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Case report

Ultra-deep sequencing provides insights into the virology of hepatitis C super-infections in a case of three sequential infections with different genotypes

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ABSTRACT

The current epidemic of Hepatitis C infection in HIV-positive men who have sex with men is associated with increasing use of recreational drugs. Multiple HCV infections have been reported in haemophiliacs and intravenous drug users. Using ultra-deep sequencing analysis, we present the case of an HIV-positive MSM with evidence of three sequential HCV infections, each occurring during the acute phase of the preceding infection, following risk exposures. We observed rapid replacement of the original strain by the incoming genotype at subsequent time points. The impact of HCV super-infection remains unclear and UDS may provide new insights.

1. Why this case is important

The continuing epidemic of hepatitis C virus (HCV) infection in HIV-positive men who have sex with men (MSM) has been well documented [1]. This is associated with increasing recreational drug use, often in combination with high-risk sexual practises (such as group sex and fisting) [2,3]. Liver disease consequent to hepatitis B and C remains a leading cause of non-AIDS morbidity and mortality in HIV-positive individuals [4].

Here we report a case of an HIV-positive MSM with evidence of three sequential HCV infections, each occurring during the acute phase of the preceding infection before viral clearance. We have used ultra-deep sequencing (UDS) analysis to demonstrate different genotypes and the rapidity at which one viral strain replaces another.

2. Case description

A 49-year old caucasian MSM was diagnosed with HIV-1 infection in 1997 with no baseline HIV drug resistance, HIV-related complications or significant medical history. He started tenofovir/emtricitabine/efavirenz single tablet regimen in 2011 when his CD4 count was 310 cells/mm^3 and HIV-RNA viral load was 98,000 copies/ml; virological suppression was achieved within 12 weeks but due to insomnia, efavirenz was switched to ritonavir boosted atazanavir with continued viral suppression.

In August 2013, he attended the sexual health clinic, asymptomatic but anxious regarding HCV, following unprotected anal intercourse and injecting crystal methamphetamine with a casual male partner, who later revealed himself to be HIV/HCV co-infected. The patient had a negative anti-HCV antibody test three months previously.

He was screened for sexually transmitted infections two weeks later. Anti-HCV was negative but HCV RNA was 13 million IU/ml, alanine aminotransaminase (ALT) 40 IU/ml (normal range 10–40 IU/ml) and HCV genotyping by Sanger sequencing of the UTR region [5] revealed genotype 4d virus (Table 1, time point 1 shown

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Table 1
Laboratory data including HCV viral load, ALT and UDS results. The k-mer analysis data are presented for each genotype in the last three columns. The shaded area indicates the time the patient was on anti HCV therapy.

Time point (Tp)	Days in follow up since HCV RNA detection	HCV RNA (IU/ml)	Anti-HCV	ALT IU/L	5′UTR region Sanger sequencing genotype	5′UTR region NGS % of genotypes present using k-mer analysis		
						4d	1c	1a
Tp1	1	13,000,000	ND*	30	4d	100%	0.0%	0.0%
Tp2	8	12,000,000	ND	31	4d	100%	0.0%	0.0%
Tp3	57	36,000,000	ND	196	1c	0.9%	99.1%	0.0%
Tp4	71	50,000,000	ND	391	1c	0.0%	100%	0.0%
Tp5	75	29,000,000	ND	1487	1c	0.0%	100%	0.0%
Tp6	111	12,500,000	Pos	1091	1a	0.0%	0.0%	100%
Tp7	162	300	Pos	375	1a	0.0%	0.0%	100%
Tp8	183	10,911,300	Pos	221	1a	0.0%	0.0%	100%
Tp9	205	9,000,600	Pos	198	1a	0.0%	0.0%	100%
Tp10	254	9,729,600	Pos	126	1a	0.0%	0.0%	100%

* Not Detected

as Tp1). He remained well and was counselled regarding HCV. Liver ultrasound scan was normal.

Eight weeks later, he returned to clinic, anxious following further exposures with two other HIV/HCV co-infected men; each had drawn up syringes of their blood and then injected the others. The sample collected at Tp3 (Table 1) revealed an increase in HCV RNA to 36 million IU/ml and now HCV genotype 1c, ALT rising to 196 IU/L.

Unfortunately he developed jaundice (Tp5) and progressive derangement of liver enzymes necessitating admission to a liver specialist unit. ALT peaked at 3900 IU/L with normal clotting and liver ultrasound. He was managed conservatively and discharged without biopsy, as liver enzymes normalised.

Two months later (Tp6) he re-presented with malaise and ALT flared to >3000 IU/L. His HCV viral load was 12.5 million and now typed as genotype 1a. Liver biopsy showed histological evidence of acute hepatitis with cholestasis in keeping with HCV-infection. Given the subfulminant hepatitis, he was commenced on telaprevir, pegylated interferon-alfa and weight-based ribavirin. At week one, the HCV RNA was 300 IU/ml (Tp7) but by week 4 (Tp8) this had risen to 10 million IU/ml. Viral sequencing revealed genotype 1a virus with the telaprevir resistance-associated mutations R155K and V36 M that were not present previously. Treatment was stopped in accordance with standard rules at failure. The patient admitted to new risks, however, the virus isolated at treatment failure was shown to be phylogenetically related to the virus isolated at Tp6 rather than a new infection. Follow up continued for a further 3 months (Tp9 and Tp10) showing persistence of a genotype 1a virus.

HCV RNA was extracted using the BioRobot MDx (Qiagen Ltd.) and HCV viral load was determined by RT-qPCR [6]. Genotyping was carried out using Sanger sequencing by an “in-house” genotyping protocol based on published primers targeting the 5′UTR region [7]. For detection of HCV NS3 protease resistance a nested PCR followed by sequencing using an “in-house” protocol.

Viral RNA was extracted from plasma at ten time points (Table 1) using the QIAamp Viral RNA Kit (Qiagen) according to manufacturer’s instructions and 80 μl of kit elution buffer. The first round RT-PCR primers are described in Supplementary data. The second round primers and PCR reagents were from a Nextera®XT DNA Sample Preparation Kit (Illumina Inc. [5]) and detailed methodology and analysis are provided as Supplementary data. Samples were then individually loaded onto a MiSeq reagent Kit V3 600 cycle (Illumina Inc.) and sequenced on a MiSeq (Illumina Inc.).

Additionally, two plasma samples from Tp1 (genotype 4d) and Tp5 (genotype 1c) were mixed at different ratios to evaluate the sensitivity of the method (0.01%) and processed as described. A reference-based alignment method followed by variant calling was

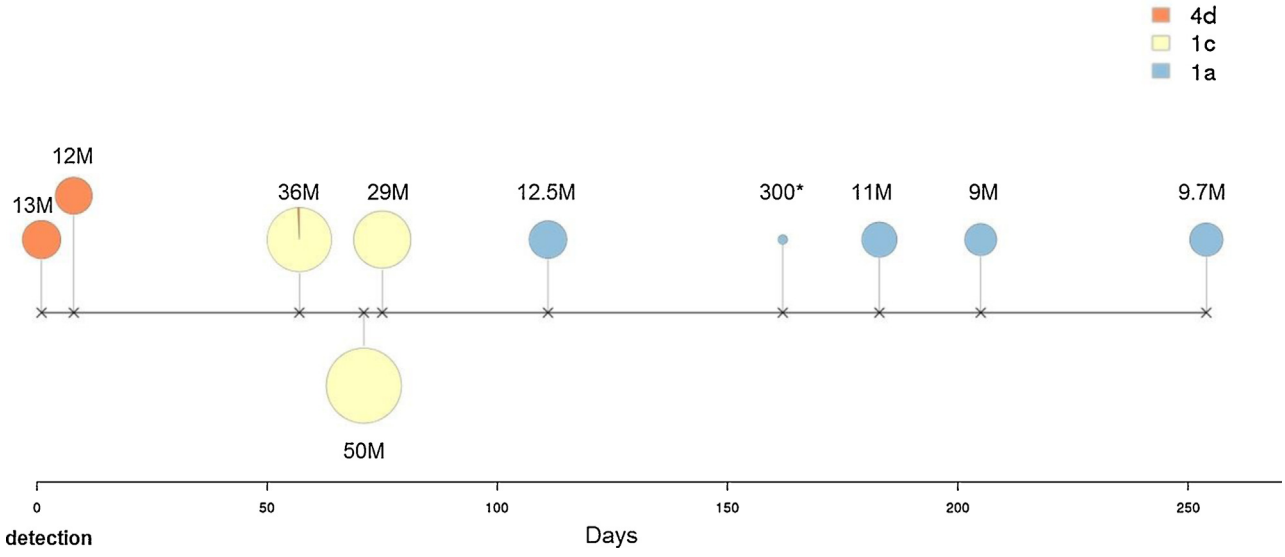

Fig. 1. Time (in days) is shown as a horizontal line, with crosses representing dates of sampling. Coloured pie charts illustrate the varying detection of different HCV genotypes (expressed as percentage) over time. The sizes of pies are proportional to HCV viral load, labeled next to each pie in IU/ml except for 300* where the scale is 50 times larger. Tp3 is a mixture of genotypes 1c and 4d while samples from all other Tp contain a single genotype.