SUPPLEMENT ARTICLE

Hepatitis C Virus Reinfection Following Treatment Among People Who Use Drugs

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Most new cases of hepatitis C virus (HCV) infections in the developed world are associated with injection drug use. However, treatment for people who inject drugs (PWID) is controversial, as successful treatment risks being followed by new infection. Reinfection after sustained virologic response has been reported, but is the risk so great that treatment should be withheld from this large HCV population? Preliminary evidence suggests that the reinfection incidence is low, but studies to date have been limited by small sample size and few cases of reinfection. In this review, we assess data from studies among PWID of HCV reinfection following treatment to give a reasonable estimate on how frequently reinfection appears and try to characterize those most at risk, The observation that spontaneous clearance of HCV reinfection following treatment occurs is suggestive of a partial protective immunity against persistent infection.

Keywords. mixed infection; substance abuse; hepatitis C virus; clearance; sequence analysis.

People who inject drugs (PWID) are at great risk of infection with hepatitis C virus (HCV), and most new HCV infections in the developed world are associated with injection drug use. With treatment, sustained virologic response (SVR) rates vary from 50% to 80% depending on genotype [1, 2]. With the arrival of new and shorter treatment regimens with direct-acting antiviral agents, SVR rates >90% are hoped for. HCV treatment response rates among PWID have been reported to be comparable with rates in patients with no active drug use [3–5]. However, treatment of HCV in active drug users is controversial owing to concerns on side effects and risk of reinfection [6, 7].

One could speculate that patients who have cleared infection upon treatment carry a protective immunity against reinfection, but several groups have reported that reinfections do occur [8–14]. Given the fact that reinfection can occur, is the risk so substantial that treatment should be withheld from this large HCV population? In this review we give an estimate on how frequently reinfection occurs and attempt to characterize those most at risk. In addition, we will define reinfection in high-risk settings and point out how multiple testing and viral sequencing can enhance the current knowledge and clinical decision making on reinfection and viral relapse following HCV treatment among PWID. The review will also briefly discuss the possibility of some degree of immunologic protection against reinfection with HCV.

INCIDENCE OF HCV REINFECTION FOLLOWING SVR

Over the past years, several case reports and studies have addressed the occurrence of HCV reinfections following treatment-induced clearance, both in PWID and men who have sex with men (MSM) populations [8–16]. In Table 1, the details of 7 such studies are presented. All studies had a prospective design and were performed in Germany [8], Norway [11], the United States [10], Canada [13], the Netherlands [12], and Australia [9, 14]. Sample size varied between 9 and 88 persons, and data on injection drug use pretreatment and during treatment were available in 3 of 7 studies. In one study,

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Table 1. Overview of Studies on Hepatitis C Virus Reinfection Following Treatment Among People Who Inject Drugs

Study	Country	Study Design	Genotyping	Sequence Analysis	No.	Median Age at Treatment Start, y	% Male	IDU Pretreatment <6 mo	IDU Post treatment	Follow-up, Median (IQR)	PY Ever PWID/ PWID Who Continue	No. of Reinfections	Reinfection Rate (95% CI) per 100 PY Ever PWID/PWID Who Continue
Backmund et al, 2004 [8]	Germany	Pros	Yes	No	18	32	61	NA	9	Mean 2.8 (SD 0.8–5.1)	50.8/23.8	2	3.94 (0.48–14.22)/ 8.4 (1.02–30.36)
Dalgard et al, 2002 [11]	Norway	Pros	Yes	No	27	30	66	0	9	5.4 (1.1–6.8)	125.0/40.0	1	0.8 (0–5)/2.5 (0–14)
Currie et al, 2008 [10]	US	Pros	No	No	9	46 (mean)	88	NA	2	3.6 (3.2–6.0)	38.0/3.5	1	2.63 (0.07–14.66) 28.57 (0.72–159.19)
Grebely et al, 2010 [13]	Canada	Pros	Yes	Yes	35	44 (mean)	86	19	16	2.0 (0.4–5.0)	62.5/37.7	2	3.20 (0.39–11.56)/ 5.30 (0.64–19.16)
Bate et al, 2010 [9]	Australia	Pros	Yes	No	57	NA	NA	NA	NA	NA	NA	5	NA
Grady et al, 2012 [12]	Netherlands	Pros	Yes	Yes	42	51	73	5[a]	11	2.5 (1.6–3.7)[b]	131.6/32.3	1	0.76 (0.04–3.73)/ 3.42 (0.17–16.90)
Grebely, 2012 [14]	Australia	Pros	Yes	Yes	88	36	72	33[a]	NA	1.2 (0.1–3.0)[b]	108	5	4.7 (1.9–11.2)

Abbreviations: CI, confidence interval; IDU, injection drug use; IQR, interquartile range; NA, specific information was not available; Pros, prospective; PWID, people who inject drugs; PY, person-years.

[a] During treatment.

[b] Follow-up from end of treatment.

reinfection was defined as HCV recurrence in at least 2 consecutive tests after achieving SVR; in the remaining studies only 1 positive test was required to define a reinfection. To confirm reinfection, 6 of 7 studies performed genotyping and 3 studies performed sequence analysis to discriminate between relapse and reinfection. In all studies, at least 1 case of reinfection was detected, which led to a total of 17 reinfections. Among 6 studies providing data on person-years (PY) of follow-up, the reinfection rate varied from 0.8 to 4.7 per 100 PY. When stratified to populations with ongoing risk behavior, the incidence rate varied from 2.50 to 28.57 per 100 PY. Recently, Aspinall et al [17] performed a meta-analysis on 5 of the 7 aforementioned studies on the incidence of reinfection after successful treatment. The pooled estimate of reinfection among all study participants was 2.36 (95% confidence interval [CI], 0.91–6.12) per 100 PY; when the analysis was stratified to those who reported injection drug use posttreatment, the pooled estimate of HCV reinfection was 6.44 (95% CI, 2.49–16.69) per 100 PY. In comparison, the incidence of new HCV infection outside the setting of treatment has been found to be 6.1–27.2 per 100 PY [18]. Thus, the data suggest a relatively low risk of reinfection following successful treatment. However, when comparing the new and reinfection incidence rates of HCV, a few factors should be taken into account. First, risk of reinfection may vary depending on the local background epidemic among the PWID population of HCV. For example, in Vancouver, Canada, the risk of reinfection after HCV treatment was found to be 3.2 cases per 100 PY [13], whereas in the same city the incidence of first HCV infections was 7.3 cases per 100 PY [19]. In Amsterdam, the risk of reinfection after HCV treatment was 0.76 cases per 100 PY, with a local background incidence of first HCV infections of 0.35 cases per 100 PY. Therefore, in communities with a higher local background HCV epidemic, treated PWID are likely to have a higher risk of reinfection. In addition, injecting behavior after treatment, as well as the implementation of a needle exchange program, influence the risk of reinfection among PWID.

Despite the limited number of cases and the limited number of follow-up years, these data suggest that the incidence of reinfection after successful HCV treatment is low. No specific group of PWID can be identified clearly at increased risk of reinfection, although there seems to be a trend favoring older patients (Supplementary Figure 1). This is in line with studies showing that older and more experienced PWID are less likely to share needles than younger drug users [20, 21].

DEFINING REINFECTION IN HCV TREATMENT SETTINGS AMONG PWID

It is not always clear whether a reinfection has occurred, and from both a clinical and a research perspective it is necessary to clarify what we mean by "reinfection." Reinfection is defined as

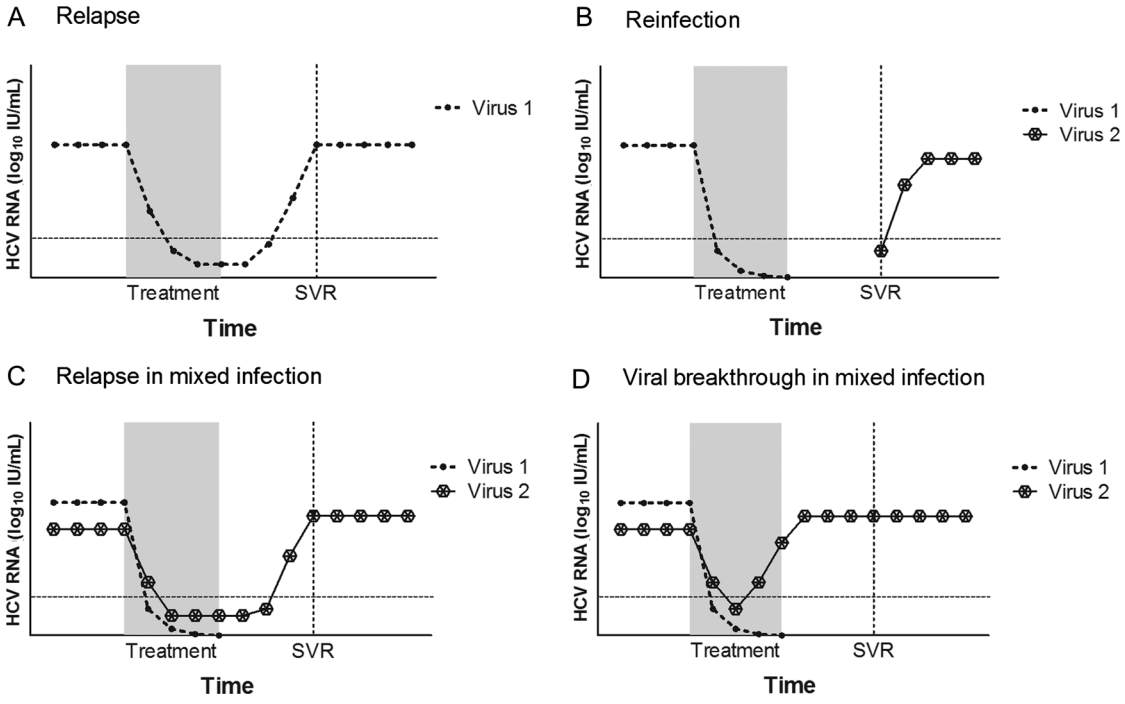

Figure 1. Scenarios for recurrence of hepatitis C virus (HCV) RNA after treatment in populations at risk for reinfection with HCV. *A*, Recurrence of HCV RNA with the same viral strain after end of treatment, reffered to as relapse. *B*, The initial infection is completely resolved prior to a reinfection. When mixed infection is present during treatment, a relapse (*C*) or viral breakthrough (*D*) could be caused by a non-responsive viral strain. Dashed horizontal line represents the detection limit for HCV RNA tests. Mixed infection (*C* and *D*) can either be a superinfection or a coinfection. Abbreviations: HCV, hepatitis C virus; SVR, sustained virologic response.

a case in which an initial infection is completely resolved prior to a subsequent infection [22]. This can be either a reinfection with a different genotype/subtype compared to the initial infection, or with the same subtype but a different strain. Classically, viral relapse following an end of treatment (EOT) response—as indicated by the absence of HCV RNA in serum—is defined as the recurrence of HCV viremia within 24 weeks of therapy cessation. However, among HCV-infected individuals in high-risk environments, several considerations need to be taken into account by clinicians when diagnosing viral relapse or even late relapse (recurrence of HCV RNA after SVR, with HCV RNA negative at EOT through SVR) based on HCV recurrence.

Two studies among MSM and 2 among PWID have shown that HCV reinfections can occur after EOT but before the SVR determining time point, 6 months later [9, 14, 15, 23]. These studies highlight the clinical need to discriminate between a relapse and reinfection (Figure 1, *A* and *B*). Further complicating the picture, in individuals with high-risk behavior and frequent exposure to HCV, multiple viral strains can be detected at a single time point. This is referred to as a mixed infection. Two types of mixed infections can be distinguished, coinfection and superinfection [22]. Coinfection can be defined as a simultaneous acquisition of 2 or more HCV strains. Superinfection occurs in individuals with chronic HCV infection, who, after reexposure to HCV, present with a new and different HCV viral strain(s). Taking a superinfection or coinfection into account, theoretically a patient may be successfully treated for virus 1 (eg, genotype 2b) but not for virus 2 (eg, genotype 1a) (Figure 1, *C* and *D*). HCV coinfection and superinfection have been documented in individuals with ongoing risk behavior, with the phenomena being detected in 2%–10% and 16%–37%, respectively [14, 24, 25]. However, these estimates are likely underestimations due to infrequent test intervals and technical limitations. One clinical trial investigated the presence of mixed infections before, during, and after treatment [14]. In 1 patient with a lack of viral clearance at week 12, a mixed infection was documented during treatment without compromising SVR. Two additional patients with mixed infection pretreatment were reported (both genotype 1a and genotype 3a). One of these patients achieved SVR but became reinfected with a different genotype 1a and similar genotype 3a strain. The other failed to clear the genotype 1a upon treatment; however, genotype 3a became undetectable. The clinical consequences of these mixed infections on progression to liver disease or reduced response to treatment have not been investigated. The distinction between (late) relapse, reinfection, and untreated

virus in mixed infection has important clinical consequences and should therefore always be considered in patients with ongoing risk behavior.

VIRAL SEQUENCE ANALYSIS FOR DIAGNOSING REINFECTIONS OR MIXED INFECTIONS

The most straightforward indication of a reinfection is the detection of HCV viremia in someone who previously cleared the virus, either treatment induced or spontaneously. However, in cases with a (late) relapse following treatment or rebound following primary infection, it may be unclear whether the recurrent viremia is caused by the primary infection or a new viral strain. No clear "cutoff" has been defined for the duration of the HCV RNA–negative interval followed by recurrent viremia, which unambiguously indicates reinfection, although an interval of 60 days has been used [26]. However, with the arrival of direct-acting antivirals, relapses occurring as late as 1 year after EOT have been documented [27]. Therefore, whenever HCV reinfection is suspected, which is the case for PWID with continuing risk behavior, or more specifically, when treatment outcome needs to be assessed in patients with a suspected relapse after EOT, sequencing is necessary to distinguish true relapse from reinfection.

For this purpose, different regions of the viral genome can be used, although given the high variability of HCV, universal assays targeting all genotypes with a single set of primers are not easily designed. However, Murphy et al [28] designed and extensively validated a polymerase chain reaction (PCR)/sequencing assay that targets a 340-bp fragment of NS5B, the gene that encodes the viral polymerase. This assay has been shown to result in good-quality sequences, using a single primer set for all genotypes (except genotype 6, where an additional primer set is used), which allows for a correct identification of viral genotype and subtype. This genotyping assay, or any other reliable genotyping assay based on viral sequencing, can also be used to investigate the possibility of reinfection, as clearly, the presence of another genotype or subtype suggests a new or "different" HCV infection. Another similar and often used genotyping assay, which targets the core/E1 region, is described by Corbet et al [29], which involves 2 consecutive rounds of amplification in a nested protocol.

Sequences derived from these genotyping assays may also be used to identify the presence of new variants from the same viral subtype. Supplementary Figure 2*A* shows pre- and post-treatment NS5B sequences of PWID, demonstrating that relapsers were indeed true relapsers, as indicated by the clustering of sequences per patient. In this patient population, this NS5B region has sufficient phylogenetic signal for discriminating reinfection with the same subtype from relapse after cessation of treatment. Nevertheless, in a recent outbreak setting, as is the case for the epidemic of HCV in human immunodeficiency virus–positive MSM, genetic diversity may be limited with a few, often highly similar clades circulating in that specific population. This is illustrated in Supplementary Figure 2*B*, where NS5B sequences from different patients are, in some cases, 100% identical, demonstrating that in this particular setting, the phylogenetic signal is insufficient to discriminate reinfection from relapse. In such epidemic settings, where similar viruses are circulating, it may be necessary to sequence a fragment of the viral envelope that contains a more variable part of the viral genome such as the hypervariable region 1. This part of E2, a gene encoding one of the envelope proteins, is the region with the greatest genetic variability (Supplementary Figure 2*C*), allowing discrimination of homologous strains, with an intrahost diversifying virus, from heterologous strains from the same viral subtype.

As described above, diagnosing reinfection is primarily based on population sequencing, which generates a consensus sequence averaging the genomic variation present. Diagnosing mixed infections is more complicated, as it involves analysis of variants that may be present as a minority population among a large population of different major variants. Population sequencing may still reveal a subpopulation of minor variants, but only when they constitute 20%–30% of the virus population. To identify mixed infections with minority variants present at a frequency below 20%–30%, additional laboratory tools are needed. To date, very few studies have systematically addressed mixed infection. Two studies, using subtype-specific PCR and sequencing, demonstrated that indeed mixed infections occur frequently in PWID [14, 25]. However, frequencies of mixed infections may be underestimated, as mixed infections with the same subtype, with 1 variant present at low frequency, cannot be detected by such subtype-specific assays. Instead, cloning and sequencing of a large number of clones, which involves labor-intensive laboratory work, is necessary to detect minor variants present at low frequency. Therefore, preferably, next-generation sequencing techniques should be used, which are able to generate thousands of single-variant sequences in one sample. To the best of our knowledge, next-generation sequencing has not been used to study the presence of mixed infections.

SPONTANEOUS CLEARANCE OF REINFECTION AFTER TREATMENT-INDUCED CLEARANCE OF THE PRIMARY INFECTION

We have shown that reinfection does occur after successful HCV treatment; however, spontaneous clearance of such reinfections may also occur. Here we report 4 patients who spontaneously resolved their reinfection after successful treatment of

the primary infection [12–14] (Supplementary Table 1). All patients were male and of young age, except case 2, who was 56 years old. All patients had received pegylated interferon with ribavirin. Reinfection occurred in 3 of 4 patients within a year after EOT. The estimated duration of primary HCV before treatment infection was stated for 2 patients and was approximately 6 months. The reinfection after treatment was with a different genotype in 2 cases. Case 2 had a reinfection with the same viral subtype as present during the primary infection. This patient reported a needlestick injury with a syringe from his HCV genotype 1a–positive partner [12]. Case 4 had a brief HCV recurrence at 7 weeks after SVR, which could not be typed. Reinfection after successful treatment does not necessarily lead to new chronic infection; thus, repeated testing should be performed in these cases.

PROTECTIVE IMMUNITY AFTER TREATMENT-INDUCED CLEARANCE OF THE PRIMARY INFECTION

Support for developing an HCV vaccine might be found in studies documenting that among patients who spontaneously clear a primary infection, duration and level of viremia following a secondary infection are reduced compared to those of the primary infection [26]. It is unknown whether the same increased control of HCV is present following treatment-induced clearance. Longitudinal studies on long-term outcome of HCV treatment among PWID in high-risk settings will provide valuable insight in HCV-specific immunity, which protects against reinfection following treatment-induced clearance.

Collectively, the data we have summarized here suggest that some degree of immunity against persistent reinfection is present. The low incidence of reinfection after treatment is in agreement with epidemiologic studies on reinfection following spontaneous clearance [30, 31], but comparable or even higher rates of reinfection compared to incident cases have been reported [24, 26, 32, 33]. However, these conflicting results are likely caused by differences in frequency of test intervals, age, risk behavior, and a lack of viral sequencing [34]. In primary HCV infection, broadly directed HCV-specific $CD4^+$ T-cell responses are generated during the acute phase, but rapid exhaustion (within 5 months) of these cells is associated with persistent infection [35]. This study by Schulze Zur Wiesch et al [35] also shows that early HCV treatment in the acute phase can lead to recovery of CD4 T-cell responses, in contrast to treatment in patients who have become chronically infected. The restoration of HCV-specific $CD4^+$ (and $CD8^+$) T-cell responses during HCV treatment in the acute phase has been confirmed [36, 37], but this functional HCV-specific T-cell response is not always complete [35, 37]. Although these results suggest a potential immunologic benefit of early treatment, it remains to be investigated whether successful treatment initiated during the acute or chronic phase leads to different outcomes upon HCV reexposure.

Even though numbers are small, it is tempting to state that the rate of spontaneous clearance of HCV reinfection seems comparable to clearance after first exposure in HCV treatment–naive cases. However, it is important to note that these 4 individuals previously failed to control HCV, and the estimated duration of the primary infection in these cases is likely longer than 5 months. Therefore, the possibility of spontaneous clearance through a restored HCV-specific adaptive immunity seems less plausible. In addition to an adaptive immune response, other host factors such as interleukin 28B genotype [38] and female sex [39] are associated with spontaneous clearance of primary infection and treatment-induced clearance. Therefore, it is surprising that individuals can resolve a reinfection even though they failed to spontaneously resolve their initial infection. Given the scarcity of data, further insight in this topic is needed to draw firm conclusions on protective immunity following treatment-induced clearance of HCV.

CONCLUSIONS

Reinfection after successful HCV treatment can occur. However, the rate of HCV reinfection is low even among persons who continue injection drug use during and after treatment, and HCV treatment should not be withheld due to concerns about reinfection alone. In high-risk populations, frequent testing and viral sequencing are necessary to discriminate between relapse and reinfection. HCV reinfection after treatment may be cleared spontaneously. Education and counseling about the risk of reinfection should be continued among PWID following successful treatment for HCV, as ongoing injection drug use following treatment appears to be quite common.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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