectively; log-rank test). HBeAg negative patients displayed higher VR rates than HBeAg positive patients at month 12 (89.9% vs 45.5%), month 24 (95.0% vs 61.5%), month 36 (98.4% vs 79.6%) and month 48 (98.4% vs 86.4%) of treatment. Even at a higher HBV DNA level (HBV DNA ≥7.0 log copies/mL), HBeAg negative patients displayed more rapid VR than HBeAg positive patients ($P < 0.001$). Seven patients did not achieve VR during the 4-year treatment, and one HBeAg positive patient developed ADV-resistant mutations without VR at month 44 of treatment. According to the results of the univariate Cox regression model, HBV DNA level and Alb level were associated with VR in HBeAg positive patients, while only the HBV DNA level was in HBeAg negative patients (HR = 0.44, 95% CI = 0.24–0.84, $P = 0.012$). In multivariate analysis, both lower HBV DNA level and lower Alb level were independent predictive factors associated with VR in HBeAg positive patients (HR = 0.26, 0.48, 95% CI = 0.15–0.44, 0.28–0.81, $P < 0.001$, $P = 0.0065$, respectively) (Table 2), while only the HBV DNA level was selected by a stepwise analysis for HBeAg negative patients.

HBeAg clearance or HBeAg seroconversion

Among 99 HBeAg positive patients, HBeAg clearance and seroconversion were achieved by 17.1% and 11.0% at month 24, by 24.3% and 14.3% at month 36 of treatment, and by 34.0% and 16.0% by the end of follow up, respectively. Except for a history of IFN therapy (OR = 2.46, 95% CI = 0.94–6.6, $P = 0.047$), none of the other baseline variables were significantly associated with HBeAg clearance, according to the results of the univariate logistic regression analysis. In multivariate analysis, serum ALT level and history of IFN therapy were independent predictive factors for HBeAg clearance (Table 3). No patient experienced a reappearance of HBeAg or reverse seroconversion to HBeAg positive status during this treatment.

Normalization of ALT levels

The mean ALT level declined from 138.2 to 24.7 IU/L by add-on ADV therapy. Furthermore, addition of ADV to LAM-resistant CHB led to normalization of ALT levels in 75.2%, 79.5% and 82.7% of the patients at months 24 and 36, and at the final follow up, respectively. We next estimated the predictive factors for ALT normalization. Univariate logistic regression analysis revealed that only the baseline Alb level was significantly related to the ALT normalization. In the multivariate model, female patients (OR = 0.19, $P = 0.037$) and lower Alb level (OR = 0.19, $P = 0.0017$) were found to be independent predictors of ALT normalization.

DISCUSSION

ADD-ON ADV therapy has been a standard rescue treatment for patients with LAM-resistant HBV, but the overall benefits of long-term add-on ADV therapy

Table 3 Univariate and multivariate logistic regression analysis of predictors of HBeAg clearance and ALT normalization

Variable	HBeAg loss, $n = 99$				ALT normalization			
	Univariate		Multivariate		Univariate		Multivariate	
	Odds ratio	*P*-value	Odds ratio	*P*-value	Odds ratio	*P*-value	Odds ratio	*P*-value
Age (years) (<45/45≤)	0.42	0.065			0.94	0.85		
Sex (male/female)	3.02	0.075	2.99	0.081	0.4	0.34	0.19	0.037
Liver disease (CH/cirrhosis)	0.76	0.59			0.54	0.73		
Duration of LAM therapy (months) (<34/34≤)	1.1	0.97			0.59	0.39		
History of IFN therapy (−/+)	2.46	0.047	2.67	0.041	1.2	0.78		
HBV DNA (log copies/mL) (<7.0/7.0≤)	0.49	0.15			0.32	0.21		
Total bilirubin (mg/dL) (<1.0/1.0≤)	1.03	0.83			1.83	0.72		
AST (IU/L) (<100/100≤)	1.52	0.47			3.99	0.075		
ALT (IU/L) (<130/130≤)	2.44	0.061	2.74	0.043	3.71	0.13		
γ-GTP (IU/L) (<70/70≤)	2.16	0.17			1.29	0.98		
Albumin (g/dL) (<4.4/4.4≤)	0.9	0.99			0.17	0.0047	0.19	0.0017
Platelet count ($\times 10^4/mm^3$) (<15/15≤)	1.21	0.82			0.52	0.39		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HR, hazard ratio; IFN, interferon; γ-GTP, γ-glutamyl transpeptidase; LAM, lamivudine.

have not yet been fully assessed. In this multicenter study of 158 patients from 21 hospitals over a mean follow-up period of 43.5 months, we tried to evaluate the long-term efficacy of add-on ADV therapy to LAM-resistant patients, and also to investigate which baseline factors were associated with VR, HBeAg clearance and ALT normalization. We found long-term add-on ADV treatment produced long-term virological and biochemical improvement. In addition, each outcome had different predictive factors; baseline HBV DNA and Alb level were predictive factors for VR in HBeAg positive patients, history of IFN therapy and ALT level for HBeAg clearance, and sex and Alb level for ALT normalization.

The rate of VR was 90.8% at 4 years of treatment. The strongest predictive factor for VR in both HBeAg positive and negative patients were confirmed by previous observations showing that add-on ADV therapy achieves more rapid and higher rates of VR when ADV is initiated in LAM-resistant patients with low viral replication levels.[11–17] We also found that lower Alb level was an independent predictive factor for VR in HBeAg positive patients. In fact, baseline Alb correlated with PLT counts ($r = 0.51$, $P < 0.001$) and T-Bil ($r = -0.38$, $P < 0.001$), indicating that a lower Alb level reflected progression of liver disease. Little attention has been given to the relation of Alb level with VR – further studies will be needed to confirm our findings and understand its underlying mechanisms – but progression of chronic hepatitis might be predictive of VR under the add-on ADV treatment. This is the first report to show the significance of baseline Alb levels as we used a time-to-event method for large populations, which is a more powerful and informative method to assess the association of factors to time-to-event outcomes.

The rate of HBeAg clearance was 34% at the end of follow up, which was compatible with previous observations.[10,18] According to the results of multivariate analysis, IFN history was the strongest predictor of HBeAg clearance. Of the 37 patients, 17 (46%) who had previously received IFN therapy achieved HBeAg loss, suggesting that previous IFN therapy might have some immune modulatory effect on the ongoing combination therapy. IFN-induced HBeAg loss has been reported to be durable after a follow-up period of 4–8 years.[19–21] In addition, baseline ALT levels were also significantly associated with HBeAg clearance in this study. Our results agree with those of many clinical studies that have shown baseline ALT levels to be the strongest predictor of HBeAg seroconversion in response to IFN therapy[22] as well as nucleos(t)ide analog therapy.[23,24]

Alanine aminotransferase normalization was achieved in 82.7% of the patients. ALT normalization and VR were independent of each other. Actually, among 24 patients who did not achieve ALT normalization, only seven had not achieved VR, suggesting that ALT elevation after sustained suppression of HBV replication might be associated with some conditions other than CHB. In addition, lower baseline Alb was revealed

to be an independent and positive predictive factor for ALT normalization. Considering that patients who did achieve ALT normalization had lower Alb levels than patients with elevated ALT at the final follow up (4.4 vs 4.6 g/dL, $P < 0.01$), and Alb levels are significantly higher in non-alcoholic fatty liver disease,[25] we speculate that fatty liver disease is related to the abnormal ALT. To clarify this, further studies by liver biopsy and/or ultrasonography will be needed.

In conclusion, long-term ADV treatment was highly effective in LAM-resistant CHB patients in terms of virological and biochemical response. In addition, the emergence of resistance to the add-on ADV therapy appears to be delayed and infrequent, in contrast to LAM. Furthermore, lower HBV DNA level and lower Alb level were significant predictive factors for better outcomes. Even though add-on ADV therapy in LAM-resistant CHB patients was highly effective in the long term, CHB patients with LAM or entecavir monotherapy need to be carefully followed-up and the optimal timing of ADV intervention should be determined on the basis of HBV DNA level and progression of liver disease.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article:

Appendix S1 Relationship of liver cirrhosis with virological response on the basis of fibrosis, using 60 out of 158 patients liver biopsy had been performed. Fibrosis was related with platelet counts but neither with albumin levels nor with the virological response.

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APPENDIX I

THE LIVER DISEASE Network Group of the National Hospital Organization consists of the following physicians and their institutions: Hiromi Ishibashi, Hiroshi Yatsuhashi, Department of Clinical Research Center, Nagasaki Medical Center; Makoto Nakamuta, Department of Gastroenterology, Kyushu Medical Center; Michiyasu Yagura, Department of Gastroenterology, Tokyo National Hospital; Hirotsugu Takano, Department of Gastroenterology, Kure Medical Center; Takeaki Satoh, Center for Liver Disease, Kokura Medical Center; Toyokichi Muro, Department of Gastroenterology, Oita Medical Center; Hideo Nishimura, Department of Gastroenterology, Asahikawa Medical Center; Hiroshi Mano, Department of Gastroenterology, Sendai Medical Center; Fujio Makita, Department of Gastroenterology, Nishigunma National Hospital; Akira Saitou, Department of Gastroenterology, Nishisaitama-chuo National Hospital; Masahiko Takahashi, Department of Gastroenterology, Tokyo Medical Center; Shigeo Hayashi, Department of Gastroenterology, National Disaster Medical Center; Tatsuji Komatsu, Department of Gastroenterology, Yokohama Medical Center; Yukio Watanabe, Department of Gastroenterology, Sagamihara National Hospital; Masakazu Kobayashi, Department of Gastroenterology, Matsumoto Medical Center; Hideo Morimoto, Hajime Ohta, Department of Gastroenterology, Kanazawa Medical Center; Masaaki Shimada, Department of Gastroenterology, Nagoya Medical Center. Toshiki Komeda, Department of Gastroenterology, Kyoto Medical Center; Taizo Hijioka, Department of Gastroenterology, Osaka Minami Medical Center; Haruhiro Yamashita, Department of Gastroenterology, Okayama Medical Center; Eiichi Takesaki, Department of Gastroenterology, Higashi Hirosima Medical Center; Toru Hayashi, Department of Gastroenterology, Zentsuji National Hospitail; Mitsuaki Koga, Department of Gastroenterology, Ureshino Medical Center; Kazuhiro Sugi, Departoment of Gastroenterology, Kumamoto Medical Center; Hironori Sakai, Department of Gastroenterology, Beppu Medical Center; Tetsuo Yamamoto, Department of Gastroenterology, Yonago Medical Center; Yukio Oohara, Department of Gastroenterology, Hokkaidou Medical Center; Michio Kato, Department of Hepatology, Minamiwakayama Medical Center; Naohiko Masaki, Department of Gastroenterology, Kohnodai Hospital.

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Original Article

Acute hepatitis B in Japan: Incidence, clinical practices and health policy

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Aim: The epidemiology of acute hepatitis B is unknown in many countries, and the clinical features of this disease remain unclear. In this study, we used the Diagnosis Procedure Combination (DPC) database to estimate the incidence of acute hepatitis B and investigate the clinical practices for acute hepatitis B in Japan.

Methods: The DPC database is a nationwide discharge abstract and administrative claims database, covering 40% of all inpatient admissions to acute care hospitals between 1 July and 31 December each year in Japan. We identified cases with a diagnosis of acute hepatitis B between 2007 and 2008. Patient characteristics, length of stay, in-hospital mortality and total charges were determined. Clinical practice patterns were examined, including drugs used and procedures performed during hospitalization.

Results: We identified 890 cases with acute hepatitis B among 5.85 million inpatients in the database. The mean age was 40.0 years old and 76% were male. The incidence of acute hepatitis B was estimated to be approximately 2100–2400/year (17–19/1 million people per year). Of 890 cases, 53 (6.0%) developed fulminant hepatitis and 36 (4.0%) died. Nucleos(t)ide analogs were prescribed for 226 cases (25.4%). Only 194 cases (21.8%) were tested for HIV status.

Conclusion: It is essential to monitor the trends of this communicable and preventable disease. The establishment and distribution of appropriate clinical evidence and guidelines are vital to improve the clinical practices for acute hepatitis B.

Key words: acute hepatitis B, Diagnosis Procedure Combination, fulminant hepatitis, hepatitis B virus, public health surveillance

INTRODUCTION

WORLDWIDE, MORE THAN 2 billion people are thought to have a history of hepatitis B virus (HBV) infection and 360 million people have chronic HBV infection.[1] Liver cirrhosis and liver cancer are associated with HBV infection and, although they progress slowly, they are life-threatening diseases responsible for an estimated 620 000 deaths worldwide annually.[2] Prevention of infection is essential to control the epidemic of these silent diseases. Universal hepatitis B vaccination for all infants is widely accepted as a fundamental strategy to protect the general public from these potentially severe diseases. In fact, the World Health Organization now recommends universal vaccination and, as of 2009, 177 countries have introduced hepatitis B vaccines into their national infant immunization schedules.[3] However, in Japan, vaccination against HBV is only provided for infants born to mothers with HBV since 1986.

Vaccination alone cannot completely control these diseases. For successful disease control, a patient-monitoring system should be established to identify, treat and track patients with new infection. However, few countries have established surveillance systems for acute hepatitis B (AHB). Even in countries with a system to report infectious diseases, there are few reliable data

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for the true incidence of AHB because many cases go unreported because reporting is not always mandatory. In the present study, we utilized a nationwide administrative claims database in Japan, the Diagnosis Procedure Combination (DPC) inpatient database, to estimate the incidence of AHB.

Although there is some evidence for effective treatments for AHB, a standardized clinical management strategy has not yet been established. Therefore, another aim of this study was to determine the actual clinical practices for AHB in real-world clinical settings in Japan using the DPC database, and to evaluate their validity in terms of consistency with the current scientific evidence. Furthermore, we discuss the health policy implications of the results in terms of essential policy innovations for national HBV control.

METHODS

Data source

THE DPC IS a Japanese casemix classification system that is similar to the Diagnosis-related Groups in the US Medicare system. This patient classification system was launched in 2002 by the Ministry of Health, Labor and Welfare of Japan, and linked with a lump-sum payment system. All the teaching hospitals are obliged to adopt the DPC system, but community hospitals can voluntarily adopt it. A survey of DPC hospitals is conducted between 1 July and 31 December every year by the DPC Research Group funded by the Ministry of Health, Labor and Welfare of Japan. Not only administrative claims data but also detailed patient data are collected for all inpatients discharged from participating hospitals between 1 July and 31 December. Data are mainly used for profiling of practice patterns, refinement of casemix classifications and health policy planning such as resource allocation. The survey began in 2003 with 82 teaching hospitals and the number of participating hospitals has increased, with 953 in 2007 and 844 in 2008. The numbers of inpatients were 2.99 million in 2007 and 2.86 million in 2008. The number of inpatients in 2008 (2.86 million) represented approximately 40% of all inpatient admissions to acute care hospitals in Japan.[4,5]

The DPC database includes the following data: patients' age and sex; main diagnoses, comorbidities at admission and complications after admission recorded with the International Classification of Diseases, 10th Revision (ICD-10) codes and text data in the Japanese language; procedures coded with the Japanese original codes; duration of anesthesia; drugs and devices used; volume of blood transfusion; length of stay (LOS); in-hospital mortality; and hospital charges. The physicians in charge are required to submit data on diagnoses with reference to medical charts. Hospital staff should record the dates when all procedures and examinations are performed and when drugs and devices are used. The physicians and hospital staff show strong compliance to data entry because they are required to submit accurate data to receive reimbursement.

This study was based on a secondary analysis of the administrative claims data. Given the anonymous nature of the data, the requirement for informed consent was waived. Study approval was obtained from the institutional review board of the University of Occupational and Environmental Health.

Case definition

First, cases with the ICD-10 code B16 (acute hepatitis B) were identified from among the 5.85 million inpatients included in the DPC database between 2007 and 2008. This process was performed using a computer-assisted text searching method running on Microsoft SQL Server 2008 software. Second, we manually checked all of the screened cases to confirm the main diagnoses of "acute hepatitis B", "fulminant hepatitis (FH) B", and combination of "hepatitis B" and "acute hepatitis or fulminant hepatitis", which were originally written in the Japanese language. Cases with "suspected acute hepatitis B" were excluded. Cases with diagnoses of chronic hepatitis, HBV carrier, liver cirrhosis, gastric and esophageal varices, or liver cancer were excluded because such cases were considered to be chronic HBV infection. Cases with diagnoses of other liver diseases were excluded. Cases with malignancy were excluded because they might be cases of reactivated HBV caused by chemotherapy. Furthermore, infants aged less than 1 year and pregnant women were also excluded because such cases were considered to be those undergoing preventive management for vertical HBV infection. Duplicate cases referred from one hospital to another were identified based on the demographic data and merged into single cases. To ensure the reliability of the search results, two authors (A. S. and H. Y.) independently performed these procedures.

Estimation of incidence of AHB

We assumed that there was no seasonality and all of the cases with AHB were almost always hospitalized. We estimated the incidence of AHB based on the number of beds in all acute care hospitals in Japan,[6] and the numbers of beds and AHB patients in the DPC hospi-

tals. Hospitals were stratified with bed volume categories. The estimated number of AHB cases each year (*Yi*) and the 95% confidence intervals (CI) were calculated with the following equation using Wald confidence intervals for the population proportion:

$$\frac{Yi}{Ni} = p \pm Z\sqrt{p(1-p)/ni}$$

where *Ni* is the number of beds in all acute care hospitals in Japan, *ni* is the number of beds in the DPC hospitals, $P = 2Xi/ni$ (*Xi* is the observed number of AHB cases in the DPC hospitals between July and December each year), and $Z = 1.96$.

FH incidence

We identified cases of FH among all of the AHB cases. To optimize the validity of the FH diagnosis, we defined true FH cases as those who underwent plasma exchange with the diagnosis of FH. This definition can be justified to eliminate over-reporting of FH and shows good validity for FH diagnoses. This definition can also minimize the possibility of underreporting because more than 90% of FH patients in Japan undergo plasma exchange.[7]

The sex distribution, average age, LOS and total charges based on fee-for-service payment system, and rates of in-hospital mortality were compared between AHB cases with and without FH.

Clinical practice

We investigated the patterns of treatments and examinations performed for all AHB cases, including blood test for HIV-1, administration of three nucleos(t)ide analogs (NA) (lamivudine, adefovir and entecavir), interferon, protease inhibitors (gabexate mesilate and nafamostat mesilate), antithrombin concentrate, injection of corticosteroids, prostaglandin E_1, glucagon, monoammonium glycyrrhizinate, and use of liver transplantation, plasma exchange, hemodialysis, continuous hemodiafiltration and blood transfusion.

Analyses

We used univariate analyses to compare patient characteristics and outcomes between AHB cases with and without FH, using Student's *t*-tests or χ^2-tests as appropriate. All statistical analyses were performed using PASW statistics ver. 18.0 software. $P < 0.05$ was considered to be significant. The exchange rate was assumed to be ¥ 100 for $US 1.

RESULTS

Estimated incidence of AHB

A TOTAL OF 890 AHB cases were identified between 1 July and 31 December in 2007 and 2008. The two authors who independently performed the search obtained the same results.

Table 1 shows the number of all acute care beds in Japan (*Ni*), the number of beds in the DPC hospitals (*ni*), and the observed number of AHB cases in the DPC hospitals between July and December each year (*Xi*). The estimated number of AHB cases in Japan was 2176 in 2007 and 2391 in 2008. Because the population of Japan was approximately 127 million, the annual incidence of AHB was estimated to be 17–19 cases/1 million people per year.

FH incidence

Fifty-three cases (6.0%) developed FH. The average age, LOS and total charge, and rate of in-hospital mortality were significantly higher among cases with FH than among cases without FH (Table 2).

Clinical practices

Table 3 summarizes the procedures used for all of the AHB cases. Most of the FH cases were prescribed with lamivudine and/or entecavir. More than half of the FH cases were provided with continuous hemodiafiltration, protease inhibitors, corticosteroids injection and fresh frozen plasma. Gabexate mesilate was administrated in 31 (58.5%) cases with FH, and nafamostat mesilate in 45 (84.9%); these drugs were rarely used in AHB cases without FH.

HIV-1 status

Thirteen cases were diagnosed with HIV-1 infection. HIV-1 status was determined in only 194 (21.8%) cases. Among 226 cases treated with lamivudine, adefovir or entecavir, only 64 cases (28.3%) were checked for HIV-1 status.

DISCUSSION

Incidence of AHB

ACCURATE DATA ON the incidence of AHB is lacking in most countries. To our knowledge, this study was the first to estimate the national incidence of AHB using a nationwide inpatient database. Although there is a reporting system for several infectious diseases in Japan, the National Epidemiological Surveillance for

Table 1 Estimated incidence of acute hepatitis B (AHB) in Japan

Bed volume	No. of acute care hospitals in Japan	No. of DPC hospitals (%)		No. of acute care beds in Japan (*Ni*)	No. of acute care beds in DPC hospitals (*ni*)		No. of AHB patients in DPC hospitals in July to December (*Xi*)		Estimated no. of all AHB patients in Japan (95% confidence interval) (*Yi*)	
		2007	2008		2007	2008	2007	2008	2007	2008
≥900	64	36 (56%)	36 (56%)	55 286	38 420 (69%)	38 420 (69%)	57	71	164 (134–194)	204 (171–238)
800–899	31	14 (45%)	14 (45%)	23 709	11 825 (50%)	11 825 (50%)	16	10	64 (42–86)	40 (23–58)
700–799	53	27 (51%)	26 (49%)	31 760	20 125 (63%)	19 403 (61%)	28	35	88 (65–111)	115 (88–141)
600–699	108	53 (49%)	47 (44%)	57 110	34 226 (60%)	30 337 (53%)	39	39	130 (101–159)	147 (114–179)
500–599	170	93 (55%)	87 (51%)	74 701	50 682 (68%)	47 258 (63%)	70	63	206 (172–240)	199 (164–234)
400–499	296	108 (36%)	97 (33%)	101 014	47 368 (47%)	42 369 (42%)	60	69	256 (210–302)	329 (274–384)
300–399	589	186 (32%)	158 (27%)	143 045	62 319 (44%)	53 116 (37%)	94	70	432 (370–493)	377 (315–439)
200–299	803	180 (22%)	159 (20%)	114 526	43 659 (38%)	38 495 (34%)	52	58	273 (220–325)	345 (282–408)
≤199	5609	256 (4.6%)	220 (3.9%)	309 087	33 001 (11%)	28 242 (9.1%)	30	29	562 (420–704)	635 (472–798)
Total	7723	953 (12%)	844 (11%)	910 238	341 625 (38%)	309 465 (34%)	446	444	2175 (2026–2324)	2391 (2227–2555)

DPC, Diagnosis Procedure Combination, $Y = \Sigma Yi = \Sigma 2Xi/ni \times Ni$.

Infectious Disease (NESID), the occurrence of AHB has remained underreported, as an NESID report stated the incidence of AHB in Japan was 510 in 1999 and 199 in 2007.[8,9] These figures are misleading because they imply that selective HBV vaccination in Japan has reduced the incidence of AHB. By contrast, a multicenter study revealed a continuous increase in the number of the AHB cases between 1991 and 2008, with a dramatic increase in number of cases with the HBV genotype A, which was formerly rare in Japan.[10] Similarly, our study has revealed underreporting of AHB in the NESID database. A similar situation may occur in other countries because non-mandatory reporting systems will inevitably result in underestimation of the occurrence of diseases, and thus impair health policy evaluation and decision making.

The DPC database has an advantage in that it includes a large proportion of inpatients in Japan. One limitation is that the sample collection in the DPC survey is not based on a random sampling method, and that the patient distribution tends to be biased toward those in larger hospitals. Stratification of patients according to hospital size (characterized by bed volume) is justifiable to adjust for such maldistribution, but the 95% CI for the estimated incidence of AHB in low bed volume hospitals had a wide range. Another limitation is the possibility of inaccurate reporting of diagnoses. Although we excluded cases with diagnoses of chronic HBV infection, some patients with a chronic status but who had an acute exacerbation might have been registered as AHB, resulting in an overestimation of the AHB incidence. Furthermore, detailed clinical data, including past history, symptoms and signs, and laboratory data are not recorded in this database. Despite these limitations, our study has demonstrated the usability of the DPC database for continuous monitoring of the incidence of diseases that require hospitalization.

Incidence of FH

Previous studies on the incidence of FH in AHB patients have shown varying results, with incidences of less than 1%,[11] less than 5%[12] or approximately 5%.[13] This variation may be due to differences in study populations, areas and definitions of FH between studies. A previous Japanese report showed that 45 (9.3%) of 485 AHB patients developed FH,[14] but the participating hospitals in that study were limited to large hospitals. Our study showed the incidence of FH was 6.0%, which may be reliable because the present study included large and small hospitals.

Table 2 Patients' age, sex and outcomes

	All (n = 890)	AHB (n = 837)	FH (n = 53)	P-value
Age (average ± SD, years)	40.0 ± 15.0	39.1 ± 14.6	53.3 ± 15.0	<0.001
Male (n, %)	678 (76.2%)	643 (76.8%)	35 (66.0%)	0.074
LOS (average ± SD, days)	20.9 ± 12.5	20.5 ± 11.6	26.7 ± 21.3	0.039
In-hospital mortality (n, %)	36 (4.0%)	8 (1.0%)	28 (52.8%)	<0.001
Total costs (average ± SD, $US)	7571 ± 12 161	5507 ± 4 564	40 116 ± 32 295	<0.001

AHB, acute hepatitis B; FH, fulminant hepatitis; LOS, length of stay; SD, standard deviation.

Treatment of AHB

Another advantage of the DPC database is that it includes records of the clinical practices performed in each patient. Thus, we can track the use of drugs, procedures and clinical examinations, including plasma exchange, NA use and HIV-1 tests.

In this study, NA was used in as many as 25% of all AHB cases. In fact, the efficacy of NA for AHB is still controversial. Some small-scale studies have suggested the efficacy of lamivudine or entecavir to prevent the progression of AHB to FH and chronic hepatitis.[15–21] Another study showed lower mortality in lamivudine users compared with the control group, but a lower seroconversion rate of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg).[22] However, one randomized placebo-controlled trial showed no significant difference in clinical outcomes and a lower rate of development of anti-HBs and anti-HBe in the lamivudine group.[23] According to clinical guidelines, NA is only indicated for patients with severe or protracted AHB and FH.[12,24] Nevertheless, approximately 20% of AHB cases without FH were prescribed with NA in this study. Hepatologists should reconsider the probability of causing persistent HBV infection by using NA.

Various treatments other than NA were provided to many AHB patients, although protease inhibitors were used in some cases as an anticoagulant during plasma exchange or continuous hemodiafiltration. However, most of these treatments were costly and are not recommended in clinical guidelines.[25,26] Thus, further evidence for these drugs in AHB and FH is urgently required.

Table 3 Clinical practices

	All (n = 890)	AHB (n = 837)	FH (n = 53)
Liver transplantation	3 (0.3)	0 (0.0)	3 (5.7)
Plasma exchange	53 (6.0)	0 (0.0)	53 (100.0)
Continuous hemodiafiltration	28 (3.1)	2 (0.2)	26 (49.1)
NA	226 (25.4)	176 (21.0)	50 (94.3)
Lamivudine	52 (5.8)	34 (4.1)	18 (34.0)
Adefovir	4 (0.4)	4 (0.5)	0 (0.0)
Entecavir	183 (20.6)	144 (17.2)	39 (73.6)
Interferons	28 (3.1)	13 (1.6)	15 (28.3)
Protease inhibitors	64 (7.2)	16 (1.9)	48 (90.6)
Antithrombin III	29 (3.3)	9 (1.1)	20 (37.7)
Prostaglandin E_1	6 (0.7)	2 (0.2)	4 (7.5)
Glucagon	26 (2.9)	17 (2.0)	9 (17.0)
Monoammonium glycyrrhizinate	296 (33.3)	270 (32.3)	26 (49.1)
Corticosteroids injection	66 (7.4)	30 (3.6)	36 (67.9)
Fresh frozen plasma	47 (5.3)	13 (1.6)	34 (64.2)
Red blood cell transfusion	20 (2.2)	4 (0.5)	16 (30.2)
Platelet transfusion	17 (1.9)	4 (0.5)	13 (24.5)
Albumin preparation	33 (3.7)	10 (1.2)	23 (43.4)

Results are n (%).
AHB, acute hepatitis B; FH, fulminant hepatitis; NA, nucleos(t)ide analogs.

Necessity for HIV-1 tests in AHB cases

Co-infection of HIV-1 and HBV is not rare in Japan because the main route of AHB transmission is through sexual contact.[27] Lamivudine and entecavir can lead to the emergence of drug-resistant HIV-1.[28,29] According to guidelines on HIV-1 and HBV, we should assess the HIV-1 status of patients with HBV before we administrate these drugs to avoid inappropriate monotherapy for HIV-1 and HBV co-infected patients.[30]

Our study showed that the proportion of patients who were checked for HIV-1 was quite low. One of the disadvantages of the DPC database is that it does not include data from outpatient departments. Therefore, some patients might be checked for HIV-1 before admission. Although the under-utilization of the HIV-1 test in our study might be exaggerated, this is a potentially serious problem, particularly among patients who used lamivudine or entecavir. The Japanese guidelines for HIV have also placed emphasis on the co-infection of HIV-1 and HBV and the need for careful prescription of lamivudine and entecavir[31–33] because omitting HIV-1 tests may cause physicians to overlook HIV infection in AHB patients and the emergence of drug-resistant HIV-1.

Health policy implications

Our study demonstrated that the incidence of AHB in Japan was substantially higher than was previously reported. Unfortunately, the burden of the disease has been undervalued. Although many countries have introduced universal HBV vaccination for all infants in accordance with the World Health Organization's recommendation, Japan has not yet included universal HBV vaccination into the national vaccination programs. Instead, many Japanese people and physicians are more concerned about the introduction of other vaccines such as for human papilloma virus. This health policy may have been attributed, in part, to the undervaluation of the burden of HBV.

Several health policy implications are highlighted by the findings of this study, which may be useful for public health policy in any country. First, this study clearly indicates the importance of establishing a strict data collection system for population-based patient monitoring. Many countries show incomplete notifiable infectious disease reporting,[34,35] including acute viral hepatitis.[36–38] Several attempts have been made to improve public health surveillance, such as using electronic medical record data or enhanced surveillance.[39,40] The DPC database complements the NESID database, but cannot fully replace it because of the lack of timely reporting. The NESID reporting system should be improved and physicians must correctly report notifiable diseases to the public health authorities as required by law. Undervaluation of the disease burden may hinder relevant health policy changes. Second, this study revealed a discrepancy between evidence-based medicine and real-world clinical practices. A broad range of expensive treatment options have been widely used, despite inadequate evidence and the possibility of harmful effects. Medical professionals should endeavor to generate and compile scientific evidence and establish strict guidelines to standardize evidence-based best practices.

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Research Article

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Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors

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Background & Aims: Pegylated interferon and ribavirin (PEG-IFN/RBV) therapy for chronic hepatitis C virus (HCV) genotype 1 infection is effective in 50% of patients. Recent studies revealed an association between the *IL28B* genotype and treatment response. We aimed to develop a model for the pre-treatment prediction of response using host and viral factors.

Methods: Data were collected from 496 patients with HCV genotype 1 treated with PEG-IFN/RBV at five hospitals and universities in Japan. *IL28B* genotype and mutations in the core and IFN sensitivity determining region (ISDR) of HCV were analyzed to predict response to therapy. The decision model was generated by data mining analysis.

Results: The *IL28B* polymorphism correlated with early virological response and predicted null virological response (NVR) (odds ratio = 20.83, p <0.0001) and sustained virological response (SVR) (odds ratio = 7.41, p <0.0001) independent of other covariates. Mutations in the ISDR predicted relapse and SVR independent of *IL28B*. The decision model revealed that patients with the minor *IL28B* allele and low platelet counts had the highest NVR (84%) and lowest SVR (7%), whereas those with the major *IL28B* allele and mutations in the ISDR or high platelet counts had the lowest NVR (0–17%) and highest SVR (61–90%). The model had high reproducibility and predicted SVR with 78% specificity and 70% sensitivity.

Conclusions: The *IL28B* polymorphism and mutations in the ISDR of HCV were significant pre-treatment predictors of response to PEG-IFN/RBV. The decision model, including these host and viral factors may support selection of optimum treatment strategy for individual patients.

Keywords: *IL28B*; ISDR; Peg-interferon; Ribavirin; Data mining; Decision tree.
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Introduction

Hepatitis C virus (HCV) infection is the leading cause of cirrhosis and hepatocellular carcinoma worldwide [1]. The successful eradication of HCV, defined as a sustained virological response (SVR), is associated with a reduced risk of developing hepatocellular carcinoma. Currently, pegylated interferon (PEG-IFN) plus ribavirin (RBV) is the most effective standard of care for chronic hepatitis C but the rate of SVR is around 50% in patients with HCV genotype 1 [2,3], the most common genotype in Japan, Europe, the United States, and many other countries. Moreover, 20–30% of patients with HCV genotype 1 have a null virological response (NVR) to PEG-IFN/RBV therapy [4]. The most reliable method for predicting the response is to monitor the early decline of serum HCV-RNA levels during treatment [5] but there is no established method for prediction before treatment. Because PEG-IFN/RBV therapy is costly and often accompanied by adverse effects such as flu-like symptoms, depression and hematological abnormalities, pre-treatment predictions of those patients who are unlikely to benefit from this regimen enables ineffective treatment to be avoided.

Recently, it has been reported through a genome-wide association study (GWAS) of patients with genotype 1 HCV that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are strongly associated with a response to PEG-IFN/RBV therapy in

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Table 1. Baseline characteristics of all patients, and patients assigned to the model building or validation groups.

	All patients n = 496	Model group n = 331	Validation group n = 165
Gender: male	250 (50%)	170 (51%)	80 (48%)
Age (years)	57.1 ± 9.9	56.8 ± 9.7	57.5 ± 10.2
ALT (IU/L)	78.6 ± 60.8	78.1 ± 61.4	79.7 ± 59.6
GGT (IU/L)	59.3 ± 63.6	58.9 ± 62.0	60.2 ± 66.9
Platelets (10^9/L)	154 ± 53	153 ± 52	154 ± 56
Fibrosis: F3-4	121 (24%)	80 (24%)	41 (25%)
HCV-RNA: >600,000 IU/ml	409 (82%)	273 (82%)	136 (82%)
ISDR mutation: ≤1	220 (88%)	290 (88%)	145 (88%)
Core 70 (Arg/Gln or His)	293 (59%)/203 (41%)	197 (60%)/134 (40%)	96 (58%)/69 (42%)
Core 91 (Leu/Met)	299 (60%)/197 (40%)	200 (60%)/131 (40%)	99 (60%)/66 (40%)
IL28B: Minor allele	151 (30%)	101 (31%)	50 (30%)
SVR	194 (39%)	129 (39%)	65 (39%)
Relapse	152 (31%)	103 (31%)	49 (30%)
NVR	150 (30%)	99 (30%)	51 (31%)

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Gln, glutamine; His, histidine; Leu, leucine; Met, methionine; Minor, heterozygote or homozygote of minor allele; SVR, sustained virological response; NVR, null virological response.

Japanese [6], European [7], and a multi-ethnic population [8,9]. The last three studies focused on the association of SNPs in the *IL28B* region with SVR [7–9] but we found a stronger association with NVR [6]. In addition to these host genetic factors, we have reported that mutations within a stretch of 40 amino acids in the NS5A region of HCV, designated as the IFN sensitivity determining region (ISDR), are closely associated with the virological response to IFN therapy: a lower number of mutations is associated with treatment failure [10–13]. Amino acid substitutions at positions 70 and 91 of the HCV core region (Core70, Core91) also have been reported to be associated with response to PEG-IFN/RBV therapy: glutamine (Gln) or histidine (His) at Core70 and methionine (Met) at Core91 are associated with treatment resistance [4,14]. The importance of substitutions in the HCV core and ISDR was confirmed recently by a Japanese multicenter study [15]. How these viral factors contribute to response to therapy is yet to be determined. For general application in clinical practice, host genetic factors and viral factors should be considered together.

Data mining analysis is a family of non-parametric regression methods for predictive modeling. Software is used to automatically explore the data to search for optimal split variables and to build a decision tree structure [16]. The major advantage of decision tree analysis over logistic regression analysis is that the results of the analysis are presented in the form of flow chart, which can be interpreted intuitively and readily made available for use in clinical practice [17]. The decision tree analysis has been utilized to define prognostic factors in various diseases [18–25]. We have reported recently its usefulness for the prediction of an early virological response (undetectable HCV-RNA within 12 weeks of therapy) to PEG-IFN/RBV therapy in chronic hepatitis C [26].

This study aimed to define the pre-treatment prediction of response to PEG-IFN/RBV therapy through the integrated analysis of host factors, such as the *IL28B* genetic polymorphism and various clinical covariates, as well as viral factors, such as mutations in the HCV core and ISDR and serum HCV-RNA load. In addition, for the general application of these results in clinical practice, decision models for the pre-treatment prediction of response were determined by data mining analysis.

Materials and methods

Patients

This was a multicentre retrospective study supported by the Japanese Ministry of Health, Labor and Welfare. Data were collected from a total of 496 chronic hepatitis C patients who were treated with PEG-IFN alpha and RBV at five hospitals and universities throughout Japan. Of these, 98 patients also were included in the original GWAS analysis [6]. The inclusion criteria in this study were as follows (1) infection by genotype 1b, (2) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (3) lack of other causes of liver disease, such as autoimmune hepatitis, and primary biliary cirrhosis, (4) completion of at least 24 weeks of therapy, (5) adherence of more than 80% to the planned dose of PEG-IFN and RBV for the NVR patients, (6) availability of DNA for the analysis of the genetic polymorphism of *IL28B*, and (7) availability of serum for the determination of mutations in the ISDR and substitutions of Core70 and Core91 of HCV. Patients received PEG-IFN alpha-2a (180 μg) or 2b (1.5 μg/kg) subcutaneously every week and were administered a weight adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg daily) which is the recommended dosage in Japan. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee. The baseline characteristics are listed in Table 1. For the data mining analysis, 67% of the patients (331 patients) were assigned randomly to the model building group and 33% (165 patients) to the validation group. There were no significant differences in the clinical backgrounds between these two groups.

Laboratory and histological tests

Blood samples were obtained before therapy and were analyzed for hematologic tests and for blood chemistry and HCV-RNA. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse-transcription and polymerase chain reaction as reported previously [4,11]. Genetic polymorphism in one tagging SNP located near the *IL28B* gene (rs8099917) was determined by the GWAS or DigiTag2 assay [27]. Homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having the *IL28B* minor allele, whereas homozygosity for the major sequence (TT) was