Table 2 Comparison of pre-treatment factors between patients with and without sustained virological response (SVR) among the model building dataset ($n = 506$)

	SVR ($n = 240$)	Non-SVR ($n = 266$)	p
Age (years)	54 (25–75)	60 (36–73)	<0.0001
Male gender[a]	151/240 (63%)	171/266 (41%)	<0.0001
Body mass index (kg/m^2)	22.5 (16.8–32.0)	22.6 (15.5–33.3)	0.244
Albumin (g/dl)	4.1 (3.2–5.0)	4 (2.7–4.9)	0.004
Creatinine (mg/dl)	0.7 (0.44–1.14)	0.69 (0.39–1.47)	<0.0001
AST (IU/l)	59 (11–370)	61 (17–261)	0.457
ALT (IU/l)	58 (11–413)	53 (11–316)	0.031
GGT (IU/l)	31 (10–322)	43 (12–328)	0.005
Total cholesterol (mg/dl)	175 (87–297)	171 (73–274)	0.184
Triglyceride (mg/dl)	105 (36–474)	105 (33–294)	0.992
White blood cell count (/μl)	4,600 (2,200–10,900)	4,425 (1,800–10,810)	0.479
Neutrophils (/μl)	2,507 (667–7,870)	2,423 (900–7,281)	0.321
Red blood cell count (/μl)	455 (336–577)	441 (313–564)	0.001
Hemoglobin (g/dl)	14.3 (10.2–17.6)	13.9 (9.4–17.9)	0.004
Hematocrit (%)	42.1 (13.3–53.7)	41.2 (30.7–52.0)	0.031
Platelets (10^9/l)	178 (81–380)	142 (60–320)	<0.0001
AFP (ng/ml)	4.3 (0.9–680)	6.4 (1.9–468)	0.041
HCVRNA (10^3 IU/ml)	1,400 (100–5,100)	1,700 (100–5,100)	0.659
Fibrosis stage: F3–4[a]	21/198 (11%)	52/219 (24%)	<0.0001

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

Table 3 Multivariate logistic regression analysis for factors associated with sustained virological response (SVR)

	Odds	95% CI	p value
Age (years)	0.96	0.94–0.98	0.001
Platelets (10^9/l)	1.09	1.04–1.14	<0.0001
ALT (IU/l)	1.01	1.00–1.01	0.001
GGT (IU/l)	0.99	0.98–0.99	<0.0001
Male gender	2.92	1.87–4.55	<0.0001

GGT gamma-glutamyltransferase

Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of response to PEG-IFN/RBV. The analysis highlighted 5 variables relevant to response: age, gender, platelet count, AFP and GGT. Classification based on these variables identified subgroups of patients with high probabilities of achieving SVR among difficult to treat genotype 1b chronic hepatitis C patients. The reproducibility of the model was confirmed by the independent internal and external validation datasets. An advantage of the decision tree analysis over traditional regression models is that the decision tree model is user-intuitive and can be readily interpreted by medical professionals without any specific knowledge of statistics. Patients can be allocated to specific subgroups with a defined rate of response simply by following the flow-chart form. Using this model, an estimate of the response before treatment can be rapidly obtained, which may facilitate clinical decision making. Thus, this model could be readily applicable to clinical practice.

According to the results of the decision tree analysis, patients were categorized into 3 groups: the rate of SVR was 23–30% for the low probability group, 44–45% for the intermediate probability group and 65–76% for the high probability group. About 30% of patients were each categorized in the high and low probability group and the remaining 40% of patients in the intermediate probability group. These results support the evidence-based approach for selecting an optimum treatment strategy for individual patients. For example, patients in the high probability group may be the most suitable candidates for PEG-IFN/RBV therapy, while patients in the low probability group may be advised to wait for a future therapy, such as the combination of protease inhibitor, PEG-IFN and RBV. However, the estimation of low probability should not be used to preclude patients from therapy, and the final decision should be made on a case-by-case basis, taking into consideration the acceptance by the patient of a low likelihood of response and the potential risk of disease progression while waiting for a future therapy.

Another important finding was that poor adherence to drugs lowered the rate of SVR in the low and intermediate probability groups, which implies that effort should be made to maintain ≥80% of the planned dose of PEG-IFN and RBV in those patients. On the other hand, the rate of SVR was high irrespective of drug adherence in the high probability group. Whether shorter duration of therapy is sufficient in this group of patients should be confirmed in future study.

The variables used in the decision tree have been previously reported to associate with the efficacy of IFN therapy. Younger age and male gender are associated with a favorable response [28]. Lower platelet count is a hallmark of advanced fibrosis in chronic hepatitis C and is reported to be associated with poor response to IFN [29]. AFP is usually used for the screening or the diagnosis of hepatocellular carcinoma, but recent studies suggest an association between higher AFP levels and poor response to IFN therapy [30–33]. Previous report speculated that higher expression of AFP by hepatic progenitor cells may be associated with non-response to therapy [30]. Another report speculated that AFP levels predict poor response to therapy through the underlining link to advanced liver fibrosis [31]. Our data support the latter speculation since advanced fibrosis was associated with elevation of AFP levels. Fibrosis of the liver is an important predictor of response, but we did not include this factor in the decision tree analysis since liver biopsy may not always be available in general practice. As a result, two predictive factors that correlate with fibrosis stage (platelet counts and AFP) were selected in the model, and three probability groups reflected the different distribution of fibrosis stage. GGT is reported to be associated with insulin resistance and hepatic steatosis [34–37], a factor that confers resistance to IFN therapy [38–44]. What is unique to the present study is the visualization of response probability by combining these factors and its high reproducibility revealed by a high-quality validation of the model by internal and external validation datasets that were completely independent of the model building dataset. Since factors used in the model were clinical parameters that are readily available by the usual workup of patients, this model could be immediately applicable to clinical practice without imposing costs for additional examinations.

A potential limitation of this study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset but are not reproducible in different populations. Although internal and external validations showed that our model had high reproducibility, we recognize that further validation on a larger external validation cohort, especially in populations other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a pre-treatment model for the prediction of virological response to PEG-IFN/RBV. Because this decision tree model was made up of simple variables, it can be easily applied to clinical practice. This model may have the potential to support decisions about patient selection for PEG-IFN/RBV based on a possibility of response weighed against the potential risk of adverse events or costs.

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References

1. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. Hepatology. 2004;39: 1147–71.
2. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347: 975–82.
3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001; 358:958–65.
4. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41:1105–9.
5. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet. 2009;41:1100–4.
6. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
7. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Investig. 1995;96:224–30.
8. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med. 1996;334:77–81.
9. Kurosaki M, Enomoto N, Murakami T, Sakuma I, Asahina Y, Yamamoto C, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology. 1997;25:750–3.
10. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, et al. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology. 2005;48:372–80.
11. Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, et al. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol. 2009;44:952–63.
12. Breiman LJH, Friedman RA, Olshen CJ, Stone CM. Classification and regression trees. Calif: Wadsworth; 1980.
13. Garzotto M, Beer TM, Hudson RG, Peters L, Hsieh YC, Barrera E, et al. Improved detection of prostate cancer using classification and regression tree analysis. J Clin Oncol. 2005;23:4322–9.
14. Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. J Epidemiol. 2002;12:243–8.
15. Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center:

implications on the future of cancer staging. Surgery. 2002;132: 589–602.
16. Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the german dermatological society. J Clin Oncol. 2004;22:3660–7.
17. Valera VA, Walter BA, Yokoyama N, Koyama Y, Iiai T, Okamoto H, et al. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. Ann Surg Oncol. 2007;14:34–40.
18. Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. Clin Cancer Res. 2005;11:5440–3.
19. Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, et al. Phosphorus ans an early predictive factor in patients with acute liver failure. Transplantation. 2003;75: 2007–14.
20. LeBlanc M, Crowley J. A review of tree-based prognostic models. Cancer Treat Res. 1995;75:113–24.
21. Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, et al. A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. Hepatol Res. 2010;40:251–60.
22. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. Hepatology. 1996;24:289–93.
23. Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. Stat Med. 1989;8:539–50.
24. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology. 2003;38:645–52.
25. Lee SS, Ferenci P. Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. Antivir Ther. 2008;13(Suppl 1):9–16.
26. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med. 2009;360:1839–50.
27. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med. 2009;360:1827–38.
28. Sezaki H, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, et al. Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci. 2009;54:1317–24.
29. Shiratori Y, Omata M. Predictors of the efficacy of interferon therapy for patients with chronic hepatitis C before and during therapy: how does this modify the treatment course? J Gastroenterol Hepatol. 2000;15(Suppl):E141–51.
30. Gad RR, Males S, El Makhzangy H, Shouman S, Hasan A, Attala M, et al. Predictors of a sustained virological response in patients with genotype 4 chronic hepatitis C. Liver Int. 2008;28: 1112–9.
31. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol. 2007;79:1686–95.
32. Males S, Gad RR, Esmat G, Abobakr H, Anwar M, Rekacewicz C, et al. Serum alpha-foetoprotein level predicts treatment outcome in chronic hepatitis C. Antivir Ther. 2007;12:797–803.
33. Bayati N, Silverman AL, Gordon SC. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. Am J Gastroenterol. 1998;93:2452–6.
34. Fraser A, Ebrahim S, Smith GD, Lawlor DA. A comparison of associations of alanine aminotransferase and gamma-glutamyltransferase with fasting glucose, fasting insulin, and glycated hemoglobin in women with and without diabetes. Hepatology. 2007;46:158–65.
35. Marchesini G, Avagnina S, Barantani EG, Ciccarone AM, Corica F, Dall'Aglio E, et al. Aminotransferase and gamma-glutamyltranspeptidase levels in obesity are associated with insulin resistance and the metabolic syndrome. J Endocrinol Investig. 2005;28:333–9.
36. Soresi M, Tripi S, Franco V, Giannitrapani L, Alessandri A, Rappa F, et al. Impact of liver steatosis on the antiviral response in the hepatitis C virus-associated chronic hepatitis. Liver Int. 2006;26:1119–25.
37. Yaginuma R, Ikejima K, Okumura K, Kon K, Suzuki S, Takei Y, et al. Hepatic steatosis is a predictor of poor response to interferon alpha-2b and ribavirin combination therapy in Japanese patients with chronic hepatitis C. Hepatol Res. 2006;35:19–25.
38. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001;33:1358–64.
39. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol. 2007;46:403–10.
40. Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology. 2003;37:600–9.
41. Mazzella G, Salzetta A, Casanova S, Morelli MC, Villanova N, Miniero R, et al. Treatment of chronic sporadic-type non-A, non-B hepatitis with lymphoblastoid interferon: gamma GT levels predictive for response. Dig Dis Sci. 1994;39:866–70.
42. Thomopoulos KC, Theocharis GJ, Tsamantas AC, Siagris D, Dimitropoulou D, Gogos CA, et al. Liver steatosis is an independent risk factor for treatment failure in patients with chronic hepatitis C. Eur J Gastroenterol Hepatol. 2005;17:149–53.
43. Villela-Nogueira CA, Perez RM, de Segadas Soares JA, Coelho HS. Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-alpha and ribavirin. J Clin Gastroenterol. 2005;39:728–30.
44. Camps J, Crisostomo S, Garcia-Granero M, Riezu-Boj JI, Civeira MP, Prieto J. Prediction of the response of chronic hepatitis C to interferon alfa: a statistical analysis of pretreatment variables. Gut. 1993;34:1714–7.

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Original Article

Case–control study for the identification of virological factors associated with fulminant hepatitis B

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Background: Host and viral factors can promote the development of fulminant hepatitis B (FHB), but there have been no case–control studies for figuring out virological parameters that can distinguish FHB.

Methods: In a case–control study, virological factors associated with the development of FHB were sought in 50 patients with FH developed by transient hepatitis B virus (HBV) infection (FH-T) and 50 with acute self-limited hepatitis B (AHB) who were matched for sex and age. In addition, 12 patients with FH developed by acute exacerbation (AE) of asymptomatic HBV carrier (ASC) (FH-C) were also compared with 12 patients without FH by AE of chronic hepatitis B (AE-C).

Results: Higher HBV DNA levels, subgenotype B1/Bj, A1762T/G1764A, G1896A, G1899A and A2339G mutation were significantly more frequent ($P < 0.05$), while hepatitis B e-antigen was less frequent in the FH-T patients than AHB. In multivariate analysis, G1896A mutation (odds ratio [OR], 13.53; 95% confidence interval [CI], 2.75–66.64), serum HBV DNA more than 5.23 log copies/mL (OR, 5.14; 95% CI, 1.10–24.15) and total bilirubin more than 10.35 mg/mL (OR, 7.81; 95% CI, 1.77–34.51) were independently associated with a fulminant outcome by transient HBV infection. On the other hand, in comparison with the patients between FH-C and AE-C groups, there was no significant difference of virological factors associated with the development of FHB.

Conclusion: A number of virological factors have been defined that may distinguish FH-T from AHB in a case–control study. The pathogenic mechanism of FHB between transient HBV infection and AE of ASC would be different.

Key words: acute exacerbation of asymptomatic hepatitis B virus carrier, fulminant hepatitis, genotypes, transient hepatitis B virus infection

INTRODUCTION

IN JAPAN, 634 patients with fulminant hepatitis (FH) were registered from 1998–2003. Of them, 41.8% were infected with hepatitis B virus (HBV) that is the most frequent cause of FH there.[1] HBV is classified into eight genotypes (A–H) based on a sequence divergence of more than 8% in the entire genome of approximately

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3200 nucleotides.[2–5] They have distinct geographical distributions and are associated with the severity of liver disease.[6,7] Furthermore, subgenotypes have been reported for HBV/A, B and C, and they are named A1/Aa (Asian/African type) and A2/Ae (European type),[8] B1/Bj (Japanese type) and B2/Ba (Asian type),[9] and C1/Cs (Southeast Asian type) and C2/Ce (East Asian type).[10,11] HBV genotypes/subgenotypes and mutations in the pre-core region and the core promoter can influence the viral replication and expression of hepatitis B e-antigen (HBeAg).[6,12]

Acute HBV infection in adulthood resolves in the most cases by far, but can induce FH or go on to become chronic in some. It has been reported that host and viral factors may influence the development of fulminant hepatitis B (FHB), but the pathogenesis of FHB remains unclear. As for virological factors associated with FHB, mutations in the core promoter (A1762T/G1764A)[13] and the pre-core region (G1896A)[14–16] have been reported in association with the development of FHB in Asia and the Middle East. Additional mutations, including T1753V, T1754V and A2339G in the core gene are implicated, also.[17,18] In regard of HBV genotypes, subgenotype B1/Bj is highly associated with the development of FHB in Japan.[15] In contrast, an association of HBV genotypes with the fulminant outcome has not been reproduced in patients from the USA and Europe.[19–22] Such a discrepancy would be attributed, at least in part, to distinct geographical distributions of HBV genotypes/subgenotypes over the world.

The original definition by Trey *et al.*[23] about fulminant hepatic failure is widely used all over the world. On the other hand, in Japan, the diagnosis of FH was contingent on a slight modification of Trey's original definition by the Inuyama Symposium (Aichi, Japan in 1981). Furthermore, the Intractable Liver Diseases Study Group of Japan modified the criteria for the etiology of FH and late-onset hepatic failure in 2002. According to the criteria of the Intractable Liver Diseases Study Group of Japan, there are two clinical entities of FHB that are induced, respectively, by transient HBV infection and acute exacerbation (AE) of an asymptomatic HBV carrier (ASC).[1]

Recently, FH developing in ASC who undergo AE is increasing in Japan.[1] In patients with hematological malignancy, in particular, rituximab and/or glucocorticoid, can reactivate HBV for the development of FHB.[24] The outcome is poor for FHB precipitating in ASC who undergo acute exacerbation,[1] but it has been difficult to identify it by clinical examinations.

As there have been no case–control studies for figuring out virological parameters that can distinguish FHB, a case–control study was conducted on the patients with FH by transient HBV infection and acute self-limited hepatitis B (AHB) in this study, for the identification of virological factors that influence a fulminant outcome. In addition, the patients with FH by AE of ASC, which is assumed as a different clinical condition from transient HBV infection, were also compared with the patients without FH by AE of chronic hepatitis B (CHB) in a case–control study.

METHODS

Patients

DURING 9 YEARS from 1998 to 2006, in twenty-six hospitals all over Japan, sera were obtained from the 50 FH patients by transient HBV infection (the FH-T group) and the 50 patients with AHB (the AHB group) who were controlled for age and sex. As the elder patients with FHB were enrolled in this study (mean age, 42.8 years), the mean age of AHB patients became relatively high (42.9 years, Table 1). Furthermore, the 12 FH patients developed by AE of ASC (the FH-C group) were also compared with the 12 patients without FH by AE of CHB who were matched by age and sex (the AE-C group).

All the serum samples tested for this study were collected at hospitalization. All 124 patients had hepatitis B surface antigen (HBsAg) in serum. Infection with hepatitis A virus and hepatitis C virus, as well as alcoholic hepatitis, were excluded in them.

The diagnosis of acute hepatitis B was based on sudden manifestation of clinical symptoms of hepatitis and detection of high-titered immunoglobulin (Ig)M anti-hepatitis B core (HBC). Patients with initial high-titered anti-HBC (>90% inhibition by a 1:200 diluted serum) were excluded. The diagnosis of FH was contingent on a slight modification by Inuyama Symposium (Aichi, Japan in 1981) of the original definition by Trey *et al.*:[23] (i) coma of grade II or higher; and (ii) a prothorombin time less than 40% developing within 8 weeks after the onset of hepatitis. To exclude AE of ASC in FH-T and AHB groups, we confirmed the negativity of HBsAg before onset of FHB or AHB and no family histories of hepatitis were found among all the patients. Furthermore, serum HBsAg in all patients with FH-T or AHB became naturally seronegative within 24 weeks. AE of ASC or CHB was defined as the elevation of alanine aminotransferase (ALT >300 IU/L) or total bilirubin (T.bil >3.0 mg/dL).[25] All 24 patients with AE of ASC or CHB could be confirmed positive for serum HBsAg before the onset of acute liver injury.

Table 1 Baseline characteristics between fulminant hepatitis B patients by transient infection (FH-T) and acute self-limited hepatitis B (AHB) patients

Features	FH-T ($n = 50$)	AHB ($n = 50$)	Differences *P*-value
Age (years)	42.8 ± 16.1	42.9 ± 14.6	Matched
Men	25 (50%)	25 (50%)	Matched
ALT (IU/L)	3788 ± 2856	2170 ± 1350	<0.001
AST (IU/L)	3131 ± 3673	1676 ± 1851	<0.05
Total bilirubin (mg/dL)	14.8 ± 8.6	9.5 ± 9.8	<0.01
Prothrombin time (%)	16.9 ± 11.2	72.8 ± 26.0	<0.001
HBeAg positive	15 (30%)	28 (56%)	<0.01
Core protein (log U/mL)	3.21 ± 1.28	3.01 ± 1.00	NS
HBcrAg (log U/mL)	5.30 ± 1.32	5.95 ± 1.13	<0.01
HBV DNA (log copies/mL)	5.97 ± 1.87	4.98 ± 1.17	<0.005
Deceased	19 (38%)	0 (0%)	<0.001

AHB, acute self-limited hepatitis B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FH-T, fulminant hepatitis B by transient HBV infection; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

Serological markers of HBV infection

Hepatitis B surface antigen, HBeAg and the corresponding antibody (anti-HBe) were determined by enzyme immunoassay (EIA) (AxSYM; Abbott Japan, Tokyo, Japan) or chemiluminescence enzyme immunoassay (CLEIA) (Fujirebio, Tokyo, Japan). Anti-HBC of IgM and IgG classes were determined by radioimmunoassay (Abbott Japan). Core protein constituting the viral nucleocapsid and HBV core-related antigen (HBcrAg), both of which correlate with HBV DNA in serum, were measured by CLEIA as described elsewhere.[26,27]

Quantification of serum HBV DNA

Hepatitis B virus DNA sequences spanning the S gene were amplified by real-time detection polymerase chain reaction (RTD-PCR) in accordance with the previously described protocol[28] with a slight modification;[8] it has a detection limit of 100 copies/mL.

Sequencing and molecular evolutionary analysis of HBV

Nucleic acids were extracted from serum samples (100 μL) using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany) and subjected to PCR for amplifying genomic areas bearing enhancer II/core promoter/pre-core/core regions [nt 1628–2364], as described previously.[29] The target of PCR covered several mutations which were associated with FHB. Amplicons were sequenced directly with use of the ABI Prism Big Dye ver. 3.0 kit in the AMI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA, USA). All sequences were analyzed in both forward and backward directions.

Hepatitis B virus genotypes were determined by molecular evolutionary analysis. Reference HBV sequences were retrieved from the DDBJ/EMBL/GenBank database and aligned by CLUSTAL X, then genetic distances were estimated with the 6-parameter method in the Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp/).[30] Based on obtained distances, phylogenetic trees were constructed by the neighbor-joining (NJ) method with the mid-point rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1000 times.

Statistical analysis

Statistical differences were evaluated by the Mann–Whitney *U*-test, Fisher's exact probability test and χ^2-test, where appropriate. Differences were considered to be statistically significant at $P < 0.05$. Multivariate analyses with logistic regression were utilized to sort out independent risk factors for FHB. STATA Software ver. 8.0 was employed for all analyses.

RESULTS

Baseline characteristics of the patients with FHB by transient HBV infection and AHB

TABLE 1 COMPARES baseline clinical characteristics of the 50 FH-T patients and the 50 AHB who

were matched for age and sex. The peak ALT, AST and T.bil levels were significantly higher (3788 ± 2856 vs 2170 ± 1350 IU/L, $P < 0.001$; 3131 ± 3673 vs 1676 ± 1851 IU/L, $P < 0.05$; and 14.8 ± 8.6 vs 9.5 ± 9.8 mg/dL, $P < 0.01$, respectively), while HBeAg was less frequent (30% vs 56%, $P < 0.01$) in the FH-T patients than AHB. The level of HBcrAg was significantly lower (5.30 ± 1.32 vs 5.95 ± 1.13 log U/mL, $P < 0.01$), while HBV DNA loads were higher (5.97 ± 1.87 vs 4.98 ± 1.17 log copies/mL, $P < 0.005$), in the FH-T patients than AHB. The level of core protein in sera tended to be higher in the FH-T patients than AHB (3.21 ± 1.28 vs 3.01 ± 1.00 log U/mL). Death occurred more often in the FH-T patients than AHB (38% vs 0%, $P < 0.001$).

HBV Genotypes and enhancer II/core promoter/pre-core/core Mutations in Patients with FHB by transient HBV infection and AHB

Figure 1(a) compares the distribution of HBV genotypes/subgenotypes between the FH-T and the AHB patients. The subgenotype C2/Ce was most prevalent in both patients with FH-T and AHB (66% and 62%, respectively), whereas B1/Bj was more frequent in the FH-T patients than AHB (22% vs 6%, $P < 0.05$). Likewise, mutations in enhancer II/core promoter/pre-core/core regions are compared between the FH-T and AHB patients in Figure 1(b). A1762T/G1764A, G1896A, G1899A and A2339G mutation were more frequent in the FH-T patients than AHB (48% vs 16%, $P < 0.001$; 62% vs 6%, $P < 0.001$; 24% vs 4%, $P < 0.001$; and 8% vs 0%, $P < 0.05$, respectively).

Figure 2(a) compares various mutations between the 11 FH-T patients and the three AHB patients who were infected with B1/Bj. Only G1896A was significantly more frequent (73% vs 0%, $P < 0.05$), while the lack of any mutations was less common (0% vs 33%, $P < 0.05$) in the FH-T patients than AHB. In comparison with the 33 FH-T patients and the 31 AHB patients who were infected with C2/Ce (Fig. 2b), A1762T/G1764A (70% vs 19%, $P < 0.001$), G1896A (61% vs 6%, $P < 0.001$) and the combination of all three mutations (A1762T/G1764A and G1896A) (45% vs 6%, $P < 0.001$) were significantly more frequent, while the lack of any mutations was less common (9% vs 70%, $P < 0.001$) in the FH-T patients than AHB. Interestingly, all the AHB patients with both G1896A and A1762T/G1764A mutations suffered acute severe hepatitis B that was defined by prothrombin time less than 40% but without coma of grade II or higher.

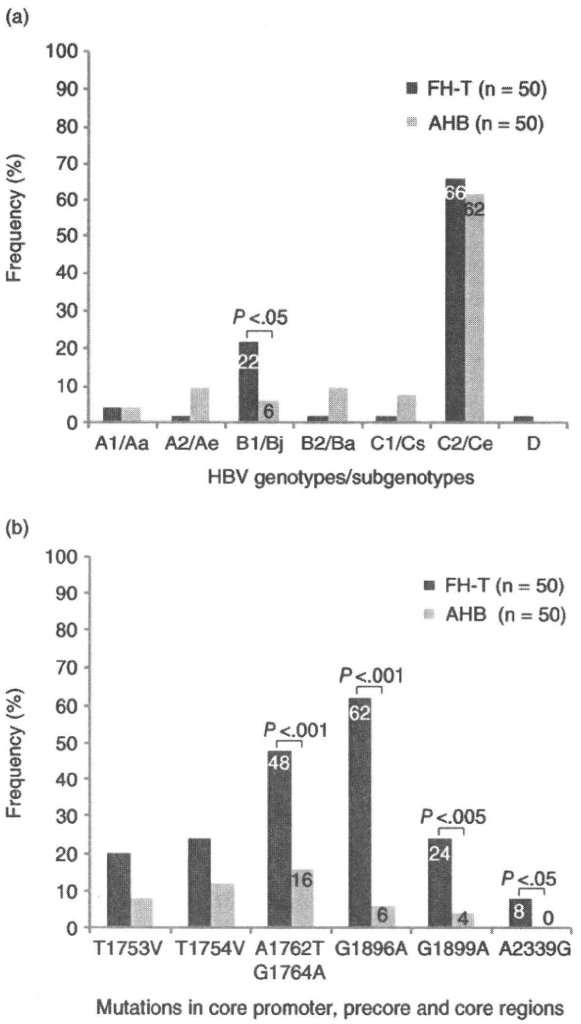

Figure 1 Genotypes/subgenotypes (a) and mutations in core promoter, pre-core and core regions (b) between the 50 transient hepatitis B virus infection (FH-T) and the 50 acute self-limited hepatitis B (AHB) patients.

Factors independently associated with the development of FHB by transient HBV infection

The following independent factors, promoting the development of FHB, were evaluated by multivariate analysis: ALT, AST, T.bil, HBeAg, HBV DNA, core protein, HBcrAg, genotypes/subgenotypes (B1/Bj or not) and mutations (T1753V, T1754V, A1762T/G1764A, G1896A, G1899A and A2339G). T.bil more than 10.35 mg/dL (OR, 7.81 [95% CI, 1.77–34.51], $P = 0.0067$), G1896A mutation (OR, 13.53 [95% CI,

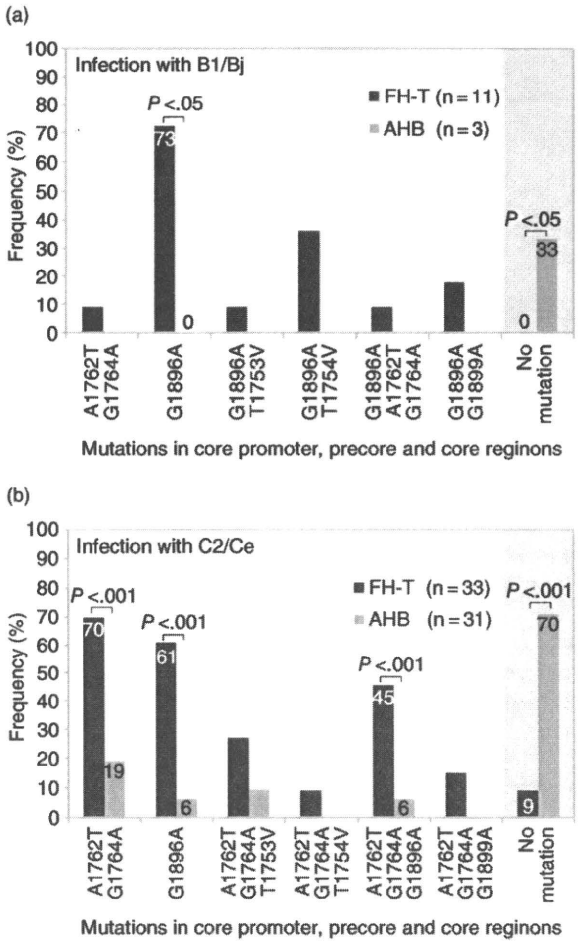

Figure 2 Frequencies of core promoter, pre-core and core mutations compared between the transient hepatitis B virus infection (FH-T) and the acute self-limited hepatitis B (AHB) patients who were infected with HBV of subgenotype B1/Bj (a) or C2/Ce (b).

2.75–66.64], $P = 0.0014$) and serum HBV DNA more than 5.23 log copies/mL (OR, 5.14 [95% CI, 1.10–24.15], $P = 0.0379$) were independent risk factors for the development of FHB by transient HBV infection (Table 2). Other mutations (T1753V, T1754V, A1762T/G1764A, G1899A and A2339G) were not significantly associated with the development of FHB by transient HBV infection, however.

Baseline clinical characteristics for distinguishing between the patients with FHB by AE of ASC (FH-C) and those without FHB by AE of CHB (AE-C)

Table 3 compares baseline clinical characteristics between the 12 FH-C patients and the 12 AE-C patients who were matched for age and sex. The levels of T.bil were significantly higher in the FH-C patients (15.0 ± 7.3 vs 7.3 ± 8.8 mg/dL, $P < 0.05$), but the peak ALT and AST levels tended to be slightly higher in the FH-C patients than AE-C (887 ± 681 vs. 641 ± 620 IU/L and 701 ± 451 vs 601 ± 753 IU/L, respectively). There were also no significant differences in levels of sera HBV DNA, core protein and HBcrAg between these two groups (7.44 ± 1.51 vs 6.60 ± 1.10 log copies/mL, 5.04 ± 1.45 vs 5.07 ± 1.07 log U/mL, and 6.35 ± 1.70 vs 6.29 ± 1.95 log U/mL, respectively).

HBV genotypes and enhancer II/core promoter/pre-core/core mutations between the patients with FH-C and those with AE-C

There were no significant differences in the frequencies of any HBV genotypes between the 12 FH-C patients and the 12 AE-C patients (Fig. 3a). In addition, there were also no significant differences in the frequencies

Table 2 Multivariate analysis for factors independently associated with fulminant hepatitis by transient HBV infection

Factors	Odds ratio	95% confidence interval	*P*-value
Total bilirubin (mg/dL)†			
<10.35	1		
≥10.35	7.81	1.77–34.51	0.0067
G1896A mutation			
Absent	1		
Present	13.53	2.75–66.64	0.0014
HBV DNA (log copies/mL)†			
<5.23	1		
≥5.23	5.14	1.10–24.15	0.0379

†Median values. HBV, hepatitis B virus.

Table 3 Baseline characteristics between patients with FH by AE of ASC (FH-C) and those without FH by AE of CHB (AE-C)

Features	FH-C ($n = 12$)	AE-C ($n = 12$)	Differences *P*-value
Age (years)	51.7 ± 14.7	49.9 ± 5.6	Matched
Male	10 (83%)	9 (75%)	Matched
ALT (IU/L)	887 ± 681	641 ± 620	NS
AST (IU/L)	701 ± 451	601 ± 753	NS
Total bilirubin (mg/dL)	15.0 ± 7.3	7.3 ± 8.8	<0.05
Prothrombin time (%)	25.8 ± 6.6	48.4 ± 21.5	<0.005
HBeAg positive	4 (33%)	3 (25%)	NS
Core protein (log U/mL)	5.04 ± 1.45	5.07 ± 1.07	NS
HBcrAg (log U/mL)	6.35 ± 1.70	6.29 ± 1.95	NS
HBV DNA (log copies/mL)	7.44 ± 1.51	6.60 ± 1.10	NS

AE, acute exacerbation; ALT, alanine aminotransferase; ASC, asymptomatic HBV carrier; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

of any specific mutations between these two groups (Fig. 3b).

DISCUSSION

THE MAGNITUDE OF liver injuries depends on the replication level of HBV and cytotoxic immune responses of the host raised against viral epitopes in general.[31] Various viral factors have been proposed that promote the development of FHB, represented by pre-core (G1896A) and core promoter (A1762T/G1764A) mutations.[13–16] Impact of virological factors on the development of FHB has remained controversial, however, especially because these mutations are rarely detected in the patients from the USA and France.[19–21] It has been argued that the development of FHB is not promoted by these mutations and is dependent on host factors including the human leukocyte antigen (HLA) environment.[22]

The expression of HBeAg is terminated by G1896A mutation in the pre-core region at the translation level,[32] and downregulated by the A1762T/G1764A double mutation at the transcription level.[33,34] Lamberts *et al.* are the first to implicate a negative influence of HBeAg on the replication of HBV.[35] Should HBeAg suppress the replication of HBV, presumably by inhibiting the encapsidation of pre-genome,[35] the lack or decrease of HBeAg would enhance the reproduction of HBV. Furthermore, HBeAg acts as a tollerogen to T cells recognizing epitopes on core protein, thereby, obviating immune injury of hepatocytes.[36,37] In the absence or decrease of HBeAg, therefore, hosts would mount vigor cytotoxic T-cell responses to core epitopes excessively presented on hepatocytes, and develop severe liver injuries culminating in FHB.[38]

There is a possibility that influence of viral factors such as HBV mutants with a HBeAg-negative phenotype, on the induction of FHB, may have been confounded by host factors and created disagreement. Therefore, the sheer influence of virological factors on FHB would need to be evaluated in case–control studies, as has been attempted to sort out the influence of HBV genotypes on development of cirrhosis and hepatocellular carcinoma.[8] These backgrounds have instigated us to identify virological factors accelerating the severity of liver disease in the 50 FHB patients by transient HBV infection and the 50 AHB patients who were of the same ethnicity and matched for age as well as sex.

In this case controlled study, A1762T/G1764A, G1896A, G1899A and A2339G mutation were significantly more frequent in the patients with FH-T than AHB, providing further corroboration of previous studies;[13–16] these mutations could enhance viral replication. Interestingly, our recent study using an *in vitro* replication model, showed that A2339G mutation in the core region enhanced viral replication and the effect of A2339G mutation may be associated with inhibition of the cleavage of the core protein by a furin-like protease, resulting in the high expression of the complete core protein.[18] Such enhanced HBV would induce significant immune response, resulting in development of FHB.

In multivariate analysis, higher levels of serum HBV DNA and G1896A mutation were independent virological risk factors for the development of FHB by transient

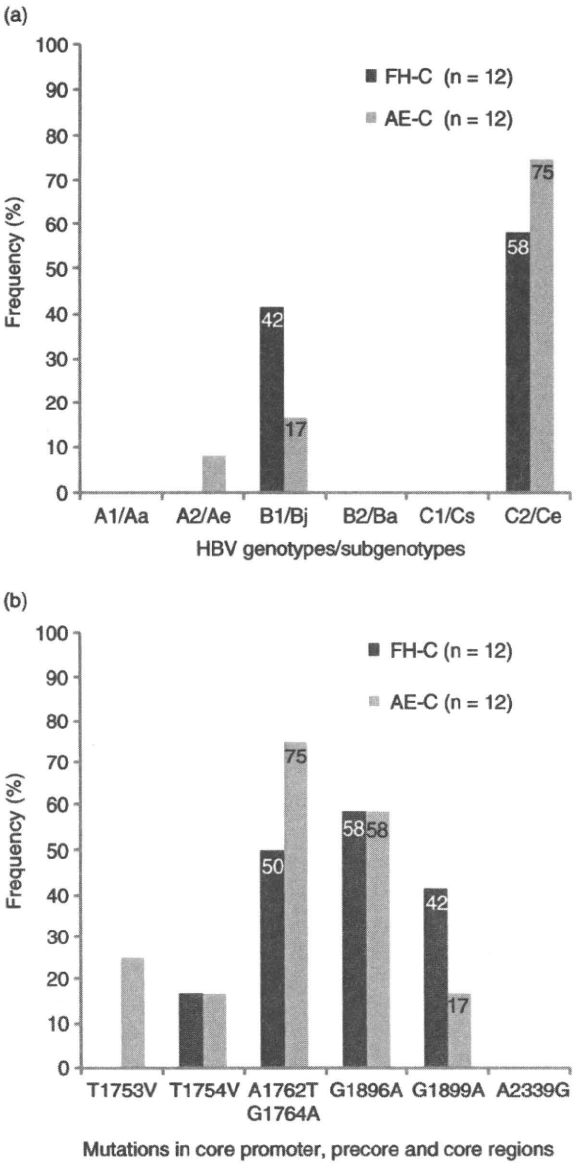

Figure 3 Genotypes/subgenotypes (a) and mutations in core promoter, pre-core and core regions (b) between the 12 transient hepatitis B virus infection (FH-T) and the 12 acute self-limited hepatitis B (AHB) patients.

HBV infection (Table 2). In particular, G1896A mutation was the most important factor associated with the development of FHB. Host responses, represented by T.bil, contributed to the development of FHB as well.

As for HBV genotypes, B1/Bj alone was significantly more frequent in the FH-T patients in univariate analysis. In the patients infected with B1/Bj, G1896A was more frequent in those with FH-T than AHB. In *in vitro* replication analysis, Ozasa *et al.*[15] observed extremely high expressions of intra- and extracellular HBV DNA in culture transfected with an HBV clone of B1/Bj genotype having the G1896A mutation; a high replication would be induced by this pre-core mutation for the induction of FHB. Our clinical results stand in support of this *in vitro* analysis. Taken altogether, chances for developing severe acute or FH would be high in the patients with acute hepatitis who are infected with HBV/B1 having the pre-core mutation. By contrast, in patients infected with C2/Ce, G1896A or A1762T/G1764A, or both was much more frequent in the FH-T patients than AHB. Of note, the co-occurrence of G1896A and A1762T/G1764A mutations was invariably accompanied by either FHB or acute severe hepatitis B in this study. Hence, these pre-core and core-promoter mutations might have addictive or synergetic effects for exacerbating hepatitis, when they emerge in the patients infected with C2/Ce. Such high-risk patients deserve special care and surveillance for signs and symptoms of fulminant or severe acute hepatitis B.

In the present study, serum levels of HBV DNA were significantly higher in the patients with FH-T than AHB. High serum levels of HBV DNA have been reported in patients with FHB;[39] they are followed by rapid decrease as the sequel of virus elimination operated by vigorous immune responses. Because of rapid and extensive elimination of HBV by the host immune system, HBV DNA in serum, in general, has decreased to low levels in patients with FHB at the presentation.[40] HBV DNA levels may be subject to the time that has elapsed from the onset of hepatitis to its measurement.[39] Also, serum levels of core protein (the product of the C gene) closely correlate with serum HBV DNA levels in patients with hepatitis B,[27] and they were compared between the FH-T patients and AHB. The core protein was determined by the newly developed CLEIA method; it is much easier and less expensive than the determination of HBV DNA. The level of core protein has turned out to be marginally higher in the FH-T patients than AHB (Table 1), and therefore might not contribute to an early diagnosis of FHB by transient infection.

Fulminant hepatitis B by AE of ASC is assumed as a different clinical condition from FHB by transient HBV infection. In this study, as there was no case–control study on virological factors associated with FHB for the patients with AE of ASC, we also attempted to identify virological factors associated with the development of FHB in the 12 FH-C and the 12 AE-C patients who were

matched for age as well as sex. Disappointingly, no differences of virological factors such as HBV genotypes and pre-core mutations, which were strongly associated with the development of FHB by transient infection, were found between the FH-C and AE-C patients (Fig. 3a,b). Furthermore, there were also no significant differences about HBeAg-positive rate and the levels of serum HBV DNA or core protein (Table 3), suggesting that several host factors may play a more important role in the development of FHB in ASC instead of virological factors. In this case–control study, however, there seems to be some problems: a small number of patients, different duration of HBV infection, different clinical stage (ASC or CHB) at the onset of AE, and HBV quasispecies complexity. Further investigations are needed to identify factors associated with FHB precipitating in asymptomatic HBV carriers.

In conclusion, virological factors associated with enhancement of viral replication seemed to be important for the development of FHB in the patients by transient HBV infection. But no virological factors were identified for differentiation of the FH-C patients from the AE-C patients. Hence, the pathogenic mechanism of FHB between transient HBV infection and AE of ASC would be different.

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REFERENCES

1 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; **38**: 646–57.
2 Norder H, Hammas B, Lofdahl S, Courouce AM, Magnius LO. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J Gen Virol* 1992; **73** (Pt 5): 1201–8.
3 Okamoto H, Tsuda F, Sakugawa H *et al.* Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988; **69**: 2575–83.
4 Stuyver L, De Gendt S, Van Geyt C *et al.* A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; **81** (Pt 1): 67–74.
5 Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; **83** (Pt 8): 2059–73.
6 Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. *Intervirology* 2003; **46**: 329–38.
7 Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. *Hepatology* 2002; **35**: 1274–6.
8 Tanaka Y, Hasegawa I, Kato T *et al.* A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. *Hepatology* 2004; **40**: 747–55.
9 Sugauchi F, Orito E, Ichida T *et al.* Hepatitis B virus of genotype B with or without recombination with genotype C over the pre-core region plus the core gene. *J Virol* 2002; **76**: 5985–92.
10 Huy TT, Ushijima H, Quang VX *et al.* Genotype C of hepatitis B virus can be classified into at least two subgroups. *J Gen Virol* 2004; **85** (Pt 2): 283–92.
11 Tanaka Y, Orito E, Yuen MF *et al.* Two subtypes (subgenotypes) of hepatitis B virus genotype C: a novel subtyping assay based on restriction fragment length polymorphism. *Hepatol Res* 2005; **33**: 216–24.
12 Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus–large-scale analysis using a new genotyping method. *J Infect Dis* 1997; **175**: 1285–93.
13 Sato S, Suzuki K, Akahane Y *et al.* Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 1995; **122**: 241–8.
14 Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the pre-core region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 1991; **324**: 1699–704.
15 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and pre-core mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; **44**: 326–34.
16 Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; **324**: 1705–9.
17 Imamura T, Yokosuka O, Kurihara T *et al.* Distribution of hepatitis B viral genotypes and mutations in the core promoter and pre-core regions in acute forms of liver disease in patients from Chiba, Japan. *Gut* 2003; **52**: 1630–7.
18 Sugiyama M, Tanaka Y, Kurbanov F, Nakayama N, Mochida S, Mizokami M. Influences on hepatitis B virus replication by a naturally occurring mutation in the core gene. *Virology* 2007; **365**: 285–91.

19 Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. Nucleotide sequence analysis of the pre-core region in patients with fulminant hepatitis B in the United States. *Gastroenterology* 1993; **105:** 1173–8.
20 Liang TJ, Hasegawa K, Munoz SJ *et al.* Hepatitis B virus pre-core mutation and fulminant hepatitis in the United States. A polymerase chain reaction-based assay for the detection of specific mutation. *J Clin Invest* 1994; **93:** 550–5.
21 Feray C, Gigou M, Samuel D, Bernuau J, Bismuth H, Brechot C. Low prevalence of pre-core mutations in hepatitis B virus DNA in fulminant hepatitis type B in France. *J Hepatol* 1993; **18:** 119–22.
22 Karayiannis P, Alexopoulou A, Hadziyannis S *et al.* Fulminant hepatitis associated with hepatitis B virus e antigen-negative infection: importance of host factors. *Hepatology* 1995; **22:** 1628–34.
23 Trey C, Lipworth L, Chalmers TC *et al.* Fulminant hepatic failure. Presumable contribution to halothane. *N Engl J Med* 1968; **279:** 798–801.
24 Ng HJ, Lim LC. Fulminant hepatitis B virus reactivation with concomitant listeriosis after fludarabine and rituximab therapy: case report. *Ann Hematol* 2001; **80:** 549–52.
25 Fujiwara K, Mochida S, Matsui A. [Prospective study for the efficiency of lamivudine for the patients with acute exacerbation of HBV carrier.] *Annual Report of Intractable Liver Disease Study Group of Japan, the Ministry of Health, Welfare and Labor* 2004. (In Japanese.)
26 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; **40:** 439–45.
27 Kimura T, Rokuhara A, Matsumoto A *et al.* New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and relation between levels of HBcAg and HBV DNA. *J Clin Microbiol* 2003; **41:** 1901–6.
28 Abe A, Inoue K, Tanaka T *et al.* Quantitation of hepatitis B virus genomic DNA by real-time detection PCR. *J Clin Microbiol* 1999; **37:** 2899–903.
29 Sugauchi F, Mizokami M, Orito E *et al.* A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol* 2001; **82** (Pt 4): 883–92.
30 Shin IT, Tanaka Y, Tateno Y, Mizokami M. Development and public release of a comprehensive hepatitis virus database. *Hepatol Res* 2008; **38:** 234–43.
31 Chisari FV, Ferrari C Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13:** 29–60.
32 Carman WF, Jacyna MR, Hadziyannis S *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; **2** (8663): 588–91.
33 Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on pre-core gene expression and viral replication. *J Virol* 1996; **70:** 5845–51.
34 Okamoto H, Tsuda F, Akahane Y *et al.* Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994; **68:** 8102–10.
35 Lamberts C, Nassal M, Velhagen I, Zentgraf H, Schroder CH. Precore-mediated inhibition of hepatitis B virus progeny DNA synthesis. *J Virol* 1993; **67:** 3756–62.
36 Chen MT, Billaud JN, Sallberg M *et al.* A function of the hepatitis B virus pre-core protein is to regulate the immune response to the core antigen. *Proc Natl Acad Sci USA* 2004; **101:** 14913–8.
37 Chen M, Sallberg M, Hughes J *et al.* Immune tolerance split between hepatitis B virus pre-core and core proteins. *J Virol* 2005; **79:** 3016–27.
38 Bocharov G, Ludewig B, Bertoletti A *et al.* Underwhelming the immune response: effect of slow virus growth on CD8+-T-lymphocyte responses. *J Virol* 2004; **78:** 2247–54.
39 Sainokami S, Abe K, Sato A *et al.* Initial load of hepatitis B virus (HBV), its changing profile, and pre-core/core promoter mutations correlate with the severity and outcome of acute HBV infection. *J Gastroenterol* 2007; **42:** 241–9.
40 Tassopoulos NC, Papaevangelou GJ, Roumeliotou-Karayannis A, Ticehurst JR, Feinstone SM, Purcell RH. Search for hepatitis B virus DNA in sera from patients with acute type B or non-A, non-B hepatitis. *J Hepatol* 1986; **2:** 410–8.

ペガシス，コペガス

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索引用語：ペグインターフェロン，ペガシス，コペガス，リバビリン

1 はじめに

本稿では，PEG-IFNの開発状況からペガシス，コペガスの国内治療成績，海外治療成績について紹介する．

2 PEG化とは

ポリエチレングリコール(PEG：polyethylene glycol)は，エチレンオキシド・サブユニットの繰り返し単位から構成される分子で，水溶性で低毒性の中性分子であることから，医薬品として広く使用されている．PEGの分子量は，サブユニットの数が多いほど大きくなり，また，その分子量により化学的特性も大きく異なる．

タンパクにPEG分子を結合させたPEGタンパクの一般的特性として，下記の4点が広く知られている．

① 分子量の増大により投与部位の皮下組織からの生体内への吸収が緩徐で持続的になる．

② 分子量の同大により体内分布が肝臓や血液などに限定的となる．

③ 酵素の分解を受けにくいため，生体内での分解が遅延する．すなわち，血中半減期が遅延され生物学的活性が持続するため，従来よりも少ない投与回数で効果が期待される．

④ PEGそのものは非免疫原性で水和していることから，免疫系から外来物質であると探知されにくくなり，抗原性が低下する．

PEG化することにより，薬物動態，薬力学的作用の点で，従来とは異なる医学的効果が発揮される．

3 IFN製剤のPEG化，PegIFNα2a（ペガシス）の開発

IFNは，抗ウイルス活性と免疫調節作用を併せ持つタンパクであり，従来のIFNは，投与後，急速に血中に移行し短時間に最高血中濃度に達する．また，小さな分子であるため体内のいたるところに広く分布した後，分解し速やかに尿中に排泄される．半減期は短く通常は約24時間以内に血中から排泄される．

B型，C型肝炎ウイルスを駆除するための

Hiroshi YATSUHASHI : Pegasys / Copegus

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表1　IFN α 2a，PegIFN α 2a，IFN α 2b，PegIFN α 2bの薬物動態の違い

	IFN α 2a	PegIFN α 2a	IFN α 2b	PegIFN α 2b
体内分布の体積	31～73 L	8～12 L	1.4 L/kg	0.99 L/kg
クリアランス	6600～29200 mL/h	60～100 mL/h	231.2 mL/h kg	22.0 mL/h kg
吸収半減期	2.3 h	50 h	2.3 h	4.6 h
消失半減期	3～8 h	65 h	-4 h	-40 h
T max （最高血中濃度までの時間）	7.3～12 h	80 h	7.3～12 h	15～44 h

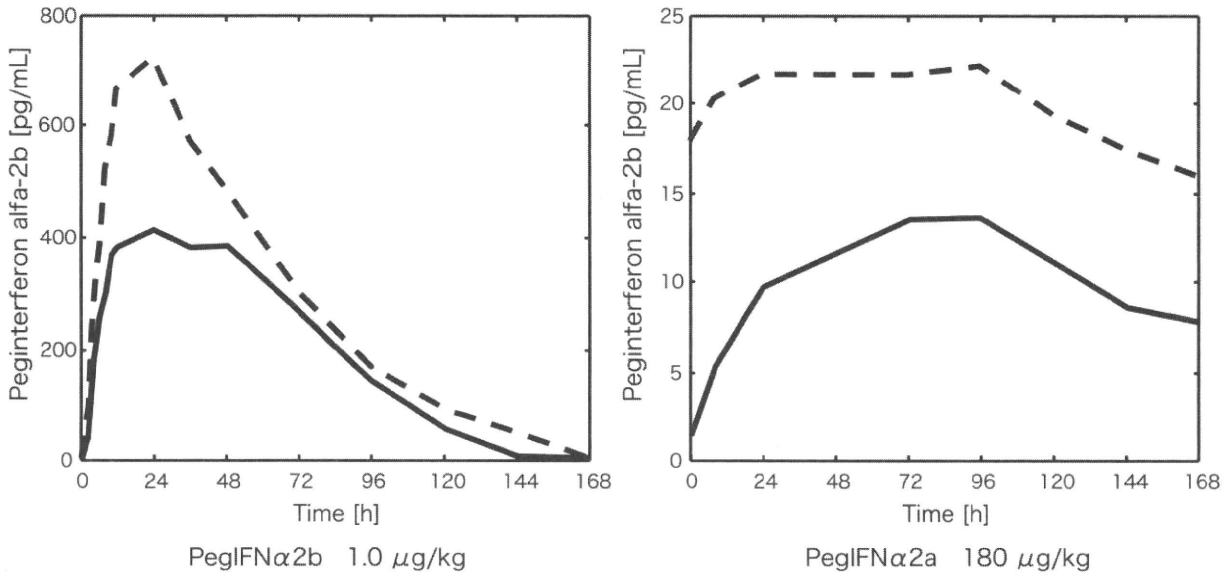

図1　PegIFN α 2bとPegIFN α 2a投与後の1週目と4週目の平均血中濃度の推移

有効血中濃度を維持するためには理論的には連日投与が必要である．わが国での従来型のIFN治療では，開始2週間は連日投与を行い，その後，週3回の間歇投与する方法が長く行われた．しかし，この週3回投与は1週間の間に血中IFN濃度が大きく変化し，血中濃度が低下した時には抗ウイルス効果が低下する可能性があり，また，IFN濃度の上昇時には，発熱，疲労感，頭痛，筋肉痛，関節痛といったインフルエンザ様症状の副作用が発現しやすくなる．

これらの問題を回避すべくIFNの有効な血中濃度を定常的に持続させることを目的として，1980年代からIFN製剤のPEG化の開発が始まった．PegIFN α 2a（ペガシス）の開発では，まず直鎖型5 kDaのPEGから検討が開始され，順にPEGの分子量を大きくしてゆき，直鎖型20 kDa，分枝型20 kDaなどのPEGが検討され，最終的に分子量20 kDaの2つのPEG鎖をウレタン結合で連結させた分枝型40 kDaのmPEG（モノメトキシPEG）を，IFN α -2aとアミド結合で結合したペガシスが開発された．

参考までにIFN α 2a，PegIFN α 2a，IFN

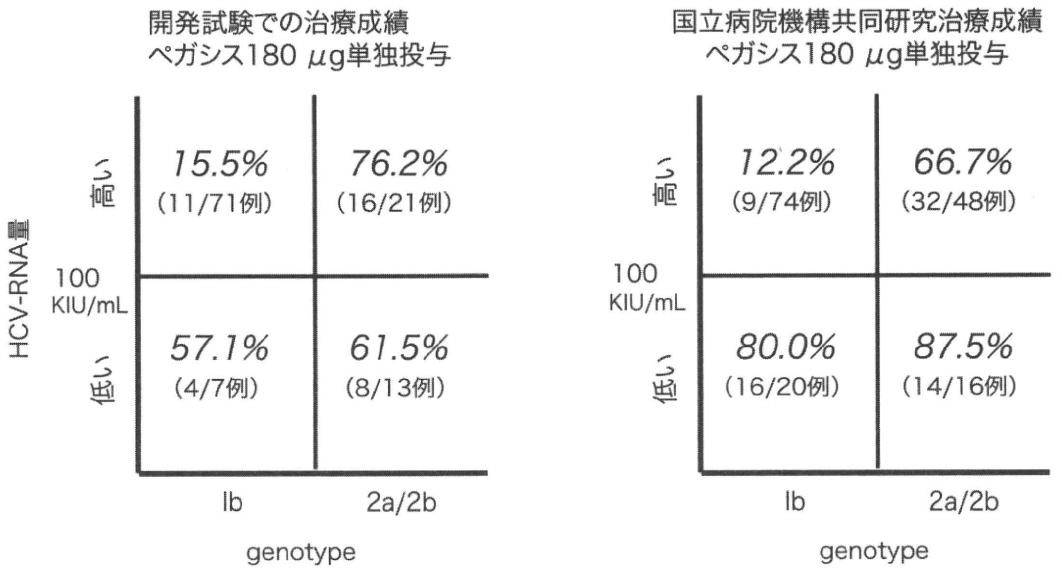

図2　HCVgenotype，HCVRNA量別，ペガシス単独投与治療成績

α 2b，PegIFN α 2bの4つの製剤の薬物動態の差異をまとめたものを表1に，PegIFN α 2aとPegIFN α 2bの2製剤の投与1週目と4週目の血中濃度の平均値の推移を図1に示す[1]．PEGの分子量，構造も異なるため，単純には比較できないが，薬物動態的にはPegIFN α 2aはPegIFN α 2bに比較して，血中濃度の上昇の仕方は緩徐であり，また半減期は長く，血中濃度が長く持続する．

4 PegIFNα2a（ペガシス）単独投与の治療成績

PegIFNの単独投与による治療法としては，PegIFN-α 2a（ペガシス）が2003年12月に保険診療で承認された．ペガシスは180 μgと90 μgの2種類があり，通常は180 μgで治療を開始し，血球数減少などがみられた場合には減量基準にしたがって90 μgに調整する．

ウイルス駆除を目指す場合のペガシス単独投与法では180 μg，週1回48本，48週間投与が基本である．HCVRNA量，HCVgenotype別の開発試験での成績，国立病院機構での多施設共同研究での治療成績を図2に示す[2]．従来型のIFNと比較して抗ウイルス効果は良好であり，HCV1b型高ウイルス群を除くと57.1～87.5％の著効率であることから，低ウイルス群やHCV2型の例，リバビリン（RBV）が適さない例に用いられている．

5 PegIFNα2a（ペガシス）による維持療法（HALT-C試験）

海外では，ペガシスの長期投与，維持療法によって肝硬変の病態改善効果，発ガン抑止効果がえられるか否かを明らかにする為に，PegIFN α 2a/RBV治療でウイルス駆除できなかった肝線維化進展例（肝硬変を含む）を対象として，ペガシス90 μgを3年間半投与した群と無治療群での比較試験が行われている（HALT-C試験）[3]．

2008年，治療後4年の時点では両群間に発ガン率の差はみられなかったと報告され，IFN維持療法の意義に関しては否定的な考え方が世界中に広まった．しかし，2010年11

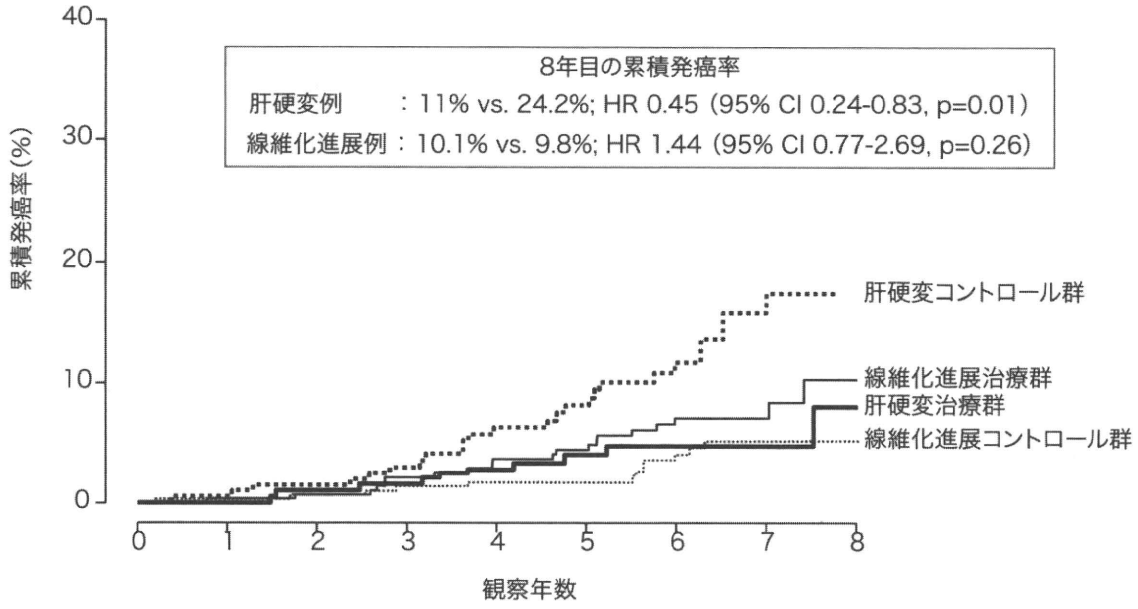

図3　ペガシスによる維持療法の有無での累積肝癌発生率の違い(HALT-C試験) 2010年AASLD発表

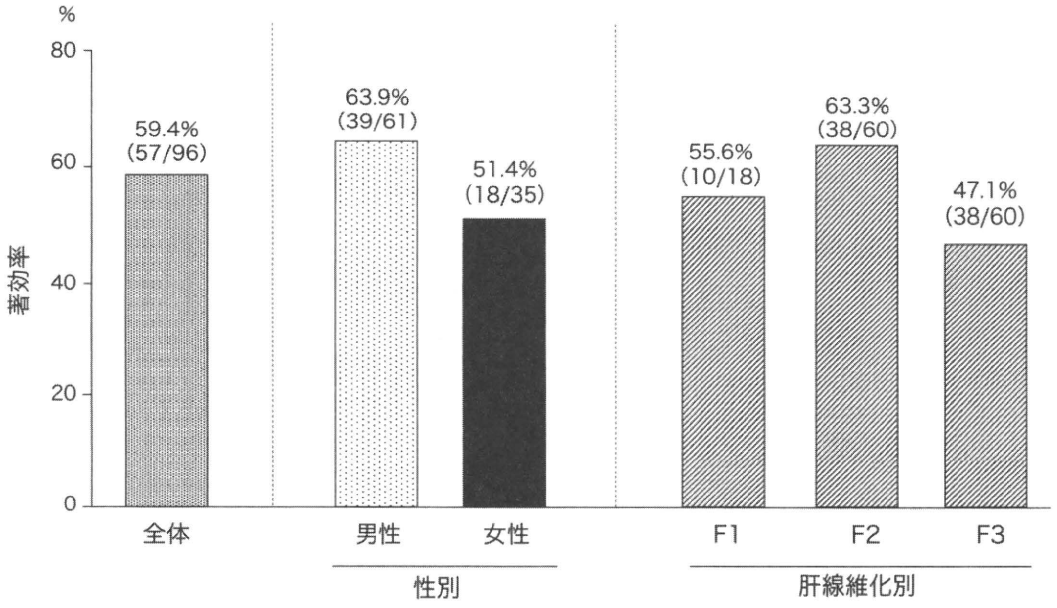

図4　ペガシス/コペガス併用療法の開発試験での治療成績(全体，性別，肝線維化別著効率)

月の米国肝臓学会においてHALT-C試験登録症例のその後の追跡結果(平均観察期間6.1年，最長8.7年)が報告された．前回よりも観察期間を延長し，また対象例を肝硬変症例と非肝硬変例に区分してサブ解析を行ったところ，肝硬変例では，無治療群に比較して維持療法をおこなった群では，有意に発ガン率が低下していたことが報告された(図3)[4]．

わが国では，高齢で，肝線維化進展したC型慢性肝炎，肝硬変症例が多く，しかも発ガンリスクも諸外国に比して高いことが明らかとなっている．概して，これらの対象者では，

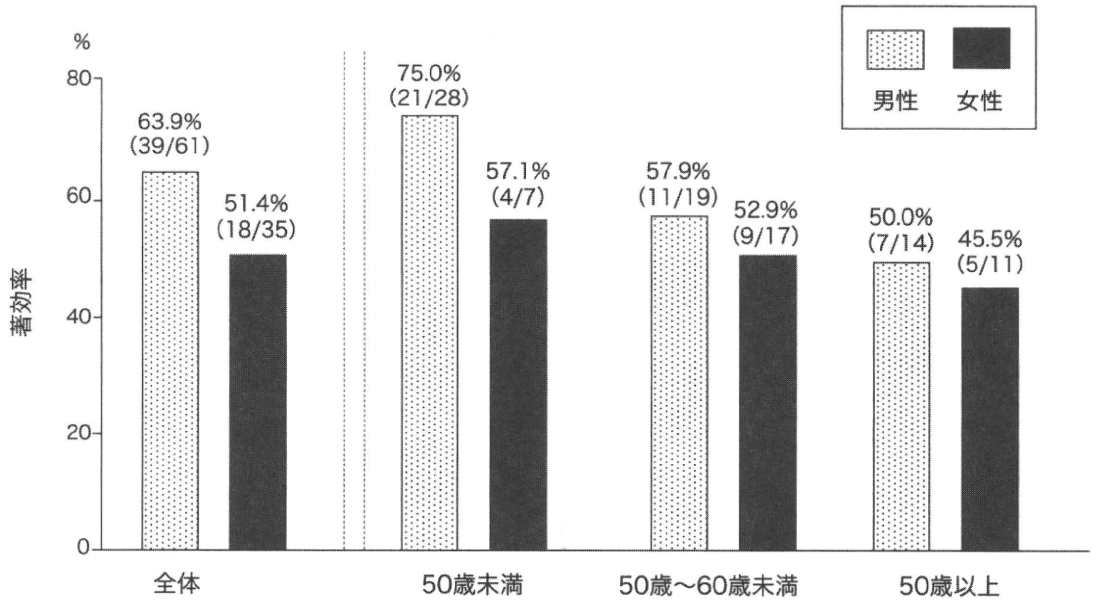

図5　ペガシス/コペガス併用療法の開発試験での治療成績(性別，年齢別著効率)

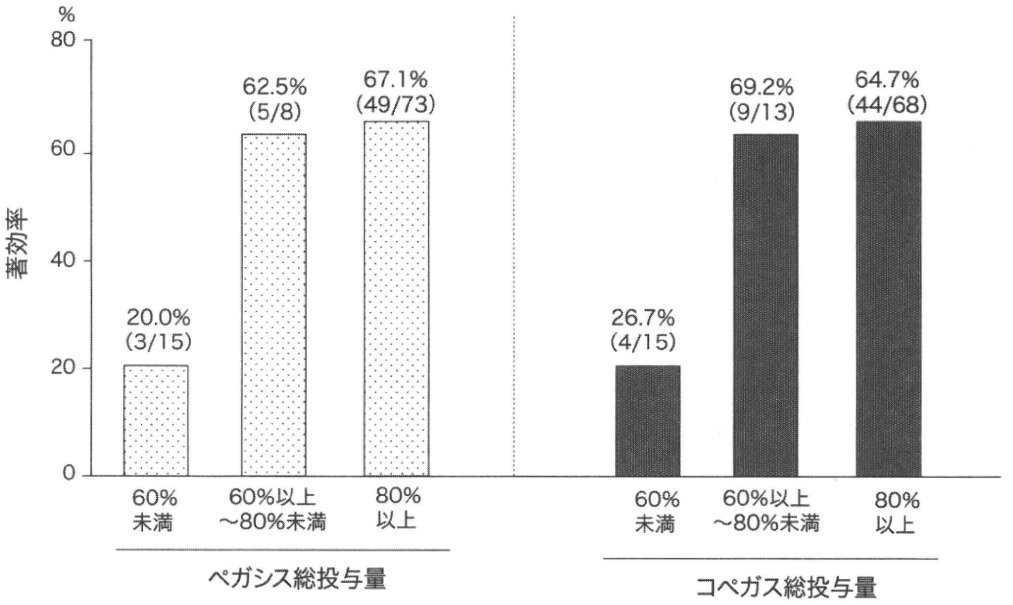

図6　ペガシス/コペガス併用療法の開発試験での治療成績(ペガシス，コペガス総投与量)

PegIFN α /RBV併用療法の導入そのものが困難な症例が多い．今後，ペガシスを用いた維持療法，IFN長期投与の意義については，わが国においても再考すべきであろう．

6　PegIFNα2a/RBV（ペガシス/コペガス）の国内治療成績

現在，C型肝炎に対するIFN治療は，PegIFN α /RBVの併用療法が標準治療である．PegIFN α 2a/RBV(ペガシス/コペガス)

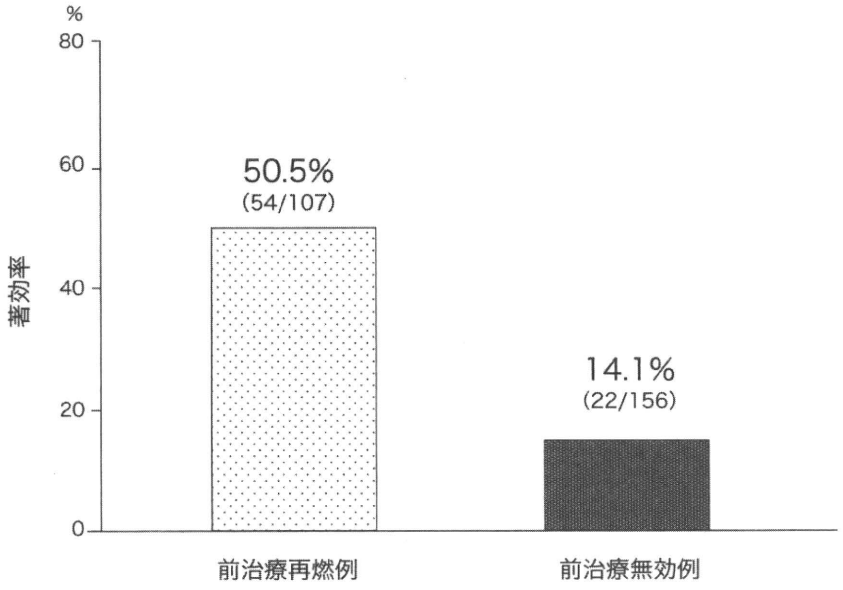

図7　PegIFN/RBV併用48週治療に対するペガシス/コペガス併用療法72週再治療成績

併用療法は，2007年3月から保険適応となった．

わが国で行われたHCV1型高ウイルス，初回治療例を対象としたペガシス/コペガス併用療法の開発試験での治療成績をまとめると[5]，

①全体の著効率は59.4％（57/96）（図4）．

②性別では，男性63.9％（39/61），女性51.4％（18/35）と女性でも比較的成績が良い（図4）．

③肝線維化別では，F1：55.6％（10/18），F2:63.3％（38/60），F3:47.1％（8/17）で，F3までは線維化進展の影響を受けにくい（図4）．

④年齢層別では，50歳以下は60歳以上よりも著効率が高い（図5）．

⑤ペガシス/コペガスともに，薬物総投与量として，最低60％以上が必要で，60％以下の場合には著効率が低下する（図6）．

市販後の治療成績については，現在，各施設から数百人規模の治療成績が各学会で発表されているが，基本的には開発試験での治療成績と類似している．

国内使用例でのペガシス/コペガス併用療法の副作用に関しては，PegIFN α 2b/RBV併用療法と基本的には同じと著者は考えている．ペガシス単独投与例では，治療中の血小板数減少症と間質性肺炎合併例の症例報告が散見されるも，2つの併用療法の間に，統計学的に，これらの発生頻度が高いか否かは現時点では確認されていない．

7　PegIFNα2a/RBV（ペガシス/コペガス）による再治療に関する海外治療成績

再治療対象者は，前治療の反応性から，再燃（Relapse）例と無効（Non-response）例に大別される．再燃例とは前回治療中に血中のHCV-RNA量が検出感度以下（50 IU/mL）に低下するも治療終了後HCV-RNAが検出される例であり，一方，無効例とは治療中に血中のHCV-RNA量が検出感度以下（50 IU/mL）にまで低下しない，治療期間中，持続的に陽性と

表2　解析対象となった12のRCTの概要

著者名	PEG-IFN用量と投与期間	リバビリン用量	リバビリン用量の調節	治療歴	HCVジェノタイプ	評価項目
Ascione (2008)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24～48週	1,000～1,200 mg/day	200 mg	なし	1, 2, 3, 4	SVR, 有害事象
Berak (2005)	α 2aは180 μg/週 α 2bは1.0 μg/kg/週 12週	体重換算	NR	NR	Non 2/3	有害事象
Bruno (2004)	α 2aは180 μg/週 α 2bは1.0 μg/kg/週 12週	1,000～1,200 mg/day	NR	なし	1, 2, 3	有害事象
Di Bisceglie (2007)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 12週	1,000～1,200 mg/day	NR	なし	1	有害事象
Kolakowska (2008)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24週	体重換算	NR	なし	3	SVR, 有害事象
Laguno (2009)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 48週	800～1,200 mg/day	NR	なし	1, 2, 3, 4	SVR, 有害事象
McHutchison (2009)	α 2aは180 μg/週 α 2bは1～1.5 μg/kg/週 24～48週	800～1,400 mg/day	200～600 mg	なし	1	SVR, 有害事象
Rumi (2008)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24～48週	800～1,200 mg/day	200 mg	なし	1, 2, 3, 4	SVR, 有害事象
Scotto (2008)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24～48週	15 mg/kg/day	4.6 mg/kg/day	治療無効	1, 2, 3, 4	SVR, 有害事象
Silva (2006)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 8週	13 mg/kg/day	認めず	なし	1	有害事象
Sinha (2004)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24～48週	1,000～1,200 mg/day	NR	なし	1, 2, 3, 4	SVR, 有害事象
Yenice (2006)	α 2aは180 μg/週 α 2bは1.5 μg/kg/週 24～48週	800～1,200 mg/day	200～600 mg	なし	1	SVR, 有害事象

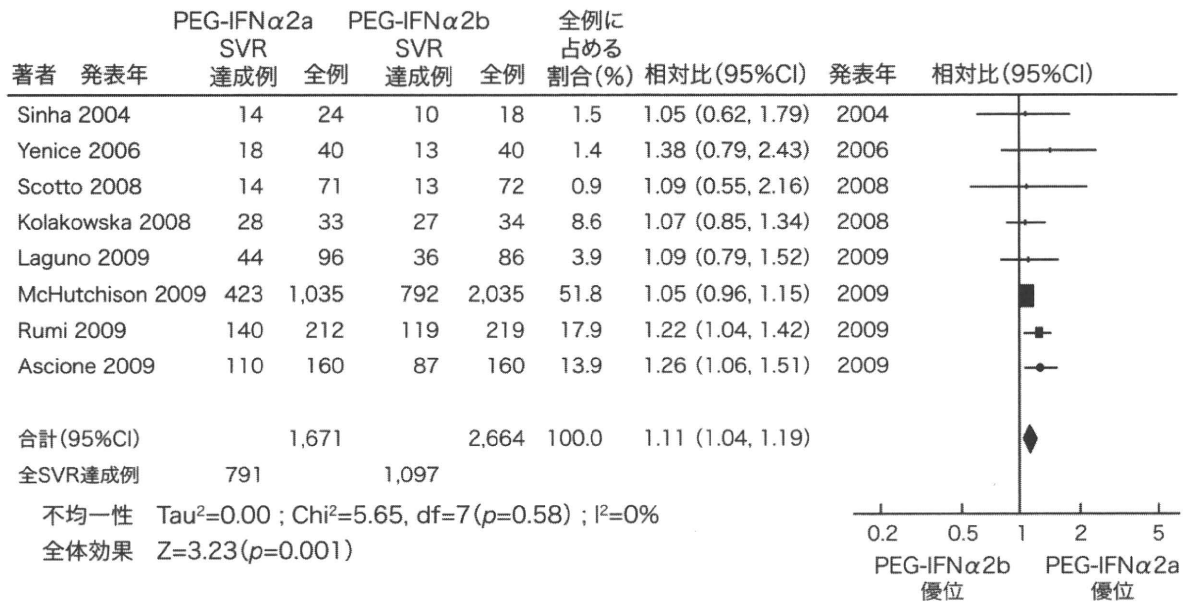

図8 著効率の比較

なった例と定義されている.

PegIFN α /RBV併用48週治療例での再燃例に対するペガシス/コペガス併用療法72週再治療での著効率は50.5％（54/107）[6]，無効例に対するペガシス/コペガス併用療法72週再治療での著効率は14.1％（22/156）[7]と報告されている(図7).

8 PegIFNα2a/RBVとPegIFNα2b/RBVの有効性と安全性の比較

PegIFN α 2a/RBVとPegIFN α 2b/RBVの両療法間の有効性を比較した大規模な無作為化比較試験としては，Genotype 1型のC型慢性肝炎3,070例を対象にMcHutchisonらが行ったIDEAL試験があり，それによると両療法間には有効性も安全性にも差が認められなかったと報告されている[8].

一方，各地域，各国においても同様の両療法の比較試験が行われているが，それらの比較試験の治療成績をまとめたメタアナリシス，Cochrane Systematic Reviewの結果が2010年にAwad Tらよって報告されている[9]. それによると12の無作為試験(表2)で5,008例を対象に解析したところ，PegIFN α 2a/RBV併用療法の著効率は47％，PegIFN α 2b/RBV併用療法では41％で，治療効果に有意差(P<0.004)を認めるも，治療中止の原因となった有害事象の発現頻度では両療法間に有意な差はみられなかったという(図8).

9 最後に

PegIFN α 2b/RBVよりもPegIFN α 2a/RBVの有効性の方が，わずかながら優っているという評価が世界の大勢となりつつある．しかしながら，実際，著者が両療法を使用した感覚としては，両療法間に大きな差があるとはいい難い．その一方でPegIFN α 2aとPegIFN α 2bの薬物動態には明白な差があることが確認されている．現時点では，症例の病態に応じて，薬物動態の違いも考慮した上で，個々の患者に最も適した薬剤を選択するように臨床医として心がけるべきであろう.