Research Article

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Hepatitis C reinfection after sustained virological response

Håvard Midgard[1,2,*], Benedikte Bjøro[3], Arild Mæland[4], Zbigniew Konopski[5], Hege Kileng[6], Jan K. Damås[7,8], Jørn Paulsen[9], Lars Heggelund[10], Per K. Sandvei[11], Jetmund O. Ringstad[11], Lars N. Karlsen[12], Kathrine Stene-Johansen[13], John H.-O. Pettersson[13], Dagny H. Dorenberg[13], Olav Dalgard[1,2]

[1]*Department of Infectious Diseases, Akershus University Hospital, Lørenskog, Norway;* [2]*Institute of Clinical Medicine, University of Oslo, Oslo, Norway;* [3]*Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway;* [4]*Department of Infectious Diseases, Oslo University Hospital, Oslo, Norway;* [5]*Department of Gastroenterology, Oslo University Hospital, Oslo, Norway;* [6]*Section of Gastroenterology, University Hospital of North Norway, Tromsø, Norway;* [7]*Centre of Molecular Inflammation Research, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway;* [8]*Department of Infectious Diseases, St. Olav's Hospital, Trondheim, Norway;* [9]*Section of Gastroenterology, Telemark Hospital Trust, Skien, Norway;* [10]*Section of Infectious Diseases, Vestre Viken Hospital Trust, Drammen, Norway;* [11]*Department of Medicine, Østfold Hospital Trust, Grålum, Norway;* [12]*Department of Medicine, Stavanger University Hospital, Stavanger, Norway,* [13]*Department of Virology, The Norwegian Institute for Public Health, Oslo, Norway*

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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* Corresponding author. Address: Department of Infectious Diseases, Akershus University Hospital, 1478 Lørenskog, Norway. Tel.: +47 908 30 071.

E-mail address: havardmi@medisin.uio.no (H. Midgard).

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.