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Natural course following the onset of cirrhosis in patients with chronic hepatitis B: a long-term follow-up study

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Abstract
Purpose To elucidate the long-term natural course following the onset of cirrhosis in patients with chronic hepatitis B.
Methods Ninety-three patients with chronic hepatitis B who had developed cirrhosis during regular follow-up were included in this long-term follow-up study. At the time of cirrhosis detection, 30% of the patients were seropositive for hepatitis B e antigen (HBeAg) and 73% had a HBV-DNA level $>10^4$ copies/ml. Follow-up studies included liver biochemistry, viral markers, α-fetoprotein and ultrasonography every 3–6 months.
Results During a mean follow-up period of 102 ± 60 (12–246; median 97) months, 32 patients (34.4%) experienced 55 episodes of hepatitis flare (7.0%/year), 15 (53.6%) of 28 HBeAg-positive patients seroconverted to anti-HBe (6.3%/yr) and 12 (12.9%) lost HBsAg (1.5%/year). Overall disease progression was observed in 25 (26.9%, 3.2%/year) patients: 12 (12.9%, 1.5%/year) hepatic decompensation, 21 (22.6%, 2.7%/year) hepatocellular carcinoma and 11 (11.8%, 1.4%/year) died. Multivariate analysis showed that age at onset of cirrhosis ($P = 0.015$) and persistent HBeAg seropositivity ($P = 0.019$) were the independent factors for overall disease progression.
Conclusions These results suggest that patients with older age at onset of cirrhosis and persistent HBeAg seropositivity following the onset of cirrhosis were independent factors for the disease progression in the first 10-year after the development of cirrhosis in patients with chronic hepatitis B.

Keywords Hepatitis flare · Hepatic decompensation · Hepatocellular carcinoma · HBeAg seroconversion · HBsAg seroconversion

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HBeAg	Hepatitis e antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDV	Hepatitis D virus
AFP	α-Fetoprotein
ALT	Alanine aminotransferase
ULN	Upper limit of normal
PCR	Polymerase chain reaction
PT	Prothrombin time

Introduction

Hepatitis B virus (HBV) affects 350–400 million people chronically worldwide [1]. Chronic HBV infection may

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lead to cirrhosis or hepatocellular carcinoma (HCC), or both in some patients [2–5]. Earlier studies showed that age, acute exacerbation, and functional status are contributing factors of outcomes and mortality in patients with HBV related cirrhosis [6–9]. However, most of the earlier studies examined patients with cirrhosis of unknown onset and the follow-up duration was relatively short. Therefore, the natural course following the onset of cirrhosis is not clearly known. We therefore conducted this long-term follow-up study.

Materials and methods

Patients

Thousands of patients with chronic HBV infection, or those seropositive for hepatitis B surface antigen (HBsAg) ≥6 months, have been followed up periodically since 1970s in our liver unit, as described earlier [2–4]. A total of 1,292 patients were histologically confirmed to have chronic hepatitis B and no evidence of liver cirrhosis before the end of 1999. After histological diagnosis, they were regularly followed up and 190 patients were found to have developed cirrhosis subsequently. Excluding 27 patients with hepatitis C virus (HCV) or hepatitis D virus (HDV) concurrent infection, 58 patients who had received antiviral therapy and 12 patients who were followed up for less than 1 year, the remaining 93 patients with HBV infection alone were included in this study. These patients included 80 males (86%) and 13 females (14%) with a mean age of 43.6 ± 10.4 (24–69, median 40.8 year) at the onset of cirrhosis. Upon diagnosis of cirrhosis, 65 (70%) were hepatitis B e antigen (HBeAg) negative and 28 (30%) were HBeAg positive. Sixty-five (73%) of 89 patients assayed showed a serum HBV-DNA level>10^4 copies/ml (Table 1).

Follow-up

Since liver cirrhosis was detected during regular periodic follow-up, the time of detection is identical or very close to the onset of cirrhosis [2]. Following detection of cirrhosis, the patients were followed up every 3–6 months, or more frequently if clinically indicated, for at least 12 months after the diagnosis of cirrhosis. Follow-up studies included clinical evaluation, liver biochemistry, virological markers and α-fetoprotein (AFP). The ultrasonography was also performed for the surveillance of HCC. Endoscopic examination was done at least once for evaluation of esophageal/gastric varices and was used to confirm variceal bleeding whenever upper gastrointestinal bleeding happened. The follow-up period following onset of cirrhosis ranged from 12 to 246 months (median 97.3 months; mean 102 ± 60 months) (Table 2).

Table 1 The demographic data at the onset of cirrhosis in patients with chronic hepatitis B

	Total $N = 93$	HBeAg (–) ($n = 65$)	HBeAg (+) ($n = 28$)	P value
Age at onset (years)[a]	43.6 ± 10.4 (24–69)	44.8 ± 10.3 (28–68)	40.6 ± 10.3 (24–69)	0.072
Male no (%)	80 (86)	53 (82)	27 (96)	0.099
HBV DNA (copies/ml)[b]				0.065
<300	15	11	4	
300–9,999	9	9	0	
10,000–99,999	12	10	2	
≥100,000	53	32	21	

[a] Data as mean ± SD (range)

[b] Excluding four patients with missing data

Methods

Liver biochemical tests and blood cell counts were performed using automatic analyzer. Episodes with alanine aminotransferase (ALT) elevation by twofold of the baseline level and over five times the upper limit of normal (ULN, 36 U/L) were considered as ''hepatitis flares'' [10–13]. Virological markers including hepatitis B surface antigen (HBsAg), HBeAg, anti-HBe and anti-HDV were assayed using commercially

Table 2 The outcomes following the onset of cirrhosis in patients with chronic hepatitis B

	Total $N = 93$	HBeAg (–) ($n = 65$)	HBeAg (+) ($n = 28$)	P
Follow-up (months)[a]	102 ± 60 (12–246)	101.9 ± 60.1 (14–246)	102.2 ± 59.9 (12–234)	0.984
HBeAg seroconversion[b]			15 (53.6)	
Hepatitis flare				
Case[b]	32 (34.4)	24 (36.9)	8 (28.6)	0.362
Episodes	55	43	12	
HBsAg clearance[b]	12 (12.9)	11 (16.9)	1 (3.6)	0.081
Decompensation[b]	12 (12.9)	7 (10.8)	5 (17.9)	0.346
HCC[b]	21 (22.6)	12 (18.5)	9 (32.1)	0.243
Liver-related death[b]	11 (11.8)	6 (9.2)	5 (17.9)	0.230
Disease progression[b]	25 (26.9)	15 (23.1)	10 (35.7)	0.321

HCC: hepatocellular carcinoma; disease progression: hepatic decompensation or HCC

[a] Mean ± SD (range)

[b] No (%)

available radioimmunoassay kits (Abbott Laboratories, North Chicago, Ill). AFP was measured by α-feto-RIA-II (Dainabot, Tokyo, Japan). Anti-HCV was tested by a commercially available third-generation enzyme immunoassay kit (AxSYM®HCV, version 3.0 Abbott Laboratories). Serum specimens stored at –70°C were assayed for HBV DNA using semi-automated quantitative polymerase chain reaction (PCR) (COBAS amplicor HBV monitor, Roche molecular system, the detection sensitivity was 300 copies/ml).

Diagnosis of cirrhosis was made histologically in 38 patients and on the basis of repeated ultrasonographic findings suggestive of cirrhotic change and complemented with clinical features such as esophageal varices, thrombocytopenia [14, 15] in 55 patients. The diagnosis of HCC was made histologically in 16 patients and based on the image findings together with an AFP level > 400 ng/ml [3] in five patients. ''Hepatic decompensation'' was defined as occurrence of ascites, clinical jaundice with prolonged prothrombin time (PT), hepatic encephalopathy or variceal bleeding [2, 14]. ''Overall disease progression'' was defined as development of either hepatic decompensation or HCC.

Statistical analysis was performed using Student *t* test, χ^2-test or Fisher exact test, Kaplan–Meier survival analysis with log-rank test and Cox hazard regression analysis, where appropriate. *P* values < 0.05 were considered significant.

Results

During 102 ± 60 (12–246; median 97) months of follow-up after the initial detection of cirrhosis, 32 patients (34.4%) experienced 55 episodes of hepatitis flare. The calculated annual incidence was 7.0% (Table 2). Forty-one (74.5%) of the 55 episodes of hepatitis flare were observed within five years after detection of cirrhosis (Fig. 1A). Hepatitis flares in five patients were associated with hepatic decompensation. Of these 93 patients, 25 (26.9%) showed overall disease progression: 12 (12.9%) hepatic decompensation (1 encephalopathy, 6 prolonged PT with clinical jaundice, 2 ascites, and 5 variceal bleeding) and 21 (22.6%) HCC. Excluding three patients died of non-liver disease, 11 (11.8%) died of liver disease (4 HCC, 3 HCC and hepatic failure, 1 variceal bleeding, 3 hepatic failure). The calculated annual incidence of hepatic decompensation, HCC and liver related mortality was 1.5%, 2.7% and 1.4%, respectively, and the cumulative incidence was 30.8%, 44% and 21.6%, respectively (Fig. 2A–C). Except variceal bleeding, most events of disease progression happened 5–10 years after the detection of cirrhosis.

Of the 28 patients seropositive for HBeAg upon detection of cirrhosis, 15 (53.6%) underwent HBeAg seroconversion and 11 (73.3%) of these 15 occurred within 5 years following detection of cirrhosis. Only 4 (26.7%) of the 15 events of HBeAg seroconversion were preceded by hepatitis flare. The calculated annual incidence was 6.3% and the cumulative incidence of HBeAg seroconversion was 65.8% (Fig.1B).

HBsAg seroclearance occurred in 12 (12.9%) of the 93 patients and 10 were observed more than 6 years following the onset of cirrhosis. The calculated annual incidence was 1.5% (Fig 1C). Although higher proportion of HBeAg-negative patients lost serum HBsAg (16.9% vs. 3.6%), the difference in the cumulative incidences was statistically non-significant between patients with different HBeAg status at presentation (Table 2).

The stored serum specimens upon cirrhosis detection were available for HBV DNA assay in 89 patients. HBV-DNA was undetectable (<300 copies/ml) in 15 (16.8%), 300–9,999 copies/ml in 9 (10.1%), 10,000–99,999 copies/ml in 12 (13.5%), and more than 100,000 copies/ml in 53 (59.6%). Hepatitis flare was documented in 24 (32.4%) of the 74 patients with serum HBV-DNA >300 copies/ml and in 7 (46.7%) of the 15 patients with HBV-DNA <300 copies/ml at entry. Most hepatitis flare were associated with HBV-DNA $>10^5$ copies/ml. There was no significant difference in cumulative incidences of hepatitis flare, hepatic decompensation, HCC, mortality and overall disease progression between different HBV DNA levels in patients with HBeAg seropositivity or seronegativity (data not shown).

The relevant variables found to be associated with HCC, hepatic decompensation and disease progression, including age, gender, HBV DNA level and HBeAg status, were analyzed using Cox hazard regression model (univariate and multivariate analysis). In the univariate analysis, age and persistent HBeAg seropositivity were associated with HCC development with marginal significance ($P = 0.057$ and $P = 0.051$, respectively) and significantly associated with overall disease progression ($P = 0.021$ and $P = 0.031$, respectively). In the multivariate analysis, age was significantly associated with HCC development ($P = 0.04$) and overall disease progression ($P = 0.015$). Persistent HBeAg seropositivity was related to HCC development with marginal significance ($P = 0.062$) but significantly associated with

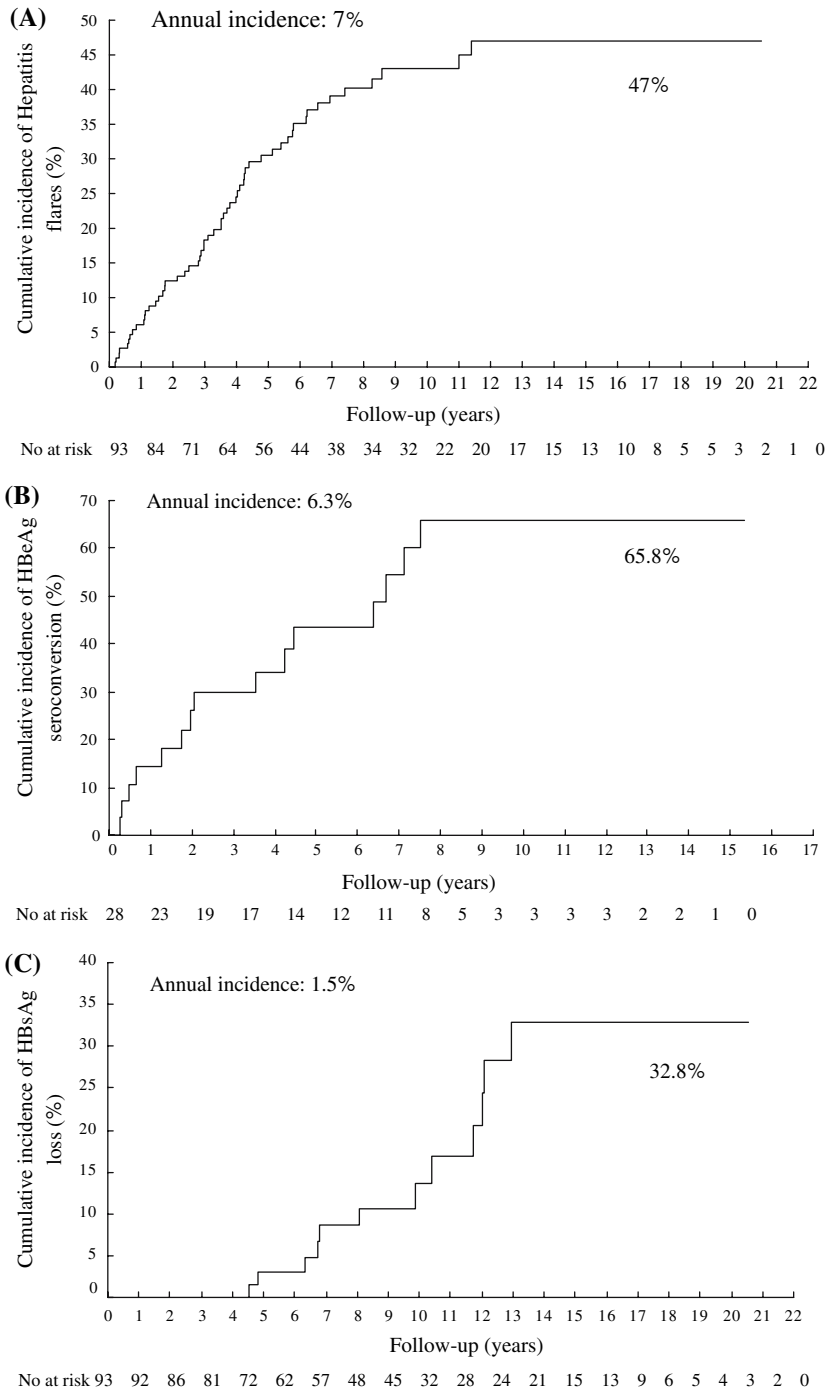

Fig. 1 The annual and cumulative incidences of hepatitis flare (**A**), HBeAg seroconversion (**B**), and HBsAg seroclearance (**C**) after the onset of cirrhosis in patients with chronic hepatitis B

hepatic decompensation ($P = 0.035$) and overall disease progression ($P = 0.019$) (Table 3).

Discussion

This long-term (1–20 years) follow-up study involved a cohort of 93 patients. The number of patients may be not big enough. However, these patients all had a well documented onset of cirrhosis. In addition, patients with concurrent HCV or HDV infection(s) were excluded. Compared with the single earlier similar study involving only 76 patients, including 15 patients with HDV superinfection and anti-HCV was not assayed then, and a mean follow-up period of only 0.5 to 7 year [4], the present study has included the greatest number of patients of this kind and followed for longest duration of time.

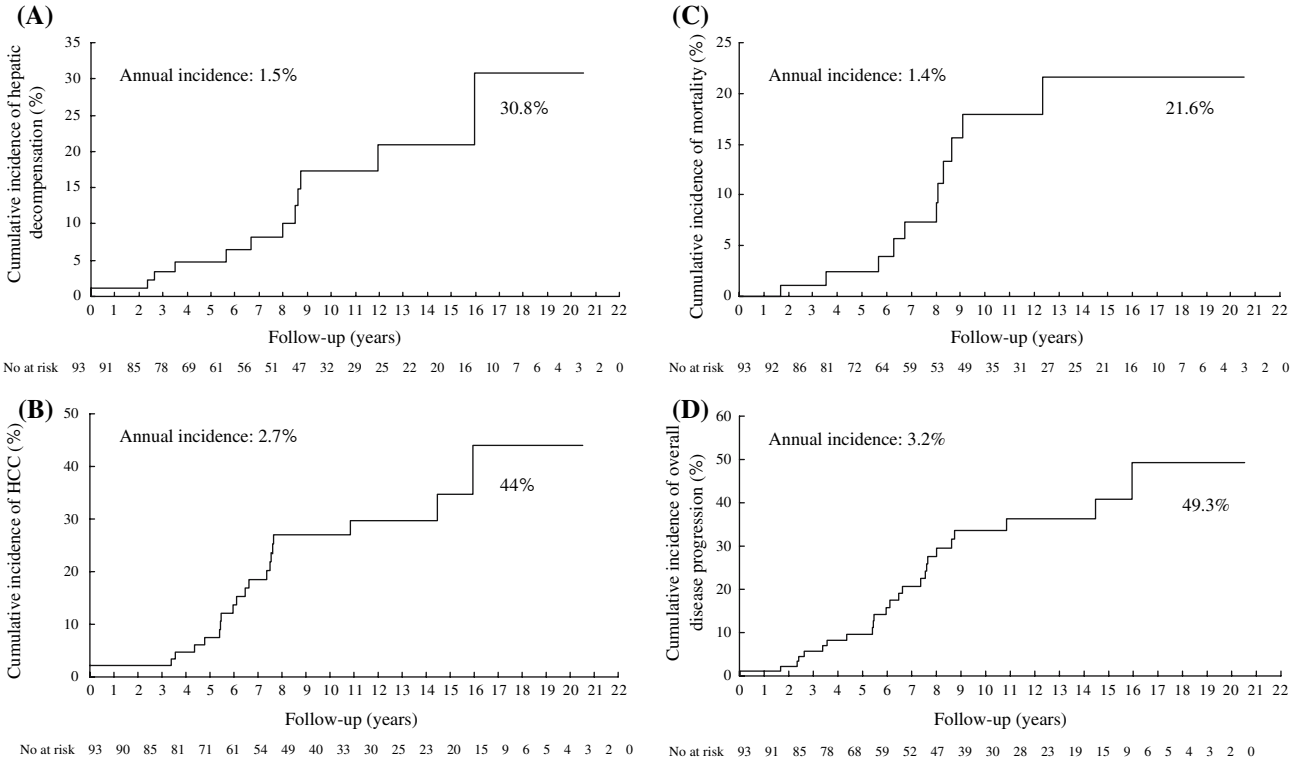

Fig. 2 The annual and cumulative incidences of hepatic decompensation (**A**), HCC (**B**), mortality (**C**), and overall disease progression (**D**) after the onset of cirrhosis in patients with chronic hepatitis B

Our results have confirmed the observation that hepatitis flares may occur, usually at the early course, after the development of cirrhosis [5, 12]. While 74.5% of the hepatitis flares occurred within 5 years following the detection of cirrhosis, 73.3% [11 in 15] of the HBeAg seroconversion in our patients also occurred during this period. However, HBeAg seroconversion were preceded by acute hepatitis flares in only 26.7% of the events, much lower than a rate of 60–70% in chronic hepatitis B [10]. These findings suggest that, unlike patients with chronic hepatitis B, HBeAg positive status in cirrhotic patients is not necessarily associated with high HBV viremia, as shown in Table 1 that only 75% of our HBeAg positive patients had a HBV-DNA level $>10^5$ copies/ml. Consistent with our earlier observation that cirrhosis is a factor for spontaneous HBsAg seroclearance [14, 16], 12 (12.9%) of our 93 patients underwent spontaneous HBsAg seroclearance as a late event.

Of note is that hepatic flares were documented not only in patients with detectable serum HBV-DNA, patients with undetectable HBV-DNA upon detection of cirrhosis also encountered a surge of HBV-DNA with hepatitis flares during follow-up. This observation strongly suggests that serum HBV-DNA is fluctuating in the course after cirrhosis detection. This may explain our finding that HBV-DNA level was not a factor for hepatic decompensation and HCC development. Using highly sensitive HBV-DNA assays, a control study showed that persistent HBV-DNA $>5 \times 10^3$ copies/ml was associated with increased risk of HCC [17] and a cohort study showed that HBV viral load was the only predictor of HCC development [18]. However, earlier studies using hybridization assays also failed to show definite correlation between the incidence of HCC development and the status of HBV-DNA at enrollment [4, 8, 19, 20]. These controversial results may be related to differences in sample size, duration of follow-up and sensitivity of HBV-DNA assays. These findings may also suggest that cirrhosis per se plays the most important role in HCC development independent of HBV-DNA level at the onset of cirrhosis.

Our results have also shown that, besides age, persistent HBeAg seropositivity tends to be associated with the risk of hepatic decompensation, HCC and overall disease progression in the first 10 years after cirrhosis development. Persistent HBeAg seropositivity is also a factor for cirrhosis and HCC development in interferon treated and untreated patients with chronic hepatitis B [21]. This underscores the

Table 3 Factors associated with HCC, hepatic decompensation, and overall disease progression after onset of cirrhosis

Factors	HCC				Hepatic decompensation				Overall disease progession			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
All patients (n = 93)												
Age of onset (per year increase)	1.044 (0.999–1.091)	0.057	1.051 (1.002–1.102)	0.040	1.048 (0.989–1.109)	0.111	1.046 (0.975–1.121)	0.210	1.048 (1.007–1.090)	0.021	1.056 (1.011–1.103)	0.015
Gender												
Female	1				1		1		1		1	
Male	1.721 (0.398–7.446)	0.467	1.843 (0.396–8.565)	0.436	0.597 (0.157–2.270)	0.449	0.726 (0.141–3.735)	0.701	1.014 (0.345–2.981)	0.979	1.105 (0.340–3.590)	0.868
HBV DNA (copies/ml)												
<300	1		1		1		1		1		1	
300–9,999	0.726 (0.066–8.044)	0.794	0.632 (0.054–7.416)	0.715	1.529 (0.224–11.328)	0.642	1.071 (0.116–9.861)	0.951	0.976 (0.162–5.868)	0.979	0.607 (0.088–4.167)	0.612
10,000–99,999	1.588 (0.223–11.339)	0.644	1.282 (0.172–9.554)	0.809	0.708 (0.064–7.845)	0.778	0.463 (0.036–5.884)	0.552	0.920 (0.153–5.536)	0.927	0.648 (0.102–4.133)	0.646
>100,000	1.955 (0.441–8.665)	0.378	1.337 (0.274–6.516)	0.720	0.824 (0.165–4.130)	0.814	0.366 (0.060–2.248)	0.278	1.358 (0.392–4.697)	0.629	0.762 (0.195–2.977)	0.696
HBeAg status												
(–) at entry	1		1		1		1		1		1	
Seroconversion after cirrhosis	1.096 (0.353–3.405)	0.874	1.201 (0.351–4.101)	0.771	1.004 (0.208–4.841)	0.996	2.319 (0.383–14.050)	0.360	0.873 (0.289–2.637)	0.810	1.226 (0.368–4.076)	0.740
Persistent (+)	2.861 (0.996–8.216)	0.051	3.048 (0.944–9.844)	0.062	3.409 (0.851–13.663)	0.083	6.037 (1.139–31.997)	0.035	2.892 (1.104–7.575)	0.031	3.645 (1.234–10.768)	0.019

importance of HBeAg as a marker of risk for disease progression [22]. Strategies to prevent cirrhosis development and to ensure earlier HBeAg seroconversion are required to improve the outcomes of chronic HBV infection.

In conclusion, the results of the present study suggest that patients with older age at onset and persistent HBeAg seropositivity following the onset of cirrhosis were independent factors for the disease progression in the first 10 years after cirrhosis development in patients with chronic hepatitis B.

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