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ORIGINAL ARTICLE

Assessment of ISGLS Definition of Posthepatectomy Liver Failure and Its Effect on Outcome in Patients with Hepatocellular Carcinoma

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Abstract
Background Posthepatectomy liver failure (PHLF) is a major complication after hepatectomy. As there was no standardized definition, the International Study Group of Liver Surgery (ISGLS) defined PHLF as increased international normalized ratio and hyperbilirubinemia on or after postoperative day 5 in 2010. We evaluated the impact of the ISGLS definition of PHLF on hepatocellular carcinoma (HCC) patients.
Methods We retrospectively analyzed 210 consecutive HCC patients who underwent curative hepatectomy at our facility from 2005 to 2010. The median follow-up period after hepatectomy was 35.2 months.
Results Thirty-nine (18.6 %) patients fulfilled the ISGLS definition of PHLF. Overall survival (OS) rates at 1, 3, and 5 years in patients with/without PHLF were 69.1/93.5, 45.1/72.5, and 45.1/57.8 %, respectively ($P=0.002$). Recurrence-free survival (RFS) rates at 1, 3, and 5 years in patients with/without PHLF were 40.9/65.9, 15.7/38.3, and 15.7/20.3 %, respectively ($P=0.003$). Multivariate analysis revealed that PHLF was significantly associated with both OS ($P=0.047$) and RFS ($P=0.019$). Extent of resection ($P<0.001$), intraoperative blood loss ($P=0.002$), and fibrosis stage ($P=0.040$) were identified as independent risk factors for developing PHLF.
Conclusion The ISGLS definition of PHLF was associated with OS and RFS in HCC patients, and long-term survival will be improved by reducing the incidence of PHLF.

Keywords International Study Group of Liver Surgery · Posthepatecomy liver failure · Hepatocellular carcinoma · Hepatectomy · Survival

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths in the world.[1] Hepatectomy, which can lead to long-term survival in patients with HCC, is widely accepted as the best treatment option for advanced HCC.[2, 3] Although surgical techniques and perioperative management have greatly improved mortality rates after hepatectomy in recent years,[4, 5] morbidity rates still remain high.[6–12]

Among several complications that can occur after surgery, posthepatectomy liver failure (PHLF) is one of the most common causes of hepatectomy-related mortality. The incidence of PHLF has been reported to vary from 4 to 19 %; this wide range can be attributed to differences in patient populations and the procedures performed[6–8, 10–12] as well as the lack of a universally accepted definition. Some studies have demonstrated that postoperative complications affect long-term survival after hepatectomy in patients with HCC,[7, 9–11] but as the definitions for postoperative complications differ, there are no widely accepted risk factors or methodologies to control these events. To standardize the definition of PHLF, the International Study Group of Liver Surgery (ISGLS) proposed a definition for PHLF based on increased international

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normalized ratio (INR) and hyperbilirubinemia on or after postoperative day 5, together with a grading system of severity considering the impact on patients' clinical management in 2010.[13] This definition was subsequently confirmed in 2011.[14] In the validation study, all patients who underwent liver resection were included in an analysis and accordingly the patients had several different liver diseases. However, as liver status is influenced by underlying disease, which may affect the incidence of PHLF itself, we considered it relevant to investigate PHLF according to underlying disease.

In the present study, we focused on patients with HCC and assessed the ISGLS definition of PHLF and the impact of PHLF on long-term prognosis and identified risk factors in this patient population.

Methods

Patients and Preoperative Factors

From January 2005 to December 2010, 279 consecutive patients with HCC who underwent an initial hepatectomy at Kobe University Hospital were included in this study. Sixty-nine patients were excluded from the study (patients with reductive hepatectomy, [15, 16] $n=43$; patients with a macroscopically positive resection margin, $n=17$; patients with distant metastasis, $n=9$). The remaining 210 patients with complete gross resection were retrospectively analyzed.

Demographic data were collected prior to surgery and included age, gender, performance status according to the Eastern Cooperative Oncology Group (ECOG),[17] body mass index (BMI), alcohol consumption, and presence of diabetes mellitus. For the evaluation of preoperative liver function, all patients had several blood tests before hepatectomy, including hepatitis B surface antigen (HBs-Ag), hepatitis C virus antibody (HCV-Ab), complete blood count, total bilirubin (normal range, 0.3–1.0 mg/dl), albumin (normal range, 4.1–5.0 g/dl), prothrombin time (normal range, 79–130 %), and retention rate of indocyanine green at 15 min ($ICGR_{15}$; normal range, ≤10 %). For the evaluation of HCC and fibrosis, abdominal ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) were applied. Preoperative upper gastrointestinal endoscopy was performed routinely to assess esophageal varices. Portal hypertension was defined as the presence of esophageal varices and/or a platelet count of $<10\times10^4$/ml in association with splenomegaly.[18] Splenomegaly was defined as length of spleen of more than 10 cm as estimated by preoperative CT.[19]

Surgical Procedures

Selection criteria for hepatectomy included the extent of comorbidity, Child-Pugh status A or B, and remnant liver volume of more than 35 % of the whole liver. For further analysis, hepatectomy was categorized into two groups: major ($n=106$) and minor ($n=104$) resection. Major resections consisted of three extended right hepatectomies (1.4 %), 36 right hepatectomies (17.1 %), ten extended left hepatectomies (4.8 %), seven left hepatectomies (3.3 %), four central resections (1.9 %), and 46 sectionectomies (21.9 %). Minor resections consisted of 104 segmentectomies or less (49.5 %). For the analysis of the incidence of ISGLS definition of PHLF categorized by extent of hepatectomy and fibrosis stage, hepatectomy was additionally categorized into three groups: segments<2 (including segmentectomy or less), 2≤segments≤3 (including left hepatectomy, central resection and sectionectomy), and segments>3 (including extended right hepatectomy, right hepatectomy and extended left hepatectomy). Blood transfusions were defined as transfusions of concentrated red blood cells, fresh-frozen plasma, or platelets but excluded albumin.

Pathologic Evaluation

For all patients, a final diagnosis of HCC was pathologically confirmed after hepatectomy using surgically removed liver

Table 1 Baseline characteristics

	Total ($n=210$)
Preoperative factors	
Age (>65/≤65 years)	132/78
Gender (male/female)	175/35
Performance status (0/1)	206/4
BMI (>22/≤22 kg/m^2)	134/76
Alcohol consumption	42
Diabetes	56
Etiology (B/C/NBNC)	41/102/69
Total bilirubin (>1/≤1 mg/dl)	44/166
Albumin (≥4.1/<4.1 g/dl)	66/144
Prothrombin time (≥79/<79 %)	182/28
$ICGR_{15}$ (>10/≤10 %)	158/52
Portal hypertension	32
Child-Pugh A/B	200/10
Intraoperative factors	
Procedures (major/minor resection)	106/104
Blood loss (>1,000/≤1,000 ml)	66/144
Blood transfusion	88
Pathologic factors	
UICC stage (I/II/IIIA/IIIB/IIIC/IVA)	84/69/24/18/13/2
Fibrosis stage (0/1/2/3/4/NA)	5/25/70/45/61/4

BMI body mass index, *B* hepatitis B surface antigen positive, *C* hepatitis C virus antibody positive, *NBNC* negative for both HBs-Ag and HCV-Ab, $ICGR_{15}$ indocyanine green retention rate at 15 min, *UICC* International Union Against Cancer, *NA* not available

tissue. Tumor stage was assessed using the seventh edition of the International Union Against Cancer (UICC) classification.[20] The degree of hepatic fibrosis was assessed by a single pathologist using the METAVIR scoring system: F0, absent; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa; and F4, cirrhosis.[21] In four patients, hepatic fibrosis stage was not available because of the limited size of the resected livers.

Postoperative Morbidity and Mortality

Overall morbidity was defined as postoperative complications which occurred during hospital stay after hepatectomy. PHLF was diagnosed based on the ISGLS definition.[13] In brief, the ISGLS definition of PHLF is increased INR and hyperbilirubinemia on or after postoperative day 5. PHLF is further categorized into three grades of severity: grade A, PHLF resulting in abnormal laboratory parameters but requiring no change in the clinical management of the patient; grade B, PHLF resulting in a deviation from the regular clinical management but manageable without invasive treatment; and grade C, PHLF resulting in a deviation from regular clinical management and requiring invasive treatment. Data on serum bilirubin levels and INR on or after postoperative day 5 were available for a total of 210 patients as blood tests were routinely performed on postoperative days 1, 3, 5, and 7 at least and thereafter based on patient condition. Therefore, the ISGLS definition was applied in 100 % of our patients. Other postoperative complications, excluding ISGLS definition of PHLF, were defined as events which deviated from the normal postoperative course and which required pharmacological, surgical, endoscopic, or radiological interventions. The surgical complication of coagulopathy (INR>1.5) was treated by the administration of FFP or vitamin K, while encephalopathy was treated with enteral lactulose and

Table 2 Postoperative complications (n=210)

	No. (%)
Overall morbidity	71 (33.8)
PHLF (ISGLS definition)	39 (18.6)
Grade A	10 (4.8)
Grade B	26 (12.4)
Grade C	3 (1.4)
Morbidity (excluding ISGLS definition of PHLF)	51 (24.3)
Pleural effusion	16 (7.6)
Wound infection	12 (5.7)
Biliary leakage	11 (5.2)
Sepsis	10 (4.8)
Cardiac arrhythmia	7 (3.3)
Intra-abdominal abscess	4 (1.9)
Cholangitis	3 (1.4)
Gastrointestinal bleeding	3 (1.4)
Pneumonia/atelectasis	3 (1.4)
Intra-abdominal bleeding	2 (1.0)
Cardiac failure	2 (1.0)
Renal failure	1 (0.5)
Ileus	1 (0.5)
Stroke	1 (0.5)
Urinary tract infection	1 (0.5)
Other	3 (1.4)
Clavien–Dindo classification	
Grade I or II	47 (22.3)
Grade IIIa	18 (8.6)
Grade IIIb	2 (1.0)
Grade IVa	1 (0.5)
Grade IVb	0 (0.0)
Grade V	3 (1.4)

Patients may have experienced more than one complication

ISGLS International Study Group of Liver Surgery, *PHLF* posthepatectomy liver failure

Table 3 Patient characteristics categorized by the presence or absence of PHLF

	No PHLF (n=171)	PHLF (n=39)	P value
Preoperative factors			
Age >65 years	113 (66.1)	19 (48.7)	0.043
Gender, male	138 (80.7)	37 (94.9)	0.032
Performance status 1	3 (1.8)	1 (2.6)	0.563
BMI >22 kg/m^2	111 (64.9)	23 (59.0)	0.486
Alcohol consumption	34 (20.0)	8 (20.5)	0.943
Diabetes	47 (27.5)	9 (23.1)	0.574
Etiology B/C	115 (67.3)	26 (66.7)	0.944
Total bilirubin >1 mg/dl	27 (15.8)	17 (43.6)	<0.001
Albumin <4.1 g/dl	114 (66.7)	30 (76.9)	0.213
Prothrombin time <79 %	20 (11.7)	8 (20.5)	0.144
$ICGR_{15}$ >10 %	128 (74.9)	30 (76.9)	0.787
Portal hypertension	21 (12.3)	11 (28.2)	0.013
Child-Pugh B	8 (4.7)	2 (5.1)	1.000
Intraoperative factors			
Major hepatectomy	73 (42.7)	33 (84.6)	<0.001
Blood loss >1,000 ml	42 (24.6)	24 (61.5)	<0.001
Blood transfusion	62 (36.3)	26 (66.7)	<0.001
Pathologic factors			
UICC stage≥III	35 (20.5)	22 (56.4)	<0.001
Fibrosis stage ≥3	86 (50.3)	20 (51.3)	0.873

Percentages are in parentheses

PHLF posthepatectomy liver failure by the definition of ISGLS, *BMI* body mass index, *B/C* hepatitis B surface antigen positive and/or hepatitis C virus antibody positive, *$ICGR_{15}$* indocyanine green retention rate at 15 min, *UICC* International Union Against Cancer

Table 4 Perioperative outcomes categorized by PHLF grade

	No PHLF (n=171)	Grade A (n=10)	Grade B (n=26)	Grade C (n=3)	P value[a]
Hospital stay, median (range), days	15 (9–121)	17 (12–24)	23 (14–98)	55 (50–92)	<0.001
In-hospital mortality[b]	0 (0.0)	0 (0.0)	1 (3.8)	2 (66.7)	<0.001
30-day mortality[b]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NC
90-day mortality[b]	0 (0.0)	0 (0.0)	2 (7.7)	2 (66.7)	<0.001
Complications excluding PHLF[b]	32 (18.7)	1 (10.0)	15 (57.7)	3 (100.0)	<0.001

PHLF posthepatectomy liver failure by the ISGLS definition, *NC* not calculated

[a] All P values for comparison between groups

[b] Values in parentheses are percentages unless indicated otherwise

branched-chain amino acids. Diuretics and albumin were used to treat massive ascites. Infectious complications were treated with antibiotics on the basis of the culture and sensitivity test results. Overall morbidities were additionally classified according to the Clavien–Dindo classification.[22] Mortality was defined as death during hospital stay after hepatectomy.

Follow-up

After discharge, all patients were followed at the outpatient clinic with laboratory tests and CT or MRI performed every 3 to 6 months for the first 5 years and thereafter at least once every 12 months until death. Recurrence of HCC was defined as the appearance of a new lesion with the radiologic features of HCC. Overall survival (OS) was defined as the interval between hepatectomy and death or the last follow-up. Recurrence-free survival (RFS) was defined as the interval from the date of resection until detection of tumor recurrence, death, or last follow-up. For patients who survived during our analysis, the date of last follow-up was set as January 31, 2012. Two patients (1.0 %) were lost to follow-up.

Statistical Analysis

Continuous data were expressed as the median and range. Differences between patients' characteristics were compared by χ^2 or Fischer's exact tests. PHLF grades were compared using χ^2 and Kruskal–Wallis tests. OS and RFS were estimated by Kaplan–Meier method, and differences in the survival curves were analyzed by log-rank test. Univariate and multivariate Cox proportional hazard regression models were performed to identify independent prognostic factors. Multivariate logistic regression analysis was used to identify independent risk factors. In this study, all factors analyzed in the univariate analysis were included in the subsequent multivariate analysis. Statistical significance was considered when the two-sided P value was <0.05. All statistical analyses were performed using JMP version 9.0 (SAS Institute Inc, Cary, NC, USA) software.

Results

Patient Characteristics and Operative Results

The characteristics of all patients are shown in Table 1. The patient population consisted of 175 men and 35 women, with a median age of 69 years (range, 32–87 years). Postoperative complications are summarized in Table 2. A total of 119 complications were observed in 71 patients (33.8 %). The most common complication was ISGLS definition of PHLF which occurred in 39 patients (18.6 %), followed by pleural effusion in 16 patients (7.6 %) and wound infection in 12

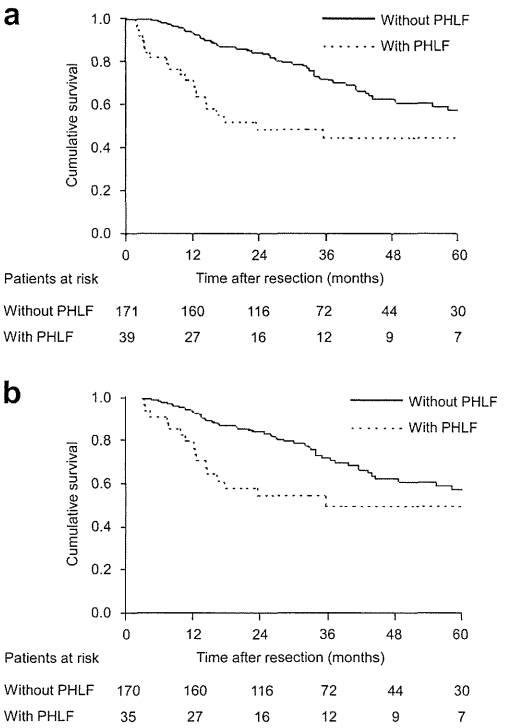

Fig. 1 Kaplan–Meier curves comparing overall survival of patients without and with posthepatectomy liver failure: **a** all patients (P=0.002), **b** excluding patients who died or were lost to follow-up within 90 days (P=0.043)

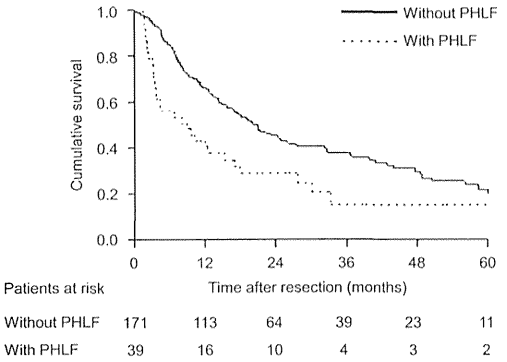

Fig. 2 Kaplan–Meier curves comparing recurrence-free survival of patients without and with posthepatectomy liver failure ($P=0.003$)

patients (5.7 %). The median hospital stay was 16 days (range, 9–121 days).

Patient characteristics according to the presence or absence of ISGLS definition of PHLF are summarized in Table 3. Factors associated with PHLF were older age, male sex, advanced stage HCC, higher preoperative bilirubin levels, pre-existing portal hypertension, and major resection with greater blood loss and transfusion requirements ($P<0.05$).

Perioperative Outcomes Categorized by PHLF Grade

Based on the ISGLS of PHLF, ten (4.8 %), 26 (12.4 %), and three (1.4 %) patients were classified as grade A, B, and C, respectively. We identified a mortality rate of 1.4 % ($n=3$) in our study. All patients who died fulfilled the PHLF criteria (grade B, $n=1$; grade C, $n=2$). Table 4 shows clinical outcomes categorized by PHLF grade. Median hospital stay was 15 days for patients without PHLF, which was significantly shorter than in those with PHLF (grade A, 17 days; grade B, 23 days; grade C, 55 days; $P<0.001$). Although ISGLS definition of PHLF was the most common complication, we also documented an additional 80 complications other than PHLF, the association with PHLF of which was also analyzed (Table 4). The incidence of these complications increased with PHLF grade ($P<0.001$).

Long-Term Prognosis for Patients with PHLF

The median follow-up period after hepatectomy was 35.2 months (range, 1.7–80.7 months). Figure 1a shows OS categorized by the presence or absence of ISGLS definition of PHLF. OS rates at 1, 3, and 5 years in patients without PHLF were 93.5, 72.5, and 57.8 %, while OS rates in patients with PHLF were 69.1, 45.1, and 45.1 %, respectively ($P=0.002$). There were four patient deaths, and one patient was lost to follow-up within 90 days after hepatectomy. OS was estimated excluding these five patients: the 1-, 3-, and 5-year OS rates in patients without PHLF were equal to the values obtained including these patients, and they were 77.0, 50.2, and 50.2 % in those with PHLF ($P=0.043$; Fig. 1b). RFS rates at 1, 3, and

Table 5 Univariate analysis of prognostic factors for overall and recurrence-free survival

	Overall survival		Recurrence-free survival	
	HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Age >65 years	1.53 (0.95–2.55)	0.079	1.32 (0.94–1.88)	0.117
Gender, male	0.96 (0.56–1.76)	0.892	0.83 (0.56–1.28)	0.380
Performance status 1	3.46 (0.57–11.21)	0.149	1.46 (0.24–4.63)	0.618
BMI >22 kg/m^2	0.71 (0.45–1.13)	0.144	0.94 (0.67–1.34)	0.738
Alcohol consumption	0.96 (0.53–1.65)	0.900	0.97 (0.63–1.44)	0.876
Diabetes	1.35 (0.83–2.16)	0.225	0.94 (0.64–1.35)	0.758
Etiology B/C	0.65 (0.41–1.05)	0.078	0.99 (0.70–1.42)	0.953
Total bilirubin >1 mg/dl	1.32 (0.76–2.17)	0.309	1.44 (0.97–2.08)	0.070
Albumin <4.1 g/dl	1.48 (0.88–2.62)	0.142	1.32 (0.92–1.93)	0.132
Prothrombin time <79 %	0.86 (0.40–1.64)	0.675	1.22 (0.75–1.88)	0.405
$ICGR_{15}$ >10 %	1.02 (0.62–1.76)	0.946	1.26 (0.86–1.90)	0.243
Portal hypertension	1.26 (0.66–2.21)	0.461	1.06 (0.66–1.64)	0.800
Child-Pugh B	1.06 (0.32–2.55)	0.918	1.02 (0.43–2.02)	0.967
Major hepatectomy	1.24 (0.79–1.94)	0.355	1.10 (0.79–1.52)	0.589
Blood loss >1,000 ml	1.46 (0.91–2.32)	0.117	0.90 (0.62–1.29)	0.576
Blood transfusion	1.99 (1.26–3.16)	0.004	1.23 (0.88–1.71)	0.218
UICC stage≥III	3.84 (2.39–6.11)	<0.001	2.46 (1.70–3.50)	<0.001
Fibrosis stage ≥3	0.97 (0.61–1.55)	0.898	1.02 (0.74–1.43)	0.891
PHLF	2.17 (1.27–3.56)	0.006	1.83 (1.20–2.70)	0.006
Complications excluding PHLF	2.44 (1.46–3.96)	0.001	1.47 (1.00–2.12)	0.048

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *B/C* hepatitis B surface antigen positive and/or hepatitis C virus antibody positive, *$ICGR_{15}$* indocyanine green retention rate at 15 min, *UICC* International Union Against Cancer, *PHLF* posthepatectomy liver failure by the definition of ISGLS

5 years in patients without PHLF were 65.9, 38.3, and 20.3 %, while RFS rates in patients with PHLF were 40.9, 15.7, and 15.7 %, respectively ($P=0.003$; Fig. 2).

As the association between ISGLS definition of PHLF and OS was newly identified, we performed univariate (Table 5) and multivariate (Table 6) Cox regression analyses to investigate other prognostic factors for OS. In the univariate analysis, UICC stage≥III, blood transfusions, PHLF, and complications other than PHLF were identified as significant prognostic factors for OS. In the multivariate analysis, however, age >65 years, UICC stage≥III, and PHLF were identified as independent prognostic factors. For RFS, univariate analysis revealed that UICC stage≥III, PHLF, and complications other than PHLF were significant prognostic factors (Table 5). Multivariate analysis revealed that UICC stage≥III and PHLF were independent prognostic factors for RFS (Table 6).

Risk Factors for Developing PHLF

As ISGLS definition of PHLF was identified as an independent prognostic factor for OS and RFS, we performed a multivariate logistic regression analysis to search for risk factors for developing PHLF. Among the prognostic factors tested, major hepatectomy, intraoperative blood loss >1,000 ml, and fibrosis stage ≥3 were identified as independent risk factors for developing PHLF (Table 7). As the extent of resection and fibrosis stage are factors which can be accessed before surgery, the incidence of PHLF categorized by these two factors was analyzed (Table 8). The incidence of PHLF increased in association with both the extent of resection and fibrosis stage.

Discussion

Posthepatectomy liver failure is a major prognostic factor for patients who have undergone hepatectomy. In 2010, the ISGLS defined PHLF as an increase in INR and concomitant hyperbilirubinemia on or after postoperative day 5.[13] In this study, we assessed the definition limited solely with HCC patients and revealed that perioperative morbidity and mortality increased with higher grades of PHLF. OS and RFS associated with the incidence of PHLF, and the extent of resection, intraoperative blood loss, and fibrosis stage were identified as independent risk factors for developing PHLF.

Using the ISGLS definition of PHLF, the first validation study was performed in 2011 with all the patients who underwent liver resection for several different liver diseases by the group who identified the definition.[14] Eleven percent of patients was reported to fulfill the ISGLS definition, and the mortality rate was 4.3 %. By contrast, the definition was met by 19 % of our study population, and the mortality rate was only 1.4 %. We speculate that these differences may have arisen from background differences in patient liver status. In the original validation study, patients mainly had metastatic disease, and accordingly 71 % of patients did not have hepatic fibrosis. In contrast, all of our patients had HCC, and accordingly half of them had a fibrosis stage of 3 or 4. This preoperative difference in hepatic fibrotic status also led to differences in the procedures performed: 80 % of their patients underwent major resection (resection of two or more anatomical segments), while 50 % of our patients underwent minor resection (resection of fewer than two segments). Despite the higher incidence and lower mortality, the ISGLS definition of

Table 6 Independent prognostic factors for overall and recurrence-free survival

	HR (95 % CI)	*P* value
Overall survival		
Age >65 years	1.88 (1.03–3.55)	0.041
UICC stage≥III	4.80 (2.58–8.98)	<0.001
PHLF	2.17 (1.01–4.59)	0.047
Recurrence-free survival		
UICC stage≥III	2.83 (1.77–4.51)	<0.001
PHLF	1.98 (1.12–3.46)	0.019

HR hazard ratio, *CI* confidence interval, *UICC* International Union Against Cancer, *PHLF* posthepatectomy liver failure by the definition of ISGLS

Table 7 Independent risk factors for PHLF

	OR (95 % CI)	*P* value
Major hepatectomy	12.21 (3.14–59.77)	<0.001
Blood loss >1000 ml	6.57 (2.02–24.60)	0.002
Fibrosis stage ≥3	3.49 (1.06–12.58)	0.040

OR odds ratio, *CI* confidence interval, *PHLF* posthepatectomy liver failure by the definition of ISGLS

Table 8 Incidence of PHLF categorized by fibrosis stage and extent of resection

	Fibrosis stage 0, 1, 2	Fibrosis stage 3, 4
Segment <2[a]	3.3 % (1/30)	7.0 % (5/71)
2≤Segment ≤3[b]	10.8 % (4/37)	26.3 % (5/19)
Segment >3[c]	39.4 % (13/33)	62.5 % (10/16)

PHLF posthepatectomy liver failure by the definition of ISGLS

[a] Segment <2 includes 101 segmentectomies or less

[b] 2≤segment ≤3 includes seven left hepatectomies, four central resections, and 45 sectionectomies

[c] Segment >3 includes three extended right hepatectomies, 36 right hepatectomies, and ten extended left hepatectomies

PHLF successfully associated with perioperative morbidity and mortality with HCC patients, thus proving the feasibility to use the definition with HCC patients.

As our findings validated the use of the ISGLS definition of PHLF in patients with HCC, we next analyzed the impact of this definition on long-term survival and clearly proved statistical associations with OS (Fig. 1a) and RFS (Fig. 2). To elucidate the impact of PHLF on long-term survival, OS excluding the patients who died or were lost to follow-up within 90 days was also estimated. A statistical difference by the presence or absence of PHLF was observed, suggesting that PHLF affects long-term survival even after the patients have recovered from liver failure (Fig. 1b). Multivariate Cox regression analysis indicated that age, UICC stage, and PHLF were independent prognostic factors for OS, and UICC stage and PHLF were independent prognostic factors for RFS (Table 6). Moreover, it is interesting to note that the occurrence of postoperative complications, excluding ISGLS definition of PHLF, was not identified as an independent predictor for poorer OS or RFS. Previous reports show that complications affect long-term survival after hepatectomy in HCC patients.[7, 9–11] Since our results show that ISGLS definition of PHLF affects long-term survival whereas complications other than PHLF do not, it should be emphasized that ISGLS definition of PHLF is a stronger predictor of prognosis than any other types of complication.

In this study, ISGLS definition of PHLF affected both OS and RFS. We speculate that PHLF-affected patients had less functional liver reserve, which limited additional treatments when recurrence was detected. However, there is no clear reason for the observed correlation between PHLF and poorer RFS. One possible explanation might be up-regulation of cytokines after liver injury.[23] Among these cytokines, hepatocyte growth factor (HGF), which is a key component for liver regeneration,[24] is implicated in the development and progression of HCC[25] and is associated with a poorer prognosis after hepatectomy.[26] Since we did not measure HGF serum levels in our study, the validity of this suggestion would be proved as future study.

As an association between ISGLS definition of PHLF and long-term prognosis was demonstrated, we finally investigated risk factors for the development of PHLF. Multivariate logistic regression analysis indicated that the extent of hepatectomy, intraoperative blood loss, and fibrosis stage were independent risk factors for developing PHLF (Table 7). The result additionally verified the feasibility of PHLF as previous studies have already demonstrated the direct relation between PHLF and extent of surgery, blood loss, and degree of fibrosis.[27] One criticism to the definition of PHLF might be that as PHLF is a postoperative event which can be identified 5 days after hepatectomy, the clinical value as a prognostic factor is comparatively low. However, by identifying the risk factors, HCC patients for hepatectomy can now be stratified based on the extent of resection and background fibrosis stage, which can be accessed before surgery (Table 8). For patients with fibrosis stage 0–2, hepatectomy can be performed with a lower risk of developing ISGLS definition of PHLF. However for patients with fibrosis stage 3 or 4, it is likely that more than 50 % of patients will develop PHLF after right hepatectomy, which is associated with poorer OS and RFS. Accordingly, the extent of hepatectomy should be decided only after a careful discussion with the patient about benefits and risk. As fibrosis stage was assessed from the resected liver in the study, prospective analysis of methods to assess fibrosis stage before surgery is in progress.

One limitation of this study is its single-center retrospective design. The other limitation is that almost half of the patients underwent minor resection. This patient population might lower the incidence of PHLF, which eventually decreases the statistical power. Our data must be confirmed with a larger population in the future.

In conclusion, we have demonstrated for the first time the validity of the ISGLS definition of PHLF, its association with OS and RFS, and the risk factors for developing PHLF in patients with HCC. Long-term survival in HCC patients will be improved by reducing the incidence of PHLF, which can be achieved by selecting patients for hepatectomy based on the extent of resection required and fibrosis stage.

Disclosures All authors listed on this manuscript have no financial disclosure.

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Hepatology Research

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Original Article

Utility of real-time shear wave elastography for assessing liver fibrosis in patients with chronic hepatitis C infection without cirrhosis: Comparison of liver fibrosis indices

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Aim: To clarify the diagnostic impact of liver fibrosis except for cirrhosis identified using shear wave elastography (SWE) in chronic hepatitis C (CHC) patients, and to compare the performance in diagnosing liver fibrosis among SWE and liver fibrosis indices.

Methods: A total of 55 CHC patients who underwent liver biopsy were analyzed. The diagnostic performance for identifying significant liver fibrosis (F2–F3) for SWE, FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI) and Forns' index was assessed using receiver–operator curve (ROC) analysis.

Results: The median SWE elasticity value, FIB-4 index, APRI and Forns' index in the F0–F1 and F2–F3 groups were 6.3 kPa and 13.1 kPa; 1.52 and 4.45; 0.41 and 1.43; and 7.69 and 8.85, respectively ($P < 0.001$ for all four methods). Multivariate analysis showed that SWE was independently associated with the presence of significant liver fibrosis (odds ratio, 2.52; 95% confidence interval, 1.49–4.28; $P < 0.001$). The area under the ROC curve for SWE in diagnosing significant liver fibrosis was 0.94, indicating high diagnostic value, compared with 0.86, 0.88 and 0.83, for the FIB-4 index, APRI and Forns' index, respectively, which corresponds to moderate diagnostic value. The accuracy of SWE, FIB-4 index, APRI and Forns' index for diagnosing significant liver fibrosis was 90.9%, 76.4%, 74.5% and 67.2%, respectively.

Conclusion: SWE has excellent ability for diagnosing significant liver fibrosis in CHC even when patients with cirrhosis are excluded. The diagnostic performance of SWE is superior to that of three liver fibrosis indices.

Key words: hepatitis C, liver fibrosis, liver fibrosis index, shear wave elastography

INTRODUCTION

PERSISTENT HEPATITIS C VIRUS (HCV) infection induces chronic hepatitis, which eventually develops into liver cirrhosis and hepatocellular carcinoma (HCC).[1] Advanced liver fibrosis in chronic hepatitis C (CHC) is associated with HCC development and complications such as esophageal variceal bleeding and liver failure.[2,3] Interferon (IFN)-based therapy has been used to treat patients with CHC. Many investigators have reported that IFN-based treatment is effective in reducing serum levels of alanine aminotransferase (ALT), eliminating circulating HCV RNA and decreasing liver fibrosis in CHC patients.[4–8] However, IFN-based therapy is associated with several severe treatment-related side-effects. Recently, direct-acting antivirals (DAA) have promised to open a new era of chronic HCV infection treatment with increased sustained virological response rates, shorter and simpler regimens, and minimal treatment-related side-effects. In Japan, the combination of daclatasvir and asunaprevir was approved as the first DAA therapy without IFN for patients with HCV genotype 1 infection in July 2014.[9,10] Other new DAA regimens without IFN for patients with HCV infection are being developed in succession. However, limitations in the workforce and societal resources may limit the feasibility of treating all patients within a short period of time. Advanced liver fibrosis in the context of CHC has one of the highest priorities for DAA treatment.[11] Thus, it is important to evaluate the degree of liver fibrosis in patients with HCV infection except for those with apparent

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cirrhosis. Liver biopsy is still considered the gold standard for the evaluation of liver fibrosis even though it is painful, costly and associated with limitations in diagnostic utility and accuracy. Moreover, because the invasiveness of liver biopsy precludes repeated examinations,[12] longitudinal evaluation of liver fibrosis is difficult.

Recently, various liver fibrosis indices based on clinical and biological data have been reported to be useful predictors of fibrosis in liver disease.[13–17] Additionally, various non-invasive imaging methods for evaluating liver fibrosis have rapidly improved as alternatives to liver biopsy. Liver fibrosis has been predicted by transient elastography (TE).[18,19] Ultrasound elastography provides objective data on tissue elasticity by representing the stiffness of tissue using grayscale or color images.[20,21] Real-time shear wave elastography (SWE) was developed on this basis; it has attracted increasing attention because it can rapidly, non-invasively, objectively and quantitatively detect the degree of fibrosis in patients with liver disease, including CHC liver disease.[21] Representative reports of non-invasive imaging methods such as SWE for evaluating liver fibrosis, however, these have included patients with cirrhosis that have high elasticity values (e.g. receiver–operator curve [ROC] analysis for fibrosis stages F0–F1 vs F2–F4 or F0–F2 vs F3–F4).[22,23]

In the present study, we clarified the diagnostic impact of SWE in CHC patients with liver fibrosis that has not reached the point of cirrhosis. In particular, we analyzed the accuracy of SWE for distinguishing between non-significant (F0–F1) and significant (F2–F3) liver fibrosis. In addition, we compared its ability to diagnose liver fibrosis with various liver fibrosis indices.

METHODS

Patients

BETWEEN DECEMBER 2012 and July 2014, liver stiffness was evaluated using SWE in 744 consecutive patients at our institution. Of these 744 patients, 67 met the following eligibility criteria: (i) detectable serum HCV RNA using a polymerase chain reaction (PCR) assay; (ii) liver biopsy was performed; (iii) liver fibrosis stage between F0 and F3; (iv) SWE evaluation performed within 2 days of liver biopsy; (v) exclusion of other causes of chronic liver disease (co-infection with hepatitis B virus, alcohol consumption of >80 g/day, severe fatty liver, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis or Wilson's disease) and hyperlipidemia on drug therapy. We excluded 12 patients due to insufficient SWE evaluation (e.g. breath-holding not possible, post-hepatectomy or severe obesity). Consequently, 55 patients were analyzed in this study (Figure 1).

The study protocol was in compliance with the Declaration of Helsinki and was approved by the institutional review board. Written informed consent was obtained from all patients for use of their laboratory data prior to the start of the study.

Clinical data and biological data

Patient age, sex, height and weight were recorded. Serum samples were collected within 1 month prior to liver biopsy. The following variables were obtained through serum sample analysis: aspartate aminotransferase (AST), ALT, γ-glutamyltransferase (γ-GT), total cholesterol and platelet count. Serum HCV RNA was measured with a real-time PCR assay (COBAS TaqMan HCV test; Roche Molecular Systems, Pleasanton, CA, USA; lower limit of detection, 1.2 $\log_{10}$ IU/mL).

Histological evaluation

All liver biopsy specimens were obtained percutaneously from the right lobe of liver using 17-G needles under ultrasound guidance. Specimens were fixed, embedded in paraffin, and stained with hematoxylin–eosin, Azan and Gitter stains. Two hepatologists with sufficient knowledge and experience in hepatic pathology (H. T. and T. K., with 23 and 37 years of experience, respectively) who were blinded to clinical and SWE data evaluated the liver biopsy samples. If there was a discrepancy, they discussed the results and reached a consensus. When length and number of portal tracts of liver biopsy specimens of less than 2.0 cm and five, the case was to be excluded from subsequent statistical analysis. Fibrosis was staged according to the METAVIR scoring system:[24] F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4 (excluded in this study), cirrhosis. Necroinflammatory activity was graded on a scale of 0–3: A0, no activity; A1, mild activity; A2, moderate activity; and A3, severe activity.

Liver fibrosis was considered significant when it was spread out in the portal tract (stage 2, 3 or 4). It was considered non-significant when it was absent or restricted to the portal tract (stage 0 or 1).[16]

Liver fibrosis indices

In the present study, we used the FIB-4 index, AST/platelet ratio index (APRI) and Forns' index as indices of liver fibrosis. These indices were previously reported to have utility in liver fibrosis diagnosis. The FIB-4 index was calculated as: AST (IU/L) × age (years) / platelet count (10^9/L) × ALT (IU/L)$^{1/2}$.[17] The APRI was calculated as:

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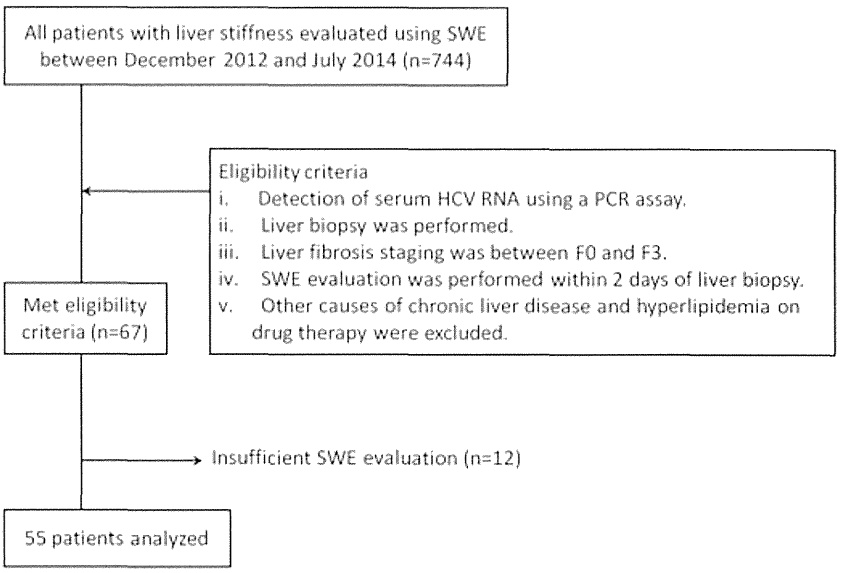

Figure 1 Patient selection criteria. HCV, hepatitis C virus; PCR, polymerase chain reaction; SWE, shear wave elastography.

(AST [IU/L] / upper limit of normal AST [IU/L]) × 100 / platelet count [10^9/L].[14] The Forns' index was calculated as: 7.811 − 3.131.ln (platelet count [10^9/L]) + 0.781.ln (γ-GT [IU/L]) + 3.467.ln (age) − 0.014 (cholesterol [mg/dL]).[15]

SWE

Shear wave elastography was performed using the Aixplorer ultrasound system (Super Sonic Imagine, Aix-en-Provence, France) and an SC6-1 convex array probe at a frequency of 6 MHz. Patients were in the supine position with their right upper extremity lifted. The detection site was fixed at 1.0–2.0 cm beneath the right liver capsule, away from the intrahepatic vessels and the gallbladder. When the elasticity imaging mode was selected, the patient held his or her breath for 3–5 s for imaging. When the target area was located, the operator initiated the SWE sequence measurements. After the color images were obtained, a circular quantitative sampling frame with a diameter of 10 mm was used to measure the elastic modulus of the liver in the region of interest (ROI). Next, the system automatically calculated the mean elastic modulus (in kPa) within the ROI. The mean of three consecutive measurements during a single examination session was used for statistical analysis.

Two sonologists from our institution (N.T. and Y.I., with 8 and 22 years of experience, respectively) performed the SWE examinations. They were blinded to the patient's histological and clinical data.

Statistical analysis

Continuous variables are expressed as medians (range). The Mann–Whitney *U*-test was used for continuous variables, and the χ^2-test with Yates' correction or Fisher's exact test was used for categorical variables. In the present study, we compared patients with and without a diagnosis of significant liver fibrosis. The diagnostic performance of SWE, FIB-4 index, APRI and Forns' index was assessed using ROC and the area under the ROC analysis. The area under the ROC was expressed as Az. Diagnostic value was classified as low (Az = 0.50–0.70), moderate (Az = 0.70–0.90) and high (Az = 0.90–1.0).[25] The paired Student's *t*-test was used to compare Az. Sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) were calculated using maximum (sensitivity + specificity − 1) as the cut-off level[26,27] in the ROC analysis. Multiple logistic regression analysis with the forward selection method was used to identify predictors of significant liver fibrosis.

Data analysis was performed using JMP statistical software version 10 (Windows version; SAS Institute, Cary, NC, USA). All *P*-values were derived from two-tailed tests, with $P < 0.05$ considered statistically significant.

RESULTS

Patients characteristics

THE CHARACTERISTICS OF all 55 patients are listed in Table 1. F0–F1 was diagnosed in 37 cases (67.3%) and F2–F3 in 18 (32.7%). There were no cases that were difficult to make a precise evaluation due to length and number of portal tracts of liver biopsy specimens.

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Table 1 Baseline characteristics of the study patients ($n = 55$)

Age (years)*	61.0 (24.0–78.0)
Sex (female/male)	32/23
BMI (kg/m^2)*	21.3 (16.9–31.6)
AST (IU/L)*	38 (10–262)
ALT (IU/L)*	42 (9–337)
γ-GT (IU/L)*	38 (10–195)
Total cholesterol (mg/dL)*	169 (91–248)
Platelet count (×10^3/μL)*	184 (89–738)
HCV RNA ($\log_{10}$ IU/mL)*	6.6 (1.2–7.8)
Fibrosis stage (F0/F1/F2/F3)	3/34/14/4
Histological activity (A0/A1/A2/A3)	1/37/15/2
Length of liver biopsy specimens (cm)*	4.5 (2.5–6.3)
No. of portal tracts of liver biopsy specimens*	13 (7–21)

*Data expressed as medians (range).
γ-GT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus.

Patient characteristics when dichotomized according to liver fibrosis stage F0–F1 versus F2–F3

The characteristics of the 55 patients dichotomized to F0–F1 (non-significant liver fibrosis) versus F2–F3 (significant liver fibrosis) are listed in Table 2. In the F2–F3 group, AST, ALT and γ-GT were significantly higher than in the F0–F1 group. Conversely, the platelet count in the F2–F3 group was significantly lower than in the F0–F1 group. In addition, the F2–F3 group had significantly higher necroinflammatory activity grades than the F0–F1 group.

SWE, FIB 4-index, APRI and Forns' index by liver fibrosis stage F0–F1 versus F2–F3

The median (range) SWE elasticity value, FIB-4 index, APRI and Forns' index in the F0–F1 and F2–F3 groups were 6.3 kPa (4.2–11.5) and 13.1 kPa (7.1–25.2); 1.52 (0.46–5.59) and 4.45 (1.75–7.70); 0.41 (0.14–2.35) and 1.43 (0.50–3.95); and 7.69 (6.21–9.20) and 8.85 (7.80–9.33), respectively (Fig. 2). For all four indices, the F2–F3 group scored significantly higher than the F0–F1 group ($P < 0.001$).

Factors associated with the presence of significant liver fibrosis

Multiple logistic regression analysis using the covariates of SWE, FIB-4 index, APRI and Forns' index showed that SWE was independently associated with the presence of significant liver fibrosis (odds ratio [OR], 2.52 for each 1 kPa increase in elasticity; 95% confidence interval (CI), 1.49–4.28; $P < 0.001$).

ROC analysis and diagnostic value

The ROC of all four indices for the diagnosis of significant liver fibrosis (F2 or F3) are shown in Figure 3. The Az value for SWE was greater than 0.9, indicating high diagnostic value. Conversely, the Az value for each of the three liver fibrosis indices was 0.8, indicating moderate diagnostic value. When comparing the Az values of these measures, the Az value of SWE was significantly higher than the Az value for the Forns' index ($P = 0.038$) (Table 3).

In addition, we analyzed the platelet counts as one of the simple markers for diagnosis of liver fibrosis.[28] The Az value

Table 2 Characteristics of patients dichotomized by liver fibrosis stage F0–F1 versus F2–F3

	F0–F1 group (n=37)	F2–F3 group (n=18)	p value
Age (years)*	60.0 (24.0–78.0)	64.5 (46.0–73.0)	0.323
Sex (female/male)	23/14	9/9	0.517
BMI (kg/m^2)*	21.3 (17.8–31.6)	21.3 (16.9–25.2)	0.943
AST (IU/L)*	33 (10–140)	79 (27–262)	<0.001
ALT (IU/L)*	24 (9–230)	58 (28–337)	<0.001
γ-GT (IU/L)*	26 (10–195)	63 (18–115)	<0.001
Total cholesterol (mg/dL)*	179 (91–248)	162 (143–203)	0.051
Platelet count (×10^3/μL)*	189 (94–738)	127 (89–280)	0.003
HCV RNA ($\log_{10}$ IU/mL)*	6.7 (1.2–7.8)	6.5 (4.8–7.5)	0.461
Histological activity (A0/A1/A2/A3)	1/30/6/0	0/7/9/2	0.004

*Data expressed as medians (range).
γ-GT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus.

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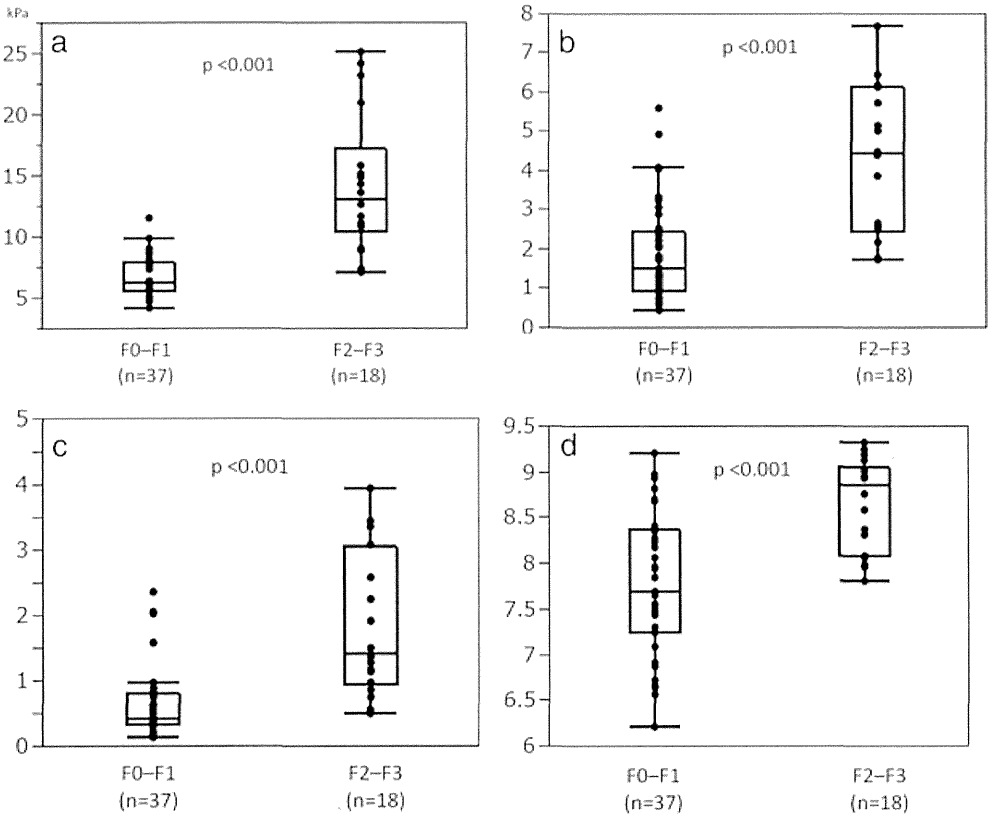

Figure 2 Correlation between the degree of liver fibrosis (F0–F1 vs F2–F3) and the four methods for assessing liver fibrosis. The bottom and top of each box represent the 25th and 75th percentiles, respectively (i.e. the interquartile range). The line though the box indicates the median. The error bars indicate minimum and maximum non-extreme values. (a) Correlation between the degree of liver fibrosis and SWE elasticity value. The F2–F3 group had a significantly higher SWE elasticity value than the F0–F1 group ($P < 0.001$). (b) Correlation between the degree of liver fibrosis and the FIB-4 index. The FIB-4 index in the F2–F3 group was significantly higher that in the F0–F1 group ($P < 0.001$). (c) Correlation between the degree of liver fibrosis and the APRI. The APRI in the F2–F3 group was significantly higher that in the F0–F1 group ($P < 0.001$). (d) Correlation between the degree of liver fibrosis and Forns' index. Forns' index in the F2–F3 group was significantly higher that in the F0–F1 group ($P < 0.001$). APRI, aspartate aminotransferase-to-platelet ratio index; SWE, shear wave elastography.

for platelet counts was 0.75 (95% CI, 0.58–0.87), and the Az value of SWE was significantly higher than the Az value of platelet counts ($P = 0.017$).

Performance of the four methods for diagnosing significant liver fibrosis

The sensitivity, specificity, accuracy, PPV and NPV for diagnosing significant liver fibrosis are shown in Table 4. The cut-off level for SWE, FIB-4 index, APRI and Forns' index according to the Youden index[26,27] was 8.8 kPa, 2.09, 0.49 and 7.69, respectively. Using these cut-off levels, the accuracy of SWE for diagnosing significant liver fibrosis was superior to the three liver fibrosis indices.

DISCUSSION

IN THE ROC curve analysis, the Az value for diagnosing significant liver fibrosis except for cirrhosis was over 0.9 for a high elasticity value as determined by SWE; this Az value is considered to indicate high diagnostic value. Conversely, the Az values for the FIB-4 index, APRI and Forns' index were 0.8, which is considered to indicate moderate diagnostic value. Additionally, there was a significant difference in Az values for diagnosing significant liver fibrosis between SWE and the Forns' index. Further, the Az value of SWE was significantly higher than the Az value for the platelet counts as one of the simple liver fibrosis markers.

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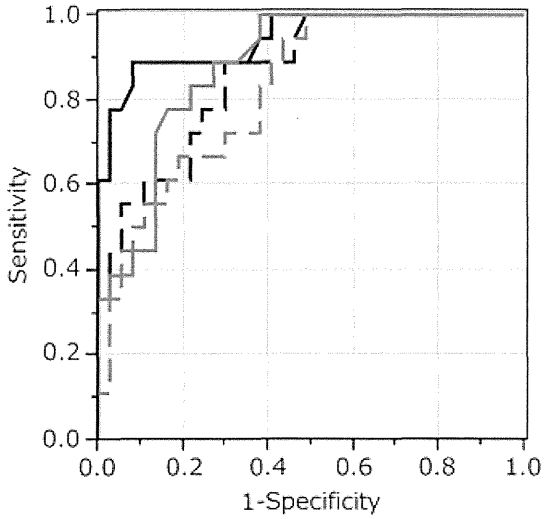

Figure 3 ROC curves for the four methods for diagnosing significant liver fibrosis (F2 or F3). (———) SWE, (- - - - -) FIB-4 index; (———) APRI, (- - - - -) Forns' index. APRI, aspartate aminotransferase-to-platelet ratio index; ROC, receiver-operator curve; SWE, shear wave elastography.

Table 3 Az values for SWE, FIB 4-index, APRI and Forns' index

			P-value, compared with:		
	Az value	95% CI	FIB 4-index	APRI	Forns' index
SWE	0.94	0.84–0.98	0.092	0.164	0.038
FIB-4 index	0.86	0.74–0.93		0.563	0.264
APRI	0.88	0.76–0.94			0.209
Forns' index	0.83	0.69–0.91			

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; SWE, shear wave elastography.

Table 4 Performance of significant liver fibrosis diagnosis

	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
SWE	8.8 kPa	88.9	91.9	90.9	84.2	94.4
FIB-4 index	2.09	88.9	70.3	76.4	59.3	92.9
APRI	0.49	100	62.2	74.5	56.3	100
Forns' index	7.69	100	51.4	67.2	50.0	100

APRI, aspartate aminotransferase-to-platelet ratio index; NPV, negative predictive value; PPV, positive predictive value; SWE, shear wave elastography.

Ferraioli *et al.*[23] assessed the accuracy of SWE versus TE in 121 patients with CHC using liver biopsy as the reference standard. They found that liver elasticity increased in parallel with the degree of liver fibrosis as determined by SWE and TE. In comparisons of F0–F1 versus F2–F4, F0–F2 versus F3–F4 and F0–F3 versus F4, the Az values were 0.92 for SWE and 0.84 for TE ($P=0.002$); 0.98 for SWE and 0.96 for TE ($P=0.14$); and 0.98 for SWE and 0.96 for TE ($P=0.480$), respectively. Therefore, SWE was more accurate than TE for assessing significant fibrosis (≥F2). Their study included all stages of liver fibrosis. In fact, approximately 20% (24/121) of patients with high elasticity values had cirrhosis (F4). The Az value for diagnosing of F4 was 0.98, reflecting extremely high diagnostic value. However, our study, which excluded patients with cirrhosis, found that SWE still had high diagnostic performance. In other words, SWE, a non-invasive and repeatable imaging method, can provide high-quality imaging assessment of the degree of liver fibrosis in CHC patients.

A remarkable feature of SWE is that it can show viscoelastic properties in all areas in an ROI with a color look-up table and, thus, is expected to overcome the limitations of TE by which liver stiffness cannot be measured accurately in patients with severe obesity, thick subcutaneous fat and ascites.[29] Further, acoustic radiation force impulse imaging (ARFI) and real-time elastography (RTE) have also been developed as non-invasive imaging methods for evaluating liver fibrosis. ARFI has been implemented on an ultrasound system as another shear-wave-based elastography technique. This approach differs from SWE, in that it is limited to a quantitative estimate of liver stiffness at a single location. In addition, the ROI of ARFI is relatively small as compared with that of SWE. Like SWE, RTE can display tissue elasticity images and conventional grayscale ultrasound images at the same time but is unable to calculate the elastic modulus. Because only a few studies of SWE were published, more information is needed for comparing SWE and these methods in clinical practice.

Recently, methods using blood test data, including the AST/ALT ratio,[13] FIB-4 index,[17] APRI,[14] Forns' index[15] and the Fibro test[16] have been reported to be useful for evaluating liver fibrosis. In the present study, we used the FIB-4 index, APRI and Forns' index as three representative liver fibrosis indices. According to published reports, the Az values for diagnosing significant liver fibrosis in patients with HCV infection for the FIB-4 index, APRI and Forns' index (both FIB-4 index and Forns' index, METAVIR score ≥F2; APRI, Ishak score ≥3)[30] were 0.85, 0.88 and 0.81, respectively. In the present study for diagnosing significant liver fibrosis that has not yet reached cirrhosis, the Az values for these three indices were 0.8, indicating moderate diagnostic value, and not as high

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as that for SWE. In addition, although the cut-off levels for the four methods were calculated automatically by statistical software according to the Youden index[26,27] and blood testing and SWE did not occur on the same day, the accuracy for diagnosing significant liver fibrosis with SWE was superior to that for the three liver fibrosis indices. Moreover, SWE was independently selected as a marker of significant liver fibrosis by multivariate analysis that included these four variables as covariates (OR, 2.52 for each 1 kPa increase in elasticity). One possible reason for this difference is that these three indices, especially the FIB-4 index and the Forns' index, include age. They are useful for predicting severe liver fibrosis in the form of cirrhosis. According to Vallet-Pichard *et al.*, the Az value for diagnosing of cirrhosis in patients with HCV infection using the FIB-4 index was greater than 0.9, indicating high diagnostic value.[31] In the present study, however, the median age of patients with non-significant and significant liver fibrosis was over 60 years, with no significant difference between the two groups.

In the present study, we excluded patients with cirrhosis that had high elasticity values. Recently, with advances in imaging techniques, the diagnosis of cirrhosis using gray-scale ultrasonography with typical findings such as superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly, >120 mm; dilated portal vein diameter, >12 mm; patent collateral veins or ascites)[32–34] without SWE has become relatively satisfactory. Therefore, we evaluated the degree of liver fibrosis except for cirrhosis, classifying patients as having non-significant or significant liver fibrosis. Although the hepatitis C guidelines of the American Association for the Study of Liver Diseases recommends that patients with a METAVIR score of F3 or more as among those with the highest priority for DAA treatment,[11] we compared CHC patients with F0–F1 and F2–F3 in the present study to prevent overlooking the appropriate timing for optimal therapeutic intervention.[16,35]

The main limitations of this study include the comparatively small number of CHC patients and its retrospective nature. In particular, the size of the group with significant liver fibrosis was small. Additionally, the hyaluronic acid levels as one of the simple liver fibrosis markers, due to the small number of patients in whom these data were available, we were unable to analyze the diagnostic value of it. Further prospective studies with a larger number of patients are warranted. Another limitation of our study was that our analysis of elasticity as evaluated by SWE did not take into account the necroinflammatory activity grades in each group. The group with significant liver fibrosis had significantly higher necroinflammatory activity grades compared with the group with non-significant liver fibrosis. In several studies, influences of necroinflammatory activity, jaundice and/or congestion on TE, ARFI or SWE measurements have been found.[36–41] Namely, liver stiffness is increased when inflammation, jaundice and/or congestion are present in the liver. Further studies evaluating how to divide patients with varying degrees of liver fibrosis by necroinflammatory activity grades that involve a larger number of CHC patients are also warranted.

In conclusion, SWE has a high ability to diagnose significant liver fibrosis not including cirrhosis in patients with HCV infection. In addition, SWE has high diagnostic performance for liver fibrosis compared with liver fibrosis indices such as the FIB-4 index, APRI and Forns' index. Further studies are warranted to confirm this finding in other populations.

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Hepatocellular carcinoma with steatohepatitic features: a clinicopathological study of Japanese patients

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Hepatocellular carcinoma with steatohepatitic features: a clinicopathological study of Japanese patients

Aims: The aim of this study was to investigate the clinicopathological significance of steatohepatitic features in hepatocellular carcinomas (HCCs) using a large-scale analysis.

Methods and results: Retrospective clinicopathological analysis was performed on HCCs treated surgically at the University of Tokyo Hospital between 2005 and 2010. The diagnosis of HCC with steatohepatitic features (SH-HCC) was made if the tumour fulfilled four of the following five criteria: steatosis (>5% tumour cells), ballooning or Mallory–Denk body formation, interstitial fibrosis and inflammatory infiltrates. There were 120 HCCs (31.4%) from 106 patients (36.3%) that met the criteria of SH-HCC. Patients with SH-HCC were characterized by a higher frequency of diabetes mellitus and hypertension, along with higher serum levels of cholesterol and triglycerides, than those with conventional HCC ($P < 0.01$). The background liver of SH-HCC patients showed steatosis and steatohepatitis more frequently ($P < 0.01$). SH-HCCs were smaller, relatively more differentiated and had a higher frequency of bile duct invasion ($P < 0.05$). Multivariate analysis failed to show prognostic significance of steatohepatitic features in HCCs.

Conclusions: SH-HCC is a subcategory of HCC associated with the patient's metabolic condition and the presence of steatosis or steatohepatitis in the background liver. Steatohepatitic features were not a significant prognostic factor for HCCs.

Keywords: alcohol, diabetes mellitus, hepatitis C virus, hepatocellular carcinoma, metabolic syndrome, non-alcoholic fatty liver disease, steatohepatitis

Introduction

Liver cancer is the sixth most commonly diagnosed cancer and the third leading cause of cancer mortality worldwide,[1] and hepatocellular carcinoma (HCC) is the most common histological type of primary liver cancer. Despite the various aetiologies[2] and number of gene signatures identified recently,[3] the histological features of HCC have been considered to have little variation; although several architectural patterns and cytological variants have been documented they usually have little, if any, clinical significance.[2]

Salomao *et al.*[4] reported recently that a subset of hepatitis C virus (HCV)-related HCCs have features of steatohepatitis (steatohepatitic HCC, SH-HCC) and are associated with concurrent non-alcoholic fatty liver disease (NAFLD). A subsequent report from the same group revealed that SH-HCCs occurred almost exclusively in patients with underlying steatohepatitis with or without viral hepatitis. Several issues regarding SH-HCC remain to be clarified. The seminal studies by Salomao *et al.*[5] investigated mainly western

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patients. Because genetic background and regional lifestyle influence an individual's metabolic state, epidemiological studies of SH-HCC involving different ethnic cohorts are of interest. Histological characteristics of SH-HCC, especially those associated with prognosis, should also be assessed in more detail. Although steatohepatitic features had little prognostic impact in the study by Salomao *et al.*, the prognostic significance of SH-HCC should be determined on a larger scale of analysis. Proper categorization of SH-HCC, whether it is a distinct entity or just a histological pattern, should be determined based on its clinicopathological significance.

We conducted a study involving a large Japanese cohort of HCC patients to determine the clinicopathological characteristics of SH-HCC in detail.

Materials and Methods

CASES

Consecutive HCC patients treated surgically at Tokyo University Hospital from 1 January 2005 to 31 December 2010 were the focus of this study. Patients undergoing initial surgery for HCC, without non-surgical treatment for HCC more than 3 months earlier, were included. Patients with pre-operative transarterial therapy or portal embolization within 3 months of surgery were not excluded only if a sufficient portion of the tumours remained viable.

CLINICAL DATA

Clinical data, retrieved from medical charts, included serum data immediately before surgery, highest pre-operative plasma levels of tumour markers, status of hepatitis virus infection, presence or absence of diabetes mellitus, hypertension and hyperlipidaemia, and history of alcohol intake (20 g or more of alcohol per day for at least 1 year) and heavy drinking (80 g or more of alcohol per day for more than 5 years). Body mass index (BMI) was calculated from height and weight on admission. In this study, patients were considered to be positive for hepatitis B virus (HBV) or HCV if they had HBsAg or HCV Ab, respectively.

All patients were screened regularly for recurrence through monitoring of the plasma tumour markers every 1–2 months, ultrasonography every 2 months, and dynamic computed tomography every 4 months. Recurrence was defined as the appearance of a new lesion with radiological features compatible with HCC, confirmed using at least two imaging modalities.

Overall survival was defined as the interval between the date of surgery and death, whereas disease-free survival was defined as the interval between the date of surgery and recurrence. If recurrence was not diagnosed, patients were censored on the date of death or last follow-up.

PATHOLOGY

Pathology reports and all tissue slides were reviewed for all patients. Tumour location and size, histological grade, presence or absence of vascular invasion, bile duct involvement and intrahepatic metastasis were re-evaluated. Superficial (abutting the hepatic surface) or deep parenchymal location was determined for small tumours up to 3 cm in diameter. The following histological criteria were applied to define multicentric HCC according to the World Health Organization (WHO) classification:[2] (i) multiple early HCCs or concurrent early HCCs and classical HCCs; (ii) the presence of peripheral areas of well-differentiated HCC in smaller lesions; and (iii) multiple HCCs of obviously different histology.

The diagnosis of SH-HCC was based on the following five criteria: the presence of steatosis (more than 5% of tumour cells), ballooning, Mallory–Denk bodies, interstitial fibrosis and intratumoral inflammatory cell infiltration (Figure 1). Tumours were considered to be SH-HCCs if they fulfilled at least four criteria, regardless of whether the steatohepatitic features were focal or predominant in the tumour. Strictly defined SH-HCC, designated as typical SH-HCC, fulfilled all five criteria, and the steatohepatitic features were predominant within the tumour.

The fibrosis stage of background liver was evaluated according to the METAVIR system[6] for cases associated with viral hepatitis, or the Nonalcoholic Steatohepatitis Clinical Research Network (NASH-CRN) scoring system[7] for non-viral cases. The degree of steatosis (grade 0, <5%; grade 1, 5–33%; grade 2, 34–66%; grade 3, ≥67%), with or without features suggestive of steatohepatitis, was also recorded.

STATISTICAL ANALYSIS

Quantitative variables were compared using Student's *t*-test or the Mann–Whitney *U*-test. Categorical variables were compared with the χ^2 or Fisher's exact tests. Overall and disease-free survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. To determine prognostic factors, multivariate regression analysis was performed using the Cox proportional hazards model

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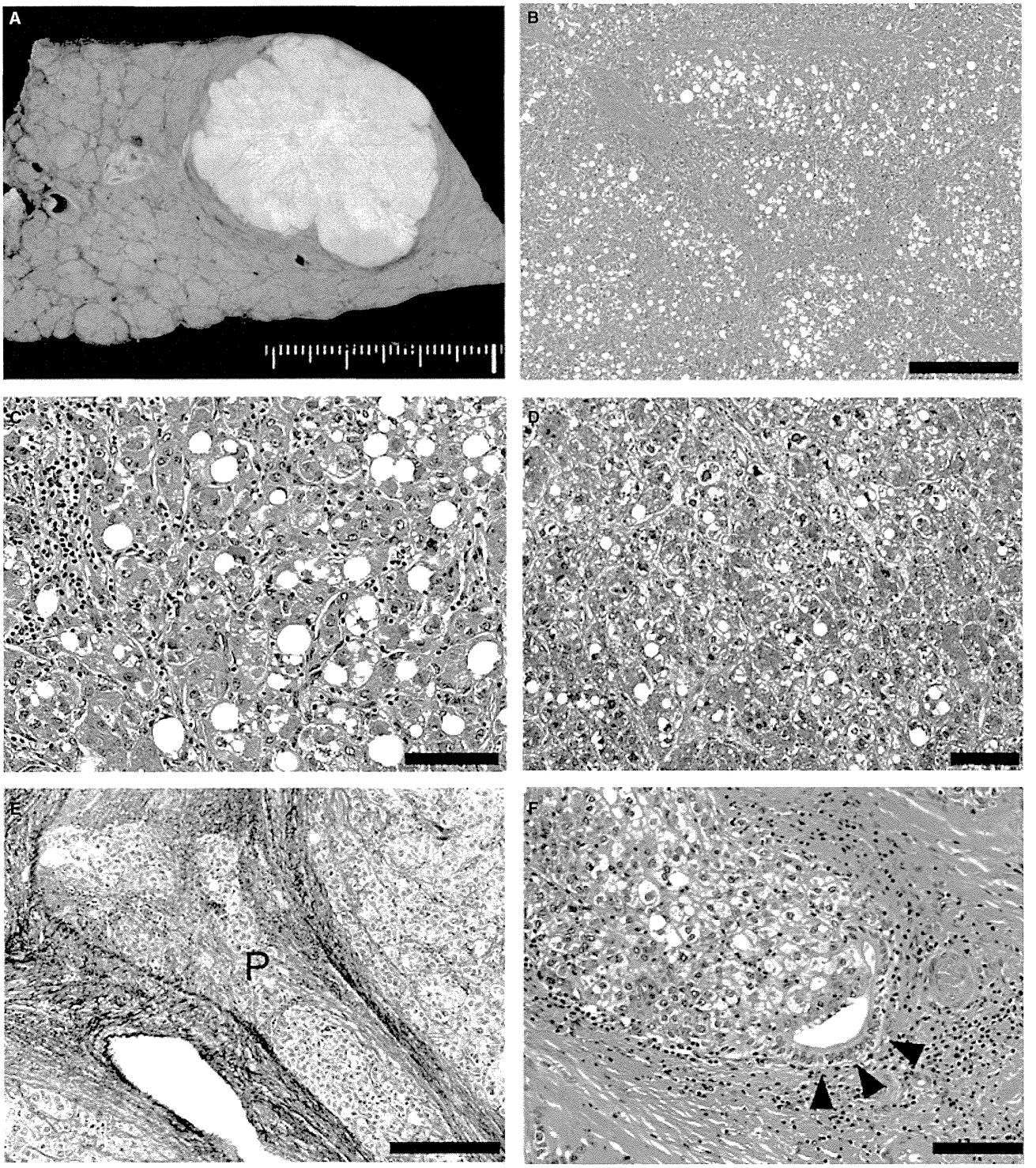

Figure 1. Histological features of steatohepatitic hepatocellular carcinoma (SH-HCC). **A**, SH-HCC typically forms a well-demarcated yellow or yellowish-green lobulated mass. Scar-like fibrosis is discernable. **B**, Steatosis and septal fibrosis are seen on low power. Haematoxylin and eosin (H&E) stain (bar, 500 μm). **C**, Macrovesicular steatosis, ballooning of tumour cells, as well as Mallory–Denk bodies, are characteristic of SH-HCC. Mixed inflammatory infiltration is also characteristic of SH-HCC. H&E stain (bar, 100 μm). **D**, Interstitial fibrosis in SH-HCC. Azan–Mallory stain (bar, 100 μm). **E**, Vascular invasion (P, portal vein). Elastica Van Gieson stain (bar, 250 μm). **F**, Bile duct involvement (arrowhead, bile duct epithelium). H&E stain (bar, 100 μm).

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