Past history of hepatocellular carcinoma is an independent risk factor of treatment failure in patients with chronic hepatitis C virus infection receiving direct-acting antivirals

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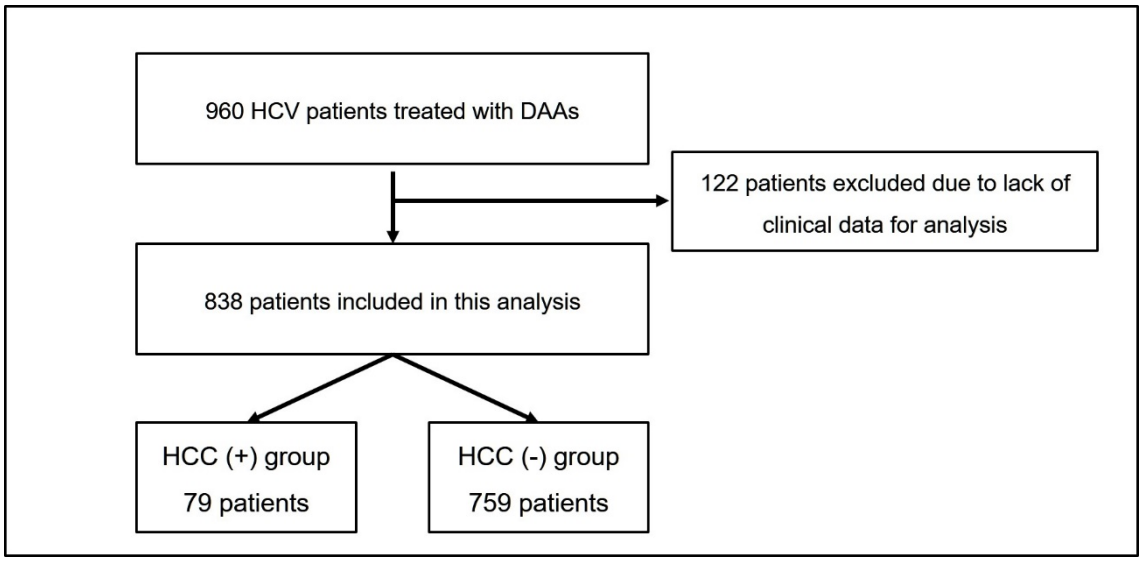
Supplementary Figure 1.

Selection flowchart of patients enrolled in this study.



Abbreviations: DAA, direct-acting antiviral; HCC, hepatocellular carcinoma

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Abstract

Direct-acting antiviral (DAA) treatment can achieve a high sustained virological response (SVR) rate in patients with hepatitis C virus (HCV) infection regardless of a history of hepatocellular carcinoma (HCC [+]). We examined 838 patients (370 men, median age: 69 years) who were treated with DAAs for comparisons of clinical findings between 79 HCC (+) (9.4%) and 759 HCC (-) (90.6%) patients and associations with treatment outcome. Male frequency was significantly higher in the HCC (+) group (60.8% vs. 42.4%, p = 0.006). There were significant differences between the HCC (+) and HCC (-) groups for platelet count (115 vs. 152 x10^9/L, p < 0.001), baseline AFP (9.9 vs. 4.5 ng/ml, p < 0.001), and the established fibrosis markers of FIB-4 index (4.7 vs. 3.0, p < 0.001), APRI (1.1 vs. 0.7, p = 0.009), M2BPGi (3.80 vs. 1.78 COI, p < 0.001), and autotaxin (1.91 vs. 1.50 mg/L, p < 0.001). The overall SVR rate was 94.7% and significantly lower in the HCC (+) group (87.3 vs. 95.5%, p = 0.001). Multivariate analysis revealed that a history of HCC was independently associated with DAA treatment failure (odds ratio: 3.56, 95% confidence interval: 1.32-9.57, p = 0.01). In conclusion, patients with chronic HCV infection and prior HCC tended to exhibit more advanced disease progression at DAA commencement. HCC (+)

77 status at the initiation of DAAs was significantly associated with adverse
78 therapeutic outcomes. DAA treatment for HCV should therefore be started as
79 early as possible, especially before complicating HCC.

80

1 Introduction

With an estimated 130–170 million people chronically infected worldwide including 1.5 million cases in Japan, hepatitis C virus (HCV) infection has become a global health concern, Chronic long-term HCV infection eventually results in severe liver disease manifesting as advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1-4]. HCV eradication is the most effective treatment to halt disease progression. During the late 1990s and early 2000s, major advances in interferon (IFN) and combinations of IFN or pegylated IFN and ribavirin (RBV) were approved for chronic HCV infection to increase sustained virological response (SVR) rates from 5% to 40–80% [5,6]. Progress in the understanding of viral kinetics has provided tools to identify patients most likely to attain a SVR, and insights into the HCV genome and proteins has also improved the efficacy and tolerability of HCV treatment, culminating in the development of multiple direct-acting antivirals (DAAs) that target specific steps within the HCV life cycle [7]. The approval of DAAs has revolutionized therapy against HCV infection, with current SVR rates of over 90% despite factors like advanced age or the presence of cirrhosis [8].

Liver cirrhosis caused by chronic HCV infection is a leading risk factor

for the development of HCC, with an annual incidence rate of 1–8% per year [9]. Although IFN therapy has been contraindicated for patients with cirrhosis and/or HCC due to several side effects, DAAs have shown high tolerability and SVR rates for such patients. Advanced fibrosis is a known risk factor of DAA treatment failure [8,10-12], but there remains debate on the clinical impact of a history of HCC on DAA outcome. This study aimed to uncover the clinical features of patients with prior HCC and determine the influence of this status on the therapeutic results of DAAs in patients with chronic HCV infection.

2 Patients and Methods

2.1 Patients

In this retrospective, multi-center, cohort analysis across Nagano prefecture, Japan, a total of 960 patients with chronic HCV infection underwent DAA therapy at Shinshu University Hospital (Matsumoto, Japan) or its affiliated institutions between April 2015 and October 2017. After excluding cases lacking sufficient clinical data for analysis, 838 patients chronic HCV infection were ultimately enrolled (supplementary figure 1). The racial background of all patients was Japanese. The diagnosis of chronic hepatitis C was based on

previously reported criteria as the presence of serum HCV antibodies and detectable HCV RNA [13]. The presence of chronic HCV infection was defined as detectable HCV RNA by the real-time polymerase chain reaction at the initiation of therapy.

This study was reviewed and approved by the Institutional Review Board of Shinshu University School of Medicine (approval number: 3244) and its affiliated hospitals. Written informed consent was obtained from all participating subjects. The study was conducted according to the principals of the Declaration of Helsinki.

2.2 Study design

All patients in this cohort were registered upon commencing DAAs for age, gender, history of IFN treatment, history of a HCC complication, and comorbidities such as hypertension, diabetes, and hyperlipidemia.

The patients were treated with DAA regimens that included daclatasvir + asunaprevir (DCV+ASV) for 24 weeks [14] or ledipasvir/sofosbuvir (LDV/SOF), ombitasvir/paritaprevir/ritonavir (OBV/PTV/r), or elbasvir + grazoprevir (EBR+GZR) for 12 weeks for patients infected with HCV genotype 1, or with

SOF+RBV for 12 weeks for those with genotype 2, based on guidelines from the Japan Society of Hepatology [15]. Since a resistance-associated substitution (RAS) at position 93 of the HCV NS5A region (NS5A-Y93H) was reported to reduce the effectiveness of DCV+ASV [16], patients with this variant were advised to wait for next generation DAA therapies for as long as possible. Individuals who were unable to postpone treatment due to clinical reasons including progression to liver cirrhosis or advanced age were treated with DCV+ASV. A SVR12 was defined as undetectable HCV RNA at 12 weeks after completion of DAA therapy. Treatment failure was defined as detectable HCV RNA during treatment or within 12 weeks of completion or discontinuation of DAAs.

2.3 Laboratory testing

All laboratory data, such as hemoglobin, platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alpha fetoprotein (AFP), were determined using standard methods at respective institutions.

2.4 Fibrosis markers

The fibrosis-4 (FIB-4) index and AST-to-platelet ratio index (APRI) were respectively calculated as: age (years) × AST (IU/L) / (platelet count [10^9/L] × ALT [IU/L]$^{1/2}$) [17] and (AST / upper limit of normal; 40 IU/L) × (100 / platelet count [10^9 /L]) [18,19]. Isolated blood samples were immediately stored at -20°C until testing. Serum autotaxin (ATX) antigen concentration was simultaneously measured using frozen serum samples by a specific two-site enzyme immunoassay with an AIA-2000 system (Tosoh Co., Tokyo, Japan) as described previously [20-22]. The recently established macrophage galactose-specific lectin-2 binding protein glycosylation isomer (M2BPGi) fibrosis marker was quantified as earlier described [23].

2.5. Resistance testing of NS5A-Y93H for DCV+ASV therapy

The NS5A-Y93H RAS was detected by RT-PCR as described previously [24], with a value of 20% or more defined as NS5A-Y93H-positive.

2.6 Statistical analysis

Statistical analysis and data visualization were carried out using StatFlex ver. 6.0 (Artech Co., Ltd., Osaka, Japan). Continuous baseline data are

171 expressed as the median ± interquartile range and statistically evaluated by
172 means of the Mann-Whitney U test. Categorical variables are presented as the
173 frequency (percentage) and were analyzed using the chi-square test. Cutoff
174 values were identified by the Youden index, and the nearest clinically applicable
175 value to the cutoff was considered as the optimal threshold for clinical
176 convenience. Multivariate analysis was performed using regression analysis with
177 stepwise method after categorizing continuous variables to minimize
178 interference. All statistical tests were two-sided and evaluated at the 0.05 level of
179 significance.
180

181 **3 Results**

182 **3.1 Baseline clinical characteristics**

183 The baseline clinical characteristics in this study are summarized in
184 Table 1. Of the 838 enrolled patients, 370 (44.2%) were male and 468 (55.8%)
185 were female and median age was 69 years. Roughly half of patients were
186 complicated with hypertension, followed next by diabetes mellitus and
187 dyslipidemia. Seventy-nine (9.4%) patients had a history of HCC and were
188 placed into the HCC (+) group, while 759 (90.6%) had no prior HCC and were

189 classified as HCC (-). The number of patients who were treated with DCV+ASV,
190 LDV/SOF, OBV/PTV/r, EBV+GRZ, and SOF+RBV was 288, 267, 22, 60, and
191 201, respectively. The overall SVR rate was 94.7% in our cohort.

192

193 **3.2 Comparisons between HCC (+) and HCC (-) groups**

194 Comparisons of the clinical characteristics of the HCC (+) and HCC (-)
195 groups are presented in Table 1. The HCC (+) group was significantly older ($p <$
196 0.001), and the frequency of male HCC (+) patients was significantly higher
197 (60.8 vs. 42.4%, $p = 0.002$). Other significant differences for the HCC (+) group
198 included lower platelet count (115 vs. 152 $x10^9$ /L, $p < 0.001$), higher baseline
199 AFP (9.9 vs. 4.5 ng/ml, $p < 0.001$), and higher scores for FIB-4 index (4.7 vs. 3.0,
200 $p < 0.001$), APRI (1.1 vs. 0.7, $p = 0.009$), M2BPGi (3.80 vs. 1.78 COI, $p < 0.001$),
201 and ATX (1.91 vs. 1.50 mg/L, $p < 0.001$). Interestingly, the overall SVR rate was
202 significantly lower in the HCC (+) group than in the HCC (-) group (87.4 vs.
203 95.4%, $p = 0.001$).

204

205 **3.3 Comparisons of DAA treatment failure and SVR groups**

206 Comparisons of clinical characteristics between DAA failure and SVR

groups are shown in Table 2. There were significant differences for platelet count (138 vs. 151 x10^9/L, p = 0.012), albumin (3.9 vs. 4.1 g/dL, p = 0.002), AST (50 vs. 37 U/L, p = 0.002), FIB-4 index (3.9 vs. 3.0, p < 0.001), APRI (1.2 vs. 0.7, p < 0.001), M2BPGi (2.38 vs. 1.86 COI, p = 0.004), and ATX (1.80 vs. 1.51 mg/L, p = 0.001). The frequency of HCC (+) was significantly higher in the DAA failure group than in the SVR group (22.7 vs. 8.7%, p = 0.001).

3.4 Predictive ability of clinical markers for DAA treatment failure

We assessed the ability of clinical markers to predict DAA treatment failure using receiver operating characteristic (ROC) analysis for continuous variables. As shown in Figure 1, the area under the ROC curve (AUROC) for platelet count, albumin, AST, FIB-4 index, APRI, M2BPGi, and ATX was 0.680, 0.630, 0.602, 0.684, 0.672, 0.564, and 0.635, respectively. Based on determined AUROC values, the optimal cutoff value, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in relation to DAA treatment failure were calculated and summarized in Table 3. HCC history showed the highest accuracy in terms of DAA treatment failure prediction.

225 **3.5 Predictors of DAA treatment failure in univariate and multivariate**
226 **analysis**

227 The univariate predictors of HCV treatment failure presented in Table 4
228 identified significant associations for platelet count < 152 x 10^9 /L (DAA failure vs.
229 SVR: 49.2 vs. 31.8%, p = 0.02), albumin < 4.0 g/dL (72.8 vs. 41.7%, p < 0.001),
230 FIB-4 index ≥ 3.25 [25] (54.1 vs. 28.6%, p = 0.02), APRI ≥ 1.0 (68.1 vs. 42.9%, p =
231 0.02), M2BPGi ≥ 2.2 COI (56.2 vs. 45.0%, p = 0.16), ATX ≥ 2.2 mg/L [20] (80.3 vs.
232 64.9%, p = 0.02), and HCC (+) (22.7 vs. 8.7%, p = 0.001).

233 Multivariate analysis confirmed that HCC (+) status (odds ratio [OR]:
234 3.56, 95% confidence interval [CI] 1.32-9.57) was an independent risk factor
235 predicting DAA treatment failure (Table 4).

236

237 **3.6 Comparisons between DAA treatment failure and SVR patients without**
238 **HCC history**

239 Since a history of HCC was the highest independent DAA failure factor,
240 we excluded all patients with prior HCC and compared the clinical characteristics
241 of DAA failure and SVR groups (Table 5). The DAA failure group showed
242 significantly lower albumin, higher AST, and higher established fibrosis markers

than did the SVR group, suggesting that clinically progressed disease could also be associated with DAA outcome in the cohort.

4 Discussion

This study identified two important clinical features of a history of HCC in chronic HCV under DAA treatment: 1) patients with prior HCC receiving DAAs exhibited more advanced pre-treatment liver disease progression than those without, and 2) a history of HCC was an independent risk factor of treatment failure with oral DAAs. These findings have important clinical implications on the optimal timing of chronic HCV infection treatment.

The patients with a history of HCC in this cohort were significantly older than those without and were more frequently male. These results were consistent with a previous report that showed independent predictive factors of complicating HCC in HCV infection to be male and over 60 years of age [26]. The subjects with prior HCC also exhibited lower platelet count, lower albumin, higher AST, and higher fibrosis marker scores for FIB-4 index, APRI, M2BPGi, and ATX, indicating more progressed liver fibrosis. It is important to understand the natural history of HCV infection, whereby chronic HCV infection slowly but

261 significantly progresses to HCC over time [1] in the absence of eradication therapy
262 [27]. Moreover, a HCC history was more frequent in patients with genotype 1 HCV
263 than in those with genotype 2, suggesting that genotype 1 led to more advanced
264 disease progression in support of previous reports [26,28]. Thus, patients with prior
265 HCC may require more intensive care during DAA treatment considering their
266 disease status.

267 To date, it remains uncertain whether a history of HCC influences DAA
268 outcome. Although active HCC at the initiation of HCV therapy has been
269 significantly associated with DAA treatment failure [29], such treatment is not
270 approved in Japan and so no patient had active HCC at the commencement of
271 DAAs. Our results demonstrated that subjects with past HCC achieved a lower
272 SVR rate than did those without, which was confirmed by multivariate analysis.
273 Several factors are reportedly associated with DAA treatment failure, including
274 fibrosis, cirrhosis, and drug regimen and adherence [8,16,30-33]. The present
275 findings indicate that a history of HCC should be included as a failure risk factor
276 as well.

277 The molecular and biological mechanisms of DAA failure in relation to
278 HCC history remain unresolved. There were significant differences in M2BPGi

279 and ATX between the study groups, suggesting the involvement of multiple
280 mechanisms since M2BPGi and ATX reflected both fibrosis and hepatitis activity
281 [20,34] and have been considered to exhibit pleiotropic functions. Genetic
282 polymorphisms, such as interleukin 28B and HCV core-region substitutions,
283 have been linked to IFN treatment outcome and HCC complications [35,36]; indeed,
284 the frequency of IFN treatment failure in our cohort was significantly higher in the
285 DAA failure group (53.7%) than in the SVR group (37.0%; p = 0.032). Moreover,
286 tumor-associated antigen (TAA)-specific $CD8^+$ T-cell responses have been
287 correlated to impaired IFN-gamma production in patients with HCC, which
288 indicated exhaustion of TAA-specific $CD8^+$ T cells [37]. The exhaustion of
289 HCV-specific $CD8^+$ T cells by mechanisms involving the expression of inhibitory
290 receptors has been associated with HCV eradication as well [38]. Taken together,
291 there are likely other unknown molecular and biological mechanisms modulating
292 the influence of prior HCC on DAA failure that merit future study. Meanwhile,
293 HCC history represents an important indicator easily obtained in medical
294 interviews that may reliably predict DAA failure.

295 In certain populations, testing for pre-existing RASs is considered
296 beneficial prior to the use of certain regimens, such as DCV+ASV. Our strategy

297 was that if patients harboring the NS5A-Y93H RAS could no longer postpone
298 treatment due to age, disease progression, or other clinical reasons, they
299 commenced DCV+ASV instead of waiting for next-generation DAAs. Accordingly,
300 the DCV+ASV cohort showed lower platelet count and higher AFP that did the
301 other regimen groups (median platelet count: 132 vs. 158 $x10^9$/ L, $p < 0.001$, and
302 median AFP: 6.2 vs. 4.5 ng/mL, $p = 0.037$), indicating more advanced disease
303 progression. As reported previously [16], RAS was an independent and the
304 strongest failure risk factor in DCV+ASV therapy (OR: 2.15, 95% CI 1.37-3.37, p
305 < 0.001). Indeed, RASs should be considered in DAA treatment planning to
306 maximize SVR rates.

307 Our study has several limitations apart from its retrospective design.
308 Since patients with Child–Pugh class B and C cirrhosis were not approved for
309 DAA therapy in Japan were not included, the risk factors and optimal timing of
310 DAAs for such patients require further investigation. Second, the merits of
311 treating patients before advanced progression of hepatic disease have been
312 clearly shown, with several-fold decreases in the risk of death and development
313 of HCC [39]. It was also reported that a past history of HCC was independently
314 associated with HCC recurrence after achieving a SVR [40]. Therefore, the

long-term outcome of HCC history requires attention in the future.

In conclusion, chronically infected HCV patients with a history of HCC showed more advanced disease progression at the onset of DAA therapy. As prior HCC at the initiation of DAAs was significantly associated with treatment failure, DAA treatment for HCV should be induced as early as possible, especially before complicating HCC.

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Additional information

Conflict of interest: Koji Igarashi is an employee of TOSOH Corporation. The remaining authors declare that they have nothing to disclose regarding funding from industries or other conflicts of interest with respect to this

manuscript.

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Table 1. Baseline characteristics and comparisons of patients with or without HCC past history.

	All patients (n=838)		HCC (+) (n=79)		HCC (-) (n=759)		HCC (+) vs. HCC (-)
	Median	IQR	Median	IQR	Median	IQR	p value
Age at enrollment (years)	69	(16-90)	72	(49-84)	69	(16-90)	<0.001
Gender (male / female)	370 / 468		48 (61%)		322 (42%)		0.002
Laboratory data							
WBC (μ/L)	4510	(590-12,690)	4,100	(590-7,300)	4,600	(1,680-12,690)	<0.001
Hb (g/dL)	13.7	(5.6-18.8)	13.2	(9.2-16.9)	13.8	(5.6-18.8)	0.019
Platelet count (x10^9 /L)	150	(10-410)	115	(34-277)	152	(27-410)	<0.001
Albumin (mg/dL)	4.1	(2.4-5.1)	3.9	(2.4-4.6)	4.2	(2.4-5.1)	<0.001
AST (U/L)	38	(10-370)	45	(21-174)	37	(10-370)	0.09
ALT (U/L)	38	(7-673)	48	(13-142)	37	(7-673)	0.526
AFP (ng/mL)	4.9	(0.9-381.0)	9.9	(1.7-381.0)	4.5	(0.9-162.9)	<0.001
eGFR (mL/min/1.73m^2)	70.1	(0.55-131.5)	69.9	(42.0-102.0)	71.0	(0.55-131.5)	0.794
Fibrosis markers							
FIB-4 index	3.0	(0.52-38.5)	4.7	(1.4-34.3)	3.0	(0.5-38.5)	<0.001
APRI	0.7	(0.13-21.5)	1.1	(0.2-8.3)	0.7	(0.1-21.5)	0.009
M2BPGi (COI)	1.85	(0.24-19.1)	3.80	(0.73-19.11)	1.78	(0.24-16.22)	<0.001
Autotaxin (mg/L)	1.51	(0.53-5.33)	1.91	(0.60-5.33)	1.50	(0.53-4.28)	<0.001
Cormorbidities							
Hypertension	40.3%		54.5%		38.6%		0.04
Diabetes	16.3%		11.4%		16.7%		0.35
Dyslipidemia	7.4%		5.4%		7.6%		0.48
Experienced							
Prior IFN	37.9%		54.1%		36.2%		0.002
Prior DAA	0.35%		0.00%		0.39%		0.44
RAS (Y93H)*	26.9%		3.6%		23.3%		0.72

Regimens, number (%)				
Genotype 1				
DCV+ASV	288 (34.3)	35 (44.3)	253 (33.3)	0.772
LDV/SOF	267 (31.9)	27 (34.2)	240 (31.6)	
OBV/PTV/r	22 (2.6)	2 (2.5)	20 (2.7)	
EBV+GRZ	60 (7.2)	5 (6.3)	55 (7.2)	
Genotype 2				
SOF+RBV	201 (24.0)	10 (12.7)	191 (25.2)	
SVR (%)				
Overall	94.7	87.3	95.5	0.001
Genotype 1 (all)	94.5	87.0	95.4	0.003
First generation DAAs				
DCV+ASV	91.7	88.5	92.0	0.47
Second generation DAAs (all)	96.8	85.3	98.1	<0.001
LDV/SOF	97.0	85.1	98.3	<0.001
OBV/PTV/r	95.5	50.0	100	0.001
EBR+GZR	96.7	100	96.6	0.66
Genotype 2				
SOF+RBV	95.5	90.0	95.8	0.38

*: RAS was determined by PCR-Invader assays in the DCV+ASV cohort.

Abbreviations: HCC, hepatocellular carcinoma; IQR, interquartile range; WBC, white blood cells; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; eGFR, estimate glomerular filtration rate; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; IFN, interferon; DAA, direct-acting antiviral; RAS, resistance-associated substitution; DCV+ASV, daclatasvir+asunaprevir; LDV/SOF, ledipasvir/sofosbuvir; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; EBR+GZR, elbasvir+grazoprevir; SOF+RBV, sofosbuvir+ribavirin; SVR, sustained virological response

Table 2. Clinical comparisons of DAA treatment failure and SVR groups.

	DAA failure (n=44)		SVR (n=794)		
	Median	IQR	Median	IQR	p value
Age at enrollment (years)	69	(43-82)	69	(16-90)	0.255
Gender (male / female)	16 / 28	(36.4 / 63.6%)	354 / 440	(44.6 / 55.4%)	0.285
Laboratory data					
WBC (μ/L)	4,185	(590-8,400)	4,530	(1,290-12,690)	0.070
Hb (g/dL)	13.4	(8.9-18.5)	13.8	(5.6-18.8)	0.179
Platelet count (x10^9 /L)	138	(27-267)	151	(10-410)	0.012
Albumin (mg/dL)	3.9	(3.0-4.5)	4.1	(2.4-5.1)	0.002
AST (U/L)	50	(13-276)	37	(10-370)	0.002
ALT (U/L)	42	(11-199)	37	(7-673)	0.420
AFP (ng/mL)	7.0	(1.8-50.0)	4.8	(0.9-381.0)	0.970
eGFR (mL/min/1.73m^2)	64.9	(0.60-102.0)	70.5	(0.55-131.5)	0.467
Fibrosis markers					
FIB-4 index	3.9	(1.6-23.1)	3.0	(0.0-34.3)	<0.001
APRI	0.7	(0.3-5.2)	0.7	(0.0-8.5)	<0.001
M2BPGi (COI)	2.38	(0.41-18.52)	1.86	(0.24-19.11)	0.004
Autotaxin (mg/L)	1.80	(0.87-3.98)	1.51	(0.53-5.33)	0.001
Comorbidities					
Hypertension	20 (50.0%)		239 (39.6%)		0.198
Diabetes	5 (22.7%)		74 (15.9%)		0.399
Dyslipidemia	4 (9.3%)		51 (7.3%)		0.624
Past history of HCC	10 (22.7%)		69 (8.7%)		0.001
Experienced					
Prior IFN	22 (53.7%)		279 (37.0%)		0.032
Prior DAA	1 (2.3%)		2 (0.3%)		0.028
RAS (Y93H)*	4.7%		22.2%		<0.001
Regimens, number (%)					

DCV+ASV	24 (54.5)	264 (33.2)	0.083**
LDV/SOF	8 (18.2)	259 (32.6)	
OBV/PTV/r	2 (2.3)	21 (2.7)	
EBV+GRZ	1 (4.5)	58 (7.3)	
SOF+RBV	9 (20.5)	192 (24.2)	

*: RAS was determined by PCR-Invader assays in the DCV+ASV cohort.
**: The frequency of HCC (+) in the DCV+ASV group was significantly higher than that of the other combined regimens: 24 of 288 (8.3%) vs. 20 of 530 (3.8%), p = 0.004

Abbreviations: DAA, direct-acting antiviral; SVR, sustained virological response; IQR, interquartile range; WBC, white blood cells; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; eGFR, estimate glomerular filtration rate; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; HCC, hepatocellular carcinoma; IFN, interferon; RAS, resistance-associated substitution; DCV+ASV, daclatasvir+asunaprevir; LDV/SOF, ledipasvir/sofosbuvir; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; EBV+GRZ, elbasvir+grazoprevir; SOF+RBV, sofosbuvir+ribavirin

Table 3. Diagnostic performance of clinical markers related to DAA failure.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Albumin < 4.0 g/dL	58	72	9	97	72.7
Platelet count < 152 x 10^9 /L	68	49	7	90	50.2
AST ≧ 40 U/L	28	47	7	97	53.8
FIB-4 index ≧ 3.25	71	54	7	97	54.9
APRI ≧ 1.0	57	68	8	97	67.7
M2BPGi ≧ 3.0 COI	43	73	8	96	71.2
Autotaxin ≧ 2.2 mg/L	35	80	9	96	77.9
Past history of HCC	23	91	13	96	87.7

Abbreviations: DAA, direct-acting antiviral; PPV, positive predictive value; NPV, negative predictive value; AST, aspartate aminotransferase; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; HCC, hepatocellular carcinoma

Table 4. Multivariate predictors of DAA treatment failure in the study population.

	Univariate		Multivariate	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Albumin < 4.0 g/dL	3.75 (1.71-8.21)	<0.001	2.35 (0.95-5.77)	0.06
Platelet count < 152 x 10^9 /L	2.07 (1.09-3.93)	0.02	2.50 (0.93-6.70)	0.06
AST ≧ 40 U/L	2.82 (1.11-7.14)	0.02		
FIB-4 index ≧ 3.25	2.95 (1.17-7.44)	0.02		
APRI ≧ 1.0	2.84 (1.20-6.67)	0.01		
M2BPGi ≧ 3.0 COI	1.84 (0.98-3.48)	0.05		
Autotaxin ≧ 2.2 mg/L	2.20 (1.11-4.38)	0.02		
Past history of HCC	3.09 (1.51-6.30)	0.001	3.56 (1.32-9.57)	0.01

Abbreviations: DAA, direct-acting antiviral; CI, confidence interval; AST, aspartate aminotransferase; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; HCC, hepatocellular carcinoma

Table 5. Comparisons of clinical characteristics between DAA failure and SVR patients in subjects without HCC history

	DAA failure (n=34)		SVR (n=725)		
	Median	IQR	Median	IQR	p value
Age at enrollment (years)	72	(43-81)	68	(16-90)	0.231
Gender (male / female)	10 / 24	(29.4 / 70.6%)	312 / 413	(43.0 / 57.0%)	0.116
Laboratory data					
WBC (μ/L)	4,285	(1,960-8,400)	4,600	(1,680-12,690)	0.277
Hb (g/dL)	14.4	(8.9-18.5)	15.3	(5.6-18.8)	0.258
Platelet count (x10^9/L)	107	(27-267)	117	(10-410)	0.075
Albumin (mg/dL)	3.8	(3.0-4.5)	4.2	(2.4-5.1)	0.001
AST (U/L)	54	(26-124)	36	(21-370)	0.001
ALT (U/L)	39	(11-199)	37	(7-673)	0.488
AFP (ng/mL)	6.5	(1.8-39.3)	4.5	(0.9-162.9)	0.559
eGFR (mL/min/1.73m^2)	64.4	(0.6-97.0)	71.0	(0.5-131.5)	0.106
Fibrosis markers					
FIB-4 index	4.0	(1.06-38.5)	3.0	(0.52-19.4)	<0.001
APRI	1.6	(0.1-21.5)	0.6	(0.1-7.8)	<0.001
M2BPGi (COI)	1.55	(0.41-16.22)	1.77	(0.24-15.53)	0.011
Autotaxin (mg/L)	1.90	(0.87-3.98)	1.48	(0.53-4.28)	0.002
Cormorbidities, number (%)					
Hypertension	16 (51.6)		207 (38.0)		0.129
Diabetes	5 (31.3)		69 (16.2)		0.113
Dyslipidemia	4 (11.8)		47 (7.4)		0.350
Experienced					
Prior IFN	18 (56.3)		243 (35.3)		0.016
Prior DAA	1 (20.0)		2 (4.5)		0.172
Regimens, number (%)					

DCV+ASV	20 (58.8)	233 (32.1)	0.052
LDV/SOF	4 (11.8)	236 (32.6)	
OBV/PTV/r	0 (0.0)	20 (2.8)	
EBV+GRZ	2 (5.9)	53 (7.3)	
SOF+RBV	8 (23.5)	183 (25.2)	

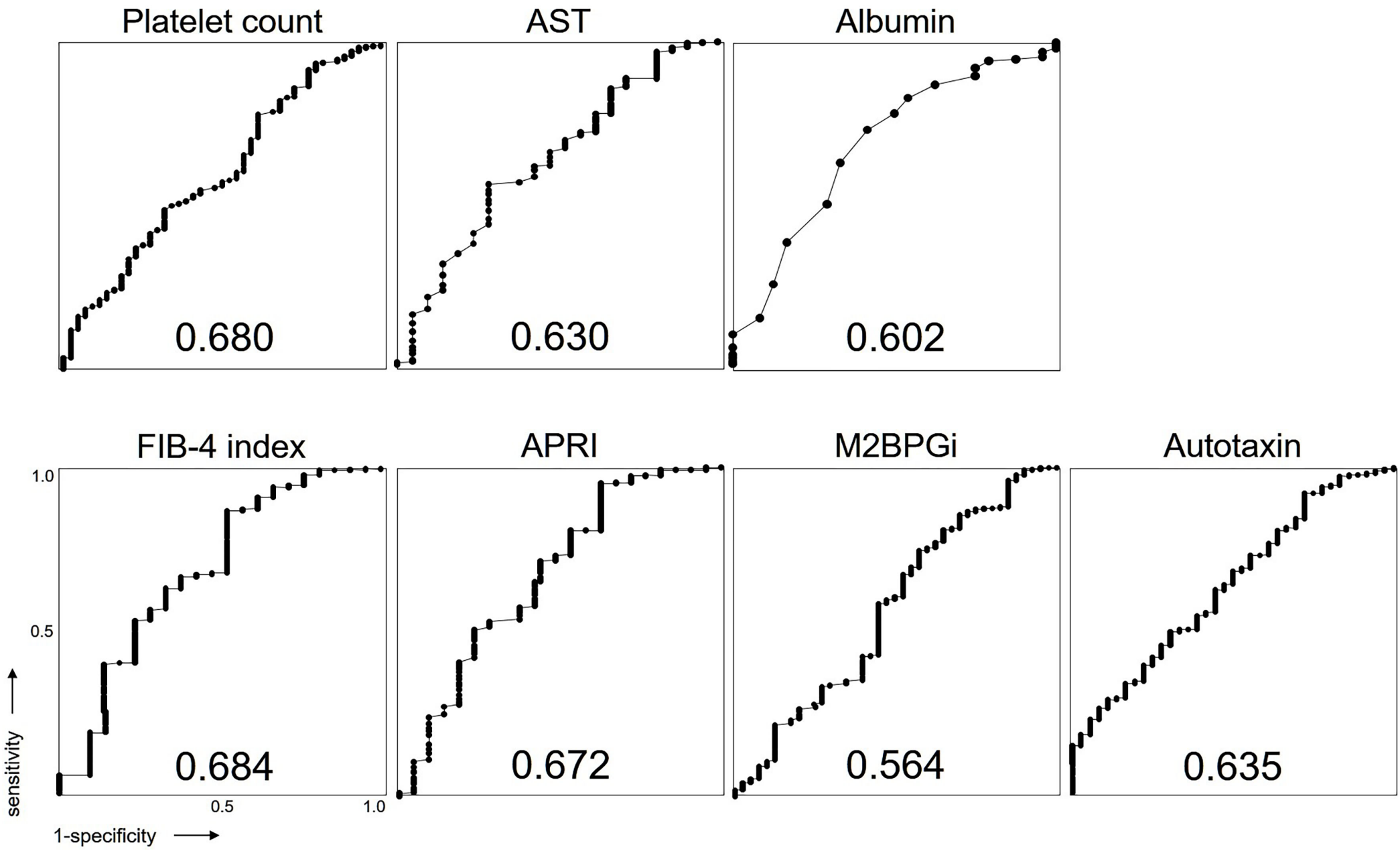
Abbreviations: DAA, direct-acting antiviral; SVR, sustained virological response; HCC, hepatocellular carcinoma; IQR, interquartile range; WBC, white blood cells; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; eGFR, estimate glomerular filtration rate; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; IFN, interferon; DCV+ASV, daclatasvir+asunaprevir; LDV/SOF, ledipasvir/sofosbuvir; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; EBR+GZR, elbasvir+grazoprevir; SOF+RBV, sofosbuvir+ribavirin

Figure Legends

Figure 1.

Diagnostic ability of platelet count, AST, albumin, FIB-4, APRI, M2BPGi, and autotaxin to predict DAA treatment failure in HCV patients. The area under the receiver operating characteristic curve for each marker is shown.

Abbreviations: AST, aspartate aminotransferase; FIB-4, fibrosis-4 index; APRI, aspartate aminotransferase-to-platelet ratio index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; DAA, direct-acting antiviral; HCV, hepatitis C virus


Platelet count
0.680
AST
0.630
Albumin
0.602
FIB-4 index
0.684
APRI
0.672
M2BPGi
0.564
Autotaxin
0.635
1.0
0.5
0.5
1.0
sensitivity
1-specificity