Hepatocellular Carcinoma Risk Assessment for Patients With Advanced Fibrosis After Eradication of Hepatitis C Virus

Nobuharu Tamaki,1,2 Masayuki Kurosaki,1 Yutaka Yasui,1 Nami Mori,3 Keiji Tsuji,3 Chitomi Hasebe,4 Kouji Joko,5 Takehiro Akahane,6 Koichi Furuta,7 Haruhiko Kobashi,8 Hiroyuki Kimura,9 Hitoshi Yasisawa,10 Hiroyuki Marusawa,11 Masahiko Kondo,12 Yuji Kojima,13 Hideo Yoshida,14 Yasushi Uchida,15 Toshifumi Tada,16 Shinichiro Nakamura,16 Satoshi Yasuda,17 Hidenori Toyoda,17 Rohit Loomba16,2 and Namiki Izumi1

The identification of patients with advanced fibrosis who do not need any further hepatocellular carcinoma (HCC) surveillance after the eradication of hepatitis C is pivotal. In this study, we developed a simple serum-based risk model that could identify patients with low-risk HCC. This was a nationwide multicenter study involving 16 Hospitals in Japan. Patients with advanced fibrosis (1,325 in a derivation cohort and 508 in a validation cohort) who achieved sustained virological responses at 24 weeks after treatment (SVR24) were enrolled. The HCC risk model at any point after SVR24 and its change were evaluated, and subsequent HCC development was analyzed. Based on the multivariable analysis, patients fulfilling all of the factors (GAF4 criteria: gamma-glutamyl transferase < 28 IU/L, alpha-fetoprotein < 4.0 ng/mL, and Fibrosis-4 Index < 4.28) were classified as low-risk and others were classified as high-risk. When patients were stratified at the SVR24, and 1 year, and 2 years after SVR24, subsequent HCC development was significantly lower in low-risk patients (0.5-1.1 per 100 person-years in the derivation cohort and 0.9-1.1 per 100 person-years in the validation cohort) than in high-risk patients at each point. HCC risk from 1 year after SVR24 decreased in patients whose risk improved from high-risk to low-risk (HCC incidence: 0.6 per 100 person-years [hazard ratio (HR) = 0.163 in the derivation cohort] and 1.3 per 100 person-years [HR = 0.239 in the validation cohort]) than in those with sustained high risk. Conclusion: The HCC risk model based on simple serum markers at any point after SVR and its change can identify patients with advanced fibrosis who are at low HCC risk, and these patients may be able to reduce HCC surveillance. (Hepatology Communications 2022;6:461-472).

Hepatitis C virus infection could lead to cirrhosis, hepatocellular carcinoma (HCC) development, and liver failure.1 Direct-acting antiviral (DAA) treatment makes it possible to eradicate the hepatitis C virus in nearly all patients.2-8 The HCC development rate decreases in patients who achieve sustained virological response (SVR), but some patients develop HCC even after SVR.9-13

Abbreviations: AFP, alpha-fetoprotein; DAA, direct-acting antiviral; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; HR, hazard ratio; IQR, interquartile range; ROC, receiver operating characteristic; SVR, sustained virological response; SVR24, sustained virological response at 24 weeks after treatment.

Received July 8, 2021; accepted September 25, 2021.
Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1833/suppinfo.
Supported by Usui Memorial Foundation (2019-0021), Japan Agency for Medical Research and Development (JP20fg0210067b0001), National Center for Advancing Translational Sciences (SUL1TR001442), National Institute of Environmental Health Sciences (5P42ES010337), National Institute of Diabetes and Digestive and Kidney Diseases (U01DK061734, R01DK106419, R01DK121378, and R01DK124318), National Institute on Alcohol Abuse and Alcoholism (U01AA029019), National Heart, Lung, and Blood Institute (P01HL147835), and DOD PRCPR (W81XWH-18-2-0026).
© 2021 The Authors. Hepatology Communications published by Wiley Periodicals LLC on behalf of American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
Patients with advanced fibrosis have a higher risk of HCC development even after SVR; therefore, these patients are recommended to continue HCC surveillance. On the other hand, continuing regular HCC surveillance in all patients with advanced fibrosis is not cost-effective, and identification of patients with low-risk HCC is an important clinical issue. However, a method for identifying patients at low risk of HCC among patients with advanced fibrosis has not been well established. Furthermore, there are studies that assess HCC risk at the time of SVR, but HCC risk changes over time after SVR. Therefore, risk stratification at any point after SVR is necessary, and studies investigating the association between HCC development and risk stratification at any point after SVR are limited.

Serum tests and serum-based fibrosis markers are widely available; the utility of these markers for the stratification of HCC risk has been previously reported. Furthermore, one advantage of these convenient methods is that they are suitable for repeat measurements that could assess the change in HCC risk. However, there are limited data that identify patients at low risk of HCC by serum markers and the association between change in serum markers and change in the risk of HCC development. Hence, in this multicenter cohort study, we developed a simple serum-based risk stratification model that could identify patients at low risk of HCC at any point after SVR and investigated changes in the risk model and changes in the rate of HCC development.

Patients and Methods

STUDY DESIGN

A nation-wide multicenter prospective registry cohort involving 14 institutes from the Japanese Red Cross Hospital Liver Study Group was registered as a derivation cohort. Two institutes were enrolled in the study as a validation cohort after the HCC
A risk model was developed. The study flow chart is shown in Fig. 1. Patients who received DAA treatment from September 2014 to July 2019 were investigated. Patients without advanced fibrosis (defined by Fibrosis-4 Index [FIB-4] < 3.25\(^{21,22}\) or histological fibrosis stage 0–2) before treatment were excluded, and no patient with decompensated cirrhosis at the beginning of the DAA treatment was registered. The following categories of patients were also excluded: (1) those who did not achieve SVR; (2) those who had co-infection of hepatitis B virus or human immunodeficiency virus; (3) those with past history of HCC development; and (4) follow-up periods within 6 months. Patients who may have developed HCC before SVR, and patients who developed HCC within 6 months after SVR at 24 weeks after treatment (SVR24) were excluded (29 patients in the derivation cohort and 22 patients in the validation cohort). Patients with data missing at the entry were also excluded. Finally, 1,325 patients with advanced fibrosis (266 diagnosed by liver biopsy and 1,059 diagnosed by FIB-4 ≥ 3.25) were enrolled in the derivation cohort. Using the same inclusion and exclusion criteria, 508 patients in the validation cohort were registered in the study. The HCC risk model was developed in the derivation cohort using serum markers at SVR24, and subsequent HCC development was examined. The HCC risk model was assessed at 1 year and 2 years after SVR24, and subsequent HCC development was also examined. Furthermore, the association between changes in the HCC risk and the rate of HCC development was investigated. Patients who fulfilled all of the gamma-glutamyltransferase (GGT), alpha-fetoprotein (AFP), and FIB-4 (GAF4) criteria (GGT < 28 IU/L, AFP < 4.0 ng/mL, and FIB-4 < 4.28) were classified as low-risk (detailed in the Results section), and others as high-risk. In the high-risk group at baseline (SVR24), patients who fulfilled the low-risk criteria at the last observation were classified as belonging to the improvement group. Patients who persistently fulfilled the high-risk criteria were classified as belonging to the non-improvement group. Written informed consent was obtained from each patient before enrollment into the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. The study was approved by the institutional ethics review committee (approval number 2022).

**FIG. 1.** Study flow chart. Abbreviations: HBV, hepatitis B virus; and HIV, human immunodeficiency virus.
Hepatology Communications, March 2022

CLINICAL AND LABORATORY DATA

The ages and genders of the patients were recorded at entry into the study. Serum samples were collected at SVR24, and 1, 2, and 3 years after SVR24. The FIB-4 index was calculated according to the following formula: FIB-4 = age [years] × AST [aspartate aminotransferase; IU/L] / (platelets [10^9/L] × ALT [alanine aminotransferase; IU/L]1/2). (21)

HCC SURVEILLANCE AND DIAGNOSIS

Ultrasonography and blood tests, including tests for tumor markers, were performed at the start of DAA treatment and every 3-6 months for HCC surveillance. When tumor marker levels rose abnormally and/or abdominal ultrasonography suggested any lesion suspicious of HCC, contrast-enhanced computed tomography, magnetic resonance imaging, or angiography were performed. HCC was diagnosed for tumors displaying vascular enhancement at the early phase and washout at the later phase according to guidelines published by the American Association for the Study of Liver Diseases (AASLD) and the Japan Society of Hepatology. (23,24) Tumor biopsy was used to diagnose tumors with nontypical imaging findings.

STATISTICAL ANALYSIS

Patient characteristics between the derivation cohort and the validation cohort were compared using Mann-Whitney U test or Fisher’s exact test. A receiver operating characteristic (ROC) curve analysis and Youden index were used to determine an optimal threshold of serum markers for HCC development. The association between HCC development and serum risk factors was evaluated using the Cox proportional hazard model. All serum factors using for the investigation are listed in Tables 1 and 2. Factors with P < 0.05 on univariate analysis were selected for multivariable backward stepwise regression analysis. The cumulative incidence of HCC was evaluated using the Kaplan-Meier method, and the differences between groups were analyzed by the log-rank test. Changes in serum markers were analyzed by the Wilcoxon rank-sum test. Values of P < 0.05 were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Shimototsuke, Japan), (25) a graphical user interface for R version 3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

PATIENT CHARACTERISTICS

A total of 1,325 and 508 patients with advanced fibrosis were enrolled in the derivation cohort and the validation cohort, respectively (Fig. 1). Patient characteristics at SVR24 are provided in Table 1. The median (interquartile range [IQR]) age was 72 (64-77) years in the derivation cohort and 74 (67-79) in the validation cohort. AST, ALT, GGT, and AFP levels were within the upper limit of the normal in both cohorts, and there were no significant differences between the two cohorts. The median (IQR) FIB-4 was 3.41 (2.7-4.6) in the derivation cohort and 3.63 (2.9-4.8) in the validation cohort.

Note: Continuous data are shown in median (IQR). P value indicates difference between the derivation cohort and the validation cohort.

Abbreviations: ALT, alanine aminotransferase; and AST, aspartate aminotransferase.

P < 0.05 were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Shimototsuke, Japan), (25) a graphical user interface for R version 3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1. PATIENT CHARACTERISTICS

|                     | Derivation Cohort | Validation Cohort | P Value |
|---------------------|-------------------|-------------------|---------|
| Age, years          | 72 (64-77)        | 74 (67-79)        | <0.001  |
| Gender, male (%)    | 533 (40.2%)       | 210 (41.3%)       | 0.7     |
| AST, IU/L           | 26 (21-31)        | 26 (22-33)        | 0.2     |
| ALT, IU/L           | 17 (13-23)        | 16 (12-24)        | 0.6     |
| Albumin, g/dL       | 4.2 (4.0-4.6)     | 4.2 (4.0-4.5)     | 0.7     |
| Bilirubin, mg/dL    | 0.8 (0.6-1.0)     | 0.8 (0.6-1.0)     | 0.5     |
| GST, IU/L           | 21 (16-31)        | 22 (16-33)        | 0.09    |
| Platelet counts, 10^9/L | 131 (102-160)    | 129 (102-155)     | 0.2     |
| AFP, ng/mL          | 4.0 (2.7-5.6)     | 3.6 (2.3-6.2)     | 0.2     |
| FIB-4               | 3.41 (2.7-4.6)    | 3.63 (2.9-4.8)    | 0.002   |
| Follow-up, years    | 2.96 (1.9-3.5)    | 3.65 (2.3-4.5)    | <0.001  |

Note: Continuous data are shown in median (IQR).
Hepatology Communications, Vol. 6, No. 3, 2022

TAMAKI ET AL.

periods were 3.65 (2.3-4.5) years, and 54 patients developed HCC during the observation periods in the validation cohort.

HCC RISK MODEL DEVELOPMENT

The association between serum factors at SVR24 and HCC development were investigated in the derivation cohort (Table 2). The threshold value for each marker was determined by ROC analysis and Youden index, and the thresholds of GGT ≥ 28 IU/L, AFP ≥ 4.0 ng/mL, and FIB-4 ≥ 4.28 for HCC development within 3 years after SVR24 were selected. In the univariate analysis, GGT ≥ 28 IU/L, AFP ≥ 4.0 ng/mL, FIB-4 ≥ 4.28, albumin, and bilirubin were significantly associated with HCC development, and these factors were chosen for the multivariable backward stepwise regression analysis. AST, ALT, and platelet counts were not used for the multivariable analysis, as these factors were included in FIB-4. In the multivariable analysis of the validation cohort, GGT ≥ 28 IU/L (HR: 2.57, 95% CI: 1.4-4.6, P = 0.001), AFP ≥ 4.0 ng/mL (HR: 2.36, 95% CI: 1.2-4.5, P = 0.01), and FIB-4 ≥ 4.28 (HR: 2.25, 95% CI: 1.3-3.9, P = 0.003) were independent factors significantly associated with HCC development similar to the derivation cohort. Based on the results, patients fulfilling all of the following GAF4 criteria were classified into the low-risk group: GGT < 28 IU/L, AFP < 4.0 ng/mL, and FIB-4 < 4.28. Others were classified into the high-risk group.

RISK MODEL AND SUBSEQUENT HCC DEVELOPMENT IN ANY YEAR

Patients were stratified into two groups based on data at SVR24, and 1 and 2 years after SVR24; subsequent HCC development was investigated in the derivation cohort. At SVR24, 375 patients (28.3%) were classified into the low-risk group: GGT < 28 IU/L, AFP < 4.0 ng/mL, and FIB-4 < 4.28. Others were classified into the high-risk group.

Table 2. Factors Associated with HCC Development

| Factor                  | Derivation Cohort | Validation Cohort |
|-------------------------|-------------------|-------------------|
|                         | Univariable Analysis | Multivariable Analysis | Multivariate Analysis |
|                         | Hazard Ratio 95% CI | P Value | Hazard Ratio 95% CI | p Value | Hazard Ratio 95% CI | p value |
| AST ≥ 25 IU/L           | 3.02 1.7-5.3 <0.001 |         | 2.57 1.4-4.6 0.001 |
| ALT ≥ 23 IU/L           | 2.03 1.4-2.9 <0.001 |         | 2.36 1.2-4.5 0.01  |
| Albumin ≤ 4.3 g/dL      | 2.28 1.2-4.5 0.02  |         | 2.33 1.5-3.7 <0.001|
| Bilirubin ≥ 1.0 mg/dL   | 2.06 1.3-3.3 0.002  |         | 2.25 1.3-3.9 0.003 |
| GGT ≥ 28 IU/L           | 2.04 1.3-3.2 0.002  | 1.88 1.2-3.0 0.01 | 2.36 1.2-4.5 0.01 |
| Platelet count ≤ 114 (10^9/L) | 2.44 1.5-3.9 <0.001 | 1.97 1.2-3.3 0.01 | 2.25 1.3-3.9 0.003 |
| AFP ≥ 4.0 ng/mL         | 2.23 1.3-3.7 0.002  |         | 2.33 1.5-3.7 <0.001|
| FIB-4 ≥ 4.28            | 2.38 1.5-3.8 <0.001  |         | 2.25 1.3-3.9 0.003 |

Note: Factors with P < 0.05 in the univariate analysis were used for the multivariable analysis. AST, ALT, and platelet counts were not used for the multivariable analysis because these factors were included in FIB-4. The threshold of each factor for HCC development within 3 years was defined by ROC analysis.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; and CI, confidence interval.
and HCC development was 1.0 per 100 person-years in the low-risk group. When patients were stratified using serum markers at 1 year after SVR24, 33.8% of them were classified into the low-risk group. The 1-year, 2-year, and 3-year HCC development rates (starting at 1 year after SVR24) were 1.4%, 1.8% and 3.6%, respectively, in the low-risk group (1.1 per 100 person-years) and 2.0%, 5.1% and 8.7%, respectively, in the high-risk group \((P = 0.01; \text{Fig. 2B})\). Similarly, 37.4% of patients were classified into the low-risk group using serum markers at 2 years after SVR24. The 1-year and 2-year HCC development rates (starting at 2 years after SVR24) were 0.0% and 1.7%, respectively, in the low-risk group (0.5 per 100 person-years) and 3.5% and 6.8%, respectively, in the high-risk group \((P = 0.001; \text{Fig. 2C})\). The cumulative rate of HCC development was significantly lower in patients belonging to the low-risk group (HCC development: 0.5-1.1 per 100 person-years) than in those belonging to the high-risk group at any point in the derivation cohort.

**CHANGES IN HCC RISK AND RATE OF HCC DEVELOPMENT**

Changes in HCC risk and rate of HCC development were investigated in the derivation cohort. In the high-risk group, at entry, patients who fulfilled the low-risk conditions (GAF4 criteria: GGT < 28 IU/L, AFP < 4.0 ng/mL, and FIB-4 < 4.28) at the last observation were classified into the improvement group. Patients who persisted in the high-risk conditions were classified into the non-improvement group. Approximately 21.8% of the high-risk patients improved to the low-risk level and were classified into the improvement group. The 1-year, 2-year, and 3-year incidence of HCC development was 1.1%, 1.1% and 1.1%, respectively, in the improvement group (0.6 per 100 person-years), and 2.3%, 6.0% and 10.9%, respectively, in the non-improvement group \((P = 0.004; \text{Fig. 3A})\). HCC development risk reduced in the improvement group with HR = 0.163 (95% CI: 0.04-0.67, \(P = 0.01\)).

**HCC RISK MODEL IN THE VALIDATION COHORT**

The clinical significance of the HCC risk model was validated in the validation cohort. When patients were stratified using the HCC risk model at SVR24 (Fig. 2D), 1 year after SVR24 (Fig. 2E), and 2 years after SVR24 (Fig. 2F), the cumulative rate of HCC development was significantly lower in patients belonging to the low-risk group than in those belonging to the high-risk group at any point. HCC development of the low-risk group in the stratification as of SVR24, 1 year after SVR24, and 2 years after SVR24 were 0.9, 1.1, and 1.0 per 100 person-years, respectively.

When examined changes in the HCC risk and rate of HCC development in the validation cohort, the 1-year, 2-year, and 3-year incidence of HCC development was 0%, 1.3% and 2.9%, respectively, in the improvement group (1.3 per 100 person-years), and 4.1%, 9.8% and 14.8%, respectively, in the non-improvement group. The HCC development rate was significantly lower in patients with the improvement group than those with the non-improvement group \((P = 0.009; \text{Fig. 3B})\). HCC development risk decreased in the improvement group with HR = 0.239 (95% CI: 0.07-0.78, \(P = 0.02\)).

**CHANGES IN VARIABLES OF GAF4 CRITERIA**

Changes in GGT, AFP, and FIB-4 in the non-improvement group and the improvement group of the whole cohort were investigated. The median (IQR) AFP at SVR24, and 1 and 2 years after SVR24, were 5.0 (3.7-7.0), 4.6 (3.3-6.3), and 4.3 (3.0-6.0) ng/mL.
in the non-improvement group, and 4.0 (3.0-5.0), 3.2 (2.5-4.0), and 3.0 (2.3-3.6) ng/mL in the improvement group, respectively (Fig. 4A). AFP values had improved significantly over time in both groups, but the median value of AFP at each point was higher than the threshold of AFP of 4 ng/mL in the non-improvement group. Similarly, The median (IQR) FIB-4 values at SVR24, and 1 and 2 years after SVR24, were 4.04 (2.8-5.6), 3.76 (2.7-5.3), and 3.71 (2.5-4.9) in the non-improvement group, and 3.60 (2.8-4.5), 3.27 (2.6-4.0), and 3.08 (2.4-3.7), respectively (Fig. 4B), and FIB-4 values had improved significantly over time in both groups. The median (IQR) GGT values at SVR24, and 1 and 2 years after SVR24, were 28 (20-41), 27 (19-42), and 28 (18-43) mg/dL in the non-improvement group, and 21 (16-30), 19 (15-25), and 18 (14-23) mg/dL in the improvement group (Fig. 4C). GGT values had improved in the improvement group over time, but no significant improvement was found in the non-improvement group.

**SUBGROUP ANALYSIS BY AGE AND SEX**

Subgroup analyses were conducted by age and sex in the whole cohort. Patients were stratified by age of <70, 70-79, and ≥80 years. In patients with age <70 years, the HCC incidence was 0.9 per 100 person-years in the low-risk group, and 2.8 per 100 person-years in the high-risk group, respectively (Supporting Fig. S1A). Similarly, in patients with age of 70-79 years, the HCC incidence was 1.2 per 100 person-years in the low-risk group, and 3.3 per 100 person-years in the high-risk group (Supporting Fig. 1B), and in patients with age ≥80 years, the HCC incidence was 0 per 100 person-years in the low-risk group, and 2.7 per 100 person-years in the high-risk group, respectively (Supporting Fig. S1C). The HCC incidence was significantly lower in the low-risk groups. When patients were stratified by sex, the HCC incidence in males was 1.7 per 100 person-years in the low-risk group, and 3.7 per 100 person-years in the high-risk group (Supporting Fig. S2A). The HCC incidence in females was 0.5 per 100 person-years in the low-risk group, and 2.4 per 100 person-years in the high-risk group, respectively (Supporting Fig. S2B).

**Discussion**

**MAIN FINDINGS**

In this multicenter nation-wide study, we demonstrated that the simple HCC risk model (GAF4 criteria) consisting of GGT, AFP, and FIB-4 at any point after
FIG. 4. Changes in AFP (A), FIB-4 (B), and GGT (C) after SVR. The bar chart indicates the median value of valuables, and the error bar indicates 75 percentiles.
SVR was associated with HCC development among patients with advanced fibrosis. Patients with low HCC risk (HCC development: 0.5-1.1 per 100 person-years in the derivation cohort and 0.9-1.1 per 100 person-years in the validation cohort) could be easily identified by GAF4 criteria, and these patients may be able to reduce HCC surveillance. The HCC incidence was especially low (0.5 per person-years) in the low-risk group of females, and HCC surveillance may be able to stop in these patients. Furthermore, even if patients had a high risk of HCC at entry, the HCC risk decreased in patients who improved to the low-risk level at the subsequent assessment. These risk-improvement patients could also reduce regular HCC surveillance. Because the risk model can be assessed easily and repeatedly, the model provides an HCC surveillance strategy after the eradication of the hepatitis C virus.

IN CONTEXT OF PUBLISHED LITERATURE

This study found that the HCC risk model based on simple serum markers at any point after SVR is associated with HCC development in patients with advanced fibrosis, and patients at low HCC risk can be identified by the model. HCC surveillance is necessary even after SVR because the lack of HCC surveillance leads to advanced HCC development and poor prognosis. However, it is not cost-effective to screen all patients who achieved SVR, and the identification of patients at low HCC risk is an important clinical issue. GGT, AFP, and FIB-4 after SVR are known as factors associated with HCC development. However, when these factors were used alone, patients at low HCC risk cannot be identified sufficiently. In this study, we found that patients with low HCC risk are able to be identified by combining these simple serum factors (GAF4 criteria).

Recently, some studies demonstrated that liver stiffness or serum markers are associated with HCC development after SVR, and low-risk patients could be detected by combining these factors. However, one limitation of these studies is that the models are calculated based on the time of SVR. Liver stiffness that correlates fibrosis in the liver is associated with HCC risk. Because liver stiffness changes over time, not only during DAA treatment but also after SVR, this indicates that HCC risk changes over time. Therefore, HCC risk should be evaluated not only at the time of SVR but also at any point after SVR. In addition, some patients cannot evaluate the HCC risk at SVR due to insufficient data on SVR. In this study, we demonstrated that the HCC risk model at any time was associated with HCC development, and the significance of GAF4 criteria is that it can be applied whenever laboratory data are measured.

One advantage of serum markers is that it is easy to repeat measurements. We previously reported that time-course changes in serum markers are associated with changes in HCC risk. In this study, we demonstrated that if the HCC risk improves from high risk to low risk, HCC development rate also decreases in these patients. Recent studies also demonstrated that changes in FIB-4 or liver stiffness are associated with changes in HCC risk, and our results espouse these findings. Therefore, patients at high risk of HCC are still at high risk of HCC and should continue HCC surveillance; however, if the risk improves to the low level at a subsequent point, these patients could afford to reduce HCC surveillance. Furthermore, the HCC development in female patients with GAF4 low-risk criteria was significantly low (0.5 per person-years), and HCC surveillance may be stopped in these patients. One advantage of our model is that observing a change in the risk model can identify patients who could afford to reduce or stop HCC surveillance, and this point was not established in previous studies.

STRENGTHS AND LIMITATIONS

This study was a multicenter nation-wide cohort study, which included over 1,800 patients with advanced fibrosis. Because our HCC risk model needs only standard laboratory tests, there is no examiner dependency like liver stiffness measurement. It is easy to evaluate, and risk assessment at any point after SVR and repeat assessment is associated with HCC development. Therefore, this risk model can be adapted to another cohort easily and immediately without specific equipment. Although patient characteristics and HCC development rate were significantly different between the derivation cohort and the validation cohort, GAF4 criteria were able to identify patients at low risk of HCC development, even in the validation cohort. This indicates that GAF4 criteria have generalities. However, this study was conducted only in Japan, and relatively elderly patients were enrolled. FIB-4 was used as a screening method for patients with advanced fibrosis, but the diagnostic
accuracy is affected by age and liver fibrosis may be overestimated in elderly patients. On the other hand, age is a risk factor for HCC development, and FIB-4 (>3.25) is associated with a high risk of HCC development\(^{(22,43)}\), therefore, using FIB-4 as a surrogate marker for patients with a high risk of HCC is thought to be valid. However, to strengthen the utility of the risk model, verification by a cohort in another region with a different age proportion is necessary. Moreover, the observation period of the study was short, and a further long-term follow-up study is necessary to demonstrate the utility of GAF4 criteria.

**FUTURE IMPLICATIONS AND DIRECTIONS**

In this study, we demonstrated patients within GAF4 criteria (GGT < 28 IU/L, AFP < 4.0 ng/mL, and FIB-4 < 4.28) at any point after SVR are at low risk of HCC development (HCC development: 0.5-1.1 per 100 person-years in the derivation cohort and 0.9-1.1 per 100 person-years in the validation cohort). Furthermore, if the HCC risk improves to the low-risk level in high-risk patients at baseline, these patients also reduce the HCC risk (HCC development: 0.6 per 100 person-years in the derivation cohort and 1.3 per 100 person-years in the validation cohort). Patients with the annual incidence of HCC risk <1.5% are not recommended HCC surveillance in the AASLD guideline.

Furthermore, a previous study indicated that HCC screening after SVR in patients with the annual incidence of HCC < 1.32% is not cost-effective. Therefore, patients who were at low risk of the model at any time and improved from the high-risk level to the low-risk level may be able to reduce regular HCC surveillance.

The significance of the model is that GAF4 criteria do not need evaluation at a specific time point and can be applied whenever laboratory data are measured. Because this strategy is easy to adapt to detect patients at low risk of developing HCC, these data have important implications for HCC surveillance in patients with the eradication of the hepatitis C virus and help all physicians engaged in the management of liver disease.

Several studies demonstrated that liver stiffness by elastography or complication status (e.g., diabetes, alcohol intake) are associated with HCC development after SVR.\(^{(10,13,15,35)}\) These data were not collected and evaluated in the study. GGT value is associated with diabetes or alcohol intake, and non-improvement of GGT value observed in the non-improvement group may be associated with the presence of these complications.⁴⁴ Therefore, more accurate risk estimation may be possible by combining these factors with GAF4 criteria, and further studies are needed.

In conclusion, the HCC risk model based on simple serum markers (GGT, AFP, and FIB-4) at any point after SVR and its change can identify patients at low risk of HCC, and these low-risk patients may be able to reduce HCC surveillance.

**REFERENCES**

1. Westbrook RH, Dusheiko G. Natural history of hepatitis C. J Hepatol 2014;61:558–568.
2. Afdhal N, Zeuzem S, Kwo P, Chojkier M, Griffin N, Puoti M, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014;370:1899–1908.
3. Zeuzem S, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, et al. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. N Engl J Med 2014;370:1993–2001.
4. Akahane T, Kurosaki M, Itakura J, Tsuji K, Joko K, Kimura H, et al. Real-world efficacy and safety of sofosbuvir + ribavirin for hepatitis C genotype 2: a nationwide multicenter study by the Japanese Red Cross Liver Study Group. Hepatol Res 2019;49:264–270.
5. Mashiba T, Joko K, Kurosaki M, Ochi H, Hasebe C, Akahane T, et al. Real-world efficacy of elbasvir and grazoprevir for hepatitis C virus (genotype 1): a nationwide, multicenter study by the Japanese Red Cross Hospital Liver Study Group. Hepatol Res 2019;49:1114–1120.
6. Asahina Y, Liu C-J, Gane E, Ishiy库 H, Kawada N, Ueno Y, et al. Twelve weeks of ledipasvir/sofosbuvir all-oral regimen for patients with chronic hepatitis C genotype 2 infection: integrated analysis of three clinical trials. Hepatol Res 2020;50:1109–1117.
7. Ogawa E, Furuyosu N, Nakamaluma M, Nomura H, Satoh T, Takahashi K, et al. Glecaprevir and pibrentasvir for Japanese patients with chronic hepatitis C genotype 1 or 2 infection: results from a multicenter, real-world cohort study. Hepatol Res 2019;49:617–626.
8. Japan Society of Hepatology guidelines for the management of hepatitis C virus infection: 2019 update. Hepatol Res 2020;50:791–816.
9. Calvaruso V, Cabibbo G, Gacciola I, et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. Gastroenterology 2018;155:411–421.e4.
10. Asahina Y, Tsuchiya K, Nishimura T, Muraoaka M, Suzuki Y, Tamaki N, et al. α-fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. Hepatology 2013;58:1253–1262.
11. Nagaoki Y, Imamura M, Teraoka Y, Morio K, Fujino H, Ono A, et al. Impact of viral eradication by direct-acting antivirals on the risk of hepatocellular carcinoma development, prognosis, and portal hypertension in hepatitis C virus-related compensated cirrhosis patients. Hepatol Res 2020;50:1222–1233.
12. Tada T, Toyoda H, Yasuda S, et al. Long-term prognosis of liver disease in patients with eradicated chronic hepatitis C virus: an analysis using a Markov chain model. Hepatol Res 2020;50:936–946.
13. Asahina Y. JSH guidelines for the management of hepatitis C virus infection, 2019 update; protective effect of antiviral therapy against hepatocarcinogenesis. Hepatol Res 2020;50:775–790.
14) Farhang Zangneh H, Wong WWL, Sander B, Bell CM, Mumtaz K, Kowgier M, et al. Cost effectiveness of hepaticellular carcinoma surveillance after a sustained virologic response to therapy in patients with hepatitis C virus infection and advanced fibrosis. Clin Gastroenterol Hepatol 2019;17:1840-1849.e16.

15) Yamada R, Hiroamasu N, Ozoe T, Uraabe A, Tahata Y, Morishita N, et al. Incidence and risk factors of hepaticellular carcinoma change over time in patients with hepatitis C virus infection who achieved sustained virologic response. Hepatol Res 2019;49:570-578.

16) Ioannou GN. HCC surveillance after SVR in patients with F3/F4 fibrosis. J Hepatol 2021;74:458-465.

17) Yasui Y, Kurosaki M, Komiyama Y, et al. Wisteria floribunda agglutinin-positive Mac-2 binding protein predicts early occurrence of hepaticellular carcinoma after sustained virologic response by direct-acting antivirals for hepatitis C virus. Hepatol Res 2018;48:1131-1139.

18) Osawa I, Tamaki N, Kurosaki M, Kirino S, Watakabe K, Wang W, et al. Wisteria floribunda agglutinin-positive Mac-2 binding protein but not α-fetoprotein as a long-term hepaticellular carcinoma predictor. Int J Mol Sci 2020;21:3640.

19) Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, El-Serag HB. Long-term risk of hepaticellular carcinoma in HCV patients treated with direct acting antiviral agents. Hepatology 2020;71:44-55.

20) Tamaki N, Kurosaki M, Yasui Y, Mori N, Tsuji K, Hasebe C, et al. Change in Fibrosis 4 Index as predictor of high risk of incident hepaticellular carcinoma after eradication of hepatitis C virus. Clin Infect Dis 2021 Feb 5. https://doi.org/10.1093/cid/ciaa1307. [Epub ahead of print]

21) Sterling RK, Lissen E, Chmeneck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-1325.

22) Tamaki N, Kurosaki M, Matsuda S, Muraoka M, Yasui Y, Suzuki S, et al. Non-invasive prediction of hepaticellular carcinoma development using serum fibrosis marker in chronic hepatitis C patients. J Gastroenterol 2014;49:1495-1503.

23) Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecasis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 2018;68:723-750.

24) Kukudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, Shimada M, et al. Clinical practice guidelines for hepatocellular carcinoma: the Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. Hepatol Res 2019;49:1109-1113.

25) Kanda Y, Investigation of the freely available easy-to-use software ‘EZRI’ for medical statistics. Bone Marrow Transplant 2013;48:452-458.

26) Toyota H, Kumada T, Tada T, Mizuno K, Hiraoka A, Tsuji K, et al. Impact of hepaticellular carcinoma aetiology and liver function on the benefit of surveillance: a novel approach for the adjustment of lead-time bias. Liver Int 2018;38:2260-2268.

27) Toyota H, Tada T, Tsuji K, Hiraoka A, Tachi Y, Itobayashi EI, et al. Characteristics and prognosis of hepaticellular carcinoma detected in patients with chronic hepatitis C after the eradication of hepatitis C virus: a multicenter study from Japan. Hepatol Res 2016;46:734-742.

28) Alonso López S, Manzano ML, Gea F, Gutiérrez ML, Ahumada AM, Devesa MJ, et al. A model based on noninvasive markers predicts very low hepaticellular carcinoma risk after viral response in hepatitis C virus-advanced fibrosis. Hepatology 2020;72:1924-1934.

29) Ide T, Koga H, Nakano M, Hashimoto S, Yatoshushi H, Higuchi N, et al. Direct-acting antiviral agents do not increase the incidence of hepaticellular carcinoma development: a prospective, multicenter study. Hepatol Int 2019;13:293-301.

30) Degasperi E, D’Ambrosio R, Iavaroni M, Sangiovanni A, Aghemo A, Soffredini R, et al. Factors associated with increased risk of de novo or recurrent hepaticellular carcinoma in patients with cirrhosis treated with direct-acting antivirals for HCV infection. Clin Gastroenterol Hepatol 2019;17:1183-1191.e7.

31) Audureau E, Carrat F, Layese R, Cagnot C, Asselah T, Guyader D, et al. Personalized surveillance for hepaticellular carcinoma in cirrhosis—using machine learning adapted to HCV status. J Hepatol 2020;73:1434-1445.

32) Yasui Y, Abe T, Kurosaki M, Higuchi M, Kiyomiya Y, Yoshida T, et al. Elastin fiber accumulation in liver correlates with the development of hepaticellular carcinoma. PLoS One 2016;11:e0154558.

33) Yasui Y, Abe T, Kurosaki M, Matsuoka K, Higuchi M, Tamaki N, et al. Non-invasive liver fibrosis assessment correlates with collagen and elastic fiber quantity in patients with hepatitis C virus infection. Hepatol Res 2019;49:33-41.

34) Higuchi M, Tamaki N, Kurosaki M, Watakabe K, Osawa L, Wang W, et al. Prediction of hepaticellular carcinoma after sustained virological responses using magnetic resonance elastography. Clin Gastroenterol Hepatol 2019;17:2616-2618.

35) Tamaki N, Higuchi M, Kurosaki M, Kirino S, Osawa L, Watakabe K, et al. Risk assessment of hepaticellular carcinoma development by magnetic resonance elastography in chronic hepatitis C patients who achieved sustained virological responses by direct-acting antivirals. J Viral Hepat 2019;26:893-899.

36) Higuchi M, Tamaki N, Kurosaki M, Inada K, Kirino S, Yamashita K, et al. Changes of liver stiffness measured by magnetic resonance elastography during direct-acting antivirals treatment in patients with chronic hepatitis C. J Med Virol 2021;93:3744-3751.

37) Singh S, Faciorusso A, Loomba R, Falcó-Ytter YT. Magnitude and kinetics of decrease in liver stiffness after antiviral therapy in patients with chronic hepatitis C: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2018;16:27-38.e4.

38) Tamaki N, Kurosaki M, Kuno A, et al. Wisteria floribunda agglutinin positive human Mac-2 binding protein as a predictor of hepaticellular carcinoma development in chronic hepatitis C patients. Hepatol Res 2015;4:1582-1588.

39) Tamaki N, Kurosaki M, Loomba R, Izuini N. Clinical utility of Mac-2 binding protein glycosylation isomer in chronic liver diseases. Ann Lab Med 2021;41:16-24.

40) Pons M, Rodríguez-Tajes S, Esteban JL, Marínio Z, Vargas V, Lenz S, et al. Non-invasive prediction of liver-related events in patients with HCV-associated compensated advanced chronic liver disease after oral antivirals. J Hepatol 2020;72:472-480.

41) Castera L, Foucher J, Bernard P-H, Carvalho F, Aliax D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828-835.

42) Tamaki N, Higuchi M, Kurosaki M, Kirino S, Osawa L, Watakabe K, et al. Wisteria floribunda agglutinin-positive Mac-2 binding protein as an age-independent fibrosis marker in nonalcoholic fatty liver disease. Sci Rep 2019;9:10109.

43) Ashaisha Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, et al. Effect of aging on risk for hepaticellular carcinoma in chronic hepatitis C virus infection. Hepatology 2010;52:518-527.

44) Zhao W, Tong J, Liu J, Liu J, Li J, Cao Y. The dose-response relationship between gamma-glutamyl transferase and risk of diabetes mellitus using publicly available data: a longitudinal study in Japan. Int J Endocrinol 2020;2020:5356498.

Author names in bold designate shared co-first authorship.

Supporting Information
Additional Supporting Information may be found at onlineibrary.wiley.com/doi/10.1002/hep.4.1833/supinfo.