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Original Research

The Daclatasvir/Asunaprevir/Beclabuvir Combination Therapy for Chronic Hepatitis C Patients Experiencing Failure of IFN-Free DAA-Based Therapies

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Abstract

Background: The combination of daclatasvir/asunaprevir/beclabuvir (DCV/ASV/BCV) has been available in Japan for the treatment of chronic hepatitis C patients with genotype 1 (CHG1). The efficacy and safety of this combination for patients experiencing treatment failure with interferon (IFN)-free direct-acting antiviral agent (DAA)-based therapies have not yet been fully evaluated.

Methods: We evaluated the efficacy and safety of 12-week DCV/ASV/BCV combination therapy for CHG1 patients experiencing failure with DCV/ASV and/or sofosbuvir/ledipasvir (SOF/LDV) combinations.

Results: Nine patients were eligible for inclusion in this study. The previous IFN-free DAA-based therapies included DCV/ASV (n = 7), SOF/LDV (n = 1), and both DCV/ASV and SOF/LDV (n = 1). The sustained virologic response at post-treatment week (PTW) 24 (SVR24) rate was 33.3% (3/9) and an SVR24 was obtained in patients who previously received DCV/ASV combination therapy. The virologic relapse occurred at PTW16 or 18 in two patients. We detected "D168E or D168H in addition to Y93H", "presumed L31 deletion (L31del) or P32del", and "P29del", which may cause resistance to DCV/ASV/BCV combination therapy. One patient developed Grade 3 liver dysfunction but treatment could be completed with dose modification.

Conclusions: The DCV/ASV/BCV combination therapy was relatively well-tolerated in patients who experienced treatment failure with IFN-free DAA-based combination regimens, although the efficiency was unsatisfactory. In two cases, virologic relapse occurred after PTW12, therefore, an SVR24 may be more suitable for judging viral eradication than an SVR12. The role of resistance-associated substitutions in patients who experienced treatment failure with DCV/ASV/BCV combination therapy should be further evaluated.

Keywords

Daclatasvir; asunaprevir; beclabuvir; chronic hepatitis C; genotype 1

1. Introduction

According to the recent version (ver. 7.0) of the Japan Society of Hepatology (JSH) guidelines, combination therapy with sofosbuvir and ledipasvir (SOF/LDV), elbasvir and grazoprevir (EBR/GZR), and glecaprevir and pibrentasvir (GLE/PIB) is recommended as the first-line treatment of chronic hepatitis and compensated liver cirrhosis in patients with hepatitis C virus (HCV) genotype 1 infection [1]. However, as per the former version (ver. 5.4) of the JSH guidelines for the management of HCV infection, daclatasvir/asunaprevir/beclabuvir (DCV/ASV/BCV) had been the first choice of therapy for chronic hepatitis and compensated liver cirrhosis [2] and in the subsequent version (ver. 6.0) of the JSH guidelines, DCV/ASV/BCV combination therapy was recommended as the first choice of therapy exclusively for chronic hepatitis [3]. Nevertheless, the relatively high frequency of liver dysfunction compared to other DAA regimens and the necessity of weekly monitoring of laboratory data may prevent the widespread adoption of DCV/ASV/BCV combination therapy as standard therapy.

Combination therapy with GLE/PIB or SOF/velpatasvir (VEL) along with ribavirin (RBV) is reportedly effective for patients experiencing treatment failure with DAA regimens, such as DCV/ASV combination therapy [4, 5]. However, the efficacy of DCV/ASV/BCV combination therapy in patients experiencing treatment failure with DAA regimens has not been fully evaluated, although a few studies have been reported [6, 7]. We tried DCV/ASV/BCV combination as a treatment for patients experiencing DAA failure when GLE/PIB or SOF/VEL combination therapy was not available and evaluated the efficacy and safety of this combination.

This case series is a part of a prospective, multi-institutional study evaluating the efficacy and safety of DAA-based therapy for patients infected with HCV genotype 1 at our university hospital and its affiliated hospitals.

2. Patients and Methods

This study was approved by the Institutional Review Board at each institute. We obtained written informed consent from each participant. This prospective study complies with the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan and is registered in Japan's UMIN-CTR (a clinical trial registry) as UMIN000019212.

This case series focused on patients receiving DCV/ASV/BCV combination therapy in this prospective study.

2.1 Patients

Overall patients from Japan were enrolled from October 2015 to March 2018 in the original prospective study. It was required that patients should be chronically infected with HCV genotype 1 and have viremia. Patients with chronic hepatitis C or compensated cirrhosis were included. All subjects were intended to receive 12 weeks of treatment with DCV (60 mg, per day)/ASV (400 mg, per day)/BCV (150 mg, per day) administered orally twice daily. After starting combination therapy, we principally checked the laboratory data and adverse events (AEs) each week. On-treatment assessments included standard laboratory testing, serum HCV RNA and symptom-directed physical examinations. Clinical laboratory testing was carried out at the time of visits during the treatment period and after the end of the treatment period. AEs were evaluated according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) [8]. In this study, the inclusion was restricted only to patients receiving DCV/ASV/BCV combination therapy due to the failure of previous combination therapies using DCV/ASV and/or SOF/LDV**.**

2.2 Quantitation of HCV RNA

HCV RNA was quantified by a TaqMan HCV Test, version 2.0, real-time PCR assay (F. Hoffmann-La Roche Ltd, Basel, Switzerland). The lower limit of qualification was 15 IU/mL, and the range of quantitation was 1.2–8.0 log IU/mL.

2.3 HCV Genotyping

Genotypes of HCV were determined in commercial laboratories (SRL, Inc., Tokyo, Japan or BML, Inc., Tokyo, Japan).

2.4 Interleukin 28B (IL28B) Single Nucleotide Polymorphisms (SNPs) Analysis

IL28B SNPs were genotyped by the Invader Plus assay conducted in commercial laboratories (SRL, Inc., Tokyo, Japan).

2.5 Analysis of Resistance-Associated Substitutions (RASs)

Determination of amino acid sequences of NS3-V36, T54, Q80, R155, A156, D168, V170, NS5A-P29, R30, L31, P32, F37, Q54, Y93, and NS5B-L159, S282, C316, L320, V321 RASs was done by direct sequencing (LSI Medience Corporation, Tokyo, Japan). The primers of the RASs have been described previously [9].

3. Results

3.1 Baseline Patient Demographics and Characteristics

All nine eligible patients were Japanese (age 59 – 84 years) and were infected with HCV/1b before the initiation of DCV/ASV/BCV combination therapy; six (66.7%) of the patients were female. Based on the laboratory data and imaging, the severity of liver disease was judged as chronic hepatitis in six patients and liver cirrhosis in three patients. Among the subjects, three patients (33.3%) presented a history of malignant liver tumor treatment. HCV RNA levels in all patients were < 7 log IU/mL. The previous IFN-free DAA-based therapies included DCV/ASV (n = 7), SOF/LDV (n = 1), and SOF/LDV following DCV/ASV combination therapy (n = 1). The baseline patient demographics and characteristics of all patients are mentioned in Table 1.

3.2 Overall Efficacy

The overall sustained virologic response at post-treatment week (PTW) 24 (SVR24) rate was 33.3% (3/9). An SVR24 was obtained exclusively in patients experiencing failure with DCV/ASV. Among the eight patients who experienced failure with DCV/ASV, the SVR24 rate was 37.5% (3/8), regardless of whether they subsequently received SOF/LDV. Treatment failure included one virologic breakthrough and five virologic relapses. There were no cases of non-response, and virologic relapse occurred at PTW 16 and 18 in two cases. The patient characteristics and antiviral efficacy are summarized in Table 2.

3.3 Overall Safety

General fatigue and gingivitis were observed in one patient each. Both symptoms developed during the treatment period and disappeared after treatment. Based on CTCAE v5., in either case, the grade was designated as grade 1., and these symptoms were considered to be treatment-emergent adverse events (TEAEs). One patient developed an increased transaminase level in the 4th week of the therapy. The increase was maintained and the peak serum ALT level was grade 3. Thereafter, the dose of DCV/ASV/BCV was reduced by 50% in the 6th week of therapy, which led to the improvement in increased transaminase levels. In the 10th week of the therapy, the dose of DCV/ASV/BCV was then increased to 75% of the original dose and treatment was completed, although mild serum ALT abnormality was maintained during therapy. The other patient who

developed an increased transaminase level (grade 1) completed the therapy without dosing modification. Other laboratory abnormalities included increased serum creatine phosphokinase, alkaline phosphatase, and bilirubin levels (all grade 1). Furthermore, increased LDL-cholesterol was observed in one case. In all cases with a history of primary liver tumor treatment, HCC or intrahepatic bile duct carcinoma recurred during therapy or within 12 weeks after the completion of therapy, although these AEs were not considered to be TEAEs. The AEs are summarized in Table 3.

Table 1 Baseline demographics and patient characteristics.

Number	9
Gender, female; n (%)	6 (66.7)
Age, years; median (range)	66 (59–84)
Liver cirrhosis, n (%)	3 (33.3)
Past history of treatment for malignant liver tumor, n (%)	3 (33.3)
HCV RNA level, $\log_{10}$ IU/mL; median (range)	6.1 (5.2–6.9)
Genotype 1a/1b	0/9
Prior Therapy (DAA-based), n (%)	
DCV/ASV	7 (77.8)
SOF/LDV following DCV/ASV	1 (11.1)
SOF/LDV	1 (11.1)
Prior effect of DCV/ASV (Partial response/Breakthrough/Relapse)	1/2/5
Prior effect of SOF/LDV (Relapse)	2
IL28B rs8099917 (TG/unknown)	3/6

ASV, asunaprevir; DAA, direct-acting antiviral agent; DCV, daclatasvir; IL28B, interleukin 28B; LDV, ledipasvir; SOF, sofosbuvir.

Table 2 Patient characteristics and antiviral efficacy of DCV/ASV/BCV combination therapy.

Case	Age	Sex	CH/LC	IL28B*	Prior IFN-based therapies	Prior IFN-free DAA-based therapies	Prior history of the treatment of liver tumor	Change of HCV RNA (Log IU/mL)†											Viral efficacy
								Baseline	4W	8W	12W	PTW4	PTW8	PTW12	PTW16	PTW18	PTW20	PTW24	
1	79	F	CH	TG	IFN, PEG-IFN, PEG-IFN+RBV, SMV+PEG-IFN+RBV	DCV/ASV	No	5.7	<1.2	-	<1.2	5.4	NA	NA	NA	NA	NA	NA	BT
2	65	M	CH	NA	naïve	DCV/ASV	No	6.4	-	-	-	4.3	NA	NA	NA	NA	NA	NA	Relapse
3	81	F	LC	NA	naïve	DCV/ASV	Yes	5.2	-	-	-	5.4	NA	NA	NA	NA	NA	NA	Relapse
4	65	F	CH	TG	PEG-IFN, Extended duration of small amount of IFN, PEG-IFN+RBV,TVR +PEG-IFN+RBV, SMV+PEG-IFN+RBV	DCV/ASV, SOF/LDV	Yes	5.7	<1.2	-	-	4	NA	NA	NA	NA	NA	NA	Relapse
5	59	M	LC	TG	TVR+PEG-IFN+RBV	SOF/LDV	Yes	5.8	-	-	-	-	-	-	-	3.1	NA	NA	Relapse
6	66	F	LC	NA	PEG-IFN/RBV	DCV/ASV	No	6.9	1.4	-	-	-	-	-	1.7	NA	NA	NA	Relapse
7	84	M	CH	NA	naïve	DCV/ASV	No	6.1	-	-	-	-	-	-	-	NA	-	-	SVR24
8	73	F	CH	NA	SMV+PEG-IFN+RBV	DCV/ASV	No	6.1	-	-	-	-	-	-	-	NA	-	-	SVR24
9	65	F	CH	NA	PEG-IFN/RBV	DCV/ASV	No	6.1	-	-	-	-	-	-	-	NA	-	-	SVR24

ASV, asunaprevir; BCV, beclabuvir; BT, breakthrough; CH, chronic hepatitis; DCV, daclatasvir; HCV, hepatitis C virus; IFN, interferon; IL28B, interleukin 28B; LC, liver cirrhosis; LDV, ledipasvir; NA, not available; PEG-IFN, peginterferon; PTW, Post-treatment week; RBV, ribavirin; SMV, simeprevir; SOV, sofosbuvir; SVR24, sustained virological response at post-treatment week 24; TVR, telaprevir; W, week. *IL28B rs8099917. †The results of HCV RNA were described until they became clearly positive.

Table 3 Adverse events.

Subjective symptoms	Frequency	Grade
Fatigue, n (%)	1 (11.1)	1
Gingivitis, n (%)	1 (11.1)	1
Laboratory abnormality		
Increase of ALT, n (%)	2 (22.2)	1 and 3
Increase of CPK, n (%)	2 (22.2)	1
Increase of ALP, n (%)	2 (22.2)	1
Increase of Total bilirubin, n (%)	1 (11.1)	1
Increase of LDL-chol, n (%)	1 (11.1)	1
Dose reduction due to liver dysfunction, n (%)	1 (11.1)	NA

ALP, Alkaline phosphatase; ALT, alanine aminotransferase; CPK, creatine phosphokinase; LDL-chol, low-density lipoprotein cholesterol; NA, not applicable.

3.4 The Association of RASs with DCV/ASV/BCV Combination Therapy

Prior to DCV/ASV/BCV combination therapy, all three patients who achieved an SVR24 were detected with Y93H and two of them had L31M-F37L-Y93H or L31M-Q54H/Y-Y93H representing NS5A-RASs (Table 4). None of the three patients harbored NS3-RASs before combination therapy (Table 4). Two of the six patients with virologic failure harbored D168E representing NS3-RASs before combination therapy, which was not detected in one of the patients after combination therapy (Table 4). Further, two patients developed D168 amino acid substitutions after combination therapy (Table 4). On considering NS5A-RASs, the "presumed L31del or P32del" indicating deletion at L31 or P32, detected by direct sequencing were found to change drastically to L31V after treatment failure in Case 4, and the change in NS5A-RASs was only subtle after treatment failure in all other cases. The P29del representing NS5A-RASs to DCV was detected prior to, and after DCV/ASV/BCV combination therapy in Case 1. Two of six patients with virologic failure were not detected with NS5B-RASs (Table 4), and for the other patients, data were not available. Noticeably, none of the six patients were detected with NS5B-RASs after DCV/ASV/BCV combination therapy. However, NS5B-RASs to BCV that are P495A/S/L [10] were not determined in this study**.**

Table 4 Change in RASs after combination therapy with DCV/ASV/BCV.

Case	NS3		NS5A		NS5B*	
	Before	After	Before	After	Before	After
1	None	D168E	P29del	P29del	NA	None
2	D168E	D168E	R30Q L31L/I F37L Y93H	R30Q L31L/I F37F/L Y93H	NA	None
3	None	None	L31I/M F37L Y93H	L31M F37L Y93H	NA	None
4	D168D/E	None	Presumed L31del or P32del† F37L Y93Y/H	L31V F37L Y93H	None	None
5	None	None	L31M Y93H	L31M Y93H	None	None
6	None	D168D/H	L31F Q54H Y93H	L31L/F Q54H Y93H	NA	None
7	None	NA	L31M Q54H/Y Y93H	NA	NA	NA
8	None	NA	L31M F37L Y93H	NA	NA	NA
9	None	NA	Y93Y/H	NA	NA	NA

ASV, asunaprevir; BCV, beclabuvir; DCV, daclatasvir; del, deletion; NA, not available; RASs, resistance-associated substitutions (associated with DCV, ASV, or NS5B nucleotide inhibitors). *RASs were not associated with BCV. †Deletion at L31 or P32 were presumed in direct sequencing.

4. Discussion

The efficacy of DCV/ASV/BCV combination therapy in patients experiencing failure of combination therapy with IFN-free DAA-based regimens was observed to be unsatisfactory, although the therapy was relatively well-tolerated. Interestingly, two of the six patients with virologic failure showed virologic relapse after PTW12 of the combination therapy.

In Japan, as per the most recent version (ver. 7.0) of the JSH guidelines, the current recommendable regimens for patients with chronic hepatitis or liver cirrhosis with the treatment failure of IFN-free DAA-based therapies are 12-week GLE/PIB or 24-week SOF/VEL plus RBV combination therapy [1]. Pre-approval phase III clinical studies have demonstrated that an SVR12 was achieved in 31/33 (93.9%) and 58/60 (97%) HCV genotype 1/2-infected patients who had treatment failure with DAA-based regimens following 12-week GLE/PIB combination therapy [4] and 24-week SOF/VEL plus RBV combination therapy [5], respectively. In comparison, in our study, significantly low efficacy of DCV/ASV/BCV combination therapy was observed in patients who experienced treatment failure with IFN-free DAA-based regimens, although our sample size was relatively small.

DCV/ASV/BCV combination therapy is a combined BCV and DCV/ASV therapy and was predicted to be relatively resistant to the treatment failure of DCV/ASV combination therapy. We also presumed that add-on BCV might overcome the insufficient efficacy of DCV/ASV combination therapy. That is, virologic relapse after DCV/ASV therapy was preferred and the null response should be avoided in this condition. However, we eventually decided the indication of the DCV/ASV/BCV combination therapy considering strong preference of the patients, the presence of liver cirrhosis, and prior history of liver tumor treatment, because GLE/PIB or SOF/VEL plus RBV combination therapy was not available in those days and waiting for desirable treatment would have aggravated liver stage or led to the development of liver tumor. In view of the effect of add-on BCV, two patients who showed partial response and breakthrough by DCV/ASV therapy showed better treatment response as virologic relapse after DCV/ASV/BCV combination therapy, although they could not achieve SVR24.

Teraoka et al. [6] showed that only one out of four patients (25%) with HCV genotype 1b and history of DAA therapy achieved an SVR12 after receiving DCV/ASV/BCV combination therapy. Three of these patients showed virologic relapse within PTW12 [6]. Likewise, Takaguchi et al. [7] showed that DCV/ASV/BCV combination therapy achieved an SVR12 in 17/55 (30.9%) HCV genotype 1b-infected patients in their study, who had previously experienced treatment failure with DAA-based regimens, although the rate and timing of virologic relapse were not available. The antiviral efficacy (33%) in our study is congruent with that reported in previous studies [6, 7]. The primary difference between the previous studies [6, 7] and our study was that in previous studies, virological eradication was assessed using SVR12 and the evaluated amino acid positions were different from those observed in our study. Virological relapse after PTW12 in pleural patients was observed for the first time in our study. The information about P29del and that D168E changed to wild type even after virological failure in one case were also original findings in our study.

On assessing the timing of virologic relapse, HCV-infected mice showed virologic relapse at one to four weeks after the completion of DCV/ASV/BCV combination therapy [6]. Therefore, the timing of the virologic relapse varied. In our study, virologic relapse was observed at 16 and 18 weeks (in two cases) after the completion of DCV/ASV/BCV combination therapy. These two cases

here may be rare because SVR12 has currently been substituted for SVR24 as a measure of viral eradication and possible cause for this phenomenon may be the insufficient antiviral efficacy of DCV/ASV/BCV combination therapy somewhat short of viral eradication. That is, HCV might be eradicated through the extension of the treatment period with this combination therapy. Extending eight-week GLE/PIB combination therapy to 12 weeks eradicated HCV in a non-cirrhotic genotype 2a patient who showed virologic relapse at PTW13 after eight-week combination therapy [11]. In fact, in patients who experienced treatment failure with IFN-free DAA-based therapies, the efficacy of SOF/VEL plus RBV combination therapy was clearly better in those who received 24-week therapy than in those who received 12-week therapy [5]. A pre-approval phase III clinical study of DCV/ASV/BCV combination therapy included patients with treatment failure after IFN-based regimens and not those with treatment failure after DAA-based regimens [12]. Adding BCV to DCV/ASV combination therapy increased the antiviral efficacy. However, the treatment period, which is reduced by 50%, might be insufficient for patients with DAA-based regimen treatment failure.

D168E or D168H, and Y93H representing NS3-RASs to ASV and NS5A-RAS to DCV, respectively, may potentially be a risk factor for treatment failure in patients receiving DCV/ASV/BCV combination therapy because the patient who achieved an SVR24 was not detected with D168E before treatment; moreover, two patients developed either of the substitutions after treatment failure. With the exception of Case 1, all patients were detected with Y93H before treatment. Thus, in Cases 2 and 4, the coexistence of D168E might have increased resistance to DCV/ASV/BCV combination therapy. However, after treatment failure, D168E was not detected in Case 4 although drastic changes were observed in presumed L31del or P32del representing NS5A-RASs to DCV [13]. The dynamic change of the NS5A-RASs in Case 4 indicates that the presumed L31del or P32del and Y93H are unlikely to coexist [13]. We repeated the evaluation of RASs approximately five months later to prepare another DAA-based therapy and found no change in RASs when compared to the previous determination. Thus, “presumed L31del or P32del” was hypothesized to have lower viral fitness compared to L31V in the presence of Y93H. The loss of D168E might have been affected by the drastic change of the NS5A-RASs; however, further evaluation is needed to establish this. Importantly, although P29del in Case 1 did not change after treatment failure, this substitution may be a potential risk factor for treatment failure in patients receiving DCV/ASV/BCV combination therapy. P29del is known to be a rare RAS that develops in patients who experienced treatment failure with DCV/ASV combination therapy, especially simeprevir/peginterferon/RBV therapy [14]. In fact, the patient had received this regimen before DCV/ASV combination therapy. Thus, the patient in Case 1 here was not detected with NS3-RAS before the DCV/ASV/BCV combination therapy, but P29del might confer relatively strong resistance, similar to that conferred by the presumed L31del or P32del. However, one must exercise caution while interpreting the role of NS5A-RASs in treatment failure in patients receiving DCV/ASV/BCV combination therapy because in this study, even the patient who achieved an SVR24 (Case 7) had L31M-Q54H/Y-Y93H as NS5A-RASs, which conferred considerably high resistance to DCV [15, 16] before DCV/ASV/BCV combination therapy. The RASs before DCV/ASV treatment was measured through invader assay [17, 18] in seven cases, or a combination method of direct sequencing and cycleave PCR (different from direct sequencing) [19] in one case. Thus, the measurement of the RASs before DCV/ASV treatment was different from that after/before DCV/ASV/BCV treatment. Besides, the evaluated amino acid positions before DCV/ASV treatment were different from those observed in the current

study. Thus, it is difficult to simply compare the RASs before DCV/ASV treatment and those before/after DCV/ASV/BCV treatment. However, Y93, L31 as NS5A-RASs and D168 as NS3-RASs substitutions could be evaluated for the RASs before DCV/ASV treatment. Considering these three RASs, no particular critical difference was observed between the SVR and non-SVR groups before DCV/ASV treatment. While we did not investigate NS5B-RASs before DCV/ASV/BCV combination therapy in some cases, none of the patients with treatment failure after combination therapy harbored NS5B-RASs. However, we did not examine NS5B-RASs to BCV including P495A/S/L [10] in this study; therefore, the role of NS5B-RASs to BCV in treatment failure should be further evaluated in patients receiving DCV/ASV/BCV combination therapy. On the other hand, the causes of treatment failure were unclear in Cases 3, 5, and 6 in terms of the RASs. Here, the disease stage of these patients was liver cirrhosis, two of them had a history of malignant liver tumor treatment, thus, the antiviral effects may have been affected by the host factors.

Takaguchi et al. [7] reported that HCV RNA levels and a history of DCV/ASV combination therapy are baseline factors associated with achieving an SVR in patients who experienced treatment failure with DAA-based therapy. We contemplated that patients with high baseline HCV RNA levels (>7 log IU/mL) were unlikely to achieve an SVR based on DCV/ASV/BCV combination therapy results in a pre-approval phase III clinical study [12], and all the subjects in our study had HCV RNA levels of <7 log IU/mL, which was considered favorable. However, the efficacy exhibited in our study was lower than expected. Eight of the nine (89%) patients here had a history of DCV/ASV combination therapy, which supports the findings by Takaguchi et al. [7].

The grade of all AEs was mild with the exception of the increase in serum ALT level and overall the treatment was well tolerated. Increased serum ALT level necessitated dose modification in Case 2, but the treatment period was maintained. However, one of the causes of virologic relapse in this case may be decreased drug adherence. Notably, elevated serum ALT level was not observed during the DCV/ASV combination treatment period although the treatment was discontinued because of partial response in Case 2. The other patient (Case 6) with grade 1 also showed elevated serum ALT levels during the DCV/ASV combination treatment period at the same grade. However, the data of only a few patients were available, thus, increased risk of the elevation of serum ALT level due to DCV/ASV/BCV combination therapy compared to DCV/ASV combination therapy was not conclusive. However, compared to DCV/ASV combination therapy, the DCV/ASV/BCV combination therapy might increase the risk of an increase in serum ALT level although the grade of this AE might vary in patients.

Unfortunately, the DCV/ASV/BCV tablet is not commercially provided any more in Japan and in several other countries and our results cannot be replicated. However, the information on RASs, the delayed virologic relapse pattern observed after DCV/ASV/BCV combination therapy, and the presumed importance of the treatment duration based on our study might contribute in overcoming the rare but critical intractable cases such as those of the treatment failure of GLE/PIB or SOF/VEL plus RBV combination therapies. Therefore, we believe that our study is scientifically valuable in this respect.

Recently, we have published the results of retreatment with DAAs for the treatment failure of DCV/ASV combination therapy in hepatitis C patients [20]. In this report, some patients received DCV/ASV/BCV treatment and were also included in our current study. However, there are significant differences between our earlier report [20] and our current study: 1) the earlier report [20] was a retrospective study, whereas our current study is the prospective one, 2) the earlier

report [20] used SVR12 but not SVR24 to assess virological response. In this study, we used SVR24 to evaluate viral eradication and state that SVR12 was not enough to evaluate viral eradication in our subjects, 3) amino acid substitutions that are not always RASs were described in the earlier report [20], 4) total amino acid substitutions were described in certain DAA failure groups in our earlier report [20]; however, the change of RASs in each case was not available, and 5) the adverse events were not described in our earlier report [20], unlike in this study.

The present study has a few limitations. The sample size was relatively small, the NS5B-RASs to BCV were not evaluated, and IL28B SNPs were not evaluated in some patients.

5. Conclusions

In conclusion, the efficacy of DCV/ASV/BCV combination therapy was unsatisfactory in patients who experienced therapeutic failure with IFN-free DAA-based combination therapies, although the therapy was relatively well-tolerated. The occurrence of virologic relapse at a relatively later period after the completion of therapy suggests that a longer duration of therapy, such as 24-week DCV/ASV/BCV therapy, might overcome this issue. Nevertheless, combination therapy with GLE/PIB or SOF/VEL plus RBV instead of DCV/ASV/BCV has now taken over this role and is currently available in Japan. Further, the role of “D168E or D168H in addition to Y93H” representing NS3-RASs, “L31del or P32del” and “P29del” representing NS5A-RASs, and NS5B-RASs to BCV in treatment failure in patients receiving DCV/ASV/BCV combination therapy should be evaluated in future studies.

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Author Contributions

K.S. drafted the article. K.S., A.N., S.S., Y.Y., N.H., and S.K. recruited the patients. K.S., A.N., S.S., T.K., H.T., H.Oh., and H.Ok. analyzed and interpreted the data. T.U. and H.Ok. gave a critical advice. H.Ok. edited the article and approved the final version to be published. All authors read and approved the final manuscript.

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Competing Interests

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