Hepatitis C virus infection: Spread and Impact in the Netherlands

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Trends in hepatitis C virus infections among men who have sex with men attending an STI clinic; 1995-2010

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Abstract

Background: Since 2000 there is growing evidence that HCV infection has emerged as an STI among HIV-positive MSM. Here we present a 15-year overview of the HCV epidemic among MSM visiting a large STI-clinic in the Netherlands.

Methods: During bi-annual cross-sectional anonymous surveys (1995-2010), participants were interviewed and tested for HIV- and HCV-antibodies. Additional HCV RNA tests were performed in all HIV-positives. Determinants of HCV infection were analyzed using logistic regression. HCV incidence was estimated using the window period from HCV RNA detection to HCV-antibody development. Phylogenetic analysis provided evidence for sexual transmission.

Results: HCV prevalence among HIV-positive MSM increased from 1995 onwards (5.6%) and peaked in 2008 (20.9%). Estimated HCV incidence peaked in 2006 (14.0/100 PY; 95%CI: 5.02-37.69) and thereafter decreased, although not significantly. Prevalent HCV infection was more strongly associated with fisting in 2007/2008 (aOR 2.85, 95%CI: 1.19-6.82) than in 2009/2010 (aOR 0.92, 95%CI:0.42-2.02). In addition, HCV infection was independently associated with Chlamydia, injecting drug use, unprotected anal intercourse, and older age. Phylogenetic analysis revealed a high degree of MSM-specific clustering from 2000 onwards. HCV prevalence among HIV-negative MSM remained low (0.5%).

Conclusions: HCV prevalence among HIV-positive MSM significantly increased over calendar time but appears to level off in recent years, possibly due to increased awareness, saturation in the population, decreased risk behaviour, and earlier HCV screening and treatment. The association with fisting became less strong over time, but our analyses continue to support sexual transmission. Monitoring HIV-positive and HIV-negative MSM remains needed to guide prevention efforts.
Introduction
Infection with hepatitis C virus (HCV) is transmitted primarily by blood and occurs frequently in injecting drug users (IDU) (1). Since 2000, acute HCV infections have increasingly been reported internationally among HIV-positive men who have sex with men (MSM) (2-9). Phylogenetic analysis confirms the existence of MSM-specific HCV transmission networks. The absence of reported parenteral routes of transmission suggests that most of these infections were acquired sexually. (2;5;6;9-11).

HCV incidence data from HIV-seroconverter cohorts (CASCADE) and molecular-clock calculations based on circulating MSM-specific HCV strains, indicate that the HCV-epidemic among HIV-positive MSM in Europe arose with the introduction of combination antiretroviral therapy (cART) for HIV in 1996, with a substantial increase after 2002 (11-13).

In the Netherlands, an alarming HCV prevalence of 17% among HIV-infected MSM was found in a biannual cross-sectional survey conducted in 2007/2008 at a large city STI outpatient clinic in Amsterdam (14). Multiple MSM-specific HCV clusters of genotypes 1a and 4d were revealed by phylogenetic analysis. HCV infection was independently associated with HIV infection, IDU, fisting and noninjecting recreational drug use (14).

Adequate longitudinal data on acute and chronic HCV infections among HIV-positive and HIV-negative MSM is needed to determine the past and current state of this epidemic, reveal possible causes, and assist prevention and case-finding strategies. In the Netherlands, our first report on HCV spread in HIV-infected MSM (14) led to prevention measures focused on the sexual transmission of HCV. Even before publication, our finding of a high HCV prevalence spurred the Amsterdam STI clinic to start routine HCV-antibody testing for all MSM clients with positive or unknown HIV status.

To put our earlier results on the HCV epidemic among HIV-positive MSM (2007-2008) (14) in a larger timeframe, we retrospectively tested stored serum samples of HIV-infected MSM participating in the same biannual cross-sectional survey at the Amsterdam STI clinic during the period 1995-2004, and complemented the dataset with newly recruited HIV-positive and HIV-negative MSM participating in the period 2008-2010. Combined HCV RNA and HCV-antibody testing allowed us to estimate HCV incidence over time. Phylogenetic analysis was used to gain insight into transmission networks and the emergence of MSM-specific HCV clusters.

Methods
Subjects
Since 1991, biannual cross-sectional and anonymous HIV surveys have been performed at the STI outpatient clinic in Amsterdam (15). Each spring and autumn, consecutive clinic visitors are asked to participate until one thousand are included. Upon informed consent, their blood is drawn, tested, and stored. As participation is anonymous, multiple visits of one visitor cannot be linked and therefore the number of possible duplicate visits during the surveys is unknown. Participants are interviewed about risk factors for blood-borne and sexually transmitted infections (STI), using a standardized questionnaire. Since 2007, the interview has addressed traditional HCV-related risk behaviour (e.g. blood transfusion prior to 1992) and HCV status. Over the years, the participation rate of the clinic population has varied between 65% to 95%, depending on changes in screening procedures. Looking at 2007-2010, characteristics of survey participants and non-participants were comparable; participation was refused more often by HIV-positive MSM than by HIV-negative MSM (overall \(P=0.005\)).

in 19 surveys (the spring survey of 2004 was not performed). We excluded 54 MSM who had no or insufficient serum samples available for HCV-testing. Samples from the remaining 777 MSM were tested for the presence of both HCV antibodies and HCV RNA. Additionally, we tested all HIV-negative MSM participating in the surveys from 2007 to 2010 (n=1513) for anti-HCV: 358 from 2007, 383 from 2008, 400 from 2009, and 372 from 2010. The biannual survey and basic data collection (e.g. age, sexual preference) were performed according to the STI clinic’s standard protocol, described elsewhere (16;17). Briefly, all MSM were tested for Chlamydia trachomatis, Neisseria gonorrhoea, and antibodies to Treponema pallidum. Since 2004, they have been tested for hepatitis B virus except when vaccinated or natural immunity has been previously documented. HIV testing was optional through 2006; since 2007, an opt-out system allows testing of all HIV-negative or HIV-unknown clients who do not expressly refuse it.

Laboratory testing
HCV antibody testing is performed using a third-generation commercial microparticle EIA system (AxSym HCV version 3.0; Abbott) with Immunoblot confirmation (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics). All anti-HCV positive MSM are tested for HCV RNA using transcription-mediated amplification (TMA: VERSANT® HCV RNA Qualitative Assay, Siemens), with a detection limit of 5 IU/ml.

Testing procedure and HCV definition
As HIV-infected individuals may have prolonged windows of seroconversion (18-20), all HIV-positive participants of our surveys were screened for HCV RNA regardless of their HCV antibody status. Those confirmed positive for HCV antibodies and/or HCV RNA are considered HCV-positive. Acute HCV infection is defined as detectable HCV RNA in the absence of HCV antibodies or as having detectable HCV RNA in the presence of a weak anti-HCV response (AxSYM ratio below 5) with negative or indeterminate Immunoblot. This definition of acute HCV infection was also used in our previous paper where a subset of these data has been described (14).

HCV RT-PCR, sequencing and phylogenetic analysis
HCV 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 (21). Viral genotype was determined by phylogenetic analysis of NS5B sequences obtained from the study participants, along with GenBank reference sequences (22). HCV phylogenetic trees were constructed by the maximum-likelihood approach using the Hasegawa-Kishino-Yano substitution model with a γ distribution of among-site rate heterogeneity (HKY-γ) implemented in PHYML 3.0 software (23). Bootstrap values (n=1000) were calculated to analyze the stability of the tree topology. Phylogenetic trees were constructed for HCV genotypes 1,2,3 and 4 separately.

Statistical analysis
Given two surveys per year, HCV prevalence was calculated per survey and not per year. To evaluate trends in HCV prevalence over time, both outcomes were modelled with calendar year as a continuous variable, using restricted cubic splines (24). The R statistical package, version 2.13.0 was used for these analyses (25). Determinants of HCV infection were evaluated using logistic regression analysis restricted to data from 2007-2010 because data on behaviour before 2007 was not fully available. Correlations between the variables were examined using the Spearman correlation test. Multivariate logistic regression models were built, using backward stepwise techniques and considering variables with
a univariate P-value ≤0.25 as potential independent determinants. To examine changes in effects over calendar time, we forced calendar time into the model. In addition, we evaluated interaction between calendar time and the variables within the final model. A P-value of <0.05 was considered statistically significant. For logistic regression analysis, SPSS 19.0 was used.

To estimate the annual HCV incidence, we used the formula P=TI, describing the relation between prevalence (P) and incidence (I) in case of low prevalence. Here, P is the prevalence of acute infection, or the number of persons with acute infection (n), divided by the susceptible population, i.e., all HCV-negative plus acutely infected MSM (N). T is the window period until antibody develops: 91 days as described (20), thus I = [(n/N)/(91/365.25)] × 100. A comparable approach has been used to estimate annual HIV incidence for the biannual clinic survey (26). To determine 95% confidence intervals (CI) for the incidences, we used the Bonferroni principle as described earlier (i.e., using 97.5% CIs) but without the corrected values based on the CIs of the window period, since this information was unavailable (27;28). Hence, for HCV incidence estimates obtained, CIs will be too narrow and P-values too small. Similar to HCV prevalence, trends in HCV-incidence over time were modelled with calendar year as a continuous variable using restricted cubic splines in the R statistical package version 2.13.0.

**Results**

Among the 777 HIV-positive MSM we surveyed, the median age was 40 years (Inter quartile range (IQR) 34-47 years); 71% were born in the Netherlands, and only 3.5% had ever injected drugs. The median number of sex partners per lifetime was 200 (IQR 95-998). Data collected between 2007-2010 showed that 74% (383/517) used recreational non-injecting drugs (e.g., GHB, XTC, cocaine) in the previous 6 months; questions added in 2008 have shown that 75% (287/383) of these MSM used such drugs shortly before or during sexual activities.

**HCV prevalence in HIV-positive MSM**

Of the 777 HIV-positive MSM in our study, 91 (11.7%, 95% CI: 9.6%-14.2%) tested positive for HCV antibodies and/or HCV RNA. Of the HIV/HCV-coinfected MSM, 10/91 (11%) reported injecting drug use.

Among HIV-positive MSM, the observed HCV prevalence gradually increased from 0-5.6% in the 1995 surveys to 9.4% (3/32) in the second survey of 2003. In 2004, HCV prevalence increased to 13.3% (4/30) and increased further to 20.9% (14/67) in 2008. In 2010, HCV prevalence slightly decreased (see Figure 1).

Overall, HCV prevalence among HIV-positive MSM increased from 1995/1996 until 2007. Prevalence was significantly higher (P<0.001) in 2007 compared to the first survey period tested (1995/1996), with an odds ratio (OR) of 9.54 (95%CI 2.65-34.3). HCV prevalence showed decreased from 2008 until 2010, but the prevalence was not significantly lower in 2010 (OR 0.57, 95% CI 0.27-1.17).

**HCV prevalence in HIV-negative MSM**

Of the HIV-negative MSM, 10/1513 (0.6%) tested positive for anti-HCV from 2007 through 2010. In 2007, the biannual surveys yielded 0.5% (1/195) and 0.6% (1/163); in 2008, 0% (0/174) and 0.5% (1/209); in 2009, 1.7% (4/234) and 0.6% (1/166), and in 2010, 1.1% (2/178) and 0% (0/194). There was no significant calendar time effect found in HCV prevalence among HIV-negative MSM (P=0.54). Only 6/10 (60%) of anti-HCV positive MSM without HIV tested positive for HCV RNA.
Acute HCV and estimated incidence

Of the 91 HCV/HIV-positive MSM (15.3%), 14 were defined as having an acute HCV infection (i.e., anti-HCV negative and HCV RNA-positive). During the study-period, the percentage of HIV-positive MSM with acute infections fluctuated between 0-4%, and we did not find a significant increase or decrease in the number of acute infections over time (lower dashed line, figure 1) (1995: n=0, 1996: n=1, 1999: n=0, 2000: n=1, 2003: n=1, 2004: n=1, 2007: n=2, 2008: n=5, 2009: n=0, 2010: n=3). The estimated HCV incidence increased from 1.9/100 person years (PY) (95% CI: 0.11-31.2) in 1995 to 14.0/100 PY (95% CI: 5.02-37.69) in 2006 (Figure 2). After 2006, it decreased gradually to 6.9/100 PY (95% CI: 2.63-17.6) in 2010. Despite variation in the estimated HCV incidence over time, no statistically significant effect could be demonstrated (p=0.72).

It must be noted that due to the anonymous and cross-sectional design of the study we were unable to test sequential samples of one participant. A verified diagnosis of acute HCV, meaning anti-HCV seroconversion in two sequential samples drawn 6 months apart, could therefore not be made.

HCV and risk behaviour

In univariate analysis, older age, number of sexual partners in lifetime, fisting (active and/or passive), ever injecting drugs, using GHB, Chlamydia diagnosis, and unprotected anal intercourse (UAI, passive and active) were significantly associated with HCV infection in HIV-positive MSM in the period of 2007-2010 (table 1).

Because GHB use might be a proxy for unmeasured sexual risk behaviour, we ran two separate multivariate models, one including the interaction between fisting and calendar time (P=0.087) and one including GHB use and calendar time (P=0.025). In both models, older age, ever-IDU, Chlamydia diagnosis, and UAI were independently associated with HCV infection (Table 1). The OR for fisting was 2.85 (95% CI: 1.19-6.82) in 2007-2008 and became less strong in 2009-2010 (OR 0.92 (95% CI 0.42-2.02). Likewise, the OR for GHB use declined from 4.38 (95% CI: 2.03-9.48) to OR 1.18 (95% CI: 0.55-2.55) in that period.

Genotyping and phylogenetic analysis

HCV RNA was detected in 64/91 (70%) of HIV-infected MSM defined as ‘HCV-positive’. HCV genotyping and sequencing succeeded for 59/64 (92%) of the HCV RNA-positive samples. HCV genotypes 1a (41/59; 69%) and 4d (12/59; 20%) predominated. Only 6/59 (10%) were infected with genotypes 3a (n=3), 1b (n=2), and 2b (n=1) (Table 2).

Of the HIV-negative MSM, 10/1513 (0.6%) tested positive for anti-HCV, of whom 6/10 (60%) were positive for HCV RNA. These 6 were infected with HCV genotypes 1b (n=4), 1a (n=1), and 4d (n=1).

Phylogenetic trees were constructed for HCV genotypes 1,2,3 and 4 separately, and included 75 HCV sequences from HIV-positive MSM; 59 MSM from the STI-clinic cohort (see Table 1) and 16 MSM participating in the Amsterdam Cohort Studies (ACS) during the period 1985-2003 (6). Of the MSM participating in the ACS, 7 were diagnosed with acute 9 had a prevalent HCV-infection. Phylogenetic trees were also supplemented with 12 HCV sequences from HIV-negative MSM (6 MSM from the STI-clinic cohort, and 6 from the ACS) and 79 HCV sequences from acute HCV infections (including re- and superinfection) among 59 DU who seroconverted in the ACS in the period 1985-2005 (29). ACS MSM samples were added to expand our sample and put our STI-clinic samples in a larger timeframe and ACS DU sequences were added to distinguish MSM-specific HCV strains from those circulating in other high-risk groups.
Phylogenetic analysis revealed 5 strongly supported monophyletic clusters (bootstrap >70) of MSM-specific strains that have no overlap with IDU-clusters containing a total of 53 HIV-positive MSM and 1 MSM without HIV; 2 homologous pairs of HCV sequences obtained from HIV-positive MSM, and 28 singleton MSM sequences that were more closely related to HCV strains obtained from IDU than to another MSM strain (Figure 3). MSM-specific clusters ranged from 3-17 sequences; four clusters were subtype 1a and one subtype 4d.

Among HIV-positive MSM, phylogenetic clustering showed a distinct pattern over time (Table 3). None of the 11 HCV sequences from HIV-positive MSM before 2000, all obtained from the ACS, were part of an MSM-specific cluster. In contrast, 8/11 (73%) and 45/53 (85%) of HCV sequences from HIV-positive MSM in the periods 2000-2005 and 2006-2010, respectively, belonged to MSM-specific clusters (Figure 3). The 5 MSM-specific phylogenetically robust clusters illustrate the, independent introductions of 5 different HCV lineages in the Amsterdam MSM population during the study period. Among our subjects, the earliest representatives of the five MSM-specific clusters were identified in 2000 for Cluster I, 2003 for Cluster II, 2007 for Clusters III and IV, and 2009 for Cluster V.

Interestingly, only 1/12 (8.3%) of HIV-negative MSM was part of an MSM-specific HCV cluster (Cluster II, genotype 4d), compared to 53/75 (71%) of HIV-positive MSM. The 11 HIV-negative MSM with non-MSM-specific sequences included 8 MSM infected with HCV 1b, the most common genotype among unpaid blood donors in the Dutch population (30). The three others each harboured a different genotype: 1a, 2b, and 4d.

Discussion
This study examined the HCV epidemic among HIV-positive MSM in the period 1995-2010 and among HIV-negative MSM in the period 2007-2010. Among HIV-infected MSM, HCV prevalence significantly increased until 2007. After 2007 no further increase was observed. On the contrary, our data suggest a decrease after 2007, but this was not statistically significant. Consistent with out HCV prevalence data, the estimated HCV incidence among HIV-positive MSM peaked in 2006 (14.0/100 PY; 95% CI: 5.0-37.2), although no statistically significant trend could be demonstrated, probably as a result of small numbers. The HCV incidence we observed in our study exceeds the HCV incidences of those studies reviewed by van de Laar et al. (13). This might be explained by the fact that our participants were included at the STI-clinic and reported very high risk behaviour. Previous studies suggest that the current HCV epidemic started between 1996-2000, with HCV-incidence rising substantially since 2000-2002 (12;13), which is in line with our findings. The most recent estimate of HCV incidence includes data from the Swiss HIV cohort study up to 2011 (31) and suggest still an increasing incidence in contrast to our finding of a levelling off of the epidemic. Larger incidence studies, including recent data among high-risk MSM, are needed to gain more insight into the ongoing HCV epidemic.

Traditionally, the acute-phase of HCV infection is defined as 6 months following infection acquisition (32). However, the anonymous and cross-sectional design of our study did not allow us to analyse sequential samples of one individual, leaving us with having to base the moment of HCV infection, on the estimated window between HCV RNA seroconversion and the development of HCV antibodies. This same definition was used as in our earlier report (14). In HIV-infected individuals, the estimated seroconversion period is 91 days, and is based on one relatively small study (20). It must be noted that the approach used to estimate HCV-incidence gives rise to a number of uncertainties that might lead to over- as well and underestimation of the true HCV-incidence in our population. If the window period is longer than 91 days, our approach might have
introduced bias and caused an overestimation of incidence. Moreover, loss or non-development of antibodies in the absence of HCV RNA could lead to an underestimation of the HCV prevalence in our study and others (33). Furthermore, our approach does not account for HCV-reinfection. Hence there will be no measurable antibody seroconversion period. Since the rate of HCV reinfection among HIV-positive MSM in Amsterdam is high (34), our approach will underestimate true HCV-incidence, especially in the recent years given the growing number of HIV/HCV coinfected MSM successfully treated for HCV.

Using a phylogenetic approach, we confirmed the presence of at least 5 MSM-specific HCV strains circulating among HIV/HCV-coinfected MSM in Amsterdam. Over time, multiple independent introductions of HCV genotypes 4d and 1a into a susceptible MSM community have led to ongoing transmission of HCV among HIV-positive MSM. Identification of another new MSM-specific HCV lineage (Cluster V) and the finding of acute HCV infection in established HCV clusters during recent years argue for the ongoing transmission of HCV among HIV-positive MSM. The introduction and ongoing circulation of multiple HCV lineages among HIV-positive MSM suggests that HCV emergence is caused by behavioural change, rather than evolution of the virus into a more virulent variant (13).

In contrast to a study from Australia (8), where half of the MSM also inject drugs, we found no overlap between MSM clusters and IDU clusters (data from the Amsterdam Cohort Studies). The percentage of co-infected MSM who reported IDU in the Netherlands was only 11% compared to the 50% in Australia. Moreover, in the Netherlands, the HCV epidemics among IDU and among HIV-positive MSM have occurred in separate timeframes. Drug injection behaviour severely declined as a result of harm reduction campaigns, eventually halting the HCV epidemic among IDU in Amsterdam with sustained low HCV-incidence rates since the late 90s (35). In contrast, new HCV infections among MSM have occurred mainly after 2000. Australia has an ongoing HCV epidemic in IDU, with new infections occurring in both MSM and IDU at the same time. Together with the higher prevalence of injecting drugs reported by Australian MSM, this might explain the discrepant findings as to overlapping clusters (8;14).

Our observation that the HCV prevalence in Amsterdam has levelled off in recent years might reflect an increased awareness among MSM, leading to reduced risk behaviour and increased uptake of early screening and treatment. In November 2007, routine HCV testing was introduced at the STI clinic and also HIV specialists started routine HCV screening with elevated ALT levels (36). Alternatively, HCV saturation in the highest-risk groups might explain our finding. The effect of fisting and GHB use became less strong in 2009-2010, compared to 2007-2008, possibly related to behavioural change associated with these practices. As our data did not include the precise moment of infection, our study of risk behaviour was limited. It should preferably be studied in longitudinal studies on HCV incidence.

Although HIV infection is not a prerequisite for sexually transmission of HCV (37), HIV-negative MSM remain largely unaffected by this outbreak. Only few MSM were diagnosed with HCV in the absence of HIV infection, comparable to the general Amsterdam population (38), and we did not observe an increase over time. All except one of the HIV-negative MSM were infected with HCV strains not related to strains among HIV-positive MSM (37;39) or to strains of other HIV-negative MSM.

Monitoring of HCV in both HIV-positive and HIV-negative MSM remains needed to guide prevention, in order to halt further spread. Early detection and treatment of HCV during the acute phase improve treatment outcome (40). Co-infection with HCV among HIV-positive MSM has serious clinical implications, reducing rates of spontaneous viral HCV clearance, complicating
treatment, and accelerating progression to liver disease (41). Successful treatment prevents secondary infections in the patient’s HIV-positive sexual contacts and could prevent possible spill-over to the HIV-negative population (4). Unfortunately, early detection of acute infection requires RNA testing, which is still expensive.

In conclusion, HCV prevalence among HIV-positive MSM significantly increased until 2007, but appears to be levelling off, at least in Amsterdam. Reasons are unclear but several factors may play a role, including reduced risk behaviour, earlier testing and treatment, or perhaps saturation within the highest-risk group. HCV prevalence among HIV-negative MSM remained stable. The association with pronounced HCV risk factors declined over the years, but both risk factor analysis and phylogenetic analysis continue to support ongoing sexual transmission of HCV among HIV-positive MSM. Monitoring of HCV prevalence and incidence therefore remains important to follow trends and possible future epidemics. The costs of HCV RNA screening might hamper adequate diagnosis of HCV in its earliest stage, as often HCV antibody testing is performed which in HIV-positives might be false-negative for a prolonged period of time. As factors shaping the current epidemic remain unclear, it remains needed to evaluate interventions to halt further transmission to HIV-negative MSM and others with lower-risk profiles in the MSM community.

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References


HCV epidemiology among subpopulations in the general Dutch population


(36) van Rooijen MS, Heijman T, de Vrieze NHN, Urbanus AT, van Leeuwen P, de Vries HJC, Prins M. Earlier HCV diagnosis by the introduction of routine HCV testing for HIV positive and MSM opting out for HIV in a large STI outpatient clinic. In preparation.


Figure 1: Modelled and observed HCV prevalence among HIV-positive and HIV-negative MSM included in the STI clinic survey 1995-2005.
Upper solid line: modelled HCV prevalence among HIV-positive MSM positive for anti-HCV and/or HCV RNA; gray area 95% confidence interval (CI)
Upper dashed line: modelled HCV prevalence among HIV-positive MSM (positive for anti-HCV and negative for HCV RNA); gray stripes: 95% CI
Lower dashed line: modelled HCV prevalence among HIV-positive MSM negative for anti-HCV and positive for HCV RNA; gray stripes: 95% CI
Lower solid line: modelled HCV prevalence among HIV-negative MSM (positive for anti-HCV and/or HCV RNA); gray area 95% CI
Asterisks: observed HCV prevalence among HIV-positive and HIV-negative MSM including 95% CI.
Stripes on x-axis: time points of observed data
Figure 2: Estimated HCV incidence among HIV-positive MSM included in the STI clinic survey 1995-2010. Gray area: 95% CI.
Table 1. Univariate and multivariate associations between risk behaviour, other characteristics, and HCV infection among 517 HIV-positive MSM participating in the Amsterdam STI clinic biannual surveys, 2007–2010.

<table>
<thead>
<tr>
<th>HCV status</th>
<th>2007-2010 Univariate analysis</th>
<th>2007-2010 Multivariate analysis (with fisting)</th>
<th>2007-2010 Multivariate analysis (with GHB use)</th>
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<tbody>
<tr>
<td></td>
<td>Negative (N=438)</td>
<td>Positive (N=79)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤35 years</td>
<td>126 (28.8%)</td>
<td>11.4%</td>
<td>1</td>
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<td>36-42 years</td>
<td>115 (26.3%)</td>
<td>30 (38.0%)</td>
<td>3.65 (1.66-8.02)</td>
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<td>43-48 years</td>
<td>100 (22.8%)</td>
<td>17 (24.5%)</td>
<td>2.38 (1.02-5.57)</td>
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<td>≥49 years</td>
<td>97 (22.1%)</td>
<td>23 (29.1%)</td>
<td>3.32 (1.47-7.50)</td>
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<td>Number of sex partners over lifetime</td>
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<td>0-95</td>
<td>74 (16.9%)</td>
<td>6 (7.6%)</td>
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<td>96-200</td>
<td>61 (13.9%)</td>
<td>17 (21.5%)</td>
<td>3.44 (1.28-9.26)</td>
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<td>201-998</td>
<td>63 (14.4%)</td>
<td>15 (19.0%)</td>
<td>2.94 (1.08-8.02)</td>
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<td>&gt;998</td>
<td>179 (40.9%)</td>
<td>23 (29.1%)</td>
<td>1.59 (0.62-4.05)</td>
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<td>Calendar period and fisting*</td>
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<tr>
<td>None reported 2007/2008</td>
<td>117 (30.8%)</td>
<td>18 (25.7%)</td>
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<tr>
<td>Fisting reported 2007/2008</td>
<td>26 (6.8%)</td>
<td>14 (20.0%)</td>
<td>3.50 (1.55-7.93)</td>
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<td>13 (18.6%)</td>
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<td>142 (33.0%)</td>
<td>15 (19.2%)</td>
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<td>44 (10.1%)</td>
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<td>23 (29.5%)</td>
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<td>GHB use reported 2009/2010</td>
<td>68 (15.8%)</td>
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<td>1.69 (0.83-3.42)</td>
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<td>427 (97.5%)</td>
<td>70 (88.6%)</td>
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<td>11 (2.5%)</td>
<td>9 (11.4%)</td>
<td>4.99 (1.99-12.48)</td>
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<td>Chlamydia found at visit</td>
<td>No</td>
<td>369 (84.2%)</td>
<td>56 (70.9%)</td>
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<td>Yes</td>
<td>69 (15.8%)</td>
<td>23 (29.1%)</td>
<td>2.20 (1.27-3.80)</td>
</tr>
<tr>
<td>Unprotected anal intercourse*</td>
<td>No</td>
<td>113 (27.3%)</td>
<td>7 (9.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>301 (72.7%)</td>
<td>71 (91.0%)</td>
<td>3.81 (1.70-8.26)</td>
</tr>
</tbody>
</table>

† Question asked only from October 2007 onwards.
* active and/or passive.
<table>
<thead>
<tr>
<th>Number tested</th>
<th>1995 (n=36)</th>
<th>1996 (n=41)</th>
<th>1999 (n=42)</th>
<th>2000 (n=58)</th>
<th>2003 (n=53)</th>
<th>2004# (n=30)</th>
<th>2007 (n=90)</th>
<th>2008 (n=142)</th>
<th>2009 (n=139)</th>
<th>2010 (n=146)</th>
<th>Total (n=777)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV+*</td>
<td>1 (2.8%)</td>
<td>1 (2.4%)</td>
<td>0</td>
<td>3 (5.2%)</td>
<td>3 (5.7%)</td>
<td>4 (13%)</td>
<td>14 (16%)</td>
<td>26 (18%)</td>
<td>24 (17%)</td>
<td>15 (10%)</td>
<td>91 (12%)</td>
</tr>
<tr>
<td>RNA+**</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>0</td>
<td>2 (67%)</td>
<td>2 (67%)</td>
<td>3 (75%)</td>
<td>10 (71%)</td>
<td>22 (85%)</td>
<td>14 (58%)</td>
<td>9 (60%)</td>
<td>64 (70%)</td>
</tr>
<tr>
<td>1a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (50%)</td>
<td>1 (33%)</td>
<td>8 (80%)</td>
<td>15 (68%)</td>
<td>11 (85%)</td>
<td>5 (63%)</td>
<td>41 (69%)</td>
</tr>
<tr>
<td>1b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
<td>0</td>
<td>1 (13%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>3a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (10%)</td>
<td>2 (9%)</td>
<td>0</td>
<td>0</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>4d</td>
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<td>0</td>
<td>0</td>
<td>1 (100%)</td>
<td>1 (50%)</td>
<td>2 (67%)</td>
<td>1 (10%)</td>
<td>3 (14%)</td>
<td>2 (15%)</td>
<td>2 (25%)</td>
<td>12 (20%)</td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

*Positive for anti-HCV and/or HCV RNA, % calculated from the number of HIV-positive MSM.

**HCV RNA-positive, % calculated from the number of co-infected MSM.

# Only one of the usual two surveys was performed in 2004.
HCV epidemiology among subpopulations in the general Dutch population

Origin of data
- ■ strains obtained from ACS
- ● strains obtained from bi-annual survey

Time period
- ● <2000
- ● 2000-2005
- ● >2005

Risk groups
- ○ HIV-negative MSM
- ● HIV-positive MSM
- ▲ MSM who reported IDU

Other
- + Acute infection in MSM

Figure 3: HCV NS5B phylogenetic tree comparing HCV-infected MSM with and without HIV coinfection visiting the STI-clinic in the period 1995-2010 along with HCV-infected MSM and IDU participating in the Amsterdam cohort studies (ACS).
**Figure 3:** HCV NS5B phylogenetic tree comparing HCV-infected MSM with and without HIV coinfection visiting the STI-clinic in the period 1995-2010 along with HCV-infected MSM and IDU participating in the Amsterdam cohort studies (ACS).
Table 3: Distribution over time of MSM-specific HCV strains in HIV-positive MSM from both STI-clinic surveys and the Amsterdam Cohort Studies (ACS).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>Cluster I (4d)</td>
<td>-</td>
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<td>3</td>
<td>4</td>
<td>4</td>
<td>15*</td>
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<tr>
<td>Cluster II (1a)</td>
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<td>-</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Cluster III (1a)</td>
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<td>-</td>
<td>10</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Cluster IV (1a)</td>
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<td>-</td>
<td>-</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Cluster V (1a)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Unrelated sequence</td>
<td>11</td>
<td>2</td>
<td>-</td>
<td>6</td>
<td>2</td>
<td></td>
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</tbody>
</table>

* Total sequences in Cluster I is 15, as it includes one HIV-negative MSM.