HLA-DP gene polymorphisms and hepatitis B infection in the Japanese population

KIYOSHI MIGITA, SEIGO ABIRU, MASASHI OHTANI, YUKA JIUCHI, YUMI MAEDA, SUNG KWAN BAE, SHIGEMUNE BEKKI, SATORU HASHIMOTO, KAKHARMAN YESMEMBETOV, SHINYA NAGAOKA, MINORU NAKAMURA, ATSUMASA KOMORI, TATSUKI ICHIKAWA, KAZUHIKO NAKAO, HIROSHI YATSUHASHI, HIROMI ISHIBASHI, and MICHIO YASUNAMI

OMURA AND NAGASAKI, JAPAN

The mechanisms underlying the different outcomes of hepatitis B virus (HBV) infection are not fully understood.[1] Kamatani et al[2] identified an association of the single nucleotide polymorphisms (SNPs) human leukocyte antigen (*HLA*)-*DPA1* (rs3077) and *HLA-DPB1* (rs9277535) with chronic HBV infection in a genome-wide association study (GWAS). Additional studies confirmed that rs3077 and rs9277535 were associated with chronic HBV infection in the Han-Chinese population and strengthened the findings from previous GWAS.[3-6] Furthermore, Hu et al[7] reported that SNPs in *HLA-DP* (rs3077 and rs9277535) were associated with both HBV clearance and hepatocellular carcinoma (HCC) development. To investigate the association of these *HLA-DP* variants with the disease progression of HBV infection, we genotyped the 2 SNPs (rs3077 and rs9277535) in different clinical stages of liver disease in Japanese HBV carriers.

CLINICAL SUMMARY

A total of 241 HBV carriers (positive for hepatitis B surface antigen) who visited the clinics for liver diseases at the Nagasaki University Hospital or Nagasaki Medical Center between 1999 and 2007 were enrolled. As controls, 143 healthy Japanese volunteers (56 men and 87 women aged 16–63 years, with a mean age of 31.3 ± 8.9 years) without any history of liver disease were enrolled. All patients did not have any other types of liver diseases, such as chronic hepatitis C, alcoholic liver disease, autoimmune liver disease, or metabolic liver disease. The study protocol was approved by the Ethics Committees of National Nagasaki Medical Center, and informed consent was obtained from each individual. Of the 241 HBV carriers, 69 were considered to be asymptomatic carriers on the basis of sustained normalization of the serum alanine aminotransferase (ALT) levels together with seropositivity for anti-hepatitis Be antigen throughout the study. On the other hand, 172 of the 241 HBV carriers were considered to have chronic liver disease, such as chronic hepatitis (57), cirrhosis (65), or HCC (50) manifested by elevated ALT levels and by clinical or histologic findings on examination of liver tissue during the follow-up period. Of the 50 patients with HCC, 6 (12%) were found to have chronic hepatitis and 44 (88%) had cirrhosis. All patients were regularly followed with measurements of serum ALT and HBV markers, such as hepatitis B surface antigen, hepatitis Be antigen, anti-hepatitis Be antibody, and HBV-DNA. A total of 79 patients had undergone liver biopsy during the study to assess the degree of liver fibrosis. However, liver biopsy was not performed in patients who had apparent biochemical, endoscopic, and ultrasound features of liver cancer. Tumor markers such as alpha-fetoprotein and des-γ-carboxy-prothrombin were measured with ultrasonography of the liver every 6 months to detect HCC in an early stage. The diagnosis of HCC was made by several imaging modalities in all patients and confirmed histologically by sonography-guided fine-needle tumor biopsy specimens. The genotype of rs3077 (*HLA-DPA1*) and rs9277535 (*HLA-DPB1*) was determined by direct sequencing. The apolipoprotein B mRNA-editing enzyme catalytic peptide 3G (*APOBEC3G* H186R) genotyping was performed on the basis of the report by An et al.[8]

The frequencies of the 2 SNPs of *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277535) are listed in Table I. There was a significant difference in the frequencies between these 2 SNPs between Japanese HBV carriers and healthy subjects, as described previously.[3] We divided HBV carriers into 2 groups: a nonadvanced group (asymptomatic carriers or chronic hepatitis, n = 115) and an advanced group (liver cirrhosis or HCC, n = 126). The frequencies of CC (rs3077) or GG (rs9277535) genotypes were higher in the advanced group compared with those in the nonadvanced group; however, the difference was not significant (Table I). Next, we stratified the HBV carriers for the presence or absence of the *APOBEC3G* H186R variant and examined the effects of *HLA-DP* polymorphisms on the progression of HBV-related liver disease. Both C and G alleles of rs3077 and rs9277535 significantly increased the risk for advanced liver disease in HBV carriers lacking the H186R variant (Table II).

A 2-stage GWAS identified SNPs including rs3077 and rs9277535 located in *HLA-DPA1* and *HLA-DPB1*, which were associated with a susceptibility to chronic HBV infection.[2] After the first Japanese GWAS, 5 studies replicated the association of these 2 *HLA-DP* SNPs (rs3077 and rs9277535) and chronic HBV infection in the Han-Chinese population.[3-7] Among these studies, an association between HBV-related HCC and rs9277535 or rs3077 was demonstrated.[7] In this study, we examined whether these 2 SNPs (rs3077 and rs9277535) in *HLA-DP* genes were associated with the disease progression and susceptibility to HBV infection in a Japanese population. As demonstrated previously, we reconfirmed that rs3077 and rs9277535 in the *HLA-DPA1* and *HLA-DPB1* genes were significantly associated with HBV infection. Although some differences in the frequencies of rs3077 and rs9277535 genotypes between HBV carriers with advanced liver disease (liver cirrhosis and HCC) and those without advanced liver disease were observed, these differences were not statistically significant.

Recent evidence suggests that APOBEC3G inhibits HBV production by interfering with HBV replication through hypermutation of the majority of the HBV genome.[8] Because of the *APOBEC3G* gene's ability to regulate HBV replication, mutations of the gene may cause a deleterious variation that may affect the outcome of HBV infection. Among the SNPs identified in the *APOBEC3G* gene, H186R variant was strongly associated with a decline in $CD4^+$ T-cell numbers and accelerated progression to acquired immune deficiency syndrome–defining conditions in human immunodeficiency virus–infected individuals.[9,10] Viral disease outcome is influenced by host variability in immune response genes and genes that control viral replication or mutation rate.[11] *APOBEC3G* coding region variant might influence the progression of HBV infection by inducing the replication of HBV.[12] Therefore, genetic diversity of immune response genes, such as *HLA*, and genes that control viral replication, such as *APOBEC3G*, could contribute to the variability in outcome of HBV infection. To minimize the effects

From the Clinical Research Center, NHO Nagasaki Medical Center, Omura, Japan; Department of Gastroenterology, Nagasaki University Hospital, Nagasaki, Japan; Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan.

Conflict of interests: None

Funding: This study was supported by a grant from the National Hospital Organization (Liver-net work study).

Submitted for publication May 25, 2012; revision submitted June 8, 2012; accepted for publication June 9, 2012.

Reprint requests: Kiyoshi Migita, MD, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan; e-mail: migita@nmc.hosp.go.jp.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2012.06.003

Table I. Association between *HLA-DP* polymorphisms (rs3077, rs9277535) and HBV infection

SNP ID	HBV carrier n = 241 (%)	Healthy subjects n = 143 (%)	*P* value*	OR (95% CI)	Advanced HBV carrier n = 115 (%)	Nonadvanced HBV carrier n = 126 (%)	*P* value*	OR (95% CI)
rs3077								
C/C	148 (61.4)	47 (32.9)			77 (67.0)	71 (56.3)		
C/T	79 (32.8)	72 (50.3)			33 (28.7)	46 (36.5)		
T/T	14 (5.8)	24 (16.8)			5 (4.3)	9 (7.1)		
C allele (allele frequencies)	375 (77.8)	166 (58.0)	<0.0001	2.533 (1.843–3.483)	187 (81.3)	188 (74.6)	0.077	1.480 (0.957–2.290)
rs9277535								
G/G	143 (59.3)	45 (31.5)			73 (63.5)	70 (55.6)		
A/G	82 (34.0)	72 (50.3)			36 (31.3)	46 (36.5)		
A/A	16 (6.6)	26 (18.2)			6 (5.2)	10 (7.9)		
G allele (allele frequencies)	368 (76.3)	162 (56.6)	<0.0001	2.471 (1.804–3384)	182 (79.1)	186 (73.8)	0.170	1.345 (0.880–2.056)

Abbreviations: CI, confidence interval; *HBV,* hepatitis B virus; *OR,* odds ratio; *SNP,* single-nucleotide polymorphism.
**P* values were calculated using the chi-square test.

Table II. Association between *HLA-DP* polymorphisms (rs3077, rs9277535) and the outcome of HBV infection in HBV carrier without H186R variant

SNP ID	Advanced HBV carrier n = 90 (%)	Nonadvanced HBV carrier n = 108 (%)	*P* value*	OR (95% CI)
rs3077				
C/C	64 (71.1)	60 (55.6)		
C/T	22 (24.4)	40 (37.0)		
T/T	4 (4.4)	8 (7.4)		
C allele (allele frequencies)	150 (83.3)	160 (74.1)	0.026	1.750 (1.065–2.874)
rs9277535				
G/G	5 (5.6)	10 (9.3)		
A/G	24 (26.7)	39 (36.1)		
A/A	61 (67.8)	59 (54.6)		
G allele (allele frequencies)	146 (81.1)	157 (72.7)	0.049	1.614 (1.000–2.604)

Abbreviations: CI, confidence interval; *HBV,* hepatitis B virus; *OR,* odds ratio; *SNP,* single-nucleotide polymorphism.
**P* values were calculated using the chi-square test.

of viral factors, such as APOBEC3G-mediated HBV editing, and evaluate the effect of *HLA-DP* more precisely, we focused on the subjects without the H186R variant. Because the *APOBEC3G* coding region variant might influence the progression of HBV infection,[11] we investigated the effect of *HLA-DP* polymorphisms on the outcome of HBV infection in HBV carriers lacking the H186R variant.

Our results showed that *HLA-DP* polymorphisms were associated with the progression of HBV infection and that this association was significant in Japanese HBV carriers lacking H186R variants. Our data demonstrated that *HLA-DP* polymorphisms are important in determining the susceptibility and the progression of HBV infection in the Japanese population.

One limitation of our study is the lack of information of HBV genotypes in the patients studied. Another limitation is that the number of HBV carriers (n = 241) is relatively small. Larger studies are needed to confirm the results of our study.

CONCLUSIONS

We confirmed that rs3077 and rs9277535 SNPs in the *HLA-DP* locus are associated with the susceptibility and progression of HBV infection in the Japanese population. Further functional analyses are warranted to validate the biological plausibility of these SNPs in chronic HBV infection.

REFERENCES

1. Ganem D, Prince AM. Hepatitis B virus infection–natural history and clinical consequences. N Engl J Med 2004;350:1118–29.
2. Kamatani Y, Wattanapokayakit S, Ochi H, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 2009;41:591–5.
3. An P, Winkler C, Guan L, O'Brien SJ, Zeng Z. HBV Study Consortium. A common HLA-DPA1 variant is a major determinant of hepatitis B virus clearance in Han Chinese. J Infect Dis 2011;203:943–7.
4. Guo X, Zhang Y, Li J, et al. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology 2011;53:422–8.
5. Wang L, Wu XP, Zhang W, et al. Evaluation of genetic susceptibility loci for chronic hepatitis B in Chinese: two independent case-control studies. PLoS One 2011;6:e17608.
6. Li J, Yang D, He Y, et al. Associations of HLA-DP variants with hepatitis B virus infection in southern and northern Han Chinese populations: a multicenter case-control study. PLoS One 2011;6:e24221.
7. Hu L, Zhai X, Liu J, et al. Genetic variants in HLA-DP/DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. Hepatology 2012;55:1426–31.
8. An P, Bleiber G, Duggal P, et al. APOBEC3G genetic variants and their influence on the progression to AIDS. J Virol 2004;78:11070–6.
9. Suspène R, Guétard D, Henry M, Sommer P, Wain-Hobson S, Vartanian JP. Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. Proc Natl Acad Sci U S A 2005;102:8321–6.
10. Do H, Vasilescu A, Diop G, et al. Exhaustive genotyping of the CEM15 (APOBEC3G) gene and absence of association with AIDS progression in a French cohort. J Infect Dis 2005;191:159–63.
11. Martin MP, Carrington M. Immunogenetics of viral infections. Curr Opin Immunol 2005;17:510–6.
12. Bonvin M, Greeve J, Hepatitis B. modern concepts in pathogenesis–APOBEC3 cytidine deaminases as effectors in innate immunity against the hepatitis B virus. Curr Opin Infect Dis 2008;21:298–303.

© Med Sci Monit, 2012; 18(12): CR698-705
PMID: 23197230

WWW.MEDSCIMONIT.COM
Clinical Research

Received: 2012.05.12
Accepted: 2012.09.03
Published: 2012.12.01

Prediction of early HBeAg seroconversion by decreased titers of HBeAg in the serum combined with increased grades of lobular inflammation in the liver

Authors' Contribution:
-·- Study Design
- - Data Collection
-- Statistical Analysis
·-· Data Interpretation
-·- Manuscript Preparation
-·- Literature Search
·-· Funds Collection

Sung Kwan Bae[1,2ABCDEF], Hiroshi Yatsuhashi[1,2ABDEF], Satoru Hashimoto[1,2BD], Yasuhide Motoyoshi[1,2BD], Eisuke Ozawa[1,2BD], Shinya Nagaoka[1,2BD], Seigo Abiru[1,2BD], Atsumasa Komori[1,2BD], Kiyoshi Migita[1,2D], Minoru Nakamura[1,2D], Masahiro Ito[1,2BD], Yuzo Miyakawa[3DEF], Hiromi Ishibashi[1,2ABDEF]

[1] Clinical Research Center, National Hospital Organization (NHO), Nagasaki Medical Center, Nagasaki, Japan
[2] Department of Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[3] Miyakawa Memorial Research Foundation, Tokyo, Japan

Source of support: Self financing

Summary

Background: Hepatitis B e antigen (HBeAg) seroconversion is an important hallmark in the natural course of chronic hepatitis B. This study was designed to predict early HBeAg seroconversion within 1 year, by not only biochemical and virological markers, but also pathological parameters in patients with chronic hepatitis B.

Material/Methods: In a retrospective cohort study, 234 patients with HBeAg were reviewed for demographic, biochemical, virological and pathological data at the time of liver biopsy. Then, the patients who accomplished HBeAg seroconversion within 1 year thereafter were compared with those who did not, for sorting out factors predictive of early HBeAg seroconversion.

Results: Early HBeAg seroconversion occurred in 58 (24.8%) patients. In univariate analysis, factors predictive of early HBeAg seroconversion were: alanine aminotransferase (ALT) (p=0.002), IP-10 (p=0.029), HBsAg (p=0.003), HBeAg (p<0.001), HBV DNA (p=0.001), HBcrAg (p=0.001), core-promoter mutations (p=0.040), fibrosis (p=0.033) and lobular inflammation (p=0.002). In multivariate analysis, only serum HBeAg levels <100 Paul Ehrlich Institute (PEI) U/ml and grades of lobular inflammation >2 were independent factors for early HBeAg seroconversion (odds ratio 8.430 [95% confidence interval 4.173–17.032], p<0.001; and 4.330 [2.009–9.331], p<0.001; respectively).

Conclusions: HBeAg levels < 100 PEIU/ml combined with grades of lobular inflammation >2 are useful for predicting early HBeAg seroconversion. In patients without liver biopsies, high ALT levels (>200 IU/L) can substitute for lobular inflammation (grades >2).

key words: **alanine aminotransferase • chronic hepatitis • hepatitis B virus • hepatitis B e antigen • lobular inflammation • seroconversion**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=883595

Word count: 2920
Tables: 4
Figures: 4
References: 33

Author's address: Hiroshi Yatsuhashi, Clinical Research Center, National Nagasaki Medical Center, Kubara 2-1001-1, Omura, Nagasaki 856-8562, Japan, e-mail: yatsuhashi@nmc.hosp.go.jp

CR

Background

Worldwide, an estimated 350 million people are infected with hepatitis B virus (HBV) persistently [1,2]. HBV infection is a major global concern, because up to 40% of patients can develop grave complications, such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [3]. In the natural course of chronic hepatitis B, HBeAg seroconversion, defined by the loss of HBeAg and development of the corresponding antibody (anti-HBe), is an important hallmark, because it is highly correlated with a favorable long-term outcome. Seroconversion is usually followed by sustained suppression of HBV DNA, normalization of alanine aminotransferase (ALT) levels, and clinical remission accompanied by ameliorated necro-inflammatory activities in the liver [4–6].

To date, a number of factors have been found to predispose patients to spontaneous HBeAg seroconversion [7–19]. However, few studies have evaluated pathological factors for predicting early HBeAg seroconversion. In a small series of patients from Spain, the Knodell's index of histological activity was one of the independent predictors of early HBeAg seroconversion [14]. Recently, novel markers of the replication of HBV were introduced, such as levels of HBsAg, HBeAg and HBcrAg (HBV core-related antigen), which can replace HBV DNA levels. These serological markers of HBV replication have been evaluated for sensitive and reliable prediction of early HBeAg seroconversion [20–23]. In the present study, an attempt was made to select factors predictive of early HBeAg seroconversion, from among many biochemical, virological and pathological parameters, based on the data of 234 HBeAg-positive patients with chronic hepatitis B.

Material and Methods

Patients and study design

This is a retrospective cohort study with use of stored sera and liver biopsy specimens from patients with chronic hepatitis B who were taken care of in the Hepatology Department, Nagasaki Medical Center, Japan, during 1991 through 2005. The clinical database was reviewed to identify consecutive patients who underwent liver biopsies and had been followed for longer than 1 year. The inclusion criteria were presence of hepatitis B surface antigen (HBsAg) for 6 months or longer, positivity for HBeAg at the time of liver biopsy, and lack of antiviral treatments before receiving liver biopsies. The exclusion criteria were co-infection with hepatitis C virus (HCV) or human immunodeficiency virus type-1, serological markers suggestive of autoimmune disease, daily intake of alcohol >50 g, recent exposure to hepatotoxic drugs, and no stored sera available. They were followed every 3 months or more frequently, if indicated clinically, and their serum samples were monitored for liver biochemistry and serologic markers of HBV infection, including HBsAg, HBeAg, anti-HBe, HBV DNA and HBcrAg. Serum samples had been stored at –20°C until use.

Antiviral therapy was commenced immediately in the patients with: (1) significant fibrosis/cirrhosis detected by liver biopsy; and (2) evidence of decompensation, such as ascites, varices and hepatic encephalopathy.

To identify predictors of early HBeAg seroconversion, clinical, biological, virological and pathological data at the time of liver biopsy were compared between patients who did and who did not achieve early HBeAg seroconversion, within 1 year after receiving liver biopsies, by univariate and multivariate analyses. Further, patients were stratified by independent factors for HBeAg seroconversion, and the cumulative incidence of HBeAg seroconversion was compared between groups using the Kaplan-Meier method. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of the institution. Each patient gave a written informed consent before participating in this study.

Routine laboratory tests for HBV markers

Quantitative measurements of HBsAg and HBeAg were carried out using commercial enzyme-linked immunosorbent assay (ELISA) kits in the ARCHITECT ANALYSER i2000 (Abbott Japan Co., Ltd., Tokyo, Japan) in accordance with the manufactures' instructions in Nagasaki Medical Center. The sensitivity of HBsAg assay ranged from 0.05 to 250 IU/ml. Sera with HBsAg >250 IU/ml were serially diluted 100-fold so as to include them within the dynamic range. HBeAg was quantified by a two-step immunoassay with use of chemiluminescence microparticles. Briefly, undiluted samples were mixed with paramagnetic beads coated with anti-HBe. After a washing step, conjugate and reactants were added for exciting emission of the light that is proportional to the concentration of HBeAg. The result was expressed by the ratio of relative light unit (RLU) of the sample to the cut-off RLU (S/CO). Samples with S/CO values >1.0 were regarded positive for HBeAg. Then, serial dilutions of the reference standard of PE HBeAg (Paul-Ehrlich Institute, Langen, Germany) were used to define the linear range of the assay and create a reference curve for linear regression. The linear range was 0.024–100 PEIU/ml. A standard curve was produced, and linear regression was used to convert assay results into appropriate units (PEIU/ml). For samples that fell outside the linear range of the assay, the assay was performed on serial dilutions to ensure the linearity.

HBV DNA and HBcrAg

HBV DNA was determined by the COBAS Taqman HBV test (Roche Diagnostics K.K., Tokyo, Japan). Values under or over the detection range were recorded as 2.1 or 9.1 log copies/ml. HBcrAg was measured by the CLEIA HBcrAg assay kit (Fujirebio, Inc., Tokyo, Japan) in a fully automated analyzer (Lumipulse system, Fujirebio, Inc.). Values under or over the detection range were recorded as 3.0 or 7.0 log copies/ml. Assays for HBV DNA and HBcrAg were performed in a commercial clinical laboratory (SRL, Inc., Tokyo, Japan). Sera with values over the detection range were diluted to include them within the dynamic range.

Interferon-inducible protein 10 (IP-10)

IP-10 was quantified by the Invitrogen Human IP-10 ELISA (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's protocol in Nagasaki Medical Center.

HBV genotyping

HBV DNA was extracted from serum (100 µl) with use of the SMITEST EX R&D extraction kit (MBL Co., Ltd., Nagoya, Japan). It was amplified for determination of genotypes by

Table 1. Histological evaluation of liver biopsy specimens.

(A) Fibrosis staging	
Stage	**Fibrosis**
0	None
1	Enlarged, fibrotic portal tracts
2	Periportal or portal-portal septa but intact architecture
3	Fibrosis with architectural distortion without obvious cirrhosis
4	Probable or definite cirrhosis

(B) Inflammation grading			
Grade	**Portal/periportal activity**		**Lobular inflammation**
	Piecemeal necrosis	**Lymphocyte aggregation**	
0	None or minimal	None	None
1	Inflammation only	< 1/3 in portal triad	Inflammation alone
2	Mild	1/3–2/3 in portal areas	Focal necrosis or acidphil bodies
3	Moderate	> 2/3 in portal areas	Severe focal cell damages
4	Severe	Entire portal triad	Damage with bridging necrosis

the SMITEST HBV Genotyping Kit (MBL Co., Ltd.) based on hybridization with type-specific probes immobilized on a solid-phase support [24].

Precore stop codon (G1896A) and core promoter (A1762T/G1764A) mutations

A1896 mutation in the precore (PreC) region was detected by the enzyme-linked minisequence assay (SMITEST HBV PreC ELMA, Roche Diagnostics, Tokyo, Japan), and mutations in the core promoter (CP) region for T1762/A1764 by the enzyme-linked specific probe assay (SMITEST HBV Core Promoter Mutation Detection Kit, Roche Diagnostics K.K.). The results were recorded as "the wild-type" and "mutant types" dominantly expressed by HBV isolates [25].

Histological examination

Liver biopsy was taken by fine-needle aspiration (16G sonopsy) guided by ultrasonography. Biopsy specimens were fixed in 10% neutral formalin, cut at 3- to 4-μm thickness, and stained with Hematoxyline-Eosin and Azan-Mallory, as well as for silver to visualize reticuline fibers. Tissue sections were examined independently by two senior liver pathologists. For each biopsy specimen, a protocol was filled out for grading necro-inflammation and staging fibrosis by the criteria of Desmet et al. [26] and Scheuer [27] (Table 1). As for the portal activity, not only piecemeal necrosis, but also lymphocytic aggregation was categorized into 5 (0–4) grades in the respective area involved.

Statistical analysis

Continuous variables were compared between groups by the Mann-Whitney *U* test, and categorical variables by χ^2 and Fisher's exact tests. The cumulative incidence of HBeAg seroconversion was calculated using the Kaplan-Meier method, and the difference was evaluated by the log-rank test. Multiple logistic regression analysis was performed to identify independent factors in significant association with early HBeAg seroconversion. A p value <0.05 was considered significant. Statistical analyses were performed using the SPSS version 17.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of patients

Among the 673 patients with HBsAg who had received liver biopsies in our hospital during 1991 through 2005, 234 (34.8%) patients who met the inclusion criteria were enrolled in this study. Demographic and laboratory characteristics at the time of liver biopsy are listed in Table 2. They had a median age of 37 years (range: 12–74), and 161 (69%) were men. Of them, 231 (99%) were infected with HBV of genotype C. The median serum ALT level at the baseline was 141 IU/l (range: 13–2644 IU/l), and the median duration of follow-up was 86.5 months (range: 12.0–213.0 months). During the follow-up, 91 (39%) received antiviral treatment, with interferon (IFN) or lamividine, or the combination thereof.

Comparison of clinical features between patients with and without early HBeAg seroconversion

Early HBeAg seroconversion, within 1 year after receiving liver biopsies, was achieved by 58 of the 234 (24.8%) patients. In univariate analysis, factors predictive of early HBeAg seroconversion were: ALT (p=0.002), IP-10 (p=0.029), HBsAg (p=0.003), HBeAg (p<0.001), HBV DNA (p=0.001), HBcrAg (p<0.001), CP mutations (p=0.040), fibrosis (p=0.033) and lobular inflammation (p=0.002). Other factors including age, albumin, platelets, AFP, PreC mutation, cell infiltration and

Table 2. Baseline characteristics of patients.

Features	Total (n=234)
Demographic data	
Age (years)	37 (12–74)
Men (%)	161 (69)
Biolochemical markers	
Albumin (g/dl)	4.1 (2.5–5.0)
Platelets ($\times 10^3/mm^3$)	179 (43–338)
ALT (IU/l)	141 (13–2644)
AFP (ng/ml)	7 (0–1863)
IP-10 (ng/ml)	214 (66–3253)
Virological markers	
HBV genotypes: A/B/C (%)	1/2/231 (0/1/ 99)
HBsAg (IU/ml)	8039 (2–261647)
HBeAg (PEIU/ml)	245.3 (0.01–3179.7)
HBV DNA (log copies/ml)	7.7 (3.6–8.9)
HBcrAg (log U/ml)	7.8 (5.4–9.2)
PC mutations: wild/mix/ mutant (%)	132/100/2 (56/43/1)
CP mutations: wild/mix/ mutant/others (%)	55/50/126/3 (24/21/54/1)
Pathological features	
Fibrosis stages: 0/1/2/3/4 (%)	15/73/54/38/54 (7/31/23/16/ 23)
Lymphocytic aggregation: 0/1/2/3/4 (%)	6/65/107/45/11 (2/28/46/19/5)
Piecemeal necrosis: 0/1/2/3/4 (%)	59/52/57/58/8 (25/22/24/25/4)
Lobular inflammation: 0/1/2/3/4 (%)	4/91/104/32/3 (2/39/44/14/1)
Antiviral treatments	
Within 1 year of biopsy (%)	91 (39)
Antiviral agents: 1/2/3/4* (%)	44/33/13/1 (49/36/14/1)
Duration of follow up (months)	86.5 (12.0–213.0)

Qualitative variables are expressed in the number with percentage in parentheses, and quantitative variables are expressed in the median with range in parentheses. ALT – alanine aminotransferase; AFP – alpha-fetoprotein; IP-10 – the interferon-gamma inducible protein-10; HBV – hepatitis B virus; HBsAg – hepatitis B surface antigen; HBeAg – hepatitis B e antigen; HBcrAg – hepatitis B virus core-related antigen; PC – precore; CP – core promoter. * 1, Interferon alpha; 2, lamivudine; 3, lamivudine plus interferon-alpha; 4, entecavir.

piecemeal necrosis in the liver, as well as treatments within 1 year after the entry and type of antiviral agents, were not associated with early HBeAg seroconversion (Table 3).

Evaluation of HBV markers for predicting early HBeAg seroconversion

HBV markers were compared for sensitivity and specificity in predicting early HBeAg seroconversion by the receiver operating characteristic analysis (Figure 1). HBeAg at the time of liver biopsy was the best predictor of early HBeAg seroconversion, with the widest area under the curve of 0.750; it was larger than those of HBcrAg (0.708), HBV DNA (0.650) and HBsAg (0.630). Hence, HBeAg was selected as the best HBV marker predictive of early seroconversion. Based on the receiver operating characteristic curve, HBeAg titers were dichotomized by 100 PEIU/ml in the immunoassay.

Independent predictors for early HBeAg seroconversion

A multivariate logistic regression analysis was performed to select independent predictors of early HBeAg seroconversion from among variables significant in the univariate analysis (Table 4). Of all factors, including histological characteristics, HBeAg <100 PEIU/ml and grades ≥2 lobular inflammation remained as independent factors predictive of early HBeAg seroconversion (Table 4A). Of factors exclusive of histological parameters, HBeAg <100 PEIU/ml and ALT ≥200 IU/ml remained as independent factors for early HBeAg seroconversion (Table 4B).

Combinations of two independent factors for predicting early HBeAg seroconversion

Two combinations of independent factors were evaluated for the performance in predicting early HBeAg seroconversion. The patients who had two predictors in combination, HBeAg <100 PEIU/ml and grades ≥2 lobular inflammation, achieved early HBeAg seroconversion in the highest frequency at 66.0% (31/47). In a remarkable contrast, merely 6.9% (4/58) of the patients without either of these predictors achieved early HBeAg seroconversion (Figure 2A).

Likewise, early seroconversion was achieved by 18 of the 30 (60.0%) patients with the other combination of independent factors, exclusive of pathological parameters, HBeAg <100 PEIU/ml and ALT ≥200 IU/l. By contrast, only 6 of the 99 (6.1%) patients without either of them achieved early HBeAg seroconversion (Figure 2B).

Sensitivity, specificity, positive predictive value and negative predictive value of predicting early HBeAg seroconversion are: 74.5% (31/58), 90.9% (160/176), 66.0% (31/47) and 85.6% (160/187), respectively, for the combination of HBeAg <100 PEIU/ml and grades ≥2 lobular inflammation; and 31.0% (18/58), 93.2% (164/176), 60.0% (18/30) and 80.4% (164/204), respectively, for the combination of HBeAg <100 PEIU/ml and ALT ≥200 IU/l.

Long-term clinical outcomes

Besides the 58 patients with early HBeAg seroconversion, an additional 97 patients achieved HBeAg seroconversion during a median follow-up period of 86.5 months. Cumulative

Table 3. Univariate analysis of risk factors for early HBeAg seroconversion.

Variables	Early HBeAg seroconversion		p value
	Achieved (n=58)	Not achieved (n=176)	
Demographic data			
Age (years)	36 (17–69)	37 (12–74)	0.303
Men (%)	41 (71)	120 (68)	0.721
Biolochemical markers			
Albumin (g/dl)	4.1 (2.8–4.8)	4.1 (2.5–5.0)	0.877
Platelets ($\times 10^3$/mm^3)	171 (43–291)	186 (57–338)	0.487
ALT (IU/l)	227 (18–2072)	121 (13–2644)	0.002
AFP (ng/ml)	12 (1–1863)	6 (0–683)	0.070
IP-10 (ng/ml)	259 (77–1743)	204 (66–3253)	0.029
Virological markers			
HBV genotypes A/B/C (%)	0/0/58 (0/0/100)	1/2/173 (1/1/98)	1
HBsAg (IU/ml)	5127 (8–261647)	9033 (2–128511)	0.003
HBeAg (PEIU/ml)	20.9 (0.01–1985.0)	377.1 (0.01–3179.7)	<0.001
HBV DNA (log copies/ml)	7.2 (3.7–8.7)	7.8 (3.6–8.9)	0.001
HBcrAg (log U/ml)	7.2 (5.7–9.2)	8.0 (5.4–9.1)	<0.001
PC mutations: wild/mix/mutant (%)	26/31/1 (45/53/2)	106/69/1 (60/39/1)	0.075
CP mutations: wild/mix/mutant/others (%)	8/9/40/1 (14/15/69/2)	47/41/86/2 (27/23/49/1)	0.040
Pathological features			
Fibrosis stage: 0/1/2/3/4 (%)	1/12/18/14/13 (2/21/31/24/22)	14/61/36/24/ 41 (8/35/20/14/23)	0.033
Lymphocytic aggregation: 0/1/2/3/4 (%)	0/11/27/17/3 (0/19/47/29/5)	6/54/80/28/8 (3/31/45/16/5)	0.087
Piecemeal necrosis: 0/1/2/3/4 (%)	7/12/18/19/2 (12/21/31/33/3)	52/40/39/39/6 (30/23/22/22/3)	0.068
Lobular inflammation: 0/1/2/3/4 (%)	0/13/29/15/1 (0/22/50/26/2)	4/78/75/17/2 (2/44/43/10/1)	0.002
Antiviral treatments within 1 year after biopsy (%)	28 (48)	63 (36)	0.091
Antiviral agents: 1/2/3/4* (%)	18/5/5/0 (64/18/18/0)	26/28/8/1 (41/44/13/2)	0.051

Qualitative variables are expressed by the number of patients with percentage in parentheses, and quantitative variables are expressed by the median with range in parentheses. ALT – alanine aminotransferase; AFP – alpha-fetoprotein; IP-10 – the interferon-gamma inducible protein-10; HBV – hepatitis B virus; HBsAg – hepatitis B surface antigen; HBeAg – hepatitis B e antigen; HBcrAg – hepatitis B virus core-related antigen; PC – precore; CP – core promoter. * 1, Interferon alpha; 2, lamivudine; 3, lamivudine plus interferon-alpha; 4, entecavir.

rates of HBeAg seroconversion at 1, 3, 5, 7 and 10 years were 24.8%, 50.1%, 66.3%, 71.3% and 73.1%, respectively, during the follow-up >10 years after liver biopsies (Figure 3). Of note, HCC developed in 18 of the 234 (7.7%) patients during the follow-up.

Figure 4A compares cumulative HBeAg seroconversion rates stratified by HBeAg titers and grades of lobular inflammation. The patients, who had the combination of HBeAg <100 PEIU/ml and lobular inflammation grades ≥2, gained an HBeAg seroconversion rate higher than those having 3 other combinations. Likewise, cumulative HBeAg seroconversion rates stratified by HBeAg titers and ALT levels are compared in Figure 4B. HBeAg seroconversion rate of the patients, who had the combination of HBeAg <100 PEIU/ml and ALT ≥200 IU/l, was higher than those with 3

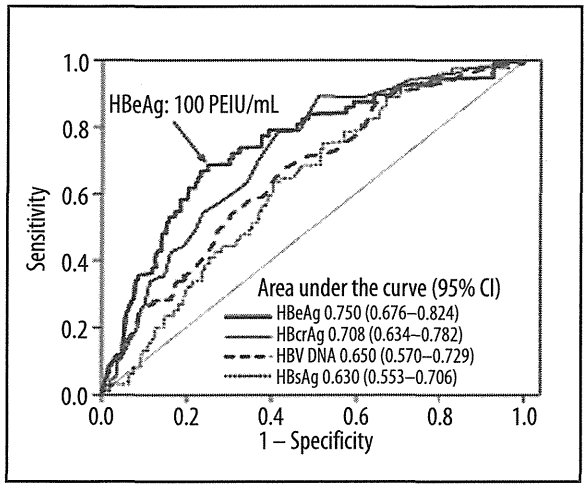


Figure 1. Receiver operating characteristic curves for evaluation of the power of predicting early HBeAg seroconversion.

other combinations, with definitive (p=0.003 and p<0.001) or marginal (p=0.061) significance.

Discussion

HBeAg seroconversion is important as a clinical target in the management of chronic hepatitis B. In the absence of therapeutic interventions, HBeAg seroconversion occurs spontaneously at a rate of 0.8–15% per year [28]. To date, many factors have been found in association with HBeAg seroconversion, including older age, high ALT levels, genotype B (compared with C), the Knodell's index of histologic activities, the amount of HBV core antigen in the liver, high serum AFP levels, increased immunoglobulin-M anti-HBc titers, increased serum β_2-microglobulin concentrations, enhanced expression of HLA-antigens on the membrane of hepatocytes, non-vertical transmission modes, low HBV DNA levels, and high serum levels of IL-10 as well as IL-12 [7–19].

It would be clinically useful to predict early HBeAg seroconversion, because antiviral treatments can be withheld in the patients in whom HBeAg disappears and anti-HBe develops within a certain time limit, perhaps 1 year. In the present study, the majority of patients (99% of the 234 examined) were infected with HBV of genotype C. Patients with persistent HBV infection in Japan are infected with HBV of either genotype B or C, with an increasing gradient of C toward the south [29,30]. All

Table 4. Multivariate analysis for the risk of early HBeAg seroconversion.

Variables	Odds ratio	95% confidence interval	p value
(A) All factors including histological characteristics			
HBeAg (<100 PEIU/ml)	8.430	4.173–17.032	<0.001
Lobular inflammation (≥2)	4.330	2.009–9.331	<0.001
(B) Factors exclusive of histological characteristics			
HBeAg (<100 PEIU/ml)	7.327	3.703–14.497	<0.001
ALT (≥200 IU/l)	3.093	1.562–6.127	0.001

HBeAg – hepatitis B e antigen; ALT – alanine aminotransferase.

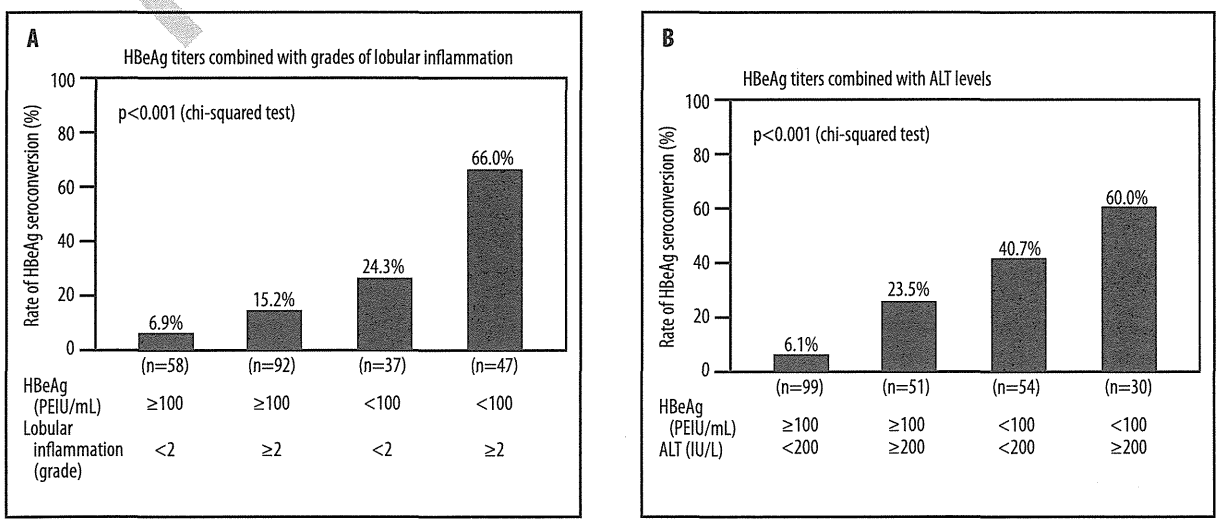


Figure 2. Probability of early HBeAg seroconversion. (**A**) The rate of early HBeAg seroconversion assessed by HBeAg titers and grades of lobular inflammation. (**B**) The rate of early HBeAg seroconversion assessed by HBeAg titers and ALT levels.

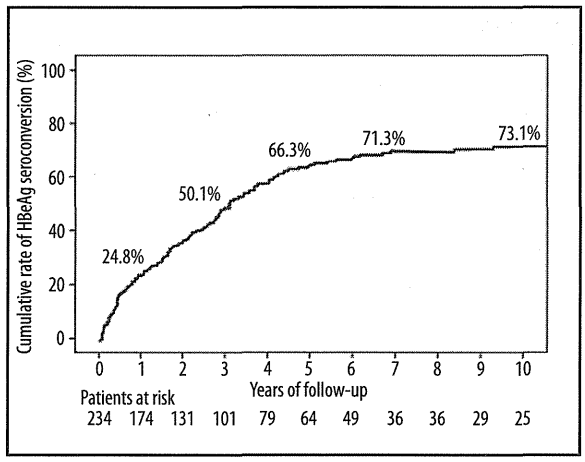


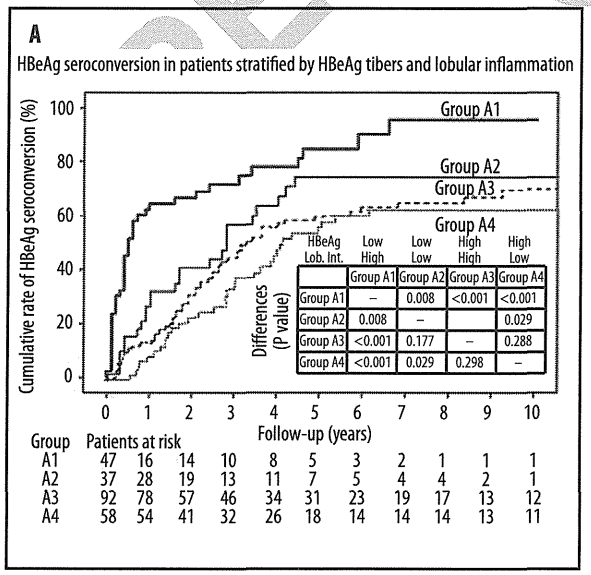
Figure 3. Cumulative rates of HBeAg seroconversion in the 234 patients during 10 years. Cumulative rates of HBeAg seroconversion at 1, 3, 5, 7 and 10 years were 24.8%, 50.1%, 66.3%, 71.3% and 73.1%, respectively, during the follow-up.

the 234 patients had received liver biopsies before they were started to be followed for HBeAg seroconversion. The present study is unique in that, not only serological variables, but also histological parameters were evaluated for the association with early HBeAg seroconversion within 1 year. By univariate analysis, many factors that have been reported in association with HBeAg seroconversion predicted early HBeAg seroconversion. Among them, only HBeAg (<100 PEIU/ml) and lobular inflammation (grades ≥2) remained as independent factors for early HBeAg seroconversion by multivariate analysis.

Previous clinical studies have indicated that serial monitoring of HBsAg, HBeAg and HBV DNA levels during antiviral treatments is useful for predicting HBeAg seroconversion [20–23]. Although the determination of HBV DNA in sera remains as an important tool for monitoring outcomes of patients with chronic hepatitis B, it is technically challenging, costly, and subject to inconsistency. Hence, three serological markers of HBV replication, HBsAg, HBeAg and HBcrAg, were quantitated for evaluating the performance in predicting early HBeAg seroconversion, in comparison with HBV DNA levels. In the receiver operating characteristic analysis, HBeAg levels performed the best amongst these four replication markers, with an area under curve wider than those of the other three. Since the quantitation of HBeAg is relatively easy, fast, and inexpensive, HBeAg would be qualified as a sensitive and practical predictor of early HBeAg seroconversion [20–23].

The histological activity has been reported to predict early HBeAg seroconversion in previous studies [14,31]. Therefore, pathological parameters including the stage of fibrosis, as well as grades of portal inflammation, piecemeal necrosis and lobular inflammation, were evaluated in this study. By multivariate analysis, lobular inflammation of grades ≥2, represented by focal necrosis or acidophil bodies, was identified as an independent factor for early seroconversion. Hence, portal inflammation without necrosis would not be enough, but instead, severe lobular inflammation may be required for predicting early seroconversion.

Many previous studies have identified a variety of factors associated with HBeAg seroconversion [7–19], but a combination of serum markers of HBV with pathological parameters was evaluated rarely. Therefore, the combination of HBeAg <100 PEIU/ml and grades ≥2 lobular inflammation was evaluated for the predictability of early HBeAg seroconversion. Patients with neither HBeAg <100 PEIU/ml nor grades ≥2 lobular inflammation had a minimal chance for early HBeAg seroconversion (6.9% [4/58]), whereas a high proportion of patients with both of these predictors did accomplish early seroconversion (66.0% [31/47]) (Figure 2A). Thus, the combination of histologic activity and serum HBV marker would be very useful for predicting early HBeAg seroconversion, and serve in decision making whether or not



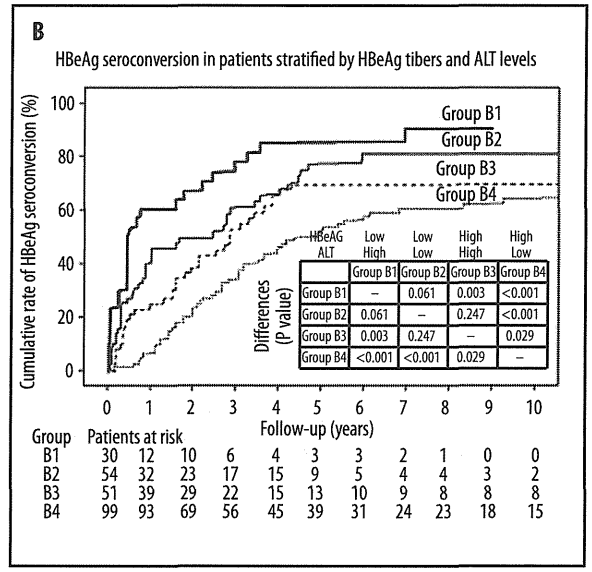


Figure 4. Cumulative rates of HBeAg seroconversion in four groups of patients. (**A**) Cumulative rates of HBeAg seroconversion stratified by HBeAg titers and grades of lobular inflammation. (**B**) Cumulative rates of HBeAg seroconversion stratified by HBeAg titers and ALT levels. HBeAg titers were dichotomized into low (<100 PEIU/ml) or high (≥100 PEIU/ml); lobular inflammation grades into low (<2) or high (≥2); and ALT levels into low (<200 IU/l) or high (≥200 IU/l).

to commence antiviral treatments in HBeAg-positive patients with chronic hepatitis B. Although some patients received antiviral treatments, they would not have influenced the evaluation to any serious extent. Within the first 1 year of follow-up, antiviral treatments were given comparably frequently to patients with and without early HBeAg seroconversion (48% *vs.* 36%, p=0.091). In addition, HBeAg seroconversion is achieved by at most 12–27% of patients who had received antiviral treatments during the first year [28].

Although liver biopsy is essential for defining the stage of disease progression, it has some limitations, in that it is invasive and accompanies the risk of complications. By multivariate analysis, exclusive of pathological factors, ALT ≥200 IU/l remained as an independent factor (Table 4). ALT ≥200 (IU/l), corresponding to 5 × the upper limit of normal [ULN], coincided with the cut-off point recognized by the receiver operating characteristic curve (data not shown). In previous studies, also, ALT levels ≥5 × ULN were predictive of early HBeAg seroconversion [19,32–33]. Present results are in line with these observations, and point to the capability of ALT ≥200 IU/l to replace lobular inflammation of grades ≥2 in the patients in whom liver biopsy is not feasible.

Conclusions

The results of this study indicate that the combination of low HBeAg titers and high grades of lobular inflammation is clinically useful for predicting early HBeAg seroconversion in patients with chronic hepatitis B. When and if liver biopsy is not to be performed, ALT can substitute for lobular inflammation. The combination of low HBeAg titers, with either high grades of lobular inflammation or elevated ALT levels, predicted not only early, but also long-term HBeAg seroconversion.

Acknowledgments

The authors are grateful to Rumiko Hamada, Mika Fukuda, Yoko Tamada, Rumiko Nakao, and Dr. Osami Inoue for valuable supports, and thank Dr. Koji Yano for his suggestions and encouragement in the preparation of this manuscript.

References:

1. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol, 2009; 50: 227–42
2. Lee WM: Hepatitis B virus infection. N Engl J Med, 1997; 337: 1733–45
3. Yuen MF, Lai CL: Natural history of chronic hepatitis B virus infection. J Gastroenterol Hepatol, 2000; 15(Suppl.): E20–24
4. Fattovich G, Rugge M, Brollo L et al: Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology, 1986; 6: 167–72
5. Hoofnagle JH, Dusheiko GM, Seeff LB et al: Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med, 1981; 94: 744–48
6. Realdi G, Alberti A, Rugge M et al: Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology, 1980; 79: 195–99
7. Chu CM, Karayiannis P, Fowler MJ et al: Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. Hepatology, 1985; 5: 431–34
8. Kao JH, Chen PJ, Lai MY, Chen DS: Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. J Med Virol, 2004; 72: 363–69
9. Kim HS, Kim HJ, Shin WG et al: Predictive factors for early HBeAg seroconversion in acute exacerbation of patients with HBeAg-positive chronic hepatitis B. Gastroenterology, 2009; 136: 505–12
10. Liaw YF, Chu CM, Huang MJ et al: Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. Liver, 1984; 4: 301–6
11. Livingston SE, Simonetti JP, Bulkow LR et al: Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F. Gastroenterology, 2007; 133: 1452–57
12. Lok AS, Lai CL, Wu PC et al: Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology, 1987; 92: 1839–43
13. Pignatelli M, Waters J, Brown D et al: HLA class I antigens on the hepatocyte membrane during recovery from acute hepatitis B virus infection and during interferon therapy in chronic hepatitis B virus infection. Hepatology, 1986; 6: 349–53
14. Sanchez-Tapias JM, Costa J, Mas A et al: Analysis of factors predicting early seroconversion to anti-HBe in HBeAg-positive chronic hepatitis B. J Hepatol, 1988; 6: 15–22
15. Sjogren M, Hoofnagle JH: Immunoglobulin M antibody to hepatitis B core antigen in patients with chronic type B hepatitis. Gastroenterology, 1985; 89: 252–58
16. Wu JF, Wu TC, Chen CH et al: Serum levels of interleukin-10 and interleukin-12 predict early, spontaneous hepatitis B virus e antigen seroconversion. Gastroenterology, 2010; 138: 165–72 e161–63
17. Yuen MF, Fung SK, Tanaka Y et al: Longitudinal study of hepatitis activity and viral replication before and after HBeAg seroconversion in chronic hepatitis B patients infected with genotypes B and C. J Clin Microbiol, 2004; 42: 5036–40
18. Yuen MF, Sablon E, Yuan HJ et al: Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology, 2003; 37: 562–67
19. Yuen MF, Yuan HJ, Hui CK et al: A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut, 2003; 52: 416–19
20. Fried MW, Piratvisuth T, Lau GK et al: HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. Hepatology, 2008; 47: 428–34
21. Jang JW, Kim MS, Lee SY et al: Pre- and post-treatment predictors of the early achievement of HBeAg loss in lamivudine-resistant patients receiving adefovir therapy. J Gastroenterol Hepatol, 2007; 22: 1092–97
22. Park NH, Shin JW, Park JH et al: Monitoring of HBeAg levels may help to predict the outcomes of lamivudine therapy for HBeAg positive chronic hepatitis B. J Viral Hepat, 2005; 12: 216–21
23. Tangkijvanich P, Komolmit P, Mahachai V et al: Comparison between quantitative hepatitis B surface antigen, hepatitis B e-antigen and hepatitis B virus DNA levels for predicting virological response to pegylated interferon-alpha-2b therapy in hepatitis B e-antigen-positive chronic hepatitis B. Hepatol Res, 2010; 40: 269–77
24. Kato H, Orito E, Sugauchi F et al: Frequent coinfection with hepatitis B virus strains of distinct genotypes detected by hybridization with type-specific probes immobilized on a solid-phase support. J Virol Methods, 2003; 110: 29–35
25. Asahina Y, Izumi N, Uchihara M et al: Core promoter/pre-core mutations are associated with lamivudine-induced HBeAg loss in chronic hepatitis B with genotype C. J Hepatol, 2003; 39: 1063–69
26. Desmet VJ, Gerber M, Hoofnagle JH et al: Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology, 1994; 19: 1513–20
27. Scheuer PJ: Classification of chronic viral hepatitis: a need for reassessment. J Hepatol, 1991; 13: 372–74
28. Liaw YF, Lau GK, Kao JH, Gane E: Hepatitis B e antigen seroconversion: a critical event in chronic hepatitis B virus infection. Dig Dis Sci, 2010; 55: 2727–34
29. Orito E, Ichida T, Sakugawa H et al: Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology, 2001; 34: 590–94
30. Yamishita Y, Kurashina S, Miyakawa Y, Mayumi M: South-to-north gradient in distribution of the r determinant of hepatitis B surface antigen in Japan. J Infect Dis, 1975; 131: 567–69
31. Perrillo RP, Lai CL, Liaw YF et al: Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology, 2002; 36: 186–94
32. Liaw YF: Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. J Gastroenterol Hepatol, 2003; 18: 246–52
33. Lok AS, Lai CL: Acute exacerbations in Chinese patients with chronic hepatitis B virus (HBV) infection. Incidence, predisposing factors and etiology. J Hepatol, 1990; 10: 29–34

shortcut though impairs the analysis in many different ways, limiting our full understanding of the phenomenon being modelled and ultimately our ability to accurately assess 'value for money' beyond the simple 'average'. This paper explores the value of access to individual patient data for cost-effectiveness modelling, structuring the discussion of the topic around three interrelated questions. First, what benefits can access to IPD bring to cost-effectiveness modelling? Second, what are the challenges for the simultaneous statistically synthesis of AD plus IPD to derive input parameters for a cost-effectiveness model? Third, what is the value of access to IPD compared to AD for cost-effectiveness modelling? Using two different case studies, the above questions will be addressed and discussed in the context of the debate around CEA of individualised treatment decisions.

DISEASE-SPECIFIC STUDIES

GASTROINTESTINAL DISORDERS - Clinical Outcomes Studies

PGI1

THE EFFECTIVENESS AND TOLERABILITY OF COMBINED TREATMENT WITH PEGINTERFERON ALPHA-2A OR ALPHA-2B AND RIBAVIRIN IN THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C: RESULTS BASED ON THE NATIONWIDE HEPATITIS REGISTRY IN JAPAN

Shimbo T, Miyaki K, Song Y, Masaki N, Study Group Developing Nationwide Database of Hepatitis Japan
National Center for Global Health and Medicine, Shinjuku-ku, Tokyo, Japan

OBJECTIVES: When comparing combined therapy with peginterferon alpha-2a or alpha-2b and ribavirin to treat chronic hepatitis C (CHC), the results of clinical trials, observational studies, and meta-analyses have been inconsistent. Their effectiveness and tolerability were investigated using the nationwide database of chronic hepatitis patients who received interferon therapy in Japan. **METHODS:** The proportion with a sustained virologic response (SVR) and the dropout rate due to adverse events (AEs) were compared between alpha-2a and alpha-2b. All patients also received ribavirin. Multivariate logistic regression was conducted with adjustment for age, sex, platelet counts, ALT, viral load, genotype, and whether the patient was treatment-naïve, which are associated with effectiveness and tolerability. **RESULTS:** By December 2011, the database included 7820 patients. CHC patients treated with either alpha-2a (n=1737) or alpha-2b (n=4495) were analyzed. The mean (SD) age was 58.1 (10.4) years, and 3131 (50.2%) were female. In total, 2503 (41.0%) patients had a platelet count <150x103, 2503 (40.5%) had ALT > 60 IU/L, and 5765 (93.2%) had a high viral load. The numbers with genotype 1, 2, and 3 were 4291 (69.2%), 1838 (29.6%), and 76 (1.2%), respectively. Overall, 4434 (71.2%) patients were treatment-naïve. SVR was achieved in 53.5% (95% CI: 51.1-55.9%) with alpha-2a and 61.6% (95% CI: 60.2-63.1%) with alpha-2b (p<0.001). The dropout rate due to any AEs was 10.3% (95%CI: 8.9-11.8%) and 9.3% (95%CI: 8.5-10.2%) for alpha-2a and alpha-2b, respectively (p=0.226). After adjustment for possible confounders, no differences in effectiveness or tolerability were observed between the therapies, and the odds ratio of alpha-2a for SVR was 0.97 (95% CI: 0.86-1.10), and its odds ratio for dropout due to any AEs was 0.96 (95% CI: 0.79-1.17). There was no significant interaction of genotype and therapy. **CONCLUSIONS:** Alpha-2a and alpha-2b in combination with ribavirin showed comparable effectiveness and tolerability in clinical settings.

PGI2

INFLIXIMAB REDUCES THE RISK OF SURGICAL INTERVENTIONS AND HOSPITALIZATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Costa J[1], Alarcão J[1], Caldeira D[2], Borges M[1], Vaz Carneiro A[1]
[1]*Center for Evidence Based Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal,* [2]*Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal*

OBJECTIVES: In addition to the pharmacological efficacy of infliximab therapy in inflammatory bowel disease (IBD), it is also important to evaluate its impact on other health outcomes, particularly in the rate of surgical interventions and hospitalizations, which have high economic burden and are believed to represent a marker of IBD severity. We aimed to estimate the impact of infliximab in these outcomes in patients with IBD. **METHODS:** Systematic review and meta-analysis of experimental (clinical trials) and observational studies comparing infliximab with any other control group in IBD. Studies were identified by searching Medline and Cochrane from inception to April 2012. Search results and studies characteristics were assessed independently. Subgroup analyses were done according to IBD type: Crohn disease (CD) and ulcerative colitis (UC). Pooled estimates were performed separately for clinical trials and observational studies. Odds ratios (OR) and 95% confidence intervals (CI) were derived by random-effects meta-analysis. Heterogeneity was assessed with I^2 test. **RESULTS:** Nine trials and 9 observational studies were included. Infliximab significantly decreased risk of gastrointestinal surgery in experimental studies (OR 0.36; 95%CI: 0.18-0.71), both in DC (OR 0.25; 95%CI: 0.10-0.63) and UC (OR 0:55; 95%CI: 0.40-0.76). In absolute terms, there was a 9% reduction in the rate of surgery (95%CI: 1-19%). Observational studies also showed a reduced risk of surgery, which was significant in the case of CD (OR 0.42; 95% CI: 0.22 to 0.78). Infliximab significantly reduced the risk of hospitalization, both in experimental (OR 0.48; 95%CI: 0.34-0.66) and observational (OR 0:38; 95%CI: 0.24-0.58) studies, with a decrease of 9% in hospitalization rate (95%CI: 5-14%). Mean duration of hospitalization was shorten by 4.2 days (95%CI: 1.9-6.5) in infliximab treated patients. **CONCLUSIONS:** Based on the best available evidence, infliximab therapy is associated with a reduced risk of gastrointestinal surgery and hospitalization rates in patients with IBD.

PGI3

TREATMENT OF CHRONIC HEPATITIS C GENOTYPE 1 IN POLAND – REAL-LIFE DATA

Kaczor MP[1], Pawlik D[2], Wójcik R[2], Rolka M[2], Maniszewska-Weyher I[3], Tronczynski K[3]
[1]*Jagiellonian University Medical College, Kraków, Poland,* [2]*Aestimo s.c., Kraków, Poland,* [3]*Janssen-Cilag Polska, Warsaw, Poland*

OBJECTIVES: To describe health outcomes, the course of treatment and the demographic and clinical characteristics of HCV adult patients infected with genotype 1 receiving interferon-alfa+ribavirin therapy in Poland. **METHODS:** A retrospective analysis of anonymous data of patients treated in the HCV therapeutic programme of the National Health Fund was performed. Data was gathered from three medical centres and included demographic and clinical characteristics (sex, age, body weight, initial HCV RNA level, disease staging and grading) as well as treatment course (first line/retreatment, posology, treatment duration, outcomes and discontinuations). **RESULTS:** A total of 813 HCV genotype 1 adult patients' records [586 treatment-naïve (N) and 227 treatment-experienced (E)] were included in the analysis. 55% were male (N: 53%, E: 60%), mean age at the beginning of therapy was 48 (SD:13) years. Mean body weight was 68,0 (SD:11,8) kg in females and 82,4 (SD:12,3) kg in males. Mean initial HCV RNA was 5,9 (SD:0,8) $\log_{10}$IU/mL and 46% of patients had HCV RNA<800 000 IU/mL. A total of 85% records included data on disease staging (Sheuer 0-2: 67%; stage 3: 19%; stage 4: 14%). 96% of patients received pegylated interferons (pegylated interferon-alfa2a: 54%), 97% with ribavirin. A total of 15% of patients discontinued therapy prematurely (N: 14%, E: 18%) after a mean of 6 months, and mean treatment duration was 44 weeks for all patients. Overall SVR (sustained viral response) was achieved in 42% of patients (N: 45%, E: 33%). Among treatment-naïve patients not fully responding to therapy, 41% had relapse, 21% were partial responders and 38% were null-responders. **CONCLUSIONS:** The real-life results of HCV genotype 1 treatment, with SVR rates below 50% in treatment naïve patients, are unsatisfactory, especially when in Poland the prevalence of this difficult-to-treat genotype is one of the most highest in Europe. Forthcoming triple therapy with HCV protease inhibitors are promising and anticipated options for these patients.

PGI4

EVALUATION OF THE EFFICACY AND INCONTINENCE RATE OF BIOMATERIALS IN COMPARISON TO CONSERVATIVE AND OTHER INTERVENTIONAL THERAPIES IN TREATMENT OF PERIANAL FISTULA. A META-ANALYSIS

Mirfazaelian H[1], Nikfar S[2], Derakhshani S[3], Abdollahi M[1]
[1]*Tehran University of Medical Sciences, Tehran, Iran,* [2]*Tehran University of Medical Sciences, Tehran, Tehran, Iran,* [3]*Parsian Hospital, Tehran, Iran*

OBJECTIVES: This meta-analysis of randomized controlled trials was conducted to evaluate the efficacy and incontinence rate of biomaterials (fibrin glue and fibrin plug) in comparison to conservative and other interventional therapy in the treatment of perianal fistula. **METHODS:** PubMed, Embase, Scopus, Google Scholar, and Web of Science were searched for clinical trial studies investigated the effects of biomaterials in the treatment of fistul in-ano. Clinical response and incontinence were the key outcomes of interest. Data were searched from the time period of 1966 through June 2012. **RESULTS:** Eight randomized placebo-controlled clinical trials that met our criteria (six comparing biomaterial with conservative treatment and two with other interventions) were included in the analysis. Pooling of data showed biomaterials effectiveness in comparison to other interventions was non significant with relative risk (RR) of 1.23 (95% CI of 0.31-4.84, P= 0.77). The RR for biomaterials comparing with conservative was non significant (RR= 0.73 with 95% CI = 0.31-0.89, P= 0.096). The incontinence rate RR in biomaterials and intervention was also non significant with RR of 0.35 (95% CI = 0.05-2.28, P = 0.27). **CONCLUSIONS:** This meta-analysis demonstrates that the effectiveness of biomaterials and conservative treatment was not different. The biomaterials in comparison to other interventional therapies did not show any difference in regard to effectiveness and also incontinence rate.

PGI5

EFFECTIVENESS AND SAFETY OF ANTACIDS IN PREGNANT WOMEN SUFFERING FROM GERD/HYPERACIDITY SYMPTOMS

Donde S
Pfizer India, Mumbai, India

OBJECTIVES: Symptoms of gastro-oesophageal reflux disease are estimated to occur in 30-50% of pregnancies, with the incidence approaching 80% in some populations. Indian studies have shown prevalence ranging upto 50 % . As with many other conditions in pregnancy, medical therapy with pharmaceutical agents is a concern, as the potential teratogenicity of medications is not well known. Though prevalence numbers are high, many patients have mild and infrequent symptoms, which often respond to lifestyle and dietary modifications. However, some patients report very severe symptoms of hyperacidity affecting their Quality of Life which need treatment. The safety of H2 Receptor antagonists and PPIs in pregnancy is not well established. Antacids could be a good option as their systemic toxicity is low and safety profile is enhanced. **METHODS:** Data has been collected from practicing gynaecologists in a hospital located in Southern India . 50 female patients suffering from heartburn and/or dyspepsia as symptoms of hyperacidity and GERD were treated with antacids containing aluminium hydroxide, magnesium hydroxide and dimethicone. The patients were prescribed antacids for atleast 7 days and the effectiveness and safety profile of antacids was studied. The patients were followed up after one week and the response along with adverse effects were documented. **RESULTS:** The effectiveness was achieved in 85 % of women who took only antacids atleast for 1 week. In 10 % of patients, Proton Pump Inhibitor was also added to achieve the desired response. Apart from 2 cases of mild diarrhea, no other significant side effects were noted. **CONCLUSIONS:** Antacids containing aluminium hydroxide , magnesium hydroxide and dimethicone can be a good therapy option

J Gastroenterol
DOI 10.1007/s00535-012-0679-8

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Risk factors for the exacerbation of esophageal varices or portosystemic encephalopathy after sustained virological response with IFN therapy for HCV-related compensated cirrhosis

Yuko Nagaoki · Hiroshi Aikata · Tomoki Kobayashi · Takayuki Fukuhara · Keiichi Masaki · Mio Tanaka · Noriaki Naeshiro · Takashi Nakahara · Yohji Honda · Daisuke Miyaki · Tomokazu Kawaoka · Shintaro Takaki · Masataka Tsuge · Akira Hiramatsu · Michio Imamura · Hideyuki Hyogo · Yoshiiku Kawakami · Shoichi Takahashi · Hidenori Ochi · Kazuaki Chayama

Received: 18 June 2012 / Accepted: 3 September 2012

Abstract
Background We aimed to identify risk factors contributing to the exacerbation of esophageal varices (EV) or portosystemic encephalopathy after hepatitis C virus (HCV) eradication with interferon (IFN) therapy in patients with compensated cirrhosis. Also, the prognosis after HCV eradication was analyzed.
Methods Fifty-two patients with sustained virological response to IFN treatment for HCV-related compensated cirrhosis were enrolled in this retrospective cohort study.
Results At the achievement of HCV eradication, in 31 of the 52 patients (60 %), feeding vessels for EVs (left gastric vein, posterior gastric vein, short gastric vein) were shown, and in 18 patients (35 %) there were extrahepatic portosystemic shunts (paraesophageal vein, paraumbilical vein, and splenorenal shunt). Although the HCV eradication was successful, significant improvements were not observed in portosystemic collateral vessels 1 year after HCV eradication, and EVs were exacerbated in 19 (36 %) patients. The cumulative 1- and 3-year rates of EV exacerbation were 13 % and 49 %, respectively. By multivariate analysis, the existence of feeding vessels for EVs at HCV eradication was an independent predictive factor for the exacerbation of EVs ($P = 0.009$). Seven patients who had an extrahepatic portosystemic shunt at HCV eradication developed portosystemic encephalopathy during follow up. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy were 6, 21, and 34 %, respectively. The cumulative 5-year survival rate of the cohort was 81 %. Two patients died of hepatocellular carcinoma (HCC).
Conclusions Our findings suggest that the existence of radical portosystemic collateral vessels at successful HCV eradication increases the risk of the exacerbation of EVs and the incidence of portosystemic encephalopathy in patients with HCV-related cirrhosis.

Keywords HCV-related cirrhosis · Interferon therapy · Sustained virological response · Esophageal varices · Portosystemic encephalopathy · Portosystemic collateral vessels

Introduction

Chronic hepatitis C is a common disease that may progress to cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Longitudinal evaluation of patients with compensated cirrhosis caused by HCV infection has clearly shown that the yearly development rate of HCC ranges between 2 and 4 %, and HCC is the most frequent liver-related complication in these patients [3]. Interferon (IFN)-based therapy in patients with chronic hepatitis C results in the amelioration of hepatic inflammation and fibrosis, improvement of serum alanine aminotransferase levels, decrease of circulating HCV-RNA levels, and a decrease in the incidence of HCC development. Accordingly, it has been considered that IFN therapy and HCV eradication would be an

Y. Nagaoki · H. Aikata (✉) · T. Kobayashi · T. Fukuhara · K. Masaki · M. Tanaka · N. Naeshiro · T. Nakahara · Y. Honda · D. Miyaki · T. Kawaoka · S. Takaki · M. Tsuge · A. Hiramatsu · M. Imamura · H. Hyogo · Y. Kawakami · S. Takahashi · H. Ochi · K. Chayama
Programs for Applied Biomedicine, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
e-mail: aikata@hiroshima-u.ac.jp

Published online: 05 October 2012

effective cancer chemoprevention strategy for patients with chronic hepatitis C [3–5].

A reduced incidence of HCC in HCV-associated cirrhosis brought about by IFN therapy has been reported by many investigators [6–10]. Also the prognosis of patients with cirrhosis has improved markedly in recent years through advances in clinical management, such as IFN therapy, long-term supplementation with branched-chain amino acids, and the use of a low protein and low salt diet [11–14]. In 2011, antiviral therapy using pegylated-interferonα2b (PEG-IFNα-2b) combined with ribavirin was accepted by the Japanese health insurance system for the treatment of liver cirrhosis. In the future, this therapy is expected to lead to benefits such as the regression of cirrhosis and the reduction and prevention of cirrhosis-related complications.

At present, hemorrhage from esophageal or gastric varices is still one of the main causes of death in patients with liver cirrhosis. The reported prevalence of esophageal varices (EVs) in patients with cirrhosis ranges from 80 to 90 % [15, 16], and, annually, 10–30 % of patients with EVs develop variceal hemorrhage [17]. Despite substantial improvements in the early diagnosis and treatment of variceal hemorrhage, the associated mortality remains high (20–35 %) [1, 12, 18]. Proper management of EVs would be expected to improve the prognosis of patients with liver cirrhosis.

The decreased incidence of HCC and the attenuation of liver fibrosis in cirrhotic patients with HCV eradication is now well documented [3–5], even though we often experience the development of HCC, exacerbation of EVs, and portosystemic encephalopathy in these patients. However, the risk factors contributing to the exacerbation of EVs and portosystemic encephalopathy after HCV eradication have not been fully investigated. In this study, we explored the risk factors that contribute to the exacerbation of EVs and portosystemic encephalopathy after HCV eradication in patients with liver cirrhosis, and we also analyzed the prognosis of the patients.

Patients, materials, and methods

Patients

We retrospectively reviewed 362 consecutive patients with hepatitis C virus (HCV)-related compensated cirrhosis who received IFN therapy at Hiroshima University Hospital between January 2001 and December 2010. HCV eradication was achieved in 63 of these 362 patients (17.4 %). In brief, the HCV eradication rate was 12.5 % in patients with genotype 1b, and 38.2 % in patients with genotypes 2a, 2b, and other genotypes. All patients were negative for hepatitis B surface antigen. Of the 63 patients with compensated cirrhosis after HCV eradication, 52 patients who received regular surveillance such as liver function tests, ultrasonography, dynamic computed tomography (CT), and endoscopic examinations periodically after IFN therapy were enrolled in this study.

The diagnosis of cirrhosis was based on histological findings and/or clinical findings. Compensated cirrhosis was defined as cirrhosis with no history of ascites, jaundice, hepatic encephalopathy, or variceal bleeding at entry into the study.

Endoscopic examination for assessing EVs

The endoscopic findings of EVs were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [19]. The form (F) of EVs was classified as complete eradication after treatment (F0), small straight (F1), enlarged tortuous (F2), and large coiled-shaped (F3). The red color sign (RC) was also classified based on the criteria of the Japanese Society for Portal Hypertension and Esophageal Varices [19]. Endoscopic examinations were carried out at least annually. Worsening of the F and the RC sign compared with baseline findings on follow-up endoscopy was defined as exacerbation of EVs.

Computed tomography (CT) examination

CT examinations were done in the high-quality scanning mode, with 1.25-mm slice thickness, and reconstruction intervals of 0.625-mm for portal venous phase images. The first and second acquisitions were used for hepatic artery phase images, the third acquisition for portal venous phase images, and the fourth acquisition for hepatic venous phase images. The left gastric vein, posterior gastric vein, short gastric vein, paraesophageal vein, paraumbilical vein, and splenorenal shunt were considered as portosystemic collateral vessels. Evaluation of portosystemic collateral vessels was done by dynamic CT. The diameters of the portosystemic collateral vessels were measured, with the largest portion of the vessel being recorded in all cases. Cutoff diameters of 6, 4, 2, 4, 3, and 13 mm, respectively, represented the median values of these vessels. All patients received regular annual surveillance by dynamic CT, to check for extrahepatic portosystemic shunt and HCC.

Diagnosis of HCC

Hepatocellular carcinoma was diagnosed based on a hypervascular staining pattern in the arterial phase and a hypovascular staining pattern in the portal phase, shown by dynamic CT, magnetic resonance imaging, and/or

angiography. Tumors without enhancement upon imaging were diagnosed by fine-needle biopsy.

Follow up of enrolled patients

All 52 patients received regular surveillance, including annual blood examinations, endoscopic examinations, and dynamic CT scans after HCV eradication. Monitoring for the presence of hepatic encephalopathy and HCC was precisely evaluated at each examination. The rate of exacerbation of EVs, the incidence of portosystemic encephalopathy and HCC, and the prognosis after HCV eradication were evaluated based on these findings.

Statistical analysis

Statistically significant differences in quantitative data were determined using the Mann–Whitney *U*-test and the Wilcoxon rank-sum test, when applicable. Overall survival and EV exacerbation rates were calculated by the Kaplan–Meier method. Multivariate analysis was conducted with a Cox proportional hazard model using the stepwise selection of variables or two logistic analyses. All statistical analyses were performed using an SPSS software package (version 12.0 for Windows; SPSS, Chicago, IL, USA), with $P < 0.05$ denoting statistical significance.

Table 1 Clinical characteristics of 52 patients before interferon (IFN) therapy

Age (years, range)	58 (39–73)[a]
Sex (male/female)	36/16
Genotype 1b/others	36/16
Child–Pugh grade A/B	45/7
Diabetes mellitus (with/without)	19/33
Alcohol intake (with/without)	19/33
Total bilirubin (mg/dl, range)	0.9 (0.4–3.9)[a]
AST (IU/l, range)	62 (29–265)[a]
ALT (IU/l, range)	64 (22–321)[a]
Albumin (g/dl, range)	3.9 (2.8–5.0)[a]
Platelet count ($\times 10^4/\mu l$, range)	7.3 (3.4–24.6)[a]
Prothrombin time activity (%, range)	85 (36–121)[a]
Total cholesterol (mg/dl)	151.2 (90–2275)[a]
Body mass index (kg/m^2, range)	22.5 (16.3–30.4)[a]
Past history of HCC (with/without)	26/26
Esophageal variceal form (F1/F2)	22/5

[a] Median

Alcohol intake, ≥80 g/day for more than 5 years

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCC* hepatocellular carcinoma, *Variceal form* form according to the criteria of the Japanese Society for Portal Hypertension and Esophageal Varices

Results

Clinical characteristics of enrolled patients

The clinical characteristics of the 52 patients are shown in Table 1. The median age of the patients was 58 years (range 39–73 years); 36 patients were men and 16, women; 36 patients had HCV genotype 1b; and 45 patients were classified as Child–Pugh grade A before IFN therapy. Before the IFN therapy, 25 (48 %) patients did not have EVs, and among the 27 patients with EVs, those in 22 patients (42 %) were classified as F1 and those in 5 patients (10 %) as F2. The RC was not observed in any of the enrolled patients (Table 1). In this study, in regard to portosystemic collateral vessels, the left gastric vein, posterior gastric vein, and short gastric vein were defined as feeding vessels for EVs, and the paraesophageal vein, paraumbilical vein, and splenorenal shunt were defined as extrahepatic portosystemic shunts. The portosystemic collateral vessels are listed in Table 2.

Changes in portosystemic collateral vessels after HCV eradication

Among the 52 patients, 31 patients had feeding vessels for EVs and 18 patients had an extrahepatic portosystemic shunt at HCV eradication. We compared portosystemic collateral vessels at HCV eradication with those seen 1 year after HCV eradication. Significant improvements were not observed in the portosystemic collateral vessels (Fig. 1).

We also compared changes in portosystemic collateral vessels between those seen at HCV eradication and those seen 1 year after HCV eradication in patients who showed exacerbation of EVs and those who did not show exacerbation, and in patients with and without portosystemic hepatic encephalopathy. The diameter of the portosystemic collateral vessels had worsened in some patients. However,

Table 2 Portosystemic collateral vessels at the time that hepatitis C virus (HCV) eradication was achieved

Feeding vessels for EVs (number)	$n = 31/52$
Left gastric vein	18
Posterior gastric vein	20
Short gastric vein	28
Extrahepatic portosystemic shunt (number)	$n = 18/52$
Paraesophageal vein	13
Paraumbilical vein	9
Splenorenal shunt	9

EVs esophageal varices

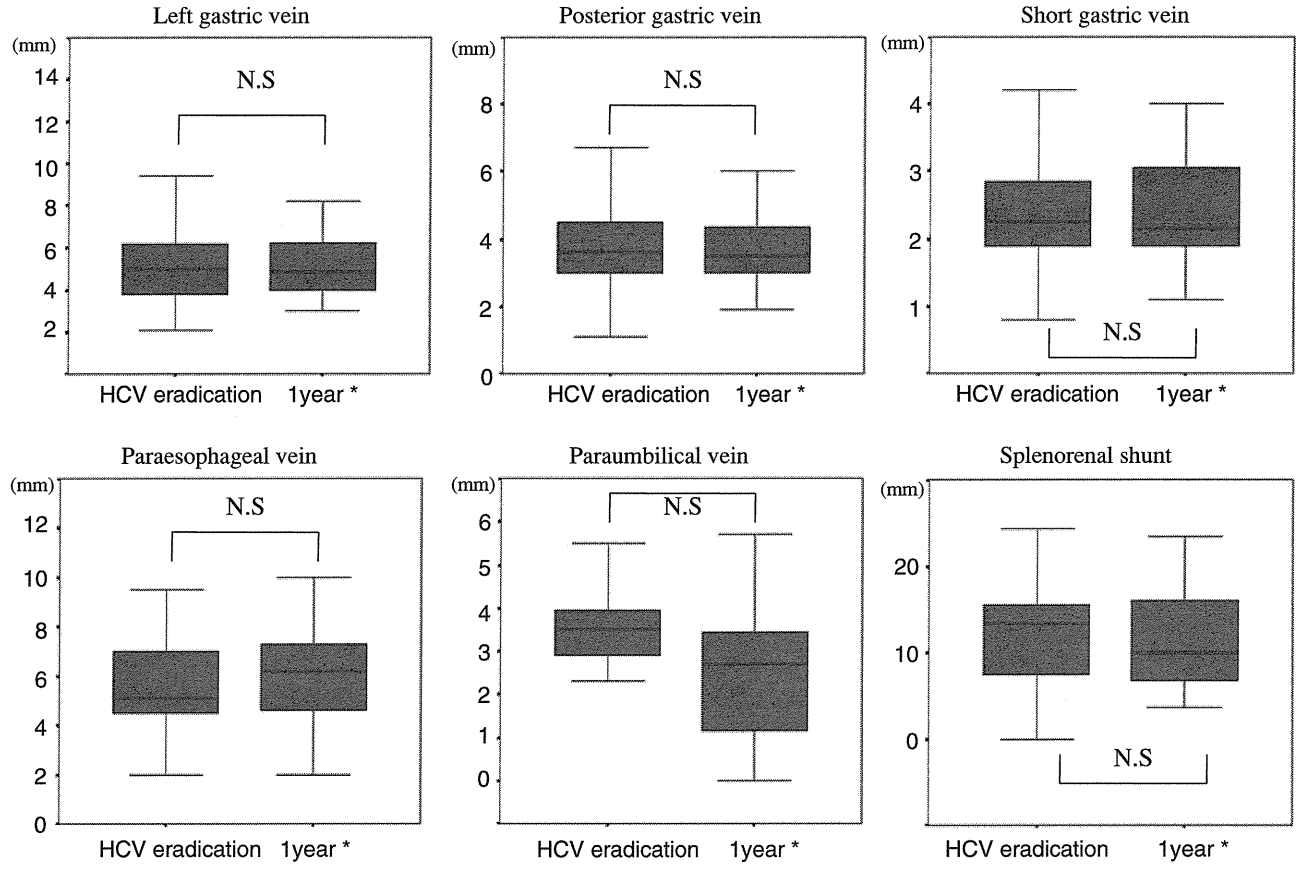


Fig. 1 Changes in the diameters of portosystemic collateral vessels in patients at hepatitis C virus (*HCV*) eradication and 1 year after HCV eradication. *1 year** 1 year after HCV eradication. *N.S Not significant*

significant improvements were not observed in portosystemic collateral vessels at 1 year after HCV eradication.

Risk factors for exacerbation of EVs after HCV eradication

Esophageal varices were exacerbated in 19 patients. The median period until exacerbation after HCV eradication was 13 months (range 4–36 months). The cumulative 1- and 3-year exacerbation rates were 13 and 49 %, respectively (Fig. 2). One year after HCV eradication, EVs were exacerbated from F1 to F2 in 13 patients, from F1 to F3 in 2 patients, and from F2 to F3 or development of the RC sign in 4 patients; a representative case is shown in Fig. 3. By univariate analysis, serum albumin <4.0 g/dl, past history of HCC, recurrence or emergence of HCC, and existence of feeding vessels for EVs at HCV eradication were significantly associated with the exacerbation of EVs during the follow-up period (Table 3). By multivariate analysis, the existence of feeding vessels for EVs at HCV eradication was an independent predictive factor for the exacerbation of EVs (risk ratio 3.719, 95 % confidence interval [CI] 1.387–89.975, $P = 0.009$).

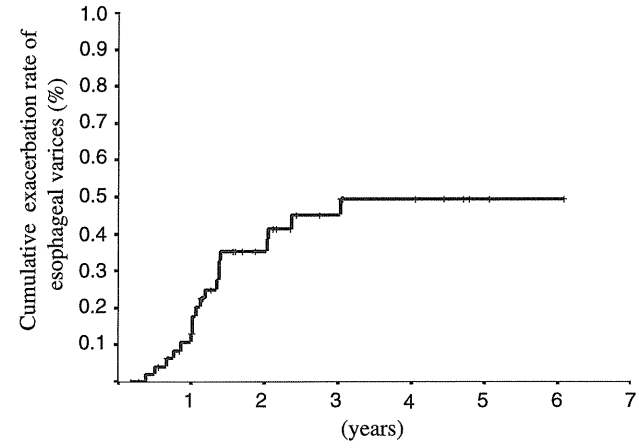


Fig. 2 The cumulative exacerbation rate of esophageal varices (EVs) after HCV eradication with interferon (IFN) therapy

Incidence of portosystemic encephalopathy after HCV eradication

Among the 52 patients, 7 patients who had an extrahepatic portosystemic shunt at HCV eradication developed portosystemic encephalopathy during follow up. The clinical

characteristics of these patients are shown in Table 4. Six of these patients were Child–Pugh grade A. All seven patients had a splenorenal shunt as the extrahepatic portosystemic shunt at the study entry. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy were 6, 21, and 34 %, respectively (Fig. 4). The median period from HCV eradication until the development of portosystemic encephalopathy was 15 months (range 8–59 months). Representative changes in an extrahepatic portosystemic shunt are shown in Fig. 5.

Comparison of liver function in patients before IFN therapy and 1 year after HCV eradication

Liver function was compared between the patients with or without feeding vessels for EVs and extrahepatic portosystemic shunt before IFN therapy and 1 year after HCV eradication. Regardless of the presence of portosystemic collateral vessels, liver function was improved at 1 year after HCV eradication, as shown in Tables 5 and 6.

Development of HCC after HCV eradication

Among the 26 patients with no history of previous HCC, 3 (12 %) patients developed HCC after HCV eradication. The median period from the time of HCV eradication to HCC development was 53 months (range 21–56 months), and the cumulative 1-, 3-, and 5-year development rates were 0, 6, and 34 %, respectively. Sixteen of the 26 patients with a past history of HCC before IFN therapy developed tumor recurrence after HCV eradication.

Overall survival after HCV eradication

The median observation period after HCV eradication was 21 months (range 1–79 months). The cumulative 5-year survival rate was 81 % (Fig. 6). Two patients died of HCC and four patients died of extrahepatic complications (two from septic shock, one from cancer of the maxilla, and one from heart failure). No patients died from hemorrhage from EVs or from liver failure.

Discussion

The prognosis of patients with HCV-related cirrhosis is greatly affected by the development of HCC, hemorrhage from esophageal or gastric varices, and hepatic encephalopathy, especially during the compensation period. A previous report by Kasahara et al. [4] demonstrated that the

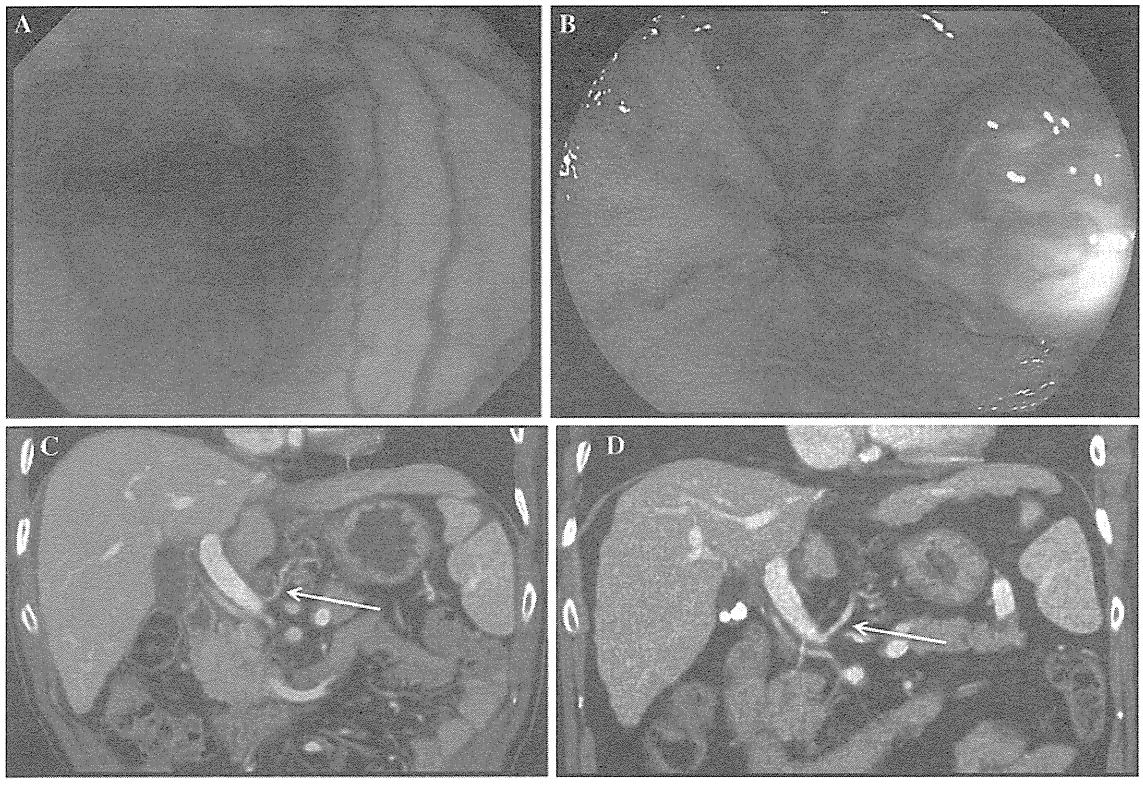


Fig. 3 Representative case: findings in a 56-year-old man with compensated HCV-related cirrhosis. Gastroendoscopic and dynamic computed tomography (CT) findings at the study entry (**a**, **c**) and 1 year after HCV eradication (**b**, **d**). EVs were exacerbated from F1 to F2. *Arrows* show the left gastric vein, and this was the feeding vessel responsible for the exacerbation

development of HCC in patients with chronic hepatitis C was suppressed by IFN therapy. Likewise, HCC was reported to be significantly inhibited by IFN therapy in patients with HCV-related cirrhosis [6–10]. However, the risk factors contributing to the exacerbation of EVs, the incidence of portosystemic encephalopathy, and the overall survival rates after HCV eradication in cirrhotic patients have not been fully elucidated. Accordingly, the present study aimed to elucidate these points.

In our study, no improvement of portosystemic collateral vessels was observed at 1 year after HCV eradication, but exacerbation of EVs was observed in 37 % of the patients. The cumulative 1- and 3-year exacerbation rates were 13 and 49 %, respectively. The existence of feeding vessels for EVs at HCV eradication was the only independent predictive factor for the exacerbation of EVs shown by multivariate analysis (risk ratio 3.719, 95 % CI 1.387–89.975, $P = 0.009$). Thirteen percent of all patients developed portosystemic encephalopathy after HCV eradication. They all had a splenorenal shunt as the extrahepatic portosystemic shunt. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy after HCV eradication were 6, 21, and 34 %, respectively.

Regarding liver function, several studies have reported that IFN therapy in patients with HCV-related cirrhosis

Table 3 Univariate analysis of risk factors associated with the exacerbation of esophageal varices after HCV eradication with IFN therapy

At HCV eradication	Category	*P* value
Age (years)	≥60/<60	0.8297
Sex (male/female)	Male/female	0.7322
Genotype 1b/others	1b/others	0.7271
Diabetes mellitus	With/without	0.0666
Alcohol intake	With/without	0.2465
Child–Pugh grade	A/B	0.1192
Past history of HCC	With/without	0.0097
Recurrence or emergence of HCC	With/without	0.0112
Total bilirubin (mg/dl)	≥1.0/<1.0	0.1402
AST (IU/l)	≥50/<50	0.9826
ALT (IU/l)	≥50/<50	0.7608
Albumin (g/dl)	≥4.0/<4.0	0.0343
Platelet count ($\times 10^4/\mu l$)	≥12/<12	0.7238
Prothrombin time activity (%)	≥80/<80	0.2028
Total cholesterol (mg/dl)	≥180/<180	0.7429
Body mass index (kg/m^2)	≥25/<25	0.4416
Feeding vessels for EVs	With/without	0.0010
Extrahepatic portosystemic shunt	With/without	0.5156

Alcohol intake, ≥80 g/day for more than 5 years

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCC* hepatocellular carcinoma, *EVs* esophageal varices

Table 4 Clinical characteristics of the 7 patients who developed portosystemic encephalopathy

Case no.	Sex	Age (years)	Child–Pugh score	At HCV eradication					At the time portosystemic encephalopathy occurred						
				T-bil (mg/dl)	Alb (g/dl)	Plt ($\times 10^4/\mu l$)	PT (%)	Shunt	Period (months)[a]	Child–Pugh score	T-bil (mg/dl)	Alb (g/dl)	Plt ($\times 10^4/\mu l$)	PT (%)	NH_3 (μg/dl)
1	F	70	5	0.6	4.2	23.3	89	PE, SR	8	8	0.6	43.3	19.8	59	131
2	M	77	5	0.9	3.0	10.5	74	PE, SR	12	9	0.9	2.5	15.0	42	99
3	M	66	7	1.3	2.9	6.8	85	PU, SR	13	8	1.3	3.6	5.5	51	62
4	M	77	5	0.9	4.5	13.4	86	SR	15	5	0.6	4.3	11.3	96	127
5	M	56	5	1.2	4.1	6.5	80	PE, PU, SR	23	5	1.0	4.2	8.5	54	116
6	M	70	5	1.0	3.7	9.6	93	PE, SR	36	5	1.0	4.1	10.0	97	82
7	F	60	5	1.2	3.5	7.3	85	PE, SR	59	5	1.5	3.9	10.3	73	109

T-bil total bilirubin, *Alb* albumin, *Plt* platelet count, *PT* prothrombin time activity, *NH_3* ammonia, *PE* paraesophageal vein, *PU* paraumbilical vein, *SR* splenorenal shunt

[a] Period from time of HCV eradication to development of portosystemic encephalopathy

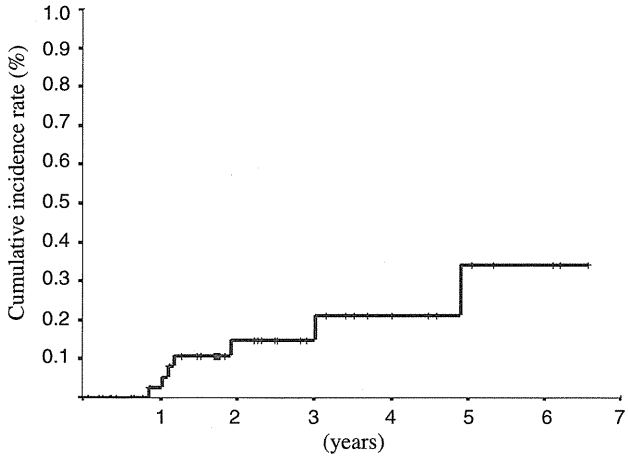


Fig. 4 The incidence rate of portosystemic encephalopathy after HCV eradication with IFN therapy

significantly reduces fibrosis and significantly improves liver function [20–22]. Similarly, we found a significant improvement of liver function in patients with cirrhosis with IFN therapy 1 year after HCV eradication. These improvements were observed irrespective of the existence of portosystemic collateral vessels. These results indicated that HCV eradication did not influence the improvement of portosystemic collateral vessels that already existed at the time of HCV eradication.

IFN therapy may produce clinically significant reductions in the hepatic venous pressure gradient (HVPG) in patients with HCV-related cirrhosis after HCV eradication [23, 24]. IFN therapy is reported to reduce the progression of hepatic fibrosis irrespective of the virological response [25–27]. The authors of these reports speculated that the reduction of portal vein pressure via a reduction of the

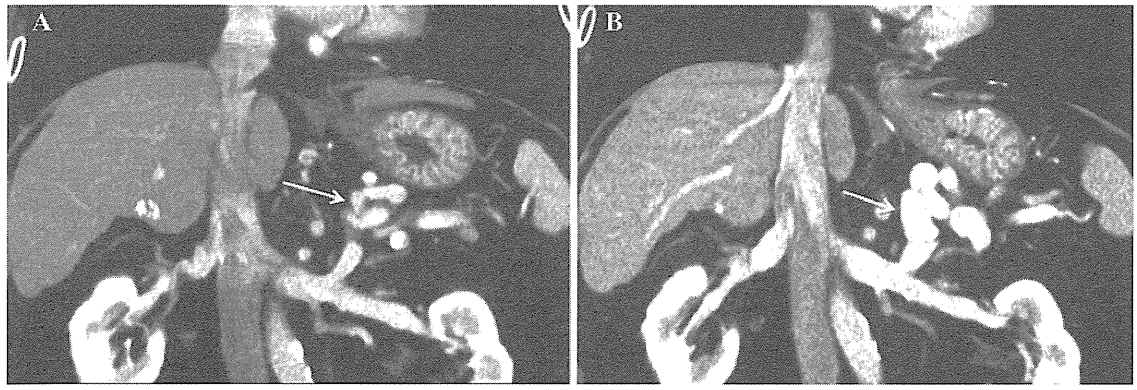


Fig. 5 Findings in a 77-year-old man who developed hepatic encephalopathy 15 months after HCV eradication with IFN therapy for HCV-related compensated cirrhosis (case 4 in Table 4). Dynamic CT showed exacerbated splenorenal shunt (*arrow*) 15 months after HCV eradication (**b**), compared with findings at HCV eradication (**a**)

Table 5 Liver function test results before IFN therapy and 1 year after HCV eradication in patients with and without feeding vessels for EVs

	Feeding vessels for EVs					
	With ($n = 31$)			Without ($n = 21$)		
	Before IFN therapy	1 Year after HCV eradication	*P* value	Before IFN therapy	1 Year after HCV eradication	*P* value
Total bilirubin (mg/dl)	1.4 ± 1.8	0.9 ± 0.5	0.258	1.0 ± 0.4	0.8 ± 0.4	0.034
AST (IU/l)	68 ± 46.0	51 ± 28.5	0.002	83 ± 60.0	45 ± 30.2	0.002
ALT (IU/l)	68 ± 52.8	42 ± 23.3	0.001	97 ± 73.8	47 ± 32.1	<0.001
Albumin (g/dl)	3.8 ± 0.8	4.0 ± 0.6	0.024	3.9 ± 0.7	4.5 ± 0.4	<0.001
Platelet count ($\times 10^4/\mu l$)	8.1 ± 3.0	11.6 ± 5.6	0.003	9.5 ± 4.3	13.0 ± 5.3	0.001
Prothrombin time activity (%)	86 ± 8.9	80 ± 16.5	0.192	90 ± 11.0	96 ± 11.3	0.043
Total cholesterol (mg/dl)	151 ± 41.5	180 ± 39.0	0.001	157 ± 32.3	177 ± 31.0	0.012

Values are means ± SD

AST aspartate aminotransferase, *ALT* alanine aminotransferase

P value, Wilcoxon rank-sum test

Table 6 Liver function test results before IFN therapy and 1 year after HCV eradication in patients with and without extrahepatic portosystemic shunt

	Extrahepatic portosystemic shunt					
	With ($n = 18$)			Without ($n = 34$)		
	Before IFN therapy	1 Year after HCV eradication	*P* value	Before IFN therapy	1 Year after HCV eradication	*P* value
Total bilirubin (mg/dl)	0.9 ± 2.1	0.9 ± 0.6	0.458	1.1 ± 0.7	0.7 ± 0.4	<0.001
AST (IU/l)	56 ± 48.2	34 ± 23.1	0.020	55 ± 47.3	31 ± 28.5	<0.001
ALT (IU/l)	57 ± 50.5	33 ± 17.8	0.018	61 ± 50.5	32 ± 29.9	<0.001
Albumin (g/dl)	3.8 ± 0.7	4.1 ± 0.6	0.045	3.9 ± 0.7	4.6 ± 0.5	0.001
Platelet count ($\times 10^4/\mu l$)	8.7 ± 3.3	10.9 ± 4.9	0.029	7.5 ± 3.3	11.8 ± 5.2	<0.001
Prothrombin time activity (%)	83 ± 14.6	86 ± 16.5	0.055	88 ± 12.4	92 ± 15.7	0.067
Total cholesterol (mg/dl)	132 ± 45.5	179 ± 49.5	0.007	153 ± 33.8	195 ± 43.6	0.001

Values are means ± SD

AST aspartate aminotransferase, *ALT* alanine aminotransferase

P value, Wilcoxon rank-sum test

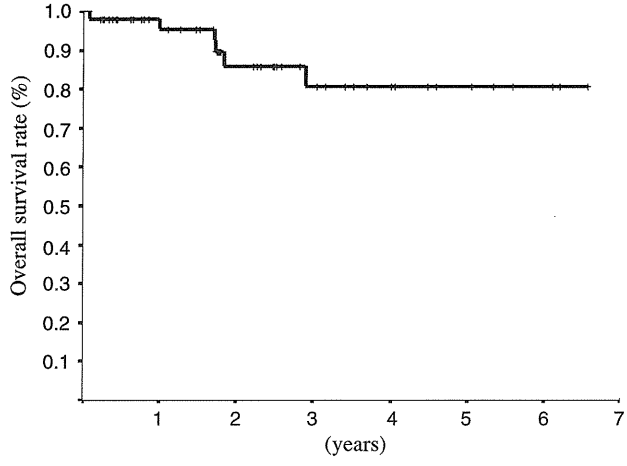


Fig. 6 Overall survival rate after HCV eradication with IFN therapy

sinusoidal space and a reduction of pressure in the peripheral branch of the portal vein led to an improvement of the fibrosis activity grade [25–27]. With our findings, similar these reported results, we expected that portosystemic collateral vessels would be improved by the IFN therapy. However, our results showed no improvement of cirrhosis-related complications such as EVs and portosystemic encephalopathy. These findings might be attributable to the existence of advanced fibrosis and/or increased blood flow volume in the portosystemic collateral vessels before the IFN therapy. Accordingly, we would recommend assessing portosystemic collateral vessels in cirrhotic patients before instituting IFN therapy; this assessment can be done by using CT portography, which would provide beneficial findings, as previously reported [28, 29].

The cumulative development rates of HCC at 5, 10, and 15 years were reported to be 32.5, 59.6, and 77.4 %, respectively, in Japanese patients with HCV-related cirrhosis [30]. In our study, 12 % of cirrhotic patients with no history of previous HCC developed HCC after HCV eradication. The cumulative 1-, 3-, and 5-year HCC development rates in these patients were 0, 6, and 34 %, respectively. The rate of development of HCC was not changed by HCV eradication in our patients. These findings indicate the need for long-term surveillance for HCC even after HCV eradication.

The cumulative 5-year survival rate in our study was 81 % and this was similar to the rate in previous reports of similar patient cohorts [31, 32]. Two patients in our study cohort died of HCC, and four others died of extrahepatic complications.

In our patient group, the results of treatment with endoscopic injection sclerotherapy (EIS) and/or surgical ligation for exacerbated EVs and portosystemic encephalopathy were adequate, probably because of the well-maintained liver function achieved by HCV eradication. As a result, there were no deaths from EV hemorrhage or liver failure in the studied patients during follow up.

In conclusion, our findings indicate that the existence of portosystemic collateral vessels increases the risk of the exacerbation of EVs and the incidence of portosystemic encephalopathy in patients with HCV-related cirrhosis, even after successful HCV eradication. We suggest that portosystemic collateral vessels should be evaluated in cirrhotic patients before IFN therapy is instituted.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Sangiovanni A, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, et al. The natural history of compensated cirrhosis due to hepatitis C virus: a 17-year cohort study of 214 patients. Hepatology. 2006;43:1303–10.
2. Bruno S, Zuin M, Crosignani A, Rossi S, Zadra F, Roffi L, et al. Predicting mortality risk in patients with compensated HCV-induced cirrhosis: a long-term prospective study. Am J Gastroenterol. 2009;104:1147–58.
3. Benvegnú L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut. 2004;53:744–9.
4. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology. 1998;27:1394–402.
5. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. Hepatology. 1999;29:1124–30.
6. Yoshida H, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, et al. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut. 2004;53:425–30.
7. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346:1051–5.
8. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. Lancet. 1998;351:1535–9.
9. Hu KQ, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. Hepatology. 1999;29:1311–6.
10. Jeong S, Aikata H, Katamura Y, Azakami T, Kawaoka T, Saneto H, et al. Low-dose intermittent interferon-alpha therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma. World J Gastroenterol. 2007;13:5188–95.
11. Ikeda K, Saitoh S, Koboyashi M, Suzuki Y, Suzuki A, Tsubota A, et al. Long-term interferon therapy for 1 year or longer reduces the hepatocellular carcinogenesis rate in patients with liver cirrhosis caused by hepatitis C virus: a pilot study. J Gastroenterol Hepatol. 2001;16:406–15.
12. Sarin SK, Lamba GS, Kumar M, Misra A, Murthy NS. Comparison of endoscopic ligation and propranolol for the primary prevention of variceal bleeding. N Engl J Med. 1999;340:988–93.
13. Moriwaki H, Miwa Y, Tajika M, Kato M, Fukushima H, Shiraki M. Branched-chain amino acids as a protein and energy source in liver cirrhosis. Biochem Biophys Res Commun. 2004;313:405–9.
14. Gundling F, Teich N, Strebel HM, Schepp W, Pehl C. Nutrition in liver cirrhosis. Med Klin. 2007;102:435–44.
15. D'Amico G, Pagliaro L, Bosch J. The treatment of portal hypertension: a meta-analytic review. Hepatology. 1995;22:332–54.
16. Lay CS, Tsai YT, Teg CY, Shyu WS, Guo WS, et al. Endoscopic variceal ligation in prophylaxis of first variceal bleeding in cirrhosis patients with high-risk esophageal varices. Hepatology. 1997;25:1346–50.
17. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterology. 2001;120:726–48.
18. Pagliaro L, D'Amico G, Sorensen TI, Lebrec D, Burroughs AK, Morabito A, et al. Prevention of first bleeding in cirrhosis: a meta-analysis of randomized trials of nonsurgical treatment. Ann Intern Med. 1992;117:59–70.
19. The Japan Society for Portal Hypertension and Esophageal Varices. The general rules for study of portal hypertension. 2nd ed. Tokyo: Kanehara; 2004. p. 37–8 (in Japanese).
20. Cammà C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001;34:593–602.
21. Heathcote EJ, Shiffman ML, Cooksley WG, Samuel S, Lee SS, Balart L, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med. 2000;343:1673–80.
22. Cammà C, Di Bona D, Schepis F, Heathcote J, Stefan Zeuzem S, Pockros PJ, et al. Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. Hepatology. 2004;39:333–42.
23. Tanabe J, Izawa A, Takemi N, Miyauchi Y, Torii Y, Tsuchiyama H, et al. Interferon-beta reduces the mouse liver fibrosis induced by repeated administration of concanavalin A via the direct and indirect effects. Immunology. 2007;122:562–70.
24. Inagaki Y, Nemoto T, Kushida M, Sheng Y, Higashi K, Ikeda K, et al. Interferon alfa down-regulates collagen gene transcription and suppresses experimental hepatic fibrosis in mice. Hepatology. 2003;38:890–9.
25. Rincon D, Ripoll C, Lo Iacono O, Salcedo M, Catelina MV, Alvarez E, et al. Antiviral therapy decreases hepatic venous pressure gradient in patients with chronic hepatitis C and advanced fibrosis. Am J Gastroenterol. 2006;101:2269–74.
26. Roberts S, Gordon A, McLean C, Pedersen J, Bowden S, Thomson K, Augus P. Effect of sustained viral response on hepatic venous pressure gradient in hepatitis C-related cirrhosis. Clin Gastroenterol Hepatol. 2007;5:932–7.
27. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everthart JE, Wright EC, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 2008;359:2429–41.
28. Kodama H, Aikata H, Takaki S, Azakami T, Katamura Y, Kawaoka T, et al. Evaluation of portosystemic collaterals by MDCT-MPR imaging for management of hemorrhagic esophageal varices. Eur J Radiol. 2010;76:239–45.
29. Kodama H, Aikata H, Takaki S, Takahashi S, Toyota N, Ito K, et al. Evaluation of patients with esophageal varices after endoscopic injection sclerotherapy using multiplanar reconstruction MDCT images. AJR Am J Roentgenol. 2009;192:122–30.
30. Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, et al. Natural history of compensated cirrhosis in the Child–Pugh class A compared between 490 patients with hepatitis C and 167 with B virus infections. J Med Virol. 2006;78:459–65.
31. Bruno S, Stroffolini T, Colombo M, Bollani S, Benvegnú L, Mazzella G, et al. Sustained virological response to interferon-α is associated with improved outcome in HCV-related cirrhosis: a retrospective study. Hepatology. 2007;45:579–87.
32. Morgan TR, Ghany MG, Kim HY, Snow KK, Shiffman ML, De Santo JL, Lee WM, et al. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. Hepatology. 2010;52:833–44.