

Non-Structural 5A Resistance-Associated Substitutions and Interleukin28B in HCV Genotype 3b Decompensated Cirrhosis Patients with Sofosbuvir Plus Velpatasvir

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1. Abstract

1.1. Background: Hepatitis C Virus (HCV) Genotype (GT) infection is still regarded as one of the more “difficult-to-treat” GTs in the era of IFN-free treatments. The viral factors involved in this unfavorable outcome have yet to be clarified.

GT3 HCV strains consist of various subgenotypes, and only a few studies on genome sequences of GT3 HCV have been published, since universal primers suitable for any subgenotype strain cannot be designed.

1.2. Case Summary: We describe two cases of HCV GT 3b decompensated liver cirrhosis treated with sofosbuvir plus velpatasvir (SOF/VEL) for 12 weeks resulting in breakthrough and relapse.

Case1 (breakthrough): A 71-year-old man with Child-Pugh-Turcotte (CPT) score 7 demonstrated negative HCV RNA after 4 weeks of treatment and at 8 weeks thereafter; however, HCV RNA turned positive 12 weeks post-treatment.

Case2 (relapse): A 60-year-old man with CPT score 8 demon-

strated negative HCV RNA after 12 weeks of treatment; 16 weeks post-treatment, HCV RNA turned positive. Despite breakthrough and relapse, hepatic reserve functions including albumin, bilirubin and prothrombin time improved in both cases without change in the CPT score.

Analysis of both cases, revealed no resistance-associated substitutions (RAS) within NS3 or NS5B, whereas non-structural protein 5A (NS5A) paired RASs (A30K, L31M), except M28 and Y93, were in evidence indicating strong resistance to any NS5A; interleukin28B (IL28B) single nucleotide polymorphisms (SNP) was heterogenous (rs8099917 TG).

1.3. Conclusion: To the best of our knowledge, this is the first analysis of RAS and IL28B SNP in GT3b decompensated cirrhosis patients demonstrating breakthrough and relapse after SOF/VEL treatment.

2. Introduction

In Phase 3 studies, Sofosbuvir (SOF) (400 mg/day)/Velpatasvir (VEL) (100 mg/day) treated for 12 weeks has been evaluated in

57 patients with hepatitis C virus (HCV) genotypes (GTs) and decompensated liver cirrhosis [Child-Pugh-Turcotte (CPT)] class B or C [1].

Of the 51 patients enrolled without ribavirin administration, 77%, 16% and 1% show GT 1, 2 and 3 HCV infection, respectively, and 77% and 20% show CPT class B and C cirrhosis, respectively. Sustained Viral Response (SVR)12 rates are 98%, 89% and 0% in GT 1, 2 and 3 respectively [1]. The number of GT3 patients being very small (2 patients) and the SVR rate being 0%, exact data such as CPT class, subtype, Resistant Associated Substitutions (RAS) and Interleukin28B (IL28B) Single Nucleotide Polymorphisms (SNP) regarding GT3 is unclear.

In 2019, SOF/VEL is approved in Japan, and Japan Society of Hepatology guidelines recommend SOF/VEL treatment for patients with decompensated cirrhosis.

We encountered two cases of HCV GT3b decompensated liver cirrhosis treated with SOF/VEL for 12 weeks resulting in breakthrough and relapse, and analyzed RAS and IL28B SNP (rs8099917).

3. Case Reports

3.1. Case1 (breakthrough)

A 71-year-old treatment-naive man was referred to our hospital

for the treatment of HCV-related liver cirrhosis.

SOF (400 mg/day) plus VEL (100 mg/day) was fully prescribed for 12 weeks without interruption. HCV RNA was negative at weeks 4 of treatment and persisted for 8 weeks thereafter, however, HCV RNA turned positive 12 weeks after the treatment. Laboratory data at baseline and at 12 weeks are shown in Table 1.

3.2. Case2 (relapse)

A 60-year-old treatment-naive man was referred to our hospital for the treatment of HCV-related liver cirrhosis.

SOF (400 mg/day) / VEL (100 mg/day) was fully prescribed for 12 weeks without interruption. HCV RNA was negative after 12 weeks of treatment, however, HCV RNA turned positive 16 weeks post-treatment. Laboratory data at baseline and at 16 weeks are shown in Table 1.

In both cases, hepatic reserve functions improved, however, the CPT B class did not change (score: 7 for case 1 and 8 for case 2) (Table 1).

The patients had no past history of blood transfusion or drug abuse, and did not take drugs, such as proton-pump inhibitors, that impact the efficacy of SOF/VEL. They had never been abroad, making the transfection route unclear.

IL28B SNP was heterogenous (TG) in both patients (Table 1).

Table 1: Laboratory data

Parameters	Case 1		Case 2	
	At baseline	12 weeks post-treatment	At baseline	16 weeks post-treatment
Total protein	6.8 g/dl	6.8 g/dl	6.8 g/dl	7.0 g/dl
Albumin	3.2 g/dl	3.3 g/dl	2.5 g/dl	2.9 g/dl
Total Bilirubin	1.98 mg/dl	2.45 mg/dl	3.09 mg/dl	2.5 mg/dl
Prothrombin time	82.30%	83.80%	64.40%	63.40%
Child-Pugh-Turcotte class	B (score 7)	B (score 7)	B (score 8)	B (score 8)
Genotype	3b		3b	
IL28B	heterogenous (CT)		heterogenous (CT)	
HCV RNA	5.8 Log IU/ml	1.4 Log IU/ml	5.7 Log IU/ml	5.3 Log IU/ml

IL28B: interleukin28B.

RAS analysis

Methods and Results

Briefly, the method for RAS analysis was as follows:

Viral RNA was extracted and reverse transcribed as described.

Primers GT3.5AF2 and GT3.5AR2 were used for non-structural protein 5A (NS5A) regions as described.

The NS5B regions were amplified into two overlapping regions with two pairs of primers, the former (from S76 of NS5B to A338); forward: 5'- AAGGAGGTGAAGGAGCGAGCA-3', reverse: 5'-GTACCTGGTCATAGCCTCCGTG-3', and the latter (from T227 of NS5B to S549); forward: 5'- TGATACCCGCTGYTTT-GACTC-3', reverse: 5'- GTGATAAATGTCGTTCCCGCC-3'.

Amplified products were purified with magnetic beads AMPure XP (Beckman Coulter, Indiana, United States), and terminated

with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) according to the manufacturer's protocol.

The terminal products were sequenced with 3500 Genetic Analyzer (Thermo Fisher Scientific) after purification with magnetic beads (Agencourt CleanSEQ Beckman Coulter). The resulting nucleotide sequence data were assembled with the use of ATGC ver.6 (GENETYX, Tokyo, Japan).

Paired RASs (A30K + L31M) in NS5A region were observed in both patient's post-treatment (case 1: 12 weeks after the treatment, case 2: 16 weeks after the treatment) and at baseline.

No other RAS in NS5A region, such as M28 Y93, was observed in either patient post-treatment, or at baseline.

No RASs, including LI59, S282, or V323 in NS5B region was observed post-treatment or at baseline (Figure 1).



Figure 1: NS5A class RAS at baseline and at post-treatment in the breakthrough and relapse patients.

4. Discussion

SOF/VEL is recommended in the US, the European Union, and other areas for the treatment of GTs 1-6 chronic HCV infection in patients with or without compensated liver cirrhosis, and for use with ribavirin in patients with decompensated liver cirrhosis [2, 5]. The ASTRAL-4 study assesses 12 and 24 weeks of treatment with SOF/VEL with or without ribavirin in HCV-infected patients with CPT class B decompensated liver cirrhosis in the US [5]: SVR12 in 83% of patients taking 12 weeks of SOF/VEL, in 94% of patients taking 12 weeks of SOF/VEL plus ribavirin, and in 86% of patients taking 24 weeks of SOF/VEL, SVR rates are lower in HCV GT3 patients than in those with the other GTs, particularly in treatment-experienced liver cirrhotic patients [2, 3, 6].

HCV GT3 is the second-most prevalent genotype, accounting for 25% of all infected patients, and has a particularly high percentage among European drug abusers and in Southern Asia [7, 8]. Moreover, recent data indicate that HCV GT3 infection is related to higher rates of hepatic steatosis, more rapid fibrosis progression, and occurrence of hepatocellular carcinoma compared to infection with other HCV genotypes [9, 11].

From view point of history, HCV GT3 infection treated with IFN is thought an easy-to-treat GT with SVR rates of about 70% after 24-48 weeks of IFN-based therapy in non-liver cirrhotic patients [12, 13]. Most first-generation HCV protease and NS5A inhibitors are, however, less effective in GT3 infection, especially with other negative predictive factors, including liver cirrhosis, previous IFN therapy failure and/or Resistance-Associated Substitutions (RASs) [8, 14].

Thus, HCV GT3 infection is still estimated as one of the more “difficult-to-treat” GTs in the age of IFN-free treatments. The viral factors involved in this unfavorable result remain to be clarified.

GT3 HCV strains are composed of various subgenotypes, and a few studies on genome sequences of GT3 HCV have been reported, since universal primers suitable for any subgenotype strain cannot be developed [15].

HCV-resistant patients in a network of the world of cohorts of GT3a infected patients, DAA-naïve patients (n=315, 51.8%), show no NS5B RASs, whereas NS3 RASs are demonstrated in

8.9% (e.g. Q80K, Q168Q/R, A166T) and NS5A RASs in 25.1% of patients (with A30K and Y93H, at 4.4% and 6.0% respectively) [16]. Of DAA-treated patients (n=293, 48.2%) failing NS5A inhibitor-based therapy, 78.8% harbored NS5A RASs at failure of all NS5 inhibitor-containing regimens, except ledipasvir, followed by NS5A A30K/S (17%). A combination of NS5A A30K and y93H is seldom detected. The percentage of NS5B S282C/T is low (1.6%) in patients failing SOF-containing regimens. NonNS5B polymorphisms (including at newly reported positions 150 and 206) are related to SOF-containing regimen failures [16]. The authors did not, however, include GT3b in the study.

The A30K + L31M combination is shown in all samples of GTs 3b and 3g, demonstrating high frequencies of RASs in non-structural protein 5A inhibitors of GT3 HCV and in vitro analysis shows that these subtypes may be inherently resistant to all approved non-structural protein 5A inhibitors for GT3 HCV [17, 20].

In the present study, the paired A30K and L31M substitutions were observed at baseline, as in the previous reports. The significance of such substitutions has, however, not yet been clarified in clinical practice because of the difficulty in genome sequencing of GT3 HCV [15].

Nowadays, clinical trials with SOF/VEL have been performed for difficult-to-treat GT3 patients with first generation HCV inhibitors and non-structural protein 5A inhibitors.

Of 478 patients (GT3a 472, GT3b 3, GT3g 2, GT3k 1) with GT3 infections, 12% have NS5A class RASs at baseline. The SVR rate is 93% (53/57) and 98% (411/420) in patients with and without baseline NS5A class RASs. Of the tested SVR rates in the presence of Y93H in patients with GT3 infections and liver cirrhosis, only 1.2% (6/478) with GT3 are liver cirrhotic, however, and harbor Y93H at baseline. Four of these six patients obtain SVR after SOF/VEL therapy. In the context of GT3b, the SVR rate is 100% (3/3) [6].

The authors demonstrate also 100% of SVR12 in 5 GT3 patients treated with SOF/VEL for 12 weeks, regardless of baseline NS5A RASs, such as A30K and L31M. Data with respect to decompensated liver cirrhosis and GT3b is not clear [6].

Analysis of 293 GT3 patients shows 25.3% as liver cirrhotic and

21.8% as treatment-given, including 4.1% with DAA experience. Baseline NS5A RASs (Y93H, A30K, L31M) are found in 11.2%. Ribavirin (RBV) added in 5% of non-liver cirrhotic and 58.9% of liver cirrhotic patients shows SVR12 rates for SOF/VEL/RBV at 95.9% (mITT) and 99.5% (PP). Only 1 virological relapse experiences in a liver cirrhotic patient previously treated with SOF/RBV [21].

However, they did not include GT3b patients in the study.

A landmark discovery of SNPs in or near the IFNL3 and IFNL4 loci [22, 23], first reported as IL28B SNP, is strongly related to the response to IFN-based therapy for chronic hepatitis C [22, 25], and with spontaneous clearance of HCV [26, 28]. In the age of IFN-free treatment, a few papers reported IL28B SNP CC (major) as related to favorable early viral kinetics and effectiveness of DAA therapy of HCV GT1 patients [29, 30].

5. Conclusion

In our study, 3 factors were related to the breakthrough and relapse cases.

First, paired NS5A (A30K, L31M) at baseline was in evidence in both cases, indicating strong resistance to any NS5A inhibitors.

Second, decompensated cirrhosis itself (CPT B score 7 and score 8) may be related.

Third, IL28B SNP heterogenous may be a factor, although IL28B SNP analysis of GT3b cases has not been reported.

In addition, regarding the factors related to the breakthrough case, NS5B mutation, including 150, 206 and 282 may have occurred in the course of treatment, although NS5B mutation was not observed at baseline or post treatment in our breakthrough and relapse patients.

To the best of our knowledge, this is the first analysis of RAS and IL28B SNP in decompensated cirrhosis patients, demonstrating breakthrough and relapse with SOF/VEL treatment.

Further study is needed to clarify the reason for treatment failure in GT3b decompensated liver cirrhosis patients.

6. Author Contributions:

Kim SK conceived the study and wrote the manuscript; Kim SR, Ogawa S and Tanaka Y observed the clinical course of the patients and made the figures; Fujii T, Fujii Y, Kobayashi H, Hayakumo T, Okuda T, Nakai A, Yuasa K, Takami M, Kim KI, Ohtani A, Nakao K and Saijo Y observed the clinical course of the patient; Ogawa S and Tanaka Y performed RAS and IL28B SNP analyses.

7. Conflicts of interest:

Tanaka Y. Research support: Janssen and Gilead, Speaker: Gilead. The other authors have no conflicts of interest to declare.

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