Molecular epidemiology of hepatitis C virus
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Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission

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Abstract

We retrospectively screened 1836 men who have sex with men (MSM) participating in the Amsterdam Cohort Studies (1984-2003) for hepatitis C virus (HCV) antibodies. HCV incidence was 0.18/100 person years (PY) in human immunodeficiency virus (HIV)-positive MSM (8/4408 PY [95% confidence interval (CI), 0.08-0.36]), but was 0/100 PY in MSM without HIV (0/7807 PY [95%CI 0.00-0.05]). After 2000, HCV-incidence among HIV-positive men increased 10-fold to 0.87/100 PY (5/572 PY [95%CI 0.28-2.03]). Additional hospital cases (n=34) showed that MSM in Amsterdam who acquired HCV infection after 2000 reported high rates of ulcerative sexually transmitted infections (59%) and rough sexual techniques (56%), denied injection drug use, and were infected mainly with the difficult-to-treat HCV genotypes 1 (56%) and 4 (36%). Phylogenetic analysis showed three monophyletic clusters of MSM-specific HCV strains. The emergence of an MSM-specific transmission network suggests that HIV-positive MSM with high-risk sexual behavior are at risk for sexually acquired HCV. Targeted prevention and routine HCV screening among HIV-positive MSM is needed to deter the spread of HCV.
Introduction

Hepatitis C virus (HCV) infection occurs mainly by parenteral exposure, particularly injection drug use (IDU) [1]. Its association with sexual transmission remains controversial. Even in the presence of HIV coinfection, HCV is rarely transmitted by heterosexual intercourse [2]. HCV-serodiscordant partners in long-term monogamous heterosexual relationships show only slightly higher rates of HCV infection than the general population [3]. Data obtained from men who have sex with men (MSM) are more conflicting [4,5]. Previous cross-sectional and cohort studies have reported increased HCV prevalence among MSM and have highlighted unprotected anal intercourse, multiple sex partners, rough sexual techniques, and coinfection with HIV-1 and other sexually transmitted infections (STIs) as potential risk factors [6-10]. However, these associations between sexual risk factors and HCV did not reach statistical significance, and were often confounded by concurrent IDU [4].

A growing number of case reports from Europe [11,12], the United States [13], and Canada [14] have suggested that HCV is emerging as an STI among HIV-1 positive MSM [15-17]. The availability of highly active antiretroviral therapy (HAART) in Western countries has been followed by an increase in sexual risk behavior among MSM and, thereby, increased infection rates of STIs such as rectal gonorrhoea and syphilis [18,19]. A decrease in the perceived threat of HIV/AIDS appears to have weakened the safe-sex attitudes of MSM. High-risk sexual networks have emerged that are susceptible to the introduction of HCV and other infectious agents for which sexual contact is actually an inefficient route of transmission [20,21].

Our study investigated HCV prevalence and incidence in a large prospective longitudinal cohort of both HIV-positive and HIV-negative MSM in Amsterdam, the Netherlands, over the course of nearly two decades (1984-2003). Additionally, 5 Amsterdam hospitals provided serum samples and data on parenteral and sexual risk behaviours of MSM given the diagnosis of an acute HCV infection after 2000. Phylogenetic analysis was used to obtain evidence of sexual transmission of HCV in MSM.

Materials and Methods

Study population I – the Amsterdam Cohort Studies (ACS) of MSM

The ACS of MSM is an open, ongoing prospective study [20]. Recruitment was started in October 1984, and uses “convenience sampling” (advertisements: brochures at the STI-clinic and social venues for MSM) and “chain referral sampling” (recruitment by participants among their acquaintances). The local ethical committee approved the ACS and all MSM participated only after they provided informed consent. Until 1995, the ACS included MSM of
all ages with at least two male sex partners during the 6 months preceding enrolment, regardless of HIV-status. After 1995, participation was restricted mainly to HIV-negative MSM, aged ≤ 30 years with at least one male sexual encounter during the preceding 6 months. Participants complete a self-administered standardized questionnaire at ACS visits every 6 months (if HIV-positive, every 3 months), about personal health and sexual risk behavior, and blood was drawn for HIV testing and storage at -80°C.

Our study included all ACS participants who made at least 2 study visits between October 1984 and January 2003 (n=1836). The last available serum sample predating February 2003 was tested for HCV antibodies, using a third-generation commercial microparticle EIA system (AxSym HCV version 3.0; Abbott). HCV-positive test results were confirmed by transcription-mediated nucleic acid amplification (Versant, Bayer Healthcare) and/or Immunoblot (Chiron RIBA HCV 3.0 SIA, Ortho-Clinical Diagnostics). When a participant was found to be HCV positive, his sample from ACS entry and succeeding visits were tested to determine whether he was positive at entry or, if not, between which visits HCV seroconversion occurred.

**Study population II – hospital case reports**

In the Netherlands, the law requires that all acute HCV infections (until 2003, all newly diagnosed HCV infections) be reported to the local health service to enable source tracing and to prevent the further spread of HCV. Through review of these reports and through contact with physicians treating HCV in 5 major hospitals, including the STI-clinic in Amsterdam, we identified 34 MSM who acquired HCV after the year 2000 and for whom blood was stored. Of these, 32 men had a confirmed seroconversion of anti-HCV antibodies and detection of HCV RNA after a previously HCV-negative sample. The other two men lacked a previous HCV-negative sample but acute HCV-infection (jaundice, elevated liver enzymes, and exclusion of hepatitis A and B) had been diagnosed by their physicians. To identify the probable route of transmission, additional data on possible IDU, blood-blood contact, STI coinfection, and sexual risk behaviours were obtained by review of medical charts and notification reports. For all patients, informed consent had been obtained by their treating physician.

**HCV RNA isolation and RT-PCR methods**

Using the first HCV-seropositive sample obtained for ACS participants and hospital patients, RNA isolation was performed on 100 µL of serum by the TriPure method (Roche Diagnostics). Each RNA isolate was used as input for 2 nested multiplex RT-PCRs. The first PCR, which targets the conserved HCV core region, was devised as a genotyping system to amplify HCV RNA of subtypes 1a, 1b, 2a, 2b, 3a, 4, 5a and 6a. The second PCR, which
targets the NS5B region, was used for phylogenetic analysis. Conditions and primers for both RT-PCRs have been described elsewhere [22].

**Sequencing and phylogenetic analysis**

The sequencing reaction was performed as by Van de Laar et al. [22]. First, NS5B PCR products were ethanol precipitated. Sense and antisense strands were separately cycle-sequenced using the BigDye Terminator system (version 1.1; Perkin Elmer). Sequence products were purified using DyeEx spin kits (Qiagen) and analyzed on an ABI-310 automated sequencer (Applied Biosystems). Sequence alignment of the 436-bp NS5B fragment was performed using the BioEdit software package [23]. Viral genotype was confirmed after phylogenetic analysis of the NS5B sequences obtained (GenBank accession numbers DQ897975-DQ898026 and EF123061-EF123064) along with 5 GenBank reference sequences [24]. Mega software (version 3.1; available at: http://www.megasoftware.net) was used to construct a phylogenetic tree by the neighbour-joining method, using the Tamura-Nei substitution model \( \gamma \)-distribution \((\alpha=0.40)\). Bootstrap values \((n=1000)\) were calculated to analyze the stability of tree topology. HCV sequences from MSM participants were compared to HCV sequences from HIV-positive European IDUs [25] Amsterdam (injecting) drug users [22,26], and Dutch blood donors [26].

**Statistical analysis - study population I**

Differences in potential determinants of HCV-infection at ACS entry (i.e., prevalent HCV) were tested univariately between HCV-positive and HCV-negative participants using Pearson’s \( \chi^2 \) test with, if necessary, Fisher’s exact test (for non continuous variables) or student’s t-test (for continuous variables). Variables tested were age (in years), a history of IDU during the preceding 5 years (yes/no), history of blood transfusion (yes/no), HIV-status (positive/negative), number of lifetime sex partners (1-20, 20-100, or >100), number of sexual partners in the preceding 5 years (1-20, 20-100, and >100) and self-reported history of syphilis (yes/no). To identify factors that were independently associated with a positive HCV serostatus at ACS entry, all variables were introduced in a multivariate. \( P< .05 \) was considered to be statistically significant.

HCV incidence per annum was calculated as the number of new infections divided by total person-years (PY) under observation for all ACS participants at risk and for HIV-positive and HIV-negative ACS participants separately. The midpoint between the last HCV-negative and first HCV-positive test was defined as the seroconversion date. Differences in HCV incidence before and after 2000 were analyzed using Poisson-regression. That year was chosen as the cut-off because increases in sexual risk behaviours [20], STIs [18], and HCV incidence among MSM [15] were apparent thereafter.
Results

Study population – ACS

The study population included 1836 MSM with ≥ 2 ACS visits between October 1984 and January 2003. Mean age at entry was 31.8 ± 8.1 years, 92% was of northwest European nationality, and 28% had at least a college degree. Median follow-up time in the ACS was 6.3 years (interquartile range [IQR]: 2.8 – 11.2 years). These 1836 men made a total of 44.107 visits at a median interval of 91 days (IQR, 49-162 days). Most (89%) identified themselves as (almost) exclusively MSM. Reported rates of risk behavior were high; at ACS entry, 69% had had >20 sex partners during the preceding 5 years, 28% tested positive for HIV-1 and 17% had a history of syphilis. Only 4 ACS participants (0.2%) reported IDU.

Table 1: Risk factors associated with positive hepatitis C virus (HCV) serostatus at Amsterdam Cohort Studies entry

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HCV status</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>seronegative</td>
<td>seropositive</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>31.7 ± 8.1</td>
<td>36.1 ± 9.1</td>
<td>1.1 (1.0-1.1)*</td>
</tr>
<tr>
<td>History of IDU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1693 (99)</td>
<td>18 (90)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>2 (0.1)</td>
<td>2 (10)</td>
<td>94.1 (12.5-705)†</td>
</tr>
<tr>
<td>History of blood transfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1761 (99)</td>
<td>23 (96)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (1)</td>
<td>1 (4)</td>
<td>60.5 (7.4-495)†</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1311 (73)</td>
<td>11 (46)</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>491 (27)</td>
<td>13 (54)</td>
<td>3.8 (0.5-29.7)</td>
</tr>
<tr>
<td>History of syphilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1474 (83)</td>
<td>22 (92)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>305 (17)</td>
<td>2 (8)</td>
<td>4.1 (1.6-10.4)*</td>
</tr>
<tr>
<td>Lifetime sex partners, no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-19</td>
<td>(505 missing)</td>
<td>(6 missing)</td>
<td>1</td>
</tr>
<tr>
<td>20-100</td>
<td>284 (22)</td>
<td>3 (17)</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>357 (27)</td>
<td>6 (33)</td>
<td>0.6 (0.2-2.5)</td>
</tr>
<tr>
<td>Sex partners in the preceding 5 years, no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-19</td>
<td>(730 missing)</td>
<td>(4 missing)</td>
<td>1</td>
</tr>
<tr>
<td>20-100</td>
<td>342 (32)</td>
<td>4 (20)</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>352 (33)</td>
<td>11 (55)</td>
<td>2.7 (0.8-8.5)</td>
</tr>
<tr>
<td></td>
<td>388 (36)</td>
<td>5 (25)</td>
<td>2.7 (0.8-8.5)</td>
</tr>
</tbody>
</table>

Data are no. (%) of subjects, unless otherwise indicated. CI = confidence interval; IDU = injection drug use; OR = odds ratio
* P<.01
† P<.001

HCV prevalence - ACS

At study entry, 24 (1.3%) of 1836 MSM tested positive for HCV antibodies. Of these, 2 had injected drugs and 1 had received a blood transfusion before the introduction of standard
Men who have sex with men

HCV-screening of blood donors (<1991). HCV-positive MSM were older than HCV-negative MSM (36.1 vs. 31.7 years; P=.008), and were more likely to have a history of IDU (10% vs. 0.1%; P<.001) or to test positive for HIV (54% vs. 27%; P=.005). In multivariate analysis, only a history of IDU (odds ratio [OR], 60.5, [95% confidence interval {CI}, 7.4-495]) and HIV-positivity (OR, 4.1, [95%CI, 1.6-10.4]) were significantly associated with a positive HCV status at ACS entry. No other factors reflecting sexual risk behavior - such as the number of partners or a history of syphilis - were associated with HCV prevalence (table 1).

HCV-incidence - ACS

Between October 1984 and January 2003, 8 MSM seroconverted for HCV, and after December 2000, 5 seroconverted; all 8 HCV seroconverters were HIV-positive; their median age was 38.7 years (IQR, 34.5-42.6 years). Overall, HCV incidence was 0.07/100 PY (95% CI, 0.03-0.13/100 PY); it was 0/100 PY among HIV-negative men (95% CI, 0-0.05/100 PY) and 0.18/100 PY among HIV-positive men (95% CI, 0.08-0.36/100 PY). The HCV incidence among HIV-positive MSM increased significantly after the year 2000, compared with previous years - 0.87/100 PY in 2000-2003 (95% CI, 0.28-2.03/100 PY) compared with 0.08/100 PY in 1984-1999 (95% CI 0.02-0.23/100 PY); P=.001 (table 2). The total number of HCV seroconverters in the ACS was too small to allow for a statistical analysis on sexual risk factors.

**Table 2**: Hepatitis C virus (HCV) incidence among HIV-positive and HIV-negative men who have sex with men (MSM) participating in the Amsterdam Cohort Studies over time

<table>
<thead>
<tr>
<th></th>
<th>No. PY</th>
<th>Incidence 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-negative MSM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984-1999</td>
<td>0</td>
<td>0.00, 0.00-0.05</td>
</tr>
<tr>
<td>2000-2003</td>
<td>0</td>
<td>0.00, 0.00-0.37</td>
</tr>
<tr>
<td>1984-2003</td>
<td>0</td>
<td>0.00, 0.00-0.05</td>
</tr>
<tr>
<td>HIV-positive MSM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984-1999</td>
<td>3</td>
<td>0.08, 0.02-0.23</td>
</tr>
<tr>
<td>2000-2003</td>
<td>5</td>
<td>0.87, 0.28-2.03</td>
</tr>
<tr>
<td>1984-2003</td>
<td>8</td>
<td>0.18, 0.08-0.36</td>
</tr>
<tr>
<td>Total MSM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984-1999</td>
<td>3</td>
<td>0.03, 0.01-0.08</td>
</tr>
<tr>
<td>2000-2003</td>
<td>5</td>
<td>0.32, 0.10-0.74</td>
</tr>
<tr>
<td>1984-2003</td>
<td>8</td>
<td>0.07, 0.03-0.13</td>
</tr>
</tbody>
</table>

CI = confidence interval; PY = person-years

Hospital case-reports

For the 34 hospital participants who had a diagnosis of HCV in the 5 participating hospitals in Amsterdam, the median duration between the last HCV-negative test and the first HCV-
positive test was 201 days (IQR, 98-472 days). All but 1 subject were HIV positive at the moment of HCV seroconversion; their median age was 40.0 years (IQR, 35.3-46.6 years). Medical chart reviews, performed for 27 of 34 hospital cases, found no IDU during the 6 months preceding seroconversion; 1 MSM reported possible blood-blood contact after assisting at an accident involving a presumed drug user. Sexual risk behavior among the hospital subjects was high; at least 16 (59%) of 27 had 1 or even 2 ulcerative coinfections within the 6 months preceding HCV seroconversion: lymphogranuloma venereum (LGV; 10/27), syphilis (8/27), and/or herpes simplex virus 2 (3/27). Other STIs mentioned were rectal gonorrhoea (10/27), acute hepatitis B virus (2/23), rectal non-LGV Chlamydia trachomatis (3/27), and genital warts (3/27). Of the 27 men, 15 (56%) practiced anal fisting. In total, 23 (85%) of 27 reported either an ulcerative coinfection and/or anal fisting; 2 of the remainder reported neither IDU, blood-blood contact nor high-risk sexual contact.

Table 3: Hepatitis C virus (HCV) genotype distribution among seropositive men who have sex with men (MSM) in Amsterdam over time

<table>
<thead>
<tr>
<th>HCV infections among MSM over time</th>
<th>After 2000: ACS and hospital cases, incident, no (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV subset</td>
<td>Prevalent, no</td>
</tr>
<tr>
<td>RNA-positive*</td>
<td>15/24 (63)</td>
</tr>
<tr>
<td>HCV subset</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2</td>
</tr>
<tr>
<td>1b</td>
<td>5</td>
</tr>
<tr>
<td>2b</td>
<td>2</td>
</tr>
<tr>
<td>3a</td>
<td>4</td>
</tr>
<tr>
<td>4d</td>
<td>2</td>
</tr>
<tr>
<td>Untypeable</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
</tr>
</tbody>
</table>

ACS = Amsterdam Cohort Studies
* Data are no. positive/total no. of subjects (%)

Genotyping and phylogenetic analysis

All HCV seroconversion samples (8 ACS cases) and samples obtained at the time of HCV diagnosis (34 hospital cases) tested positive for HCV RNA, as did 15 (63%) of 24 MSM who were positive at ACS entry. Table 3 shows the HCV genotype distribution among Amsterdam MSM infected before and after 2000. HCV infections predating 2000 were mainly of subtypes 1b and 3a (56%), whereas, after 2000, subtypes 1a and 4d predominated (85%).

Figure 1a represents a phylogenetic tree of 56 partial HCV NS5B sequences obtained from the Amsterdam MSM participating in the study. MSM who acquired HCV infection after 2000 had a high degree of clustering, showing 3 monophyletic clusters (cluster I-III) and, additionally 3 pairs (a-c) of nearly identical HCV strains. When MSM who contracted HCV before 2000 were compared with those who contracted HCV after 2000, none of the 17 HCV
isolates from before 2000 and 33 (85%) of 39 HCV isolates from after 2000 had at least 1 nearly identical HCV isolate. The 2 MSM (S11 and S39) with recent HCV-infection who reported no IDU, blood-blood contact or high-risk sexual contact, did not have a clustered or paired HCV isolate, suggesting unrelated routes of transmission. The MSM (S18) who assisted in the accident with the presumed IDU, who also reported unprotected sex, was part of cluster III.

Figure 1: LEFT, NS5B phylogeny of prevalent (P) and incident (S) HCV infections among men who have sex with men (MSM) in Amsterdam. A neighbour-joining tree based on the Tamura-Nei substitution model with $\gamma$-distribution ($\alpha = .40$) is shown. Isolates from MSM who acquired HCV after the year 2000 are outlined (n=34; hospital cases) or outlined and shaded (n=5; Amsterdam Cohort Studies [ACS] participants). Isolates not outlined were from ACS participants who contracted HCV before the year 2000 (n=17). Each isolate code also contains the year of sampling. * Two hospital case patients diagnosed with an acute HCV infection who had no previous HCV-seronegative sample available; † 2 hospital case patients who reported no injection drug use (IDU), blood-blood contact, or high-risk sexual contact. RIGHT, HCV NS5B phylogeny of HCV genotype 4 infections among different risk groups. A neighbour-joining tree based on the Tamura-Nei substitution model with $\gamma$-distribution ($\alpha = .40$) is shown. Isolates from MSM are outlined, from IDUs are light gray, and from Dutch immigrants are dark gray. Isolate codes contain the city and year of sampling. * Dutch MSM who also admitted to a history of IDU; † Dutch donors without any reported risk behavior; § non-injection drug user from Surinam; ¶ Dutch IDU without any known connection with Egypt. Ali/Cas, Alicante/Castellon (Spain); Ams, Amsterdam (the Netherlands); Inn, Innsbruck (Austria); Mar, Marseille (France); Tur, Turin (Italy).
The largest cluster (III) includes 14 MSM infected with HCV genotype 4d. To address whether their HCV strains resemble strains circulating in other risk groups, we constructed a phylogenetic tree (figure 1b) of all 51 HCV genotype 4 sequences in our database [26]: from MSM in this study (n=16), IDUs in Amsterdam (n=17) and other European cities (n=10), and Dutch blood donors (n=8). For genotype 4, but also for genotype 1a (data not shown), different risk groups can be distinguished based on phylogeny. Although MSM and IDUs both were infected with strains of HCV subtype 4d, the 14 MSM (Cluster III) who contracted HCV infection after 2000 form a separate cluster within this subtype (bootstrap value, 91). Sequences obtained from 2 MSM who acquired HCV infection before 2000 (P2 and P7) were interspersed with sequences of IDUs from Amsterdam; both of them had reported IDU. Interestingly, geographic clustering was observed of IDUs from Amsterdam, and those from Southern-Europe.

Discussion

A rapidly increasing number of case reports from Europe [11,12], the United States [13], and Canada [14] have suggested recent outbreaks of sexually transmitted HCV among HIV-1 infected MSM [15-17]. To our knowledge, this article is the first to suggest an increase in HCV incidence over time among HIV-positive but not HIV-negative MSM on the basis of 20 years of prospective longitudinal cohort data. Additional phylogenetic analysis of HIV/HCV-coinfected cohort and hospital cases strongly supports the presence of an MSM-specific transmission network, probably sexual, in Amsterdam.

As in London, Paris, and Rotterdam [11,17,27], phylogenetic analysis of incident HCV infections among MSM in Amsterdam showed at least three distinct clusters of independent cocirculating HCV lineages; 83% of Amsterdam MSM infected after 2000 harboured a strain present in at least 1 other MSM in this study. This degree of clustering is typical of a common source of infection and confirms sustained HCV transmission among HIV-positive MSM over the past few years. The low evolutionary distances among recent HCV isolates, the shift in genotypes over time (1b and 3a before 2000 vs. 1a and 4d after 2000), and the lack of clustering among isolates predating 2000 all imply a recent spread of HCV that was absent before 2000. The fact that multiple introductions of different HCV genotypes took place suggests behavioural change in MSM rather than evolution of the virus into a variant that is more easily transmitted.

No sexual transmission of HCV has been reported in recent HCV screenings among MSM in Seattle (n=833) [28] and Montreal (n=1085) [4]; 35 (85%) of 41 infections could be traced to IDU. However, persons screened were HIV-negative MSM, a sub-population almost unaffected by the current HCV outbreaks [29]. Moreover, HCV screening stopped before the onset of the HCV outbreaks reported in Europe. By contrast, Amsterdam MSM infected after
2000 reported no IDU, and like Parisian MSM [11], they carried MSM-specific HCV strains that differed significantly from those circulating among IDUs. Thus, IDU as an underlying mechanism of HCV transmission among Amsterdam MSM is highly unlikely. In a Swiss cohort study among HIV-infected MSM, increased HCV incidence was associated with sexual risk-taking and inconsistent condom use, especially in younger MSM [5]. Most likely, the current outbreak of HCV is triggered by the increase in sexual risk-taking observed among MSM after the introduction of HAART [20,21].

How and when might sexual transmission of HCV occur? Among persons infected with HCV alone, >95% intermittently shed HCV RNA in saliva [30], and 42% of HIV/HCV co-infected subjects shed HCV RNA in semen [31]. The rare event of sexual transmission among MSM, however, suggest that the rectal mucosa is relatively resistant to HCV-infection. HIV-1 infection facilitates HCV-transmission by increasing both (1) viral infectiousness due to higher viral loads, and (2) viral susceptibility through its detrimental effects to the gastrointestinal-associated immune system [32,33]. Moreover, ulcerative STIs can enhance sexual transmission of both HIV-1 and HCV [8,34] by creating a portal of entry through the mucosal barrier. Quite possibly, HIV infection in itself makes the host vulnerable to HCV infection in combination with the above [35]. The observation that ulcerative STIs facilitate the transmission of HCV infection is consistent with the upsurge of STIs among MSM in countries where HAART is generally available [19,20] and the high number of ulcerative coinfections reported by MSM with acute HCV-infection [16,17,27]. However, STIs and sexual risk-taking have no higher incidence now than in the early eighties, when the sexual transmission of HCV seemed absent. What else has changed?

First, current outbreaks of HCV coincide with the recent epidemic of LGV, a “new” STI that manifests as anorectal ulcers, hemorrhagic proctocolitis, and hyperplasia of intestinal and perirectal lymphatic tissue. Both HCV and LGV are strongly associated with a positive HIV-status [36], and, in the Netherlands, acute HCV is reported among MSM coinfected with LGV [27]. This suggests a potential role for LGV in the outbreak of HCV reported in the Netherlands. Second, successive outbreaks of syphilis, rectal gonorrhoea, *Shigella sonnei*, LGV, and acute HCV among MSM in major European cities [16,18,19,27,36,37] points to the presence of a high-risk “core group” of HIV-positive MSM. This “core group” functions as a reservoir for STIs and is highly susceptible to new or even inefficiently transmitted infections such as LGV and HCV. The popularity of the Internet, trans-European MSM weekend tourism, and organized sex parties may have increased contacts among HIV-positive MSM who share risky sex and are relatively unconcerned about acquiring “treatable” STIs [38]. Lesions in the rectal endothelial lineage could occur especially with rough sexual techniques such as anal fisting, the use of toys, and group sex, which favour blood-blood contact and, hence, HCV-transmission [14,17,27,39]. In 2005, anal bleeding during sex was reported by 10% of young (<35 years old) HIV-negative participants in the ACS. The use of psychoactive substances, to enhance sexual pleasure and anal sphincter relaxation during receptive anal...
intercourse or to remove inhibition during rough sexual practices, might also cause mucosal trauma or bleeding, especially when substances are applied anally [40].

The increased HCV incidence among MSM has clinical implications. HIV/HCV coinfection is associated with lower rates of spontaneous HCV clearance, accelerated liver disease, and less favourable HCV treatment outcome [41]. HCV combination therapy achieves a sustained virological response in only 17% of HIV-positive individuals infected with genotypes 1 or 4, [42] - the HCV strains that affect the majority (91%) of MSM in Amsterdam. In 90%-98% of HIV-negative patients, however, HCV chronicity can be prevented when treatment is initiated during the acute phase of infection [43]. The results of early interferon treatment in HIV-positive MSM infected with HCV genotypes 1 and 4 are conflicting but promising [11,42,44].

Several limitations should be considered while interpreting our results. Despite a significant rise in HCV incidence after the year 2000, the low number of HCV incident cases among ACS participants makes it necessary to investigate whether this trend continued after 2002. All HIV-positive ACS participants with extended follow-up after 2002 will be tested for HCV prospectively (or retrospectively at their last ACS visit). The high number of hospital cases collected after 2002, however, indicates sustained HCV transmission among HIV-positive MSM. Second, hospital cases were collected in a clinical setting, not for epidemiological research. Although high rates of concurrent STIs and fisting among hospital cases are suggestive for permucosal transmission of HCV during sexual contact, a control group is needed to establish whether these rates are significantly higher than are those in HIV-positive MSM without HCV coinfection. Additionally, HIV-positive MSM visit a physician more frequently, and thus are more likely to receive HCV and STI testing than those who are HIV-negative. Testing therefore might have occurred on indication and might have biased our conclusions about HCV and high levels of STIs. ACS data confirm, however, that HCV mainly circulates among HIV-positive MSM, given that no HCV seroconversions were identified among HIV-negative MSM. Third, retrospective data collection based on self-reported behavior may be compromised by report and recall bias. However, all 39 recently infected MSM in this study denied IDU, which accounts for the vast majority of new HCV infections in western Europe. Moreover, phylogenetic analysis confirmed a non-IDU related common source of infection. Data on possible other confounders (e.g. tattooing/piercing, acupuncture or various nosocomial transmission routes) were not always available, but such general routes of incidental transmission cannot explain the common source of HCV infection observed among MSM. Potential subculture-related confounders such as intranasal/anal drug and enema use need further investigation.

Despite these limitations, the present results indicates that HCV appears to be emerging as an STI among a restricted "core group" of HIV-positive MSM who practice risky sex. Given the absence of clear clinical symptoms during acute HCV infection, a significant proportion
of the HCV epidemic may still remain unnoticed. Routine HCV screening among HIV-positive MSM is needed to identify the magnitude and character of the current epidemic and to enhance treatment. Although the exact biological mechanism of transmission has yet to be determined and should be examined in a prospective cohort or retrospective case-control study, targeted prevention can deter further spreading and increase awareness in HIV-positive MSM about the additional risks associated with acquiring serious bloodborne coinfections.

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References

Chapter 4


Men who have sex with men


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