HEPATITIS C VIRUS LATE RELAPSE AFTER SUSTAINED VIROLOGIC RESPONSE
FROM INTERFERON AND RIBAVIRIN TREATMENT CONFIRMED BY RNA SEQUENCING

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Hepatitis C (HCV) viremia is unusual (<5%) after successful treatment, defined as sustained virologic response (SVR) or undetectable HCV PCR 12-24 weeks after therapy. We present a case of late virologic relapse (de novo infection excluded by RNA sequencing) after SVR followed by spontaneous viral clearance.

CASE REPORT

A 49 year old woman without significant past medical history was evaluated for HCV genotype 3a cirrhosis. Her only identified risk factor was intravenous gamma globulin transfusion in 1972. Her presenting HCV viral load was $2.64 \times 10^5$ IU/mL. Serum ALT was 82 U/L, AST 113 U/L, albumin 25 g/L, bilirubin 26 µmol/L, INR 1.3 and platelets were 49 x 10⁹/L. Initial HCV treatments using weight based dosing failed twice due to relapse, defined as HCV PCR being negative on treatment but then positive after stopping treatment. Treatment #1: January 2001 with standard interferon (IFNα2b) 3 MU three times per week and Ribavirin (RBV) 1200 mg od x 48 weeks. Treatment #2: March 2003 with pegylated (PEG) IFNα2b 120 µg weekly and RBV 1000 mg od x 24 weeks. Both treatments were complicated by neutropenia leading to dose reductions. Treatment #3: November 2006 with PEG-IFNa2b 100 µg weekly and RBV 1200 mg od x 22 weeks. Treatment stopped early, with no dose reduction on therapy, due to an episode of spontaneous bacterial peritonitis. Nonetheless, she achieved SVR with negative HCV PCR in November 2007, 29 weeks post treatment with normalization of ALT.

It was not routine clinical practice to repeat HCV PCR testing after SVR. However, ALT became abnormal again, peaking at 109 U/L (figure 1) in February 2009. HCV PCR testing was repeated then and confirmed HCV infection with genotype 3a. A detailed history revealed no risk factors for re-infection. HCV sequencing performed on stored sera from November 2006 (pre-treatment) and December 2009 (post-treatment) confirmed relapse after SVR rather than new infection. The clinical course was reminiscent of acute infection with wildly fluctuating viral loads, followed by spontaneous resolution without antiviral therapy.
HCV RNA was extracted from 250 μL of pre- and post-treatment sera using the automated nucleic acid extraction system NucliSENS easyMag (BioMerieux Inc, Durham, US) and was amplified by reverse transcription PCR using primers specific for 5’ non-coding region, the Core, E1, and NS5B genes. Sequence data obtained were used to determine the HCV genotype and phylogenetic relatedness. Genetic distances were estimated by Kimura two-parameter analysis, and a phylogenetic tree was constructed by the maximum likelihood method. Significant taxonomic relationships were obtained by bootstrap resampling analysis (200 replicates) (confidence values of 70% or greater are considered significant).

A phylogenetic tree of 50 HCV strains (Figure 2) was prepared to determine genetic relatedness. Forty-two randomly selected HCV subgenotype 3a strains from three Canadian provinces were aligned with four strains belonging to the same subgenotype as our patient. To better illustrate the difference between re-infection and relapse after SVR, three additional HCV strains from a patient with re-infection (initially suspected to be late relapse) were also included. A HCV subgenotype 1a sequence was used as an outlier.

Older sequences from our patient before treatment (2003 & 2006) were very similar (99% sequence homology). The ones obtained after the relapse post SVR (February and August 2009) exhibited even higher sequence homology – 99.8% (Figure 2). The same phylogenetic relatedness between the HCV strains was observed with sequences from the Core and NS5B genes (data not shown). All four sequences cluster together by branching on the same sub-node. Relatedness is supported by a very high bootstrap value (node bootstrap of 93) (Figure 2). That well defined cluster is located apart from the rest of the HCV subgenotype 3a isolates.

In contrast, there is no phylogenetic relatedness comparing the pre-treatment HCV isolates from the 37 year old male patient with HCV genotype 3a included in figure 2 and his 2008 HCV isolate indicating de novo infection. It is of interest that the viral strains from these two patients were quite stable with minimal change during the last three years before treatment based on the sequence homology mentioned above. This is an important consideration when analyzing phylogenetic relatedness between different HCV strains because the virus/host interaction in some cases speeds up the mutation rate leading to increased variability of the virus genome.
which makes it difficult to assess phylogenetic relationships without intermediary samples throughout the years.

SVR is accepted as a durable treatment endpoint in HCV (1, 2). Cases of serum HCV RNA detected after SVR have been reported (3-8). However, most studies do not distinguish between relapse after SVR and *de novo* re-infection. Detection of serum HCV RNA should further be differentiated from the more frequently documented – yet of uncertain clinical significance – “occult HCV” where HCV RNA is undetectable in serum but detectable in hepatocytes or peripheral blood mononuclear cells (7).

Veerapu *et al.* report a series of 98 patients treated with IFN and RBV, where 15% of patients had detectable serum HCV RNA after SVR. (9) In all cases, with one exception, RNA was detectable only when using nested PCR rather than conventional PCR. Moreover, the authors analyzed the highly conserved 5’ untranslated region, which is less sensitive for distinguishing relapse from *de novo* infection within the same genotype subtype.

We found two cases of late relapse confirmed through sequencing of high variability regions; one in a patient with hypogammaglobulinemia after IFN,(10) and the other after IFN and RBV in which late relapse progressed to chronic infection.(11)

This case is unique in that we were able to not only confirm relapse after SVR following IFN and RBV treatment, but also document spontaneous resolution. It is furthermore important to acknowledge the complex interplay between several factors such as genotype, age, and duration of therapy that influenced response to treatment in this patient leading to HCV clearance (12-14). In this individual, SVR represented HCV suppression rather than HCV eradication. Furthermore, relapse was asymptomatic and only detected after investigation of newly elevated transaminase levels. The possibility that viremia after SVR occurred before the rise in ALT cannot be eliminated. It is possible that late events might happen more frequently but not recognized. We
speculate that SVR achieved using new IFN-free regimens may be more durable due to viral eradication rather than immune suppression.

SVR is a durable and clinically significant endpoint of interferon-based HCV therapy. Relapse after SVR is rare but potentially unrecognized as it is likely clinically silent and viremia can be self-limited.

REFERENCES


FIGURE LEGENDS

Figure 1. Evolution of Serum HCV RNA Viral Load through Time
HCV treatment from November 2006 to April 2007: SVR was followed by late relapse in February 2009, and spontaneous clearance in October 2009. * Lower limit of HCV RNA detection =15 IU/mL; ** Normal range ALT ≤ 40 U/L.

Figure 2. Phylogenetic Analysis of 50 HCV E1 Coding Region Sequences
Phylogenetic tree constructed using sequences from 50 HCV genotype 3a strains and one from genotype 1a used as outlier. Each sequence is denoted by a code number and the year. Ovals: Pre-treatment samples (H4068 in 2003 and H4081 in 2006) are closely related to probable late relapse samples (H4082 and H4083 in 2009). Diamonds: For contrast, pre-treatment samples (H6488 in 2000 and H6489 in 2003) are not related to sample H6490 in 2008 indicating a re-infection rather than relapse. AB= Alberta; ON=Ontario; BC=British Columbia

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