Enhanced liver fibrosis score as a predictive marker for hepatocellular carcinoma development after hepatitis C virus eradication

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Abstract. Advanced liver fibrosis is the most important risk factor for hepatocellular carcinoma (HCC) development after achieving sustained virological response (SVR) by direct-acting antiviral (DAA) treatment in patients with chronic hepatitis C. *Wisteria floribunda* agglutinin-positive Mac-2-binding protein (M2BPGi), enhanced liver fibrosis (ELF) score, type IV collagen and fibrosis-4 (FIB-4) index have been reported as non-invasive biomarkers for liver fibrosis. In the present study, the possibility of using fibrosis biomarkers and other parameters to predict the development of HCC was evaluated. A total of 743 patients infected with hepatitis C virus who achieved SVR by using DAA were retrospectively enrolled. Of these, 122 patients whose blood samples were stored were selected. The aforementioned four fibrosis biomarkers were analyzed at baseline, at the end of treatment (EOT) and at post-treatment week 24 (PTW24). Tumor markers and laboratory tests were also analyzed. The baseline/EOT/PTW24 values for each fibrosis biomarker were as follows: ELF score: 11.5±1.2/10.8±1.1/10.4±1.0; type IV collagen: 213±85/190±67/174±55 ng/ml; M2BPGi: 4.8±3.5/2.7±2.0/2.2±1.8; and FIB-4 index: 5.31±3.82/4.36±2.79/4.24±3.09. Of the 122 patients, 23 developed HCC. A high baseline ELF score (P=0.0264), PTW24 ELF score (P=0.0003), PTW24 α-fetoprotein level (P=0.0133), baseline FIB-4 index (P=0.0451) and low baseline prothrombin time (P=0.0455) were risk factors for HCC development based on univariate analyses. Based on the multivariate analysis, a high PTW24 ELF score was the only risk factor for HCC development (P=0.0035). The ELF score after DAA therapy was strongly associated with HCC development; therefore, it may be a useful marker for predicting HCC.

Introduction

The advent of direct-acting antivirals (DAAs) revolutionized the treatment of hepatitis C virus (HCV) and high sustained virological response (SVR) rates may be achieved. The SVR rates were reported to be 89.9% with daclatasvir/asunaprevir (1), 95.8% with sofosbuvir/ribavirin (2), and 98.5% with sofosbuvir/ledipasvir (3). Accumulating evidence suggests that SVR with DAA treatment reduces the incidence of hepatocellular carcinoma (HCC) development (4-6). However, even after achieving SVR, some patients may develop HCC.

Advanced liver fibrosis was reported to be the most important risk factor for HCC development after SVR (7); therefore, evaluation of the degree of liver fibrosis is important. Regarding the evaluation of fibrosis, non-invasive methods (serum markers or transient elastography) were recently adopted instead of invasive liver biopsy. Representative fibrosis markers include type IV collagen (8), *Wisteria floribunda* agglutinin-positive Mac-2-binding protein (M2BPGi) (9), and fibrosis-4 (FIB-4) index (10,11). On the other hand, an enhanced liver fibrosis (ELF) score composed of three liver fibrosis markers was developed to evaluate liver fibrosis (12). The ELF score was confirmed to be useful in patients with non-alcoholic fatty liver (13), primary biliary cholangitis/cirrhosis (14) and chronic hepatitis C (15). It was recently reported that the ELF score was comparable with transient elastography in detecting advanced fibrosis (F≥3) in treatment-naïve patients with chronic HCV infection (16). In addition, the usefulness of ELF score as a predictor of HCC in the general population,

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Abbreviations: AFP, α-fetoprotein; DAA, direct-acting antiviral; ELF score, enhanced liver fibrosis score; FIB-4 index, fibrosis-4 index; HCC, hepatocellular carcinoma; M2BPGi, *Wisteria floribunda* agglutinin-positive Mac-2-binding protein; SVR, sustained virological response

Key words: hepatocellular carcinoma, chronic hepatitis C, sustained virological response, direct-acting antiviral, enhanced liver fibrosis score

particularly in predicting non-viral-related HCC, was previously reported (17).

Among these fibrosis markers, M2BPGi and FIB-4 index were reported to be useful markers for the risk of HCC development after HCV eradication (18,19). On the other hand, other than fibrosis markers, α-fetoprotein (AFP) was also reported to be useful for predicting HCC development after HCV eradication (20). However, to the best of our knowledge, there is no report confirming the usefulness of the ELF score for predicting HCC development after HCV eradication. The aim of the present study was to assess fibrosis markers, including ELF score, type IV collagen, M2BPGi and FIB-4 index, tumor markers, and biochemical tests associated with HCC development after viral eradication. The time course of the changes in fibrosis markers during and after DAA treatment was also examined.

Materials and methods

Subjects. Patients with chronic hepatitis C or liver cirrhosis from three hospitals in Japan (Kurume University Hospital, Yame General Hospital and Chikugo City Hospital) who were initiated on DAA therapy between October 2014 and September 2016 were selected. A total of 999 patients with chronic hepatitis C or liver cirrhosis were treated with DAA (daclatasvir plus asunaprevir, or ledipasvir plus sofosbuvir, or sofosbuvir plus ribavirin). SVR was achieved in 743 patients. The diagnosis of liver cirrhosis was comprehensively made based on biochemical test results, imaging findings and physical findings on a case-by-case basis. Among the patients who achieved SVR, 122 patients (50 male and 72 female patients, with a mean age ± SD of 68.7±8.8 years; range, 32-83 years) whose blood samples were stored were enrolled (Fig. 1). All the patients were positive for HCV antibody as determined using chemiluminescence immunoassay (Architect®; Abbott Japan Co., Ltd.). The HCV RNA levels were measured using a COBAS Taq Man test (Roche Diagnostics). Patients who had hepatitis B surface antigen, a history of HCC prior to DAA therapy, or developed HCC within 24 weeks after DAA therapy were excluded. Imaging surveillance (ultrasonography, computed tomography or magnetic resonance imaging) were undertaken every 3-6 months. Patients were followed up until HCC development or the last visit before October 2018. The mean observation period was 2.7 years after the initiation of DAA therapy. The present study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committees of Kurume University School of Medicine (approval no. 14178), Yame General Hospital (approval no. 19-005), and Chikugo City Hospital (approval no. 2019-09). Written informed consent was obtained from all patients.

DAA therapy. The HCV treatment regimen of daclatasvir (60 mg once daily) plus asunaprevir (100 mg twice daily) for 24 weeks, or ledipasvir (90 mg) plus sofosbuvir (400 mg) for 12 weeks, was administered to patients with HCV genotype 1. Sofosbuvir (400 mg) plus ribavirin (weight-based dosing: 600 mg daily for patients weighing ≤60 kg, 800 mg daily for patients weighing >60 and ≤80 kg, and 1,000 mg daily for patients weighing >80 kg) for 12 weeks was administered to patients with HCV genotype 2. SVR was defined as undetectable serum HCV RNA at 24 weeks after completing DAA therapy.

Table I. Baseline characteristics of patients (n=122) with chronic hepatitis C.

Characteristics	Values
Male, n (%)	50 (41.0)
Cirrhosis/chronic hepatitis, n	36/86
Genotype (1/2), n	119/3
Treatment regimen, n	
Daclatasvir + asunaprevir	113
Ledipasvir + sofosbuvir	6
Sofosbuvir + ribavirin	3
Age (years)	68.7±8.8
Aspartate aminotransferase (U/l)	53±27
Alanine aminotransferase (U/l)	51±36
Albumin (g/dl)	3.9±0.4
Total bilirubin (mg/dl)	0.8±0.3
Prothrombin time (%)	91±16
Platelet count ($x10^4/\mu l$)	12.4±5.0
α-Fetoprotein (ng/ml)	16.9±30.5
Des-γ-carboxy prothrombin (mAU/ml)	20.4±18.1

Values are presented as mean ± SD, unless otherwise indicated.

Liver fibrosis markers. Four fibrosis biomarkers (type IV collagen, M2BPGi, FIB-4 index and ELF score) were assessed in 122 patients. For all patients, the biomarkers were analyzed at baseline, at the end of treatment (EOT) and at post-treatment week 24 (PTW24). Type IV collagen was measured using a JCA-BM 8000 series automated immune analyzer (Japan Electron Optics Laboratory Ltd.). M2BPGi was measured using a HISCL-5000 (Sysmex Corporation). The FIB-4 index was calculated as follows: Age (years) x aspartate aminotransferase (AST; U/l)/platelet count (10^9/l) x alanine aminotransferase (ALT; U/l)$^{1/2}$ (10). The ELF score consists of three fibrosis markers: Hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase type-1 (TIMP-1). The ELF score (12) was measured using an ADVIA Center XP automated immunoanalyzer and calculated automatically using the following equation: ELF score=2.278 + 0.851 $\ln(C_{HA})$ + 0.751 $\ln(C_{PIIINP})$ + 0.394 $\ln(C_{TIMP1})$. Three biomarkers (type IV collagen, M2BPGi and ELF score) were measured using stored blood samples. All collected blood samples were stored at -30˚C until analysis.

Parameters associated with HCC. The following parameters were analyzed to identify the factors associated with HCC: Sex, cirrhosis, age, type IV collagen, M2BPGi, FIB-4 index, ELF score, AST, ALT, γ-glutamyl transpeptidase, albumin, total bilirubin, prothrombin time, platelets, AFP and des-γ-carboxy prothrombin. For all patients, all parameters were assessed at baseline and PTW24.

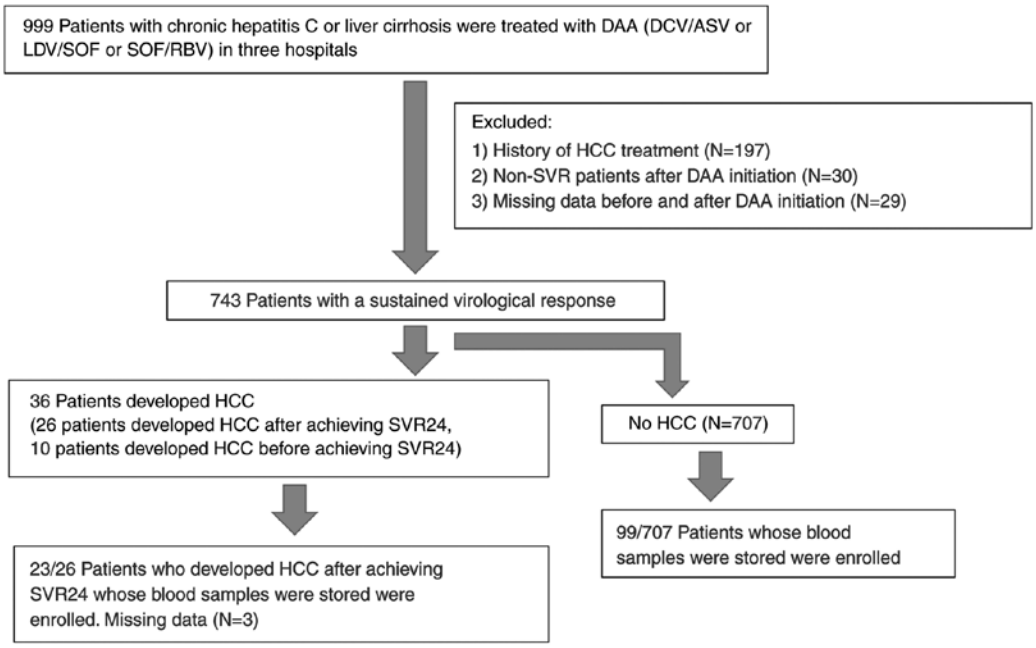

Figure 1. Flow chart of subject inclusion in the study. DAA, direct-acting antiviral; DCV, daclatasvir; ASV, asunaprevir; LDV, ledipasvir; SOF, sofosbuvir; RBV, bibavirin; HCC, hepatocellular carcinoma; SVR, sustained virological response; SVR24, SVR at 24 weeks.

Statistical analysis. Statistical analysis was performed using the JMP software package (release 13; SAS Institute, Inc.). Mean values and SDs were calculated for continuous data. For comparison of variables, the Wilcoxon signed-rank test was performed as appropriate. Factors associated with HCC risk were determined using the Cox proportional hazard regression analysis. $P<0.05$ was considered to indicate statistically significant. Diagnostic accuracy was assessed using time-dependent receiver operating characteristics (ROC) curves by examining the area under the ROC curve (AUROC).

Results

Patient characteristics. The characteristics of the patients are summarized in Table I. Of the 122 patients, 50 (41%) were male and 72 (59%) were female, with a mean age of 68.7±8.8 years. A total of 36 (30%) patients were diagnosed with cirrhosis clinically.

Changes in fibrosis biomarkers and their association with HCC risk. The baseline/EOT/PTW24 fibrosis biomarkers (type IV collagen, M2BPGi, FIB-4 index and ELF score including its individual components, namely HA, PIIINP and TIMP-1) are shown in Table II. There was a significant decrease in all biomarkers at PTW24 compared with those at baseline ($P<0.0001$). First, the correlations among the four fibrosis markers were investigated. As a result, correlations were identified among the four fibrosis markers, albeit weak. The strongest correlation observed was between M2BPGi and type IV collagen ($r=0.5998$). A scatterplot matrix is shown in Fig. 2. Second, factors associated with the risk for HCC development were investigated (Table III). Of the 122 patients, 23 (19%) developed HCC. A high baseline ELF score ($P=0.0264$), PTW24 ELF score ($P=0.0003$), baseline FIB-4 index ($P=0.0451$), PTW24 AFP level ($P=0.0133$), and baseline prothrombin time ($P=0.0455$) were identified as risk factors for HCC development based on the univariate analyses. A multivariate analysis was performed using the four factors that were found to be significant in the univariate analysis: PTW24 ELF score, baseline FIB-4 index, PTW24 AFP level and baseline prothrombin time. Based on the multivariate analysis, a high PTW24 ELF score was the only significant risk factor for HCC development ($P=0.0035$; hazard ratio=1.89; 95% confidence interval: 1.24-2.85).

Diagnostic accuracy of fibrosis biomarkers for predicting HCC development. As the ELF score is composed of three fibrosis markers, it was examined which component was mostly involved. The diagnostic accuracy of the PTW24 ELF score, PTW24 HA, PTW24 PIIINP and PTW24 TIMP-1 for predicting HCC development is shown in Table IV. The cut-off value of the PTW24 ELF score of 10.96 had a sensitivity of 65.2% and specificity of 82.8% for predicting HCC development. PTW24 TIMP-1 had a high sensitivity (100%) but low specificity (44.4%). The AUROC of PTW24 ELF and PTW24 TIMP-1 was 0.75 and 0.76, respectively. The ROC curves of PTW24 ELF, PTW24 HA, PTW24 PIIINP and PTW24 TIMP-1 are shown in Fig. 3.

Discussion

The objective of the present study was to measure fibrosis markers, tumor markers and biochemistry parameters in patients with chronic hepatitis C who achieved SVR with DAA therapy in order to identify useful markers for predicting HCC development. The ELF score at 24 weeks after the completion of DAA therapy was demonstrated to be such a marker. In addition, the time course of the changes in fibrosis markers during DAA therapy was investigated, and the levels of all the markers decreased after the completion of therapy.

Table II. Changes in biomarkers measured at baseline, end of treatment, and at 24 weeks after DAA therapy. P-values are for the comparison between baseline and 24 weeks after treatment.

Biomarkers	Baseline	End of treatment	24 weeks after treatment	P-value
Type IV collagen (ng/ml)	213±85	190±67	174±55	<0.0001
M2BPGi	4.8±3.5	2.7±2.0	2.2±1.8	<0.0001
FIB-4 index	5.31±3.82	4.36±2.79	4.24±3.09	<0.0001
ELF score	11.5±1.2	10.8±1.1	10.4±1.0	<0.0001
HA (ng/ml)	534±780	350±687	224±272	<0.0001
PIIINP (ng/ml)	15.3±7.7	12.7±8.3	10.6±5.3	<0.0001
TIMP-1 (ng/ml)	338±291	248±74	233±69	<0.0001

Values are presented as mean ± SD. M2BPGi, *Wisteria floribunda* agglutinin-positive Mac-2-binding protein; ELF, enhanced liver fibrosis score; FIB-4, Fibrosis-4; HA, hyaluronic acid; PIIINP, amino-terminal propeptide of type-III procollagen; TIMP-1, tissue inhibitor of metalloproteinase type-1.

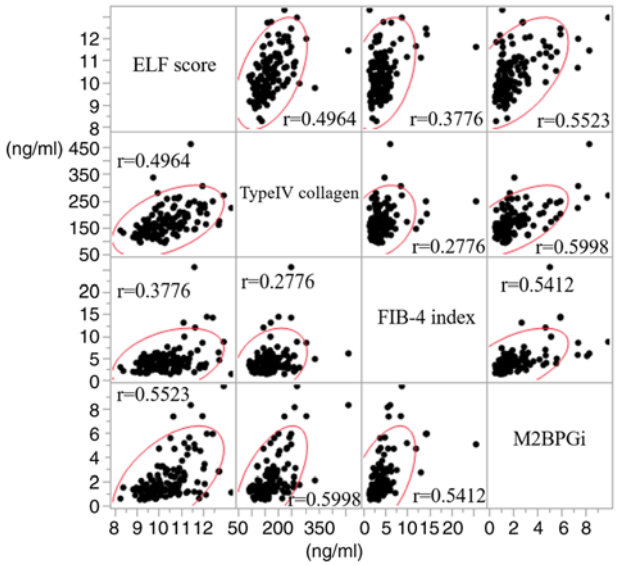

Figure 2. Scatterplot matrix of the correlations among the four fibrosis markers. The correlations among the four fibrosis markers were found to be weak. ELF, enhanced liver fibrosis; M2BPGi, *Wisteria floribunda* agglutinin-positive Mac-2-binding protein; FIB-4, Fibrosis-4.

As regards important markers for the prediction of HCC development after SVR, among fibrosis markers, M2BPGi (9) after the completion of DAA therapy was reported to be useful (18,21). In addition, a pre-FIB-4 index of 3.25 was previously shown to exhibit a significant association with HCC development (19). In addition to fibrosis markers, the usefulness of AFP after treatment was also previously reported (20).

Therefore, M2BPGi, FIB-4 index and AFP, which were previously reported to be useful for predicting HCC development after SVR, were investigated in the present study. However, only the ELF score at 24 weeks after treatment was extracted by multivariate analysis and demonstrated to be the most useful marker for predicting HCC development.

The ELF score (12) is calculated from 3 hepatic fibrosis markers, HA, PIIINP, and TIMP-1, and these markers have been reported to be useful for evaluating fibrosis in varying etiologies of chronic liver disease such as non-alcoholic fatty liver disease (NAFLD), primary biliary cholangitis/cirrhosis and chronic hepatitis C (13-15). HA is a glycosaminoglycan involved in fibrogenesis by hepatic stellate cells, PIIINP is a marker of inflammation and early fibrogenesis, and TIMP-1 inhibits fibrinolysis, thereby increasing fiber deposition. The usefulness of HA, PIIINP and TIMP-1 as fibrosis markers in NAFLD was previously reported (22-24). Furthermore, HA, PIIINP and TIMP-1 have all been reported to be useful for the evaluation of fibrosis in chronic hepatitis C (25-27). Therefore, an increase in HA, PIIINP and TIMP-1 levels is considered to reflect fibrosis in NAFLD and chronic hepatitis C. Individual parameters may coincidentally increase due to the presence of other diseases; however, evaluation of liver fibrosis based on the ELF score is stable, as this score is calculated by summing up all three fibrosis markers (12). This may be the reason why only the ELF score was identified as the predictor of HCC development by multivariate analysis.

The longitudinal change in fibrosis markers during DAA therapy were next investigated, and all biomarkers were found to be significantly decreased 24 weeks after DAA therapy.

In patients who achieved SVR by DAA therapy, M2BPGi (28), ELF score (29) [and its components HA (28,29) and TIMP-1 (29)], type IV collagen (28) and FIB-4 index (30-32) were found to be significantly decreased after therapy, consistent with the findings of the present study. In our study, PIIINP also significantly decreased after therapy. The decreases in the levels of these fibrosis markers may be due to the improvement of fibrosis. On the other hand, regarding the improvement of fibrosis stage based on histological examination of liver biopsy, it improves by 0.282 stages per year in patients who achieve SVR by IFN therapy (33), indicating that a long time is required for the improvement of liver fibrosis at the tissue level. The fibrosis markers investigated in the present study had decreased by 10-58% at 24 weeks after the end of therapy. The reduction rate in the levels of fibrosis markers from baseline to the end of therapy was higher compared with that from the end of therapy to 24 weeks after the end of therapy, suggesting that the decrease in fibrosis markers from baseline to the end of DAA therapy may reflect both inflammation and

Table III. Factors associated with the risk for HCC development.

			Univariate analysis			Multivariate analysis
Factors	HCC^+ (n=23)	HCC^- (n=99)	HR	95% CI	P-value	P-value, HR, 95% CI
Sex (male/female), n	10/13	40/59	1.17	0.50-2.67	0.707	
Liver cirrhosis/chronic hepatitis, n	9/14	27/72	1.57	0.65-3.59	0.299	
Age, years	69.6±7.3	68.5±9.0	1.00	0.95-1.06	0.915	
Pre AST (U/l)	64.1±38.5	50.9±22.7	1.01	1.00-1.02	0.070	
PTW24 AST (U/l)	28.9±10.2	26.9±9.0	1.03	0.99-1.08	0.153	
Pre ALT (U/l)	61.3±49.8	48.9±31.4	1.01	1.00-1.01	0.210	
PTW24 ALT (U/l)	19.6±8.6	19.6±11.5	1.01	0.97-1.05	0.581	
Pre γ-GTP (U/l)	47±32	40±40	1.01	1.00-1.02	0.100	
PTW24 γ-GTP (U/l)	32±25	26±27	1.01	1.00-1.02	0.155	
Pre Alb (g/dl)	3.8±0.4	4.0±0.4	0.39	0.14-1.07	0.067	
PTW24 Alb (g/dl)	4.2±0.3	4.3±0.3	0.48	0.16-1.53	0.210	
Pre T.Bil (mg/dl)	0.9±0.3	0.8±0.3	1.34	0.33-4.50	0.669	
PTW24 T.Bil (mg/dl)	0.8±0.3	0.9±0.3	0.39	0.07-1.61	0.215	
Pre PT[a] (%)	84.6±14.8	92.5±15.6	0.97	0.95-1.00	0.0455	
PTW24 PT (%)	87.8±15.4	92.4±13.9	0.98	0.95-1.01	0.217	
Pre Plt ($x10^4/\mu l$)	11.2±5.2	12.5±4.7	0.95	0.86-1.04	0.254	
PTW24 Plt ($x10^4/\mu l$)	12.0±5.6	13.1±4.3	0.96	0.87-1.05	0.388	
Pre AFP (ng/ml)	23.7±39.4	15.2±27.8	1.00	0.99-1.01	0.522	
PTW24 AFP[a] (ng/ml)	8.1±11.0	4.4±2.6	1.06	1.02-1.09	0.0133	
Pre DCP (mAU/ml)	20.1±8.3	20.5±19.6	1.01	0.97-1.04	0.457	
PTW24 DCP (mAU/ml)	23±10	21±16	1.03	1.00-1.05	0.0758	
Pre type IV (collagen (ng/ml)	225±62	210±89	1.00	1.00-1.01	0.522	
PTW24 type IV collagen (ng/ml)	192±42	169±56	1.00	1.00-1.01	0.120	
Pre M2BPGi	5.43±3.02	4.69±3.58	1.05	0.93-1.16	0.417	
PTW24 M2BPGi	2.98±1.61	2.05±1.76	1.18	0.98-1.39	0.0764	
Pre FIB-4 index[a]	7.2±6.1	4.9±2.9	1.08	1.00-1.15	0.0451	
PTW24 FIB-4 index	5.6±5.0	3.9±2.3	1.09	0.99-1.17	0.0879	
Pre ELF score	12.0±1.1	11.3±1.2	1.47	1.05-2.10	0.0264	
PTW24 ELF score[a]	11.2±1.0	10.3±1.0	1.96	1.38-2.77	0.0003	0.0035, 1.89, 1.24-2.85
PTW24 HA (ng/ml)	414±434	180±193	1.00	1.00-1.00	0.0007	
PTW24 PIIINP (ng/ml)	13.4±5.9	10.0±5.0	1.08	1.02-1.13	0.009	
PTW24 TIMP-1 (ng/ml)	277±63	223±66	1.01	1.00-1.01	0.0033	

[a]Multivariate analysis was performed using the four factors which were found to be significant in the univariate analysis: PTW24 ELF score, baseline FIB-4 index, PTW24 AFP level and baseline PT. Values are presented as mean ± SD unless otherwise indicated. HCC, hepatocellular carcinoma; Pre, before treatment; PTW24, post-treatment week 24; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GTP, glutamyl transpeptidase; Alb, albumin; T.Bil total bilirubin; PT, prothrombin time; Plt, platelet count; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; ELF, enhanced liver fibrosis; M2BPGi, *Wisteria floribunda* agglutinin-positive Mac-2-binding protein; FIB-4, Fibrosis-4; HA, hyaluronic acid; PIIINP, amino-terminal propeptide of type-III procollagen; TIMP-1, tissue inhibitor of metalloproteinase type-1.

fibrosis in liver tissue. Indeed, in a previous study comparing the degree of liver fibrosis and fibrosis markers in patients with chronic hepatitis C, the ELF score, M2BPGi and FIB4-index were considered to reflect both fibrosis and inflammation (34). These findings indicate that the fibrosis markers at PTW24 may reflect the fibrosis level with greater precision. Previous reports have demonstrated that hepatocarcinogenesis was closely associated with the degree of liver fibrosis rather than that of inflammation (35-37). Therefore, the ELF score at PTW24 was selected as a predictor of HCC development after HCV eradication.

As regards the limitations of the present study, the total number of patients in the study was relatively small, but the HCC development rate was high (19%). Age and cirrhosis were not found to be associated with HCC development, although these are known risk factors associated with the development of this type of cancer. In addition, a pre-FIB-4 index of 3.25 was previously found to exhibit a significant

Table IV. Diagnostic accuracy of the PTW24 ELF score, PTW24 HA, PTW24 PIIINP and PTW24 TIMP-1 for predicting hepatocellular carcinoma development.

Diagnostic measures	Cut-off	Sensitivity	Specificity	AUC	PPV	NPV	P-value
PTW24 ELF score	10.96	0.652	0.828	0.749	0.469	0.911	0.0002
PTW24 HA (ng/ml)	153.6	0.783	0.657	0.733	0.346	0.928	0.0009
PTW24 PIIINP (ng/ml)	10.5	0.783	0.687	0.717	0.367	0.932	0.0104
PTW24 TIMP-1 (ng/ml)	199.1	1	0.444	0.762	0.295	1	0.0013

PTW24, post-treatment week 24; ELF, enhanced liver fibrosis; HA, hyaluronic acid; PIIINP, amino-terminal propeptide of type-III procollagen; TIMP-1, tissue inhibitor of metalloproteinase type-1; AUC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.

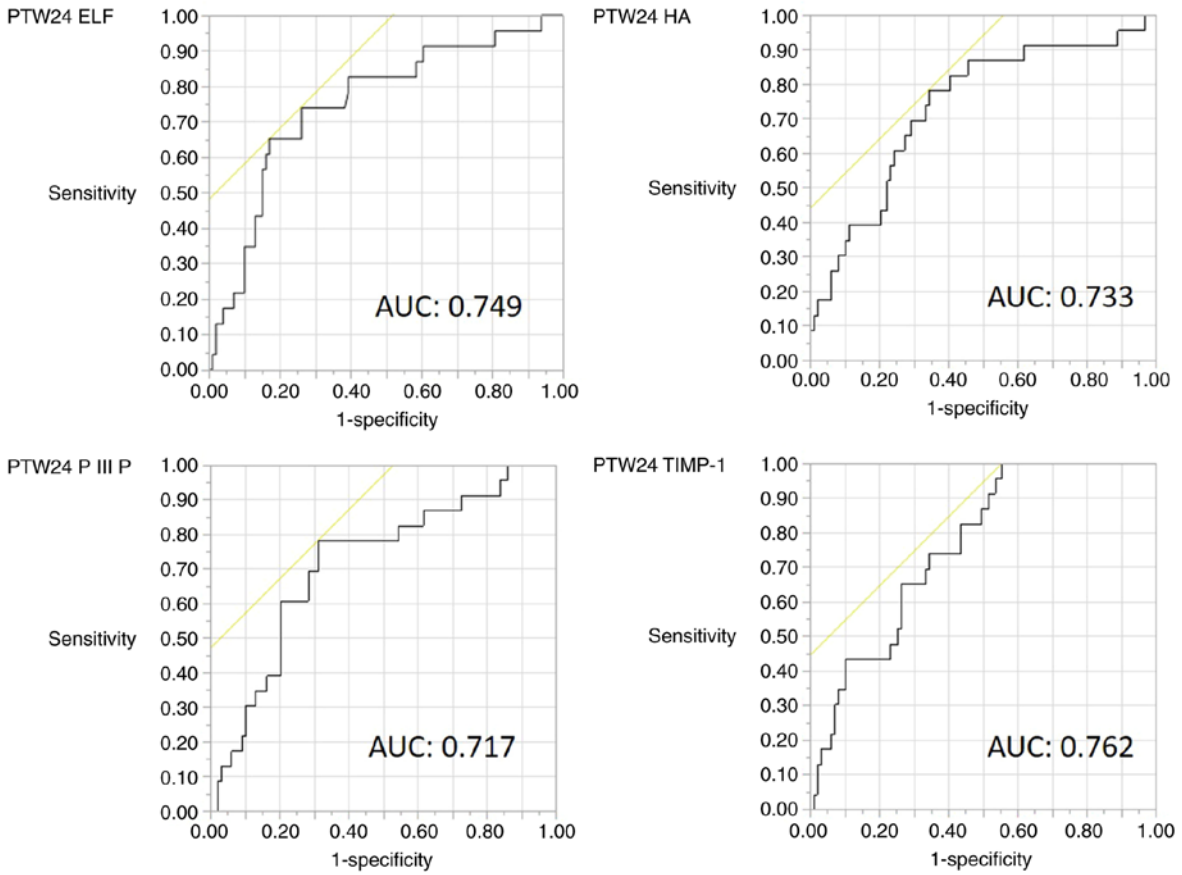

Figure 3. ROC curve of PTW24 ELF, PTW24 HA, PTW24 PIIINP and PTW24 TIMP-1 for prediction of hepatocellular carcinoma. AUC, area under the ROC curve; ROC, receiver operating characteristic; ELF, enhanced liver fibrosis; HA, hyaluronic acid; TIMP-1, tissue inhibitor of metalloproteinase type-1; PIIINP, amino-terminal propeptide of type-III procollagen; PTW24, post-treatment week 24.

association with HCC development (19). Finally, there may exist selection bias, as only patients with stored serum samples were selected.

In conclusion, the most useful parameter for predicting hepatocarcinogenesis after DAA therapy for chronic hepatitis C was found to be the ELF score at 24 weeks after therapy. In addition, the four investigated fibrosis markers decreased after DAA therapy. The number of patients who achieve SVR by DAA therapy is expected to increase in the future; therefore, the incidence of HCC development after SVR is also expected to increase. Although DAA administration improves inflammation and fibrosis, careful follow-up is required for patients with a high ELF score ≥10.96 at 24 weeks after DAA therapy owing to the high risk for HCC development.

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Availability of data and materials

The datasets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TK and TI contributed to the study concept and design; TK, TI, KA, TAH, RK, TS, SM, NO and TT contributed to data acquisition and analysis; TT revised the manuscript. TK and TI have seen and can confirm the authenticity of the raw data. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was reviewed and approved by the Ethics Committee of Kurume University School of Medicine (approval no. 14178), Yame General Hospital (approval no. 19-005), and Chikugo City Hospital (approval no. 2019-09). Written informed consent was obtained from all the patients enrolled in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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