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CASE REPORT

Hepatitis C virus recurrence in two patients who achieved sustained viral response with interferon-free direct-acting antiviral therapy: reinfection or relapse?

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Abstract

We experienced two patients with chronic hepatitis C (HCV) in whom it was difficult to distinguish between relapse and reinfection after interferon-free direct-acting antiviral (DAA) therapy. Case 1 was a 55-year-old man infected with HCV genotype 1b, at 5.6 log IU/mL, with a history of injecting drug use. He was treated with ombitasvir/paritaprevir/ritonavir for 12 weeks. After DAA therapy, recurrent HCV showed the genotype 2a differed from the baseline genotype. Close examination for baseline sample showed that he was coinfected with HCV genotype 1b and 2a. Case 2 was a 60-year-old woman with HCV2b, at 5.7 log IU/mL. She was treated with sofosbuvir/ribavirin for 12 weeks and achieved a sustained virological response (SVR) at 24 weeks. Even after SVR24, her serum alanine aminotransferase levels remained fluctuated. HCV RNA was detected again at 48 weeks. She had a sexual partner who was also infected with HCV2b. The phylogenetic tree analysis revealed a high degree of homology among the three strains: pre- and post-HCV treatment, and her partner. After HCV recurrence, HCV RNA level decreased spontaneously below the limit of detection and serum ALT levels normalized. It is important to make a precise diagnosis regarding reemerged HCV after DAA therapy.

Keywords Direct-acting antivirals · Hepatitis C virus · Late relapse · Reinfection · Sustained virological response

Introduction

The development of direct-acting antivirals (DAAs) has markedly improved the rate of viral eradication in patients with chronic hepatitis C virus (HCV) infection. However, HCV can still recur and it is important to distinguish between reinfection with HCV after achieving a sustained virological response (SVR) and late viral relapse. In the era of interferon-based therapy, late relapse was reportedly rare [1], and testing for an SVR 12 weeks after completion of therapy (SVR12) was shown to be as effective at predicting SVR as testing at 24 weeks (SVR24) [2]. The European Association for the Study of the Liver (EASL) guidelines recommend that patients who achieve an SVR should be retested for HCV RNA 48 weeks after completing the treatment, and that the infection can be considered to be definitely cured when HCV RNA is undetectable up to that time. It has been recommended that, following an SVR, HCV reinfection should be monitored with an annual HCV RNA assessment in high-risk patients, such as injecting drug users (IDUs), men who have sex with men, and those engaging in other high-risk behaviors [3]. The American Association for the Study of Liver Diseases (AASLD) guidelines recommend assessing HCV relapse or reinfection in patients at risk of HCV infection, or with unexplained hepatic dysfunction [4]. Higher rates of reinfection may be expected in the era of DAA treatment due to increasing treatment access for those with ongoing high-risk behavior and reduced concerns over adverse effects of treatment [5]. Between September 2014 and November 2018, 689 patients (mean age 67 ± 12 years; 309 males, 380 females) treated with DAAs were followed up beyond 48 weeks after the end of treatment. We

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experienced two cases of suspected reinfection among 16 non-SVR patients.

Case report

Case 1

A 55-year-old man was infected with HCV genotype 1b, at 5.6 log IU/mL. Laboratory data at baseline are shown in Table 1. He had been an IDU. He was treated with ombitasvir (25 mg), paritaprevir (150 mg), and ritonavir (100 mg) for 12 weeks. Although HCV RNA was undetectable at 4 weeks after starting treatment and SVR4 was achieved, HCV RNA reappeared 12 weeks after the end of treatment. The HCV genotype at treatment failure was 2a and differed from the baseline genotype. Phylogenetic analysis of NS5B also revealed that the HCV genotype at treatment failure differed from that at baseline. These results strongly suggested that this patient had been reinfected with another HCV genotype after the initial DAA therapy. However, the existence of two types of HCV at baseline was revealed after separating several clones amplifying them with the conventional method, and analyzing the sequences (Fig. 1a). We concluded that the ombitasvir/paritaprevir/ritonavir therapy eradicated genotype 1b HCV, but genotype 2a HCV remained. We confirmed the emergence of a resistance-associated substitution in NS5A in genotype 2a after the initial DAA therapy. Retreatment with glecaprevir (100 mg) and pibrentasvir (40 mg) for 12 weeks achieved SVR12 (Fig. 1b).

Case 2

A 60-year-old woman was infected with HCV genotype 2b, at 5.7 log IU/mL (Table 1). She did not have a history of injecting drug use and a family history for HCV. The single-nucleotide polymorphism (SNP) of *IL28B* rs8099917 was the major type, TT [6]. She was treated with sofosbuvir (400 mg) and ribavirin (600 mg) for 12 weeks and achieved SVR24. Even after SVR24, her serum alanine aminotransferase (ALT) levels remained elevated. HCV RNA was detected again at 48 weeks after the end of treatment (Fig. 2a). She had a sexual partner who was also infected with HCV. To discriminate between relapse and reinfection from the partner, we compared the HCV NS5B sequences of three samples: her HCV at baseline and at treatment failure, and her partner's HCV. Phylogenetic tree analysis based on HCV NS5B sequences spanning 339 nt (nucleotide position 8278–8618) was performed. The analysis revealed a high degree of homology among the three strains: pre- and post-HCV treatment, and her partner (Fig. 2b). Using HCV sequence analysis, it was difficult to differentiate between reinfection and late relapse. After recurrence of HCV, the HCV RNA level decreased spontaneously below the limit of detection at 24 weeks after the repeat detection and the

Table 1 Clinical data at baseline and treatment outcome

	Case 1	Case 2
Age (years)	55	60
Gender	Male	Female
Cirrhosis/non-cirrhosis	Non-cirrhosis	Non-cirrhosis
Liver stiffness (kPa)	9.9	13.3
Past IFN-based therapy	–	–
WBC (/μL)	5700	3200
Hb (g/dL)	15.2	13.7
Plt ($\times 10^4$/μL)	18.9	12.6
T-Bil (mg/dL)	0.5	0.6
ALT (IU/L)	87	220
FIB-4 index	2.06	4.40
AFP (ng/mL)	5.8	47.2
HCV RNA viral load ($\log_{10}$ IU/mL)	5.6	5.7
Serotype	1	2
Genotype	1b	2b
Interleukin 28B rs8099917 SNP	TT	TT
DAA regimen	OMV + PTV + r	SOF + RBV
Outcome after DAA therapy	Relapse	Suspected reinfection

IFN interferon, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *T-Bil* total bilirubin, *ALT* alanine aminotransferase, *FIB-4 index* fibrosis-4 index, *AFP* α-fetoprotein, *HCV* hepatitis C virus, *SNP* single-nucleotide polymorphism, *DAA* direct-acting antiviral, *OMV* ombitasvir, *PTV* paritaprevir, *r* ritonavir, *SOF* sofosbuvir, *RBV* ribavirin, *GLE* glecaprevir, *PIB* pibrentasvir, *SVR* sustained viral response

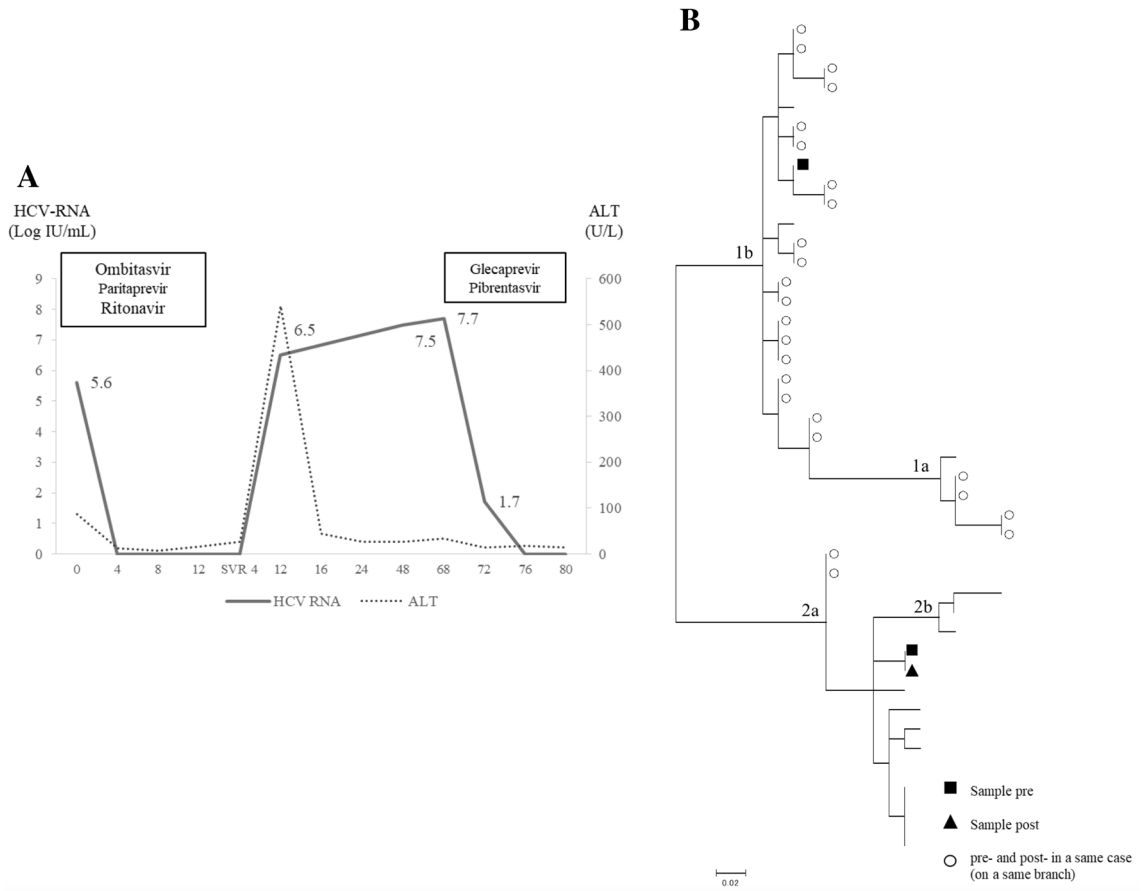

Fig. 1 **a** Clinical course of the serum HCV RNA and ALT levels in case 1. **b** Phylogenetic tree based on HCV NS5B sequences spanning 339 nucleotide positions (8278–8618) in case 1. Two strains were detected at baseline: GT1b and GT2a. The black squares and triangles indicate the strains detected pre- and post-treatment, respectively. White circles indicate the pre- and post-treatment strains from different cases. The pre- and post-treatment strains in each case are classified on the same branch

serum ALT levels normalized, similar to the typical clinical course of HCV infection-induced acute hepatitis. After recurrence of HCV, her partner achieved an SVR by combination with sofosbuvir and ribavirin therapy.

Discussion

We presented two patients who were suspected HCV reinfection after interferon-free DAA therapy. In case 1, the HCV genotype at baseline differed from that at failure. In addition, the patient had a history of IDU. Midgard et al. reported that HCV reinfection was common, with an incidence of 1.7/100 person-years (95% CI 0.8–3.1) among individuals who returned to IDU after treatment [5]. Therefore, we strongly suspected HCV reinfection in this case. However, sub-cloning analysis revealed coinfection with HCV genotypes 1b and 2a at baseline. Commercial HCV genotyping had some limitations with regard to the limit of detection of viral load and the inability to genotype individual HCVs. Although sequence analysis costs more and takes more time, it remains the gold standard for genotyping of HCV [7]. Combination therapy with ombitasvir/paritaprevir/ritonavir for DAA-naïve patients with HCV genotype 1 achieved a high SVR rate [8]. However, the phase II trial with these DAAs for 12 weeks resulted in SVR 12 rate of 72% for HCV genotype 2-infected Japanese. It was speculated that this regimen was not effective for patients with genotype 2, although base HCV viral load was low [9]. Now, a combination with glecaprevir/pibrentasvir, pan-genotypic regimen is available as one of the first recommended options in Japan. In our knowledge, there is no report that one of the coinfected HCV emerged after pan-genotypic DAA therapy. Okamoto et al. reported that 4 (1.6%) of 256 patients were infected with two different HCV genotypes [10]. It is important to be aware that IDUs have the opportunity for infection with more than one HCV genotype. There are three patterns of HCV RNA recurrence in SVR patients: relapse, persistence/emergence of a pre-existing minority variant, and

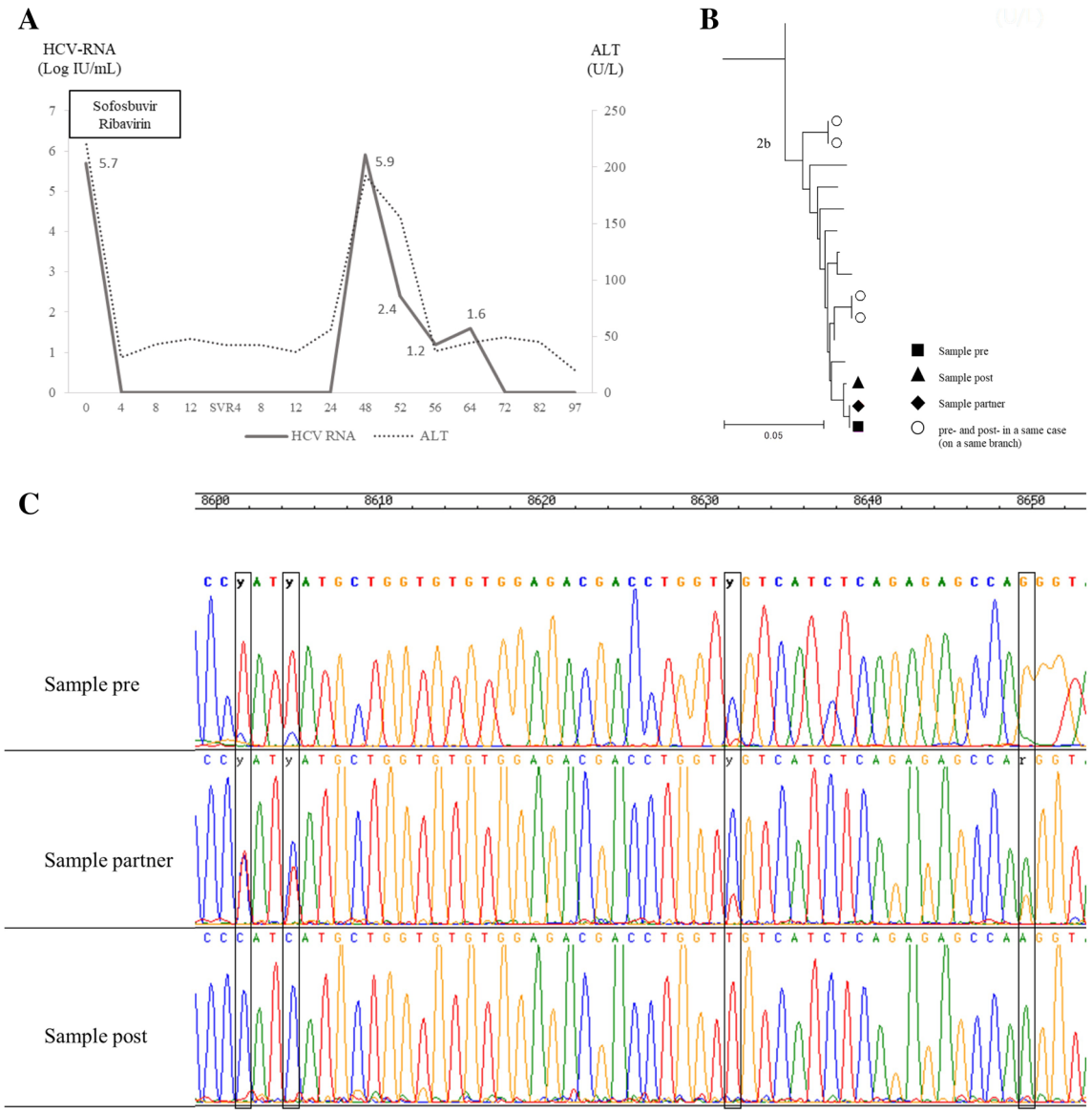

Fig. 2 **a** Clinical course of changes in serum HCV RNA and ALT levels in case 2. **b** Phylogenetic analysis based on HCV NS5B sequences spanning 339 nt (nucleotide positions 8278–8618). Pre- and post-treatment strains obtained in case 2 were compared with those of other relapsed patients or reference sequences retrieved from the EMBL/DDBJ/GenBank database using MEGA6.0 software to analyze their evolutionary relationships [16]. White circles on the same branch indicate the same patient at pre-treatment and at relapse. Black squares, triangles, and diamonds indicate the pre- and post-treatment strains of case 2 and those of her partner, respectively. Phylogenetic analysis showed that these three strains were closely related. **c** The upper and lower sequences are for pre- and post-treatment HCV from case 2. The middle sequence shows HCV from her partner. These three sequences showed a high degree of identity

reinfection [11]. Case 1 presented herein corresponded to the emergence of a pre-existing minority variant: HCV genotype 2a.

In case 2, HCV RNA was detected again at 48 weeks after the end of sofosbuvir/ribavirin treatment. The ALT levels fluctuated continuously during the SVR. Therefore, we tested for HCV RNA repeatedly. Following SVR48, the serum ALT level increased abruptly and HCV RNA became detectable. A late relapse was suspected. However, the patient had an HCV-infected sexual partner, whose HCV RNA sequence showed a high degree of homology to those of the patient at both baseline and reemergence. In sexually transmitted infections, the same pathogen can pass to and from patients. It has been reported that the incidence rate of HCV infection in seronegative partners of subjects with chronic HCV infection was 0–0.6% by prospective cohort studies of monogamous heterosexual couples [12, 13]. Although sexual transmission of HCV was reported to

be extremely rare, we could not distinguish between late relapse and reinfection based on a comparison of HCV RNA sequences. The reemerged HCV disappeared spontaneously, similar to the clinical course of acute hepatitis C. HCV infection is often self-limited in patients with the *IL28B* major type [14]. Therefore, we strongly suspected that our patient had been reinfected with HCV from her partner.

No patient in the cohort seen at our university hospital had definite HCV reinfection after DAA therapy. Across 11 phase III clinical trials of sofosbuvir-based regimens, only 12 of 3004 patients (0.40%) had detectable HCV RNA following SVR12. Of these 12 patients, 7 were reinfected with a different HCV genotype after treatment [15]. Most studies have analyzed recurrence after treatment with interferon-based therapy, and there is no evidence to support the hypothesis that recurrence rates differ in DAA-based treatment regimens [1]. In the era of DAA treatment, resistance-associated substitutions were detected in the relapsed HCV, apart from natural HCV. To determine the DAA regimen for retreatment, it is necessary to distinguish between relapse and reinfection.

In conclusion, we encountered one case of coinfection with two HCV genotypes and another with a high possibility of reinfection following DAA-based treatment. It is important to make a precise diagnosis regarding reemerged HCV after DAA therapy.

Compliance with ethical standards

Conflict of interest Dr. N. Kawada and Dr. A. Tamori and Dr. Y. Tanaka have received lecture fees from Gilead Sciences, and AbbVie Inc. The other authors declare that they have no conflict of interest.

Human rights All the procedures followed have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Informed consent was obtained from the patient for being included in the study.

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