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Serum Growth Differentiation Factor 15 Can Be a Novel Biomarker to Predict the Prognosis of Patients With Hepatitis C Virus Cirrhosis After Virus Elimination

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Keywords: decompensated cirrhosis | HCV | liver cancer

ABSTRACT

Background and Aims: Although direct-acting antivirals (DAAs) achieve high sustained virologic response (SVR) rates, the long-term outcomes of patients with hepatitis C virus (HCV)-related cirrhosis remain variable. Growth differentiation factor 15 (GDF15), a stress-induced cytokine, has emerged as a potential biomarker for liver disease progression. This study aimed to

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; BMI, body mass index; C-index, concordance index; CT, computed tomography; DAA, direct-acting antiviral; ELF, enhanced liver fibrosis test; ELISA, enzyme-linked immunosorbent assay; EOT, end of treatment; EPV, events per variable; FIB-4, Fibrosis-4 index; GDF15, growth differentiation factor 15; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IQR, interquartile range; M2BPGi, Mac-2 binding protein glycosylation isomer; MASLD, metabolic dysfunction-associated liver disease; MRI, magnetic resonance imaging; OS, overall survival; p24w, 24 weeks after DAA therapy; ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis; SVR, sustained virologic response; TGF-β, transforming growth factor-β.

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evaluate the prognostic value of serum GDF15 levels in predicting hepatocellular carcinoma (HCC), hepatic decompensation, and mortality in patients with HCV-related cirrhosis.

Methods: We retrospectively analyzed 196 patients with HCV-related cirrhosis who achieved SVR at 18 Japanese institutions. Serum GDF15 levels were measured at baseline (BL) and 24 weeks posttreatment (p24w). A previously validated cutoff of 1.75 ng/mL was applied. The clinical outcomes included HCC occurrence, hepatic decompensation, and all-cause mortality.

Results: During a median follow-up of 46.2 months, 75 patients developed HCC, 28 experienced hepatic decompensation, and 25 died. Hepatic decompensation occurred significantly less frequently in the BL GDF15-low group. Importantly, no deaths occurred in the GDF15-low group, whereas the 5-year survival rate in the GDF15-high group was 71.7%. Similar trends were observed for GDF15 levels at p24w. In the overall cohort, GDF15 was not significantly associated with incident HCC; among HCC-naïve patients, a nonsignificant trend toward lower 3-year HCC incidence was observed in the GDF15-low group.

Conclusions: Serum GDF15 is a promising prognostic biomarker for post-SVR outcomes in patients with HCV-related cirrhosis. Its ability to predict decompensation and mortality through a validated cutoff supports its use for risk stratification and long-term management in patients with chronic liver disease.

1 | Introduction

Chronic hepatitis C virus (HCV) infection remains a major cause of cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Direct-acting antivirals (DAAs) achieve high sustained virologic response (SVR) rates including in cirrhosis [3]. Despite viral clearance, long-term outcome in HCV-related cirrhosis is heterogeneous [4, 5] and reliable biomarkers for predicting clinical outcomes are limited.

Growth differentiation factor 15 (GDF15) is a stress-responsive cytokine involved in the transforming growth factor-β (TGFβ) super family [6]. Elevated circulating GDF15 has been linked to cardiovascular disease, cancer, and liver disorders [7, 8]. We and other groups reported the association of serum GDF15 levels and clinical outcomes in patients with chronic liver disease, including hepatitis C [9], hepatitis B [10], and metabolic dysfunction-associated liver disease (MASLD) [11]. These observations suggest that GDF15 may reflect pathways relevant to disease progression.

Here, we evaluated whether serum GDF15—measured at baseline (BL) and 24 weeks after DAA therapy (p24w)—predicts HCC, hepatic decompensation, and mortality in patients with HCV-related cirrhosis who achieved SVR. This may help identify novel biomarkers that facilitate risk stratification and guide management strategies for this high-risk population.

2 | Materials and Methods

2.1 | Study Design

A total of 478 patients with HCV infection who were treated with DAAs at 33 institutions in Japan between February 2019 and December 2021 were prospectively registered. The clinical course of these patients has been reported previously [12–14]. Among them, 446 patients achieved a sustained virologic response (SVR), and baseline serum samples were available for 200 patients. One patient was excluded from further analysis because of the presence of a malignant tumor at baseline, and three patients were excluded because of a lack of follow-up imaging for HCC surveillance, resulting in a final cohort of 196 patients from 18 institutions included in the present study (Figure 1A). Written informed consent was obtained from all participants, and the study was conducted in accordance with the principles of the Declaration of Helsinki as revised in 2013. The research protocol was approved by the institutional review board of the University of Osaka Hospital and those of all the participating institutions [IRB No. 22285]. Patient management and treatment were conducted in accordance with the Japanese clinical practice guidelines for chronic hepatitis C virus infection.

2.2 | Monitoring of Liver Cancer and Decompensation Events

Before treatment, all patients were screened using ultrasonography, computed tomography (CT), and/or magnetic resonance imaging (MRI) and HCC. The clinical information and blood test results were collected at baseline (BL), at the end of treatment (EOT), and at 24 weeks after treatment (p24w). After EOT, imaging and blood tests were conducted every 6 months. HCC was diagnosed by typical contrast-enhanced CT imaging and/or MRI following the recommendation of the Japan Society of Hepatology [15, 16].

2.3 | Serum GDF15 Examination

Patient sera were stored at −80°C at each institution, and the GDF15 level was analyzed through an enzyme-linked immunosorbent assay (ELISA) kit for human GDF15 (DGD150, R&D Systems, Minneapolis, MN). A cutoff value of 1.75 ng/mL was used to stratify patients into high- and low-GDF15 groups according to previous reports [11].

2.4 | Clinical Outcome and the Initiation of Observation

The initiation of antiviral therapy was defined as the start of the observation period for analyses using baseline parameters. For analyses based on posttreatment parameters at p24w, the observation period was 24 weeks after the end of therapy.

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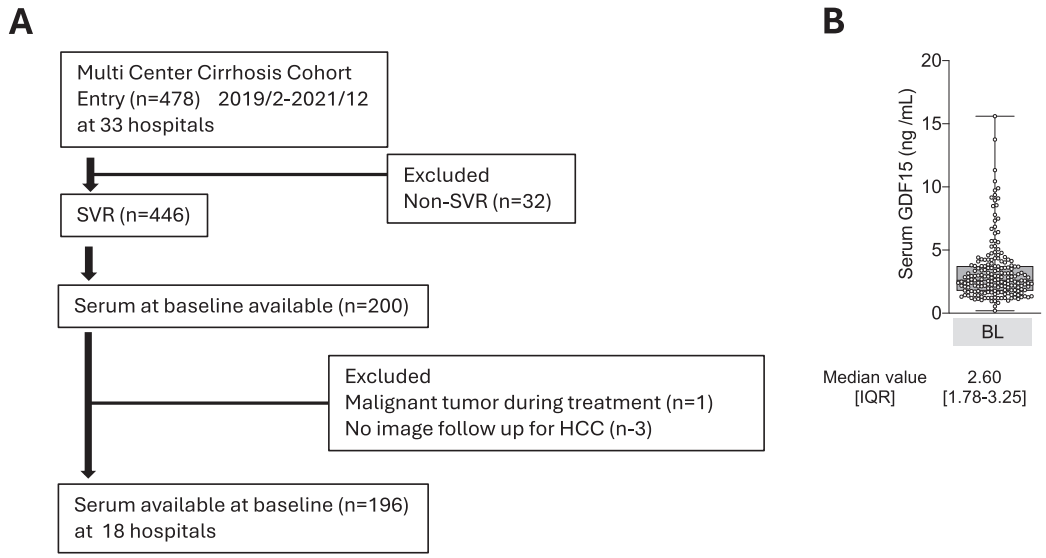

FIGURE 1 | Study overview and patient cohort. (A) Consort diagram for this study. (B) GDF15 levels in all the available serum samples were analyzed through ELISA. The box plots show the baseline GDF15 level ($n = 196$).

For the analysis of HCC occurrence, the date of diagnosis by imaging was considered the date of HCC occurrence, and for patients without HCC development, the end of follow-up was defined as the date of the most recent liver imaging.

Decompensated cirrhotic events were defined as those requiring hospitalization for the worsening of ascites or hepatic encephalopathy, rupture of esophageal or gastric varices, and the onset of spontaneous bacterial peritonitis (SBP), sepsis, or acute kidney injury [17]. For survival analysis, overall survival—including both liver-related and nonliver-related deaths—was assessed without censoring patients who underwent liver transplant.

The final follow-up date for analyses of decompensation events and survival was defined as the date of the most recent hospital visit in patients who remained alive during the observation period. The observation period for each patient was calculated from the defined start date to the occurrence of the event of interest or the last available follow-up date, whichever came first.

2.5 | Time-Dependent ROC Analysis

Time-dependent receiver operating characteristic (ROC) analyses were carried out to evaluate the prognostic performance of GDF15 at specific time points (e.g., 3-year and 5-year outcomes). For each analysis, patients without sufficient follow-up beyond the target time point were excluded to avoid bias from incomplete observation. The cumulative/dynamic definition of cases and controls was applied: patients who experienced the event of interest before the target time were considered cases, whereas those who remained event-free beyond that time were considered controls. The time-dependent area under the curve (AUC) was then calculated, and the optimal cutoff value at each time point was determined using Youden's index.

2.6 | Statistical Analysis

For statistical analysis, PRISM (GraphPad, ver 8.4.3), JMP Pro (JMP statistical discovery LLC, ver 17.0.0), and R studio were used. For the comparison of two groups, the Student *t*-test was used for the parametric test and the Mann–Whitney *U*-test was used for the nonparametric test. For the analysis of HCC incidence and decompensation events, we constructed Kaplan–Meier curves using the start date of DAA therapy as the index and followed patients until the occurrence of HCC, death, or the last day of HCC surveillance before March 2025.

The log-rank test was used to compare the risk of HCC occurrence and decompensation events. We applied the Cox proportional hazards model and likelihood analysis test to compare the risk of HCC occurrence, decompensation events, and death. We constructed parsimonious multivariable Cox models ensuring an events-per-variable (EPV) > 10, in which clinically relevant covariates reported in previous studies were used [17].

To quantify model discrimination, we computed Harrell's concordance index (C-index) from Cox proportional hazards models. Overall survival (OS) and time to decompensated events in months were the time scale, with deaths/decompensated events coded as events and others censored. Predictors were the Child–Pugh score and serum GDF15; GDF15 was analyzed on the natural-log scale, and the Child–Pugh score was entered as a point score. We prespecified three models: Child–Pugh alone, ln-GDF15 alone, and the combined model (Child–Pugh A/B/C + GDF15 high/low). For each model, we derived the linear predictor and calculated Harrell's C on the analysis dataset. Given the limited number of events, model complexity was constrained by events-per-variable considerations. We applied bootstrap internal validation (1000 resamples) to obtain optimism-corrected C-indices and to reduce optimism due to model fitting on the same dataset. Differences in C-index between models were assessed using the compareC test. Analyses were conducted in R (survival, rms, and compareC).

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3 | Results

3.1 | Patient Characteristics and GDF15 Levels

The patients' baseline characteristics are summarized in Supporting Information S3: Table 1. The median observation period was 46.2 months (interquartile range (IQR): 30.0–57.2). Among the 196 patients, 109 (55.6%) had compensated cirrhosis and 87 (44.4%) had decompensated cirrhosis at baseline. A total of 123 patients (62.8%) had no history of HCC, whereas the remaining 73 patients (37.2%) had a prior history of HCC.

Serum levels of GDF15 were quantified in all 196 patients through a validated enzyme-linked immunosorbent assay (ELISA). The median GDF15 concentration at baseline was 2.60 ng/mL (IQR: 1.78–3.25) (Figure 1B). No significant differences in GDF15 levels at SVR24 were observed between patients with compensated and decompensated cirrhosis, between those with or without a prior history of HCC or across Child–Pugh classes, although there was a trend toward higher levels in patients with higher Child–Pugh scores (Supporting Information S2: Figure S1A,B). The 196 patients were divided into two groups based on baseline GDF15 levels, with a previously validated cutoff of 1.75 ng/mL established in a MASLD cohort for the prediction of liver disease progression, including liver carcinogenesis, hepatic decompensation, and mortality [11]. The baseline clinical characteristics of the high- and low-GDF15 groups are summarized in Table 1. There was no significant difference in the frequency of decompensated cirrhosis between the two groups. Compared to those in the low-GDF15 group, patients in the high-GDF15 group had a lower body mass index (BMI), lower serum albumin levels, lower platelet counts, more severe ascites, and higher FIB-4 index values.

3.2 | Baseline Serum GDF15 Levels and Hepatic Decompensation Events After SVR

During the follow-up period, 28 patients were hospitalized due to hepatic decompensation (Supporting Information S3: Table 2). Patients in the high-GDF15 group had a significantly greater risk of hepatic decompensation than those in the low-GDF15 group did (Figure 2A). The five-year incidence rate was 22.8% in the high-GDF15 group and 4.5% in the low-GDF15 group. In

TABLE 1 | Patient's background of GDF15 high and low patients.

		GDF15 BL high (*n*−149)			GDF15 BL low (*n*−47)			
		Median	IQR (25%–75%)	Missing value (*n*)	Median	IQR (25%–75%)	Missing value (*n*)	*p* value
Observation time	Months	46.2	28.0–57.3	0	46.2	26.5–57.2	0	0.9308
Age	Years old	71	61.5–79	0	68	62–75	0	0.2996
Sex	M/F	83/66		0	25/22		0	0.894
HCV-serogroup	1/2/others	89/52/8		0	26/17/4		0	0.893
HCV-RNA	Log IU/mL	6	5.3–6.4	0	5.9	5.3–6.4	0	0.5067
BMI	kg/m^2	23.5	21.4–25.9	1	25	22.7–27.1	1	0.0317
Status of cirrhosis	Comp/decomp	78/71		0	31/16		0	0.142
HCC prehistory	Yes/no	91/58		0	32/15		0	0.488
AST	U/L	54	39–81	0	52	35–71	0	0.8008
ALT	U/L	39	24–68	0	36	24–72	0	0.6407
GGT	U/L	38	24–78	0	33	21–60	0	0.545
T-bil	mg/dL	0.9	0/6–1.45	0	0.9	0.7–1.4	0	0.8828
AFP	ng/mL	8.8	4.4–17.9	0	5.2	3.7–11	0	0.4565
Albumin	g/dL	3.4	3–3.7	0	3.6	3.4–4	0	0.0021
Platelet	× 104/μL	8.9	6.4–11.6	0	10.9	7.5–14.5	0	0.0004
Child–Pugh score	A/B/C	78/60/11		0	32/14/1		0	0.1208
Esophagogastric varix	Yes/no/unknown	68/36/45		0	12/23/12		0	0.1208
Ascites	1/2/3	81/55/13		0	37/8/2		0	0.012
Encephalopathy	1/2/3	135/14/0		0	45/2/0		0	0.2618
Fib-4 index		7.07	5.33–9.55	0	5.41	3.59–6.98	0	0.0025

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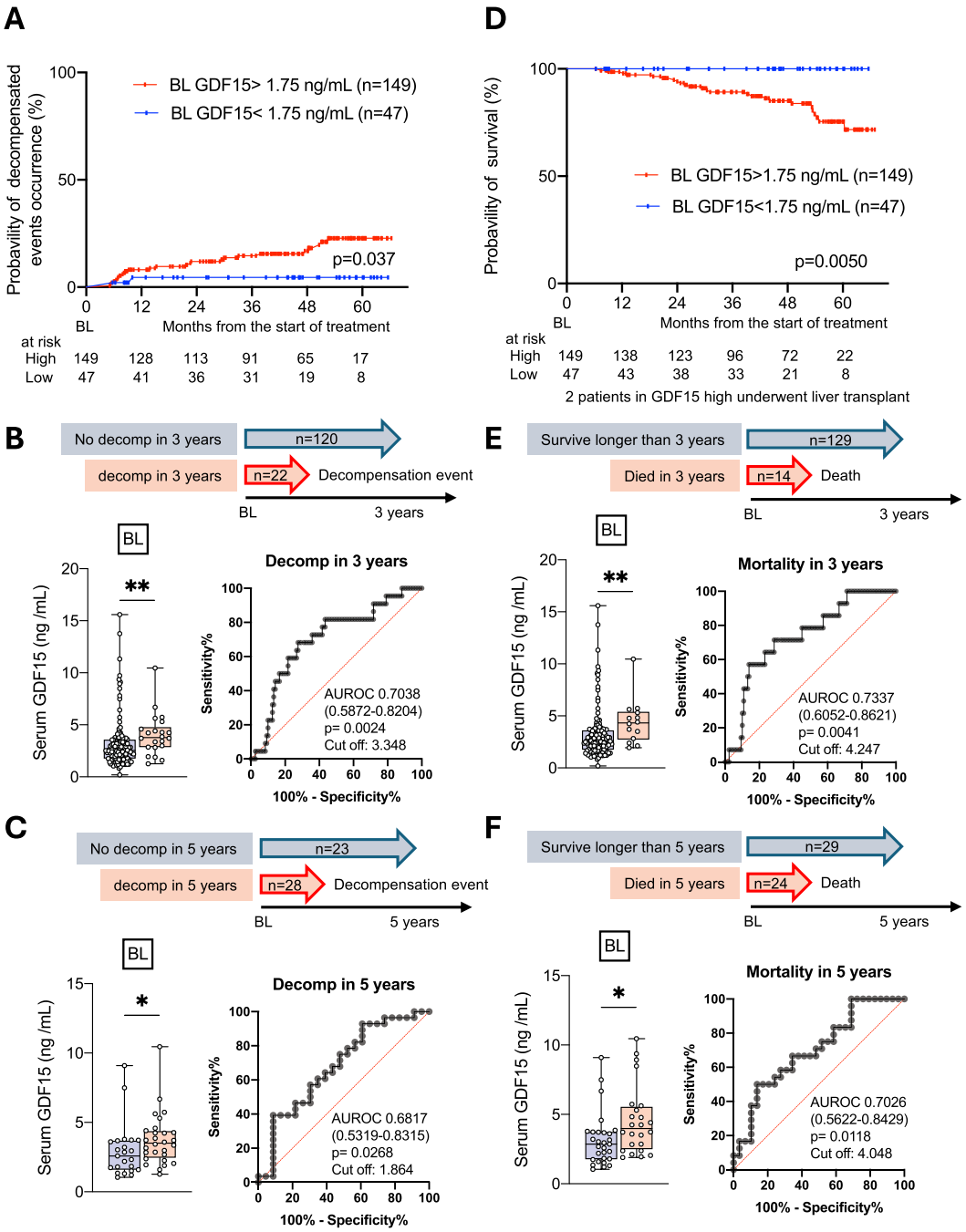

FIGURE 2 | Baseline GDF15 levels can also predict the prognosis of patients with HCV-related cirrhosis in the HCC-naïve cohort. (A) Kaplan–Meier curve for hepatic decompensation in the entire cohort with available serum samples (n = 196). Patients were stratified by the baseline GDF15 cutoff of 1.75 ng/mL. The log-rank test was used for statistical analysis. (B) Baseline serum GDF15 levels measured by ELISA. Box plots show GDF15 levels in patients who developed hepatic decompensation within 3 years and those who did not. ROC curves illustrate the ability of baseline GDF15 to discriminate patients who developed hepatic decompensation within 3 years from those who remained event-free, with the area under the curve (AUC) showing predictive accuracy. Statistical analysis was carried out using the Mann–Whitney U test. (C) Baseline serum GDF15 levels measured by ELISA. Box plots show GDF15 levels in patients who developed hepatic decompensation within 5 years and those who did not. ROC curves illustrate the discriminatory ability of baseline GDF15 for 5-year hepatic decompensation, with the AUC showing predictive accuracy. Statistical analysis was carried out using the Mann–Whitney U test. (D) Kaplan–Meier curve for overall survival in the entire cohort (n = 196) stratified by the baseline GDF15 cutoff of 1.75 ng/mL. The log-rank test was used for statistical analysis. (E) Baseline serum GDF15 levels measured by ELISA. Box plots show GDF15 levels in patients who died within 3 years and those who survived. ROC curves illustrate the ability of baseline GDF15 to discriminate 3-year mortality, with the AUC showing predictive accuracy. Statistical analysis was carried out using the Mann–Whitney U test. (F) Baseline serum GDF15 levels measured by ELISA. Box plots show GDF15 levels in patients who died within 5 years and those who survived. ROC curves illustrate the ability of baseline GDF15 to discriminate 5-year mortality, with the AUC showing predictive accuracy. Statistical analysis was carried out using the Mann–Whitney U test. All the x-axes of the Kaplan–Meier curves describe the months from the start of treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, and ****$p < 0.001$.

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the univariate Cox regression analysis, the platelet count, total bilirubin level, albumin level, prothrombin time, Child–Pugh score, presence of esophagogastric varix, presence of ascites, presence of encephalopathy, and GDF15 level were associated with the incidence of hepatic decompensation events (Table 2). The serum GDF15 level remained significant in the multivariable analysis after adjusting for the Child–Pugh score (Table 2). Furthermore, baseline GDF15 levels were significantly higher in patients who experienced hepatic decompensation within 3 or 5 years than in those who did not (Figure 2B,C). We assessed the predictive performance of GDF15 for hepatic decompensation at 3-year and 5-year using time-dependent ROC analysis. For 3-year outcomes, the AUC was 0.704 (95% CI, 0.587–0.821), with a Youden index–derived cutoff of 3.348 ng/mL (Figure 2B); for 5-year outcomes, the AUC was 0.6817 (95% CI, 0.532–0.832), with a cutoff of 1.864 ng/mL (Figure 2C). Stratification at 3.3 ng/mL identified a high-risk group with significantly worse outcomes in survival analyses (Supporting Information S2: Figure S1C).

3.3 | Baseline Serum GDF15 Levels and Mortality After SVR

During the follow-up period, 25 patients died and 2 patients underwent liver transplantation. Patients in the high-GDF15 group had a significantly greater risk of death than those in the low-GDF15 group (Figure 2D). No deaths or liver transplantations occurred in the low-GDF15 group, whereas the five-year cumulative incidence of death in the high-GDF15 group was 28.3%. According to the univariate Cox regression analysis, albumin levels, the presence of ascites, the presence of encephalopathy, and GDF15 levels were associated with mortality (Table 3). The association between the GDF15 level and mortality remained significant in the multivariable analysis after adjusting for the Child–Pugh score (Table 3). Baseline GDF15 levels were significantly higher in patients who experienced 3-year or 5-year mortality compared with those who survived (Figure 2E,F). We next evaluated the predictive performance of GDF15 for all-cause mortality at 3 and 5 years. For 3-year mortality, the AUC was 0.734 (95% CI, 0.605–0.862) with a Youden index–derived cutoff of 4.247 ng/mL (Figure 2E); for 5-year mortality, the AUC was 0.722 (95% CI, 0.624–0.820), with the same cutoff of 3.468 ng/mL (Figure 2F). Stratification at 4.2 ng/mL similarly identified a high-risk group with significantly worse outcomes (Supporting Information S2: Figure S1D).

3.4 | Baseline Serum GDF15 Levels and HCC Occurrence After SVR

During the follow-up period, 75 patients developed HCC. No statistically significant difference in overall HCC incidence

TABLE 2 | Factors related to decompensated events occurrence.

Factors	Time point			Missing data	Univariate analysis hazard ratio	*p* value	Multivariate analysis hazard ratio	*p* value
Age	BL	years old	> 70/≤ 70	0	0.721	0.3897		
Sex	BL		Male/female	0	1.108	0.7887		
HCV serogroup	BL		1/2	0	1.513	0.3094		
HCV RNA	BL	Log IU/mL	> 6.0/≤ 6.0	0	0.5063	0.0776		
Platelet	BL	× 104/μL	> 10/≤ 10	0	0.4092	0.0294		
AST	Bl	U/L	> 30/≤ 30	0	1.017	0.9785		
ALT	BL	U/L	> 30/≤ 30	0	0.6448	0.2574		
GGT	BL	U/L	> 30/≤ 30	0	0.7741	0.513		
Total bilirubin	BL	mg/dL	> 0.7/≤ 0.7	0	2.948	0.0424		
Albumin	BL	g/dL	> 3.6/≤ 3.6	0	0.228	0.0062		
PT	BL	%	> 90/≤ 90	0	0.3141	0.0321		
AFP	BL	ng/mL	> 5/≤ 5	0	1.113	0.7968		
GDF15	BL	ng/mL	> 1.75/≤ 1.75	0	4.092	0.0186	3.85	0.02589
Child–Pugh	BL		A,B/C	0	0.1651	0.0014	0.191	0.0029
Esophagogastric varix	BL		Presence/absence	0	5.354	0.0066		
	BL		Unknown/absence	0	1.385	0.6697		
Ascites	BL		Presence/absence	0	6.07	< 0.0001		
Encephalopathy	BL		Presence/absence	0	5.885	< 0.0001		
Fib4-index	BL		> 3.25 ≤ 3.25	0	1.664	0.4554		

Note: Events $n = 28$. *p* values were calculated by effect likelihood ratio tests.

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TABLE 3 | Factors related to mortality.

Factors	Time point			Missing data	Univariate analysis hazard ratio	*p* value	Multivariate analysis hazard ratio	*p* value
Age	BL	years old	> 70/≤ 70	0	1.788	0.1184		
Sex	BL		Male/female	0	1.976	0.0761		
HCV serogroup	BL		1/2	0	1.774	0.1493		
HCV RNA	BL	Log IU/mL	> 6.0/≤ 6.0	0	0.5357	0.0929		
Platelet	BL	× 104/μL	> 10/≤ 10	0	0.8712	0.7103		
AST	Bl	U/L	> 30/≤ 30	0	0.6447	0.3942		
ALT	BL	U/L	> 30/≤ 30	0	0.7216	0.3889		
GGT	BL	U/L	> 30/≤ 30	0	0.8745	0.7255		
Total bilirubin	BL	mg/dL	> 0.7/≤ 0.7	0	1.505	0.3835		
Albumin	BL	g/dL	> 3.6/≤ 3.6	0	0.41	0.0346		
PT	BL	%	> 90/≤ 90	0	0.6237	0.2559		
AFP	BL	ng/mL	> 5/≤ 5	0	0.7657	0.4891		
GDF15	BL	ng/mL	> 1.75/≤ 1.75	0	Not estimable[a]	0.00002	Not estimable[a]	0.00028
HCC prehistory	BL		Yes/no	0	1.884	0.115		
Child–Pugh	BL		A,B/C	0	0.332	0.078	0.3673	0.1048
Esophagogastric varix	BL		Presence/absence	0	2.643	0.0842		
	BL		Unknown/absence	0	1.531	0.1492		
Ascites	BL		Presence/absence	0	2.418	0.0349		
Encephalopathy	BL		Presence/absence	0	3.467	0.0082		
Fib4-index	BL		> 3.25 ≤ 3.25	0	0.9507	0.92544		

Note: 25 events. *p* values were calculated by effect likelihood ratio tests.
[a]Hazard ratio not estimable due to absence of events in the low-GDF15 group. *p* value calculated by log-rank test.

was observed between the high- and low-GDF15 groups (Figure 3A). Univariate Cox regression analysis revealed that a history of HCC, platelet count, aspartate aminotransferase (AST) level, Child–Pugh score, presence of ascites, presence of encephalopathy, and albumin level, but not GDF15 level, were significant factors associated with HCC occurrence (Supporting Information S3: Table 3). Patients with a history of HCC presented a significantly higher cumulative incidence of HCC than did HCC-naïve individuals (Supporting Information S2: Figure S1E). Among patients without a history of HCC, none in the low-GDF15 group developed HCC during the three-year follow-up, whereas 19.3% of those in the high-GDF15 group developed HCC within three years, although the difference was not statistically significant (Figure 3B). We assessed the predictive performance of GDF15 for HCC occurrence at 3-years using time-dependent ROC analysis. For 3-year outcomes, the AUC was 0.6667 (95% CI, 0.4784–0.8549), with a Youden index–derived cutoff of 3.510 ng/mL (Supporting Information S2: Figure S1F). Stratification at 3.5 ng/mL identified a high-risk group with significantly worse outcomes in survival analyses (Supporting Information S2: Figure S1G).

In contrast, GDF15 levels did not distinguish HCC risk among patients with a prior history of HCC (Figure 3C).

3.5 | Combination Scoring Using Child–Pugh Score and GDF15

We developed a score combining the baseline Child–Pugh score and baseline GDF15 level and evaluated its predictive performance using Harrell's C-index. For all-cause mortality, the optimism-corrected C-indices were 0.637 for Child–Pugh alone, 0.705 for GDF15 alone, and 0.722 for the combined model. The combined model showed significantly better discrimination than Child–Pugh alone ($p = 0.043$), whereas the improvement over GDF15 was modest and not significant ($p = 0.548$). For hepatic decompensation, the corrected C-indices were 0.737 for Child–Pugh alone, 0.669 for GDF15 alone, and 0.775 for the combined model. The combined model discriminated significantly better than Child–Pugh alone ($p = 0.014$), whereas the difference from GDF15 alone was not significant ($p = 0.278$). Kaplan–Meier analyses further confirmed that this score effectively stratified risks

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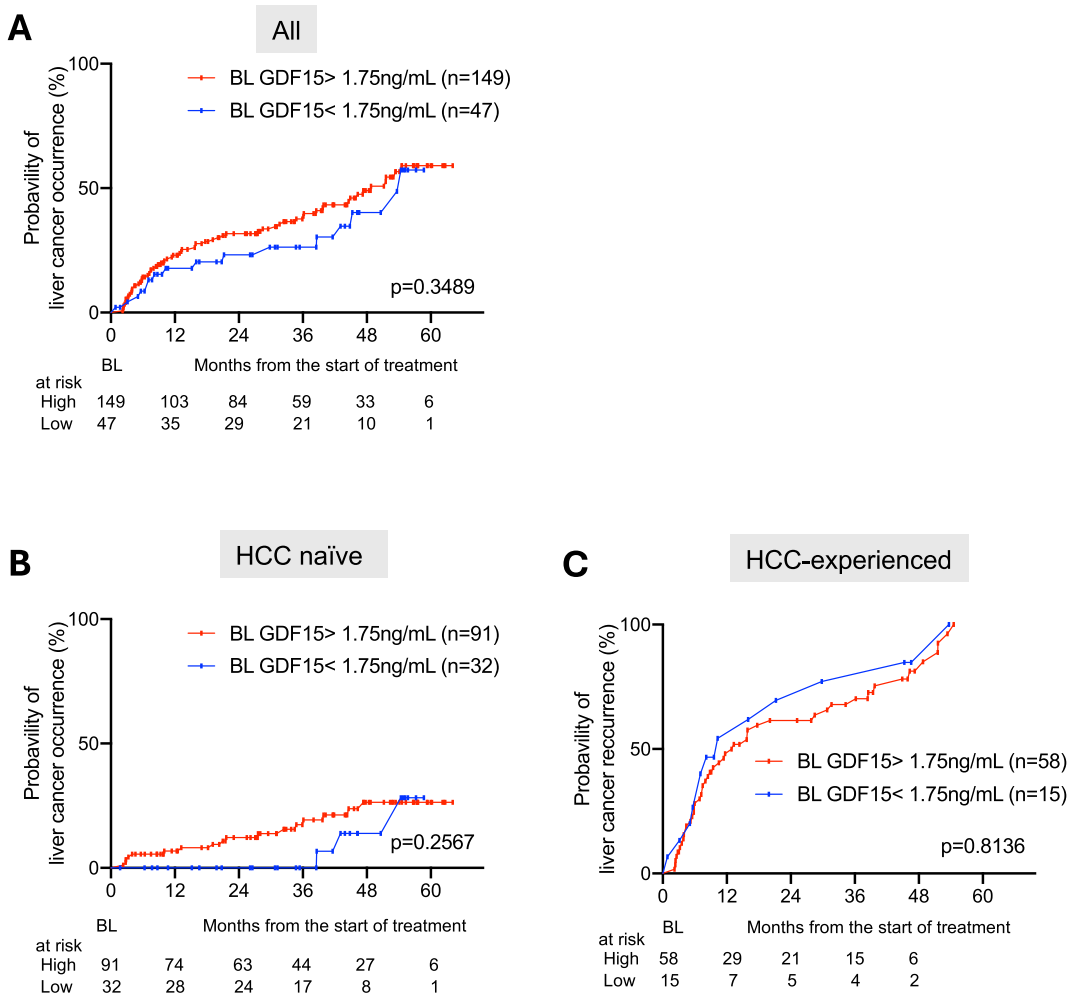

FIGURE 3 | Baseline GDF15 levels predict liver cancer occurrence in patients with HCV-related cirrhosis in the HCC-naïve cohort. (A) Kaplan–Meier curve showing the cumulative incidence of HCC in the entire cohort with available baseline serum samples and follow-up imaging data (n = 196). Patients were stratified by a baseline GDF15 cutoff value of 1.75 ng/mL. Statistical significance was assessed through the log-rank test. (B) Kaplan–Meier curve showing HCC development in the HCC-naïve cohort (n = 123) stratified by baseline GDF15 levels (cutoff = 1.75 ng/mL). The log-rank test was used for statistical analysis. (C) Kaplan–Meier curve for HCC recurrence in the HCC-experienced cohort (n = 73) stratified by baseline GDF15 levels (cutoff = 1.75 ng/mL). The log-rank test was used for statistical analysis.

of both mortality and hepatic decompensation (Supporting Information S2: Figure S2A,B).

3.6 | Serum GDF15 Levels After SVR24 and Liver Disease Events

We assessed whether GDF15 levels measured after viral elimination retained prognostic value. Among the 196 patients, 11 patients died 24 weeks after achieving SVR. Serum samples at both time points—baseline and 24 weeks after achieving SVR—were available for 185 patients. The median serum GDF15 concentrations at baseline and at SVR24 were 2.67 ng/mL (IQR: 1.81–3.76) and 2.29 ng/mL (IQR: 1.37–3.77), respectively. There was a significant decrease in GDF15 levels following viral elimination (Figure 4A).

Patients were stratified using the threshold of 1.75 ng/mL for the cutoff value [11]. We analyzed hepatic decompensation events, all-cause mortality, and the incidence of HCC occurring after p24w in 185 patients. For the analysis of hepatic decompensation events or the incidence of HCC, 11 patients who had experienced hepatic decompensation by p24w were excluded as were 38 patients who had developed HCC by p24w.

At p24w, GDF15 levels rose with increasing Child–Pugh class and score showing a stronger trend than that observed at baseline (Supporting Information S2: Figure S3A).

Patients in the high-GDF15 group had a significantly greater incidence of hepatic decompensation events (n = 174). At 24 weeks, decompensation events occurred in 12% of patients in the high-GDF15 group, whereas none were observed in the low-GDF15 group (Figure 4B). The GDF15 level was an independent risk factor for developing decompensation events from the Child–Pugh score (Table 4). Similarly, OS was significantly shorter in patients with high GDF15 levels at p24w (n = 185) (Figure 4C). The GDF15 level was an independent risk factor for mortality from the Child–Pugh score (Table 5).

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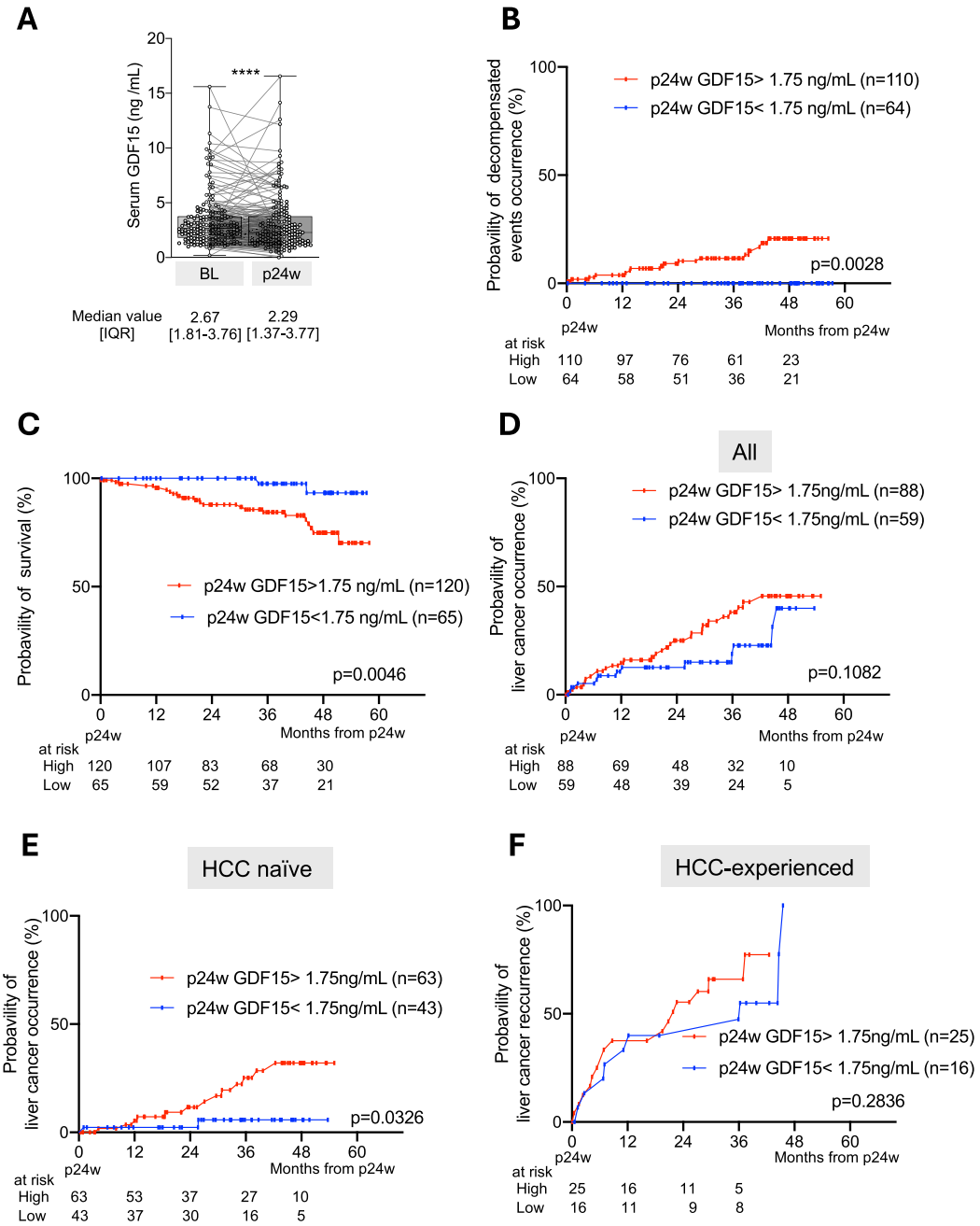

FIGURE 4 | GDF15 levels after virus elimination can also predict the prognosis of patients with HCV-related cirrhosis. (A) Box plots showing the GDF15 levels at baseline (BL) and p24w (24 weeks after EOT) in patients whose serum was available at these three time points (n = 185). The Wilcoxon matched-pairs signed-rank test was used for the statistical test. (B) Kaplan–Meier curve for decompensated event occurrence in the entire cohort with available serum samples (n = 174). Patients were divided by the cutoff value of GDF15 = 1.75 ng/mL at p24w. The log-rank test was used for statistical analysis. (C) Kaplan–Meier curve for overall survival in the entire cohort with available serum samples (n = 185). Patients were divided by the cutoff value of GDF15 = 1.75 ng/mL at p24w. The log-rank test was used for statistical analysis. (D) Kaplan–Meier curve for HCC occurrence in the entire cohort with available serum samples and follow-up imaging data (n = 147). Patients were divided by the cutoff value of GDF15 = 1.75 ng/mL at p24w. The log-rank test was used for statistical analysis. (E) Kaplan–Meier curve for HCC occurrence in the HCC-naïve cohort with available serum samples and follow-up imaging data (n = 106). Patients were divided by the cutoff value of GDF15 = 1.75 ng/mL at p24w. The log-rank test was used for statistical analysis. (F) Kaplan–Meier curve for HCC recurrence in the HCC-experienced cohort with available serum samples and follow-up imaging data (n = 41). Patients were divided by the cutoff value of GDF15 = 1.75 ng/mL at p24w. The log-rank test was used for statistical analysis. All the x-axes of the Kaplan–Meier curves describe the months from the Day 24 weeks after the end of treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, and ****$p < 0.001$.

At 24 weeks posttreatment, GDF15 levels did not significantly stratify the risk of HCC occurrence (n = 147) similar to the findings at baseline (Figure 4D, Supporting Information S3: Table 4). However, among the HCC-naïve patients, those with GDF15 levels ≥ 1.75 ng/mL had a significantly greater incidence of HCC (Figure 4E), whereas

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TABLE 4 | Factors related to decompensated events occurrence after the virus elimination.

Factors	Time point			Missing data	Univariate analysis hazard ratio	*p* value	Multivariate analysis hazard ratio	*p* value
Age	BL	Years old	> 70/≤ 70	0	0.45	0.1242		
Sex	BL		Male/female	0	1.078	0.8817		
HCV serogroup	BL		1/2	0	2.665	0.094		
Platelet	p24w	× 104/μL	> 10/≤ 10	2	0.417	0.0915		
AST	p24w	U/L	> 30/≤ 30	2	1.618	0.3471		
ALT	p24w	U/L	> 30/≤ 30	2	1.35	0.7027		
GGT	p24w	U/L	> 30/≤ 30	2	2.346	0.0922		
Total bilirubin	p24w	mg/dL	> 0.7/≤ 0.7	2	4.791	0.055		
Albumin	p24w	g/dL	> 3.6/≤ 3.6	2	0.229	0.00389		
PT	p24w	%	> 90/≤ 90	9	0.88	0.799		
AFP	p24w	ng/mL	> 5/≤ 5	8	1.91	0.2122		
GDF15	p24w	ng/mL	> 1.75/≤ 1.75	0	Not estimable[a]	0.00001	Not estimable[a]	0.00011
Child–Pugh	p24w		A,B/C	8	0.0962	0.0905	0.0878	0.08855
Fib4-index	p24w		> 3.25 ≤ 3.25	2	2.558	0.1645		

Note: Events $n = 16$. *p* values were calculated by effect likelihood ratio tests.
[a]Hazard ratio not estimable due to absence of events in the low-GDF15 group. *p* value calculated by log-rank test.

TABLE 5 | Factors related to mortality after the virus elimination.

Factors	Time point			Missing data	Univariate analysis hazard ratio	*p* value	Multivariate analysis hazard ratio	*p* value
Age	BL	Years old	> 70/≤ 70	0	1.787	0.1623		
Sex	BL		Male/female	0	2.069	0.0917		
HCV serogroup	BL		1/2	0	1.983	0.128		
Platelet	p24w	× 104/μL	> 10/≤ 10	2	0.982	0.965		
AST	p24w	U/L	> 30/≤ 30	2	1.201	0.6662		
ALT	p24w	U/L	> 30/≤ 30	2	2.777	0.0428		
GGT	p24w	U/L	> 30/≤ 30	2	1.646	0.2248		
Total bilirubin	p24w	mg/dL	> 0.7/≤ 0.7	2	1.238	0.6642		
Albumin	p24w	g/dL	> 3.6/≤ 3.6	2	0.3207	0.0068		
PT	p24w	%	> 90/≤ 90	9	0.6369	0.2938		
AFP	p24w	ng/mL	> 5/≤ 5	9	1.236	0.6057		
GDF15	p24w	ng/mL	> 1.75/≤ 1.75	0	6.215	0.0015	5.768	0.0029
HCC prehistory	BL		Yes/no	0	2.049	0.0812		
Child–Pugh	p24w		A,B/C	8	0.0407	0.00052	0.0401	0.00006
Fib4-index	p24w		> 3.25 ≤ 3.25	2	1.017	0.971		

Note: 24 events. *p* values were calculated by effect likelihood ratio tests.

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no association was observed in the HCC-experienced group (Figure 4F).

Finally, we analyzed whether the change in GDF15 from baseline to posttreatment (ΔGDF15) had prognostic significance. Patients with increased GDF15 after treatment had higher mortality and a greater risk of developing hepatic decompensation (Supporting Information S2: Figure S3B,C).

4 | Discussion

In this multicenter cohort of patients with HCV-related cirrhosis after SVR, higher serum GDF15 levels were associated with worse clinical outcomes. Specifically, GDF15 related to hepatic decompensation and mortality at both BL and p24w.

Prior work—including our own—has linked GDF15 with hepatic inflammation, fibrosis severity, and future adverse outcomes across viral and metabolic liver disease [8–11, 18]. The findings in the present study extend prior work to post-SVR cirrhosis and show that higher GDF15 is associated with increased risks of decompensation and mortality.

Although preliminary findings suggest that GDF15 might provide complementary prognostic information to established noninvasive tools (e.g., FIB-4, M2BPGi, ELF, and Child–Pugh score), its current role in clinical practice is uncertain. Further prospective studies in larger cohorts, ideally with head-to-head comparisons and predefined cutoffs, will be needed to clarify whether and how GDF15 could be incorporated into risk stratification or surveillance strategies.

When patients intervene with DAA therapy, the pretreatment Child–Pugh class becomes less important as a prognostic factor for patients with HCV cirrhosis; rather, the posttreatment Child–Pugh class becomes the defining patient prognostic factor [12]. In the present study, GDF15 values after viral elimination correlated well with the Child–Pugh class at that time. Although the precise mechanisms linking GDF15 with posttreatment liver function remain unclear, GDF15 likely reflects systemic stress, inflammation, and metabolic dysregulation. Notably, GDF15 predicted decompensation and mortality independently of posttreatment Child–Pugh, suggesting that it provides complementary prognostic information beyond conventional clinical scores.

There are several limitations in this study. Although the study used a prospective multicenter design ($n = 196$), the number of outcome events was limited (deaths: $n = 25$; decompensated events: $n = 28$; and HCC occurrence: $n = 75$), which reduces statistical power and widens confidence intervals. To minimize overfitting, we restricted model complexity according to events-per-variable considerations and conducted bootstrap internal validation (1000 resamples) to obtain optimism-corrected performance estimates. Accordingly, our results should be interpreted with caution and confirmed in larger independent cohorts. Because death and de novo HCC may act as competing risks, future studies with sufficient events should also consider competing-risk methods.

In conclusion, elevated serum GDF15 levels following viral elimination are associated with poor prognosis and may serve as a predictive biomarker for liver-related adverse outcomes in cirrhosis patients treated with DAAs.

Conflicts of Interest

Hayato Hikita received lecture fees from Gilead Sciences. Takahiro Kodama received lecture fees from Gilead Sciences. Nobuharu Tamaki received lecture fees from Gilead Sciences and AbbVie. Tetsuo Takehara received research grants from Gilead Sciences Inc. and AbbVie GK and is on the speakers' bureau for Gilead Sciences Inc. and AbbVie GK.

Data Availability Statement

This work does not include sequencing data. The data used in this study are available from the corresponding author upon request.

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