Original Article

Clinical significances of several fibrotic markers for prognosis in hepatocellular carcinoma patients who underwent hepatectomy

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Background: Progression of chronic liver fibrosis and related increased fibrotic markers are associated with functional liver reserves or patient prognosis as well as tumor factors in hepatocellular carcinoma (HCC) patients. The aim of this study was to newly clarify the relationship between fibrotic markers and HCC malignant behaviors or its long-term postoperative prognosis by the retrospective cohort study.

Methods: We examined the relationship between tumor-related factors or six liver fibrosis-associated parameters, including platelet count, hyaluronic acid (HA), Mac-2 binding protein glycosylation isomer (M2BPGi), type IV collagen 7S (T4C7), aspartate aminotransferase-to-platelet ratio index (APRI), fibrosis-4 (Fib-4) index, and clinicopathological parameters, surgical records, and postoperative prognosis in 130 HCC who underwent curative hepatectomy.

Results: Histological fibrosis stage 4 as cirrhosis was in 31%. The platelet count significantly decreased in stage 4 fibrosis and correlated with grade B liver damage ($P<0.01$). HA levels were significantly increased in multiple HCC, stage 4 fibrosis, and grade B liver damage ($P<0.01$). T4C7 was significantly increased in patients with post-hepatectomy tumor recurrence compared to those without ($P<0.01$). Additionally, M2BPGi was significantly higher in stage 4 fibrosis and liver damage grade B, and was significantly associated with poor prognosis ($P<0.05$). Fib-4 index was significantly higher in patients with liver damage B ($P<0.05$), and T4C7 alone did not correlate with other five fibrosis markers. Stage 4 fibrosis, higher T4C7, higher M2BPGi, and increased tumor size were significantly associated with shorter cancer-free, overall, and cancer-specific survivals. Higher T4C7, non-met Milan criteria, liver damage B, blood transfusion, and curability C were independently associated with cancer-specific survivals ($P<0.05$).

Conclusions: Type IV collagen 7S (T4C7) may reflect not only impaired liver function but also HCC malignant behaviors and patient survivals.

Keywords: Hepatocellular carcinoma (HCC); hepatectomy; fibrosis marker; malignancy of HCC; patient prognosis

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Introduction

Background

In case of surgical intervention as radical hepatectomy for hepatocellular carcinoma (HCC), several factors are significantly associated with short-term outcomes after surgery, and hepatic fibrosis is an important potential factor of impaired functional causes for chronic liver injury (1-7). Thus, in HCC, cancer-related and non-cancerous background factors of the liver had similar disease and treatment effects in each patient survival, including the first author's related studies on HCC (5,8).

Rationale and knowledge gap

Except for needle biopsy or resected specimen histology, representative direct liver fibrosis-associated surrogate markers in serum samples, such as type IV collagen, hyaluronic acid (HA) (9-13), and Mac-2 binding protein glycosylation isomer (M2BPGi), have been investigated for decades (14,15). HA level was associated with prognosis in HCC patients by our previous study (12) and, however, other sensitive markers may need to be considered for recent diagnostic management at this stage. Serum alpha fetoprotein (AFP) also showed not only cancer malignancy but also chronic liver injured severity as well (16). In a noninvasive scoring system based on liver functional parameters, decreased platelet count (17), increased aspartate aminotransferase-to-platelet ratio index (APRI), and Fib-4 index are often used to evaluate hepatic fibrosis and patient prognosis (18). The hepatic fibrosis can be accurately examined by several measurement mechanisms using individual ultrasound elastography, which is more useful than the use of surrogate markers (9-18). We previously found that shear wave elastography fibrosis reflects postoperative complications after hepatectomy for HCC (19): however, this modality was not routinely applied in this series.

As described above, although the principal author previously reported the significance of HA level with overall survival in patients with HCC undergoing hepatectomy (11,12), the recent study since 2015 might have advanced perioperative management and non-surgical treatments as Deb-TACE technique for recurrent HCC (20). Furthermore, we comprehensively examined five types of surrogate fibrotic markers and investigated the most significant marker related to cancer malignancy and disease-free or overall survival in patients with HCC. We hypothesized that the deterioration of liver function and coexisting hepatocyte injury due to increased fibrosis would influence tumor progression and subsequent carcinogenesis. Furthermore, in HCC patients, survival is influenced not only by cancer-related factors but also by postoperative deterioration of liver function, as indicated by the Japan Integrated Staging (JIS) or Cancer of the Liver Italian Program (CLIP) score combined with tumor prognostic factors (21).

Type IV collagen is a classical fibrotic marker of the liver, and the marker of serum HA is a well-used in both hepatic fibrosis and endothelial dysfunction of the hepatic sinusoid (12,13). The APRI and Fib-4 index have also been clinically used to evaluate the degree of fibrosis using serum blood parameters (16). Additionally, M2BPGi is a novel marker for assessing hepatic fibrosis that induces inflammatory cytokines and increases extracellular collagen or fibronectin levels (15). Stellate cell is a source of M2BPGi, and this level reflects this activation of these cells according to the process of liver fibrosis (14). Furthermore, increased biological activity of m2bpgi is associated with

Highlight box

Key findings

- Liver fibrosis is important factors for operative indication and the surrogate fibrotic marker except liver biopsy are required reflecting degree of liver dysfunction. Several markers have been proposed so far and, however, each marker may have its own role of liver functional reserve or oncological role associated with overall prognosis in hepatocellular carcinoma (HCC) patients.

What is known and what is new?

- The liver fibrotic markers were known as good serum markers reflecting chronically impaired liver functional reserves and, however, influences to patient prognosis with HCC after surgery has not been fully clarified.
- Newly finding of this study was to clarify the relationship between fibrotic markers and HCC malignant behaviors or its long-term postoperative prognosis by the retrospective cohort study. Particularly, type IV collagen 7S (T4C7) may reflect not only impaired liver function but also HCC malignant behaviors and postoperative patient survival.

What is the implication, and what should change now?

- Use of combined several fibrotic markers would predict surgical outcomes including complications or long-term prognosis regrading recurrence pattern, recurrent-free survival, overall survival including liver dysfunctional cause of death, and cancer-specific overall survivals.

the development of HCC (22). Our previous study showed that M2BPGi showed a close relationship with post-hepatectomy complications (23). To our knowledge, at this stage, the relationship between these fibrosis markers and disease-free or overall survivals in patients with HCC has not been fully elucidated as in the present study.

Objective

Therefore, we aimed to clarify which fibrotic marker is significantly related to the tumor-related factors of HCC using our surgical specimens or could be applied to predict shorter patient survival. We collected data on fibrosis parameters, followed-up patient clinical outcomes, and retrospectively investigated the association between these blood marker levels and clinicopathological factors, morbidities, tumor relapse. 130 consecutive patients with HCC who underwent hepatectomy at a single academic cancer institute at the University of Miyazaki, Japan, for approximately eight years. We present this article in accordance with the STROBE reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-94/rc).

Methods

Patients

This retrospective cohort study consisted of 130 consecutive HCC patients who were administrated to the Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, University of Miyazaki Faculty of Medicine, Miyazaki, Japan for 7.5 years between April 2015 and September 2022. Patients with distant metastasis or double cancer during surgery were excluded from the present study.

Before and after primary treatment, serum tumor levels of AFP and protein induced by vitamin K absence or antagonist II (PIVKA-II) were measured as HCC tumor markers per 3–6 months, and enhanced computed tomography was performed at 6 months after hepatectomy to monitor tumor relapse. The postoperative follow-up period for HCC was 12 months (range, 12–83 months). Anesthesia and patient data were retrieved from the University of Miyazaki Hospital database. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Human Ethics Review Board of the University of Miyazaki Faculty of Medicine (approval number: O-1469; December 22, 2023). Informed consent was obtained from all individual participants prior to treatment, and for their data to be used for research purposes. Informed consent was obtained by the opt-out procedure at the university website but no disclaimer.

Measurement of tumor and histological markers

Clinicopathological data, including PIVKA-II (Sanko Junyaku Co., Tokyo, Japan) and AFP levels, were retrieved from the archives at our institute. In our institute, the normal upper range of AFP and PIVKA-II are set at 20 ng/mL and 40 mAU/mL, respectively. Tumor-related factors were compared with histopathological findings of the specimen. For assessment, we used the rules established by the Liver Cancer Study Group of Japan for the classification of primary liver cancer (24). Tumor staging (0–4) of hepatic fibrosis and its histological activity index (HAI) score, by Knodell *et al.* (25), were applied for histological findings of non-tumorous liver parenchyma.

Measurement of fibrotic markers

Blood samples were collected from each patient before surgery when the patient was stable. This sample was centrifuged at 3,000 rpm for 15 min, and the serum was stored at –80 ℃. HA was assayed using the sandwich binding protein assay by SRL Inc. (Tokyo, Japan) (11). The normal serum HA level reported by SRL Inc. is <50 ng/mL. Further, M2BPGi was measured by a chemiluminescent enzyme immunoassay with anti-*Wisteria floribunda* agglutinin and anti-M2BP antibodies by an automated HSCL-2000i Immunoanalyzer (Sysmecs Co., Hyogo, Japan) (23). The upper cut-off value was set at 1 cut-off index (COI) by the company data. The serum type IV collagen 7S concentration was measured by an RIA kit (Diaiatron Co., Tokyo, Japan), which uses a polyclonal antibody against the human 7S domain of type IV collagen (T4C7). The normal value was set at <8.0 ng/mL, according to company data (26). The FIB-4 score was calculated as follows: age (years) × aspartate aminotransferase (AST) (U/L)/platelet count ($\times 10^9$/L) × alanine transaminase (1/2 IU/L). According to the literature, the FIB-4 cut-off level for fibrosis or cirrhosis (F3 and F4) is set at 3.25 (27). The APRI score was calculated by the AST level divided by 30 (upper limit of normal at our institute), multiplying it by 100, and divided the total by the platelet count. The validated cut-off point with the

presence of advanced fibrosis was 1.5, and a cut-off value of 0.5 is predicted the absence of fibrosis.

Statistical analysis

Differences in categorical data between groups were evaluated using the chi-square test, Fisher's exact test, or Dunnett's multiple comparison test. Differences in the continuous data between groups were assessed by the Student's *t*-test or the Mann-Whitney test. Disease-free and overall survival were calculated using the Kaplan-Meier method, and differences between groups were tested for significance by the log-rank test. Multivariate analysis was performed by the Cox proportional hazards regression model. A two-tailed P value of <0.05 was considered significant. Statistical analyses were performed using statistical software (SPSS software version 23; Statistical Package for the Social Science, Inc., Chicago, IL, USA).

Results

Perioperative parameters

Basic patient data were indicated as follows: patients included 103 (79%) man and 27 (21%) women and the mean age was 70.7±8.7 years (± standard deviation; range, 42–87 years). Thirty patients showed normal livers, and others had diseased livers including metabolic-associated steatotic liver dysfunction (MASLD) in 44 patients, primary biliary cholangitis in two, and chronic viral hepatitis in 54 (including hepatitis B in 30 and hepatitis C in 24). Cirrhosis was observed in 41 (32%) patients. Operative procedures included hemihepatectomy or more extensive hepatectomy in 14 patients, central bisectionectomy in three, segmentectomy or sectionectomy in 65, and partial resection in 48. Since 2017, laparoscopic hepatectomy was performed in 53 (41%) patients, including limited resection in 39 (74% of laparoscopic hepatectomies), segmentectomy in 13 (25%), and sectionectomy in one (1%). Curative hepatectomy was undergone, and the hepatic tumors were completely resected without macroscopic tumor margin

The 129 (99%) patients were classified as Child-Pugh A, and one patient was Child-Pugh B. The mean AFP level was 659±5,316 μg/mL [median 5; interquartile range (IQR) 3–20.5], and the mean PIVKA-II level was 2,002±7,831 ng/mL (median 52; IQR 23.3–207). According to the Liver Cancer Study Group of Japan, the pathological tumor-node-metastasis (TNM) stage of HCC was stage I in 20 (15%) patients, stage II in 71 (55%), stage III in 31 (24%), and stage IVA in 8 (6%). The mean blood loss was 741±838 mL (median, 480 mL; IQR 158–993), and blood loss >1,500 mL was observed in 19 (15%) patients. Allogeneic blood transfusions were administered to 22 (17%) patients. After the hepatectomy, hepatic failure occurred in 1 (1%) patient, and uncontrolled ascites was observed in 7 (5%).

Relationship between clinicopathological factors and each fibrotic marker

Fibrotic parameters were shown as follows: the mean and median HA levels were 102±94 and 76 ng/mL (IQR 46–134), respectively. The mean and median platelet counts were 17.9±6.9 and 17×10^4/mL (IQR 13–22), respectively. The mean and median T4C7 were 7.9±18.5 and 5.5 ng/mL (IQR 4.4–7.2), respectively. The mean and median M2BPGi levels were 1.21±0.93 and 0.90 (IQR 0.57–1.54) COI, respectively. The mean and median APRI scores were 0.77±0.85 and 0.56 (IQR 3.74–8.45), respectively. The mean and median Fib-4 index were 2.93±3.21 and 2.45 (IQR 1.77–3.21), respectively. The calculation of APRI and Fib-4 index were described in Method section.

In the 130 patients, relationships between the six types of fibrotic markers or scores and clinicopathological, surgical, or cancer recurrence after hepatectomy are shown in *Table 1*. Platelet count was significantly higher in tumor size >5 cm in size (P<0.01). The platelet count was significantly decreased in stage 4 of the histological HAI score, equivalent to cirrhosis (P<0.01), and in the group with grade B liver damage (P<0.01). The serum HA level was significantly higher in the multiple HCC group than that in the solitary HCC group (P<0.01), in stage 4 histological fibrosis 4 compared to each stage (P<0.01), in patients with liver damage grade B compared to A (P<0.01), and in patients who received allograft red cell transfusion compared to those who did not (P<0.01). T4C7 was significantly increased in patients with post-hepatectomy tumor recurrence compared to those without (P<0.01). The M2BPGi in stage 4 was significantly higher compared to that of each stage (P<0.01) and in patients with liver damage grade B compared with grade A (P<0.05), and was significantly associated with worse prognosis (P<0.05). The APRI in histological fibrotic stage 4 was significantly higher than that of stage 2 alone (P<0.05) and liver damage grade B compared to stage A (P<0.05), and was significantly related to worse prognosis (P<0.01). The Fib-4 index was significantly higher in patients with grade B liver damage

Table 1 Relationship between fibrotic markers and HCC parameters, surgical records, post-hepatectomy complications, and tumor recurrence (N=130)

Parameters	Platelet count (10^4/mL)	HA (ng/mL)	Type4 collagen 7S (ng/mL)	M2BPGi (COI)	APRI	Fib-4 index
Data differences						
Number of HCC						
Solitary (n=107)	18±7.1	96±98	8.1±20.5	1.18±0.96	0.77±0.92	2.9±3.5
Multiple(n=23)	17.4±6.0	129±68**	6.6±1.6	1.33±0.81	0.76±0.36	3.1±1.7
Size of HCC						
<20 mm (n=24)	16.2±7.2	110±83	5.4±2.7	1.06±0.73	0.63±0.40	2.3±1.1
20–50 mm (n=74)	16.3±5.2	103±102	9.3±25.4	1.26±0.96	0.85±1.01	3.3±4.0
>50 mm (n=32)	21.5±9.3**	91±84	6.7±3.0	1.17±1.01	0.63±0.54	3.5±1.9
Milan criteria						
Met (n=94)	17.1±6.1	105±100	7.1±3.6	1.17±0.89	0.78±0.94	3.0±3.6
Non-met (n=36)	19.9±8.5	92±78	8.1±22	1.30±1.06	0.73±0.56	2.8±1.8
Vascular involvement						
No (n=103)	17.0±5.7	103±101	8.1±20.9	1.22±0.96	0.78±0.92	3.0±3.6
Yes (n=27)	21.1±9.8	97±55	6.5±3.8	1.17±0.67	0.74±4.78	2.6±1.2
Fibrotic stage						
0 (n=13)	22.5±9.1	67±55	4.6±1.2	0.67±0.48	0.53±0.31	2.5±1.2
1 (n=26)	21.7±6.0	62±45	13.5±41.6	0.91±0.55	0.48±0.35	2.0±0.9
2 (n=30)	17.7±7.2	81±56	6.0±3.8	0.48±0.69	1.90±0.43	2.5±1.1
3 (n=21)	17.0±3.9	77±44	6.1±3.9	0.16±0.39	0.86±0.29	2.6±1.1
4 (n=40)	14.6±5.9**	162±131**	7.5±2.0	1.54±1.24**	1.19±1.35	4.3±5.4
Macroscopic finding						
SN (n=65)	16.9±6.0	109±114	9.4±26.3	1.05±0.65	0.83±1.1	3.2±4.3
SNEI (n=40)	20±7.9	87±60	6.3±4.0	1.40±1.05	0.71±0.65	2.4±1.5
CN (n=24)						
Tumor factor number	17.7±7.2	99±75	5.9±1.9	1.34±1.35	0.69±0.46	3.1±1.6
1 (n=20)	17.7±6.3	94±64	5.1±2.7	0.99±0.61	0.64±0.52	2.1±1.0
2 (n=70)	16.8±5.6	102±116	9.1±25.6	1.24±1.11	0.83±1.07	3.3±4.2
3 (n=32)	19.2±8.2	106±52	6.8±3.1	1.23±0.69	0.76±0.43	2.8±1.4
4 (n=8)	22.9±11	95±60	8.4±5.2	1.33±0.76	0.52±0.30	2.5±1.3
Liver damage grade						
A (n=117)	18.4±6.9	97±97	7.9±19.3	1.10±0.72	0.67±0.45	2.6±1.4
B (n=13)	13.0±5.1**	147±60**	6.4±2.6	2.13±1.82*	1.67±2.17*	6.0±9.0*
Preoperative TAE						
No (n=109)	17.8±7.1	100±97	8.2±20.2	1.12±0.84	0.78±0.89	2.9±3.4
Yes (n=21)	18.1±6.2	109±79	5.7±2.1	1.25±1.84	0.69±0.59	3.0±1.7

Table 1 *(continued)*

Table 1 *(continued)*

Parameters	Platelet count (10^4/mL)	HA (ng/mL)	Type4 collagen 7S (ng/mL)	M2BPGi (COI)	APRI	Fib-4 index
Blood transfusion						
No (n=110)	18.0±6.6	93±93	5.8±2.7	1.16±0.95	0.77±0.91	2.9±3.5
Yes (n=19)	17.5±8.5	138±91**	9.9±43.2	1.40±0.84	0.74±0.45	3.1±1.5
Tumor recurrence						
No (n=92)	17.4±8.8	64±96	7.0±2.4	1.0±1.4	0.76±0.63	5.3±8.2
Yes (n=38)	18.1±6.1	104±104	8.2±22.4**	0.9±1.1	0.75±0.95	7.5±9.5
Patient prognosis						
Alive (n=91)	18.0±5.9	99±102	8.2±22.2	1.1±0.9	0.66±0.51	2.7±1.4
Recurrent alive (n=24)	21.8±13.6	84±57	6.5±1.1	1.2±0.5	1.00±0.76	2.4±2.4
Death of HCC (n=9)	14.4±7.9	150±89	7.2±0.6	2.3±0.8*	2.36±3.34**	9.6±14.4**
Other death (n=5)	16.8±6.8	107±73	6.9±2.6	1.4±1.2	0.75±0.48	2.8±1.4
Correlations (r)						
Blood loss	–0.003	0.136	0.165	0.115	0.004	0.018
Fibrotic parameters						
Platelet count	–	–0.265**	–0.054	–0.327**	–0.342**	–0.319**
HA	–0.265**	–	0.04	0.351**	0.298**	0.233**
Type IV collagen 7S	–0.054	0.04	–	0.067	–0.009	–0.003
M2BPGi	–0.327**	0.351**	0.067	–	0.418**	0.327**
APRI	–0.342**	0.298**	–0.009	0.418**	–	0.898**
Fib-4 index	–0.319**	0.233**	–0.003	0.327**	0.898**	–
Immuno-nutritional index and HCC marker						
Albumin	0.0003	–0.345**	47	–0297**	–0.113	–0.109
Total cholesterol	0.083	–0.08	–0.002	–0.135	–0.075	–0.014
Lymphocyte count	–0.054	0.023	0.0003	0.152	–0.023	–0.007
AFP	–0.03	–0.057	–0.0009	0.08	0.103	–0.01
PIVKA-II	0.12	0.009	–0.018	0.084	0.031	–0.006

The continuous data was indicated by mean ± SD. Clinicopathological findings and tumor factor number of Japan Tumor-Node-Metastasis (TNM) classification were based on the *General Rules for the Clinical and Pathological Study of Primary Liver Cancer* (24). *, P<0.05; **, P<0.01. HCC, hepatocellular carcinoma; HA, hyaluronic acid; M2BPGi, Mac2 binding protein glycosylation isomer; COI, cut-off index; APRI, aspartate aminotransferase-to-platelet ratio index; SN, simple nodule; SNEI, simple nodule with extracapsular infiltration; CN, confluent nodule; TAE, transarterial chemoembolization; AFP, alpha fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II.

than that in those with grade A liver damage (P<0.05), and it was significantly associated with a worse prognosis (P<0.01). Among the fibrotic parameters, except for T4C7, they tended to correlate with each other. Higher HA levels and M2BPGi significantly correlated with lower preoperative albumin levels (P<0.01).

Relationship between preoperative fibrotic parameter levels and post-hepatectomy disease-free and overall survivals

Table 2 shows the cancer-free, overall, and cancer-specific overall rates and the differences in each clinicopathological parameter. The one- and three-year cancer-free survival

Table 2 Relationship between fibrotic markers or HCC parameters, and patient survivals by the log-rank test under Kaplan-Meier survival curves

Parameters	Tumor-free survival (1/3/5 years)	Overall survival (3/5/8 years)	Cancer-specific overall survival (3/5/8 years)
Cause of chronic liver injury			
Normal (n=30)	75/63/63	88/88/88	88/88/88
MASLD (n=44)	84/74/62	93/78/58	98/90/68
Viral (B) (n=30)	94/69/58	96/86/86	96/86/86
Viral (C) (n=24)	92/67/62	100/94/94	100/100/100
PBC (n=2)	100/100/0	100/100/100	100/100/100
Number of HCC			
Solitary (n=107)	89/74/62	94/88/81	96/92/85
Multiple (n=23)	73/49/49$^{\#}$	89/73/54	96/86/64
Size of HCC			
<20 mm (n=24)	92/74/60	95/88/59	100/67/67
20–50 mm (n=74)	87/67/67	97/90/86	100/97/93
>50 mm (n=32)	73/55/46	84/76/57*	84/76/57**
Milan criteria			
Met (n=94)	87/74/62	95/87/79	99/97/85
Non-met (n=36)	83/57/51	89/78/65	89/84/70
Vascular involvement			
No (n=103)	85/72/65	95/83/69	99/91/76
Yes (n=27)	89/61/44	89/89/89	89/89/89
Fibrotic stage, 0 (n=13)	76/76/76	76/76/76	76/76/76
1 (n=26)	92/69/69	100/89/89	100/89/89
2 (n=30)	93/84/70	97/97/80	97/88/88
3 (n=21)	95/78/78	100/100/100	100/100/100
4 (n=40)	74/54/19*	89/81/48	97/93/56
Platelet count (10^4/mL)			
<17 (n=60)	87/65/53	94/80/60	100/97/66
≥17 (n=70)	88/75/65	91/87/87	91/91/91
HA (ng/mL),			
<76 (n=63)	85/73/64	93/78/78	95/84/84
≥76 (n=67)	87/64/48	95/91/65	98/98/70
Type IV collagen 7S (ng/mL)			
<5.5 (n=57)	93/74/69	94/94/94	94/94/94
≥5.5 (n=59)	76/60/45*	92/77/63$^{\#}$	96/87/71
M2BPGi (COI)			
<1 (n=75)	91/73/65	97/94/90	97/94/94
≥1 (n=55)	81/66/53	89/78/59*	95/87/65

Table 2 *(continued)*

Table 2 *(continued)*

Parameters	Tumor-free survival (1/3/5 years)	Overall survival (3/5/8 years)	Cancer-specific overall survival (3/5/8 years)
APRI			
<0.56 (n=64)	85/68/64	92/92/92	92/92/92
≥0.56 (n=66)	86/71/54	95/80/62	100/97/70
Fib-4 index			
<2.45 (n=65)	85/69/60	92/87/56	92/89/78
≥2.45 (n=65)	89/72/60	98/94/77	100/95/84
Macroscopic finding			
SN (n=65)	74/62/62	98/86/77	100/92/83
SNEI (n=40)	88/74/64	91/82/65	91/87/69
CN (n=25)	90/69/56	89/89/89	96/96/96
Tumor factor number			
1 (n=20)	90/76/59	94/94/63	100/100/86
2 (n=70)	88/81/69	96/77/77	98/91/61
3 (n=32)	80/48/42	92/92/76	97/97/81
4 (n=8)	75/56/56	71/71/71	71/71/71
Albumin (g/dL)			
<4 (n=55)	79/65/51	93/84/72	96/87/65
≥4 (n=75)	91/73/64	94/85/77	96/94/84
Lymphocyte (/mm^3)			
<1500 (n=58)	91/70/60	94/86/72	98/90/75
≥1500 (n=72)	81/69/63	92/84/76	95/91/83
AFP (ng/mL)			
<100 (n=112)	85/72/62	93/85/79	97/92/86
≥100 (n=19)	89/59/45	94/84/72	94/84/42*
PIVKA-II (mAU/mL)			
<54 (n=65)	88/72/62	98/94/85	100/96/86
≥54 (n=65)	83/65/61	88/74/64*	93/85/73*
Liver Damage grade			
A (n=117)	87/61/59	94/84/78	96/90/84
B (n=13)	77/62/62	92/92/62	100/100/67
Preoperative TAE			
No (n=109)	86/76/62	95/85/72	97/91/77
Yes (n=21)	86/41/41	83/83/83	91/91/91
Blood transfusion			
No (n=110)	87/69/57	94/89/78	97/91/79
Yes (n=19)	81/73/73	88/49/49*	95/95/95

Table 2 *(continued)*

Table 2 *(continued)*

Parameters	Tumor-free survival (1/3/5 years)	Overall survival (3/5/8 years)	Cancer-specific overall survival (3/5/8 years)
Curability			
A (n=75)	91/80/63	94/91/81	97/93/83
B (n=50)	82/58/54	94/80/70	96/89/78
C (n=5)	60/60/48#	75/75/65#	75/75/65#
Tumor recurrence			
No (n=92)	-	97/91/91	99/99/99
Yes (n=38)		88/75/54*	91/77/55**

Data was indicated by percentage (%). *, P<0.05; **, P<0.01; #, 0.05<P<0.1. Clinicopathological findings and the tumor factor number by Japanese TNM classification were based on the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (24). Prognoses: alive without recurrence (n=91), alive with HCC recurrence (n=24), death from HCC (n=9), and other related deaths (n=5). HCC, hepatocellular carcinoma; MASLD, metabolism-associated steatotic liver dysfunction; PBC, primary biliary cholangitis; HA, hyaluronic acid; M2BPGi, Mac2 binding protein glycosylation isomer; COI, cut-off index; APRI, aspartate aminotransferase-to-platelet ratio index; SN, simple nodule; SNEI, simple nodule with extracapsular infiltration; CN, confluent nodule; AFP, alpha fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II; TAE, transarterial chemoembolization.

rates were 86%, 70%, and 59%, respectively, with a median survival of 58 months. The three-, five-, and eight-year overall survival rates were 94%, 85%, and 75%, respectively, with a median survival period of 79.7 months. The three-, five-, and eight-year overall survival rates were 96%, 91%, and 81%, respectively, and the median survival period was 98.2 months.

With respect to tumor-free survival, patients with histological fibrotic stage 4 (cirrhosis) and higher T4C7 showed the worst survival (P<0.05), respectively. With respect to the overall survival rates, patients with higher M2BPGi, PIVKA-II, intraoperative blood transfusion, and tumor relapse showed significantly worse survival (P<0.05). With respect to the HCC-specific survival rate, tumor size >5 cm (P<0.01), AFP or PIVKA-II levels (P<0.05), and tumor relapse (P<0.01) were significantly associated with worse survival. Curability C tended to show worse overall survival rate, but this was not significant (P=0.06, 0.09, and 0.09, respectively).

Table 3 shows the Cox multivariate analysis using significant prognostic factors identified by univariate analysis with respect to cancer-free, overall, or cancer-specific overall survival rates. A tumor size ≥5 cm was an independently associated parameter with decreased disease-free survival (P<0.05). Higher T4C7 ≥5.5 ng/mL, liver damage grade B, and curability C were an independently associated parameter with decreased overall survival (P<0.05). The higher T4C7 ≥5.5 ng/mL, non-met Milan criteria, liver damage grade B, allograft blood transfusion, and curability C were independently associated with cancer-specific overall survival (P<0.05).

Discussion

Summary of the present study findings; We investigated a retrospective and consecutive analysis of the outcomes of 130 patients with HCC undergoing curative hepatectomy, by an analysis of the six preoperative fibrotic markers or conventional clinicopathological parameters and patient surgical outcomes. Among several fibrotic markers, type IV collagen 7S was not significantly correlated with other fibrotic markers; however, higher levels were related to the postoperative tumor relapse rate and significantly associated with poor overall or cancer-specific overall survival, as an independent parameter in multivariable analysis.

PIVKA-II, AFP, and L3 fractions are commonly used in Japan for the diagnosis of HCC or the evaluation of tumor aggressiveness (28-30). High levels of these preoperative markers after hepatectomy reflects poor prognosis or early tumor recurrence (31). As described above, our previous study showed that not only tumor-associated markers but also background liver dysfunction or liver functional reserve were significantly associated with poor prognosis in patients with HCC undergoing hepatectomy. (8) Increased blood loss per weight, uncontrolled ascites, and liver damage grade B were associated with tumor relapse (P<0.05). The

Table 3 Multivariate analysis of prognostic factors influencing tumor-free survival and overall survival using Cox proportional hazard test

Variables of selected parameters	Tumor-free survival		Overall survival		Cancer-specific overall survival	
	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value
Number of HCC (solitary *vs*. multiple)	2.27 (0.89–5.76)	0.09	0.78 (0.41–1.51)	0.46	0.79 (0.43–1.49)	0.48
Size of tumor (cm) (<5 *vs*. ≥5)	3.68 (1.27–10.7)	0.02	1.44 (0.73–2.84)	0.30	1.24 (0.63–2.44)	0.53
Milan criteria (met *vs*. non-met)	1.03 (0.37–2.79)	0.96	0.58 (0.031–1.10)	0.09	1.08 (1.01–1.65)	0.047
Staging (fibrosis) (0–3 *vs*. 4)	1.99 (0.83–4.80)	0.13	1.57 (0.86–2.88)	0.15	1.69 (0.94–3.03)	0.08
Type IV collagen 7S (ng/mL) (<5.5 *vs*. ≥5.5)	1.13 (0.47–2.71)	0.78	2.18 (1.29–3.68)	0.003	2.05 (1.23–3.40)	0.006
M2BPGi (COI) (<1 *vs*. ≥1)	1.84 (0.87–3.89)	0.11	0.93 (0.58–1.49)	0.76	0.935 (0.59–1.49)	0.78
Liver damage grade (A *vs*. B)	1.76 (0.49–6.28)	0.39	1.12 (1.03–1.84)	0.045	2.56 (1.06–6.25)	0.04
Allograft blood transfusion (no *vs*. yes)	1.56 (0.84–3.69)	0.09	1.89 (1.05–3.43)	0.04	2.23 (1.28–3.89)	0.005
Alpha fetoprotein (μg/mL) (<100 *vs*. ≥100)	1.42 (0.54–3.74)	0.48	1.24 (0.63–2.44)	0.53	1.19 (0.61–2.34)	0.61
PIVKA-II (ng/mL) (<54 *vs*. ≥54)	0.73 (0.34–1.78)	0.55	1.18 (0.74–1.89)	0.50	1.32 (0.83–2.07)	0.24
Curability (A, B *vs*. C)	1.12 (.012–2.90)	0.08	4.22 (1.23–14.48)	0.02	4.55 (1.34–15.53)	0.02

Prognosis: alive without recurrence (N=91), alive with HCC recurrence (N=24), death from HCC (N=9), and other related deaths (N=5). RR, risk ratio; CI, confidence interval; HCC, hepatocellular carcinoma; M2BPGi, Mac2 binding protein glycosylation isomer; COI, cut-off index; PIVKA-II, protein induced by vitamin K absence or antagonist II.

uncontrolled post-hepatectomy ascites was identified as an independent risk factor in this study (8). We also reported that serum HA, a hepatic fibrosis marker, is associated with poor survival, and an increased serum HA level is known to reflect non-parenchymal liver dysfunction or the possibility of post-hepatectomy uncontrolled ascites (11). HA and its protein complex are increased in HCC and other malignancies (32,33). The mechanism of the relationship between HA and tumor progression has not been fully elucidated; however, physiological stress in the liver parenchyma may stimulate the production of liver-derived growth factors and induce subsequent tumor progression (34). Based on these results, it is hypothesized that increased liver fibrosis and its related biomarkers enhance the progression of liver dysfunction and remnant cancer progression or new multiple carcinogenesis of the liver, which would influence the prognosis of patients with HCC by physiological stress after hepatectomy.

Key findings

In the present study, five fibrotic parameters plus platelet count, which are routinely examined in every patient with liver disease, were retrospectively examined in consecutive 130 consecutive patients with HCC, with a minimum follow-up period of one year after hepatectomy, to clarify the relationship between these parameters and patient prognosis. First, although it was clarified that lower platelet counts and higher HA or M2BPGi were obviously increased in histological cirrhosis (stage 4) in comparison with stage 0–3, serum levels of T4C7, APRI, and Fib-4 index were not correlated in contrast to previous reports (35). This inconsistent result is unclear, but high T4C7 levels are significantly associated with shorter survival and tumor relapse rates. T4C7 is common in various tumors, and a positive correlation between T4C7 expression and tumor metastasis has been reported (36,37). Native T4C7 induced an epithelial-to-mesenchymal transition-like process, thereby increasing matrix metalloproteinase-2, focal adhesion kinase, and nuclear factor kappa-light-chain-enhancer of activated B cells activation, cell migration, and invasion in MCF10A human mammary epithelial cells, which were suitable for HCC progression (38). Thus, beyond the influence of hepatic biochemical markers, type IV collagen may be related to not only degree of liver fibrosis or hepatocyte damage but also cancer-specific progressive parameters under above oncological point of view, contrary to our expectations.

With respect to patient survival in our present study, the overall survival progressed due to improved liver function or nutritional status, leading to longer survival due to the recent development of management of patients with chronic

liver diseases, such as anti-inflammatory drug therapy (39,40). The multivariable survival analysis by selecting the parameters identified using univariate analysis showed some knowledge of the present results. Regarding cancer-free survival, larger tumor size was an independent factor. Multiple HCC, curability C, and use of intraoperative blood transfusion tend to be associated; thus, tumor-related factors or surgical quality might influence shorter periods of cancer relapse (1-5). Among the non-tumor-related factors, higher type IV collagen, grade B liver damage, and blood transfusion were independent risk factors for overall and cancer-specific survival. Allogeneic blood transfusion was still a poor prognostic factor in our previous studies among patients with HCC undergoing hepatectomy (8,41). Although the mechanism underlying poor prognosis following blood transfusion has not been fully elucidated, increased blood loss may be a cause for transfusion. Various attempt to reduce blood loss by using hemostatic devices, IVC half-clamping, or Trendelenburg position and so on have been our long-term policy and, therefore, prediction of liver fibrosis is one of important parameter before hepatectomy (42,43). Thus, to achieve no blood transfusion is always aiming in our proposition. Please realize this issue. It has been speculated that liver injury stress or transfusion-induced immunodeficiency may occur (44). Nevertheless, a decrease in blood loss or transfusion should be avoided during the initial hepatectomy in HCC patients with impaired liver function. Careful follow-up over a short period or adjuvant drug therapy is required in patients with severe liver damage (grade B liver damage). The clinical significance of higher T4C7 levels for prognosis in patients with HCC undergoing hepatectomy was clearly elucidated in this study; therefore, the progressive oncological mechanism of this factor should be clarified in the next step, as well as the degree of tissue damage or fibrosis of background liver cells (45).

Strengths and limitations

Strengths were supposed to be the first report by the comprehensive analysis using liver fibrotic markers to analyze the long-term prognosis in HCC patients to our knowledge. This result may be contributed that the HCC patients died not only tumor progression but also progressive background liver damage and its related decreased functional liver reserve. Limitations were considered as follows: (I) this study was performed among a consecutive cohort with a relatively small number of participants (130 patients with HCC); (II) we did not perform elastography evaluation for detecting the degree of hepatic fibrosis in all the patients with HCC in this study, which seems to be a more reliable and less-invasive parameter for evaluating fibrosis and surgical outcomes (19); (III) the relationship between the precise histological mechanism of liver fibrosis and each parameter was not clarified, regardless of fibrotic markers in each because T4C7 was not significantly correlated with other fibrosis markers in our results; and (IV) already-known specific biomarkers for HCC, such as AFP or PIVKA-II, are not independent risk parameters for survival. In our study, the median values of AFP and PIVKA-II were relatively too lower (5 and 54, respectively) than those in other studies; thus, these tumor markers might not affect patient prognosis in comparison to background liver injury or fibrotic markers. However, these unexpected and contradictory results need to be confirmed in a larger number of participants.

Conclusions

By the comprehensive prognostic analysis focusing liver fibrotic parameters, Type IV collagen 7S (T4C7) may reflect not only impaired liver function but also HCC malignant behaviors and patient survivals. To clarify both the cancer-associated mechanisms and liver injury associated with type IV collagen 7S, future studies with larger number of patients are necessary.

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uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-94/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Human Ethics Review Board of the University of Miyazaki Faculty of Medicine (approval number: O-1469; December 22, 2023). Informed consent was obtained from all individual participants prior to treatment, and for their data to be used for research purposes. Informed consent was obtained by the opt-out procedure at the university website but no disclaimer.

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