Hepatitis C virus infections among HIV-infected men who have sex with men: an expanding epidemic

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\textbf{Background:} Since 2000 outbreaks of sexually transmitted hepatitis C Virus (HCV) infections have been reported among HIV-infected men who have sex with men (MSM). We studied the prevalence and determinants of HCV-infection among MSM attending a large sexually transmitted infection (STI) clinic in the Netherlands.

\textbf{Methods:} In 2007–2008, 3125 attendees of the STI clinic Amsterdam, including 689 MSM, participated in an anonymous biannual crosssectional survey. Participants were interviewed and screened for HIV and HCV antibodies. Additionally, all anti-HCV positive and HIV-infected individuals were tested for HCV RNA. Using phylogenetic analysis, HCV strains of the STI clinic attendees were compared with those isolated from MSM with acute HCV in 2000–2007. Determinants of HCV-infection were analysed using logistic regression.

\textbf{Results:} Two of 532 (0.4%) HIV-negative MSM and 28 of 157 (17.8%) HIV-positive MSM were infected with HCV. Over the study period, HCV prevalence among HIV-infected MSM increased (14.6%–20.9%). Seven of 28 (25.0%) HIV/HCV coinfected MSM had acute HCV infection. Only five of 28 (17.9%) HIV/HCV coinfected MSM ever injected drugs (IDU). HIV-infection, IDU, fisting and gamma hydroxy butyrate (GHB)-use were significantly associated with HCV-infection. Phylogenetic analyses revealed a high degree of MSM-specific clustering.

\textbf{Conclusion:} We found a high and increasing HCV prevalence in HIV-infected MSM. Though not statistically significant, this trend, and the relatively large proportion of acute infections suggest ongoing transmission of HCV in HIV-positive MSM. Regardless of IDU, rough sexual techniques and use of recreational drugs were associated with HCV-infection; phylogenetic analysis supported sexual transmission. Targeted prevention, like raising awareness and routine testing, is needed to stop the further spread among HIV-infected MSM, and to prevent possible spillover to HIV-negative MSM.

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\textbf{Keywords:} coinfection, epidemiology, hepatitis C, homosexual, men, risk factors, sexual behaviour, sexually transmitted infection
Introduction

Hepatitis C virus (HCV) infection is primarily a blood-borne infection and is highly frequent among injecting drug users (IDU) [1]. Sexual transmission of HCV is inefficient and is rarely reported among discordant heterosexual couples [2,3]. Since 2000, acute cases of HCV among HIV-positive men who have sex with men (MSM) have been reported from several high-income countries [4–9]. In the majority of these cases, no HCV parental transmission routes were identified and sexual transmission is the most likely route of infection. Additionally, phylogenetic analysis has shown the presence of an MSM-specific transmission network, supporting sexual transmission [5,8–11]. Following this recent outbreak of HCV in MSM, several reports have provided behavioural data on HCV/HIV coinfected men but without comparing these with the behaviour of HCV-negative MSM. This design is therefore not appropriate to evaluate whether specific characteristics are associated with HCV. Only one case–control study (HCV/HIV coinfecition versus HIV monoinfection) with detailed data on risk behaviour has examined its independent relation with HCV [5]. Although limited by a retrospective design, Danta et al. [5] suggest that high-risk mucosal traumatic sexual practices (in the context of recreational drug use) are associated with acute HCV infection. Better insight into factors associated with HCV infection in MSM is important to introduce targeted screening and prevention.

In the current study, we examined the prevalence and determinants of HCV infection among MSM attending a large STI clinic in Amsterdam, the Netherlands. Data were obtained from an anonymous biannual survey. We collected detailed data about sexual risk behaviour and risk factors for HCV together with samples tested for HIV, HCV and other STIs. Phylogenetic analysis was used to obtain evidence of the transmission routes of HCV in MSM.

Methods

Study population and study procedures

In a total of three waves – in May and November 2007 and April 2008 – 4829 attendees of the STI outpatient clinic were asked to participate in a biannual anonymous survey that has been undertaken for a number of years [12]. After an informed consent, only Dutch or English-speaking participants were interviewed about risk factors for blood-borne and sexually transmitted infections. In the second and third wave, some questions were added.

Of the total of 4829 attendees, 3154 (65.3%) Dutch or English-speaking individuals agreed to participate in the anonymous survey. Eventually, 27 participants had to be excluded due to lacking laboratory results, whereas another two were under age (<16 years). Of the remaining 3125 participants, 689 (22.0%) were MSM, which is our study population.

The participants were screened anonymously for HCV and HIV antibodies by means of a third-generation commercial microparticle EIA system (AxSym HCV version 3.0; Abbott respectively AxSym HIV Ag/Ab Combo; Abbott). HCV-positive and HIV-positive test results were confirmed by Immunoblot (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics respectively INNO-LIA HIV 1/II Score; Innogenetics N.V.). All anti-HCV positive MSM were tested for HCV RNA (the VERSANT(r) HCV RNA Qualitative Assay, Siemens), which has a detection limit of 5 IU/ml. Since prolonged seroconversion windows have been observed among HIV-infected individuals [13–15], all HIV-positive participants were screened for HCV RNA regardless of their anti-HCV status. All participants who had a confirmed anti-HCV positive test result and/or HCV RNA were considered HCV-positive. Acute HCV infection was defined as detectable HCV RNA in the absence of HCV antibodies, or as detectable HCV RNA in the presence of a weak anti-HCV response (AxSym ratio below 5) and negative (or indeterminate) immunoblot.

STI screening and basic data collection (such as age, sexual preference) were undertaken according to the clinic’s standard protocol, which has been described in detail elsewhere [16,17]. As part of the standard protocol, all MSM were tested for Chlamydia trachomatis, hepatitis B, gonorrhoea, syphilis and HIV.

Hepatitis C virus reverse transcriptase-PCR, sequencing and phylogenetic analysis

For all HCV RNA positive samples, RNA isolation was performed on 100 μl of serum using the TriPure method (Roche Diagnostics). A 436-nucleotide fragment of the HCV NS5B region was amplified and sequenced using previously described methods [18]. The viral genotype was determined after phylogenetic analysis of the NS5B sequences obtained from the study participants, along with established GenBank reference sequences [19]. A HCV phylogenetic tree was constructed by the neighbour-joining method in Mega version [20], using the Tamura-Nei substitution model with γ-distribution (α=0.40). Bootstrap values (n=1000) were calculated to analyse the stability of the tree topology. The HCV sequences of MSM attending the STI clinic were compared with those obtained from 57 MSM with sexually acquired acute HCV in Amsterdam and Rotterdam during the period 2000–2007 [12] as well as with sequences from injecting drug users participating in the Amsterdam Cohort Studies [18].
Statistical analysis
Firstly, by means of the χ²-test for categorical and dichotomous variables and the Mann–Whitney test for continuous variables, the characteristics of the survey participants (MSM) and those who refused were compared, as well as the characteristics of HCV-positive and HCV-negative MSM participants. The χ²-test for trend was used to evaluate the time trend in HCV prevalence.

Secondly, the determinants of HCV infection were evaluated using logistic regression analyses. Multivariate logistic regression models were built using backward-stepwise techniques considering variables with a univariate P-value less than 0.10 as potential independent risk factors. A P-value less than 0.05 was considered statistically significant. The correlations between the variables were examined using the Spearman correlation test. If potential risk factors had a correlation of at least 0.3, those variables were not included in the same multivariate model due to multicollinearity. Finally, we checked for confounding and interaction between variables in the final models.

The variables tested included sociodemographic variables (age, nationality, education), risk factors for HCV (e.g., haemophiliacs, blood transfusions, IDU, having tattoos and/or piercings, surgery in HCV high-risk countries), sexual behaviour (e.g., a history of IDU, surgical bleeding during sex and therefore not considered in the same multivariate model due to multicollinearity), other possible risk factors like the use of recreational drugs in the past 6 months, and the presence of STI, including HIV, at time of visit.

Results
In total, 689/919 (75.0%) MSM attending the STI clinic during the three waves participated in this anonymous survey. The characteristics of MSM who refused to participate and those who did participate were comparable, except that MSM attendees who refused to participate were younger (P = 0.021) and more often of non-Dutch nationality (P < 0.001).

The median age of the participating MSM was 37.0 years (IQR 30.0–45.0), 75.3% were of Dutch nationality and 63.1% had a university degree. Furthermore, 58.2% reported that they had used recreational drugs in the previous 6 months; questions added to the third survey indicated that over 80% used one of these drugs shortly before or during sexual activities (119/147). The proportion reporting injecting drug use was very low (2.2%). The median number of lifetime sex partners was 80 (IQR 25–250).

Of the 689 MSM participating in the surveys, 30 (4.4%, 95% CI 3.05–6.17) were HCV positive; 24 of 30 (80%) had detectable HCV RNA. In total, seven of 24 (29.2%) HCV RNA-positive MSM were defined as having acute HCV infection; four were anti-HCV negative, the other three had a weak HCV antibody response combined with a negative (n = 1) or indeterminate (n = 2) immunoblot. Questions concerning previous HCV testing were only asked in the second and third wave of the survey. Of the 22 HCV-infected MSM of the second and third wave, seven (31.8%) were unaware of their infection, four of whom were categorized as having acute HCV infection.

In total, 157 of 689 (22.8%) MSM were HIV infected. Of these, 28 of 157 (17.8%, 95% CI 12.6–24.6) were coinfected with HCV, whereas only two of 532 (0.4%, 95% CI 0.10–1.36) of MSM without HIV tested HCV-positive. Of the two HCV-positive MSM without HIV, one reported ever-injecting drug use (IDU), whereas only five of 28 (17.9%) HCV/HIV coinfected MSM reported ever-IDU. Two out of these five HIV/HCV coinfected men reported IDU in the early 1990s; the other three had injected drugs after 2000.

Although not significant, the HCV prevalence increased over time: In May 2007, seven of 48 (14.6%) HIV infected MSM were HCV positive; in November 2007, seven of 42 (16.7%) and in May 2008, 14 of 67 (20.9%) (P for linear trend = 0.38). We also found a not significant increasing trend in respect of acute HCV: in May 2007, one of seven (14.3%) of the HCV infected MSM had an acute infection; in November 2007, one of seven (14.3%) and in May 2008, five of 14 (35.7%) (P for linear trend = 0.24).

Hepatitis C virus and risk behaviour
Variables that were significantly associated with HCV infection in MSM using univariate analysis (see Table 1) were: a history of IDU, a history of STI, having been treated for STI, having been diagnosed with STI, having sex with a non-Dutch partner, number of lifetime sex partners, unprotected sex with IDU, unprotected anal intercourse, GHB (gamma hydroxy butyrate) use, tattoo/piercing in a non-Dutch country, HIV infection and fisting. In multivariate analysis, HIV infection (OR 42.8, 95% CI 8.49–215.1), IDU (OR 27.0, 95% CI 4.50–162.3), recent fisting (OR 12.6, 95% CI 3.51–45.5), and the use of GHB (OR 5.0, 95% CI 1.90–13.1). Fisting was strongly correlated with the use of sex toys, group sex, and bleeding during sex and therefore not considered in the same multivariate model. GHB use was strongly correlated with the use of other recreational drugs like XTC, poppers and cocaine.

In an additional analysis, in which we included only MSM without a history of IDU (n = 674), HIV infection, fisting and the use of GHB were again found to be associated with HCV infection.

We also performed an analysis in which we only included HIV-infected MSM (n = 157). In this analysis IDU
fisting (adjusted OR: 13.4; 95% CI: 1.56–115.7), fisting (adjusted OR: 10.6; 95% CI: 2.78–40.7) and the use of GHB (adjusted OR: 4.6; 95% CI: 1.62–13.0) were again associated with HCV infection. Interestingly, those having sex with a non-Dutch partner (adjusted OR: 3.8; 95% CI: 0.95–15.0) were at increased risk for HCV, although the effect was borderline significant ($P = 0.059$).

**Genotyping and phylogenetic analysis**

Amplification and sequencing of the HCV NS5B fragment succeeded in all 24 HCV RNA positive MSM. The majority of isolates were HCV genotype 1a (17/24, 71%) and 4d (4/24, 17%), with some 3a (2/24, 8%) and 1b (1/24, 4%). A phylogenetic tree was constructed that included the 24 HCV RNA positive

**Table 1. Univariate and multivariate associations between risk behaviour, other characteristics and HCV infection among 689 MSM participating in the Amsterdam STI clinic bi-annual surveys, 2007–2008.**

<table>
<thead>
<tr>
<th>HCV status</th>
<th>Seronegative</th>
<th>Seropositive</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>659/689 (95.6%)</td>
<td>30/689 (4.4%)</td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>530/659 (80.4%)</td>
<td>2/30 (6.7%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>129/659 (16.6%)</td>
<td>28/30 (93.3%)</td>
<td>57.5 (13.5–244.6)</td>
<td>42.8 (8.49–215.1)</td>
</tr>
<tr>
<td>Number of sex partners lifetime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–80</td>
<td>218/659 (33.1%)</td>
<td>1/30 (3.3%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>81–250</td>
<td>101/659 (15.3%)</td>
<td>7/30 (23.3%)</td>
<td>15.1 (1.83–124.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;250</td>
<td>94/659 (14.3%)</td>
<td>14/22 (64.7%)</td>
<td>32.5 (4.21–250.5)</td>
<td></td>
</tr>
<tr>
<td>History of STI lifetime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>275/659 (41.7%)</td>
<td>1/30 (3.3%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>384/659 (58.3%)</td>
<td>29/30 (96.7%)</td>
<td>20.7 (2.81–153.3)</td>
<td></td>
</tr>
<tr>
<td>Treated for STI in past 6 months</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>584/659 (88.6%)</td>
<td>15/30 (50.0%)</td>
<td>1</td>
<td></td>
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<tr>
<td>Yes</td>
<td>75/659 (11.4%)</td>
<td>15/30 (50.0%)</td>
<td>0.62 (0.45–0.87)</td>
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<tr>
<td>Number of sex partners lifetime</td>
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<td></td>
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<td>0–80</td>
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<td>29/30 (96.7%)</td>
<td>20.7 (2.81–153.3)</td>
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</tr>
</tbody>
</table>

*AI, anal intercourse; CI, confidence interval; GHB, gamma hydroxy butyrate; HCV, hepatitis C virus; IDU, injecting drug use; OR, odds ratio; PAI, protected anal intercourse; UAI, unprotected anal intercourse.

**Questions only asked from 2nd survey onwards.**

*Overall $P$ value at least 0.001.

**Overall $P$ value at least 0.01.
MSM cases of this survey, as well as 57 previously recognized cases of acute HCV among Dutch MSM during the period 2000–2007 [9] (see Fig. 1). Phylogenetic analysis revealed four monophyletic clusters of MSM-specific strains, two homologous pairs of genetically similar HCV sequences and 10 singleton MSM sequences that were not closely related to any other strain circulation in this study population (like IDU strains). The four clusters ranged in size from 10–23 sequences, and contained 21 of 24 (88%) of our study participants and 46 of 57 (81%) previously described MSM cases (see Fig. 1).

All the survey participants infected with HCV genotypes 1a and 4d were part of MSM-specific clusters: nine were part of cluster III (genotype 1a), five of cluster IV (genotype 1a), four of cluster I (genotype 4d) and three of cluster II (genotype 1a). All MSM in the MSM-specific clusters were HIV-infected except one HIV-negative survey participant who reported IDU in MSM cluster I. Only three MSM in our survey had singleton HCV sequences (two HIV-positive MSM with genotypes 3a and one HIV-negative MSM with genotype 1b), suggesting unrelated routes of transmission. However, these three MSM (two of Dutch and one of Russian origin) reported the use of recreational drugs in the previous 6 months, had no history of IDU, reported a high number of sexual partners (90–1000), had an STI in the past and practised rough sexual techniques, indicating that sexual transmission was likely.

Most of the acute HCV infections were in MSM cluster III (n = 4 genotype 1a), two were in MSM cluster IV (genotype 1a) and one was in MSM cluster I (genotype 4d). All MSM with acute HCV infection were HIV positive.

IDU was reported by four HIV-positive MSM who tested positive for HCV RNA. These four MSM were infected with HCV genotype 1a but did not cluster with IDU sequences of genotype 1a [18] (data not shown); three MSM were in MSM-specific cluster III and one in MSM-specific cluster IV.

**Discussion**

We found a high and increasing HCV prevalence in HIV-infected MSM attending the Amsterdam STI clinic. Though not statistically significant, this trend, and the
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relatively large proportion of acute infections strongly indicate a rapid and recent spread of HCV among high-risk HIV-positive MSM. Before 2000, the estimated HCV prevalence among HIV-positive MSM was very low (1–4%) [9]. In addition to injecting drug use and HIV infection, recreational drug use and practicing rough sexual techniques were associated with HCV, indicating that transmission is driven by high-risk sexual behaviour.

In the additional analysis, excluding HIV-negative MSM, sex with a non-Dutch partner showed a borderline association with HCV. This result supports recent findings of an international HCV transmission network among HIV-infected MSM in Europe [11] with outbreaks reported in major cities including London, Paris, Amsterdam and Berlin [7,8,11]. Madrid has reported no outbreak yet [21], but ongoing HCV transmission in Amsterdam and the presence of a European transmission network, suggests a further spread to HIV-positive MSM in other countries [11]. Outbreaks of HCV are also reported outside Europe, including New York and Sydney [22,23].

Phylogenetic analysis showed at least four distinct clusters of independent cocirculating HCV lineages, strongly supporting the presence of MSM-specific HCV transmission networks in Amsterdam. The fact that multiple strains of different genotypes cocirculate in this population, suggests that HCV has emerged as an STI among HIV-positive MSM due to a change in risk behaviour rather than evolution of one single virus into a more virulent variant. As 17.9% of HIV/HCV coinfected MSM reported IDU, separate introductions of HCV into the MSM-population probably originate from overlap with the IDU scene. Genotypes 1a and 4d, which predominate among MSM, account for approximately 50 and 10% of new HCV infections among IDU in the Netherlands [24]. Phylogenetic analysis of HCV genotype four strains in Amsterdam confirmed that the MSM-specific HCV-4d strain originates from the HCV-4d strain circulating among European IDU in the late 1980s or early 1990s [25].

We found much higher HCV prevalence in HIV-infected MSM than in HIV-negative MSM. HIV infection might facilitate HCV transmission by increasing both viral infectiousness due to higher HCV viral loads in blood and semen [26] and viral susceptibility through HIV-impaired immunological control [26,27]. HIV-infected MSM tend to have unprotected sexual contact with other HIV-infected MSM (serosorting), further fuelling HCV transmission [28]. Finally, HIV-infected MSM generally display more high-risk behaviour than HIV-negative MSM [29]. The practice of rough sexual techniques such as fisting, group sex and the sharing of toys might facilitate blood-to-blood contact by damaging the anal mucosal barrier [5]. However, a core group of HIV-negative MSM sometimes practise high-risk sexual behaviour with HIV-infected MSM [28], raising concerns about possible spillover of HCV (and HIV) to the HIV-negative MSM population. Indeed non-IDU acquired HCV has been reported among HIV-negative MSM in Brighton [30].

About one-third of MSM in our study were unaware of their HCV status, however, the majority was diagnosed with an acute HCV infection. Given the prolonged windows of HCV seroconversion in HIV-infected individuals [15], HIV specialists should routinely test MSM for HCV antibodies and HCV RNA. Testing will enable timely discovery of acute HCV infections and its treatment in the early phase, improving HCV treatment outcome [31]. On the basis of preliminary results from the first wave of this survey, the Amsterdam STI clinic has implemented a protocol by which all HIV-infected MSM and all MSM who opt out for HIV testing are offered HCV testing.

One limitation of this study lies in the high correlations between potential risk factors. Consequently, it was difficult to distinguish between different sexual techniques and recreational drugs particularly and transmission probably results from combined factors.

In conclusion, prevalence of sexually acquired HCV infection among HIV-infected MSM is high and increasing, although this trend is not statistically significant. Targeted preventions like raising awareness and internationally widespread routine testing are needed to minimize further spread among HIV-infected MSM, and spillover to HIV-negative MSM.

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