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Impact of antibody to hepatitis B core antigen on the clinical course of hepatitis C virus carriers in a hyperendemic area in Japan: a community-based cohort study

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Abstract

Aim—Subjects positive for antibody to hepatitis B core antigen (HBcAb) and negative for hepatitis B surface antigen (HBsAg) are considered to have occult hepatitis B virus (HBV) infection. The aim of this study was to determine the impact of occult HBV infection on aggravation of the clinical course in hepatitis C virus (HCV) carriers.

Methods—A prospective cohort study was performed in 400 subjects who were positive for anti-HCV antibody and negative for HBsAg. Among these subjects, 263 were HCV core antigen-positive or HCV RNA-positive (HCV carriers). We examined whether the presence of HBcAb affected the clinical course in these HCV carriers from 1996 to 2005.

Results—The HBcAb-positive rates were 53.6% and 52.6% in HCV carriers and HCV RNA-negative subjects, respectively. There were no differences in the incidence of hepatocellular carcinoma (HCC) and cumulative mortality associated with liver-related death between HCV carriers who were positive and negative for HBcAb. In multivariate analysis, age (≥ 65 years old) and alanin aminotransferase level (≥ 31 IU/L) emerged as independent risk factors for HCC development and liver-related death, but the HBcAb status was not a risk factor. In addition, increased serum hepatic fibrosis markers (measured from 2001 to 2004) were not associated with HBcAb status.

Conclusion—In our cohort study, the presence of HBcAb had no impact on HCC development, liver-related death and hepatic fibrosis markers in HCV carriers. Thus, our results indicate that occult HBV infection has no impact on the clinical course in HCV carriers.

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Keywords

antibody to hepatitis B core antigen; occult hepatitis B virus infection; hepatitis C virus; hepatocellular carcinoma; mortality; hepatic fibrosis

Introduction

Hepatitis B surface antigen (HBsAg) is the most common serum marker for simple detection of hepatitis B virus (HBV) infection. It has previously been thought that HBsAg-positive individuals were HBV carriers and that HBsAg-negative individuals who were anti-hepatitis B core antibody (HBcAb)-positive and/or anti-HBs antibody (HBsAb)-positive had a history of the infection, but were not HBV carriers. However, it has recently been established that HBV DNA is occasionally detected in blood and/or liver tissue in individuals who are HBcAb-positive and/or HBsAb-positive and HBsAg-negative. Thus, HBsAg-negative patients who are HBcAb- and/or HBsAb-positive are regarded to have occult HBV infection.^{1,2} Such patients are suspected HBV carriers, even if they are negative for HBsAg,³ and serum HBcAb is considered to be a serologic marker of occult HBV infection.^{4,5}

HBcAb is present in 9-25% of healthy individuals,^{2,6,7} but in 46-78% of patients with hepatitis C virus (HCV)-related chronic liver disease.^{3,5,8} The HBcAb-positive rate in HCV carriers with hepatocellular carcinoma (HCC) has been reported to be higher than that in HCV carriers without HCC.⁹ However, there is a debate concerning the effects of occult HBV infection on the pathology of diseases such as HCC in patients with HCV-related chronic liver disease. Previous studies of these effects have been performed in patients who visited hospitals for treatment of advanced chronic liver disease,^{1,3,8} but not as cohort studies in community residents. Tanaka et al. found that an HBcAb-positive status was a significant risk factor for development of HCC in a 12-year, but small-scale (n=74), prospective study in anti-HCV antibody (HCVAb)-positive community residents.¹⁰ Thus, a large-scale cohort study is needed to clarify whether occult HBV infection affects the natural course of HCV infection, and a long-term prospective study in community residents is desirable. To address this issue, we performed a community-based prospective cohort study in an HCV hyperendemic area of Japan to investigate the effects of occult HBV infection on prognosis, development of HCC, and progression of hepatic fibrosis in community residents with HCV infection.

Materials And Methods

Study population

The subjects were residents in town C, an HCV hyperendemic area of Japan in which we performed a cohort study from 1993 to 2005.¹¹⁻¹³ Town C is a small town located in the midwest of Miyazaki Prefecture in Kyushu, Japan. It has a HCVAb-positive rate of 20.6%, which is higher than those in surrounding areas.¹³ During a 10-year period beginning in 1995, 1125 anti-HCV-seropositive residents of Town C were enrolled in the previous study¹¹ and followed for mortality through 2005. Among the HCVAb-positive subjects, 231 deaths occurred over a mean follow-up period of 8.2 years. Liver-related deaths occurred more frequently among HCV carriers, whereas the rates of other causes of death did not differ between HCV carriers and non-carriers.¹¹ The current study included 400 HCVAb-positive and HBsAg-negative subjects in whom HBcAb was measured before August 1996.

Follow-up

This study was started in September 1996 and completed on December 31, 2005. Subjects with HCC shown by abdominal ultrasonography at the time of HBcAb measurement were excluded from the study. Abdominal ultrasonography was performed once a year when possible during follow-up. During the course of this study, two subjects moved to other areas and follow-up was stopped at this point. No other subjects were lost to follow-up. Cause of death was based on information from death certificates. In 2001 and 2004, serum hyaluronic acid and type IV collagen 7S, which are hepatic fibrosis markers, were measured in 129 subjects.

Informed consent was obtained from subjects at the time of enrollment. The study was approved by the ethics committees of the University of Miyazaki, Faculty of Medicine, and the Kagoshima University Graduate School of Medical and Dental Sciences. In the study, HCV carriers were defined as individuals positive for HCV core antigen (HCVcAg) or HCV RNA. Subjects with occult HBV infection were defined as those positive for HBcAb, but negative for HBsAg.

Serum markers

HCVAb was detected using a second-generation enzyme immunoassay kit (Immunocheck F-HCVAb, International Reagents, Kobe, Japan). Serum levels of HCVcAg were determined by a fluorescence enzyme immunoassay (Immunocheck F-HCVAg Core, International Reagents). For HCVAb-positive subjects with HCVcAg levels <8 pg/ml, HCV RNA was examined before 1996 by a qualitative reverse transcription polymerase chain reaction assay (Amplicore HCV, Roche Diagnostics, Tokyo, Japan). The serologically defined genotype (serotype) of HCV was determined using a serological genotyping assay kit (Immunocheck F-HCV Grouping, International Reagents). Detection of HBcAb was performed by radioimmunoassay (Dainabott, Tokyo, Japan). Hyaluronic acid and type IV collagen 7S were measured by a latex bead agglutination assay (LPIA-ACE HA, Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan; normal range: <50 ng/ml) and a radioimmunoassay (Type IV collagen 7S kit, Mitsubishi Kagaku Iatron; normal range: <6.0 ng/ml), respectively.

Liver stiffness evaluated by elastography

Liver stiffness was evaluated by transient elastography (Fibroscan®; Echosens, Paris, France) for 144 subjects in 2004. This is a rapid, non-invasive method that gives results that are correlated with the fibrotic stage of liver disease.¹⁴ The measurements were made on the right lobe of the liver. The results are expressed in kilopascals (kPa).¹⁵

Statistical analysis

A χ^2 , Fisher's exact, Student's *t* or Mann-Whitney *U* test was used to compare frequencies or means, as appropriate. Cumulative survival curves were constructed using the Kaplan-Meier method and analyzed by log-rank test. For multivariate analysis, logistic regression analysis or Cox proportional hazards models were used. Statistical analyses were performed using SPSS v.18 software (SPSS Inc., Chicago, IL), with *P*<0.05 considered significant.

Results

Characteristics of patients at enrollment

Among 400 subjects who were positive for HCVAb, 263 were HCV carriers (positive for HCVcAg or HCV RNA), including 141 HBcAb-positive (53.6%) and 122 HBcAb-negative (46.4%) subjects (Table 1). A total of 137 subjects were negative for both HCVcAg and

HCV RNA, including 72 HBcAb-positive (52.6%) and 65 HBcAb-negative (47.4%) subjects. Among the HCV carriers, the HBcAb-positive subjects were older than those who were HBcAb-negative ($P=0.02$) (Table 1). The HBcAb-positive rates showed no sex differences in HCV carriers and HCV RNA-negative subjects (Table 1, Fig. 1A), and these rates in subjects in their 50s to 70s were 40-60% regardless of the status of HCV infection (Figures 1B). There were also no significant differences in other characteristics between the two groups (Table 1).

Factors influencing the development of HCC

During the follow-up period, HCC developed in 35 of 263 HCV carriers, including 22 and 13 who were HBcAb-positive and HBcAb-negative, respectively, and in 3 of 137 HCV RNA-negative subjects. Suspected liver cancer cases were identified in our liver disease screening programs using abdominal ultrasonography and the diagnosis of HCC was subsequently confirmed by primary physicians. For 36 cases of HCC, the diagnosis was determined on the basis of information collected via biopsy and/or imaging analysis using magnetic resonance imaging, computed tomography, angiography or ultrasonography. Two additional HCC cases were identified based on death certificates. Since the exact date of HCC onset was not available, the subjects were divided into two groups based on the presence or absence of development of HCC and differences in characteristics were investigated between these groups.

Univariate analysis indicated that age ≥ 65 years old, male, platelet count $<150,000/\mu\text{l}$ and ALT ≥ 31 IU/L were significantly related to development of HCC in HCV carriers. Multivariate analysis showed that age ≥ 65 years old and ALT ≥ 31 IU/L were independent risk factors for HCC development (Table 2). In contrast, the presence of HBcAb was not a significant factor in these analyses.

Effect of occult HBV infection on death caused by liver diseases

During the follow-up period, death occurred in 67 of 263 HCV carriers and in 21 of 137 HCV RNA-negative subjects. In these deaths, liver-related death such as HCC, liver cirrhosis, and esophageal varices occurred in 33 HCV carriers, including 17 and 16 who were HBcAb-positive and HBcAb-negative, respectively, and in 3 HCV RNA-negative subjects. There was no significant difference in the cumulative survival rate calculated by the Kaplan-Meier method between HBcAb-positive and HBcAb-negative HCV carriers (Figure 2).

Risk factors for death caused by liver diseases

Analysis using the Kaplan-Meier method identified age ≥ 65 years old, platelet count $<150,000/\mu\text{l}$, and ALT ≥ 31 IU/L as significant risk factors for death caused by liver-related death. In multivariate Cox regression analysis, age ≥ 65 years old and ALT ≥ 31 IU/L remained as significant independent risk factors, while HBcAb was not a risk factor (Table 3).

Effect of occult HBV infection on serum albumin levels and hepatic fibrosis markers in HCV carriers

Serum albumin levels measured in 2001 or 2004 were compared between HBcAb-positive and HBcAb-negative HCV carriers. The mean serum albumin levels in both groups were >4 g/dl in 2001 and 2004, with no significant difference between the groups (Figure 3A). In addition, the serum albumin levels in 2004 were not significantly lower than those in 2001 in either group (62 subjects in each group) who underwent measurements in both years.

The effect of occult HBV infection on hepatic fibrosis was studied in HCV carriers using measurements made in 2001 or 2004. The platelet count, an index of hepatic fibrosis, did not differ between HBcAb-positive and HBcAb-negative HCV carriers in 2001 or 2004 (Figure 3B). There was also no significant difference in platelet counts in 2001 and 2004 in subjects in each group who underwent measurements in both years (Figure 4A). Further, there were no significant differences in the levels of hyaluronic acid and type IV collagen 7S, which are hepatic fibrosis markers, between HBcAb-positive and HBcAb-negative HCV carriers in 2001 or 2004. In contrast, the levels of these markers in 2004 were significantly greater than those in 2001 in both groups who underwent measurements in both years (Figures 4B, C), but with no dependence on the presence or absence of HBcAb.

Hepatic fibrosis was evaluated in 144 HCV carriers using elastography in 2004. Mean values of 8.48 and 8.51 kPa were obtained in HBcAb-positive (n=78) and HBcAb-negative (n=66) HCV carriers, with no significant difference between the groups ($P=0.67$).

Discussion

Occult HBV infection may influence development of HCC in patients with HCV infection, but this is an issue on which no consensus has been reached. The current study was performed as an approximately 10-year cohort study in community residents by defining occult HBV infection based on a HBsAg-negative and HBcAb-positive status. Prospective analysis of the effect of HBcAb on prognosis in 263 HCV carriers indicated that HBcAb had no effect on development of HCC, death caused by liver diseases, and progression of hepatic fibrosis in HCV carriers.

The HBcAb-positive rate is reported to be greater in HCV carriers than in healthy individuals. This study also showed a high HBcAb-positive rate (53.3%, 213/400) in HCVAb-positive subjects, in agreement with previous findings in Japan.^{3,8} However, the HBcAb-positive rate did not differ between HCV carriers and HCV-RNA negative subjects, but did vary with age, with a higher rate in elderly subjects. Thus, the incidence rate of occult HBV infection in HCV carriers might also have differed by age in the previous study, and the HBcAb-positive rate in HCVAb-negative patients living in the same area should be determined.

We found that a HBcAb-positive status was not significantly related to development of HCC. There is some debate as to whether patients with HCV-related chronic liver disease develop HCC due to the presence of HBcAb or occult HBV infection. In a case control study in 91 HCC patients with chronic hepatitis C and advanced hepatic fibrosis who did not respond to interferon (IFN) treatment (HCC group) and 182 patients with chronic hepatitis C who did not develop HCC (non-HCC group), Lok et al. found no significant differences in the prevalence of HBcAb and detection rate of HBV DNA in the liver between the two groups, and concluded that occult HBV infection was not involved in development of HCC in patients with HCV-related liver disease.¹ In a study of 140 chronic hepatitis C patients without HBsAg, Hasegawa et al. found no significant difference in the rate of development of HCC between 76 HBcAb-positive and 64 HBcAb-negative subjects.¹⁶ In contrast, in a cohort study of 123 patients with HCV-related liver cirrhosis, Adachi et al. detected serum HBcAb in 78% of the subjects and the cumulative HCC development rate was significantly higher in the HBcAb-positive group, with multivariate analysis indicating that a HBcAb-positive status was an independent risk factor for development of HCC.⁸ A prospective study in 275 patients with HCV-related liver cirrhosis indicated that HBcAb was the only factor associated with progression to HCC³ and Shi et al. reported a relative risk of HCC of 2.83 for occult HBV infection in HCV-infected patients in a meta-analysis.¹⁷ However, this meta-analysis focused on hospital-based populations and selection bias may have been

present due to inclusion of the studies of Ikeda et al.¹⁸ and Adachi et al.,⁸ which targeted only liver cirrhosis patients. Another study showed a high rate of HCC due to HBcAb found in patients with HCV infection, but this rate was significant only in patients with liver cirrhosis.³ In our study, the number of HCV carriers with liver cirrhosis was relatively low compared to previous reports, based on the platelet count measured in 1996. Therefore, occult HBV infection in HCV carriers may promote development of HCC in cases with liver cirrhosis, but occult HBV infection may have less effect on development of HCC in all HCV carriers.

HBV infection may promote hepatocarcinogenesis through integration of HBV DNA into the host genome, even in a case of occult HBV infection, and long-term expression of viral proteins such as HBx may be involved in hepatocarcinogenesis.¹⁹ Thus, longer HBV infection including occult HBV infection might have a propensity for onset of HCC, and it is plausible that the risk for HCC development due to occult HBV infection in patients with HCV infection is higher in patients with liver cirrhosis compared to those with chronic hepatitis. In contrast, chronic hepatitis C progresses at varying rates and not all infected subjects necessarily develop liver cirrhosis and HCC. Factors such as sex and age are associated with development of liver cirrhosis and HCC in HCV carriers, but there may also be unidentified confounders such as host genetic factors, including single nucleotide polymorphisms. We speculate that confounders associated with liver cirrhosis are cofactors that contribute to induction of HCC in HCV carriers with occult HBV infection. Therefore, occult HBV infection in patients with HCV infection might be significantly high only in patients with liver cirrhosis and might exert only a precipitating effect on development of HCV-related HCC.¹⁷

There are many reports on occult HBV infection and development of HCC in HCV carriers, but few have examined the long term prognosis. Our 10-year follow-up indicated that a HBcAb-positive status did not influence the long term prognosis of patients with HCV carriers. In addition, this status did not influence liver-related death, such as that due to liver failure and rupture of esophageal varices. Age and ALT levels are known to influence the occurrence of HCC and liver-related mortality,^{11,20,21} and this study also found that age ≥ 65 years old and ALT ≥ 31 IU/L were independent risk factors for development of HCC and liver-related death. These results suggest that the subjects in this study were not a special population. Therefore, occult HBV infection may not influence the long-term prognosis of HCV carriers among community residents.

Several reports have suggested effects of occult HBV infection on progression of hepatic fibrosis and liver cirrhosis in patients with HCV-related chronic liver disease. In a study of 468 patients with chronic hepatitis C or liver cirrhosis, Matsuoka et al. reported that the degree of inflammatory cell infiltration in the liver was significantly higher in HBV-DNA-positive patients than in HBV-DNA-negative patients.²² We did not perform histological evaluation in the current study, but there was no difference in ALT levels between HBcAb-positive and HBcAb-negative subjects; thus, the severity of hepatitis appears not to be influenced by HBcAb status. In addition, persistent hepatitis decreases the hepatic functional reserve, but we also found no difference in serum albumin levels, an index of hepatic functional reserve, between the HBcAb-positive and -negative groups. These findings suggest that occult HBV infection does not influence hepatic functional reserve. However, we note that the number of subjects with low ALT levels was relatively high and the period for comparison of serum albumin levels was short (2001 to 2004) in our study. Thus, longer term observation in patients with more severe hepatitis is needed to clarify our findings.

Serum levels of hyaluronic acid and type IV collagen 7S, which are both serum hepatic fibrosis markers, were determined in 2001 and 2004 to evaluate hepatic fibrosis in HCV

carriers. Neither marker showed a significant difference between the HBcAb-positive and -negative groups in either year. Evaluation of hepatic fibrosis using Fibroscan also indicated that hepatic fibrosis was not related to the presence or absence of HBcAb, and the significant increase in serum hepatic fibrosis markers from 2001 to 2004 was not related to occult HBV infection. These findings suggest that occult HBV infection does not influence hepatic fibrosis in HCV carriers.

There were some limitations in this study. First, the accuracy of the evaluation of occult HBV infection may be limited because HBV DNA in blood or liver tissue was not examined. However, serum HBcAb is a marker reflecting occult HBV infection,^{4,5,23} and occult HBV infection can be detected even in a HBV DNA-negative case. A future study is required to determine whether HBV DNA or HBcAb can accurately indicate occult HBV infection. Second, this study might not have had a sufficient number of patients with progression of hepatic fibrosis and active hepatitis. The numbers of HCV carriers with platelet count >150,000/ μ l and albumin levels ≥ 4 g/dl were 156/208 (75%) and 152/166 (92%), respectively, and only a few subjects progressed to liver cirrhosis. The number of subjects with ALT levels ≥ 30 IU/L was 117/262 (45%), which may include many HCV carriers with persistent normal ALT.^{24,25} Based on these data and the levels of hepatic fibrosis markers, the number of subjects with liver cirrhosis was small. Therefore, the 10-year follow-up period may not have been enough to show an influence of a HBcAb-positive status on hepatic fibrosis and liver-related death, and further long-term observation is needed. Third, we did not exclude the effect of IFN treatment.²⁶ However, only a small number of patients were treated by IFN and there was no significant difference in IFN treatment history between the HBcAb-positive and -negative groups, which suggests that the results were not influenced by the effects of IFN treatment. Fourth, this study included a Japanese population with a relatively low mortality rate due to HCC.¹¹ Studies on cirrhosis and HCC indicate rates at 20 years after infection of 4%-24% and 1%-7%, respectively, depending on the study population and method of cohort recruitment.²⁷ Therefore, a meta-analysis of eligible studies is needed to evaluate whether occult HBV infection or a HBcAb-positive status worsens the prognosis of HCV carriers. Fifth, the increased serum levels of hyaluronic acid seemed to indicate more severe hepatic fibrosis progression compared to those of type IV collagen 7S; and serum levels of hyaluronic acid in 2004 indicated advanced hepatic fibrosis, but type IV collagen 7S, platelet counts and elastography indicated mild hepatic fibrosis.²⁸⁻³⁰ The reason for this difference is unclear, but serum levels of hyaluronic acid are likely to be affected by other factors such as arthritis.³¹ In addition, platelet count, a hepatic fibrosis marker, did not change from 2001 to 2004. Therefore, progression of hepatic fibrosis appeared to be mild and occult HBV infection did not affect these changes. A histological evaluation is needed to confirm these findings.

In conclusion, a HBcAb-positive status did not influence hepatic fibrosis, development of HCC, or liver-related death in HCV carriers in a 10-year community based cohort study. Our results indicate that occult HBV infection has no effect on the clinical course in HCV carriers.

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A)

B)

Figure 1.

HBcAb-positive rates in HCV carriers and HCV RNA-negative subjects. The HBcAb-positive rates by gender were similar in the two groups (A) and gradually increased with age in both groups (B). HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

[Number of subjects examined].

Figure 2. Cumulative survival rates associated with liver-related death in HBcAb-positive and HBcAb-negative HCV carriers, plotted using the Kaplan-Meier method. There was no significant difference between the two groups by log-rank test. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

Figure 3. Serum albumin levels and platelet counts in HBcAb-positive and HBcAb-negative HCV carriers. Comparison of serum albumin levels (A) or platelet counts (B) obtained in 2001 or 2004. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus. [Number of subjects examined].

Figure 4.

Fibrosis markers in HBcAb-positive and HBcAb-negative HCV carriers for whom data were obtained in 2001 and 2004. Platelet counts did not differ significantly between 2001 and 2004 (A). Serum hyaluronic acid (B) and type IV collagen 7S (C) levels significantly increased from 2001 to 2004, but the increases in these levels from 2001 to 2004 did not differ significantly between the HBcAb-positive and HBcAb-negative groups. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

Table 1

Characteristics of patients at enrollment

Characters	HCV carrier [†] (n=263)			HCV RNA (-) (n=137)		
	HBcAb (+) n=141 (53.6%)	HBcAb (-) n=122 (46.4%)	P value *	HBcAb (+) n=72 (52.6%)	HBcAb (-) n=65 (47.4%)	P value *
Age (years)	65.1±9.2	62.2±9.8	0.02	65.6±7.8	64.3±8.3	0.36
Gender [male/female]	59/82	53/69	0.80	27/45	23/42	0.86
Alcohol [never/occasionally/daily/unknown]	68/18/50/5	57/23/41/1	0.29	30/16/21/5	29/13/23/0	0.17
Blood transfusion [yes/no/unknown]	18/114/9	18/111/3	0.86	12/55/5	13/51/1	0.82
Previous interferon therapy [yes/no]	26/115	22/100	1.00	6/66	6/59	1.00
HCV serotype [I/II/indeterminate/not tested]	88/41/8/4	77/38/4/3	0.83			
HCV core antigen (pg/mL) [undetectable/<100/ 100/not tested]	14/45/80/2	12/45/65/0	0.53			
Platelet count ($\times 10^4/\mu\text{L}$)	18.0±5.5 <114>	19.0±4.5 <94>	0.16	21.3±6.0	20.5±5.4	0.47
Alanine aminotransferase (IU/L)	41.4±34.3 <140>	45.3±31.3	0.34	23.2±17.5	24.7±28.4	0.70

Data are shown as a number or mean $\pm$ standard deviation <number of subjects examined>.

[†] HCV carriers were defined as subjects who were positive for HCV core protein or HCV RNA.

* Based on χ^2 test, Fisher's exact test, or Student's t test, as appropriate. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

Table 2

Univariate and multivariate analyses of variables associated with development of hepatocellular carcinoma

Variable	Status	Odds ratio	95% CI	P-value
<i>Univariate analysis</i>				
HBcAb	negative	1		
	positive	1.550	0.745-3.227	0.277
Age (years)	< 65	1		
	65	2.372	1.089-5.164	0.029
Sex	female	1		
	male	2.258	1.092-4.669	0.028
HCV core antigen (pg/ml)	undetectable	1		
	< 100	2.026	0.427-9.618	0.374
	100	1.701	0.370-7.812	0.495
Alcohol	none	1		
	occasionally	1.510	0.569-4.007	0.408
	daily	1.008	0.440-2.312	0.984
Blood transfusion	no	1		
	yes	0.583	0.168-2.025	0.589
Platelet count ($\times 10^4/\mu\text{L}$)	15	1		
	< 15	3.220	1.324-7.829	0.011
ALT (IU/L)	30	1		
	31	4.427	1.766-11.1	0.001
<i>Multivariate analysis</i> *				
Age (years)	< 65	1		
	65	4.308	1.198-15.495	0.025
ALT (IU/L)	30	1		
	31	5.803	1.625-20.727	0.007

* Based on logistic regression analysis. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus; ALT, alanine aminotransferase; CI, confidence interval

Table 3
Univariate and multivariate analyses of variables associated with liver-related death.

Variable	Status	Hazard ratio	95% CI	P-value
<i>Univariate analysis</i>				
HBcAb	negative	1		
	positive	1.035	0.522-2.054	0.922
Age (years)	< 65	1		
	65	2.157	1.026-4.534	0.043
Sex	female	1		
	male	0.982	0.488-1.975	0.959
HCV core antigen (pg/ml)	undetectable	1		
	< 100	0.588	0.152-2.275	0.442
	100	1.157	0.345-3.878	0.814
Alcohol	none	1		
	occasionally	0.255	0.060-1.090	0.255
	daily	0.519	0.23-1.172	0.115
Blood transfusion	no	1		
	yes	0.619	0.188-2.039	0.430
Platelet ($\times 10^4/\mu\text{L}$)	15	1		
	< 15	3.126	1.327-7.362	0.009
ALT (IU/L)	30	1		
	31	4.553	1.753-11.824	0.002
<i>Multivariate analysis</i> *				
Age (years)	< 65	1		
	65	5.983	1.375-26.036	0.017
ALT (IU/L)	30	1		
	31	4.337	1.255-14.986	0.020

* Based on Cox proportional hazards models. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus; ALT, alanine aminotransferase; CI, confidence interval