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Hepatitis C reinfection after sustained virological response

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Background & Aims: On-going risk behaviour can lead to hepatitis C virus (HCV) reinfection following successful treatment. We aimed to assess the incidence of persistent HCV reinfection in a population of people who inject drugs (PWID) who had achieved sustained virological response (SVR) seven years earlier.

Methods: In 2004–2006 we conducted a multicentre treatment trial comprising HCV genotype 2 or 3 patients in Sweden, Norway and Denmark (NORTH-C). Six months of abstinence from injecting drug use (IDU) was required before treatment. All Norwegian patients who had obtained SVR (n = 161) were eligible for participation in this long-term follow-up study assessing virological and behavioural characteristics.

Results: Follow-up data were available in 138 of 161 (86%) individuals. Persistent reinfection was identified in 10 of 94 (11%) individuals with a history of IDU prior to treatment (incidence rate 1.7/100 person-years (PY); 95% CI 0.8–3.1) and in 10 of 37 (27%) individuals who had relapsed to IDU after treatment (incidence rate 4.9/100 PY; 95% CI 2.3–8.9). Although relapse to IDU perfectly predicted reinfection, no baseline factor was associated with reinfection. Relapse to IDU was associated with age <30 years (*vs.* ⩾40 years) at treatment (adjusted odds ratio [aOR] 7.03; 95% CI 1.78–27.8) and low education level (aOR 3.64; 95% CI 1.44–9.18).

Conclusions: Over time, persistent HCV reinfection was common among individuals who had relapsed to IDU after treatment. Reinfection should be systematically addressed and prevented when providing HCV care for PWID.

Introduction

Injecting drug use (IDU) is the main risk factor for hepatitis C virus (HCV) transmission in high-income countries, accounting for the majority of both new and existing cases [1]. Although HCV treatment among people who inject drugs (PWID) has shown good outcomes [2] and is recommended by international guidelines [3–5], access to treatment remains limited in this population due to multiple barriers to care [6,7]. With increasing use of highly effective and tolerable direct-acting antivirals (DAAs), HCV treatment for PWID might become much more feasible in the near future. However, while a partial protective immunity may exist [8], on-going risk behaviour can lead to HCV reinfection following successful treatment.

In the first published study of HCV reinfection following sustained virological response (SVR) [9], we demonstrated low reinfection rates despite frequent relapse to drug use following a period of abstinence during treatment. Most succeeding studies [10–15] have reported similarly low rates and a meta-analysis [2] reported an incidence of 2.4/100 person-years (PY) among individuals who had injected drugs ever and moderately higher (6.4/100 PY) among those with continued risk behaviour after treatment.

Keywords: HCV; Reinfection; Incidence; PWID; Injecting drug use; Risk behaviour.

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Abbreviations: HCV, hepatitis C virus; PWID, people who inject drugs; SVR, sustained virological response; IDU, injecting drug use; DAAs, direct-acting antivirals; RNA, ribonucleic acid; OST, opioid substitution treatment; RVR, rapid virological response; E1, envelope 1; HVR1, hypervariable region 1; E2, envelope 2; RT, reverse transcriptase; PCR, polymerase chain reaction; IQR, interquartile range; CI, confidence interval; PY, person-years; HR, hazard ratio; OR, odds ratio; HIV, human immunodeficiency virus.

However, these studies have been limited either by small sample sizes, short longitudinal follow-up or lack of methods to distinguish between viral relapse and reinfection. Heterogeneity in study populations and in HCV testing intervals [16] may also have biased reinfection incidence estimates and accounted for the differences observed between studies. Furthermore, data concerning long-term reinfection outcomes, particularly clinically relevant persistent reinfections, are very limited. More data are therefore needed to resolve controversies and guide treatment decisions in a growing population of former and current PWID receiving HCV treatment.

The primary aim of this study was to assess the incidence of persistent HCV reinfection in a population of PWID who seven years earlier had achieved SVR following at least six months of abstinence from drug use prior to treatment in the NORTH-C trial. The secondary aims were to assess the proportion of PWID who had relapsed to IDU after treatment and to identify factors associated with reinfection and relapse to IDU.

Materials and methods

Patient population

In 2004–2006, we performed a randomized controlled multicentre trial to assess the effect of short treatment with pegylated interferon alpha and ribavirin in a population dominated by PWID (NORTH-C) [17]. The NORTH-C trial comprised 428 mono-infected HCV genotype 2 or 3 patients in Norway, Sweden and Denmark of which 68% were infected through IDU. Patients on opioid substitution treatment (OST) were excluded. Patients with a rapid virological response (RVR) were randomized to 14 or 24 weeks treatment and those without RVR received 24 weeks treatment. The overall SVR24 rate was 76%. At least six months abstinence from drug use was required prior to treatment, but urinary drug screening was not mandatory. All participants received standard of care information about risk reduction but were not systematically followed prospectively.

This follow-up study was performed in 2012–2014 at all 22 Norwegian study sites. Patients who had achieved SVR in the NORTH-C trial (n = 152) or following subsequent retreatment (n = 9) were eligible for inclusion.

Data collection

Patients were scheduled for a follow-up visit at their local study site for routine clinical assessment, blood samples and questionnaires. A local study nurse collected the following demographical and clinical data: age, gender, education level, occupational status, alcohol consumption and liver-specific medical history in the follow-up period. The following drug behavioural data were collected: pre- and post-treatment IDU (none, sporadic [<100 injections] or frequent [$\geqslant$100 injections]), sharing of drug equipment (needles, syringes or injecting paraphernalia [water, cookers or cotton]) and OST. In cases with discrepancy between pre-treatment drug behaviour as reported at follow-up and at baseline in the NORTH-C trial, information favouring drug use was chosen to cover the possibility of under-reporting.

Great effort was made to make contact with patients who did not meet for follow-up. A few were interviewed by telephone, but for individuals not contactable, relevant data were collected retrospectively from the patient files and from microbiological laboratories.

Virological assessments

All follow-up samples were tested for HCV RNA using COBAS AmpliPrep/COBAS Amplicor HCV Test v2.0 (Roche) with limit of detection 20 IU/ml or COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0 (Roche) with limit of detection 15 IU/ml. All samples with detectable HCV RNA were retested/confirmed on a quantitative assay (COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0) and genotyped using a line probe assay (HCV genotype 2.0 Assay (LiPA)). In cases of viral recurrence, viral sequencing (see details below) was performed on the first available HCV RNA positive sample at follow-up and if available, on stored frozen baseline samples taken prior to treatment in the NORTH-C trial. All patients with recurrence of HCV RNA were reassessed for viral persistence after minimum six months.

Viral sequencing and phylogenetics

HCV RNA was extracted and complementary DNA was generated using Superscripts One-Step PCR High Fidelity (Invitrogen) and Expand High Fidelity PCR system (Roche) with random hexamers and specific primers. A ~1500 basepairs (bp) fragment of the HCV genome covering Core, Envelope 1 (E1), hypervariable region 1 (HVR1) and Envelope 2 (E2) (bp 340–1808 with reference to HCV strain NZL1, GenBank accession number D17763.1) was amplified by a nested reverse transcriptase (RT) polymerase chain reaction (PCR) using universal and subtype specific primers as previously described [18], with additional genotype 3a specific primers designed for improved detection of this prevalent subtype (Supplementary Table 1). The PCR product was sequenced using the Sanger method.

Sequence alignment and a maximum-likelihood phylogenetic tree of the Core-E2 fragment of all available samples with genotype 3a and a set of reference sequences retrieved from GenBank were constructed using RAxML v.8.1.22 with a General Time Reversible model of nucleotide substitution, gamma model of rate heterogeneity and 100 rapid bootstrap replications. Maximum genetic distance thresholds for HCV reinfection were assessed in MEGA6 [19] and defined based on pairwise Core-E2 sequence comparison of reference sequences obtained from GenBank and the local database at the Norwegian Institute of Public Health.

Study definitions and outcomes

Mixed infection was defined as the presence of two or more subtypes in the baseline samples, either detected by sequence analysis or by a line probe assay. Confirmed reinfection was defined as recurrence of HCV RNA post SVR with a viral strain different from the strain(s) detected in the baseline sample prior to treatment. Probable reinfection was defined as recurrence of HCV RNA post SVR with lacking sequence data, but occurring in a patient who had relapsed to IDU after treatment. Persistent reinfection was defined as persistent viremia in a repeated sample taken at least six months after viral recurrence. The estimated date of reinfection was defined as the midpoint between the last negative and the first positive HCV RNA test available during follow-up.

The primary study outcome was persistent reinfection, either confirmed or probable according to the previous definitions. The time at risk was calculated from the date of SVR24 until the date of the last negative HCV RNA test or until the estimated date of persistent reinfection. Thus, individuals with spontaneous clearance following reinfection (reclearance) or uncertain reinfection outcomes were censored at the last negative HCV RNA test. However, when providing incidence rates for any reinfection, individuals with reclearance or uncertain outcomes were censored at the estimated date of reinfection.

The secondary study outcome was relapse to IDU. Information regarding the date of relapse to IDU was largely missing.

Statistical analysis

Data are summarized using frequency and percentage or median and interquartile range (IQR). Incidence rates for reinfection are presented as number of cases per 100 person-years (PY) at risk. Confidence intervals (CI) for incidence rates were calculated using Poisson distribution.

Factors associated with time to any reinfection were evaluated using Cox proportional hazards regression. Hazard ratios (HR) with corresponding 95% CI are presented. Potential predictors were determined *a priori* and included age at treatment, gender, education level, employment status at baseline, pre-treatment injection frequency, treatment duration, relapse to IDU during follow-up and OST during follow-up.

Baseline variables (see above) associated with relapse to IDU were evaluated using logistic regression analysis (due to lack of time-to-event data). Odds ratios (OR) with corresponding 95% CI are presented.

Variables significant at the 0.10 level in unadjusted analysis were included in adjusted analysis and removed using a stepwise elimination approach until only factors significant at a two-tailed p <0.05 remained in the model. All analyses were performed using Stata version 14.0 (Stata Corp, College Station, TX).

Ethics

The regional committee for medical and health research ethics in Norway approved the study and informed consent was collected. However, permission was subsequently given to collect data retrospectively from hospital patient files and microbiological laboratories without informed consent for patients who did not meet for follow-up.

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Results

Patient characteristics

Of 161 Norwegian patients who had achieved SVR in the NORTH-C trial, 106 had acquired HCV infection through IDU while 55 had other routes of transmission. Nine patients had died during the follow-up period (Supplementary Table 2). Follow-up data were available in 138 of 161 (86%) eligible individuals, either collected by questionnaire (n = 105), telephone interview (n = 2) or chart review (n = 31).

Characteristics of included patients are shown in Table 1. The median age at treatment was 36 years (IQR 28–40) in the IDU group and 39 years (IQR 32–46) in the non-IDU group, with a male predominance in both groups (61% and 57% respectively). Among individuals with a history of IDU, 80% (75 of 94) reported more than 100 lifetime injections while 20% (19 of 94) reported less than 100 lifetime injections prior to treatment. The median follow-up time was 7.1 years (IQR 6.1–7.5) and 7.5 years (IQR 6.9–8.0) in the IDU group and non-IDU group, respectively.

Table 1. Characteristics of included patients who achieved sustained virological response in the NORTH-C trial according to mode of HCV transmission (n = 138).

Characteristic	IDU (n = 94)	Non-IDU (n = 44)
Inclusion rate, %	89	80
Age at treatment, median (IQR)	36 (28-40)	39 (32-46)
Age at treatment, n (%)		
<30 years	28 (30)	8 (18)
30-39 years	42 (45)	15 (34)
≥40 years	24 (26)	21 (48)
Gender, n (%)		
Female	37 (39)	19 (43)
Male	57 (61)	25 (57)
Education level, n (%)[a]		
Secondary school or lower	45 (48)	13 (31)
High school or vocational school	40 (43)	21 (50)
Higher education	9 (10)	8 (19)
Employment status at enrolment, n (%)[b]		
Full time or part time employment	44 (47)	22 (50)
Student	13 (14)	5 (11)
Sick leave or welfare benefits	17 (18)	13 (30)
Unemployed	19 (20)	4 (9)
IDU before treatment, n (%)		
<100 lifetime injections	19 (20)	n.a.
≥100 lifetime injections	75 (80)	
Alcohol consumption, n (%)[c]		
None	38 (42)	23 (56)
0-3 units/day	50 (55)	17 (41)
>3 units/day	3 (3)	1 (2)
HCV genotype, n (%)		
2a	6 (6)	3 (7)
2b	5 (5)	4 (9)
3a	83 (88)	37 (84)
Treatment duration, n (%)		
Short (14 weeks)	35 (37)	17 (39)
Long (24 weeks)	59 (63)	27 (61)
Follow-up time after SVR, median years (IQR)	7.1 (6.1-7.5)	7.5 (6.9-8.0)
Time at risk after SVR, PY	593	313

[a]Data missing for 2 individuals. [b]Data missing for 1 individual. [c]Data missing for 6 individuals.
IDU, injecting drug use; SVR, sustained virological response; IQR, interquartile range; n.a, not applicable; PY, person-years.

Relapse to IDU

Among individuals with a history of IDU prior to treatment, 39% (37 of 94) had relapsed to IDU during follow-up. Of those, 49% (18 of 37) reported sporadic (<100) injections and 51% (19 of 37) reported frequent (⩾100) injections after treatment. Overall, 16% (15 of 94) had received OST during follow-up. Among individuals who responded completely to the behavioural questionnaire, 35% (7 of 20) reported sharing of needles, syringes or injecting paraphernalia.

Recurrence of HCV RNA

At follow-up, recurrence of HCV RNA was identified in 12 of 94 (13%) patients in the IDU group, while no recurrence of HCV RNA was observed in the non-IDU group. HCV RNA measurements had not occurred at regular intervals. Among individuals with viral recurrence, only four had performed more than one HCV RNA test between SVR24 and follow-up (median testing interval 4.4 years). Timelines for individuals with viral recurrence are shown in Fig. 1.

Of 24 potential samples (baseline and follow-up samples from 12 individuals), 18 samples were available for viral sequencing, of which sequence data were obtained in 10 samples. All samples, except two, had also been genotyped using line probe assays. In one case (ID 379) there was discrepancy between results from viral sequencing and line probe assays (2b *vs.* 2a, respectively), but no sample showed clear evidence of mixed infection.

HCV reinfections

Of the 12 recurrent cases, six were considered as confirmed reinfections with distinct viral strains. The remaining six cases had

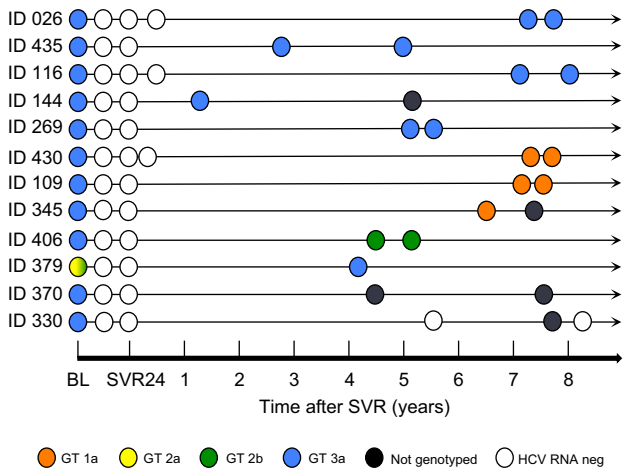

Fig. 1. Timelines for individuals with recurrence of HCV RNA following sustained virological response. Five cases (ID 026, ID 435, ID 116, ID 144 ID 269) had recurrence of the same genotype as present at baseline, five cases (ID 430, ID 109, ID 345, ID 406, ID 379) had recurrence of a different genotype and two cases (ID 330, ID 370) had detectable HCV RNA in samples that were not available for further analysis. Ten cases demonstrated persistent viremia, one case (ID 330) showed reclearance and one case (ID 379) had uncertain outcome due to loss to follow-up. BL, baseline; SVR, sustained virological response; GT, genotype.

Table 2. Demographic, behavioural and virological characteristics of individuals with HCV reinfection following sustained virological response.

ID	Gender and age	IDU before treatment	IDU post treatment	Sharing[a]	Genotype at baseline	Genotype at follow-up	Time to reinfection (years)	Confirmed or probable reinfection	Reinfection outcome
026	F 38	Frequent	Sporadic	Yes	3a[1]	3a[1,2]	3.9	Probable	Persistent
109	F 37	Frequent	Sporadic	No	3a[1]	1a[1,2]	3.6	Confirmed	Persistent
116	M 39	Frequent	Frequent	Yes	3a[1,2]	3a[1,2]	3.8	Confirmed	Persistent
144	M 27	Frequent	Frequent	n.a.[b]	3a[1]	3a[1]	0.6	Probable	Persistent
269	M 36	Frequent	Frequent	n.a.[b]	3a[1]	3a[1,2]	2.5	Probable	Persistent
330	M 27	Frequent	Sporadic	No	3a[1]	n.a.[c]	6.7	Probable	Reclearance
345	M 40	Sporadic	Sporadic	No	3a[1]	1a[1]	3.3	Confirmed	Persistent
370	M 28	Sporadic	Sporadic	Yes	3a[1]	n.a.[c]	2.3	Probable	Persistent
379	M 36	Frequent	Frequent	n.a.[b]	2a[1], 2b[2]	3a[1]	2.0	Confirmed	Uncertain
406	M 38	Frequent	Sporadic	n.a.[b]	3a[1,2]	2b[1]	2.2	Confirmed	Persistent
430	M 37	Frequent	Frequent	No	3a[1,2]	1a[1,2]	3.7	Confirmed	Persistent
435	F 18	Frequent	Frequent	n.a.[b]	3a[1]	3a[1,2]	1.4	Probable	Persistent

[1]HCV genotype 2.0 Assay (LiPA). [2]Viral sequencing of the Core-E2 fragment. [a]Needles, syringes or injecting paraphernalia. [b]Data not available. [c]Sample not available for analysis.
F, female; M, male; IDU, injecting drug use; SVR, sustained virological response; n.a., not applicable.

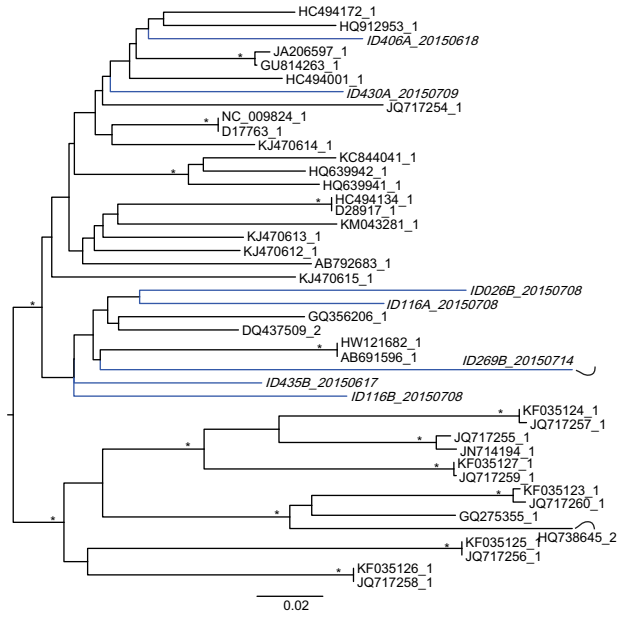

Fig. 2. Maximum-likelihood phylogenetic tree of the Core-E2 fragment of seven samples with HCV genotype 3a (shown in blue) and a set of reference sequences. Baseline and follow-up sequences were obtained in one participant (ID 116). This case was considered as a reinfection with a viral strain genetically distinct (116B) from the strain detected in the baseline sample (116A). Bootstrap values ⩾80% are indicated with an asterisk (*). The tree is midpoint-rooted only for visual purposes.

insufficient sequence data, but were considered as probable reinfections according to the study definitions. Ten cases were persistent reinfections, one case (ID 330) demonstrated reclearance, and one case (ID 379) had uncertain outcome due to loss to follow-up. No patient had received retreatment due to reinfection during the follow-up period; in fact, none of the patients who presented with reinfection were aware of their viremia. Demographic, behavioural and virological characteristics of the 12 cases are presented in Table 2. Notably, all cases of reinfection occurred in individuals who had relapsed to IDU after treatment. A maximum-likelihood phylogenetic tree of the Core-E2 fragment of the samples with genotype 3a and a set of reference sequences is shown in Fig. 2.

Consequently, after a median of 7 years of follow-up, persistent reinfection was identified in 10 of 94 (11%) individuals with a history of IDU prior to treatment (Supplementary Fig. 1) and in 10 of 37 (27%) individuals who had relapsed to IDU after treatment. The incidence of persistent reinfection was 1.7/100 PY (95% CI 0.8–3.1) among individuals with IDU prior to treatment and 4.9/100 PY (95% CI 2.3–8.9) among those who had relapsed to IDU after treatment. The incidence of any reinfection was 2.0/100 PY (95% CI 1.0–3.5) among individuals with IDU prior to treatment and 5.8/100 PY (95% CI 3.0–10.2) among those who had relapsed to IDU after treatment (Supplementary Table 3).

Factors associated with reinfection and relapse to IDU

Risk factors for any reinfection (Table 3) and relapse to IDU (Table 4) were evaluated among all 94 patients with a history of IDU prior to treatment. No baseline factor was significantly associated with reinfection. Relapse to IDU during follow-up perfectly predicted reinfection, but the hazard of reinfection was not higher among those who reported relapse to frequent IDU compared to those who reported relapse to sporadic IDU (HR 1.12; 95% CI 0.36–3.50). OST during follow-up was associated with increased hazard of reinfection (HR 7.31; 95% CI 2.35–22.7), but OST was correlated with relapse to IDU ($\rho = 0.47$; $p < 0.001$) and could also have occurred after the outcome.

Low education level (adjusted OR 3.64; 95% CI 1.44–9.18) and lower age at treatment was associated with relapse to IDU. Individuals <30 years at treatment had seven times higher odds of relapse to IDU compared to those ⩾40 years (adjusted OR 7.03; 95% CI 1.78–27.8). Gender, treatment duration, employment status or pre-treatment injection frequency was not associated with relapse to IDU.

Discussion

This study assessed HCV reinfection seven years following SVR in a population mainly comprising PWID who had been abstinent

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Table 3. Cox proportional hazards analysis of factors associated with time to any HCV reinfection among individuals with a history of injecting drug use prior to HCV treatment (n = 94).

Factor	Reinfection, n (%)	Unadjusted HR (95% CI)	*p* value
Age at treatment			
≥40 years	1 (4)	1.00	
30-39 years	7 (17)	3.99 (0.49-32.5)	0.195
<30 years	4 (14)	3.47 (0.39-31.0)	0.267
Gender			
Female	3 (8)	1.00	
Male	9 (16)	2.16 (0.58-7.98)	0.248
Low education level			
No	3 (6)	1.00	
Yes	9 (20)	3.56 (0.96-13.2)	0.057
Unemployed or welfare benefits at baseline			
No	6 (11)	1.00	
Yes	6 (16)	1.51 (0.49-4.61)	0.473
IDU before treatment			
<100 lifetime injections	2 (11)	1.00	
≥100 lifetime injections	10 (13)	1.36 (0.30-6.20)	0.693
Short treatment			
No	5 (8)	1.00	
Yes	7 (20)	2.62 (0.83-8.29)	0.099
Relapse to IDU during follow-up[a]			
No relapse	0 (0)	-	-
Sporadic (<100 injections)	6 (32)	1.00	
Frequent (≥100 injections)	6 (33)	1.12 (0.36-3.50)	0.833
OST during follow-up			
No	6 (8)	1.00	
Yes	6 (40)	7.31 (2.35-22.7)	0.001
Sharing of drug equipment			
No	4 (31)	1.00	
Yes	3 (43)	1.73 (0.39-7.77)	0.474
No data available	5 (7)	-	-

[a]Overall, IDU during follow-up (yes *vs.* no) perfectly predicted reinfection (p <0.001; Fisher Exact Test).
HR, hazard ratio; CI, confidence interval; IDU, injecting drug use; OST, opioid substitution treatment.

from drug use at least six months prior to HCV treatment. Among 94 individuals with a history of IDU prior to treatment, 39% had relapsed to IDU after treatment. Persistent reinfection was observed in 11% of individuals with a history of IDU and in 27% of those who had relapsed to IDU after treatment. The incidence of persistent HCV reinfection was 1.7/100 PY among individuals with a history of IDU and 4.9/100 PY among those who had relapsed to IDU. Although relapse to IDU perfectly predicted reinfection, no baseline factor was associated with reinfection. Younger age and low education level was associated with relapse to IDU.

Our findings are in line with previous studies of HCV reinfection in PWID where rates of reinfection post SVR have ranged from 1 to 5 cases per 100 PY in patients with IDU ever, increasing to 3 to 33 cases per 100 PY in patients with continued injecting risk behaviour [9–15]. The results are also consistent with findings from a recent meta-analysis reporting a risk of reinfection 5 years after SVR of 0.9% among low risk patients and 8.2% among PWID or prisoners [20]. However, one would expect persistent reinfection incidence to be lower than rates of reinfection previously reported, which have included infections that are cleared as well as those who become persistent. Although direct comparison with previously published estimates therefore may be challenging, the long-term reinfection outcomes provided in this study are unique and should inform HCV management among PWID.

The study population consisted of young PWID mainly with a history of heroin use, not receiving OST but still abstinent from IDU prior to HCV treatment. Nevertheless, relapse to IDU was a common event, especially in younger individuals with low education level. Reinfection however, was not associated with any baseline variable. Although probably being associated with poor social functioning as previously reported [14], prediction of reinfection could thus prove difficult in a clinical setting. Reinfection occurred more often in individuals enrolled in OST after treatment, but considering the exclusion of OST patients at baseline and the lack of detailed behavioural data during follow-up, OST

Table 4. Logistic regression analysis of factors associated with relapse to injecting drug use among individuals with a history of injecting drug use prior to HCV treatment (n = 94).

Factor	Relapse to IDU, n (%)	Unadjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Age at treatment					
≥40 years	4 (17)	1.00		1.00	
30-39 years	18 (43)	3.75 (1.09-12.9)	0.036	3.83 (1.07-13.8)	0.040
<30 years	15 (54)	5.77 (1.56-21.3)	0.009	7.03 (1.78-27.8)	0.005
Gender					
Female	14 (38)	1.00			
Male	23 (40)	1.11 (0.48-2.60)	0.808	-	
Low education level					
No	13 (27)	1.00		1.00	
Yes	24 (53)	3.16 (1.33-7.50)	0.009	3.64 (1.44-9.18)	0.006
Unemployed or welfare benefits					
No	22 (40)	1.00			
Yes	15 (39)	0.98 (0.42-2.28)	0.959	-	
IDU before treatment					
<100 lifetime injections	6 (32)	1.00			
≥100 lifetime injections	31 (41)	1.53 (0.52-4.45)	0.439	-	
Short treatment					
No	20 (34)	1.00			
Yes	17 (49)	1.84 (0.78-4.33)	0.161	-	

OR odds ratio; CI, confidence interval; IDU, injecting drug use.

is probably a confounder for high risk behaviour rather than a risk factor for reinfection.

The main strength of this study is the high inclusion rate (89%) among PWID achieved more than seven years after completion of HCV treatment. This has minimized potential selection bias and resulted in one of the largest sample sizes and the longest follow-up period to date in any study of HCV reinfection following SVR. The inclusion of a non-IDU group as controls has strengthened the study design. Taken together, this has provided robust estimates of persistent reinfection well anchored in a representative real-life setting.

There are limitations to this study. Firstly, due to the long intervals between HCV RNA tests, individuals with reclearance may have been missed. This study therefore likely underestimates the incidence of all reinfection episodes. This statement is supported by modelling data [16] and by a recent study in which the proportion of individuals with reclearance six months after reinfection was 52% [21]. However, persistent reinfections are the most clinically significant endpoint and such cases have not been overlooked. Thus, this study stresses the incidence of persistent reinfection, but also provides rates for any reinfection (which includes cases with reclearance and uncertain outcome). Yet, as a result of long HCV testing intervals, the estimated dates of reinfection are uncertain.

Secondly, this study relied on self-reported behavioural data much prone to recall bias. Also, the data collected retrospectively from patients files could be subject to information bias as injecting behaviours might have been under-reported. Although this may have impeded the detection of risk factors for reinfection, it probably has not affected the observed results considerably. Moreover, while all patients were HIV negative at baseline, data on HIV status were largely lacking at follow-up. This is unfortunate as HIV/HCV co-infection is an established risk factor for HCV reinfection [22], but HIV infection is uncommon (~1%) among Norwegian PWID [23].

Finally, the methods for viral sequencing used in this study were suboptimal, as adequate baseline and follow-up sequences were present for only two of twelve recurrent cases. This can be explained by several factors including primer mismatch, low viral load and degradation of HCV RNA due to suboptimal storage conditions for the baseline samples. Our conclusions therefore also depend on evidence of genotype switch based on line probe assays. However, both line probe assays and Sanger sequencing have shown poor sensitivity for detection of minor HCV variants (10% and 20% respectively), and our methods correspondingly failed to identify such cases. As mixed infection may be prevalent in PWID with recurrent exposure to HCV [24], late relapse of unresponsive coexisting viral strains undetected at baseline may therefore have been misinterpreted as reinfections in our study. By using more sensitive methods for detection of mixed infection, preferably deep sequencing, those issues could have been addressed more properly.

Furthermore, we cannot formally exclude the possibility of late viral relapse in the four patients with recurrence of the same genotype and lacking sequence data. However, all cases of viral recurrence occurred among patients with continued injecting risk behaviour and late viral relapse post SVR24 is a very rare event (<1%) in patients without such risk factors [25]. Also, genotype 3a is the dominating genotype in the Norwegian population of PWID (personal communication K. Stene-Johansen). One of the participants with recurrence of the same genotype (ID 116) was considered as a reinfection with a distinct viral strain (confirmed reinfection) despite insufficient robustness of the phylogenetic analysis with bootstrap threshold of 80%. Although this participant denied sharing of needles or syringes, he reported several injecting episodes with frequent sharing of paraphernalia during follow-up. This information increases the probability that this case represents an actual reinfection.

Our findings have important implications for the management of HCV infection in a growing population of former and current PWID receiving HCV treatment. As persistent reinfection becomes an important event over time, the long-term benefits of treatment for patients with continuous risk behaviour might be compromised. Given the difficulties of predicting reinfection, we would not recommend that HCV treatment should be withheld from PWID based on concerns of reinfection alone. However, all patients should be educated about the lack of protective immunity and the risk of reinfection associated with sharing of needles, syringes and injecting paraphernalia. Harm reduction should be incorporated in HCV care for active PWID with treatment being closely linked to OST and needle and syringe programs.

It is reasonable to believe that individuals at risk of reinfection are the ones most likely to transmit HCV if left untreated in the first place. Modelling studies have suggested that scaling up DAA based treatment among PWID could prevent onward transmission and lead to substantial reductions in HCV prevalence [26]. Thus, treating patients at high risk of reinfection may have great prevention potential as these patients are being "kept out of the pool" for a period and prevented from transmitting the virus. Recent guidelines [3,5] therefore recommend that HCV treatment should be prioritized in PWID. However, the prevention potential is also dependent on the HCV RNA prevalence, which is reported to be 40–50% among PWID in Norway [23]. As suggested by a recent study, treating high risk patients could yield greatest benefits in populations with HCV RNA prevalence below 50% [27].

More research concerning HCV reinfection is urgently needed in a field rapidly moving forward. Most importantly, strategies to prevent reinfection should be addressed in future controlled studies. Moreover, the incidence of reinfection following DAA based treatment is unknown and should be evaluated carefully as access to treatment among PWID increases. In such studies, deep sequencing should probably be the preferred method to distinguish reinfection from viral relapse.

In conclusion, in a population of PWID who had been abstinent from IDU prior to HCV treatment, relapse to IDU was common and an important proportion of this subgroup had a persistent HCV reinfection seven years after SVR. While reinfection may compromise treatment outcomes for individual patients, treating patients at high risk of transmitting HCV may prove a great prevention benefit at the population level. However, the risk of reinfection should be systematically addressed and prevented when providing HCV care for PWID.

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Research Article

Conflict of interest

HM has held sponsored lectures for Roche, Medivir and Abbvie. OD is a consultant/advisor and has received research grants from Gilead Sciences, Merck, Abbvie and Medivir. LNK is a consultant/advisor for Gilead Sciences and Abbvie. PKS has held sponsored lectures for Roche and Schering-Plough. JOR is a consultant/advisor for Abbvie.

Authors' contributions

HM developed the protocol, contributed to data collection, performed the statistical analyses and drafted the manuscript. BB developed the protocol, organized the data collection and revised the manuscript. AM, ZK, HK, JKD, JP, LH, PKS, JOR and LNK contributed to data collection and revised the manuscript. KSJ, JHOP and DHD performed the molecular characterisation and sequence analyses and revised the manuscript. OD developed the protocol, supervised the study and contributed to data collection, statistical analyses and development of the manuscript.

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Supplementary data

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References

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[1] Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol 2013;10:553–562.
[2] Aspinall EJ, Corson S, Doyle JS, Grebely J, Hutchinson SJ, Dore GJ, et al. Treatment of hepatitis C virus infection among people who are actively injecting drugs: a systematic review and meta-analysis. Clin Infect Dis 2013;57:S80–S89.
[3] European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2015. J Hepatol 2015;63:199–236.
[4] Grebely J, Robaeys G, Bruggmann P, Aghemo A, Backmund M, Bruneau J, et al. Recommendations for the management of hepatitis C virus infection among people who inject drugs. Int J Drug Policy 2015;26:1028–1038.
[5] Hepatitis C Guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus 2015. <http://www.hcvguidelines.org>.
[6] Alavi M, Raffa JD, Deans GD, Lai C, Krajden M, Dore GJ, et al. Continued low uptake of treatment for hepatitis C virus infection in a large community-based cohort of inner city residents. Liver Int 2014;34:1198–1206.
[7] Iversen J, Grebely J, Topp L, Wand H, Dore G, Maher L. Uptake of hepatitis C treatment among people who inject drugs attending Needle and Syringe Programs in Australia, 1999–2011. J Viral Hepat 2014;21:198–207.
[8] Grebely J, Prins M, Hellard M, Cox AL, Osburn WO, Lauer G, et al. Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine. Lancet Infect Dis 2012;12:408–414.
[9] Dalgard O, Bjoro K, Hellum K, Myrvang B, Skaug K, Gutigard B, et al. Treatment of chronic hepatitis C in injecting drug users: 5 years' follow-up. Eur Addict Res 2002;8:45–49.
[10] Currie SL, Ryan JC, Tracy D, Wright TL, George S, McQuaid R, et al. A prospective study to examine persistent HCV reinfection in injection drug users who have previously cleared the virus. Drug Alcohol Depend 2008;93:148–154.
[11] Bate JP, Colman AJ, Frost PJ, Shaw DR, Harley HA. High prevalence of late relapse and reinfection in prisoners treated for chronic hepatitis C. J Gastroenterol Hepatol 2010;25:1276–1280.
[12] Grebely J, Knight E, Ngai T, Genoway KA, Raffa JD, Storms M, et al. Reinfection with hepatitis C virus following sustained virological response in injection drug users. J Gastroenterol Hepatol 2010;25:1281–1284.
[13] **Grady BP**, **Vanhommerig JW**, Schinkel J, Weegink CJ, Bruisten SM, Lindenburg CE, et al. Low incidence of reinfection with the hepatitis C virus following treatment in active drug users in Amsterdam. Eur J Gastroenterol Hepatol 2012;24:1302–1307.
[14] **Grebely J**, **Pham ST**, Matthews GV, Petoumenos K, Bull RA, Yeung B, et al. Hepatitis C virus reinfection and superinfection among treated and untreated participants with recent infection. Hepatology 2012;55:1058–1069.
[15] Marco A, Esteban JI, Sole C, da Silva A, Ortiz J, Roget M, et al. Hepatitis C virus reinfection among prisoners with sustained virological response after treatment for chronic hepatitis C. J Hepatol 2013;59:45–51.
[16] Vickerman P, Grebely J, Dore GJ, Sacks-Davis R, Page K, Thomas DL, et al. The more you look, the more you find: effects of hepatitis C virus testing interval on reinfection incidence and clearance and implications for future vaccine study design. J Infect Dis 2012;205:1342–1350.
[17] Dalgard O, Bjoro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlund E, et al. Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology 2008;47:35–42.
[18] Jacka B, Applegate T, Krajden M, Olmstead A, Harrigan PR, Marshall BD, et al. Phylogenetic clustering of hepatitis C virus among people who inject drugs in Vancouver, Canada. Hepatology 2014;60:1571–1580.
[19] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725–2729.
[20] Hill A, Saleem J, Heath KA. Effects of sustained virological response (SVR) on the risk of liver transplant, hepatocellular carcinoma, death and re-infection: meta-analysis of 129 studies in 23,309 patients with Hepatitis C infection. Program and abstracts of the 2014 Annual Meeting of the American Association for the Study of Liver Diseases; November 7-11, 2014; Boston, Massachusetts Abstract 44 2014.
[21] Sacks-Davis R, Grebely J, Dore GJ, Osburn W, Cox AL, Rice TM, et al. Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection-the InC3 Study. J Infect Dis 2015;212:1407–1419.
[22] Martin TC, Martin NK, Hickman M, Vickerman P, Page EE, Everett R, et al. Hepatitis C virus reinfection incidence and treatment outcome among HIV-positive MSM. Aids 2013;27:2551–2557.
[23] Dalgard O, Egeland A, Ervik R, Vilimas K, Skaug K, Steen TW. Risk factors for hepatitis C among injecting drug users in Oslo. Tidsskr Nor Laegeforen 2009;129:101–104.
[24] Cunningham EB, Applegate TL, Lloyd AR, Dore GJ, Grebely J. Mixed HCV infection and reinfection in people who inject drugs–impact on therapy. Nat Rev Gastroenterol Hepatol 2015;12:218–230.
[25] Pearlman BL, Traub N. Sustained virologic response to antiviral therapy for chronic hepatitis C virus infection: a cure and so much more. Clin Infect Dis 2011;52:889–900.
[26] Martin NK, Vickerman P, Grebely J, Hellard M, Hutchinson SJ, Lima VD, et al. Hepatitis C virus treatment for prevention among people who inject drugs: Modeling treatment scale-up in the age of direct-acting antivirals. Hepatology 2013;58:1598–1609.
[27] de Vos AS, Prins M, Kretzschmar ME. Hepatitis C virus treatment as prevention among injecting drug users: who should we cure first? Addiction 2015;110:975–983.