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【 CASE REPORT 】

Late Relapse after a Sustained Virologic Response at 24 Weeks after Treatment with Daclatasvir and Asunaprevir Combination Therapy for Chronic Hepatitis C Virus Genotype 1b Infection with Liver Cirrhosis

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Abstract:
There have been few studies on relapse after a sustained virological response in hepatitis C virus (HCV) patients treated with interferon-free regimens. Thus, the risk of late relapse in patients treated with interferon-free therapy remains unclear. A 67-year-old woman with HCV genotype 1b and liver cirrhosis received oral daclatasvir and asunaprevir. Combination therapy was stopped after 4 weeks because of an episode of encephalopathy. Nonetheless, an HCV polymerase chain reaction at 24 weeks posttreatment was negative. However, HCV ribonucleic acid was detectable at approximately 62 weeks posttreatment. Very late HCV relapses may occur in patients with liver cirrhosis who receive an interferon-free regimen when the treatment period is insufficient.

Key words: asunaprevir, daclatasvir, interferon-free regimen, late relapse, liver cirrhosis

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Introduction

A sustained virological response (SVR), which reduces the risk of hepatocellular carcinoma and cirrhosis, is accepted as a durable and clinically significant treatment endpoint of hepatitis C virus (HCV) therapy (1). HCV ribonucleic acid (RNA) negativity at 12 and 24 weeks posttreatment is currently accepted as the definition of an SVR (2).

A number of studies have analyzed the rate of relapse after a SVR with interferon-based therapies in a range of patient populations (3-5). However, there have been few studies on relapse after treatment with interferon-free regimens. Thus, the risk of late relapse with interferon-free therapy remains unclear. We herein describe a case of late relapse after an SVR in a patient with liver cirrhosis who was treated with daclatasvir (DCV) and asunaprevir (ASV) combination therapy (DCV+ASV) for chronic HCV genotype 1b infection.

Case Report

A 67-year-old woman was presented to our hospital to undergo evaluation for HCV genotype 1b with liver cirrhosis. She had not previously received conventional interferon therapy. She was human immunodeficiency virus (HIV)-negative. There was no history of HCV infection in her family. On physical examination, vascular spider was observed on her subclavicular upper left thorax, without jaundice. Her laboratory data upon admission for combination therapy are shown in Table 1. HCV infection (genotype 1b) was confirmed by an HCV-polymerase chain reaction (PCR). Her plasma HCV RNA load, as quantified using a Cobas TaqMan version 2.0 assay (Roche Diagnostics, Tokyo, Ja-

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Table 1. Laboratory Data on Admission for Combination Therapy.

Complete Blood Count	Case 1	Normal range
Hemoglobin	13.5	11.5-15.0 g/dL
White blood cells	4.0	4.0-9.0 $\times 10^3/\mu L$
Neutrophils	2.1	1.7-6.4 $\times 10^3/\mu L$
Platelets	6.3	15.0-35.0 $\times 10^4/\mu L$
Coagulation		
Prothrombin	85	70-130 %
Biochemistry		
AST	85	10-35 U/L
ALT	66	5-40 U/L
Albumin	3.2	3.8-5.2 g/dL
BUN	15.1	8.0-22.0 mg/dL
Creatinine	0.5	0.40-0.80 mg/dL
Total bilirubin	1.5	0.2-1.0 mg/dL
ZTT	13.8	<4 U
TTT	25.1	2-12 U
M2BPGi	9.07	COI
Hyaluronic acid	387	<50 ng/mL
Alpha-fetoprotein	5	0-10 ng/mL
PIVKA-2	7	<40 mAU/mL
Serology		
IgA	293	93-393 mg/dL
IgM	158	50-269 mg/dL
IgG	2,490	861-1,747 mg/dL
HBs antigen	(-)	
HBs antibody	(-)	
HBc antibody	(-)	
HCV antibody	(+)	
IL28B SNP (rs8099917),	TT	
Virology		
Plasma HCV RNA load	6.1	<1.2 logIU/mL
Genotype	Ib	

PIVKA-2: protein induced by vitamin K absence/antagonist-II, M2BPGi: Mac-2 binding protein glycan isome, IL28B SNP: Interleukin-28B single nucleotide polymorphism

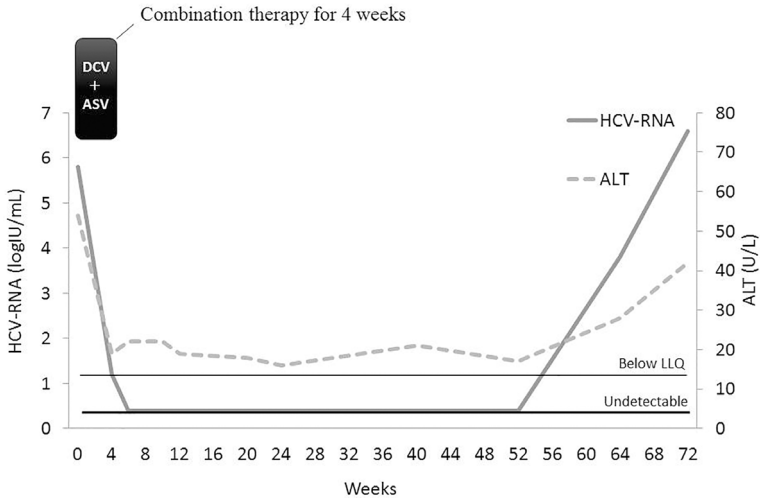

Figure 1. The time course of the hepatitis C virus ribonucleic acid levels and alanine aminotransferase (ALT) levels in the patient. Late relapse occurred in the 62nd week after treatment, after an insufficient treatment period. ASV: asunaprevir, DCV: daclatasvir, LLQ: lower limit of quantification (IU/mL) 15 IU/mL

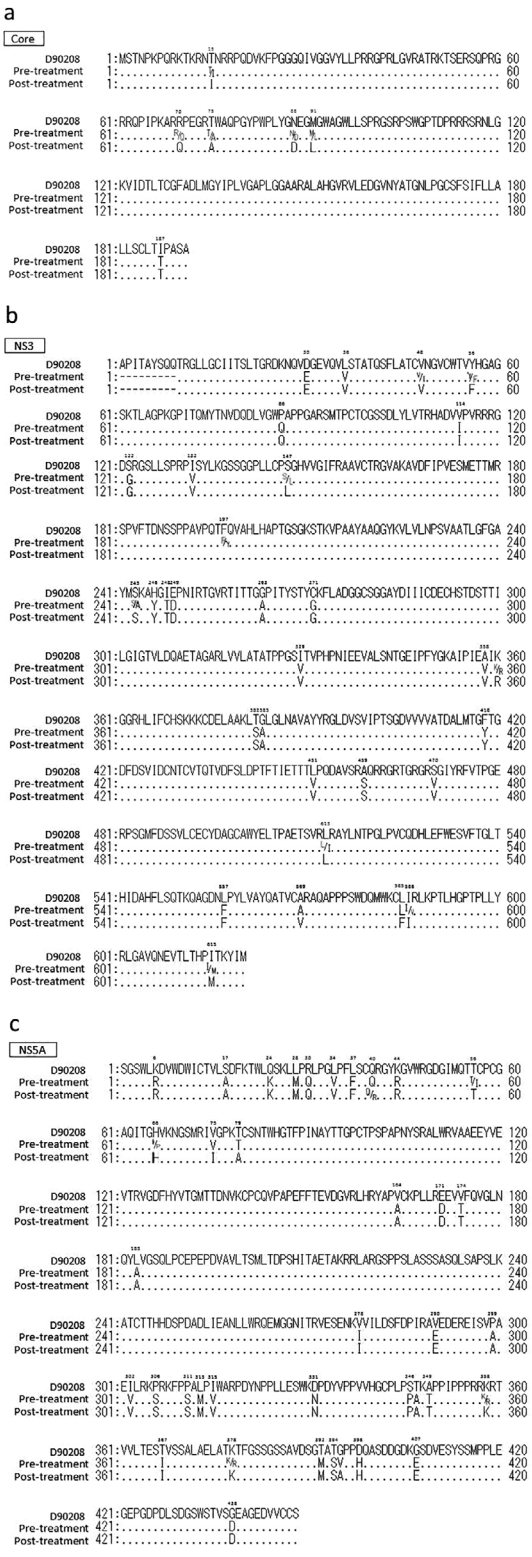

Figure 2. The amino acid alignment in the core (a), NS5A (b) and NS5B (c). The amino acid alignment was confirmed to be similar to that of a wild-type strain using the GeneBank database (D90208).

pan), was 6.1 $\log_{10}$ IU/mL. The resistance-associated variants (RAV) of NS5A-Y93, L31, P32, P58, and NS3-D168, which were identified by direct sequencing, were wild-type variants. Dynamic contrast-enhanced magnetic resonance imaging showed that the left lobe of the liver was enlarged, as well as collateral circulation from portal hypertension, and splenomegaly. A liver biopsy revealed regenerative nodules of hepatic cells that were enclosed by fibrous connective tissue bridging between the portal tracts. A moderate increase in inflammatory cells was observed in these portal tracts were moderate, and moderate piecemeal necrosis was observed in the circumference of most portal tracts. Fatty change was also observed but Mallory's hyaline was not (histology activity index: I-3, II-1, III-3, IV-4). Hepatic venous catheterization revealed a hepatic venous pressure gradient (HVPG) of 11 mmHg. She was therefore disgnosed to have clinically significant portal hypertension (6).

DCV (60 mg capsule, once daily) and ASV (100 mg tablet, twice daily) were administered orally, according to the prescription information provided by the respective manufacturers in February 2015. During treatment with DCV+ ASV, the patient experienced a rapid decline in her viral titer which fell below the limit of quantification. However, combination therapy was stopped after 4 weeks because of an episode of encephalopathy and liver enzyme elevation. Nonetheless, the patient achieved an SVR, and an HCV PCR in August 2015, at 24 weeks posttreatment, was negative.

In August 2016, at approximately 62 weeks after the treatment, an HCV PCR (routinely performed after an SVR) revealed a detectable level of HCV RNA (4.2 $\log_{10}$ IU/mL) (Fig. 1). The amino acid mutations that were present before and after the administration of combination therapy are shown in Fig. 2. RAV of NS5A and NS3, which were observed at the time of viral rebound, were wild-type variants. Detailed history taking revealed no risk factors for reinfection.

The viral nucleotide sequence homology

Total RNA was extracted from a serum sample and reverse transcribed into cDNA using a random hexamer primer. HCV RNA was confirmed in the samples by amplifying the core, NS3, and NS5A regions with a PCR and followed up by direct sequencing using an ABI 3500 Genetic Analyzer (Applied Biosystems, Thermo Fischer Scientific Corp., Foster City, USA).

Results

The viral nucleotide sequence homology between samples obtained before treatment and after relapse in the core, NS3, and NS5A was 97.7% (560/573), 93.6% (1,685/1,800) and 97.7% (1,315/1,345), respectively. The core sequence chromatograms from before treatment showed some overlapping peaks as mixed signals. Each mixed signal contained the same signal as after the relapse. Thus, the sequence similarity in the core, NS3, and NS5A was 99.6% (571/573), 99.2% (1,786/1,800), and 98.9% (1,330/1,345), respectively.

Phylogenetic analyses

The sequences for the phylogenetic analysis were re-

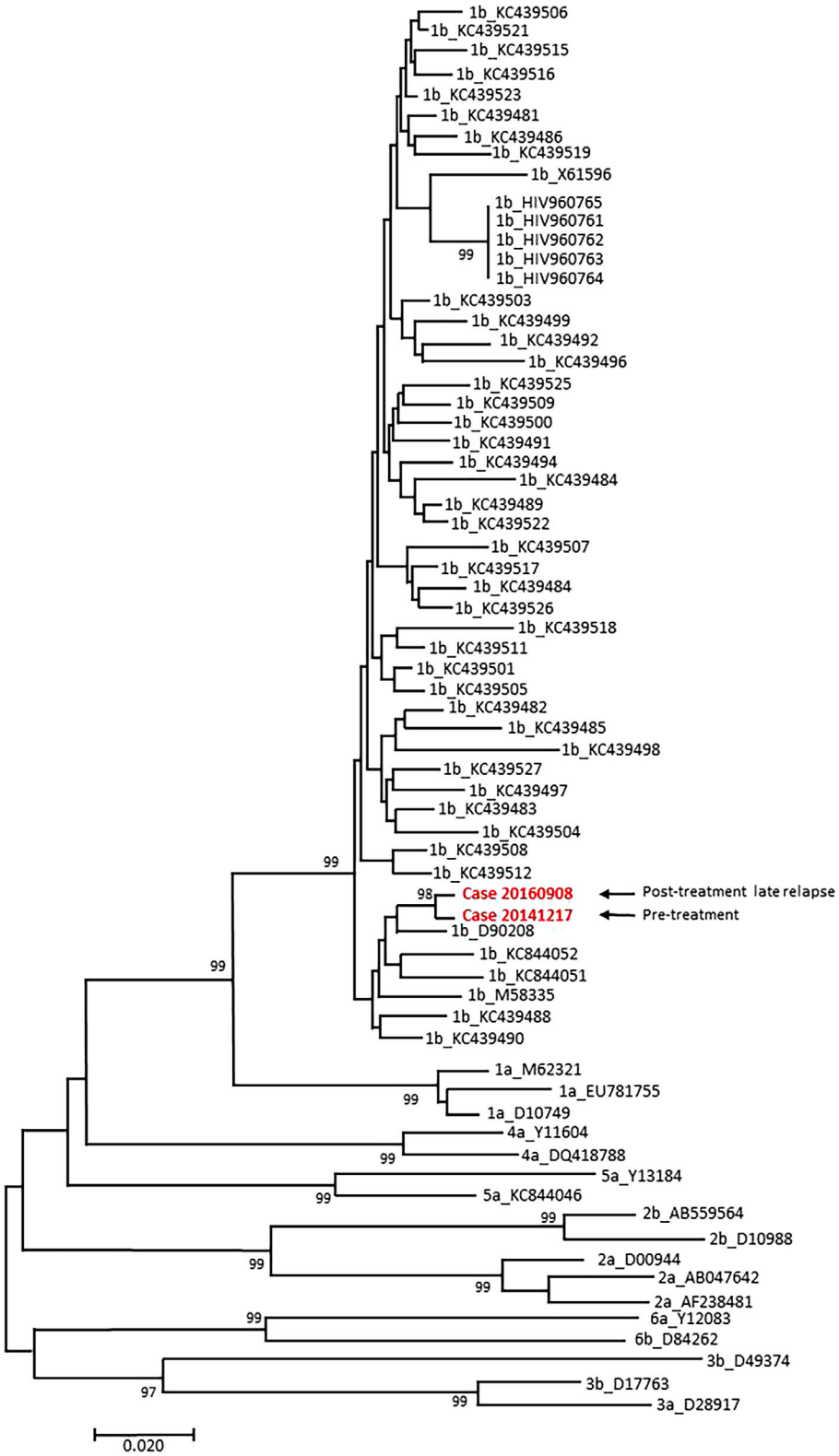

Figure 3. **The hepatitis C virus sequences recovered in this study are indicated in red. Sequences from the NCBI GenBank nucleotide sequence database are numbered with the GenBank accession no. Labels indicate the HCV genotype and subtype. Bootstrap values of >90% are shown. Nucleotide alignments were performed using the ClustalW multiple alignment tool from BioEdit v7.0.5.3. The tree was constructed using the MEGA v7.0 software program, and branch support was determined by bootstrapping with 1,000 replications. A phylogenetic analysis using the core sequences from this study and the database showed that the strains from each patient before and after treatment had significant clusters with high bootstrap scores of 98%.**

Table 2. Clinical and Biological Features with HCV Late Relapse.

Study	Interferon-free regimens	Time from HCV therapy completion at virological relapse	Age	Sex	Geno type	Cirrhosis	HIV infection	IL28B	Previous IFN therapy	RAV	
										Base line	At the time of rebound
Our case	Daclatasvir and Asunaprevir for 4 weeks	62weeks	67	Female	1b	+	-	rs8099917 TT	naive	wild	wild
Ref 16	Faldaprevir, BI-207127 plus ribavirin for 40 weeks	36 weeks	66	Male	1b	-	-	rs12979860 CT	naive	wild	wild
Ref 7	ABT-450 and ABT-072 plus ribavirin for 12 weeks	36 weeks		Male	1a	-	-	rs12979860 CC		wild	NS3 protease : A156T NS5B polymerase : Y448H, Y448R, C316R

Ref: References, RAV: resistance-associated variants

trieved from the DDBJ/EMBL/GeneBank, and corresponded to the accession numbers mentioned in the trees. Alignment was accomplished using the ClustalW multiple sequence alignment tool from BioEdit (v7.0.5.3), and a phylogenetic tree was constructed by neighbor-joining tree method using the Molecular Evolutionary Genetics Analysis (MEGA) software program (version 7.0: http://www.megasoftware.net/). To confirm the reliability of the phylogenetic tree, bootstrap resampling tests were performed 1,000 times. The strains from each patient were grouped into the same cluster (Fig. 3).

Discussion

We herein report a rare case of late relapse after an SVR in a patient with liver cirrhosis who received DCV+ASV therapy for chronic HCV genotype 1b infection. The re-emergence of HCV after antiviral therapy represents either a late relapse after SVR or *de novo* reinfection (7). Phylogenetic analyses revealed that the core sequence derived from the patient before and after treatment showed a high sequence identity, and that the strains had significant clusters with high bootstrap scores. Consequently, our case was considered to be a late relapse rather than a case of *de novo* HCV re-infection.

Late relapses upon treatment withdrawal from interferon-based therapy tend to be more common in patients who are immunocompromised due to HIV, IL28B alleles, or a high baseline serum HCV RNA level, which influence the response to treatment factors leading to the clearance of HCV by interferon-based therapy (8-10). According to a previous study, which investigated the number of events divided by the number of person years of follow-up (PYFU), the late relapse rate from interferon-based therapy was 0.82/1,000 PYFU (8). However, to our knowledge, there have been no studies on late relapse after treatment with interferon-free regimens. There are only two recently reported cases of relapse after treatment with interferon-free regimens (Table 2). If treatment-related factors are correlated with late relapse, the presence of a resistance variant may be a risk factor for late relapse when patients are treated with direct-acting antivirals. In a phase III study of a 24-week regimen of DCV+ASV, a multivariate analysis confirmed that antiviral-resistant mutations of NS5A and NS3 were independent of the factors affecting the responders to this combination therapy, and that the HCV RNA quantity, IL28B, and the extent of liver cirrhosis were not correlated with the response to this combination therapy (11-13). Actually, a case of late viral rebound at 36 weeks after treatment was recognized in a trial of an interferon-free combination with ABT-450 and ABT-072 plus ribavirin in a patient in whom an antiviral-resistant mutation was detected at the time of relapse (7).

Risk factors other than the presence of resistance variants should be considered when determining the duration of treatment and the appropriate plasma concentrations of direct-acting antiviral agents. In our case, without RAV at the time of rebound, an insufficient length of treatment and an insufficient liver function (due to liver cirrhosis) were factors that were listed in the manufacturer's prescribing information. Although this regimen was well tolerated with low rates of adverse event-related discontinuation, our patient had cirrhosis with collateral circulation from portal hypertension. This patient, who had an impaired liver function, showed an increased level of alanine aminotransferase (ALT), which is the most common side effect of this combination therapy, and which may exacerbate hepatic encephalopathy (11). As DCV+ASV undergo hepatic biotransformation requiring an intact liver function for their efficient elimination, the serum concentrations and drug activity of DCV+ASV in a patient with insufficient liver function may influence HCV clearance and the incidence of adverse events (14).

The etiology of the re-emergence of HCV in the serum after a long period in which the virus was undetectable un-

der antiviral therapy is unclear. HCV genomes may remain in a non-replicative state, evading the effects of the antivirals on the replication cycle (15, 16). It is also unknown whether there is a location in which the virus can remain hidden to survive antiviral therapy. Thrombin formation with accompanying microthrombosis of the portal vein radicles in patients with liver cirrhosis may create a suitable hiding place in the liver (17). However, these mechanisms are not clear because there is little evidence to support this hypothesis. Further detailed research on late relapse in patients receiving interferon-free therapy is warranted.

In conclusion, physicians should be alert for the occurrence of very late HCV relapses in patients with liver cirrhosis who undergo treatment with an all-oral interferon-free regimen for a short period of time. In such circumstances, close monitoring should be performed.

The authors state that they have no Conflict of Interest (COI).

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