Table 9 Clinical features of patients with HCC

	Percentage (%)	Prevalence of HCC (%)	Age of onset of HCC (years)	M : F ratio	Child–Pugh classification (A/B/C, %)	BMI	Platelet (10^3/mm^3)
Total ($n = 2438$)	100	35.9	67.2 ± 10.1	3.06	62.6/28.8/8.6	24.6 ± 4.0	127 ± 66
NASH ($n = 485$)	19.9	50.9*	70.8 ± 9.0**	1.06	66.0/28.9/5.1	26.8 ± 4.3‡‡	128 ± 61
ALD ($n = 1302$)	53.4	34.3	64.8 ± 9.4†	19.05††	60.3/29.8/9.9	24.0 ± 3.8	126 ± 66
FLD ($n = 91$)	3.7	54.5*	68.4 ± 8.8***	17.20††	82.5/15.9/1.6	25.8 ± 4.0‡‡‡	120 ± 61
PBC ($n = 79$)	3.2	14.4	68.0 ± 10.4***	0.32†††	53.2/35.9/10.9	22.3 ± 3.0	110 ± 54
Other biliary cirrhosis ($n = 4$)	0.2	6.8	–	–	–	–	–
AIH ($n = 119$)	4.9	26.0	68.8 ± 8.7**	0.23†††	42.5/42.5/15.0‡	24.3 ± 4.1	107 ± 60§
Metabolic disease ($n = 2$)	0.1	5.1	–	–	–	–	–
Congestive disease ($n = 16$)	0.7	32.0	52.0 ± 16.6	1.67	57.2/21.4/21.4	23.6 ± 3.2	127 ± 72
Parasites ($n = 3$)	0.1	30.0	–	–	–	–	–
Unknown etiology ($n = 337$)	13.8	47.5*	70.9 ± 10.9**	1.57	70.8/22.4/6.8	23.6 ± 3.7	143 ± 76

Results of age are expressed as mean ± standard deviation. *P*-values were analyzed by Mann–Whitney *U*-test and χ^2-test as appropriate.
*$P < 0.0001$, vs ALD, PBC and AIH; **$P < 0.0001$, vs ALD and congestive disease; ***$P < 0.001$, vs ALD and congestive disease; †$P < 0.001$, vs congestive disease; ††$P < 0.0001$, vs NASH, PBC, AIH and unknown etiology; †††$P < 0.0001$, vs NASH, ALD, FLD and unknown etiology; ‡$P < 0.0001$, vs NASH, FLD and unknown etiology; ‡‡$P < 0.0001$, vs ALD, PBC, AIH and unknown etiology; ‡‡‡$P < 0.0001$, vs ALD, PBC and unknown etiology; §$P < 0.001$, vs NASH and unknown etiology.
AIH, autoimmune hepatitis; ALD, alcoholic liver disease; BMI, body mass index; FLD, fatty liver disease; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; PBC, primary biliary cirrhosis.

predominantly female. Yasui *et al.* reported that NASH HCC patients were predominantly male, although the prevalence of cirrhosis among these patients was significantly lower in male patients compared with that in female patients.[10] These studies suggest that sex is implicated in the progression of fibrosis in NASH patients in Japan. In addition, the prevalence of HCC in the NASH LC patients in the present study was significantly higher compared with that in the previous nationwide survey (50.9% vs 31.5%, $P < 0.001$).[1] The incidence of NASH and NASH HCC has been gradually increasing in Japan, contrary to the decreased incidence of virus-related HCC.[4] Starley *et al.* found that as many as 4–27% of cases of NASH transform to HCC after the development of cirrhosis, and that the prevalence of HCC in NAFLD is 0–0.5%, whereas that of HCC in NASH is 0–2.8% over time periods of up to 19.5 years.[7] Yatsuji *et al.* reported the prospective evaluation of NASH LC and HCV-related LC (LC-C). They reported that NASH LC followed a course similar to that of LC-C, namely, complications of cirrhosis developed, including HCC (the 5-year cumulative rate of HCC development was 11.3% for NASH LC and 30.5% for LC-C).[11] Therefore, NASH LC patients need to be followed up carefully with respect to the occurrence of HCC, similar to LC-C patients.

Alcoholic liver disease remains the most prevalent cause of NBNC LC in Japan, accounting for approximately 55% of all NBNC LC cases. In the present study, the prevalence of HCC was significantly lower in the ALD LC patients than in the NASH LC patients, whereas the ALD LC patients were significantly younger and had a lower hepatic reserve. Regarding the comparison of outcomes with LC-C, Toshikuni *et al.* reported that the risk of HCC was lower in ALD LC than in LC-C, whereas the risk of hepatic decompensation and mortality was the same.[12] It is estimated that there are approximately 2.4 million heavy drinkers in Japan, and the number of ALD patients has been increasing because of increased alcohol consumption.[13] Therefore, ALD LC patients need to be followed up carefully with respect to the occurrence of hepatic decompensation, similar to LC-C patients. Obesity appears to be involved in the progression of ALD LC.[13] Accordingly, we investigated the risk factors associated with HCC and clarified that obesity and complication of DM could be the risk for hepatic carcinogenesis in ALD LC patients. The comparison of the clinical features between the two ALD LC groups divided based on BMI revealed that the prevalence of HCC in the ALD LC patients with obesity was significantly higher compared with that in those without obesity. Horie *et al.* also reported similar results.[14] Thus,

Table 10 Factors associated with HCC in patients with ALD

Factors	HCC (−), ($n = 2494$)	HCC (+), ($n = 1303$)	Univariate analysis, *P*-value	Multivariate analysis, *P*-value
Sex (M : F)	83.7%:16.3%	95.0%:5.0%	<0.0001	<0.0001
Age (years)	57.9 ± 11.0	64.8 ± 9.4	<0.0001	<0.0001
Body mass index (kg/m^2)	22.8 ± 3.8	24.0 ± 3.8	<0.0001	<0.0001
Hypertension (− : +)	77.4%:22.6%	61.9%:38.1%	<0.0001	0.068
Dyslipidemia (− : +)	87.0%:13.0%	81.6%:18.4%	<0.0001	0.482
Diabetes mellitus (− : +)	67.2%:32.8%	50.2%:49.8%	<0.0001	<0.0001
Child–Pugh classification (A : B + C)	38.5%:61.5%	60.3%:39.7%	<0.0001	0.188
Esophageal varices (− : +)	42.3%:57.7%	57.9%:42.1%	<0.0001	<0.0001
Ascites (− : +)	57.1%:42.9%	76.5%:23.5%	<0.0001	<0.0001
WBC (/mm^3)	6014 ± 3465	5532 ± 3484	0.001	0.547
Hemoglobin (g/dL)	11.3 ± 2.6	12.7 ± 2.2	<0.0001	<0.0001
Platelet (×10^3/mm^3)	114.6 ± 67.1	126.1 ± 65.5	<0.0001	0.104
AST (IU/L)	93 ± 209	65 ± 71	<0.0001	0.974
ALT (IU/L)	51 ± 118	45 ± 43	0.159	0.786
Bilirubin (mg/dL)	2.8 ± 3.9	1.6 ± 2.4	<0.0001	0.006
Albumin (g/dL)	3.3 ± 1.0	3.5 ± 0.7	<0.0001	0.281
PT%	69 ± 22	79 ± 19	<0.0001	0.628

Results of age are expressed as mean ± standard deviation. *P*-values were analyzed by Mann–Whitney *U*-test, χ^2-test and multivariate Cox's proportional hazard model as appropriate.
ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; PT, prothrombin time; WBC, white blood cells.

obesity appears to be involved in the progression of HCC in ALD LC. Therefore, ALD LC patients with obesity need to be followed up carefully with respect to the occurrence of HCC, similar to NASH LC and LC-C patients. Not only abstinence from alcohol but also improvement in lifestyle is important to decrease the progression of ALD LC.

In the present study, we established a new clinical etiologic criterion: FLD. According to previous clinical etiologic criteria in Japan, mild drinkers (intake of >20 g and <70 g of ethanol/day) with steatohepatitis were not diagnosed with both NASH and ALD. The prevalence of minor homozygote or heterozygote type of the aldehyde dehydrogenase-2 gene (*ALDH2*), which oxidizes acetaldehyde to acetate and is a key enzyme in alcohol metabolism, is very high in Asian countries. The enzyme activity of a minor homozygote of *ALDH2* is completely defective. Moreover, the enzyme activity of a heterozygote is only 1/16th. Our survey is the first to reveal that these FLD LC patients were observed in 2.5% of NBNC LC patients. Considering the frequencies of mild drinkers and obese people in Japan, it is thought that the frequency of FLD LC is lower than that of LC with unknown etiology. This is because there were many patients whose amounts of daily alcohol intake were unknown; therefore, some were diagnosed as having an

Table 11 Clinical features of patients with ALD LC

	BMI <25 ($n = 1915$)	BMI ≥25 ($n = 749$)	*P*-value
Sex (M : F)	1644:317 (83.4%:16.6%)	692:57 (92.4%:7.6%)	$P < 0.001$
Age	60.2 ± 11.1	61.0 ± 10.2	N.S.
Diabetes mellitus	35.1%	43.9%	$P < 0.001$
HCC	35.7%	48.3%	$P < 0.001$

Results of age are expressed as mean ± standard deviation, *P*-values were analyzed by by Mann–Whitney *U*-test and χ^2-test as appropriate.
ALD, alcoholic liver disease; BMI, body mass index; HCC, hepatocellular carcinoma; N.S., not significant.

unknown etiology. Interestingly, the clinical features of the FLD LC patients overlapped with those of the NASH LC and ALD LC patients. Because the mean age of the FLD LC patients was between that of the NASH and ALD patients, the FLD LC patients were predominantly male, similar to the ALD LC patients, and they were more likely to have DM and HCC similar to the NASH LC patients. Horie *et al.* described a category such as FLD as overlap steatohepatitis.[13,14] The most important clinical feature in FLD LC patients was that the prevalence of HCC was high, similar to that in the NASH LC patients. This finding suggests that steatohepatitis per se is a potent risk factor of HCC, irrespective of alcohol consumption.

The LC patients with unknown etiology (or cryptogenic LC) were approximately 10% of the NBNC LC patients and were more likely to have HCC similar to the NASH and FLD patients. Some FLD LC patients whose daily alcohol intake was unknown may have been included in this group, and some "burnt-out" NASH LC patients whose liver showed complete disappearance of steatosis[15] may have also been included in this group. In addition, some patients who had been HBV carriers but had become HBsAg negative or those with occult HBV may have also been included in this group. Anti-HBc positivity was significantly higher in this group than in the NASH, ALD and FLD LC groups. Several studies have suggested a high prevalence of occult HBV among cryptogenic LC and NBNC HCC patients and also the participation of occult HBV in the progression to cirrhosis and occurrence of HCC.[16,17] In the present study, anti-HBc positivity was significantly higher in the NBNC LC patients with HCC than in those without HCC; however, the role of occult HBV in the progression to cirrhosis and carcinogenesis remains unclear. Occult HBV is defined as the presence of HBV DNA in the liver (with or without detectable HBV DNA in serum) for patients testing HBsAg negative.[18] Because of the lack of a HBV DNA assay in the present study, the impact of occult HBV on carcinogenesis could not be evaluated. Thus, a HBV DNA assay in the liver is needed for the evaluation of occult HBV on carcinogenesis. Although NBNC LC seemed to include varied etiology, occult HBV should be taken into account in the prediction of future HCC development in NBNC LC.

Our nationwide survey determined the etiology of NBNC LC in Japan. Future changes in etiology must be considered for the establishment of precise diagnostic strategies. We hope that these results contribute new ideas toward understanding NBNC LC and NBNC HCC.

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APPENDIX I

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Review Article

Anticarcinogenic impact of interferon therapy on the progression of hepatocellular carcinoma in patients with chronic viral infection

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Hepatocellular carcinoma (HCC) is mainly caused by a persistent infection due to the hepatitis B or hepatitis C virus. The number of HCC cases is increasing in Asian and African countries, as well as in European and American countries. Interferon (IFN) therapy, used for type B chronic liver diseases, inhibits hepatic carcinogenesis in patients with compensated cirrhosis. However, there is insufficient evidence that IFN therapy inhibits hepatic carcinogenesis in patients with chronic hepatitis B. There are few cases of HCC due to chronic hepatitis B, and long-term follow-up periods verifying the inhibitory effect of IFN on hepatic carcinogenesis have not been obtained. To improve the prognosis of type B chronic liver diseases, it is important that hepatitis treatment follows guidelines in which a patient's age and the extent of hepatic fibrosis are taken into account. As for chronic hepatitis C, since a sustained virological response (SVR) in IFN therapy inhibits hepatic carcinogenesis and improves prognosis, treatment that aims for an SVR while taking into consideration host-sided and virus-sided factors is recommended for patients with type C chronic liver diseases. In areas with low incidence of HCC (e.g. USA), a large number of cases and a long-term follow-up period are needed before it can be accepted that IFN therapy inhibits hepatic carcinogenesis. After locally curative treatment of HCC, IFN therapy suppresses recurrence and improves survival rates.

Key words: chronic hepatitis, hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, interferon, prevention

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) ranks fifth in the number of patients worldwide who are diagnosed with cancer; its death toll ranks third.[1] Approximately 600 000 to 700 000 patients worldwide die of HCC each year; the number of HCC cases is increasing in Asian and African countries, as well as in European and American countries.[2,3] HCC is mainly derived from a persistent infection due to the hepatitis B virus (HBV) or hepatitis C virus (HCV); thus, treating viral hepatitis inhibits hepatic carcinogenesis. In clinical and epidemiological studies of patients with chronic hepatitis B, active replication of HBV is linked to progression to cirrhosis and HCC.[4] Cessation of HBV replication reduces complications and improves prognosis. If, as a result of interferon (IFN) therapy, seroclearance of hepatitis B e antigen (HBeAg) can be achieved and the patient is negative for HBV DNA, then this might reduce the chances of HCC developing.[5] IFN therapy for chronic hepatitis C helps to reduce the risk of HCC developing in patients in whom a sustained virological response (SVR) has been achieved and that therapy also helps to reduce the risk of HCC developing in patients in whom viral clearance has proven difficult.[6,7] This paper reviews clinical research studies that have focused on the inhibitory effect of IFN therapy on hepatic carcinogenesis.

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THE ANTITUMOR ACTION OF IFN

INTERFERON IS A cytokine with varied forms of bioactivity including antiviral action as well as action to inhibit cell growth, angiogenetic activity, action to regulate the immune response, and action to inhibit

telomerase activity. IFNs are generally grouped into type I IFNs, which include IFN-α, -β, and -ω, and type II IFN, which consists solely of IFN–γ.[8–10] IFN-α and -β are widely used clinically to treat viral diseases such as chronic hepatitis B and C and neoplastic diseases such as renal cell carcinoma and glioblastoma. Evidence of IFN's direct antitumor action has been reported, i.e. IFN-α and -β have been found to inhibit growth of a hepatoma cell line in a concentration-dependent manner.[11] In addition, IFN has been found to exhibit antitumor action by inducing apoptosis of tumor cells via p53 and by stopping the progression of the cell cycle.[12] Similarly, an *in vivo* study noted that an IFN dose similar to that used clinically suppressed the growth of hepatic carcinoma cells.[13] Moreover, alpha fetoprotein (AFP) levels decreased after administration of IFN to patients with chronic hepatitis C and consistently elevated AFP levels; the mechanism for this phenomenon may be antitumor action.[14] In addition, IFN is also assumed to have indirect antitumor action by immunopotentiation via natural killer cells.[15] Nevertheless, the current reality is that the mechanism of IFN's antitumor action has yet to be fully elucidated.

HBV-RELATED HCC

THERE ARE AN estimated 300 million or more HBV carriers in the world; many of them are concentrated in Asian and African areas.[1] About 15% of HCC cases in Japan are HBV-related.[16] The annual incidence of HCC in patients with type B chronic hepatitis is 0.1% to 1.0% and in patients with type B cirrhosis, 2.2% to 4.3%; the incidence of HCC is higher in Asia than elsewhere in the world.[17] A study of the natural history of HCC has reported that factors for a high risk of developing the condition are cirrhosis, being an elderly male, having genotype C or F1, having a double substitution (A1762T and G1764A) in the core promoter region, and high HBV DNA levels.[4] Since it is difficult to completely eliminate HBV, the primary goals of treatment are to eliminate or reduce HBV DNA in the blood and to normalize the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[18]

We searched the medical published reports and found that the inhibitory effect of IFN therapy on hepatic carcinogenesis in patients with type B chronic hepatitis and cirrhosis was first reported in 1996; we also found four randomized controlled trials (RCT) – three from Europe and one from Asia (Table 1).[19–22] The paper from Asia indicated that IFN therapy inhibits hepatic carcinogenesis, but the papers from Europe did not have this

Table 1 Baseline characteristics of randomized control trials assessing effect of interferon (IFN) on hepatocellular carcinoma (HCC) development in hepatitis B virus (HBV)-infected patients

Study [reference]	Year	Sample size (*n*)	Male (%)	Mean age (years)	HBeAg positive (%)	Pre-existing cirrhosis (%)	IFN regimen	Mean follow-up (years)	VR (%)	Incidence of HCC (%)
Lampertico P *et al.*[21]	1997	T21	T80	T44	T0	T19	α-2b: 6 MU 3 times a week for 24 months	3.8	T28	T5
		C21	C90	C47	C0	C14			C0	C0
Krogsgaard K *et al.*[19]	1998	T210	81	36	T100	19	α-2a: 2.5–18 MU 3 times a week for 12–24 weeks	4.7	T24	T1
		C98			C100				C20	C1
Mazzella G *et al.*[20]	1999	T33	T76	T36	T100	T0	α: 5 MU/m^2 3 times a week for 24 weeks	7.1	T36	T3
		C31	C80	C40	C100	C0		6.6	C0	C6
Lin SM *et al.*[22]	1999	T67	T100	T32	T100	T10	α: 4–6 MU/m^2 3 times a week for 12 weeks	7.4	T42	T1.5
		C34	C100	C32	C100	C14		6.5	C24	C11.8

C, control group (no treatment); HBeAg, hepatitis B e antigen; MU, million units; T, IFN treated group; VR, virological response.

finding. In a study involving 308 patients with HBe antigen (HBeAg)-positive chronic hepatitis and cirrhosis, Krogsgaard *et al.*[19] administered a 2.5 to 10 million unit (MU)/m^2 dose of IFN-α2a three times weekly for 12 to 24 weeks to 210 patients (i.e. the treatment group). During a mean follow-up period of 4.7 years, HCC occurred in two patients in the treatment group and in one patient in the control group. In a study involving 64 patients with HBeAg-positive chronic hepatitis, Mazzella *et al.*[20] administered a 5 MU/m^2 dose of IFN-α three times weekly for 24 weeks to 33 patients (i.e. the treatment group). During a mean follow-up period of 7.1 years, HCC occurred in three patients whose chronic hepatitis had progressed to cirrhosis (one patient in the treatment group and two patients in the control group). In a study involving 42 patients with HBeAg-negative chronic hepatitis, Lampertico *et al.*[21] administered a 6 MU dose of IFN-α2b three times weekly for 96 weeks to 21 patients (i.e. the treatment group). During the mean follow-up period of 3.8 years, HCC occurred in one patient in the treatment group. In a study involving 101 patients with HBeAg-positive chronic hepatitis, Lin *et al.*[22] administered a 4–6 MU dose of IFN-α three times weekly for 12 weeks to 67 patients (i.e. the treatment group). During a mean follow-up period of 7 years, HCC occurred in one patient in the treatment group and in four patients in the control group ($P = 0.043$). They believed that IFN therapy had an inhibitory effect on hepatic carcinogenesis.

On the other hand, reports from a study involving patients with HBeAg-positive cirrhosis[23] and several studies involving patients with HBeAg-positive chronic hepatitis and HBeAg-negative chronic hepatitis[19,20,24,25] deny that IFN therapy inhibits hepatic carcinogenesis. Based on their meta-analysis of seven non-randomized controlled trial (NRCT) studies involving patients with cirrhosis,[20,23,26–30] Cammà *et al.*[31] believe that IFN therapy inhibits hepatic carcinogenesis (risk difference [RD], −6.4%; confidence interval [CI], −2.8 to −10; $P < 0.001$). However, their analyses of subgroups with small variations showed no significant differences and they found that IFN therapy did not inhibit hepatic carcinogenesis. Sung *et al.*[32] in a meta-analysis of 12 papers (which included an RCT study)[5,19,20,23–26,28–30,33,34] concluded that, compared with patients in the control group, patients in the IFN therapy group had a 34% reduced risk of developing HCC. This effect was especially beneficial for patients with cirrhosis. However, in a recent meta-analysis based on two RCT studies,[20,22] Zhang *et al.*[35] concluded that IFN therapy does not necessarily reduce the development of HCC.

At the current point in time, previous reports offer conflicting results with regard to whether or not IFN therapy suppresses hepatocarcinogenesis when used to treat hepatitis B virus-related chronic liver disease. Reasons for this conflict are presumably related to discrepancies in IFN's suppression of carcinogenesis brought about by differences in the clinical characteristics of the patients studied. In other words, numerous factors, such as: (i) patient age; (ii) sex; (iii) liver function tests; (iv) differences in the mode of infection (vertical or horizontal infection); (v) stage of liver fibrosis and grade of necroinflammatory activity; (vi) positivity or negativity for HBeAg; (vii) HBV genotype; (viii) levels of HBV DNA; (ix) treatment protocol; (x) therapeutic efficacy; and (xi) follow-up period, may affect study results. In a NRCT involving 313 patients with cirrhosis due to hepatitis B, Ikeda *et al.*[26] administered 6 MU IFN-α three times a week for 40 weeks to 94 patients in a treatment group (including 61 patients who were positive for HBeAg). A follow-up lasting an average of 7 years revealed 10 patients in the treatment group and 51 of 219 patients in an untreated group developed HCC; this finding indicated that use of IFN decreased the rate of carcinogenesis. In addition, Lin *et al.*[5] reported a case-control study matching for age, sex, HBeAg, ALT, and levels of HBV DNA. Their results revealed that five patients in a group receiving IFN therapy and 16 in an untreated group developed HCC ($P = 0.025$) in a mean follow-up of 6.8 years. A follow-up of 15 years indicated that the cumulative rate of hepatocarcinogenesis was significantly lower for patients who had cirrhosis and were receiving IFN therapy in comparison to the control group, but differences between the control group and patients who did not have cirrhosis and were receiving IFN therapy were not noted. Multivariate analysis indicated that independent risk factors for the progression of HCC were age, not having undergone IFN therapy, pre-existing cirrhosis, carrying HBeAg, and having the HBV genotype C (in comparison to genotype B). Based on previous studies, IFN therapy for patients with compensated cirrhosis B should be able to suppress hepatocarcinogenesis.[5,22,23,26,31,32,36] However, the inhibitory effect of IFN therapy on hepatic carcinogenesis for patients with type B chronic hepatitis has not yet gained a sufficient consensus. One reason is that there are few cases of hepatic carcinogenesis that develop from type B chronic hepatitis; thus, researchers cannot obtain either a sufficient number of cases or long-term follow-up periods to verify that IFN therapy inhibits hepatic carcinogenesis. An HBV carrier who has a high level of HBV DNA

rapidly progresses to cirrhosis, which is associated with a high rate of HCC.[37] In patients with HBeAg seroconversion and reduced levels of HBV DNA due to IFN therapy, the progression of cirrhosis slows and development of HCC is inhibited.[5] When serum transaminase returns to normal and HBV DNA falls below detection limits due to IFN therapy given to HBeAg-negative European patients, an improved prognosis is noted but IFN therapy has not been found to suppress hepatocarcinogenesis.[38] Miyake *et al.* reported that IFN therapy has been found to suppress hepatocarcinogenesis in Asians; they also reported that IFN has an effect in populations with a 10% or greater incidence of HCC that have not undergone IFN therapy and study populations with 70% or more subjects that are positive for HBeAg.[39]

Compared with the standard IFN, pegylated-IFN (PEG-IFN) has been reported as more effective in the elimination of HBeAg, reducing HBV DNA, and normalizing the serum ALT level.[36] However, there is no report on whether PEG-IFN (in comparison with the standard IFN and nucleos(t)ide analogs such as lamivudine) more greatly reduces the risk of developing HCC. Future research is needed.

In addition, IFN for type B cirrhosis is not price-listed in Japan, and the IFN administration period for type B chronic hepatitis is 6 months. Price-listing of IFN for type B cirrhosis, the extension of the administration period, and the approval of using PEG-IFN are pending.

HCV-RELATED HCC

THE HCV WOULD not be naturally eliminated when an infection is passed to humans. About 70% of persistently infected people become carriers and necroinflammatory reactions continue; as a result, hepatic fibrosis progresses to cirrhosis.[40] However, hepatic fibrosis progression rates in persistently HCV-infected people differ significantly among individuals and are influenced by the person's age when infected, the amount of alcohol intake, gender, and the extent of liver function abnormality. It has been demonstrated that, in people who have insulin resistance and fatty livers, the hepatic fibrosis progression rate is rapid and the sustained virological response (SVR) ratio in IFN therapy is reduced.[41,42] HCC incidence rates increase in relation to the progression of hepatic fibrosis.[43] The annual incidence of HCC from type C compensated cirrhosis is reportedly 7.1% in Japan and 3.7% in both Europe and America; and the annual incidence of HCC from chronic hepatitis is 1.8% in Japan and 0% in both Europe and America.[17] When such natural courses are taken into account, the treatment goals for type C chronic hepatitis are to prevent the progression to cirrhosis and to inhibit hepatic carcinogenesis.

In 1995, we examined (using an RCT) the inhibitory effect of IFN therapy on hepatic carcinogenesis for type C cirrhosis.[44,45] Ninety patients with type C cirrhosis were divided into two groups: the IFN treatment group and the untreated group. We examined the long-term clinical effects of IFN therapy. In the IFN treatment group, an SVR occurred in seven patients and a biological response (BR) occurred in six patients. In the untreated group, the spontaneous disappearance of the HCV and sustained normalization of ALT level did not occur. During the mean follow-up period of 8.2 years, the cumulative incidence rate of HCC was significantly lower in the IFN treatment group than in the untreated group (27% vs. 73%, respectively) ($P = 0.001$). The relative risk (RR) was 0.256. A multicenter Japanese study – the Inhibition of Hepatocarcinogenesis by Interferon Therapy (IHIT) study – showed that, compared with the untreated group, the risk of hepatic carcinogenesis was inhibited by 0.51-fold in the IFN treatment group; the RR of hepatic carcinogenesis was 0.197 in patients who achieved SVR with IFN therapy.[46] To our knowledge, seven RCT papers have been published since 1995 that investigated the inhibitory effect of IFN therapy on hepatic carcinogenesis (Table 2).[44,47–52] In a study involving 99 patients with compensated cirrhosis, Valla *et al.*[47] administered a 3 MU dose of IFN-α2b three times weekly for 48 weeks to 52 patients (i.e. the treatment group). A mean follow-up period of 3.3 years showed that HCC occurred in five patients in the treatment group and in nine patients in the control group; however, there was no statistically significant difference between the groups. On the other hand, the results of a meta-analysis by Cammà *et al.*[31] confirmed that IFN therapy inhibits hepatic carcinogenesis in patients with type C cirrhosis. Their investigation of 3109 patients in three RCT studies[44,47,53] and 11 NRCT studies[28,30,46,54–61] showed that the risk of developing HCC in the IFN treatment group was reduced by 12.8% (95% CI, −8.3% to −17.2%), compared with the risk in the untreated group. They reported that, especially in patients who obtained a SVR, there was a marked inhibition of hepatic carcinogenesis (as indicated by an RD of −19.1%). Even in people who did not have a SVR, the RD was significantly reduced (at −11.8%). Miyake *et al.*[62] reported that hepatic carcinogenesis was inhibited in the IFN-treated group, compared with the untreated group (RR, 0.45; 95% CI, 0.31–0.65), based on their meta-analysis of three RCT studies[47–49] and six

Table 2 Baseline characteristics of randomized control trials assessing effect of interferon (IFN) on hepatocellular carcinoma (HCC) development in hepatitis C virus (HCV)-infected patients

Study [reference]	Year	Sample size (*n*)	Male (%)	Mean age (years)	Pre-existing cirrhosis (%)	IFN regimen	Mean follow-up (years)	SVR (%)	Incidence of HCC (%)
Nishiguchi S *et al.*[44]	1995	T45	T62	T55	T100	α: 6 MU 3 times a week for 24 weeks	T4.4	T16	T4
		C45	C51	C57	C100		C5.5	C0	C38
Valla DC *et al.*[47]	1999	T45	T73	T57	T100	α-2b: 3 MU 3 times a week for 48 weeks	3.3	NA	T11
		C49	C65	C56	C100				C18
Bernardinello E *et al.*[48]	1999	T38	T50	T56	T100	β: 6 MU 3 times a week for 24 weeks followed by 3 MIU for another 24 weeks	5	T3	T5
		C23	C61	C58	C100			C0	C4
Francesco A *et al.*[50]	2004	T30	T57	T55	T100	α-2b: 6 MU daily for 1 month followed by 3 MIU daily for 11 months plus ribavirin 1 g daily for 12 months	5	T43	T0
		C30	C60	C57	C100			C0	C30
Soga K *et al.*[49]	2005	T103	T49	T52	T0	α, α-2a or α-2b: 3–10 MU daily for 2–4 weeks and 3 times a week for total of 14–28 weeks or β; 3–6 MU daily for 6–8 weeks	7.8	T32	T5
		C30	C43	C54	C0			C0	C23
Fartoux L *et al.*[51]	2007	T51	T45	T60.5	T100	α-2a: 3 MU 3 times a week for 2 years	2	T0	T12
		C51	C45	C60.5	C100			C0	C12
Lok AS *et al.*[52]	2009	T495	T71	T50	T40	PEG-IFN α-2a: 90 μg weekly for 3.5 years	T4.6	T0	T4.6
		C510	T79	C53	C41		C4.9	C0	T4.9

C, control group (no treatment); MU, million units; NA, not available; PEG-IFN, pegylated interferon; SVR, sustained virological response; T, IFN treated group.

NRCT studies[50,55,58,63–65] published between 1989 and 2009.

The inhibitory effect of IFN is furthermore demonstrated in non-responders (NRs) to IFN therapy (RR, 0.48; 95% CI, 0.26–0.66). Zhang *et al.*[35] recently performed a meta-analysis on the effect of non-maintenance IFN therapy on hepatic carcinogenesis. They used only four RCT papers (in three papers, the subjects were patients with type C cirrhosis).[45,47–49] The results indicated that IFN therapy inhibited hepatic carcinogenesis in the IFN treatment group, compared with the untreated group (RR, 0.39; 95% CI, 0.26–0.59). The results of IFN therapy, when focusing only on patients with cirrhosis, also showed the same inhibitory effect (RR, 0.44; 95% CI, 0.28–0.68). In one study, patients who were initially NR to IFN therapy were divided into two groups: a maintenance IFN treatment group and an untreated group.[51,52] An analysis of the results showed that IFN therapy has no inhibitory effect on hepatic carcinogenesis.

In the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial,[52] 1005 patients had cirrhosis and had chronic hepatitis that progressed to fibrosis (i.e. bridging fibrosis). Of these, patients who were unresponsive to a combination therapy with PEG-IFN and ribavirin (RBV) were divided into two groups – the maintenance treatment group (PEG-IFN α-2a, 90 μg) and the untreated group. The incidence of HCC in each group was investigated. During a mean follow-up period of 4.6 years, there was no difference between the two groups in the incidence of HCC. In a continuation of the HALT-C report,[7] the mean follow-up period was extended to 6.1 years. The results showed that IFN therapy inhibited hepatic carcinogenesis in patients with cirrhosis in the maintenance IFN treatment group, compared with its inhibitory effect in the untreated group (HR, 0.45; 95% CI, 0.24–0.83). On the negative side, maintenance IFN therapy insufficiently inhibited hepatic carcinogenesis in patients with chronic hepatitis that had progressed to fibrosis. However, the incidence of HCC was reduced in these patients if their liver had a histological improvement with IFN therapy.

One report shows that maintenance IFN therapy reduces the incidence of HCC in elderly patients with chronic hepatitis, compared with patients in the untreated group.[66] Kumada *et al.* state that the administration of IFN therapy is important in normalizing serum ALT level or reducing the AFP level, even if HCV does not disappear.[6] For non-SVR patients receiving IFN therapy, a patient's age is an important risk factor for hepatic carcinogenesis, and the annual incidence of HCC in patients with chronic hepatitis and hepatic fibrosis is significantly higher in aged people than in young people.[67] It can be accordingly conjectured that the reason for a higher complication rate of HCC among HCV carriers in Japan than among HCV carriers in the USA is that Japanese carriers have a higher mean age and a higher extent of hepatic fibrosis.[68] These factors may be responsible for the difference in the inhibitory effect of IFN therapy on hepatic carcinogenesis noted between patients in Japan and patients in the USA.

Based on past studies investigating the inhibitory effect of IFN therapy on hepatic carcinogenesis in patients with type C chronic liver diseases, a consensus has been reached concerning the following three points:

- Point 1: Patients achieving SVR with IFN therapy have a reduced HCC incidence rate and an improved HCC prognosis.[46,69,70]
- Point 2: Because of the use of combination therapy (e.g. PEG-IFN and RBV) in recent years, the treatment outcome has improved and the SVR ratio is about 50% to 80%.[71] However, for patients with chronic hepatitis that has progressed to fibrosis and for patients with compensated cirrhosis, IFN therapy alone reduces the SVR ratio and reduces the incidence of complications associated with the liver (including hepatic carcinogenesis).[69]
- Point 3: IFN therapy reduces the incidence rate of HCC when a BR is achieved or when there is a histological improvement.[66,72]

Further examinations are needed to determine whether maintenance IFN therapy inhibits hepatic carcinogenesis and whether there is any difference between IFN therapy NR patients and IFN-untreated patients in the rate of hepatic carcinogenesis. Studies are needed that take into account the amount of IFN administered, the administration period, and the use of RBV and novel concurrent drugs. In addition, we expect that combination therapy with PEG-IFN and RBV for patients with compensated cirrhosis will be promptly price-listed in Japan.

RECURRENCE INHIBITION AFTER LOCALLY CURATIVE TREATMENT FOR HCC

EVEN IF LOCALLY curative treatment for HCC is performed, HCC relapses occur at an annual rate of 15% to 20%. This high rate is not caused by any other malignant neoplasms, and it results in a high mortality.[73] To improve the prognosis of patients with HCC, measures are needed that advance HCC treatment and inhibit recurrence.

Basic investigations reveal that IFN has anti-viral activity and it inhibits the growth of HCC.[74,75] In a retrospective examination, Someya *et al.* reported that singlevariate and multivariate analyses showed that IFN therapy inhibits recurrence in patients with HCC-complicated type B cirrhosis after locally curative treatment.[76] Lo *et al.*[77] performed an RCT, using as subjects 40 patients who had undergone a radical hepatic resection because of HBV-related HCC. On examining the IFN treatment group (in which patients were administered 10 MU/m^2 of IFN-α2b three times weekly for 12 weeks) and the untreated group, they found that the one-year and 5-year survival rates were 97% and 79%, respectively, in the IFN treatment group and 85% and 61%, respectively, in the untreated group. Therefore, the IFN treatment group had a better prognosis ($P = 0.137$). A multivariate analysis demonstrated that IFN therapy may reduce the risk of death (HR, 0.42; 95% CI, 0.17–1.05; $P = 0.063$). In the examination of subgroups, there was no difference between the IFN treatment group and the untreated group in the 5-year survival rate in patients at stage I/II; however, in patients at stage III/IV A, IFN therapy inhibited the early recurrence of HCC and improved the 5-year survival rate from 24% to 68% ($P = 0.038$). Sun *et al.*[78] in their RCT also reported that IFN therapy was useful after an operation for HCC and that the median overall survival time and median disease-free time were significantly longer in treated patients, compared with the untreated patients.

We found six RCT studies that examined the inhibitory effect of IFN therapy on recurrence after locally curative treatment for HCV-related HCC.[79–84] Ikeda *et al.*[79] and Kubo *et al.*[80] showed that IFN therapy significantly inhibits the recurrence of HCC. Shiratori *et al.*[81] reported no difference between the IFN-treated group and the control group with the first relapse of HCC, but noted that IFN therapy inhibits a second or later recurrence of HCC. Only Mazzaferro *et al.*[83] reported that IFN therapy shows no significant difference between the IFN treatment group and the control group; however, at the first relapse of HCC, IFN therapy inhibits recurrence in patients having a single tumor that is free from vascular invasion and has a diameter of less than 3 cm. An examination of NRCT, which were performed in Japan, also showed that IFN therapy significantly inhibits the relapse of HCC (especially in patients who receive IFN treatment aimed at eliminating HCV), achieves an SVR,[85–87] and improves survival rates.[88] Maintenance IFN therapy after the locally curative treatment of HCC reportedly inhibits recurrence.[85,89,90] Kudo *et al.*[89] reported that IFN therapy inhibits the first relapse (as well as a second or third relapse) and improves the prognosis. We also demonstrated that long-term maintenance IFN therapy, given after the combination therapy with PEG-IFN and RBV, effectively inhibits HCC recurrence and improves prognosis.[91] Singal *et al.*[92] performed a meta-analysis of five RCT papers[79,81–83,93] and five NRCT papers.[87,89,94–96] They reported that IFN therapy inhibits HCC recurrence (odds ratio [OR], 0.31; 95% CI, 0.26; $P < 0.0001$) and significantly extends the overall survival time. Furthermore, Zhang *et al.*[97] conducted a meta-analysis of six RCT papers (Two papers focused on HBV-related HCC and four papers focused on HCV-related HCC).[77,78,80–83,93] Their meta-analysis showed that IFN therapy inhibits early recurrence (OR, 0.62; 95% CI, 0.42–0.93; $P = 0.02$) and improves the one-year survival rate (OR = 3.14; 95% CI = 1.79–5.52; P (00.0001). Shen *et al.*[98] similarly performed a meta-analysis of 13 papers on HBV-related and HCV-related HCC (nine papers involved RCT[77–84,93], and four papers involved NRCT[87,89,94,99]). From this, they concluded that IFN therapy improved the one-year, 2-year, and 3-year recurrence-free survival rates in the IFN treatment group, compared with the control group.

Based on past studies investigating the inhibitory effect of IFN therapy on HCC recurrence after locally curative treatment, HCC recurrence is reduced through HCV clearance. Thus, IFN therapy for viral eradication is recommended for patients with hepatitis C if possible. Meanwhile, in patients with hepatitis B, IFN therapy after locally curative treatment may improve their prognosis. Further examinations are needed to determine whether IFN therapy after locally curative treatment reduces HCC recurrence in patients with hepatitis B.

FINAL COMMENTS

FOR PATIENTS WITH chronic hepatitis B, IFN therapy reduces the risk of hepatic events (including the inhibitory effect for developing HCC) particularly among responders to treatment in Asian, but not in European patients. The progression to cirrhosis and a high level of HBV DNA (greater than 10^5 copies/mL) are strong risk factors for hepatic carcinogenesis from type B chronic liver diseases.[37] Liaw *et al.*[100] reported that therapy with lamivudine, a nucleos(t)ide analog, significantly reduces the progression to non-compensated cirrhosis and inhibits the development of HCC. Matsumoto *et al.*[101] also had similar results in a multicenter study of Japanese patients with type B chronic hepatitis. As for inhibition of hepatic carcinogenesis from type B chronic liver diseases, measures for hepatitis

are important, after taking age, amount of HBV DNA, extent of background liver disorder, HBV genotype, and others into account, according to guidelines, use of IFN or nucleos(t)ide analogs needs to be determined.[18,37]

It is important that IFN-based therapy obtains SVR to inhibit the development of hepatic carcinogenesis from type C chronic liver diseases. Thus, IFN therapy is recommended for patients with chronic hepatitis C. Tanaka N *et al.*[102] reported single nucleotide polymorphisms (SNPs) in the IL28B locus. These polymorphisms are extremely effective for estimating the effects of IFN therapy; they provide a novel indicator to help determine a patient's therapy, and will be used clinically.[103] New anti-viral drugs are being developed for treating type C chronic hepatitis. Combination therapy using PEG-IFN, RBV, and a protease inhibitor reportedly improves the SVR rate.[68] In addition, the acyclic retinoid, studied and developed in Japan, is expected to show a strong inhibitory effect on hepatic carcinogenesis.[104]

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Original Article

Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B

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Aim: The factors associated with hepatitis recurrence after discontinuation of nucleos(t)ide analogs (NAs) in patients with chronic hepatitis B were analyzed to predict the risk of relapse more accurately.

Methods: A total of 126 patients who discontinued NA therapy were recruited retrospectively. The clinical conditions of a successful discontinuation were set as alanine aminotransferase (ALT) below 30 IU/L and serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL.

Results: Relapse of hepatitis B were judged to occur when maximal serum ALT became higher than 79 IU/L or when maximal serum HBV DNA surpassed 5.7 log copies/mL following NA discontinuation since these values corresponded with mean values of ALT (30 IU/L) and HBV DNA (4.0 log copies/mL), respectively. At least 90% of patients with either detectable hepatitis B e antigen or serum HBV DNA higher than 3.0 log copies/mL at the time of NA discontinuation relapsed within one year. In the remaining patients, higher levels of both hepatitis B surface and core-related antigens at the time of discontinuation, as well as a shorter course of NA treatment, were significantly associated with relapse by multivariate analysis.

Conclusions: It appears that negative results for hepatitis B e antigen and serum HBV DNA lower than 3.0 log copies/mL are essential for successful NA discontinuation, which may be attained by a longer treatment period. Levels of hepatitis B surface and core-related antigens are also significant factors independently associated with relapse of hepatitis.

Key words: discontinuation, hepatitis B core-related antigen, hepatitis B surface antigen, nucleos(t)ide analogs, relapse of hepatitis

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern that has an estimated 350 to 400 million carriers worldwide. Chronic infection with HBV can cause chronic hepatitis, and may eventually develop into liver cirrhosis and hepatocellular carcinoma.[1-3] Over the last decade, major advances in the treatment of chronic hepatitis B have been made with nucleos(t)ide

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analogs (NAs) such as lamivudine (LVD), adefovir dipivoxil (ADV), and entecavir (ETV).[4] NAs are orally administered and are associated with low rates of adverse effects. Treatment with NAs shows strong suppression of HBV replication and consequently rapid improvement of elevated ALT levels. Furthermore, these drugs have been reported to lower the risk of complicating cirrhosis and hepatocellular carcinoma,[5–7] and so NAs are becoming widely used to treat patients with chronic hepatitis B. On the other hand, NAs carry the risk of developing drug-resistance;[8] drug-resistant viruses emerging during treatment may be associated with hepatitis flare-ups. Hepatitis B patients are also required to undergo prolonged treatment with NAs because early discontinuance often leads to relapse of hepatitis and ensuing hepatic failure following rises in alanine aminotransferase (ALT) level.[9,10]

Serum HBV DNA is normally used to monitor the antiviral effect of NAs. HBV DNA decreases rapidly and becomes undetectable in the majority of patients who are treated with NAs,[11–13] but relapse after discontinuation is not rare.[14–17] Since it is also true that favorable virological and biochemical responses to NAs may continue indefinitely in some patients,[9,15] reliable markers that can predict relapse of hepatitis after NA discontinuation are needed. Such markers would benefit not only patients who are considering discontinuation of NA treatment, but also clinicians, hospitals, and the medical economy.

In the present study, we assessed several factors associated with relapse of hepatitis after discontinuation of NAs in patients with chronic hepatitis B, including hepatitis B viral antigens, which have been reported as new and promising markers for monitoring the effect of antiviral agents, such as interferon and NAs.

METHODS

Patients

A TOTAL OF 126 patients with chronic hepatitis B who underwent and completed NA treatment between 2000 and 2010 were enrolled in this study. Patients were recruited retrospectively from 11 hospitals across Japan (Toranomon Hospital, Hokkaido University Hospital, Nagoya City University Hospital, Shinshu University Hospital, Hiroshima University Hospital, National Hospital Organization Nagasaki Medical Center, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Japanese Red Cross Nagoya Daini Hospital, and Tokyo Women's Medical University Hospital, Sapporo Kosei General Hospital) and met the following conditions: (i) serum ALT higher than 30 IU/L and serum HBV DNA higher than 4.0 log copies/mL were observed at least twice within the 6 months prior to administration of NAs; (ii) stored serum samples at initiation and discontinuation of NAs were available for measurements of viral markers; (iii) clinical outcomes were followed for at least 6 months after the discontinuation of NAs; and (iv) tests for hepatitis C and human immunodeficiency virus antibodies were negative. Hepatitis B surface antigen (HBsAg) was confirmed to be positive on at least two occasions at least 6 months apart in all patients before treatment. Patients complicated with hepatocellular carcinoma or signs of hepatic failure at treatment discontinuation were excluded from the study. Our cohort consisted of 83 men and 43 women with a median age of 46 (range, 19 to 79) years when NA administration was discontinued. Hepatitis B e antigen (HBeAg) was positive in 64 patients (51%) at the initiation of treatment and in 24 patients (19%) at its discontinuation. HBV genotype was A in two (2%) patients, B in five (4%), C in 102 (81%), and undetermined in 17 (13%). Thirty-five of the 126 patients in this study were younger than 35 years old. Although not recommended as the first line treatment for this group by Japanese guidelines,[18] NA treatment was commenced since chronic active hepatitis had been persisting in all cases irrespective of their HBeAg status (26 positive and nine negative) at the initiation of treatment.

The decision to discontinue NAs was made by individual physicians using similar, but not uniform, conditions. Four patients who halted NAs for financial reasons were included. No patient underwent interferon treatment during or after NA treatment. The decision to recommence NA administration was also made by individual physicians, essentially when relapse of hepatitis became obvious. With few exceptions, patients were seen at least once a month during the first year after discontinuation of NAs, and at least once every several months afterwards. Stored serum samples were kept frozen at −20°C or below until assayed. This study was approved by the Ethics Committees of all participating institutions.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and antibody to HBe (anti-HBe) were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd, Tokyo, Japan; Fujirebio Inc., Tokyo, Japan; and/or Sysmex Co., Kobe, Japan) at each hospital. Quantitative measurement of HBsAg[19] was done using a chemiluminescence enzyme immunoassay

(CLEIA)-based HISCL HBsAg assay manufactured by Sysmex Corporation (Kobe, Japan). The assay had a quantitative range of −1.5 to 3.3 log IU/mL. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range.

Serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche, Tokyo, Japan),[20] which had a quantitative range of 2.6 to 7.6 log copies/mL. Serum HBV DNA was also determined using a COBAS TaqMan HBV kit (Roche, Tokyo, Japan)[21] with a quantitative range of 2.1 to 9.0 log copies/mL in 43 patients whose serum samples were available at the time of NA discontinuation. According to the manufacturer's instructions, detection of a positive signal below the quantitative range was described as a positive signal, and no signal detection was described as a negative signal. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami *et al.*[22]

Serum hepatitis B core-related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously.[23,24] Briefly, 150 μL of serum was incubated with pretreatment solution and then added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After incubation and washing, further incubation was carried out with alkaline phosphatase conjugated with two kinds of monoclonal antibodies against denatured HBcAg, HBeAg, and the 22 kDa precore protein. Following washing, a substrate solution was added to the test cartridge and then incubated. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with a quantitative range set at 3.0 to 6.8 log U/mL.

Statistical analyses

A linear regression model was used to examine for associations between mean and maximal values of both ALT and HBV DNA. Correlations between variables were calculated using the Spearman's rank correction correlation coefficient test. Each cut-off value was decided using receiver operating characteristic curve (ROC) analysis and results were evaluated by measuring the area under the curve (AUC). The Fisher's exact and Pearson's χ^2 tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U*-test was used. The Kaplan–Meier method was used to estimate rates of non-relapse observations, and the log-rank test was used to test hypotheses concerning differences in non-relapse observations between selected groups. Multivariate analyses were performed using the Cox regression model. Variables associated with a *P*-value < 0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with relapse of hepatitis after discontinuation of NAs. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc., Tokyo, Japan). *P*-values of less than 0.05 were considered to be statistically significant.

RESULTS

Definition of hepatitis relapse after discontinuation of NAs

THE CLINICAL CONDITIONS of a successful discontinuation of NAs were set at serum HBV DNA below 4.0 log copies/mL and ALT below 30 IU/L according to the Japanese guidelines for the treatment of hepatitis B.[18] However, these criteria could not be directly applied to our cohort as post-therapy fluctuations in ALT and HBV DNA were difficult to evaluate consistently. In total, 26 (76%) of 34 patients with successful discontinuation of NAs showed transient abnormal levels of ALT and/or HBV DNA, especially during the early phase after cessation. We therefore used mean and maximal values of these markers to evaluate relapse of hepatitis B in this study; mean values were used to evaluate relapse of hepatitis as a whole, and maximal values were used to dynamically assess relapse during the follow-up period after NA discontinuation. Both ALT and HBV DNA were measured 11.0 times per year on average during the first year and 4.1 times per year on average thereafter.

The mean values of HBV DNA were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.853. Similarly, the mean values of ALT were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.940 (Fig. 1). The mean HBV DNA value of 4.0 log copies/mL corresponded to a maximal HBV DNA value of 5.7 by ROC analysis (AUC = 0.930, $P < 0.001$), and the mean ALT value of 30 IU/L corresponded to a maximal ALT value of 79 IU/L (AUC = 0.988, $P < 0.001$). These results suggested that patients having serum HBV DNA higher

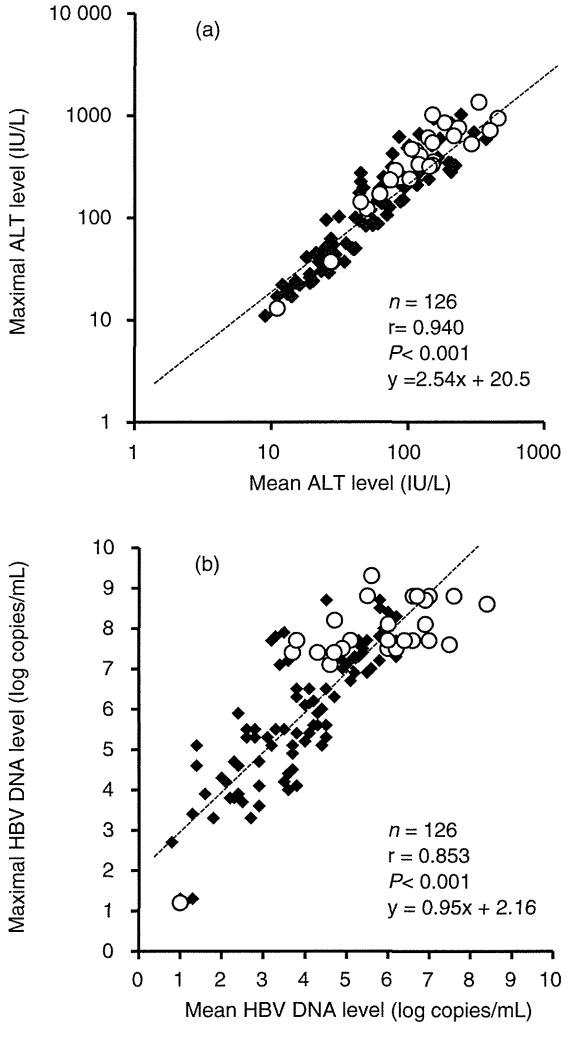

Figure 1 Correlation between maximal and mean levels of alanine aminotransferase (ALT) (a) and hepatitis B virus (HBV) DNA (b) after discontinuation of nucleos(t)ide analogs (NAs). Open circles indicate patients with detectable hepatitis B e antigen (HBeAg) and closed squares indicate patients without detectable HBeAg.

than 5.7 log copies/mL during the follow-up period after NA discontinuation were not likely to achieve the HBV DNA criterion of a successful discontinuation of below 4.0 log copies/mL. Similarly, it could be inferred that patients reaching ALT levels higher than 79 IU/L would also not likely achieve the ALT criterion of a successful discontinuation of below 30 IU/L.

Based on our findings, we judged that a relapse of hepatitis B occurred when serum ALT exceeded 79 IU/L or when serum HBV DNA exceeded 5.7 log copies/mL following NA discontinuation. Accordingly, 92 (73%) of the 126 patients enrolled in the present study showed a relapse. We set the follow-up period as discontinuation to relapse for relapse patients and as discontinuation to the last recorded examination for patients without relapse. Whereas re-administration of NAs due to relapse was commenced in 70% of relapse patients in the follow-up period, none was performed in non-relapse patients during that time.

Elimination of cases likely to show relapse of hepatitis

As it is generally believed that patients who are positive for HBeAg and/or have a higher level of HBV DNA at discontinuation of NAs are likely to relapse, these factors were assessed first. The progression of analyses in the present study and the population structure of each analysis are shown in Figure 2.

The non-relapse rate was compared using the Kaplan–Meier method between 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL and 95 patients with levels lower than 3.0 log copies/mL when NAs were discontinued (Fig. 3). The revised cut-off value of 3.0 log copies/mL was determined by ROC analysis (AUC = 0.709, $P < 0.001$). Thirty (97%) of 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL relapsed within one year of discontinuation. On the other hand, approximately 30% of patients with levels lower than 3.0 log copies/mL showed prolonged non-relapse. Thus, the 31 patients with high HBV DNA at the time of discontinuation were eliminated from the following analyses.

In the remaining 95 patients, the non-relapse rate was compared using the Kaplan–Meier method between 10 patients with detectable HBeAg and 85 patients without HBeAg when NAs were discontinued (Fig. 4). Ninety percent of patients with HBeAg experienced relapse within one year, which was significantly ($P = 0.005$) higher than in cases without HBeAg. In patients without HBeAg, the non-relapse rate decreased rapidly during the first year to approximately 45%, and then decreased relatively slowly over the following 3 years to nearly 30%. It is noteworthy that this subgroup did not relapse afterwards. Since the relapse rate was high among patients with detectable HBeAg, they were excluded from the following analyses as well.

Factors associated with relapse of hepatitis after discontinuation of NAs

Additional factors associated with relapse of hepatitis were analyzed in the remaining 85 patients who were