Alarming incidence of hepatitis C virus re-infection after treatment of sexually acquired acute hepatitis C virus infection in HIV-infected MSM

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Background: Recent data indicate that seroprevalence of sexually transmitted hepatitis C virus (HCV) infection among MSM is stabilizing in Amsterdam. However, little is known about the incidence of HCV re-infection in MSM who have cleared their HCV infection. We, therefore, studied the incidence of re-infection in HIV-infected MSM who were HCV RNA-negative following HCV treatment of acute primary infection.

Methods: Our study population comprised HIV-infected MSM at two large HIV outpatient clinics in Amsterdam, who were previously diagnosed with a sexually transmitted acute HCV infection and tested HCV RNA-negative at the end of treatment. We defined HCV re-infection as detectable HCV RNA in individuals with an undetectable HCV RNA at the end of treatment accompanied by a switch in HCV genotype or clade. Person–time methods were used to calculate the incidence of re-infection.

Results: Fifty-six persons who became HCV RNA-negative during primary acute HCV treatment were included. Five of the 56 cases relapsed and were not analysed. Eleven persons were re-infected. The incidence of HCV re-infection in this group was 15.2 per 100 person-years (95% confidence interval 8.0–26.5). The cumulative incidence was 33% within 2 years.

Discussion: An alarmingly high incidence of HCV re-infection was found in this group. This high re-infection rate indicates that current prevention measures should be discussed, frequent HCV RNA testing should be continued after successful treatment and, in case of possible relapse, clade typing should be performed to exclude re-infection.

AIDS 2011, **25**:F21–F27

Keywords: hepatitis C virus, HIV, MSM, re-infection

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Received: 5 July 2011; revised: 4 August 2011; accepted: 5 August 2011.

DOI:10.1097/QAD.0b013e32834bac44

Introduction

In the last decade, the sudden increase in incidence of acute hepatitis C virus infection (HCV) among HIV-infected MSM in Europe, Australia and the United States has led to a substantial number of studies on this new public health problem. It has become clear that transmission takes place in specific clusters of HIV-infected MSM engaging in high-risk sexual behaviour [1–3]. Subsequently, targeted prevention messages have been developed, focusing on sexual risk behaviour, recreational drug use and regular testing for HCV. Furthermore, treatment of HCV co-infection in this population has proven to be very successful in the acute phase, and recommendations on treatment have been published [4,5].

The response to this epidemic has clearly been extensive, and a recent study in Amsterdam has suggested that the prevalence of new primary HCV infections may no longer be increasing (A.T. Urbanus *et al.*, presented at AIDS Conference 2010, abstract WEPDC 104). The question remains, however, whether prevention messaging and early testing and treatment also prevents HCV re-infection in MSM co-infected with HCV and HIV who have cleared their infection.

HCV re-infection occurs frequently among IDUs who continue high-risk behaviour [6]. Incidence rates of re-infection vary depending on population, definition of re-infection and methods and frequency of testing [6–10]. Reports on HCV re-infection by sexual transmission among MSM have been published only rarely [11,12] (H.-J. Stellbrink *et al.*, presented at CROI 2011, poster 645; presented at International Congress on Drug Therapy in HIV Infection 2010, poster 200; and J. Sasadeusz *et al.*, presented at EASL 2011, poster presentation), and no specific incidence rates have been presented yet.

Therefore, the objective of the current study was to examine the incidence of HCV re-infection among HIV-infected MSM attending two HIV outpatient clinics in Amsterdam, who were HCV RNA-negative at the end of treatment for their initial acute HCV infection.

Methods

Study population

We included 56 HIV-infected MSM at the HIV outpatient clinics of two major hospitals in Amsterdam, who had been previously diagnosed with and treated for an acute HCV infection between 2003 and 2011; none had detectable HCV RNA at the end of their HCV treatment. All patients had been treated with weekly injections of peg-interferon and daily doses of ribavirin, the majority for a duration of 24 weeks [13]. In the majority of the cases, no HCV parental transmission routes were identified by clinical history and sexual transmission was the most likely mode of transmission.

Data collection

Sociodemographic, clinical and virological data, such as age, use of HAART, CD4 cell counts, HIV RNA levels, levels of alanine aminotransferase (ALT) and genotype of primary HCV infection were collected from medical files. A subset of the MSM at risk of re-infection ($n = 21$) was included in a prospective study of acute infection with HCV in MSM (MSM Observational Study of Acute Infection with hepatitis C, MOSAIC study). For these patients, additional data on risk behaviour are presented. Data collection exists of an extensive self-administered questionnaire regarding classic risk factors for HCV transmission, such as IDU and sexual risk behaviour, collected at baseline and follow-up visits.

Virological testing

All plasma samples available after the end of treatment were tested for HCV RNA with the Siemens VERSANT transcription-mediated amplification (TMA) assay which has a detection limit of 5 IU/ml. Genotyping of the first TMA-positive sample after the end of treatment was performed by amplifying and sequencing a 389 base pair fragment of NS5B, as described by Murphy *et al.* [14]. If the genotype was similar to that in the treated primary infection, a 573 base pair fragment of E2 including the hypervariable 1 region (HVR1) was amplified and sequenced directly to identify clade shifts and differentiate between relapse or re-infection.

Definition of re-infection and relapse

Re-infection was defined as having detectable HCV RNA following an undetectable level at the end of treatment, with demonstration of the presence of a different genotype compared with primary infection or, if genotype was similar, a different clade compared with primary infection, as indicated by phylogenetic analysis of the E2/HVR1 region. If in the phylogenetic pretreatment and posttreatment sequences from the same viral subtype (e.g. 1a) large genetic distances were present, as indicated by distinct clustering, this was defined as a clade switch and, therefore, as a re-infection with the same viral subtype. Relapse was defined as a positive HCV TMA after a negative HCV TMA at the end of treatment and no genotype or clade switch compared with the primary infection.

Phylogenetic analysis

Sequences were aligned using Clustal X version 2 [15]. Phylogenetic trees were inferred using maximum-likelihood methods, using a Generalized Time Reversible Model with a gamma distribution of mutations (GTR + Γ) as implemented in MEGA software package version 5 [16]. Bootstrap values were determined from 500 bootstrap resamplings of the original.

Statistical analysis

The incidence rate of re-infection was estimated by dividing the number of re-infections by the total duration of follow-up. The individuals who had relapses were excluded from this calculation. The cumulative incidence was estimated by Kaplan–Meier methods.

In case of re-infection, follow-up time was calculated as the time between the end of treatment and the date of re-infection; the latter was estimated by taking the midpoint between the last negative HCV RNA test and first positive HCV RNA test. If no re-infection occurred, the censor date for follow-up was the date of the last HCV RNA test.

We compared sociodemographic, clinical, virological and behavioural characteristics, including peak ALT levels during follow-up between patients with and without re-infection. Risk behaviour was compared between MSM with and without re-infection for whom risk questionnaires were available. Differences between the two groups were tested with the χ^2-test or Fisher's exact test for categorical variables and Student's *t*-test or Mann–Whitney *U*-test for continuous variables. Analyses were performed using SPSS (version 17.0; SPSS Inc., Chicago, Illinois, USA). The incidence rate and its confidence interval (CI) were calculated with OpenEpi [17] and are given per 100 person-years.

Results

In total, follow-up was obtained for 56 HIV-infected MSM treated for acute HCV infection who were HCV RNA-negative at the end of treatment. Patients were treated between 2003 and 2011. Patient and virological characteristics are shown in Table 1.

Five of the 56 experienced relapse, as evidenced by sequencing of the E2/HVR1 region, and were excluded from the incidence calculations.

According to our definition, 11 of the remaining 51 persons became re-infected. The total follow-up time for the 51 persons was 72.2 years [median 1.3 years, interquartile range (IQR) 0.5–1.6]. The incidence of HCV re-infection was 15.2 per 100 person-years (95% CI 8.0–26.5). Among the 11 individuals with a re-infection, the median time until re-infection was 8.4 months (IQR 3.6–19.2). The majority of re-infected patients switched from genotype 4 to 1.

Three persons became re-infected with the same genotype (clade switch). Figure 1 shows the phylogenetic tree of pretreatment and posttreatment E2-HVR1 sequences of these patients together with pretreatment and posttreatment sequences of relapse patients. Pretreatment and posttreatment sequences from patients O1, O2, O3, P06 and P44 clearly cluster together and they were, therefore, classified as 'true' relapsers, corresponding with the clinical observation of RNA rebound at the first time point available after treatment withdrawal. In contrast, pretreatment and posttreatment sequences from patients P01, P31 and P48 do not cluster and they were, therefore, considered re-infections. Although patients P01 and P31 became HCV RNA-positive again within 6 months after the end of treatment, the first sample taken at 4 weeks was

Table 1. Baseline and follow-up characteristics of hepatitis C virus–HIV-co-infected patients at risk of hepatitis C virus re-infection.

Characteristics	All ($N=51$), % (n)	Not re-infected ($N=40$), % (n)	Re-infected ($N=11$), % (n)
Baseline			
Median age in years (IQR)	44.3 (39.1–48.8)	44.6 (40.7–48.8)	42.3 (36.6–49.1)
Genotype primary infection			
Gt 1	69 (35)	73 (29)	55 (6)
Gt 2	2 (1)	3 (1)	–
Gt 3	4 (2)	5 (2)	–
Gt 4	22 (11)	15 (6)	45 (5)
Unknown	4 (2)	5 (2)	–
Median CD4 cell count at end of treatment (cells/μl)	305 (240–403)	300 (240–308)	335 (243–388)
Follow-up			
Total follow-up time in years	72.2	63.0	9.2
Median follow-up time in years (IQR)	1.3 (0.5–1.6)	1.4 (0.5–2.3)	0.7 (0.3–1.6)
Median time between tests in months (IQR)	3.0 (1.9–4.3)	3.0 (2.0–4.5)	2.7 (1.7–4.1)
cART use	75 (38)	73 (29)	82 (9)
Median maximum ALT during follow-up (U/l)	37 (26–63)	34 (25–56)	67 (28–136)
Median CD4 cell count at last HCV RNA-negative visit (not re-infected)/first HCV RNA-positive sample (re-infected) (cells/μl)	450 (400–620)	450 (400–583)	440 (345–675)
Genotype switch at re-infection			
Gt 1 → Gt 4			18 (2)
Gt 4 → Gt 1			36 (4)
Gt 1 → Gt 2			18 (2)
Gt 1 → Gt 1, different clade			18 (2)
Gt 4 → Gt 4, different clade			9 (1)

ALT, alanine aminotransferase; cART, combination antiretroviral therapy; HCV, hepatitis C virus; IQR, interquartile range.

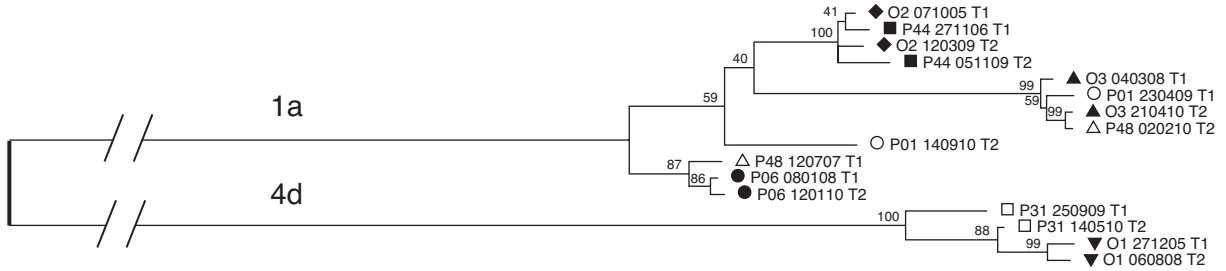

Fig. 1. Phylogenetic tree of relapsers and patients with a re-infections with the same genotype. Phylogenetic tree of sequences before and after treatment from relapsers and patients with a re-infection with the same genotype. Relapsers are presented by filled symbols and re-infections are presented by open symbols. Each patient is presented by a unique symbol. The numbers in the labels indicate sampling dates. Note: re-infections with a different genotype are not presented in this tree.

negative, supporting our phylogenetic evidence of re-infection.

The cumulative incidence of re-infection is demonstrated in Fig. 2; after 2 years, the cumulative incidence of re-infection was 33% (95% CI 16–50).

In order to examine whether ALT levels are useful for indicating a new infection, we compared peak ALT levels during follow-up in patients with and without re-infection. The peak ALT levels were in general low (with a maximum of 160 U/l), although the median ALT peak during follow-up was higher in individuals with a re-infection than in those without a re-infection ($P = 0.01$). Interestingly, in four cases with a re-infection, no increased ALT levels were observed, whereas in individuals without evidence of re-infection, ALT levels were elevated frequently (Fig. 3).

CD4 cell counts did not differ between patients with and without re-infection (Table 1). In addition, analysis of HIV load data from the eight of nine patients with a re-infection, who were on combination antiretroviral therapy (cART), showed that all patients had undetectable HIV loads around the time of HCV re-infection. From one re-infected patient on cART, no HIV load data were available.

Analysis of the 21 MSM with behavioural data revealed that re-infected MSM ($n = 7$) significantly more often reported noninjecting recreational drug use at inclusion than MSM without re-infection ($n = 14$) ($P = 0.048$). In

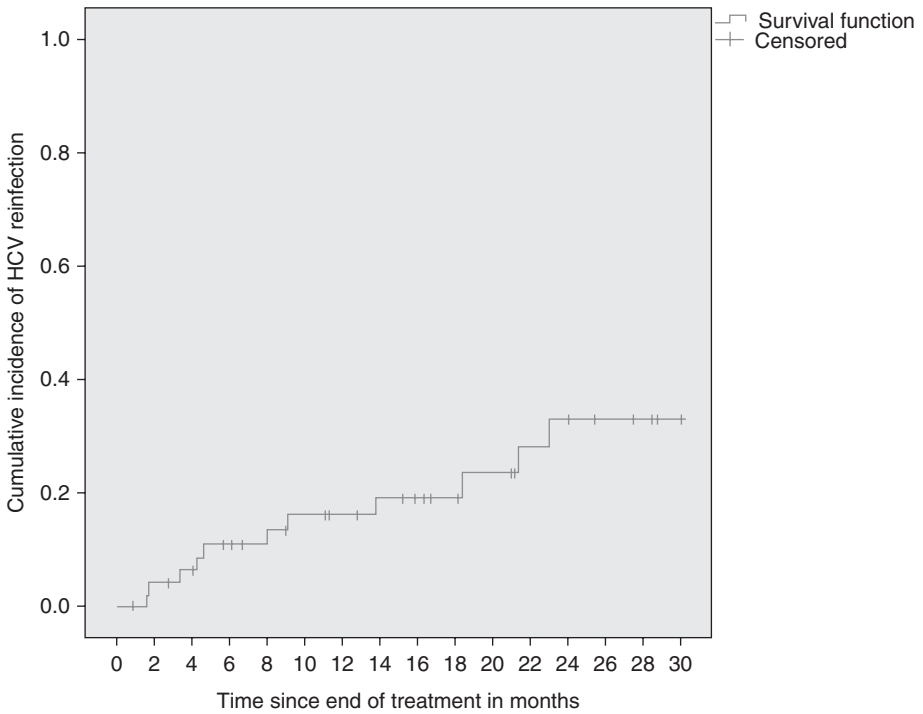

Fig. 2. Cumulative incidence of hepatitis C virus (HCV) re-infection after successful treatment of primary HCV infection.

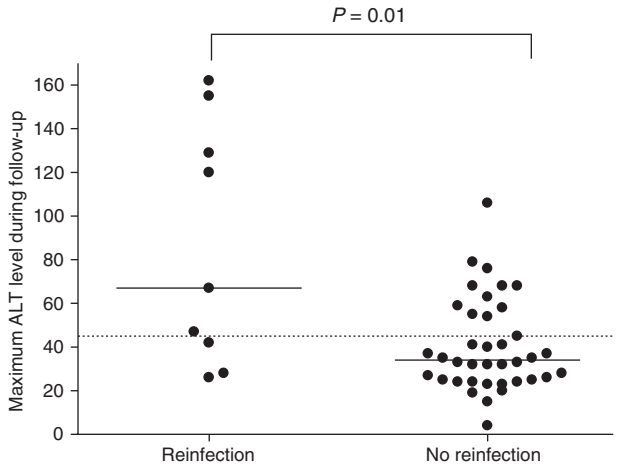

Fig. 3. Peak alanine aminotransferase levels during follow-up. Peak alanine aminotransferase (ALT) levels in units per litre during the observation period (end of treatment until last negative or first positive time point). The dotted line represents the maximum normal ALT value.

this small study population, no statistically significant differences in sexual risk behaviour were found.

Of the 11 re-infected patients, four were treated for their re-infection; of those four, two achieved a sustained virologic response (SVR), one had a relapse, and one is still in follow-up for SVR time.

Discussion

With this study, we demonstrate an alarmingly high incidence rate of sexually transmitted HCV re-infection among HIV-infected MSM previously successfully treated for primary HCV infection.

Most importantly, these findings stress the importance of repeated risk counselling for HCV transmission which should be provided not only before and during treatment but also after its completion. MSM re-infected with HCV showed higher rates of noninjecting recreational drug use. Sexual risk behaviour, including recreational drug use during sex, was highly prevalent (data not shown). Unfortunately, because of our relatively small study population with behavioural data, we were not able to examine risk behaviour more precisely and longitudinally.

The high incidence rate in this study implies that ALT levels, which can be elevated during an acute HCV infection, should be measured regularly in this population. Because these levels are not always elevated during acute infection or might not coincide with the test moment, as shown in Fig. 3, subsequent HCV RNA testing, especially in cases of high-risk behaviour, should be performed regularly, as antibodies remain in general present after successful treatment.

Along with risk behaviour, the role of biological susceptibility to HCV re-infection, although still unclear, is an important consideration. Importantly, discussion is ongoing whether previous infection with HCV can generate partial protective immunity to re-infection or increase the chance of clearance of re-infection.

Several studies that compared incidence rates of primary infection and re-infection among IDUs have presented results that argue both for [8,18,19] and against [6,7,10] this phenomenon. Differences between these studies are probably due to variations in intervals of testing, age of the participants, frequency of ongoing drug use and adjustment for behaviour in the analyses. The higher incidence of re-infection in our study compared with the incidence of primary infection in Amsterdam [2,20] and elsewhere [3] indicates that, as expected, there is no complete protection. Yet, the finding that most re-infections in this study occurred with a different genotype compared with the primary infection suggests that genotype-specific immunity may develop in some individuals.

Additionally, underlying a persons' ability to develop protective immunity, genetic profiles may play a role in susceptibility to HCV re-infection. The association of genetic variations near the interleukin-28B (IL28B) region with spontaneous HCV clearance and treatment-induced clearance has been well described for HCV-mono-infected patients [21–28]. Similar results have been found regarding HCV treatment response in persons co-infected with HIV, although in this population the association is less clear for those treated during the acute infection [29–34]. The effect of IL28B polymorphisms on HCV re-infection has not been described. In accord with the effects in primary infection, one would expect the responder genotype to be at least more likely to allow clearance of re-infection than the nonresponder genotype. Whether initial partial protective immunity is also more established in individuals with a beneficial IL28B genotype remains undetermined.

Apart from HCV-specific immune responses, HIV co-infection may play an important role. The exact role of HIV co-infection in primary sexually transmitted HCV infection is not well known. CD4 cell depletion in the gut may diminish the immune response against HCV sexual transmission [35]. Nevertheless, CD4 cell counts did not differ between patients with and without re-infection, indicating that the level of immune suppression caused by HIV co-infection does not influence the risk of re-infection following successful HCV treatment.

The results of this study may lead to a change in the current definitions of HCV relapse and re-infection.

When no further genotyping or sequencing is performed, a recurrent HCV viraemia within 6 months after a negative test at the end of the treatment is currently considered a relapse [36]. Our study demonstrates that early recurrence of HCV could well be a re-infection with another genotype or strain. This distinction has important clinical ramifications and should, therefore, be recognized by clinicians. The definition of relapse or re-infection, especially in population with a high incidence of infection, should, therefore, always be based on virological characteristics and not on a specific interval between the end of treatment and recurrence of HCV RNA in the serum.

Finally, from a clinical and cost-effective perspective, the results of this study will encourage discussion about the validity of repetitive HCV treatment in patients with numerous subsequent re-infections owing to continued risk behaviour.

Apart from small numbers, this study has other limitations. We have not studied the possible existence of HCV-mixed infections during primary infection. Therefore, we cannot entirely exclude the possibility that re-infections were previously existing infections that became detectable after a dominant strain had been cleared [37]. However, the fact that the median interval from the first HCV RNA-negative test to the first HCV RNA-positive test after treatment was 8 months, with several negative results in between, strongly suggests that all re-infections were recently transmitted infections.

Furthermore, as this was not a prospective study, time between tests was not similar for all patients, and a re-infection followed by a quick, spontaneous clearance might have been missed. Nevertheless, as the median time between tests was 3 months, we do not expect this to have significantly influenced the incidence rate.

In conclusion, a high incidence rate of HCV re-infection among HIV-infected MSM in Amsterdam was demonstrated in this study, emphasizing the need for more extensive risk behaviour counselling and secondary prevention by regular and frequent HCV testing in this population. Future research should focus on the reasons for continuing high-risk sexual behaviour in order to improve targeted prevention. In addition, research should try to elucidate the virological and host factors associated with re-infection and its outcome in HIV-infected individuals.

Acknowledgements

This study was funded by the AIDS Fund Netherlands (grant number 2 008 026).

The following persons also attribute to the MOSAIC study group: R. Countinho, H. Reesink, D. van Baarle, C. Smit, L. Gras and W. van der Veldt.

The authors would like to thank participants of the MOSAIC study. In addition, would also like to thank W. Koevoets, H. Nobel, L. Schrijnders, N. van der Meche and A. Toonen for patient inclusion and data collection; S. Koekkoek and S. Rebers for TMA testing and genotyping; M. Bakker for sample storage and handling; and S. Ebeling for language editing.

Conflicts of interest

There are no conflicts of interest.

Data from this study were presented at the CROI 2011, abstract #958.

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