model. Multivariate analysis of predictors for the development of HCC was assessed using the logistic regression test. A *P* value of less than .05 was considered to be significant. All analyses described above were performed using SPSS software (version 11, SPSS Inc., Chicago, IL).

RESULTS

Development of HCC in patients with PBC

Sixteen of the 210 patients with PBC (7.6%) developed HCC. The diagnosis of HCC was made in 11 patients (5.2%) during follow-up, whereas HCC and PBC were almost simultaneously diagnosed in the remaining 5 patients (2.4%) who had diabetes mellitus and advanced histological stages, except for a male patient aged 84 who was potentially at high risk for HCC because of his age. These 5 patients (3 males and 2 females) were excluded from analysis afterward. The clinical and histological features of the 11 patients who developed HCC during follow-up are summarized in Table 2. HCC incidence by gender was 3.6% (1/28) in males and 5.6% (10/177) in females. The mean interval between the diagnosis of PBC and the development of HCC was 11.4 ± 5.7 years. Antibody to hepatitis B core antigen (anti-HBc) was positive in 2 patients (18.2%), negative in 5 (45.4%) and unknown in 4 (36.4%), respectively. Six patients (54.5%) and 1 patient (9.1%) had advanced histological stages (III) and diabetes mellitus at diagnosis of PBC, respectively. It should be noted that the follow-up period until development of HCC was significantly longer in patients with mild histological stages (I or II) (14.8 ± 4.1 years) than in those with advanced histological stages (III) (8.5 ± 5.5 years), suggesting a potential risk for HCC development after a long period of time even in patients with a mild histological stage of PBC. Six patients had solitary tumors whose size was less than 30 mm, except for one. These tumors were treated with local ablation such as percutaneous microwave coagulation therapy, percutaneous ethanol injection or radiofrequency ablation, transarterial chemoembolization (TACE),

combination of local ablation with TACE, or liver transplantation in all the patients except one (cases 9 in Table 2) who could not be treated due to rupture of HCC.

To determine whether HCC developed as liver damage progressed, laboratory parameters (platelet count, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, γ-glutamyltransferase, total bilirubin, albumin, and prothrombin time) were compared between the two time points of PBC diagnosis and HCC diagnosis in the 11 patients. The serum albumin level, prothrombin activity and platelet count were significantly lower at the time of HCC diagnosis than at the time of PBC diagnosis, suggesting that the development of HCC largely depended on the progression of liver disease (Figure 2). Patients with PBC who developed HCC had significantly lower cumulative survival rates than those who did not. The 5-, 10-, 15- and 20-year cumulative survival rates of PBC patients with HCC and those without HCC were 91/98%, 61/87%, 51/83% and 10/80%, respectively (P<0.001, Figure 3).

Factors associated with survival in patients with PBC

We next investigated the factors associated with survival in patients with PBC to know whether HCC development actually affected their prognosis. In addition to the baseline characteristics of patients at the time of PBC diagnosis, treatment with UDCA and the development of HCC were incorporated into the parameters for analyzing the factors associated with survival of patients with PBC. However, we did not incorporate the response to treatment with UDCA into the parameters because of a lack of established biochemical criteria for the response to UDCA allowing prediction of the prognosis.

Treatment with UDCA (600 mg daily) was started within 6 months from diagnosis of PBC in 186 patients (91%). The reason why UDCA was not administered in the remaining 19 patients was unknown. The duration of UDCA treatment was almost the same as the follow-up period, since almost all the patients did not discontinue taking UDCA due to lack of moderate to severe adverse effect.

Univariate analysis identified age, portal hypertension, platelet count, AST, total bilirubin and albumin levels, prothrombin activity, advanced histological stage (Scheuer criteria III or IV), and HCC development as significant predictors for survival in patients with PBC (Table 3). Among these predictors, multivariate analysis revealed age (OR: 1.08, 95% CI: 1.03-1.13, P=0.001), total bilirubin (OR: 1.60, 95% CI: 1.09-2.36, P=0.017) and albumin levels (OR: 0.24, 95% CI: 0.10-0.56, P=0.001), and HCC development (OR: 2.97, 95% CI: 1.24-7.15, P=0.015) to be significant factors associated with survival in patients with PBC (Table 3).

Factors associated with development of HCC in patients with PBC

As the development of HCC has been demonstrated to be significantly associated with survival in patients with PBC, it is important to determine screening targets for HCC to improve the prognosis for patients with PBC. Patients with PBC who developed HCC had a lower platelet count (P=0.008), lower albumin level ((P=0.02), and more advanced histological stage (Scheuer criteria III or IV) (P=0.005) at the time of PBC diagnosis than those who did not (Table 4). Multivariate analysis identified only

advanced histological stage (Scheuer criteria III or IV) (OR: 6.27, 95% CI: 1.80-21.83, *P*=0.004) as a predictor for the development of HCC in patients with PBC (Table 5).

DISCUSSION

The frequency of HCC development in patients with PBC is estimated to be around 3% (0.7%-3.6%) according to recent and relatively large cohort studies that were conducted in European countries, the United States and Japan, even though this frequency increases as the histological stage progresses (5-10 15, 16). In this study 5.2% of patients with PBC developed HCC during follow-up and 2.4% of patients had HCC simultaneously at the time of PBC diagnosis. The incidence of HCC was slightly higher in the present study than that in previous studies, though it is unknown whether it is a significant difference. The higher incidence of HCC might be accounted by the relatively longer period of follow-up in a restricted number of institutions in the present study. Such situations potentially have less dropped-out patients during the follow-up period.

HCC was demonstrated to develop as liver damage progressed in patients with PBC, as indicated by significant decreases of the serum albumin level, prothrombin activity and platelet count at the time of HCC development. These results were consistent with the previous observation by a Japanese study group that all patients had progressed to an advanced histological stage (Scheuer criteria III or IV) by the time of HCC development (7). We also found that HCC development was one of the significant risk factors for survival in patients with PBC. Because the PBC patients have already progressed to an advanced histological stage at the time of HCC diagnosis, these results seem to be reasonable and were consistent with those of a study from Spain and Italy (9) and a recent Japanese report based on the nation-wide survey (10). In fact, the 6 PBC patients

with HCC (6/9, 67%) died of hepatic failure in the present study (Table 2). In some patients with HCC it was difficult to determine whether the cause of death was haptic failure or HCC. Thus, the deaths resulting from progressive hepatic failure related to portal venous invasion or the rapture of HCC were defined as death by HCC. The deaths resulting from progressive hepatic failure in the course of treatment for HCC without portal venous invasion were defined as death by hepatic failure.

In contrast to PBC HCC development (n=19) was not a significant risk factor for survival in patients with alcoholic liver cirrhosis (n=103) who were followed during almost the same period as followed in this study and developed haptic failure in a relatively short period of time unless they stopped drinking alcohol (unpublished data). The long-term clinical course of PBC may also account for the association of HCC development with poor prognosis in patients with PBC. In addition to HCC development, age, the albumin level and total bilirubin level at onset of PBC were selected as significant prognostic factors in patients with PBC in the present study. These results were in part consistent and in part inconsistent with those of previous studies (7, 18), probably due to the different backgrounds of the patients studied.

That the majority (90%) of patients were treated with UDCA may account for why we did not find any difference of survival between treated patients and untreated patients. However, a limitation of this study was that the response to treatment with UDCA could not be incorporated into analytic factors for survival. The response to UDCA has been reported to be associated with better prognosis in PBC patients with moderately advanced disease compared with patients with mild disease (19), who accounted for the

majority (80%) of the patients in the present study. Recent biochemical criteria for predicting outcomes of patients with early PBC at low risk of long-term development of liver cirrhosis (20) may be useful for assessing whether the response to UDCA treatment affected the survival of patients with PBC in this study.

Diabetes mellitus has been demonstrated to increase the risk of HCC in a huge cohort of patients without concomitant liver disease (12) and in a cohort of patients with hepatitis C, hepatitis B or alcoholic cirrhosis (21, 22). However, previous studies did not include diabetes mellitus as clinical parameters for assessing risk factors related to the development of HCC in patients with PBC (5-7, 9-11). We did not find any association of diabetes mellitus with the development of HCC, even though the proportion of patients with diabetes mellitus (9%) was relatively small in this study. Although the link between insulin resistance and metabolic hepatocarcinogenesis has been reported (23), the contribution of diabetes mellitus to the development of HCC in chronic liver diseases may vary according to the etiology of liver disease.

We did not identify male sex to be a significant factor associated with HCC development in patients with PBC, which was inconsistent with the results in some previous studies (5, 7, 10, 11). In particular, a discrepancy between the present study and the recent Japanese nation-wide study (10) may be explained by at least three reasons. First, the patient sample size was much smaller in this study than in the nation-wide survey. Second, the present study included more patients with advanced histological stage (Scheuer criteria III or IV) (18%) as compared with those (12%) in the nation-wide survey, even though other clinical parameters such as age, gender or the

frequency of treatment with UDCA were similar between two studies. Third, the exclusion criterion regarding history of excessive alcohol consumption (>40 g/day) in this study might be associated with this discrepancy, since the nation wide-survey has not clearly excluded the patients with history of excessive alcohol consumption. As shown in a previous study (9), we also found that only advanced histological stage (Scheuer's classification III or IV) was a risk factor associated with the development of HCC in patients with PBC. Considering that cholangiocytes, not hepatocytes, are mainly affected and liver fibrosis progresses as histological stage advances in PBC, these results indeed highlighted an important role of liver fibrosis in hepatocarcinogenesis in patients with PBC. Also, it should be noted that the follow-up period until development of HCC was significantly longer in patients with a mild histological stage (I or II) than in those with an advanced histological stage (III or IV). These results suggested a potential risk for HCC development after a long period of time even in PBC patients with a mild histological stage at the time of diagnosis.

In conclusion, the development of HCC has been demonstrated to affect the survival of Japanese patients with PBC. Considering that the prognosis of PBC has improved in general because of early diagnosis and the use of UDCA, early diagnosis of HCC is also crucial to obtain a better prognosis for patients with PBC. Therefore, strict surveillance for HCC may be necessary for patients with PBC who are in the advanced stage.

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Figure legends

Figure 1

Number of patients included and those excluded in the retrospective cohort.

Figure 2

Serum albumin levels, platelet counts and prothrombin activity at diagnosis of primary biliary cirrhosis (PBC) and hepatocellular carcinoma (HCC) in patients with PBC who developed HCC.

The results are shown as box plot profiles. The bottom and top edges of the boxes are the 25th and 75th percentiles, respectively. *: $P<0.05$

Figure 3

Cumulative survival curves for patients with primary biliary cirrhosis who developed hepatocellular carcinoma (HCC) or and those who did not.

The solid line and broken line indicate patients with HCC and those without HCC, respectively. Log-rank test $P<0.001$

Table 1 Clinical characteristics of patients at diagnosis of PBC

Characteristic	Mean ± SD or frequency (number of analyzed patients)
Age (years)	58 ± 11 (210)
Gender (Male/Female)	31/179
Body mass index (kg/m^2)	22.4 ± 3.1 (196)
Blood transfusion (+/-/unknown)	13/192/5
Diabetes mellitus (+/-)	19/191
Portal hypertension (+/-)	39/171
Platelet count (× 10^4/μL)	21.9 ± 8.2 (210)
ALT (IU/L)	58 ± 53 (210)
AST (IU/L)	56 ± 40 (207)
Alkaline phosphatase (IU/L)	523 ± 434 (207)
γ-GT (IU/L)	230 ± 233 (207)
Total bilirubin (mg/dL)	0.9 ± 0.9 (210)
Albumin (g/dL)	3.9 ± 0.5 (209)
Prothrombin time (%)	82.9 ± 16.4 (209)
IgM (mg/dL)	410 ± 279 (203)
Anti-HBc (+/-/unknown)	13/40/157
Histological stage (I or II/III or IV)	169/41
Treatment with UDCA (+/-)	189/21

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; Anti-HBc, anti-hepatitis B core antibody; UDCA, ursodeoxycholic acid;

Table 2 Characteristics of 11 patients with PBC who developed HCC

Case	Age at PBC/HCC diagnosis	Sex	BMI	DM	Anti-HBc	Histological stage at§	HCC				Cause of death
							Solitary or multiple	Maximum size (mm)‡	Vascular invasion	Therapy¶	
1	64/67	M	25.8	-	+	III	Solitary	10	-	PMCT	Sepsis
2	66/75	F	26.9	-	-	III	Multiple	45	-	PEI + TACE	Hepatic failure
3	65/74	F	23.2	-	+	III	Multiple	32	-	TACE	Hepatic failure
4	59/70	F	23.3	+	-	III	Multiple	20	-	REI + TACE	HCC
5	61/75	F	17.8	-	-	II	Multiple	30	-	TACE	Hepatic failure
6	38/55	F	19.5	-	-	I	Solitary	30	-	PEI + TACE	Hepatic failure
7	38/55	F	20.8	-	NA†	I	Multiple	15	-	TACE	Hepatic failure
8	58/76	F	23.1	-	-	I	Solitary	30	-	TACE	Hepatic failure
9	64/66	F	22.2	-	NA	III	Solitary	100	+	-	HCC rupture
10	41/49	F	27.7	-	NA	I	Solitary	10	-	Transplantation	Alive
11	48/65	F	24.6	-	NA	III	Solitary	27	-	TACE	Alive

BMI, body mass index; DM, diabetes mellitus; Anti-HBc, anti hepatitis B core antibody; †NA: unknown, §at the diagnosis of PBC

‡Tumor sizes in cases 2, 3, 4, 5 and 7 represent the largest ones among multiple tumors. ¶PMCT, percutaneous microwave coagulation therapy; PEI, percutaneous ethanol injection; TACE, transarterial chemoembolization; RFA, radiofrequency ablation

Table 3 Univariate and multivariate analyses of predictors for survival in patients with PBC

Variables	Univariate			Multivariate		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (years)	1.09	1.04-1.14	<0.001	1.08	1.03-1.13	0.001
Male	1. 65	0.63-4.34	0.31			
Body mass index (kg/m^2)	0.92	0.81-1.05	0.24			
Blood transfusion	1.19	0.28-5.03	0.62			
Diabetes mellitus	3.70	1.56-7.14	0.009			
Portal hypertension	3.34	1.93-7.04	0.002			
Platelet count (× 10^4/μL)	0.92	0.87-0.97	0.003			
ALT (IU/L)	1.00	0.99-1.00	0.64			
AST (IU/L)	1.01	1.00-1.01	0.04			
Alkaline phosphatase (IU/L)	1.00	1.00-1.00	0.24			
γ-GT (IU/L)	1.00	1.00-1.00	0.87			
Total bilirubin (mg/dL)	2.03	1.50-2.76	<0.001	1.60	1.09-2.36	0.017
Albumin (g/dL)	0.13	0.06-0.27	<0.001	0.24	0.10-0.56	0.001
Prothrombin time (%)	0.94	0.91-0.96	<0.001			
IgM (mg/dL)	1.00	1.00-1.00	0.12			
Anti-HBc	0.83	0.23-3.00	0.27			
Histological stage III or IV	5.10	2.40-10.66	<0.001			
Treatment with UDCA	3.35	0.45-24.78	0.24			
Development of HCC	5.15	2.31-11.49	<0.001	2.97	1.24-7.15	0.015

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; Anti-HBc, anti hepatitis B core antibody; UDCA, ursodeoxycholic acid; HCC, hepatocellular carcinoma

Table 4 Comparison of clinical characteristics at diagnosis of PBC between patients who developed HCC and those who did not

Variables	Patients with HCC	Patients without HCC	*P* value
Number of patients	11	194	
Age (years)	58 ± 11	58 ± 10	0.91
Gender (Male/Female)	1/10	27/167	0.54
Body mass index (kg/m^2)	23.6 ± 2.7	22.3 ± 3.1	0.10
Blood transfusion (+/-)	1/10	11/178	0.50
Diabetes mellitus (+/-)	1/10	14/180	0.58
Portal hypertension (+/-)	4/7	33/161	0.12
Platelet count (× 10^4/μL)	16.0 ± 7.5	22.5 ± 8.0	0.008
ALT (IU/L)	47 ± 29	59 ± 55	0.66
AST (IU/L)	60 ± 36	55 ± 40	0.40
Alkaline phosphatase (IU/L)	468 ± 338	518 ± 432	0.72
γ-GT (IU/L)	127 ± 86	236 ± 238	0.08
Total bilirubin (mg/dL)	1.6 ± 1.7	0.9 ± 0.6	0.11
Albumin (g/dL)	3.6 ± 0.6	4.0 ± 0.5	0.02
Prothrombin time (%)	76.8 ± 14.0	83.6 ± 16.5	0.30
IgM (mg/dL)	479 ± 393	405 ± 273	0.91
Anti-HBc (+/-/unknown)	2/6/3	11/30/153	0.65
Histological stage (I or II/III or IV)	5/6	163/31	0.005
Treatment with UDCA (+/-)	11/0	175/19	0.33

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; Anti-HBc, anti hepatitis B core antibody; UDCA, ursodeoxycholic acid

Table 5 Multivariate analysis of predictors for development of HCC in patients with PBC

Variables	Odds ratio	95% CI	*P* value
Histological stage III or IV	6.27	1.80-21.83	0.004

Figure 1

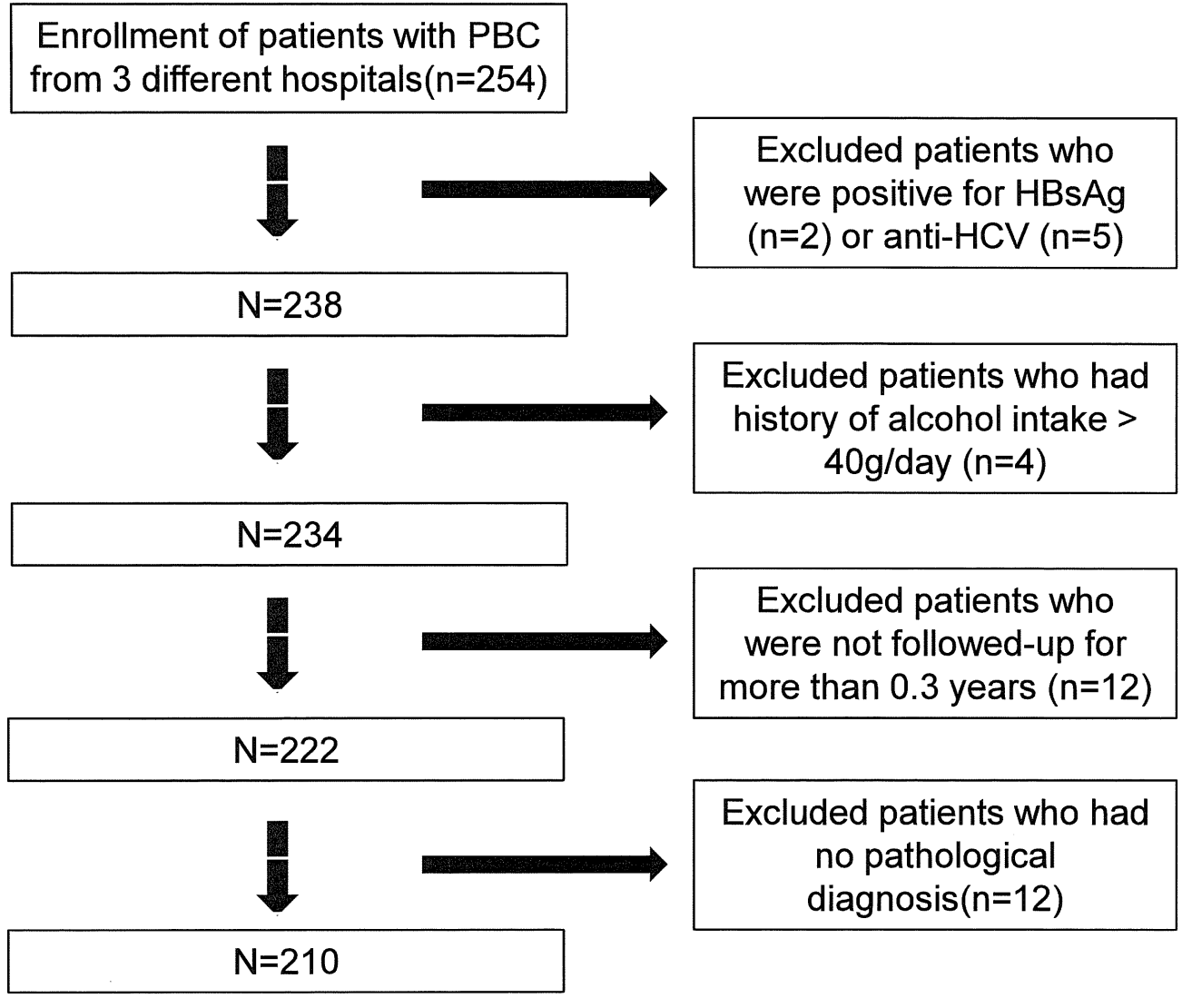
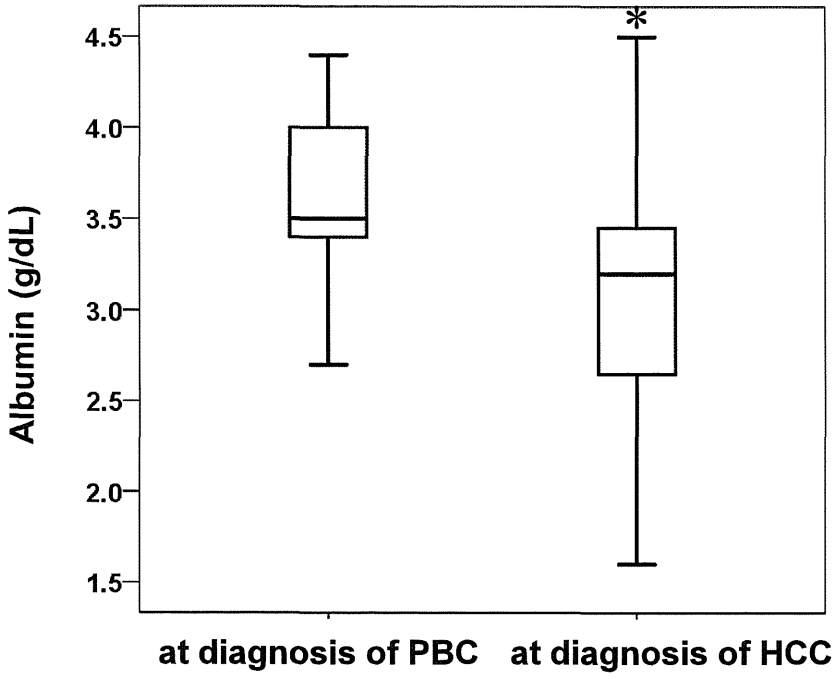
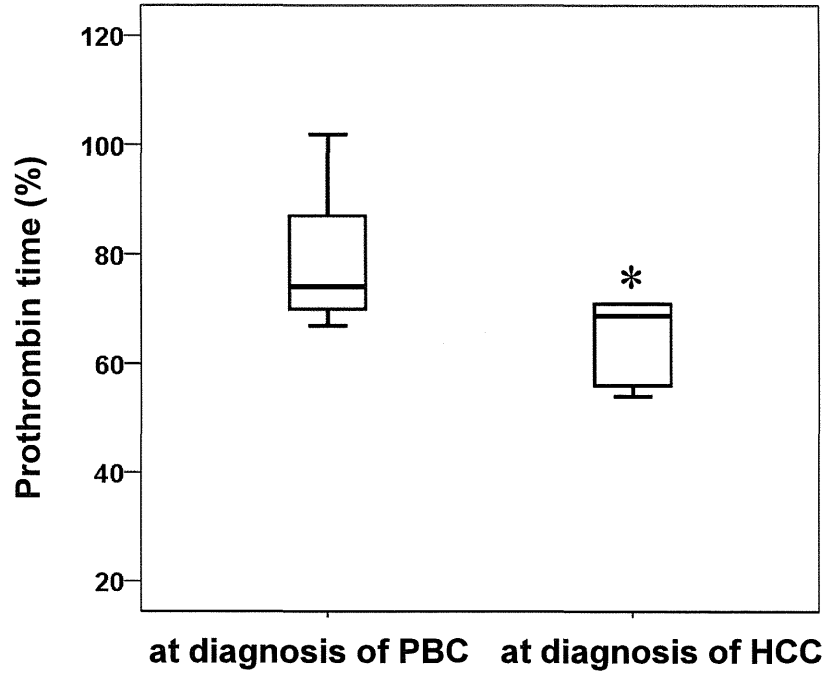
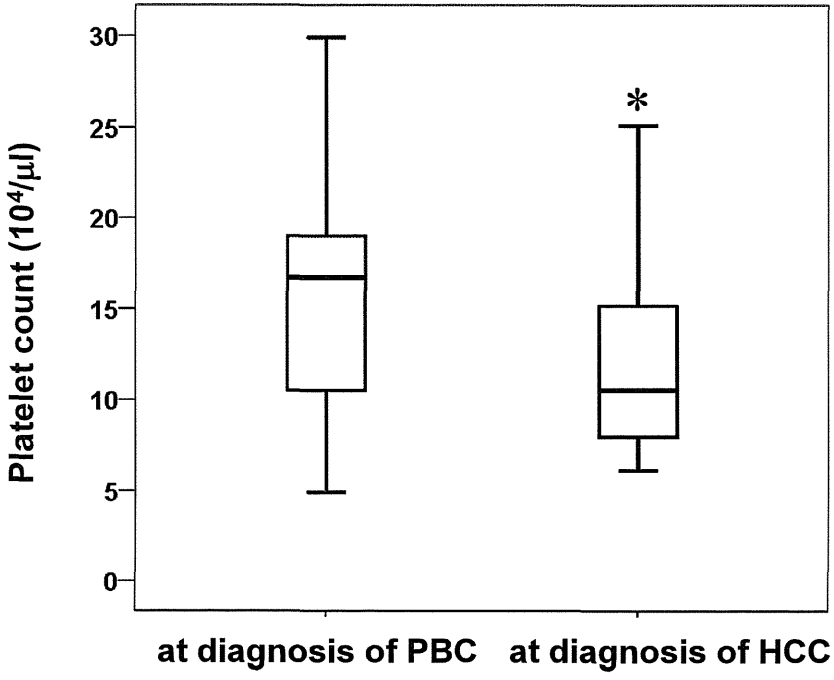

Enrollment of patients with PBC from 3 different hospitals(n=254)
Excluded patients who were positive for HBsAg (n=2) or anti-HCV (n=5)
N=238
Excluded patients who had history of alcohol intake > 40g/day (n=4)
N=234
Excluded patients who were not followed-up for more than 0.3 years (n=12)
N=222
Excluded patients who had no pathological diagnosis(n=12)
N=210

Figure 2


Albumin (g/dL)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
*
at diagnosis of PBC
at diagnosis of HCC


Prothrombin time (%)
120
100
80
60
40
20
*
at diagnosis of PBC
at diagnosis of HCC


Platelet count (10⁴/μl)
30
25
20
15
10
5
0
*
at diagnosis of PBC
at diagnosis of HCC