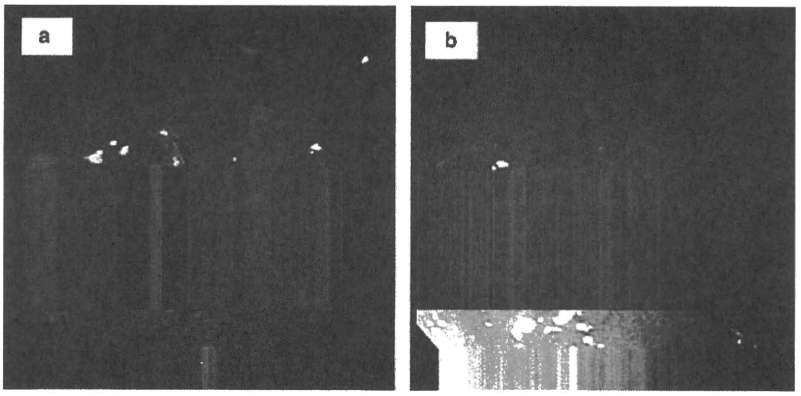

Figure 1. Endoscopic findings for the pouch mucosa. (**a**) Pouchitis induced by combination therapy before treatment with metronidazole. Erosions, friable mucosa, and purulent mucus were noted in the pouch. (**b**) No erosions were found after treatment with metronidazole.

proliferation of immature plasma cells in the inflamed pouch mucosa of patients with UC (4). In the present case, enhancement of humoral immunity by IFN-α administration could have caused dysregulation of differentiation of plasma cells that led to the development of severe pouchitis.

CONFLICT OF INTEREST

Guarantor of the article: Kenichi Morimoto, MD.

Specific author contributions: Kenichi Morimoto: planned the study; collected and interpreted data, and drafted the manuscript; Hirokazu Yamagami, Shuhei Hosomi, Mizuki Ohira, Takehisa Suekane, Noriko Kamata, Mitsue Sogawa, and Kenji Watanabe: collected and interpreted data; Kazunari Tominaga, Toshio Watanabe, Yasuhiro Fujiwara, and Akihiro Tamori: interpreted data; Nobuhide Oshitani: collected and interpreted data; Tetsuo Arakawa: interpreted data.

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Acute Hemorrhage With Retroperitoneal Hematoma After Endoscopic Ultrasound-Fine Guided-Needle Aspiration of an Intraductal Papillary Mucinous Neoplasm of the Pancreas

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To the Editor: Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) is effective for tissue diagnosis in suspected pancreatic cancer (1) and for collecting fluid from cystic tumors (2). Major complications after EUS-FNA of solid masses are rare, but cystic tumors seem to have a higher risk of infection and bleeding. In a series of 50 patients undergoing EUS-FNA of pancreatic cystic lesions, Varadarajulu and Eloubeidi (3) found acute intracystic hemorrhage at the site of cyst aspiration in three cases (frequency 6%: 95% confidence interval 1.3–16.6). There was no pancreatitis or infectious complications. Clinical history and laboratory parameters did not predict which patients were at risk for intracystic hemorrhage (3).

A 66-year-old woman presented with multiple pancreatic cysts found by chance during a transabdominal ultrasound. Computed tomography and magnetic resonance imaging confirmed an intraductal papillary mucinous neoplasm of the main pancreatic duct and side branches involving the whole pancreas. The largest cyst, measuring 3 cm, was in the tail. EUS-FNA of this cyst was done from the stomach with a 22-gauge fine needle (**Figure 1**); 10 ml of clear mucous were aspirated and the cyst collapsed completely. After removal of the needle, intracystic hemorrhage occurred at the site of aspiration, manifesting as a fine hyperechoic flow that progressed gradually to involve the entire cyst, then leaked into the retroperitoneal space along the needle path as an expanding echo-rich region (**Figure 2**). Color and power Doppler before the puncture had

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Noninvasive laboratory tests proposed for predicting cirrhosis in patients with chronic hepatitis C are also useful in patients with non-alcoholic steatohepatitis

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Abstract
Background Several noninvasive tests have been proposed to predict cirrhosis in patients with chronic hepatitis C, but not in patients with non-alcoholic steatohepatitis (NASH). We assessed whether noninvasive laboratory tests designed to predict the risk of cirrhosis in patients with chronic hepatitis C virus (HCV) infection could be used in patients with NASH.
Methods The subjects were 50 patients with biopsy-proved NASH and 100 age- and sex-matched patients with HCV. Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), age-platelet (AP) index, AST-to-platelet ratio index (APRI), cirrhosis discriminant score (CDS), and the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) model were calculated.
Results The areas under the receiver-operating characteristic curves of the AAR, AP index, APRI, CDS, and HALT-C model for predicting cirrhosis were respectively 0.813, 0.877, 0.786, 0.949, and 0.908 in patients with NASH and 0.555, 0.652, 0.761, 0.782, and 0.782 in patients with HCV. A CDS cutoff value of less than 5 misclassified none of the 9 patients with NASH who had cirrhosis, while a value of more than 8 misclassified none of the 41 patients with NASH without cirrhosis. With the HALT-C model, a cutoff value of less than 0.6 classified non-cirrhotic NASH, while a cutoff value of 0.97 or higher classified cirrhotic NASH. The use of CDS and HALT-C model could avoid liver biopsy for predicting cirrhosis in 60 and 48% of the patients with NASH, respectively.
Conclusions Noninvasive laboratory tests designed to predict cirrhosis in patients with HCV are also useful in patients with NASH.

Keywords NASH · Noninvasive test · Cirrhosis discriminant score · HALT-C · Fibrosis

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Introduction

Non-alcoholic fatty liver disease (NAFLD) has become a common diagnosis in clinical practice owing to the increasing prevalence of obesity and type 2 diabetes mellitus in the general population worldwide [1]. The spectrum of NAFLD ranges widely from simple steatosis to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis and liver failure [2, 3]. NASH is defined histologically as steatohepatitis similar to alcoholic steatohepatitis and is characterized by the presence of macrovesicular steatosis, mixed inflammatory cell infiltration, hepatocyte ballooning and necrosis, Mallory body formation, and perisinusoidal fibrosis [4, 5]. Adams et al. [6] followed 21 patients with cirrhotic-stage of NASH for a median duration of 6.8 years and reported that 62% had complications and 33% died of liver-related causes.

Liver biopsy is considered the gold standard for diagnosing chronic liver disease, grading inflammatory activity, and staging fibrosis. However, biopsy is not suitable for

repeated evaluations because it is costly, invasive, and associated with a risk of major complications (0.3–0.5%), including death (0.03–0.1%) [7, 8]. Sampling error may lead to underestimation of underlying cirrhosis, especially when biopsy specimens are small or fragmented. Thus, an inexpensive, noninvasive, and accurate method for diagnosing cirrhosis is required [9–11].

Because of the risks and limitations of liver biopsy, cirrhosis is sometimes diagnosed on the basis of imaging studies, such as ultrasonography and computed tomography, in clinical practice. In addition, several laboratory tests, indices, and scores have been proposed for the noninvasive prediction of hepatic fibrosis in patients with chronic hepatitis C. Among these, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) [12, 13], age-platelet index (AP index) [14], AST-to platelet ratio index (APRI) [15], and cirrhosis discriminant score (CDS) [16] are based on routine laboratory variables and thus can be readily determined. A new model for predicting cirrhosis has recently been developed on the basis of data derived from the large Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) cohorts (HALT-C model) [17]. The HALT-C Trial was a randomized, controlled study designed to determine whether long-term therapy with pegylated interferon can reduce the risk of progression to end-stage liver diseases in patients with chronic hepatitis C.

The aim of this study was to determine whether the noninvasive tests designed to predict the risk of cirrhosis in patients with chronic hepatitis C virus (HCV) infection (such as AAR, AP index, APRI, CDS, and HALT-C model) could also be used in patients with NASH. First, the receiver-operating characteristic curve (ROC) of each test for predicting cirrhosis was constructed in patients with NASH and in age- and sex-matched patients with HCV. Second, we estimated the percentage of patients with NASH in whom liver biopsy for the diagnosis of cirrhosis could be avoided by using noninvasive predictive tests.

Materials and methods

Patients

We studied 50 patients with NASH who underwent liver biopsy at Osaka City University Hospital between 1998 and 2007. Data from an age- and sex-matched group of 100 patients with HCV were used as a control. The diagnosis of NASH was based on the following: (1) histological features of steatohepatitis, (2) an absence of clinically significant alcohol consumption (20 g/day), and (3) no other identifiable causes of liver diseases, including drug-induced hepatotoxicity, infection with hepatitis B and C viruses, autoimmune liver diseases, Wilson's disease, hemochromatosis, and α_1-antitrypsin deficiency. Informed written consent was obtained from each patient. The study was approved by the local ethics committee and was carried out according to the provisions of the Helsinki Declaration of 1975 (2000 revision).

Laboratory assessment

AST, ALT, alkaline phosphatase, total bilirubin, total cholesterol, triglycerides, plasma glucose, prothrombin time (international normalized ratio, INR), and platelet count were routinely determined by standard procedures within 4 weeks of the liver biopsy. Patients with a fasting plasma glucose level above 125 mg/dl were given a diagnosis of diabetes mellitus. Patients with a triglyceride level above 150 mg/dl and/or a total cholesterol level above 220 mg/dl were given a diagnosis of hyperlipidemia. Patients with a blood pressure above 140/90 mmHg were regarded to have hypertension. These routine laboratory values were used to calculate AAR, AP index, APRI, CDS, and HALT-C model as follows:

1. AAR = AST/ALT
2. AP index = the sum of the age score (age expressed in years: <30 = 0; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; ≥70 = 5) plus the platelet count score [platelet count ($\times 10^9$/l): ≥225 = 0; 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; <125 = 5) (possible value 0–10)].
3. APRI = [AST/upper limit of normal)/platelet count ($\times 10^9$/l)] × 100.
4. CDS = the sum of the platelet count score [platelet count ($\times 10^9$/l): ≥340 = 0; 280–339 = 1; 220–279 = 2; 160–219 = 3; 100–159 = 4; 40–99 = 5; <40 = 6] plus the AST/ALT ratio score (>1.7 = 0; 1.2–1.7 = 1; 0.6–1.19 = 2; <0.6 = 3) plus the INR score (<1.1 = 0; 1.1–1.4 = 1; >1.4 = 2) (possible value 0–11).
5. HALT-C model = formula used to predict the probability of cirrhosis: exp (log odds)/(1+exp (log odds), where the log odds (predicting cirrhosis) = –5.56–0.0089 × platelet count ($\times 10^9$/l) + 1.26 × AST/ALT + 5.27 × INR.

Liver biopsy and histology

The liver tissues were obtained by ultrasound-guided biopsy using a 15-gauge Tru-cut needle (Hakko, Nagano, Japan). All specimens fulfilled the requirements for size as suggested by Janiec et al. [18]. Liver tissues were fixed in formalin immediately after biopsy and embedded in paraffin. Five-micrometer-thick sections were cut with a

microtome. One section of each biopsy specimen was stained with hematoxylin–eosin to assess hepatic steatosis and inflammatory activity; another section was stained with Azan–Mallory to evaluate hepatic fibrosis. Histological diagnosis was performed. Fibrosis was staged and inflammatory activity was graded according to the classification of Brunt et al. [19] in patients with NASH, and according to the classification of Desmet et al. [20] in patients with HCV.

Statistical analysis

Statistical analysis was performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). The chi-square test or Wilcoxon rank sum test was used to compare the clinical characteristics of the patients between two groups. ROC curves were constructed for the AAR, AP index, APRI, CDS, and HALT-C model. To evaluate the diagnostic accuracies of the noninvasive predictive tests, the area under the ROC curve (AUROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Individual AUROC values of noninvasive tests were compared as described by DeLong et al. [21]. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Patient characteristics

The clinical characteristics of the patients are summarized in Table 1. In the NASH group, body mass index, total cholesterol, triglycerides, fasting plasma glucose, and platelet count were significantly higher than those in the HCV group. The proportion of patients with a clinical diagnosis of diabetes or hyperlipidemia was also significantly higher in the NASH group.

Stages of fibrosis

Among the 50 patients with NASH, the stage of fibrosis according to the classification of Brunt et al. [19] was stage 1, 14 (28%); stage 2, 14 (28%); stage 3, 13 (26%); and stage 4, 9 (18%). Of the 100 patients with HCV, fibrosis was stage 1, 45 (45%); stage 2, 20 (20%); stage 3, 18 (18%); and stage 4, 17 (17%) according to the classification of Desmet et al. [20].

ROC curves

The ROC curves of the AAR, AP index, APRI, CDS, and HALT-C model for the prediction of cirrhosis in NASH and HCV are shown in Fig. 1, and the AUROC values of these noninvasive predictive tests are shown in Table 2. In both NASH and HCV, 4-variable analyses, such as the CDS and HALT-C model, had higher AUROC values for the prediction of cirrhosis than did 2-variable analyses, such as the AAR, AP index, and APRI. For all tests, the AUROC value was higher in patients with NASH than in age- and sex-matched patients with HCV.

Table 1 Patient characteristics

	NASH ($n = 50$)	HCV ($n = 100$)	
Age (years)[a]	55.8 ± 15.2	56.7 ± 13.6	Matched
Female[b]	37 (74%)	74 (74%)	Matched
Body mass index (kg/m^2)[a]	27.1 ± 3.8	22.9 ± 3.7	<0.0001
Diabetes[b]	22 (44%)	8 (8%)	<0.0001
Hypertension[b]	23 (46%)	31 (31%)	0.0721
Hyperlipidemia[b]	31 (62%)	3 (3%)	<0.0001
AST (IU/l)[c]	72 (33–332)	64 (26–340)	0.5946
ALT (IU/l)[c]	106 (24–368)	79 (20–314)	0.0858
Alkaline phosphatase (IU/l)[a]	213.6 ± 64.8	210.3 ± 85.7	0.8044
Total bilirubin (mg/dl)[a]	0.94 ± 0.35	0.87 ± 0.33	0.2325
Total cholesterol (mg/dl)[a]	209 ± 42.2	172 ± 28.3	<0.0001
Triglyceride (mg/dl)[c]	123 (49–536)	92 (34–436)	0.0005
Fasting plasma glucose (mg/dl)[a]	124.2 ± 45.7	102.3 ± 25.5	0.0002
INR[a]	1.06 ± 0.17	1.07 ± 0.13	0.4301
Platelet count ($\times 10^9$/l)[a]	192 ± 85	151 ± 59	0.0008

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *INR* international normalized ratio

[a] Mean ± SD

[b] Number (%)

[c] Median (range)

Cutoffs

The sensitivity, specificity, PPV, and NPV of CDS and HALT-C model for predicting cirrhosis in NASH with use of various cutoff values are shown in Table 3. For CDS, a cutoff predicted value of less than 5 misclassified none of the 9 patients with cirrhosis, while a cutoff predicted value of more than 8 misclassified none of the 41 patients without cirrhosis. When these cutoff values were used, cirrhosis could be excluded in 27 patients without cirrhosis and diagnosed in 3 patients with cirrhosis, meaning that liver biopsy for predicting the risk of cirrhosis could be avoided in 30 of the 50 (60%) patients with NASH. With the HALT-C model, when a cutoff value of less than 0.6 was used to exclude cirrhosis, none of the nine patients with

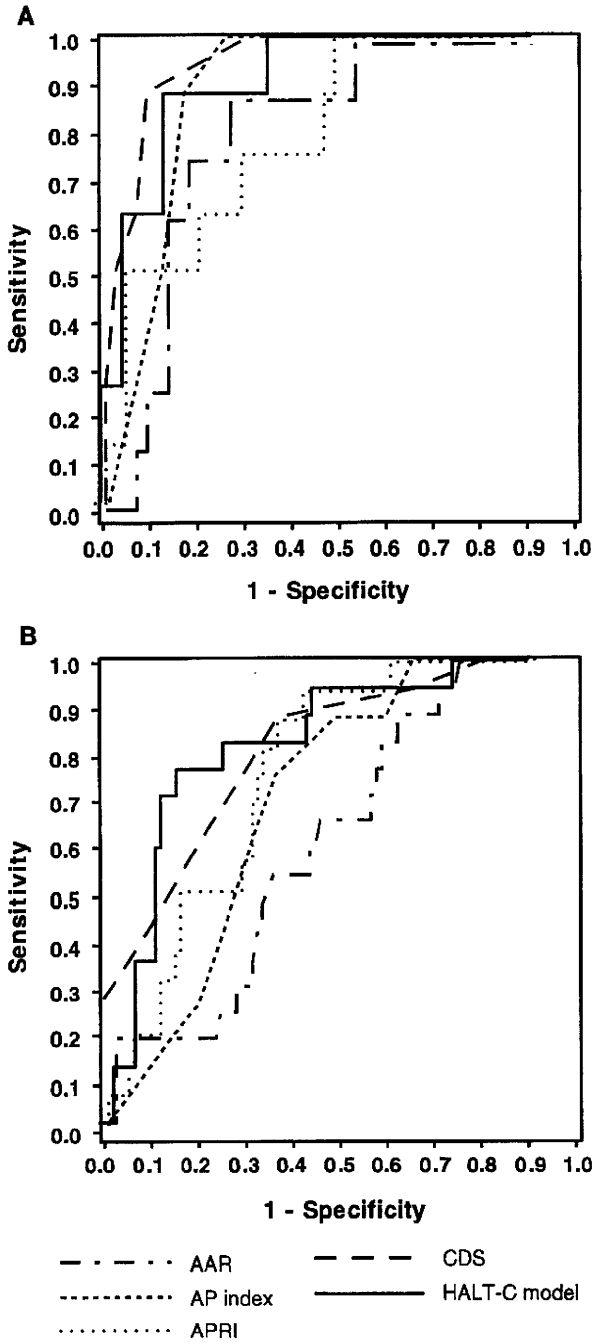

Fig. 1 ROC curves of five simple noninvasive tests for the prediction of cirrhosis constructed **a** for 50 patients with NASH and **b** for 100 age- and sex-matched patients with HCV. *AAR* AST/ALT ratio, *AP-index* age-platelet index, *APRI* AST-to-platelet ratio index, *CDS* cirrhosis discriminant score, *HALT-C model* the Hepatitis C Antiviral Long-term Treatment against Cirrhosis model

cirrhosis would have been misclassified. When a cutoff value of 0.97 or higher was used to confirm cirrhosis, none of the 41 patients without cirrhosis were mistakenly predicted to have cirrhosis. Our results indicate that use of this model might have obviated the need for a liver biopsy aimed to predict the risk of cirrhosis in 24 of the 50 (48%) patients with NASH (22 without cirrhosis and 2 with cirrhosis) (Table 4).

Discussion

Several noninvasive indices, scores, and models have been proposed for the prediction of hepatic fibrosis in patients with chronic hepatitis C [12–17]. However, most tests were based on data from white or African-American patients with HCV and remain to be validated in other ethnic groups. In our cohort of Japanese patients with HCV, the AUROC value of CDS or HALT-C model for predicting cirrhosis was 0.782, suggesting that these indices are also useful for predicting the risk of cirrhosis in Japanese patients with HCV. More importantly, the AUROC value was higher in patients with NASH than in age- and sex-matched patients with HCV. This finding suggests that the diagnostic accuracies of these noninvasive tests, originally designed for patients with HCV, were at least comparable when used in patients with NASH.

The AUROC values of the AAR, AP index, APRI, CDS, and HALT-C model for predicting advanced fibrosis (stage 3–4) were also high (0.787, 0.795, 0.838, 0.811, and 0.808; data not shown) in patients with NASH. However, we cannot directly compare the accuracies for predicting advanced fibrosis between patients with NASH and those with HCV, because the histological definition of stage 3 differs between the classification of Brunt for NASH and that of Desmet for HCV.

Negative correlations between platelet count and the degree of hepatic fibrosis have been noted previously in patients with chronic hepatitis C [22, 23]. The decreased platelet count is associated with portal hypertension, resulting in increased pooling of platelets in the spleen [24] and, to a lesser extent, with reduced production of thrombopoietin in hepatocytes [25]. Shimada et al. [26] reported that a low platelet count was a significant predictor of severe hepatic fibrosis in patients with NASH. In our study, the AUROC value of the platelet count was 0.893 in patients with NASH (data not shown). In contrast, the AUROC value of 2-variable analyses that included platelet count (such as the AP index and APRI) was smaller than that of the platelet count per se, and the addition of age or AST did not increase the predictive value.

In 1988, Williams et al. [12] reported that an AAR of 1 or more strongly suggests the presence of cirrhosis in patients with various forms of chronic hepatitis. Progression of hepatic fibrosis may reduce AST clearance, leading to increased AST levels in serum [27]. In addition,

Table 2 AUROC of noninvasive tests for the prediction of cirrhosis in patients with NASH and in patients with HCV

	NASH (n = 50)		HCV (n = 100)	
	AUROC	Confidence intervals	AUROC	Confidence intervals
AAR	0.813	(0.674–0.952)	0.555	(0.416–0.694)
AP index	0.877	(0.785–0.968)	0.652	(0.538–0.767)
APRI	0.786	(0.625–0.947)	0.761*	(0.654–0.868)
CDS	0.949*†	(0.889–1.008)	0.782**‡	(0.665–0.898)
HALT-C score	0.908	(0.811–1.004)	0.782**‡	(0.667–0.898)

AUROC the area under the receiver-operating characteristic curve

* <0.05, **<0.01 versus AAR

†<0.05 versus APRI

‡<0.05 versus AP index

Table 3 Sensitivity, specificity, PPV, and NPV of CDS for predicting cirrhosis in patients with NASH

Predicted values	No. of patients	No. (%) with cirrhosis	No. (%) without cirrhosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0	2	0 (0%)	2 (100%)	100	5	19	100
1	1	0 (0%)	1 (100%)	100	7	19	100
2	7	0 (0%)	7 (100%)	100	24	23	100
3	7	0 (0%)	7 (100%)	100	41	27	100
4	10	0 (0%)	10 (100%)	100	66	39	100
5	11	1 (9%)	10 (81%)	89	90	67	97
6	3	2 (67%)	1 (33%)	67	93	67	93
7	3	1 (33%)	2 (67%)	56	98	83	91
8	3	2 (67%)	1 (33%)	33	100	100	84
9	2	2 (100%)	0 (0%)	11	100	100	84
10	1	1 (100%)	0 (0%)				
Total	50	9 (18%)	41 (82%)				

PPV positive predictive value, *NPV* negative predictive value

Table 4 Sensitivity, specificity, PPV, and NPV of HALT-C model for predicting cirrhosis in patients with NASH

Predicted values	No. of patients	No. (%) with cirrhosis	No. (%) without cirrhosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<0.3	1	0 (0%)	1 (100%)	100	2	18	100
0.3–0.4	3	0 (0%)	3 (100%)	100	10	20	100
0.4–0.5	8	0 (0%)	8 (100%)	100	29	24	100
0.5–0.6	10	0 (0%)	10 (100%)	100	54	32	100
0.6–0.7	7	1 (14%)	6 (86%)	89	68	38	97
0.7–0.8	6	0 (0%)	6 (100%)	89	83	53	97
0.8–0.9	10	5 (50%)	5 (50%)	33	95	60	87
0.9–0.97	3	1 (66%)	2 (34%)	22	100	100	85
0.97≦	2	2 (100%)	0 (0%)				
Total	50	9 (18%)	41 (82%)				

advanced liver disease may be accompanied by mitochondrial injury, resulting in more marked release of AST, which is present in mitochondria and cytoplasm, than of ALT [28]. Although many researchers have examined the usefulness and diagnostic ability of AAR in patients with HCV, results have been conflicting [13, 29–32]. Some

studies have demonstrated that AAR is useful for predicting cirrhosis in NASH [26, 33, 34]. In our study, AAR was a better predictor of cirrhosis in patients with NASH than in patients with HCV.

In 1995, Teran et al. [35] originally described a CDS based on two clinical variables: vascular spiders and ascites. In 1997, Bonacini et al. [26] modified this score and included only objective laboratory variables, such as platelet count, AAR, and INR. As mentioned above, platelet count and AAR can be useful for predicting cirrhosis in patients with NASH. INR is directly related to the synthetic function of the liver, and worsens with progression of fibrosis and loss of hepatocyte mass. In our study, the AUROC value of CDS for predicting cirrhosis was the highest among the five noninvasive tests evaluated; with use of this score, liver biopsy for predicting the risk of cirrhosis can be avoided in 60% of patients with NASH.

The HALT-C model is also derived from platelet count, AAR, and INR [17]. It was based on data collected prospectively from more than 1,100 patients of various ethnic backgrounds. However, unlike CDS, the HALT-C Trial was not designed specially to identify predictors of cirrhosis, and the study group consisted of patients with advanced fibrosis. The HALT-C model had the second highest AUROC value for predicting cirrhosis; use of this model might obviate the need for liver biopsy for predicting the risk of cirrhosis in 48% of patients with NASH.

Fibrotest is an algorithm based on five biochemical markers: bilirubin, gamma-glutamyl transpeptidase, gamma-globulin, haptoglobin, and alpha2-macroglobulin [36]. It has been validated in patients with chronic hepatitis B, hepatitis C, and NAFLD/NASH [36–38]. Fibrotest was found to have an AUROC value of 0.80–0.85 and could prevent the need for liver biopsy in about 40% of patients. However, the algorithm includes the biomarkers that are costly to evaluate and not measured routinely.

Transient elastography is an emerging technology that is more sensitive than currently available radiologic techniques for staging hepatic fibrosis [39]. In patients with HCV, cirrhosis was differentiated from milder stages of fibrosis with an AUROC value of 0.97 [40]. Similar results were obtained from patients with primary biliary cirrhosis [41] and NASH [42]. However, elastography is not suitable for use in patients with morbid obesity, which often accompanies NASH. Moreover, the equipment is very expensive and is not available at most hospitals.

In conclusion, we demonstrated that noninvasive tests proposed for the prediction of cirrhosis in patients with HCV can also be used with a high degree of accuracy in patients with NASH. In particular, CDS and HALT-C model, which utilize four routine laboratory variables, such as platelet count, AST, ALT, and INR, can distinguish between the presence or absence of cirrhosis with sufficient reliability to be used to predict the risk of cirrhosis in patients with NASH.

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CASE REPORT

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Hepatocellular carcinoma (HCC) recurring 10 years after clearance of hepatitis B surface antigen and 20 years after resection of hepatitis B virus-related HCC

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Abstract A 62-year-old man had been followed up for chronic hepatitis B (HB) since 1973. Hepatocellular carcinoma (HCC) was detected in 1985, at the age of 42 years. Serum HB surface antigen and anti-HB envelope antibody were positive at that time. A right hepatic lobectomy was performed. In 1995, serum HB surface antigen had cleared spontaneously and liver function had normalized. In March 2005, at the age of 62 years, a 1.5-cm diameter hepatic mass was detected in the left lateral segment. At that time, he was seropositive only for anti-HB core antibody. A diagnosis of recurrent HCC was made, and partial hepatectomy was performed. Covalently closed circular HBV DNA was detected in both cancerous and noncancerous tissues by nested polymerase chain reaction (PCR). Cassette-ligation-mediated PCR showed that HBV DNA was integrated into the telomerase reverse transcriptase gene located on chromosome 5p15.

Key words Hepatocellular carcinoma · Hepatitis B virus · Human telomerase reverse transcriptase (hTERT) · Liver resection

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Introduction

It is well known that hepatitis B virus (HBV) can cause hepatocellular carcinoma (HCC). Persistent active hepatitis can result in progression to cirrhosis and the development of HCC. During the natural history of chronic hepatitis B (HB), seroconversion from HB surface antigen (HBsAg) to anti-HB surface antibody (anti-HBs) is associated with remission of active hepatitis and improvement of liver function and pathologic features.[1] Although it is thought that clearance of HBsAg from the serum indicates clinical cure and a decreased risk of carcinogenesis, HCC is sometimes detected after this seroconversion.[2–6] It has also been reported that occult HBV infection is important in the development of HCC.[4,7–10] In this report, we describe a case of HCC which recurred in 2005, 10 years after the clearance of HBsAg in 1995, and 20 years after resection of the first HCC while the patient was seropositive for HBsAg in 1985.

Case report

A 62-year-old man had been followed up for chronic HB since 1973. In 1985 (at age 42 years), a hepatic tumor was detected in the anterior superior segment (S8) by ultrasonography (US) and computed tomography (CT). Serum HBsAg was positive, serum anti-HBs was negative, HB envelope antigen (HBeAg) was negative, and anti-HB envelope antibody (anti-HBe) was positive (Table 1). Liver function tests indicated active hepatitis. Transcatheter arterial embolization and percutaneous transhepatic portal vein embolization were performed, followed by right lobectomy. He was transfused with 1500 ml whole blood and 880 ml fresh frozen plasma during the operation. The tumor measured 1.8 × 1.5 cm (Fig. 1A), and was classified as T1N0M0 according to the TNM system.[11] Pathologic examination revealed a moderately differentiated HCC and no microvascular invasion (Fig. 1B). Examination of the

Table 1. Results of laboratory tests at the times of the first and second operations

Test	First operation (Oct. 1985)	Second operation (March 2005)
Albumin (g/dl)	4.0	4.2
Total bilirubin (mg/dl)	0.6	0.6
Aspartate aminotransferase (IU/l)	50	24
Alanine aminotransferase (IU/l)	115	27
ICGR15 (%)	13.5	19.6
Prothrombin test (%)	105	113
HBeAg	–	–
Anti-HBe	+	+
HBsAg	+	–
Anti-HBs	–	–
Anti-HBc	+ (99% INH)	+ (99% INH)
HBV DNA	ND	–
Anti-HCV (titer)	ND	2.2
HCV RNA	ND	–
α-Fetoprotein (AFP; ng/ml)	57	4
AFP-L3 (%)	ND	0
PIVKA-II (AU/ml)	ND	21

ICGR15, indocyanine green retention rate at 15 min; HBeAg, hepatitis B envelope antigen; anti-HBe, anti-hepatitis B envelope antibody; HBsAg, hepatitis B surface antigen; anti-HBs, anti-hepatitis B surface antibody; anti-HBc, anti-hepatitis B core antibody; HBV DNA, hepatitis B virus DNA; anti-HCV, anti-hepatitis C virus antibody; HCV RNA, hepatitis C virus RNA; PIVKA II, protein induced by vitamin K absence or antagonist II; ND, not determined; INH, inhibition

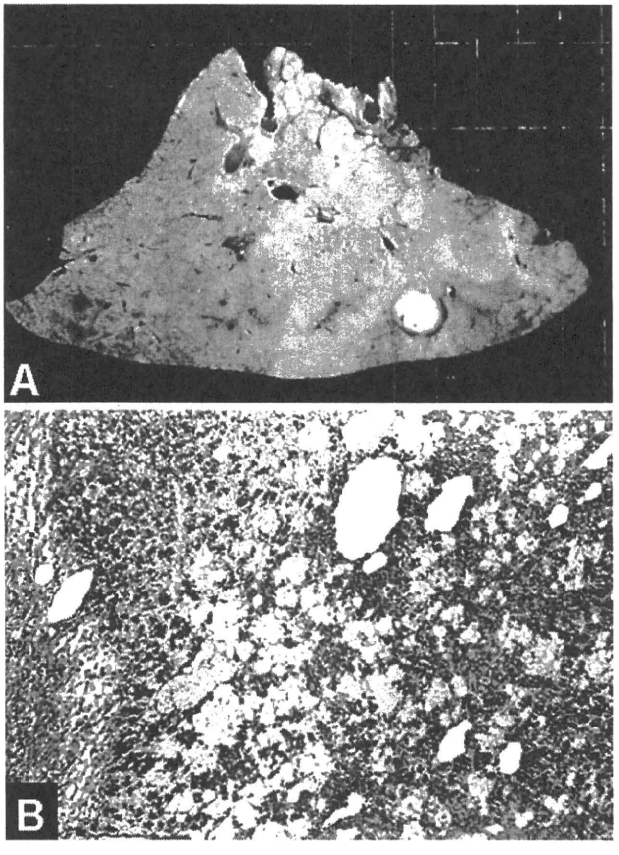

Fig. 1. **A** Resected specimen from the first operation. The tumor measured 1.8 cm × 1.5 cm and was mostly necrotic. **B** Pathologic examination showing that the tumor is a moderately differentiated hepatocellular carcinoma. H&E, ×20

nontumorous hepatic tissue showed minimal hepatitis activity (grade 1) and portal-portal septa without architectural distortion (stage 2) according to the histologic activity index score.[12] In 1995 (at age 52 years), serum HBsAg had cleared. In 1996, serum HBV DNA was negative by the polymerase chain reaction (PCR) method. Serum anti-hepatitis C virus antibody (anti-HCV; Ortho Diagnostic Systems, Tokyo, Japan) was positive, but serum hepatitis C virus RNA (Quantiplex HCV-RNA; Chiron, Emeryville, CA, USA) was negative. The anti-HCV titer was consistently low. The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) had normalized in 1995. In March 2005 (at age 62 years), a hepatic mass measuring 1.5 cm in diameter was detected in the left lateral segment by CT and US. At that time, serum HBsAg, anti-HBs, and HBV DNA were all negative (Table 1). Anti-HB core antibody was positive. The anti-HCV titer was very low. Although the indocyanine green retention rate at 15 min was 19.6%, the results of other liver function tests were within the reference ranges. Renal function tests were abnormal because of renal failure following coronary artery bypass grafting and graft replacement of the ascending aorta at the age of 59 years. The levels of the tumor markers α-fetoprotein (AFP), AFP-L3 fraction, and protein induced by vitamin K absence or antagonist II were all within the reference ranges. In May 2005, partial hepatectomy was performed with a preoperative diagnosis of recurrent HCC. The tumor measured 2.5 × 2.0 cm (Fig. 2A), and was classified as T2N0M0. Pathologic examination showed a moderately differentiated HCC and no microvascular invasion (Fig. 2B), with nonactive hepatitis (grade 0) and portal fibrous expansion (stage 1) in the noncancerous tissue. The HB surface (*HBs*) gene and covalently closed circular (ccc)

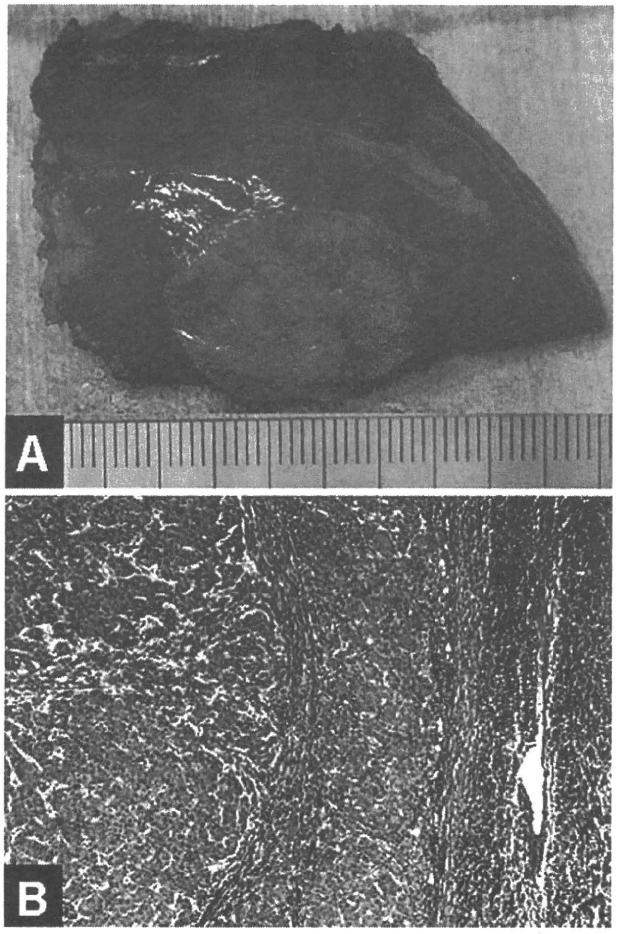

Fig. 2. **A** Resected specimen from the second operation. The tumor measured 2.5 cm × 2.0 cm. **B** Pathologic examination showing that the tumor is a moderately differentiated hepatocellular carcinoma. H&E, ×20

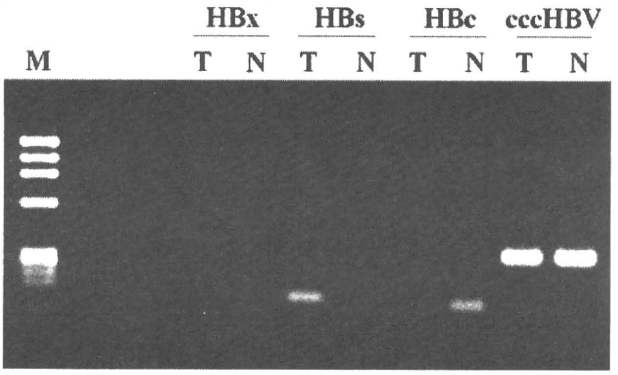

Fig. 3. Hepatitis B virus (HBV) DNA analysis of the resected sample. Detection of the *HBx* gene, *HBs* gene, *HBc* gene, and ccc HBV DNA was performed using specific primers in the tumor (*T*) and nontumorous (*N*) tissues. *M*, Marker (pBR322/AluI).

HBV DNA were detected in the tumor by PCR. The *HBs* gene, hepatitis B core (*HBc*) gene, and ccc HBV DNA were detected in the nontumorous tissue by PCR. The *HBx* gene was not detected in either tumor or nontumorous tissues by PCR (Fig. 3). Cassette-ligation-mediated PCR[10,13] showed that HBV DNA was integrated into the telomerase reverse transcriptase gene (*hTERT* gene) located on chromosome 5p15 in the tumor (Fig. 4). The patient is in good health without recurrent tumor 18 months after the second operation.

Discussion

In this report, we describe a case of recurrent HCC 10 years after the clearance of HBsAg and 20 years after resection of the first HBV-related HCC. The duration between the first HCC and the recurrent HCC is the longest reported in the literature to date. The duration between the clearance of HBsAg and the detection of the recurrent tumor is the longest except for one case reported by Yoshino et al.[6]

The mechanism of carcinogenesis in HCC associated with HBV includes mutagenesis and changes in proliferation and differentiation caused by the integration of HBV DNA.[14–17] HBx protein binds with p53 protein and inhibits p53-mediated prevention of neoplastic transformation,[18,19] and also activates cellular transcription factors.[20,21] Chronic inflammation and oxidative stress due to HBV infection induce DNA damage in hepatocytes.[22] Although we could not investigate the integration of HBV DNA at the time of the first operation in this patient, the first HCC is thought to have been caused by chronic HB, because he was seropositive for HBsAg and had active hepatitis at that time. At the time of the second operation, serum HBsAg and HBV DNA had cleared and liver histology had improved. Research on HBV DNA has shown that the *HBs* gene and ccc HBV DNA are detectable in HCC and nontumorous hepatic tissue, and that the *HBc* gene is detectable in noncancerous hepatic tissue; the *HBx* gene was not detected in either tumor or nontumorous tissues in our patient. Integration of HBV DNA into the *hTERT* gene has also been detected. Previous reports showed that HBV DNA was integrated into the *hTERT* gene, and that it also could play an important role in hepatic carcinogenesis.[23,24] These findings indicate that the patients have either occult HBV infection or integration of HBV DNA in their liver. Other possible mechanisms of hepatic carcinogenesis include inactivation of p53 by mutations and regional allelic deletions, although changes in the *p53* gene and p53 protein were not investigated in our patient.[25]

Hepatitis B antigens have been detected in the hepatic tissues of some patients after the clearance of serum HBsAg associated with the remission of active hepatitis, especially in those with a high anti-hepatitis B core antibody (anti-HBc) titer.[26,27] HBV DNA is often integrated into the host's chromosomes and plays an important role in hepatic carcinogenesis.[28] Thus, we speculate that, in our patient, the HCCs developed from multicentric origins, and that the integrated HBV DNA participated in the development of the recurrent HCC. In patients with chronic HB, screening for HCC is necessary even after clearance of serum HBsAg

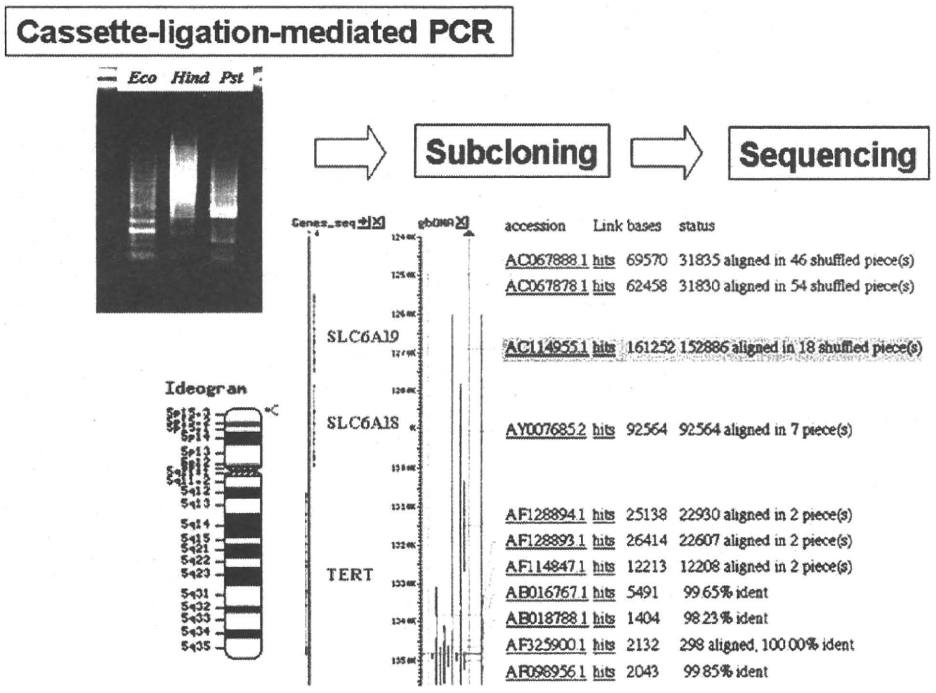

Fig. 4. Cassette-ligation-mediated polymerase chain reaction (PCR). HBV DNA is integrated into the telomerase reverse transcriptase (*hTERT*) gene located on chromosome 5p15. *Ident*, Identical

and HBV DNA and remission of hepatitis, because the oncogenic potential due to occult HBV infection or the integration of HBV DNA is considered to continue.[29]

Itsubo et al.[30] reported a case of recurrent HCC associated with HCV infection due to transfusion during the first liver resection. The first liver resection had been performed 18 years previously for HCC associated with HBV infection. In our patient, serum anti-HCV was positive and HCV RNA was negative in 1995. Although the HCV infection in the patient reported by Itsubo et al.[30] might have been caused by the transfusion during the first operation, it was transient because HCV RNA was negative and the levels of AST and ALT were within the reference ranges. Thus, the recurrent HCC was not directly caused by the HCV infection. However, an HCV superinfection might have played a role in the spontaneous HBsAg clearance in our patient.[31]

The rare case that we have reported indicates that long-term follow up is necessary for chronic HB, even after the clearance of serum HBsAg and HBV DNA and remission of hepatitis, because the oncogenic potential due to occult HBV infection or the integration of HBV DNA is considered to continue.

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Sildenafil-Induced Severe Cholestatic Hepatotoxicity

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To the Editor: Sildenafil citrate (Viagra) is a potent, orally active, cyclic guanosine monophosphate-specific phosphodiesterase type 5 inhibitor, used globally for the treatment of penile erectile dysfunction. The most common adverse effects are headache, flushing, dyspepsia, and cardiovascular events. Liver toxicity attributed to sildenafil appears to be very rare. In the English-language literature, only one case of mild hepatotoxicity induced by sildenafil has been reported earlier (1). We describe a case of severe cholestatic hepatotoxicity induced by sildenafil.

A previously healthy 58-year-old man was referred to us because of jaundice, pruritus, and malaise. The laboratory values were as follows: aspartate aminotransferase 42 IU/l, alanine aminotransferase 64 IU/l, alkaline phosphatase 476 IU/l, total bilirubin 8.5 mg/dl, direct bilirubin 6.3 mg/dl, albumin 3.6 g/dl, white blood cell count 4,300/mm^3 (with 2.8% eosinophils), hemoglobin concentration 13.3 g/dl, platelet count 239,000/mm^3, and prothrombin time 95%. Tests for immunoglobulin M anti-hepatitis A virus, hepatitis B surface antigen, immunoglobulin M anti-hepatitis B core antibodies, anti-hepatitis C virus antibodies, immunoglobulin M anti-viral capsid antigen of Epstein-Barr virus, and immunoglobulin M anti-cytomegalovirus were all negative. C-reactive protein and anti-nuclear, anti-mitochondrial, and anti-neutrophil cytoplasmic antibodies were also negative. No history of recent drug use or excessive alcohol intake was reported. Abdominal ultrasound, computed tomography, and magnetic resonance cholangiopancreatography showed no signs of bile duct obstruction. Macroscopically, the liver was green, enlarged, and had a smooth surface without nodularity on laparoscopic examination (**Figure 1**). A liver biopsy specimen revealed features of intrahepatic cholestasis; marked bile stasis was seen in canaliculi around the pericentral area, and cellular necroinflammation in the portal area was minimal (**Figure 2**).

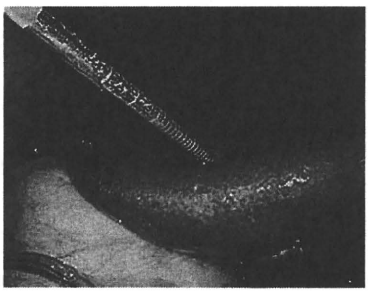

Figure 1. Laparoscopic image showing a green and enlarged liver without nodularity.

On carefully obtaining his history again, the patient admitted that he had taken sildenafil 50 mg 1 month before symptom onset. No other medications, including nonsteroidal anti-inflammatory drugs, were used. On the basis of criteria for drug-induced liver disorders (2) and the Naranjo adverse drug reaction probability scale, (3) we diagnosed probable sildenafil-induced cholestatic hepatotoxicity. The laboratory data steadily improved thereafter without any medical treatment and returned to normal 4 months after symptom onset.

Daghfous *et al.* (1) reported a case of acute hepatotoxicity attributed to sildenafil. However, the causal relation between sildenafil use and subsequent liver damage was uncertain. The alanine aminotransferase level increased to only 1.2 times the upper limit of normal, and the biluribin and alkaline phosphatase levels were normal. A liver biopsy was not undertaken. Liver injury did not recur after rechallenge with sildenafil.

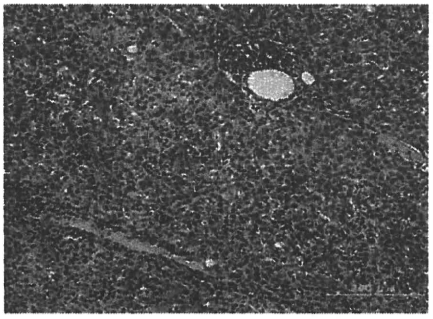

Figure 2. Liver biopsy specimen showing intrahepatic cholestasis (hematoxylin and eosin; original magnification, ×200).

Sildenafil is metabolized predominantly by the cytochrome P-450 3A4 hepatic microsomal isoenzyme. The mechanism of sildenafil-induced hepatotoxicity is unclear. In general, cholestasis can result from several mechanisms, including decreased fluidity of the sinusoidal plasma membrane, inhibition of ATP-dependent bile acid transporters, disruption of the cytoskeleton, and loss of canalicular integrity (4). It is often difficult to determine the main cause in a given case.

To our knowledge, we have documented the first reported case of severe liver toxicity ascribed to sildenafil. In our patient, sildenafil was not prescribed for a diagnosis of erectile dysfunction, but was obtained for 'recreational' use from a friend. Recently, this drug can be easily purchased from dealers or through the Internet. Patients who illicitly take sildenafil may not report a drug history, causing difficulty in the diagnosis of adverse events. Thus, the incidence of sildenafil-induced liver injury may have been underestimated; clinicians should be aware that sildenafil can cause severe liver damage.

In summary, we have described a case of sildenafil-induced severe cholestatic hepatotoxicity. Albeit rare, sildenafil is a possible cause of severe drug-induced liver toxicity.

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A Case of Rectal MALT Lymphoma Treated by Endoscopic Resection

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To the Editor: We report a case of rectal mucosa-associated lymphoid tissue (MALT) lymphoma that was treated successfully with endoscopic resection and empiric antibiotics. Extra gastric locations of MALT lymphomas include the skin, thyroid, lungs, urinary bladder, and salivary glands and rarely at other locations in the gastrointestinal tract including the rectum. The association of these with *Helicobacter pylori* is less well established.

A 65-year-old woman, in her usual state of health, had a screening colonoscopy, during which a 1.2-cm sessile rectal polyp—described as fleshy and different from a usual polyp—was resected at endoscopy with clear margins using a hot snare guillotine technique. On pathologic examination, the polyp was found to be a MALT lymphoma (**Table 1, Figure 1**). Upper endoscopy with biopsies and camplyobacter-like organism test showed chronic duodenitis and was negative for *H. pylori*. A liver function profile and a triphasic abdominal computerized tomography scan were unremarkable. She was started on empiric anti-*H. pylori* treatment (amoxicillin, lansoprazole, and clarithromycin). She remains asymptomatic and has returned for surveillance exams, four times over 4 years and has had no evidence of recurrence. The site of resection showed a clear scar on all occasions (**Figure 1**).

Mucosa-associated lymphoid tissue lymphoma, entered the literature in 1983, when Isaacson and Wright (1) described the first two cases. Most MALT lymphomas emerge from gastric lymphoid tissue looking remarkably like Peyer's patches—a paradoxical finding, as the stomach is generally devoid of lymphoid tissue (2). MALT lymphoma cells appeared to participate in immune responses and the stimulus eliciting this response remained unclear until Isaacson and co-workers described the association with *H. pylori* and later, its treatment (1–3). Today, 75% of gastric MALT lymphomas can be treated successfully by eradicating the *H. pylori*, relegating therapies such as chemo- or immunotherapy, radiation, or surgical resection to second- or third-line treatment options (4).

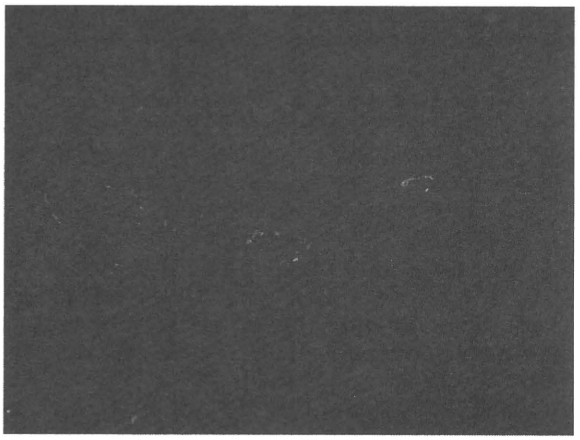
Gastric MALT lymphomas remain localized to the stomach for long periods. The *H. pylori* infection gives rise to a chronic inflammatory state in which a clone of neoplastic B cells evolves localized to the involved mucosa because of antigen-dependent growth. Subsequent mutations upregulate the production of NF-uB (1,5) and the B-cell line reproduces independently of *H. pylori* stimulation, rendering the disease no longer amenable to treatment with antibiotics

Table 1. Immunohistochemical characteristics of resected tumor

CD 3	Negative
CD 5	Negative
CD 20	Positive
CD23	Negative
CD43	Negative
BCL-1	Negative
BCL-2	Negative
Cytoplasmic Ig light chain	Suggestive, not diagnostic of i-excess

Ig, immunoglobulin.

Figure. Direct immunofluorescence revealing perivascular deposits of C3 within the affected vessels.



Original magnification, ×60.

hours at 4 °C, and cryoprecipitates were separated by centrifuge for 5 minutes. The cryoprecipitates were washed 3 times with a small volume of ice-cold, phosphate-buffered saline (pH, 7.2). Then the cryoprecipitates were dissolved in warmed (37 °C), phosphate-buffered saline for 2 hours, and the IgG anti-PS/PT antibody level was measured (at 37 °C). We detected that IgG anti-PS/PT antibody level in the cryoprecipitates was higher (120 U/mL) than that in cryoglobulin-free sera (20 U/mL) under the same dilution conditions.

The patient was treated with entecavir monotherapy, and within 6 months, HBV DNA levels decreased below the limit of detection; his purpuric rash with paresthesia and numbness disappeared; and his serum cryoglobulins, anti-PS/PT antibodies, and lupus anticoagulant also became negative.

Discussion: The mechanism of association between hepatitis infection and vasculitis is unclear. Phosphatidylserine is a constituent of cell membranes that are exposed during apoptosis or other forms of cell damage, and some reports have suggested that it is initially present in the particles produced by infected hepatocytes during HBV infection (2). Also, prothrombin binds to the surface of apoptotic cells (3). On the basis of our findings of higher anti-PS/PT antibody titers in cryoprecipitates than in cryoglobulin-free sera and of C3 deposition by direct immunofluorescence testing within the cryoglobulinemic vasculitis lesions, we propose that prothrombin induced by destroyed hepatocytes binds to phosphatidylserine produced by HBV, triggering production of anti-PS/PT antibody and other cryoglobulins; endothelial cell damage, an inflammatory cytokine cascade, and complement activation; and vasculitis. Demonstration of remission of cryoglobulinemic vasculitis by treatment with entecavir suggests that the effect of entecavir against HBV might prevent this cascade of events.

Conclusion: Entecavir treatment seemed to lead to prompt suppression of HBV replication and prompt resolution of cryoglobulinemic vasculitis.

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Entecavir to Treat Hepatitis B–Associated Cryoglobulinemic Vasculitis

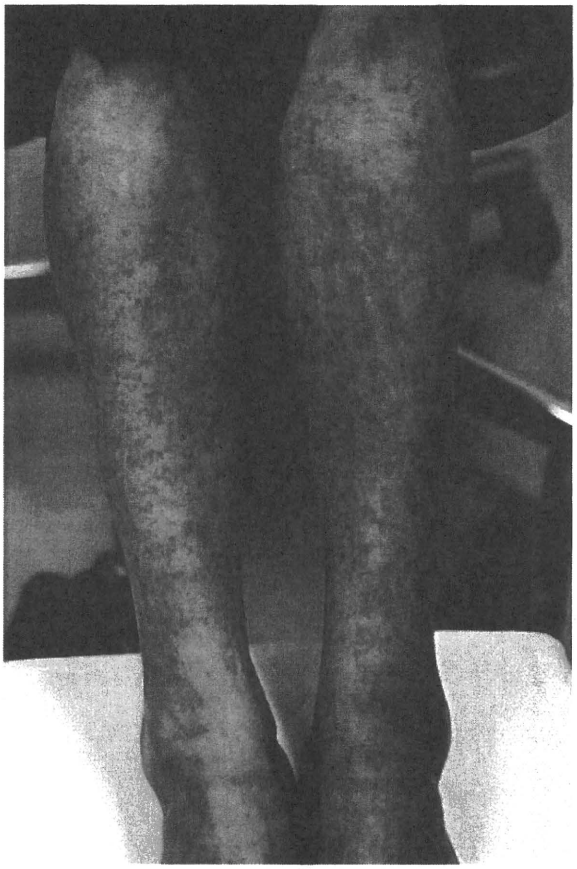
Background: Hepatitis C virus infection is associated with mixed cryoglobulinemia (1). The association of hepatitis B virus (HBV) with cryoglobulinemia is less certain, and treatment is nonspecific.

Objective: To describe a patient with HBV-associated cryoglobulinemic vasculitis that resolved with entecavir.

Case Report: A 57-year-old Taiwanese woman was referred to us because of a purpuric rash on her legs (**Figure**). Skin biopsy specimens showed leukocytoclastic vasculitis in the upper dermis. Her aspartate aminotransferase level was 143 U/L, alanine aminotransferase level was 119 U/L, γ-glutamyltransferase level was 27 U/L, total bilirubin level was 29.1 μmol/L (1.7 mg/dL), albumin level was 3.2 g/dL, creatinine level was 38.13 μmol/L (0.50 mg/dL), leukocyte count was 2.6×10^9 cells/L, hemoglobin concentration was 10.5 g/dL, and platelet count was 63×10^9 cells/L. C-reactive protein and antinuclear and antineutrophil cytoplasmic antibodies were negative. Cryoglobulins were detected by cold precipitation. Test results for antibodies to hepatitis C virus and viral RNA and anti–hepatitis B envelope antigen were negative, and results for hepatitis B surface antigen and hepatitis B envelope antigen were positive. Her HBV DNA level was 6.4 $\log_{10}$ copies/mL. The genotype of the HBV was type B. A precore stop codon mutation was found at nucleotide 1896, but no mutations were found at nucleotide 1762 or nucleotide 1764 in the basal core promoter. Abdominal ultrasonography showed no evidence of cirrhosis or hepatocellular carcinoma. A liver biopsy specimen showed moderate inflammation and severe fibrosis.

On the basis of these findings, HBV-associated cryoglobulinemic vasculitis was diagnosed, and entecavir treatment was started at a dose of 0.5 mg/d. The serum HBV DNA level decreased immediately after the start of therapy and became undetectable by polymerase chain reaction testing (<2.6 $\log_{10}$ copies/mL) by week 6. The aminotransferase activity fell to the normal range by week 12. Cryo-

Figure. Purpuric rash on the patient's legs.



globulins became undetectable by week 20, and skin lesions resolved gradually.

Discussion: Cryoglobulins are abnormal immunoglobulins that undergo reversible precipitation at low temperatures, are deposited in microvessels, and evoke vasculitis. Secondary cryoglobulinemia is best managed by treating the underlying disease. Some cases of chronic hepatitis B complicated by cryoglobulinemic vasculitis have responded to lamivudine or adefovir dipivoxil (2–4), suggesting an association between HBV infection and cryoglobulinemia. Of particular interest, Çakir and colleagues (4) described a patient whose cryoglobulinemic vasculitis responded to lamivudine, recurred owing to the emergence of lamivudine-resistant HBV, and then resolved after rescue therapy with adefovir. The suppression of HBV by antiviral agents may have resulted in decreased numbers of viral antigens that can form cryoglobulins.

Entecavir is the most potent currently available nucleoside or nucleotide analogue against HBV and has been shown to be superior to lamivudine in randomized, controlled trials (5). In addition, it is associated with the lowest rate of drug resistance. New nucleoside analogues with high antiviral potency and low resistance rates would be useful not only for treating hepatitis, but also for managing extrahepatic manifestations.

Interferon-α is an alternative treatment for chronic HBV infection, but it is not preferred to nucleoside or nucleotide analogues for the management of extrahepatic manifestations. One reason is that interferon-α produces rapid viral suppression in only some patients. Another is that interferon-α has immunomodulatory activity. The pathogenesis of extrahepatic manifestations is not completely understood, but immune-mediated mechanisms are most likely involved. The use of corticosteroids or immunosuppressive agents alone is not recommended because of possible flare-ups of viral replication.

Conclusion: Entecavir may be a first-line treatment for extrahepatic manifestations of chronic HBV infection.

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Platelet-associated IgG for the diagnosis of immune thrombocytopaenic purpura during peginterferon α and ribavirin treatment for chronic hepatitis C

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To the Editor:

Mild-to-moderate thrombocytopaenia is a common adverse event of treatment with conventional or pegylated interferon α, attributed primarily to bone marrow suppression, in patients with chronic hepatitis C. Nevertheless, severe, life-threatening immune thrombocytopaenic purpura (ITP) has rarely been associated with interferon treatment (1–7). The pathogenesis of ITP is incompletely understood, but immunoglobulin G (IgG)-type antibodies against platelet membrane glycoproteins (IIb/IIIa, Ib/IX, etc.) are involved (8). We describe a case of ITP induced by peginterferon treatment for chronic hepatitis C. Detection of platelet-associated IgG was helpful for the diagnosis.

A 69-year-old woman with chronic hepatitis C genotype 1b infection started to receive peginterferon α2b 80 μg/week and ribavirin 600 mg/day in October 2006 (Fig. 1). At the start of therapy, she was well, with a height of 155 cm and a weight of 58 kg. The laboratory values were as follows: aspartate aminotransferase 44 IU/L, alanine aminotransferase 58 IU/L, γ-glutamyltransferase 32 IU/L, bilirubin 0.9 mg/dl, albumin 4.1 g/dl, hepatitis C virus (HCV) RNA 43 kIU/ml, haemoglobin concentration 14.4 g/dl, white blood cell count 5400/mm^3 and platelet count 139 000/mm^3. A liver biopsy specimen showed moderate inflammation and mild fibrosis. After the start of therapy, the serum HCV RNA level rapidly decreased and became negative on polymerase chain reaction at the fourth week.

The platelet count gradually declined to 86 000/mm^3 by the 13th week of therapy and then rapidly declined to 14 000/mm^3 at the 16th week. She had petechiae on the upper extremities. Peginterferon and ribavirin were withdrawn. Coagulation test results were normal. A direct Coombs' test result was negative. Antinuclear and anticardiolipin antibodies were negative. Cryoglobulins were not detected. Serum was negative for antiplatelet antibody by mixed passive haemagglutination. However, the platelet-associated IgG level on the platelet surface had increased to 372 (reference range, 9.0–25.0) ng/10^7 cells as measured by an enzyme-linked immunoassay. Bone marrow

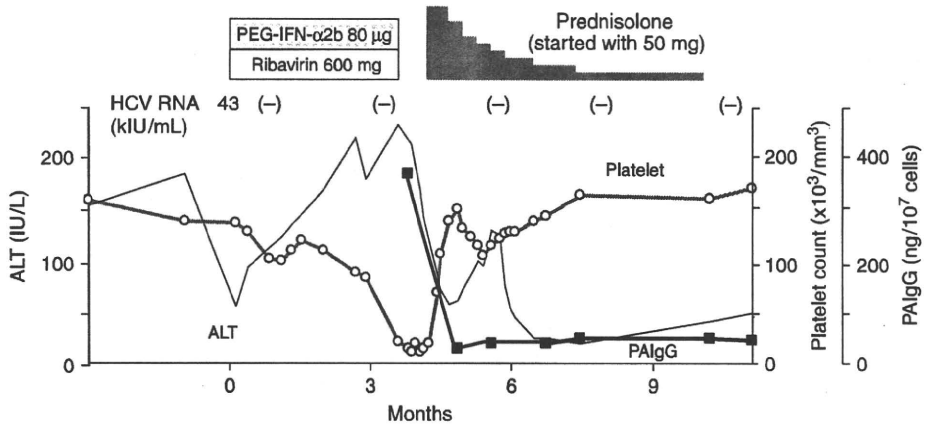

Fig. 1. Clinical course of a 69-year-old woman with chronic hepatitis C in whom immune thrombocytopaenic purpura developed during treatment with peginterferon α and ribavirin. ALT, alanine aminotransferase; PAIgG, platelet-associated immunoglobulin G; PEG-IFN, peginterferon.

aspiration demonstrated increased numbers of megakaryocytes, compatible with a diagnosis of ITP. The results of a ^{13}C-urea breath test were negative for *Helicobacter pylori* infection. Corticosteroid therapy was started with 50 mg oral prednisolone. The platelet count returned to 141 000/mm^3 in 14 days, and remained normal while tapering the dose of prednisolone. The platelet-associated IgG titre decreased in response to corticosteroid therapy. HCV RNA continued to remain negative after the withdrawal of peginterferon.

Immune thrombocytopaenic purpura is an autoimmune disorder characterized by peripheral consumption of platelets and clinical manifestations of a haemorrhagic diathesis (8). ITP is a diagnosis of exclusion, often difficult to establish. In the literature, interferon-induced ITP has developed after 4 week to 12 months of therapy (2, 4), or even 6 months after the completion of therapy (6). The ages and baseline platelet counts of patients have varied widely, ranging from 27 (6) to 73 (5) years and from 80 000 (3) to 260 000 (4)/mm^3 respectively. As demonstrated in our patient, the detection of circulating antiplatelet antibodies unbound to platelets is not sensitive enough for diagnosis. Such autoantibodies can develop in patients immunized by pregnancy, allogenic transfusions or organ transplantation and are thus not specific for ITP. In contrast, a direct assay of platelet-associated IgG (bound to platelets) is more useful for the diagnosis of ITP, with a sensitivity of 49–66% and a specificity of 78–92% (8). In our patient, platelet-associated IgG was also helpful for monitoring the response to corticosteroid therapy.

In summary, we have described a patient with chronic hepatitis C in whom ITP developed during treatment with peginterferon α and ribavirin. Albeit rare, ITP can occur any time during interferon treatment. Physicians treating patients with chronic hepatitis C should be aware that platelet-associated IgG is helpful for promptly diagnosing this potentially fatal complication.

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Hepatocellular apoptosis in polycystic liver disease

DOI: 10.1111/j.1478-3231.2008.01780.x

To the Editor:

Polycystic liver disease (PCLD) is asymptomatic in 80% of patients and often diagnosed incidentally. After diagnosis, surgical therapy is the mainstay of treatment tailored to the extent of disease for symptomatic patients. In the past several decades, there have been great advances in the knowledge of the pathogenesis, genetics and effective treatment for PCLD. These lesions in PCLD have been attributed to bile duct overgrowth after the arrest of embryogenesis and failure of the intralobar bile ducts to involute. This involutional failure results in cystic dilations that are known as biliary microhamartomas or von Meyenburg complexes (VMC) (1). However, we focused on the role of hepatocellular apoptosis in PCLD.

Hepatology Research 2008; 38: 860–868 doi: 10.1111/j.1872-034X.2008.00355.x

Original Article

Does a late evening meal reduce the risk of hepatocellular carcinoma among patients with chronic hepatitis C?

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Aim: Some studies have suggested that nutritional support might protect against the recurrence of hepatocellular carcinoma (HCC) among postoperative HCC patients. However, no epidemiological studies have evaluated the effect of nutritional support on HCC incidence. This study aimed to investigate the association between a late evening meal and HCC.

Methods: We conducted a hospital-based, case-control study comparing 73 cases with HCC to 253 matched controls among patients with chronic hepatitis C. A questionnaire elicited information on the consumption of a late evening meal, which was defined as a snack or meal within 2 h before bedtime. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by the conditional logistic regression model.

Results: After adjustment for potential confounders, patients who consumed a late evening meal had a lower OR as compared to those who did not consume one (OR, 0.08; 95% CI, 0.01–0.48). In terms of frequency of intake, a clear inverse exposure–response relationship was observed (trend $P = 0.009$). In addition, a negative association between a late evening meal and HCC was more pronounced among patients with an α-fetoprotein level of less than 20 ng/mL and those with a body mass index of less than 25 kg/m^2.

Conclusion: A late evening meal might protect against HCC, particularly among patients with a normal α-fetoprotein level and who are not obese, although these relations might be accounted for other factors, including total energy intake. Further studies with larger study sizes are needed to corroborate these findings.

Key words: case-control study, hepatitis C virus, hepatocellular carcinoma, late evening meal, risk factor

INTRODUCTION

PROTEIN–ENERGY MALNUTRITION is often observed in patients with advanced liver cirrhosis because of nutritional and metabolic abnormalities.[1–3] Several previous papers suggested that protein–energy malnutrition is significantly associated with the development of life-threatening complications and increased mortality.[4–7] In particular, nocturnal starvation in those with liver cirrhosis seems to be an important problem because a severe catabolic state is present overnight.[8] One study showed that nocturnal starvation might be a potential risk factor for the aggravation of liver disease.[9]

To improve nocturnal starvation, current guidelines recommend late evening snacks for patients with cirrhosis,[1,10] and therefore, the administration of branched chain amino acids (BCAA) or divided meal, partly consumed as a late evening snack, is now often prescribed. Previous studies have consistently demonstrated that BCAA administration corrects malnutrition in patients with cirrhosis.[11,12] Administration before bedtime seems to be most effective in terms of nutritional metabolism.[13–15] Recent studies have also suggested that BCAA might decrease mortality among patients with liver cirrhosis.[16] Before BCAA prescription for patients with cirrhosis became popular, carbohydrate-rich snacks were considered as a late evening snack. Carbohydrate-rich snacks also improve nitrogen balance and abnormal fuel metabolism in patients with cirrhosis.[8,17–19]

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