Chapter 2.2

Low incidence of reinfection with the hepatitis C virus following treatment in active drug users in Amsterdam
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European Journal of Gastroenterology & Hepatology, 2012; 24(11): 1302

Abstract
Background: More than two-thirds of hepatitis C virus (HCV) infections are associated with injecting drug use. Despite wide availability of standard treatment with pegylated interferon and ribavirin, active drug users (DU) have limited access to HCV treatment. Physicians may be reluctant to prescribe treatment because of presumed high risk of reinfection. However, data on reinfection in treated DU remains scarce.

Methods: Active DU with chronic HCV infection were treated in a multidisciplinary setting. After achieving sustained virologic response (SVR), patients were tested at 6-12 monthly intervals for HCV RNA. To distinguish between relapse and reinfection, sequence and phylogenetic analysis were performed on the NS5B region of the HCV genome. Incidence of reinfection was calculated using person-time techniques.

Results: From April 2005 to March 2010, 69 active DU treated for HCV had sufficient follow-up, median 2.5 years (interquartile range, 1.6-3.7). SVR was reached in 42 patients (61%). During follow-up, 41 cases remained HCV RNA-negative; of these, 2 patients died. During treatment 5/41 injected drugs which increased to 11/41 after end of treatment. One case of reinfection was observed, followed by spontaneous clearance of the virus. The overall incidence was 0.76 per 100 person-years (95% CI 0.04-3.73). Restricted to those reporting injecting drug use, the incidence was 3.42 per 100 person-years (95% CI 0.17-16.90).

Conclusions: We report a low incidence of HCV reinfection following treatment in DU participating in a multidisciplinary program. Active drug use, including injecting, should not preclude access to treatment for HCV.
Introduction
Hepatitis C virus (HCV) infection poses serious challenges to global health, affecting more than 170 million individuals. In high-income countries, more than two-thirds of HCV infections are associated with injection drug use [1]. About 75% of individuals infected with HCV develop chronic HCV infection [2] and are at risk for long-term sequelae, including liver cirrhosis and hepatocellular carcinoma [3]. In former and active injecting drug users (DU), HCV prevalence ranges from 30% to 95% [4, 5]. In 2010, the modelled prevalence of chronic HCV infection in the (ever) injecting DU population (n=4353) in Amsterdam was 80.7% (95% CI 66.6-89.7) [6]. Nowadays, treatment for HCV infection with pegylated interferon alpha (PEG-IFN) and ribavirin (RBV) is fairly adequate depending on genotype and more promising alternatives with direct acting antiviral agents for HCV are upcoming [7, 8]. Unfortunately, physicians seem reluctant to offer HCV treatment to DU because of their concerns about suboptimal patient adherence, potential psychiatric decompensation, the risk of premature mortality and the risk of reinfection after treatment [9-11]. We and others have shown that in a multidisciplinary program, HCV treatment uptake and response in DU are comparable to a non-drug-using population [12-14]. Moreover, modelling studies have suggested that HCV treatment could contribute to reduce the future HCV disease burden among drug users [5, 6, 15].

However, data on the risk of reinfection in DU after successful treatment are scarce. Apart from case reports, only four prospective studies among DU have been conducted. In a German study among 18 DU, 0-2 cases of reinfection were observed, resulting in a reinfection rate of 0-4.1 per 100 person-years [16]. In the other three more recent, but relatively small studies (n=9, n=27 and n=35), overall reinfection rate varied from 0.6 per 100 person years to 3.2 cases per 100 person years. However, when restricted to those returning to injection drug use the reinfection rate was higher in these studies: 1.9 cases per 100 person-years and 5.4 cases per 100 person-years [17-19].

To overcome potential barriers to treat DU, more prospective data are needed on the risk of reinfection after successful HCV treatment. The aim of this study was to evaluate the rate of HCV reinfection following end of treatment (EOT) in a prospective cohort with DU who finished HCV therapy within a multidisciplinary program. HCV strains were genotyped to discriminate between possible relapse, following HCV RNA-negativity at EOT, and true reinfection. In addition, we evaluated the mortality risk following EOT.

Materials and methods
Study population
The Amsterdam Cohort Studies (ACS) is an open and ongoing prospective cohort study among DU that was initiated in 1985 [20]. Participation is voluntary and informed consent is obtained at intake for every participant. The ACS was approved by the medical ethics committee of the Academic Medical Center. Within the ACS, the Drug Users Treatment for Chronic Hepatitis C (DUTCH-C) project was launched in December 2004 and has previously been reported in detail [12]. In brief, HCV treatment is provided to DU by ACS medical staff and a liver specialist from the Academic Medical Center, Amsterdam. Methadone and psychopharmaceutical medications are prescribed by addiction specialists and psychiatrists. Care providers from the methadone clinics provide support and observe the development of side effects. No incentives were offered, and written informed consent was required for participation. To assess hepatitis B virus (HBV) status, all patients were routinely screened for anti-HBc, HBsAg and anti-HBs. HBV vaccination was offered for those uninfected with HBV. Patients received standardized HCV treatment with PEG-IFN along with RBV. Dosages were determined according to the standard of care and individually adjusted on the basis of side effects. Treatment duration was 24 weeks (HCV genotype 2 and 3) or 48 weeks (HCV genotype 1 and 4).

Laboratory methods
Blood samples were qualitatively tested for HCV RNA using transcription-mediated amplification (TMA; Versant®, Siemens Medical Solutions Diagnostics, Munich, Germany) with a lower detection limit of 5-10 IU mL⁻¹. Successful treatment, indicated by sustained virologic response (SVR), was defined as having an undetectable HCV RNA level 24 weeks post-treatment. When HCV RNA
was detected at 24 weeks post-treatment, plasma samples taken before and after treatment were compared by sequence analysis to distinguish between reinfection and relapse. After SVR, HCV RNA was tested at 6-12 monthly intervals.

Genotyping was performed using primers and conditions as described previously by Murphy et al. [21]. Sequence alignments were created using Mega 5.0 (GenBank Accession Nos. JN426992-JN427013) along with established reference sequences [22] to determine viral genotype. For phylogenetic analysis the Tamura-3 parameter evolution model was chosen using the model test functionality in Mega 5.0 and the phylogenetic tree was constructed using the neighbour-joining method. The inferred phylogenies were tested with 1000 bootstrap replications [23].

**Causes of death**

Patients were matched against the local and national registries to obtain information about their vital status. Cause of death (COD) was actively and systematically obtained, if available, from hospitals, general practitioners or coroners. Data were collected on the primary COD, contributing COD, and underlying COD.

**Statistical analyses**

Incidence rate of reinfection was calculated using person-time techniques. Individual follow-up time was calculated from EOT date until the last HCV RNA negative test, date of HCV reinfection or death. The date of reinfection was determined as the midpoint between last HCV RNA-negative and first HCV RNA-positive visit. If spontaneous clearance of the reinfection was observed, patients were again considered at risk for another HCV reinfection from the first HCV RNA-negative visit following the previous reinfection (if confirmed by at least two HCV RNA-negative visits following the previous reinfection). Cumulative incidence curves were estimated for reinfection and death within a competing risks framework. The R language and environment for statistical computing, v2.8 [24] and SPSS v19.0 were used for data analysis.

**Results**

**General characteristics**

Between April 2005 and November 2010, 69 patients were treated for HCV infection. SVR was reached in 42/69 patients (61%). Their characteristics at start of treatment, during treatment and following EOT are described in table 1. During follow-up, 2 patients with SVR died. One patient died from pneumonia/sepsis, the other patient's death was classified as an undefined natural death, at respectively 3.6 and 2.7 years after EOT. These two patients had their last HCV RNA-negative test 26 weeks and 52 weeks before death and did not report any injection drug use during or after EOT.

During treatment, 5/42 injected drugs. This number increased to 11/42 in the period after EOT. During follow-up, 41 cases remained HCV RNA-negative. One case became HCV RNA-positive and is described in more detail later.

Of the 27/69 that did not reach SVR, 10 were defined as relapses after being HCV RNA-negative at EOT. All 10 patients were also studied for potential reinfection by phylogenetic analysis. In all cases, pre- and post-treatment sequences of genotypes 1a (n=3) and 3a (n=7), clustered closely together, which supports the notion of relapse.

**Reinfection**

One case of reinfection was observed, the case we present (study number 15895 in figure 1) concerns a 56-year-old male of Dutch origin. He has a history of ongoing injection drug use since 1974 and, in fact, injected shortly before start of treatment. Before treatment he was found to carry HCV genotype 1a at multiple visits. At start of treatment, baseline qualitative HCV RNA-test was positive, HCV RNA by quantitative testing was <1000 IU mL⁻¹ and ALT was 19 U L⁻¹. He was not infected with HIV and had previously cleared HBV infection. PEG-IFN and RBV therapy was initiated in March 2008. From week 2 onwards, qualitative HCV RNA-tests were undetectable until SVR. During treatment he was on methadone maintenance therapy and continued injecting drug use. At 40 weeks following EOT (16 weeks after SVR) he reported a needlestick incident. The
needle was from his female partner, who was HCV RNA-positive with genotype 1a and who had remained untreated. The qualitative HCV RNA-test was positive, however the viral load level was <1000 IU mL\(^{-1}\). Unfortunately, we were not able to characterize this reinfection due to the low viral load. Six weeks after the first positive test at reinfection, HCV RNA was undetectable by qualitative testing and remained undetectable (145 weeks post-EOT and 107 weeks post-reinfection).

The overall incidence of HCV reinfection was 0.76 per 100 person-years (95% CI 0.04-3.73). When restricting our analysis to those reporting injecting drug use, the incidence was 3.42 per 100 person-years (95% CI 0.17-16.90).

To investigate the likelihood of mortality and reinfection following EOT, we constructed cumulative incidence curves, as shown in figure 2. Reinfection occurred earlier in time following EOT than all-cause mortality. At 4 years after EOT, 2.4% (95% CI 0.0-6.9%) of DU were expected to have acquired a HCV reinfection and 8.6% (95% CI 0.0-19.8%) were expected to have died (all-cause mortality). Hence 89.2% were expected to be alive and reinfection-free at 4 years after EOT.

Figure 1. Phylogenetic tree of HCV NS5B sequences of one reinfection case and relapses (n=10) of DU treated for HCV in Amsterdam. One reinfection (15895, ■) was determined by TMA but could not be genotyped. This case reported a needlestick incident from his HCV-positive partner (15895 partner, ■). Among relapses (n=10), samples taken before (pre) and after (post) treatment clustered closely together, supporting the notion of relapse rather than reinfection.

HCV, hepatitis C virus; TMA, transcription-mediated amplification.
Table 1. Characteristics of patients with sustained virological response: at start of treatment, during treatment and following end of treatment (n=42).

**Characteristics**

<table>
<thead>
<tr>
<th><strong>At start of treatment</strong></th>
<th><strong>51 (47-56)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR)</td>
<td>51 (47-56)</td>
</tr>
<tr>
<td>Male Sex (n, %)</td>
<td>31 (73.8)</td>
</tr>
<tr>
<td>Dutch Nationality (n, %)</td>
<td>35 (83.3)</td>
</tr>
<tr>
<td>HCV genotype (n, %)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>2</td>
<td>12 (28.6)</td>
</tr>
<tr>
<td>3</td>
<td>22 (52.3)</td>
</tr>
<tr>
<td>4</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>HIV coinfection (n, %)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>HBV status</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc-negative, HBsAg-negative (n, %)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td>Anti-HBc-positive, HBsAg-negative (n, %)</td>
<td>25 (59.5)</td>
</tr>
<tr>
<td>Anti-HBc-positive, HBsAg-positive (n, %)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vaccinated (n, %)</td>
<td>3 (7.1)</td>
</tr>
<tr>
<td>Methadone use on prescription (n, %)</td>
<td>39 (92.8)</td>
</tr>
<tr>
<td>Alcohol, current intake &gt;5 units/day (n, %)</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Ever injected drugs (n, %)</td>
<td>41 (97.6)</td>
</tr>
</tbody>
</table>

**Active drug use during treatment**

- Injecting (n) 5
- Non-injecting drug use (n) 40

**Follow-up after end of treatment**

- Active drug use after treatment
  - Injecting (n) 11
  - Non-injecting drug use (n) 41
- Median follow-up (years, IQR) 2.5 (1.7-3.7)
- Median HCV RNA test interval (weeks, IQR) 28 (24-36)

*Patients can report on both injecting and non-injecting drug use.

HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range.
Discussion
The observed incidence rate of HCV reinfection was 0.76 to 3.42 cases per 100 person-years. These results are comparable to the reinfection rates observed in other prospective studies performed in Germany, Norway, Canada and the United States [16-19]. These studies did not include follow-up time after clearance of a reinfection. In addition, the occurrence of possible reinfection in cases initially defined as relapses was not studied. If reinfections were present among the relapses, reinfection rates in these studies might have been higher. Our observed reinfection rate is close to the average yearly incidence rate of primary HCV infection among ever-injecting DU in the ACS since 2005 (i.e., 0.35 cases per 100 person-years). The HCV reinfection rate we found among injecting DU is considerably lower than the current incidence of HCV reinfection following treatment of acute HCV infection in HIV coinfected men who have sex with men (MSM) in Amsterdam, i.e., 15.2 cases per 100 person-years [25].

We describe a case of spontaneous clearance of HCV reinfection following treatment and SVR for a chronic HCV infection. Grebely et al. described the first case of spontaneous clearance after reinfection with HCV. Their subject was treated for HCV genotype 3a and was reinfected with HCV genotype 1a, followed by spontaneous clearance [19]. Unfortunately, we were not able to genotype the reinfection of our case; however, as RNA became detectable again after SVR, this case is a reinfection by definition [26]. Based on the reported behaviour, the case we described was probably reinfected with the HCV genotype 1a from his female partner. This observation of spontaneous clearance after reinfection of presumably genotype 1a following SVR when treated for a previous chronic infection with genotype 1a, may suggest that an enhanced immune response, as a result of therapy, allowed the patient to clear the reinfection without treatment. Following spontaneous clearance of a primary HCV infection, strong and broad specific T-cell responses have been reported in spontaneous clearance of HCV reinfection [27, 28]. However, our case was not able to spontaneously resolve his primary HCV infection, which suggests that spontaneous clearance is multifactorial.

Follow-up time was calculated from EOT instead of the SVR-date, therefore our follow-up time increased with 24 weeks per individual. However, two recent studies have shown that HCV reinfections can occur in the window phase between EOT and SVR in HCV-HIV coinfected MSM treated for acute HCV [25, 29]. Of importance, these studies showed that patients originally diagnosed as late relapses should be re-evaluated for reinfections. The distinction between relapse and reinfection has important clinical consequences and should therefore prospectively be considered by clinicians. Definition of relapse or reinfection in the first 6 months after EOT should, in a population with ongoing HCV risk behaviour (e.g., injecting drug use), always be based on phylogenetic analysis. In this study we used part of the NS5B gene for this analysis, because the phylogenetic signal in this population was sufficient to distinguish between relapse and reinfection, as pre-treatment sequences were unique for each patient. In populations where highly similar viruses are circulating, for example the recent epidemic of acute HCV in HIV coinfected MSM, the genomic region analyzed in the study may not be appropriate. Formally, we cannot completely rule out that the relapse patients with clustering of pre- and post-treatment samples were reinfected by the same source. However, given the minor genetic distances between pre- and post-treatment samples and the high evolutionary rate of HCV, this seems highly unlikely.

One of the limitations of this study is that spontaneously resolved reinfections may have been missed due to 6-12 monthly testing for HCV RNA. This would lead to an underestimation of the observed HCV reinfection rate, but reinfections resulting in chronic infection would have been noticed. The results from this study are based on treatment in DU in a multidisciplinary setting with access to low-threshold comprehensive harm reduction programmes and might not be applicable for treatment in a different setting. Another limitation is that variables on drug use behaviours were self-reported and may be subjective according to socially desirable responses.

In conclusion, we found a low reinfection rate after successful treatment of HCV infection in active DU participating in a multidisciplinary HCV treatment program in a city with comprehensive harm reduction programs for DU. Active drug use, including injecting, during and after treatment was relatively common; however it should not preclude access to treatment for HCV.
Acknowledgements
The authors would like to thank all patients; research-nurses S. Moll, J. van der Werff, M. Martens, W. van der Veldt and V. Deerenburg for coordination and patient support; S. Rebers, V. Sewgobind, A. Urbanus for data acquisition; R. Molenkamp for supervising HCV RNA testing; F. Lambers for treatment supervision and data acquisition; R. Geskus for his critical appraisal and contribution to the manuscript; S. Landry for editing.

Statement of interests
Authors’ declaration of personal interests
The authors who have taken part in this study declared that they have nothing to disclose regarding funding from industry or conflict of interest with respect to this manuscript.

Declaration of funding interests
This work was supported by the Amsterdam Cohort Studies (website: http://www.amsterdamcohortstudies.org) on HIV infection and AIDS, a collaboration between the Public Health Service of Amsterdam (PHSA), the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation and the University Medical Center Utrecht, are part of the Netherlands HIV Monitoring Foundation and financially supported by the Netherlands National Institute for Public Health and the Environment.

The DUTCH-C project was funded by (1) The PHSA Research and Development foundation; (2) ZonMW (Grant 71150001); (3) Merck/Schering Plough; (4) AGIS health insurance.
References


