Epidemiology and diagnosis of acute hepatitis C virus infection

Vanhommerig, J.W.

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EPIDEMIOLOGY AND DIAGNOSIS OF ACUTE HEPATITIS C VIRUS INFECTION

Joost Vanhommerig
R.J.H. Fortuynplein 172
1019WN Amsterdam

PARANIMFEN
0651198630
martijnvnhommerig@gmail.com

Wendy van der Veldt
0638723626
wvdveldt@ggd.amsterdam.nl

Na afloop bent u van harte welkom op de receptie ter plaatse.

Joost Vanhommerig
R.J.H.-Fortuynplein 172
1019WN Amsterdam

PARANIMFEN
Martijn Vanhommerig
0651198630
martijnvnhommerig@gmail.com

Wendy van der Veldt
0638723626
wvdveldt@ggd.amsterdam.nl

UITNODIGING
EPIDEMIOLOGY AND DIAGNOSIS OF ACUTE HEPATITIS C VIRUS INFECTION

Joost Vanhommerig
EPIDEMIOLOGY AND DIAGNOSIS OF ACUTE HEPATITIS C VIRUS INFECTION

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**Universiteit van Amsterdam**

**Co-promotores:**
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INTRODUCTION AND OUTLINE OF THIS THESIS
HEPATITIS C VIRUS - A BRIEF HISTORY

After the development of diagnostic tests for hepatitis B virus (HBV) in 1963 and hepatitis A virus in 1973, it was clear that most cases of blood transfusion-associated hepatitis were not caused by these or other known viruses [1]. The causative agent of this blood-borne, non-A, non-B hepatitis virus was designated the hepatitis C virus (HCV) after its discovery in 1989. HCV is a small (50 nm), spherical, enveloped, positive sense, single-stranded RNA virus, and belongs to the genus *Hepacivirus* of the *Flaviviridae* family [2]. Soon after HCV was identified, genome sequences that became available showed a high level of genetic diversity. Currently, 7 genotypes have been described, covering at least 67 subtypes (e.g., 1a, 1b, 1c) [3,4]. HCV is considered a serious global health problem; an estimated 2.6-3.1% of the world’s population (equivalent to 169-202 million people) is chronically infected and is therefore at risk of developing liver disease [5–7]. Currently, over 350,000 deaths are attributed to chronic HCV infection each year worldwide [8]. By 2007, mortality associated with HCV infection had surpassed that from human immunodeficiency virus (HIV) infection in the USA [9].

EPIDEMIOLOGY

Worldwide
In many developed countries, the prevalence of HCV infection in the general population is less than 2% (figure 1). In several countries in Latin America, the former Soviet Union, Africa, the Middle East, and Southern Asia, the prevalence is higher (>2%). Egypt has the highest prevalence of at least 15%, due to unsafe community-wide parenteral therapy against schistosomiasis from the 1920s until the 1980s [10–13]. India has a lower prevalence of approximately 0.5-1.5% due to a (historically) underdeveloped healthcare system [14,15], but because India is the second most populous country in the world with currently over 1.2 billion inhabitants, the disease burden is among the highest [15].

The Netherlands
The most recent HCV seroprevalence estimate in the Netherlands was 0.22% (range: 0.07-0.37), corresponding to approximately 28,100 individuals (range: 9,600-48,000) [16]. The majority (i.e., 41%) of HCV positive individuals is estimated to be first generation migrants from high-endemic countries. In the Netherlands, HIV-infected men who have sex with men (MSM) are currently the main group at risk for HCV infection [17,18], while HCV seroprevalence is highest among people who inject drugs (PWID); estimates range from 46.7-78.4% for HIV-negative PWID, up to 91.6-95.9% for HIV-positive PWID [16]. The estimated prevalence of chronic HCV infection among ever-injecting PWID in Amsterdam was 80.7% in 2010 [19]. Currently, very few incident infections occur among PWID in the Netherlands, mostly because the total number of PWID, the number of new PWID, as well as injection risk behavior has
been decreasing since 1986 [20,21]. One of the major contributors to the observed decrease in incidence is a high competing mortality rate due to HIV infection in HCV-infected PWID [19]. A declining trend is also seen in the US [22], and in Australia (after 2003) [23], although the decline is not as profound as in the Netherlands.

![Figure 1](image)

**Figure 1** Global prevalence and genotype distribution of HCV infection, by Global Burden of Disease (GBD) region. The size of the pie charts is proportional to the number of seroprevalent cases [7]. Copyright © 2014 Messina et al., reprinted with permission of the publisher, John Wiley & Sons, Inc.

**TRANSMISSION**

The three main transmission routes along which HCV currently travels are: (1) sharing of injecting equipment among PWID [24], (2) nosocomial transmission, and (3) sexual transmission, which is mostly observed among HIV-infected MSM [25,26]. Mother-to-child transmission rates are estimated to be 5-7% [27,28], and are up to four times higher among HIV/HCV-coinfected mothers [28,29]. Coinfection with HIV and HCV is relatively common because both viruses share the same transmission routes; up to one third of HIV-infected individuals are estimated to be co-infected with HCV [30–32].

**People who inject drugs**

PWID are considered to be at the highest risk of HCV infection [33–35]. Worldwide, over 75% of incident infections occur among PWID [36]. Because transmission of HCV is more than 10 times more likely to occur through blood-blood exposure than transmission of HIV [37], HCV infection usually precedes HIV infection among PWID.

**Nosocomial exposure**

Before 1992, no serological screening tools were available for HCV. Patients who had a blood
transfusion (e.g., hemophiliacs) or organ transplantation prior to this year were therefore at increased risk of HCV infection. In developed countries, incidence of HCV infection after a transplant or transfusion decreased from 5.0-13.0% before 1986, to 1.5-9.0% between 1986-1990, to 0.6-3.0% thereafter [5]. In developing countries, nosocomial transmission is mostly caused by receiving blood(products) from unscreened donors, or other unsafe medical procedures [36].

**Sexual transmission**

Among serodiscordant monogamous heterosexual couples, sexual transmission of HCV is considered negligible [38]. However, in the early 2000s multiple outbreaks of HCV were reported among HIV-infected MSM in Australia [39], France [40], Germany [41], the Netherlands [42,43], Switzerland [44], the UK [45,46], and the USA [47]. Analysis of multiple, mostly European, cohorts in a collaborative study known as the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE), revealed that the incidence of HCV among HIV-infected MSM appeared to be slightly elevated already during the mid-1990s, but the main expansion started after 2002 [48]. Whether the increase in HCV incidence was due to injecting drug use of these men was, for a long time, debated [49]. The HCV epidemic among MSM seems to be largely restricted to those infected with HIV [50,51]. This may partly be a reflection of higher sexual risk behavior among MSM who acquire HIV infection compared to MSM who do not. The practice of MSM selectively having unprotected sex with men of the same HIV-status, referred to as serosorting [52], may have added to the spread of HCV infection among HIV-infected MSM [53]. However, an enhancing effect of HIV infection itself on susceptibility and/or infectivity for HCV may also exist [54]. In addition, HIV may be more efficiently transmitted through sexual contact than HCV and the prevalence of HIV was, at least at the start of the HCV epidemic, lower for HCV than HIV; HIV-negative MSM with risky sexual behavior would therefore be more likely to first acquire HIV, and later on HCV [43]. Having concomitant other, especially ulcerative, sexually transmitted infections (STI), may also increase transmissibility of HCV [50].

**VIROLOGY**

**Genome organization**

HCV consists of a single open reading frame with approximately 9,400 nucleotides, that encode for a single, polyprotein that contains slightly over 3000 amino acids (depending on the genotype). This polyprotein is post-translationally cleaved into three structural proteins (core, \( E1 \) and \( E2 \)), seven non-structural proteins (\( p7, NS2, NS3, NS4A, NS4B, NS5A, \) and \( NS5B \)), and is flanked by two highly conserved untranslated regions (UTR; figure 2). The 5’UTR consists of four highly structured domains and contains the internal ribosome entry site (IRES). The IRES is essential for the virus to use the ribosomes in the host cell for the initiation of translation. The 3’UTR consists of stem-loop structures and an internal poly(U/UC) tail [55].
Life cycle

Human hepatocytes are the main target of HCV. Viral entry occurs through receptor mediated endocytosis. Virus receptors and co-receptors on the host cell that are involved in cell entry include tetraspanin CD81, scavenger receptor class B type I (SR-B1), claudin-1, occludin, Niemann-Pick C1-like 1 (NPC1L1), and low-density lipoprotein (LDL) receptor [55–58]. Mannose-binding lectins DC-SIGN and L-SIGN may also facilitate internalization [59,60]. After viral entry, translation takes place at the ribosomes located at the endoplasmic reticulum (ER), after which the polyprotein is cleaved by activity of both cellular and virally encoded proteases, the latter including NS2, NS3, and co-factor NS4A. The NS5B protein serves as an RNA dependent RNA polymerase [57].

Replication takes place in the so-called ‘membranous web’, a web-like structure which is formed through NS5A-induced alterations of the membrane, and consists of ER-derived vesicles. All HCV proteins accumulate on the membranous web to form a viral replication complex [61]. New positive sense RNA strands are generated by the NS5B polymerase through transcription of an intermediate negative sense RNA template strand. The lack of a proofreading mechanism of the viral polymerase is the cause of a large intra-host genetic diversity, sometimes referred to as quasispecies [3]. Excess positive sense RNA may be used (again), either for translation, or as templates for replication [57]. Finally, virus particles are assembled by recruiting envelope
(E1 and E2) complexes and budding at the ER membrane. Mature virions are released by exocytosis [62].

**Diagnosis**

Standard HCV testing is performed by screening for the presence of HCV-specific immunoglobulin G (IgG) antibodies in serum. Commercial assays that can detect IgG response of HCV antibodies (anti-HCV) are enzyme immunoassays (EIA) that express recombinant proteins (i.e., core, NS3, NS4, and NS5). Anti-HCV is usually detectable within 8-20 weeks of infection [63]. Conflicting results regarding delayed seroconversion due to HIV co-infection have been published; this topic is addressed in part III of this thesis. The recombinant immunoblot assay (RIBA) was developed as a supplemental assay to enhance specificity of positive anti-HCV EIAs [64]. Currently, the primary purpose of RIBA testing is to distinguish between resolved HCV infection (EIA positive, RNA negative, RIBA positive) and false-positivity of the EIA (EIA positive, RNA negative, RIBA negative). RIBAs are rarely used in clinical practice for some years now, because clinical relevance is determined by the presence or absence of HCV RNA rather than a false-positive result of the EIA. Therefore, a positive anti-HCV test is more likely followed by an HCV RNA test. Commercially available quantitative HCV RNA assays are very sensitive and can have a lower limit of detection (LLOD) of 15 international units (IU) ml⁻¹. Another HCV-specific diagnostic assay targets the core antigen. The currently available core antigen assay is less sensitive than HCV RNA assays (LLOD: 500-3000 IU ml⁻¹) but, because it can be up to four times cheaper and results may be available sooner than RNA testing, it may be a relevant alternative for low-resource countries. The core antigen assay may also be used as a screening tool for HCV infection among HIV-infected individuals, as low HCV viral loads are rarely reported in HIV/HCV-coinfection [65]. A non-specific way of screening for HCV includes regular assessment of serum alanine aminotransferase (ALAT) concentration; elevated liver enzyme concentrations may indicate hepatic inflammation [66], even when the elevation is considered ‘mild’ [67].

**MOLECULAR EPIDEMIOLOGY**

Diversity among HCV strains

For HCV, inter- and intra-genotype sequence similarities are around 65-70% and 70-85%, respectively, depending on the genetic (sub)region of HCV [4]. When genetic similarity between different viral strains is larger, it is likely that a common source of infection and/or transmission route exists [68]. Genotype distribution differs by both geographic region (figure 1) and transmission route. Worldwide and in the Netherlands, the prevalence of HCV genotype 1 is highest, followed by genotypes 2 and 3. Genotype 4 is most prevalent in Africa and the Middle East, genotype 5 in Southern sub-Saharan Africa, and genotype 6 in South-East Asia [69,70]. Regarding differences in genotype distribution by transmission route, subtypes 1a, 3a, and
4d are commonly found among PWID [71,72], while subtypes 1b, 2a and 2b are associated with contaminated blood products [70,73–75]. Among HIV-infected MSM, subtypes 1a and 4d are the dominant genotypes [41,71,76]. In order to determine the genotype or subtype of a diagnosed infection, part of the genome can be sequenced; regions that are particularly distinctive are the core [77] and NS5B regions [78]. Alternatively, a commercial line probe assay (LiPA) [79] and a RealTime HCV genotype assay [80] are available that target the conserved 5’UTR and core regions.

Phylogenetic analysis
Because of the genetic diversity that is apparent among different genotypes and subtypes, phylogenetic analysis can be used as a tool to study the evolutionary relationship between different variants, which can be used to identify potential transmission networks. For this, the genetic distance (or divergence) between different viral strains is represented in an evolutionary tree. The absolute difference between two sequences, often referred to as p-distance, underestimates the number of (synonymous and non-synonymous) nucleotide substitutions [81]. Therefore, more complex substitution models have been developed that correct for differences in base frequency, transversion and transition rates (including reversals) [82–87]. When the divergence between sequences is small, a monophyletic cluster may be visible; a group of sequences that share a common ancestor. To determine the robustness of the inferred phylogenies, bootstrapping is a frequently used method [88]. In order to perform molecular epidemiological studies, sociodemographic information and preferably also data on risk behavior from each study subject is combined with the phylogeny of a gene of interest. For HCV, NS5B, core, and/or envelope sequences are often used for investigating its molecular epidemiology [41,71,78,89–92].

NATURAL HISTORY

Clinical course of infection
The majority of acute HCV infections (>70%) pass by clinically silent; among patients who do experience symptoms during acute infection, those most frequently reported are not specific for HCV infection: loss of appetite, fatigue, abdominal pain, nausea, and rarely icterus (jaundice) [93,94]. Approximately 25% of acute HCV infections are spontaneously cleared, usually within the first six months of infection [95–97], but clearance rates ranging from 11-49% have been reported [98,99]. While the underlying mechanisms of spontaneous clearance are not fully understood, several associations are known to positively affect the ability to clear HCV: younger age at time of infection [100,101], female gender [102–107], HBV co-infection, HIV-negative status [107], elevated bilirubin, elevated ALAT, and elevated interferon-γ-inducible protein (IP)-10 levels [108,109], favorable host single-nucleotide polymorphisms rs12979860 and/or rs8099917 in the interferon-lambda (IFNL) 3 gene
(formerly known as IL28B) [107,110], and among individuals infected with HIV: higher CD4+ T-cell count [108]. Patients that progress to chronic infection are at risk of accumulation of hepatic fibrosis and may develop cirrhosis (10-20% after 20-30 years of infection) without therapeutic intervention [95]. Hepatic fibrosis is the result of collagen production by hepatic stellate cells. Normally, hepatic stellate cells are dormant and store the majority of the body's vitamin A, but liver damage will lead to an activated state of these cells [111]. Cirrhosis is a condition in which the architectural organization of the liver is significantly altered due to hepatic fibrosis; this may lead to impaired liver function, and portal hypertension. Once the liver has become cirrhotic, risk of hepatocellular carcinoma and hepatic decompensation are respectively 1-5 and 3-6% per annum [112]. The level of fibrosis is often scored using either the METAVIR [113] or the Ishak [114] scale; these scales range from F0 or 0 (i.e., no fibrosis) to F4 or 6 (i.e., cirrhosis), respectively. HIV/HCV-coinfected individuals have an accelerated progression of liver disease and increased mortality [30,32,115]. The exact mechanisms behind the associations with accelerated disease progression in coinfected individuals compared to HCV-monoinfected individuals have not been fully elucidated. Hypotheses include a direct effect of HIV-infection on the hepatocytes and/or stellate cells, and HIV-induced immunologic dysregulation, e.g., diminished HCV specific T-cell responses, increased immune activation, and increased hepatocyte apoptosis [30].

**HCV reinfection**

A previously cleared infection (either spontaneously or treatment-induced) does not protect against reinfection, although partial protective immunity against viruses similar to the primary infection may be developed [68,91,116–118]. Chimpanzees that were re-exposed to HCV in reinfection studies have (but not consistently) shown cross-genotype immunity (i.e., protection against heterologous virus strains) [119]. Among HIV/HCV-coinfected MSM that spontaneously cleared, or were successfully treated for acute HCV infection, the incidence of reinfection has been estimated to be between 8.0-15.2 per 100 person-years (PY) of follow-up [120,121]. Among PWID treated for chronic infection, incidence of reinfection was 0.8-4.7 per 100 PY [68], and among PWID that cleared acute infection, reinfection incidence was 1.8-46.8 per 100 PY [118,122]. Reinfection is usually supported by the observation that a different viral strain is present after a patient had an undetectable viral load. Usually, sequence analysis of the NS5B gene will suffice to determine this [68]. However, in a (relatively) recent outbreak setting as is the case for HIV-infected MSM, reinfection with the same HCV genotype may only be determined (or excluded) using sequence analysis of a more variable region, e.g., the hypervariable region 1 (HVR1; also see figure 2), located in the E2 gene [91,121]. This part of the HCV genome allows for more detailed analysis because it has a faster evolution rate, driven by the host immune response, than the NS5B region.

Dual or mixed infection with two (or more) distinct HCV variants may, depending on the timing of these infections, be classified as coinfection or superinfection. Dual infection
refers to infection with two or more heterologous HCV variants simultaneously, or within a time frame in which no immunologic response to the first virus has yet been established. Superinfection is defined as infection with a heterologous virus after persistent infection of the first variant has already developed [123,124]. Few studies have examined the frequency of superinfection; three small studies among PWID reported frequencies ranging from 0% to 24% [124]. However, the impact of dual/mixed HCV infection or superinfection on clinical outcomes of the disease remains unclear [124,125].

TREATMENT

Successful treatment is indicated by a sustained virological response (SVR), meaning that no detectable HCV viral load is present 24 weeks after treatment cessation; this is regarded as a cure. Currently SVR12 is often reported, meaning 12 weeks after end of treatment (EOT) no HCV viral load can be detected. Viral relapse is defined as having a detectable HCV RNA at 24 weeks following EOT, without detectable HCV RNA at EOT. To exclude reinfection (discussed hereafter), this treatment outcome is generally supported by confirmation that the same viral strain was present before and after EOT.

Because the number of treatment options has increased enormously since 2013 and will continue to do so in the coming years, this paragraph was updated until 1 December 2015 using the Dutch HCV treatment guidelines, published by the NIV (Dutch internists association), NVHB (Dutch association for HIV care providers), NVMDL (Dutch association of gastrointestinal and liver doctors), NVH (Dutch society of hepatology), and NVZA (Dutch association of hospital pharmacists). In addition, www.hcvguidelines.org was accessed; this website is a cooperative effort from the American Association for the Study of Liver Diseases (AASLD), the Infectious Diseases Society of America (IDSA), and the International Antiviral Society-USA (IAS-USA) to constantly update recommendations for testing, managing, and treating HCV.

An increasing number of different treatment regimens can be used to treat an HCV infection. In the late 1980s, interferon α was used to treat chronic HCV infection [126,127]. The US Food and Drug Administration (FDA) approved interferon α-con1 for treatment of HCV infection in patients with compensated liver disease in 1997. Ribavirin was initially developed for treatment of HIV infection [128], but was more effective for treatment of flaviviruses. Therefore, studies were conducted to evaluate the effect of ribavirin therapy on patients with chronic HCV infection [129,130]. Ribavirin was initially developed for treatment of HIV infection [128], but was more effective for treatment of flaviviruses. Therefore, studies were conducted to evaluate the effect of ribavirin therapy on patients with chronic HCV infection [129,130]. Ribavirin therapy was found not to affect the HCV RNA load, but led to a decrease in ALT. Subsequently conducted studies showed that the combination of interferon α and ribavirin was more effective for treatment of chronic HCV infection than was therapy with either of these agents [131]. Ribavirin was approved by the FDA in 1998.
Pegylated (peg) interferon was developed in the late 1990s and was shown to be more effective than interferon [132]. Peg-interferon α-2b and peg-interferon α-2a were approved in 2001 and 2002, respectively. Since then, treatment for chronic HCV mono-infection consisted of peg-interferon and ribavirin for 12-24 weeks for genotypes 2 and 3 (response rate: 76%-80% SVR), or 24-48 weeks for genotypes 1 and 4 (response rate: 46%-60% SVR [133–135]. In 2002, ribavirin was also approved for combination therapy in HIV/HCV-coinfected patients. SVR rates obtained among HIV/HCV-coinfected patients were higher using dual therapy, but were lower than among HCV mono-infected patients [136]. Outside clinical trials, acute HCV infection among HIV-infected patients are currently treated with peg-interferon and ribavirin for 24 weeks, irrespective of the genotype [137,138]. While therapy with peg-interferon is highly effective for treatment of recently acquired HCV, the optimal timing of treatment initiation, regimen and influence of host factors remain unclear [139]. Adverse effects of treatment with peg-interferon and ribavirin may be clinically significant, and may require discontinuation of treatment; these adverse effects include severe depression, insomnia, suicide, and pancytopenia [140,141].

Recently, a new era of HCV treatment has begun, with medication known as direct acting antiviral drugs (DAA) [142]. DAAs directly target key components of the viral life cycle. When the life cycle cannot be completed, no viable virus can be produced. Viral targets of HCV DAA are: $NS3$ (protease inhibitor (PI); all have the extension -previr) [143], but also inhibitors of the viral replicase complex, e.g., (non-) nucleoside and nucleotide $NS5B$ polymerase inhibitors (all have the extension -buvir), and $NS5A$ inhibitors (all have the extension -asvir) [143]. In April 2012, two PIs were licensed in the Netherlands: telaprevir and boceprevir [144–147]. These drugs were administered in combination with peg-interferon and ribavirin and improved SVR rates, but also significantly increased side effects like rash, pruritis and anemia [148,149]. Both boceprevir and telaprevir have already become obsolete due to the development of more favorable DAAs. However, treatment of acute HCV infection among HIV/HCV-coinfected patients may be shortened to 12 weeks when triple therapy with peg-interferon, ribavirin, and telaprevir or boceprevir is given [150,151]. Currently, sofosbuvir, simeprevir, daclatasvir, ombitasvir-paritaprevir-ritonavir with or without the addition of dasabuvir, and ledipasvir-sofosbuvir have been approved for treatment of chronic HCV infection in the Netherlands, regardless of METAVIR stage [152–154]. Ombitasvir-paritaprevir-ritonavir, and daclatasvir-asunaprevir-beclabuvir are the next regimens in line for approval. The chosen regimen (and the addition of ribavirin) depends, among others, on the HCV genotype, prior response to treatment, HIV treatment regimen, the degree of fibrosis, and renal insufficiency [144,155–161].

DAAs allow for shortened, simplified treatment and are better tolerated than interferon-based treatment regimens. The proportion of patients that achieve an SVR following DAA therapy is about 95%, but may be lower for patients infected with HCV genotype 3 [162,135,149,163].
At the moment, a 12-week course of sofosbuvir-ledipasvir is, at a cost of €50,000 in the Netherlands, around 13 times more expensive than 24 weeks peg-interferon and ribavirin. Because the costs of these novel drugs are high, cost effectiveness and budget impact remain an important barrier for widespread implementation [164–170]. Drug-drug interactions with HIV PIs, as well as substance use [171], can be problematic and may require close monitoring; the latest updates on drug-drug interactions can be obtained via the website for hepatitis drug interactions [172].

PREVENTION

Currently implemented and effective primary prevention activities for HCV are: screening donors of blood, plasma, organ, and tissue; virus inactivation of plasma-derived products; sterilization of surgical and dental instruments; high coverage of comprehensive harm reduction programs including needle and syringe exchange and opiate substitution programs for PWID [173–175]. In response to the recent epidemic among HIV-infected MSM, HCV awareness campaigns have been launched. In addition, secondary prevention efforts include identification, counseling, and testing especially those who are at increased risk of infection. Timely diagnosis may be beneficial by preventing ongoing transmission through behavioral adaptation, contact tracing, and treatment initiation (i.e., treatment as prevention) [176,177].

Preventive efforts among PWID

The risk of HIV- and HCV-infection decreased notably among PWID in Amsterdam since 1990; probably partly because of implemented harm reduction programs [174,175,178]. These harm reduction programs consisted of needle exchange programs (started in the mid-1980s), opiate substitution therapy (methadone programs started in 1981) and risk education programs [179]. Programs with high uptake and a combined multi-component approach have the best potential to decrease onwards transmission of HCV [180] as well as reinfection [181]. The beneficial effect of integrated interventions in PWID on HCV was also shown in studies conducted outside the Netherlands [182–187]. Treatment of HCV with peg-interferon and ribavirin has become increasingly available for PWID and, while expensive, is considered cost-effective [174,188,189]. However, while studies have shown that response rates are comparable with other populations, HCV treatment uptake is still limited among PWID [190,191].

Preventive efforts among MSM

Several risk reduction strategies are applied by MSM to prevent infection with sexually transmitted infections (STI). Condom use is the most known and most widely practiced way of protection against STI. HIV-specific risk reduction strategies that are applied and do not include condoms are strategic positioning (top/bottom), withdrawal before ejaculation,
and serosorting [52,192]. However, especially the latter is thought to have (and have had) a
signiﬁcant effect on the transmission of HCV (and other STI) among HIV-infected MSM [50].
The impact of treatment as prevention among MSM is likely to be important in reducing
the number of incident HCV infections, as suggested in two modelling studies [193,194]. In
addition, awareness of HCV and knowledge of HCV complications increased over time [195],
as well as the number of MSM that know their status by getting tested.

In the Netherlands, awareness of HCV among MSM has been created, among others, by the
‘Man tot Man’ (i.e., Male to Male) initiative. This initiative of Soa Aids Netherlands and the
Public Health Services of Amsterdam and Rotterdam-Rijnmond, aims to provide information
on homosexuality, sexual health, sexually transmitted infections, etc. Their website [196] also
includes an extensive summary of current knowledge on HCV: e.g., symptoms, risk factors,
and options for testing and treatment. Also Poz&Proud, part of the Dutch HIV Association, has
information on their website about HCV [197] and ﬂyers are distributed since August 2009.
International examples of information providers are the Hepatitis C Trust from the UK [198],
the Centers for Disease Control and Prevention (CDC) [199], and the National AIDS Treatment
Advocacy Project (NATAP) from the USA [200], and Canada’s Source for HIV and Hepatitis C
Information (CATIE) [201].

Vaccine development
Currently no vaccine exists for the prevention of infection with HCV. The development of a
vaccine has been hampered by various obstacles, such as the high level of genetic variability,
the relatively poor immune response of the host, and the absence of small animal models
to test candidate vaccines [202]. However, a cell culture system that allows studying virus
neutralization in vitro has been introduced in 2005 [203] and more recently, full genome
HCV replication in human hepatoma cell lines has been shown [204]. The availability of these
systems will likely increase the pace in which vaccine candidates can be identiﬁed. Earlier
studies have shown that an effective vaccine would be capable of inducing cross-neutralizing
antibodies against all major HCV genotypes, as well as broad HCV-speciﬁc CD4+ T helper and
CD8+ cytotoxic responses [205]. The most promising results so far have been obtained with
the use of an adenovirus-based vector vaccine comprising envelope glycoproteins gpE1/gpE2
[206,207].
DATA USED IN THIS THESIS

The studies described in this thesis rely on data that were collected in the initiatives summarized in table 1. The background of each of these cohorts is stated here, in order of appearance in this thesis:

Amsterdam Cohort Studies
The Amsterdam Cohort Studies on HIV infection and AIDS (ACS) is a collaboration between the Public Health Service of Amsterdam, the Academic Medical Center, Sanquin Blood Supply Foundation, DC Klinieken, and the Stichting HIV Monitoring (SHM). It comprises two open and ongoing cohort studies: one among MSM that started in 1984 [208], and one among injecting and non-injecting drug users (DU) that started in 1985 [209]; these studies aimed to investigate the epidemiology, psychosocial determinants, course of infection, and pathogenesis of HIV infection, and of blood-borne infections and STI other than HIV, and to evaluate the effects of interventions. As of 31 December 2014, these cohorts consisted of 2,649 MSM and 1,680 DU. Every three to six months, participants complete a standardised questionnaire regarding demographics, medical history, sexual and drug use behaviour, underlying psychosocial determinants, healthcare use, depression, and psychological disorders. HIV-infected participants undergo a medical examination (in the past, also HIV-negative DU were examined), and blood is collected for diagnostic tests and storage.

DUTCH-C project
In December 2004 the Drug Users Treatment for Chronic Hepatitis C (DUTCH-C) project was initiated to evaluate the feasibility of HCV testing and treatment among DU. This multidisciplinary treatment unit was a collaboration between the Academic Medical Center and the Public Health Service of Amsterdam. Opiate substitution therapy was integrated, and provided by a major clinic located close to the Public Health Service of Amsterdam. The DUTCH-C project started with HCV screening of 449 ACS participants, 267 of whom were anti-HCV positive, and 134 were HCV-RNA positive [210]. Because of the successful treatment results that were obtained thereafter (65% obtained SVR in an intention-to-treat analysis), the project was extended in 2007 to also provide HCV treatment to non-ACS DU. In March 2014, the approach changed; HCV care for DU is coordinated by a social nurse of the Mental Health department of the Public Health Service of Amsterdam (MGGZ). Treatment is provided by liver specialist in the hospital in close collaboration with the MGGZ department.

MSM network study
The MSM network study recruited 2,492 MSM between August 2008 and July 2009, at the STI outpatient clinic of the Public Health Service of Amsterdam and the HIV treatment centre of the Academic Medical Center Amsterdam. All were aged ≥18 years and could understand written Dutch of English. In order to determine the spread of STIs among MSM, participants
were tested for STI and completed a detailed questionnaire. Urethral, rectal and pharyngeal Neisseria gonorrhoeae, Chlamydia trachomatis, hepatitis B and C, and HIV were tested according to the STI clinic protocol; participants could opt-out for HIV testing. The questionnaire was administered online and regarded demographics of themselves, their steady partner and/or up to three other partners during the preceding 6 months. Overall sexual behavior was assessed, as well as sexual behavior with each of the mentioned partners.

**MOSAIC study**

The MSM Observational Study for Acute Infection with hepatitis C (MOSAIC) study was initiated in December 2008 and is still ongoing. The original main objectives were to identify the frequency, clinical consequences and determinants (viral and host factors) of acquiring acute HCV infection (primary, re- or superinfection), to assess HCV treatment outcome, and to study the impact of acute HCV infection on the morbidity and mortality. The study population comprises HIV-infected MSM aged ≥18 years old that were prospectively or retrospectively diagnosed with acute HCV, and HIV-monoinfected controls (only included for prospectively identified cases) [137]. Since the revision of the research protocol in January 2015 inclusion is extended to HIV-negative MSM and HIV-infected heterosexuals with acute HCV, and the objectives (mentioned earlier) were also extended to these populations. In addition, identification of the driving factors of the HCV epidemic among MSM has been added to the objectives of the study, as well as studying the role of HIV/HCV-coinfected MSM in the ongoing HIV epidemic. Blood samples are stored and clinical outcomes, including treatment and fibroscan results, are collected during inclusion and follow-up visits that take place every six months, or more frequently during HCV treatment. Questionnaires on risk behaviour and quality of life are self-administered and also collected at each study visit. The data collection takes place during regular patient care visits at HIV outpatient clinics in Amsterdam at the Academic Medical Center, Onze Lieve Vrouwe Gasthuis (OLVG Hospital), Slotervaart Hospital, in Utrecht at the University Medical Center, and in Rotterdam at the Erasmus University Medical Center. Up to May 2015, 156 HIV-infected MSM with acute HCV infection (cases; 105 of whom were prospectively identified), and 166 HIV-infected MSM with no history of HCV infection (controls) have been included. The MOSAIC study will continue to include and follow-up participants at least until mid-2018.

**ATHENA national observational HIV cohort**

The AIDS Therapy Evaluation in the Netherlands (ATHENA) project was initiated in 1996 to investigate the effects of early adoption of the (then) newly introduced combination antiretroviral therapy for HIV infection. The valuable results obtained among 3,600 HIV-infected patients, led to the continuation and extension of the research; the Stichting HIV Monitoring (SHM) was established in 2001 to include all HIV-infected patients in the Netherlands. By May 2015, the ATHENA national observational HIV cohort comprised data from over 24,263 HIV-infected individuals, followed longitudinally in 26 HIV treatment centres in the Netherlands and one in Curaçao [211].
Bi-annual HIV surveys at the STI clinic
From 1991 until 2012, cross-sectional and anonymous bi-annual surveys were performed at the STI outpatient clinic from the Public Health Service of Amsterdam (in Dutch also known as the ‘DWAR’ studies). These surveys aimed to gain insight into the heterosexual spread of HIV infections in Amsterdam [212]. Recruitment during each survey took place until at least 1,000 STI clinic attendees (men and women; heterosexual, bisexual, and homosexual) were included and interviewed about risk factors for STI and blood-borne infections. From all participants, blood was drawn, tested and stored. Because a high HCV prevalence was found among HIV-infected MSM in May 2007 (14.6%), anti-HCV testing became available for all MSM with HIV-positive or unknown status attending the STI outpatient clinic in November 2007 [18]. Unfortunately, this service was stopped because of financial constraints in May 2014.

AIMS AND OUTLINE OF THIS THESIS
In several studies presented in this thesis, epidemiological and molecular approaches were combined to gain insight into possible linkage between HCV infections. This gives an extra dimension to the outcomes measured in our studies, as the phylogenetic profile of the HCV infections could be added to the analyses. Other studies included longitudinal data from observational cohorts, in which incident HCV (re)infection and possible predictors for HCV infection could be evaluated. Blood samples collected during multiple cross-sectional studies were used to evaluate the utility of an HCV antigen test for screening purposes. The studies in this thesis were initiated with the ultimate goal to increase our understanding of primary and recurrent acute HCV infection among two risk groups in the Netherlands: PWID and HIV-infected MSM. The results of these studies in particular may contribute to improved prevention programs, and may inform clinical decision making and testing policies (e.g., whom to test, and which test(s) should be used).

In part I the current state of the HCV epidemic among MSM and PWID is illustrated. Chapter 2 shows updated estimates of the incidence of primary HCV infection among MSM from the ACS. In Chapter 3, the incidence of HCV reinfection during follow-up among active drug users that were successfully treated in the DUTCH-C project is studied.

Part II focuses on sexual transmission of HCV among HIV-infected MSM. In chapter 4, data from the MSM network study were analysed to determine whether HCV circulated in identifiable high-risk MSM subcultures. Chapter 5 describes risk factors for HCV infection by comparing the baseline questionnaires and clinical data of cases and control patients from the MOSAIC study. Data from the MOSAIC study were combined with data from the ATHENA observational cohort in chapter 6, in which the existence of HIV-transmission networks with increased risk for HCV infection was studied using phylogenetic analysis of both HIV and HCV sequences.
Part III describes the possible use of assays for screening and diagnosis of acute HCV (re)infection in various settings. In chapter 7 HCV antibody dynamics following acute (re)infection were investigated among participants of the MOSAIC study. Chapter 8 presents a case study of an HIV-infected man with seronegative HCV infection, who also had no signs of hepatic inflammation. Chapter 9 evaluates the use of an HCV antigen assay for screening for acute and chronic HCV infection among HIV-infected MSM. For this study, data and sera collected at the bi-annual surveys at the STI outpatient clinic were used.

Finally, chapter 10 discusses the main findings of this thesis, relates these to recent literature, and gives recommendations for future studies.
Table 1 Overview of data sources used for the studies described in this thesis.

<table>
<thead>
<tr>
<th>Data source</th>
<th>Study (sub-)population used in the study that is presented in this thesis</th>
<th>Data used from study period</th>
<th>Chapter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS among MSM [208]</td>
<td>HIV-infected and HIV-uninfected MSM, followed-up at the Public Health Service of Amsterdam</td>
<td>1984-2011</td>
<td>2</td>
</tr>
<tr>
<td>DUTCH-C project [210]</td>
<td>HCV-mono-infected active drug users from the ACS that were treated for HCV</td>
<td>2005-2011</td>
<td>3</td>
</tr>
<tr>
<td>MSM network study [213]</td>
<td>HIV-infected MSM with and without HCV infection, recruited at the STI clinic of the Public Health Service of Amsterdam and at the HIV outpatient clinic of the AMC</td>
<td>2008-2009</td>
<td>4</td>
</tr>
<tr>
<td>MOSAIC study [137]</td>
<td>HIV-infected MSM with and without acute HCV infection, recruited at the HIV outpatient clinics of the AMC, OLVG Hospital, Slotervaart Hospital, UMC Utrecht, and Erasmus UMC</td>
<td>2009-2014</td>
<td>5-7</td>
</tr>
<tr>
<td>ATHENA national observational HIV cohort study [211]</td>
<td>HIV-infected individuals MSM, recruited at 26 HIV treatment centers in the Netherlands</td>
<td>1996-2014</td>
<td>6</td>
</tr>
<tr>
<td>Bi-annual HIV surveys [212]</td>
<td>HIV-infected MSM, recruited at the STI outpatient clinic of the Public Health Service of Amsterdam</td>
<td>2009-2012</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: ACS: Amsterdam Cohort Studies; AMC: Academic Medical Center; DUTCH-C: Drug Users Treatment for Chronic Hepatitis C; HCV: hepatitis C virus; HIV: human immunodeficiency virus; MOSAIC: MSM Observational Study of Acute Infection with hepatitis C; MSM: men who have sex with men; STI: sexually transmitted infection; UMC: University Medical Center.
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PART I

THE INCIDENCE OF ACUTE HCV INFECTION IN THE NETHERLANDS
LOW INCIDENCE OF REINFECTION WITH THE HEPATITIS C VIRUS FOLLOWING TREATMENT IN ACTIVE DRUG USERS IN AMSTERDAM

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Bart P.X. Grady*, Joost W. Vanhommerig*, Janke Schinkel, Christine J. Weegink, Sylvia M. Bruisten, Catherina E.A. Lindenburg, Maria Prins

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* Both authors contributed equally to the writing of this article.
ABSTRACT

Background
More than two-thirds of hepatitis C virus (HCV) infections are associated with injecting drug use. Despite the wide availability of standard treatment with pegylated interferon and ribavirin, active drug users (DU) have limited access to HCV treatment. Physicians may be reluctant to prescribe treatment because of the presumed high risk of reinfection. However, data on reinfection in treated DU remain scarce.

Methods
Active DU with chronic HCV infection were treated in a multidisciplinary setting. After achieving a sustained virologic response, patients were tested at 6-12-monthly intervals for HCV RNA. To distinguish between relapse and reinfection, sequence and phylogenetic analyses were performed on the NS5B region of the HCV genome. The incidence of reinfection was calculated using person-time techniques.

Results
From April 2005 to March 2010, 69 active DU treated for HCV had sufficient follow-up, median 2.5 years (interquartile range, 1.6-3.7). Sustained virologic response was achieved in 42 patients (61%). During follow-up, 41 patients remained HCV RNA-negative; of these, two patients died. During treatment, five out of 41 injected drugs, which increased to 11 out of 41 after the end of treatment. One case of reinfection was observed, followed by spontaneous clearance of the virus. The overall incidence was 0.76/100 person-years (95% confidence interval, CI: 0.04-3.73). For only those individuals reporting injecting drug use, the incidence was 3.42/100 personyears (95% CI: 0.17-16.90).

Conclusion
We report a low incidence of HCV reinfection following treatment in DU participating in a multidisciplinary programme. Active drug use, including injecting, should not preclude access to treatment for HCV.
INTRODUCTION

Hepatitis C virus (HCV) infection poses serious challenges to global health, affecting more than 170 million individuals. In high-income countries, more than two-thirds of HCV infections are associated with injection drug use [1]. About 75% of individuals infected with HCV develop chronic HCV infection [2] and are at risk for long-term sequelae, including liver cirrhosis and hepatocellular carcinoma [3]. In former and active injecting drug users (DU), the prevalence of HCV ranges from 30 to 95% [4,5]. In 2010, the modelled prevalence of chronic HCV infection in the (ever) injecting DU population (N=4353) in Amsterdam was 80.7% [95% confidence interval (CI): 66.6-89.7] [6].

Nowadays, treatment for HCV infection with pegylated interferon a (PEG-IFN) and ribavirin (RBV) is fairly adequate depending on the genotype, and more promising alternatives with direct acting antiviral agents for HCV are upcoming [7,8]. Unfortunately, physicians seem to be reluctant to offer HCV treatment to DU because of their concerns about suboptimal patient adherence, potential psychiatric decompensation, the risk of premature mortality and the risk of reinfection after treatment [9-11]. We and others have shown that in a multidisciplinary programme, HCV treatment uptake and response in DU are comparable with a nondrug-using population [12-14]. Moreover, modelling studies have suggested that HCV treatment could contribute towards reducing the future HCV disease burden among DU [5,15,16].

However, data on the risk of reinfection in DU after successful treatment are scarce. Apart from case reports, only four prospective studies among DU have been carried out. In a German study among 18 DU, zero to two cases of reinfection were observed, resulting in a reinfection rate of 0-4.1/100 person-years [17]. In the other three more recent, but relatively small studies (N=9, 27 and 35), the overall reinfection rate varied from 0.6/100 person-years to 3.2 cases/100 person-years. However, when restricted only to those individuals returning to injection drug use, the reinfection rate was higher in these studies: 1.9 cases/100 person-years and 5.4 cases/100 person-years [18-20].

To overcome potential barriers to the treatment of DU, more prospective data are required on the risk of reinfection after successful HCV treatment. The aim of this study was to evaluate the rate of HCV reinfection following the end of treatment (EOT) in a prospective cohort with DU who completed HCV therapy within a multidisciplinary programme. HCV strains were genotyped to discriminate between possible relapse, following HCV RNA-negativity at EOT, and true reinfection. In addition, we evaluated the mortality risk following EOT.
MATERIALS AND METHODS

Study population
The Amsterdam Cohort Studies (ACS) is an open and ongoing prospective cohort study among DU that was initiated in 1985 [21]. Participation is voluntary and informed consent is obtained at intake for every participant. The ACS was approved by the medical ethics committee of the Academic Medical Center. Within the ACS, the Drug Users Treatment for Chronic Hepatitis C (DUTCH-C) project was launched in December 2004 and has previously been reported in detail [12]. In brief, HCV treatment is provided to DU by ACS medical staff and a liver specialist from the Academic Medical Center (Amsterdam). Methadone and psychopharmaceutical medications are prescribed by addiction specialists and psychiatrists. Care providers from the methadone clinics provide support and observe the development of side-effects. No incentives were offered, and written informed consent was required for participation. To assess hepatitis B virus (HBV) status, all patients were routinely screened for anti-HBc, HBsAg and anti-HBs. HBV vaccination was offered for those uninfected with HBV. Patients received standardized HCV treatment with PEG-IFN along with RBV. Dosages were determined according to the standard of care and adjusted individually on the basis of side-effects. The treatment duration was 24 weeks (HCV genotypes 2 and 3) or 48 weeks (HCV genotypes 1 and 4).

Laboratory methods
Blood samples were tested qualitatively for HCV RNA using transcription-mediated amplification (Versant; Siemens Medical Solutions Diagnostics, Munich, Germany) with a lower detection limit of 5-10 IU/ml. Successful treatment, indicated by a sustained virologic response (SVR), was defined as having an undetectable HCV RNA level 24 weeks after treatment. When HCV RNA was detected at 24 weeks after treatment, plasma samples taken before and after treatment were compared by sequence analysis to distinguish between reinfection and relapse. After SVR, HCV RNA was tested at 6-12-monthly intervals.

Genotyping was performed using the primers and conditions as described previously by Murphy et al. [22]. Sequence alignments were created using Mega 5.0 (GenBank Accession No. JN426992-JN427013; The Biodesign Institute, Tempe, Arizona, USA) along with established reference sequences [23] to determine viral genotype. For phylogenetic analysis, the Tamura-3 parameter evolution model was chosen using the model test functionality in Mega 5.0 and the phylogenetic tree was constructed using the neighbourjoining method. The inferred phylogenies were tested with 1000 bootstrap replications [24].

Causes of death
Patients were matched against the local and national registries to obtain information about their vital status. Cause of death (COD) was actively and systematically obtained, if available, from hospitals, general practitioners or coroners. Data were collected on the primary COD,
Statistical analyses
The incidence rate of reinfection was calculated using person-time techniques. The individual follow-up time was calculated from the EOT date until the last HCV RNA-negative test, date of HCV reinfection or death. The date of reinfection was determined as the midpoint between the last HCV RNA-negative and the first HCV RNA-positive visit. If spontaneous clearance of the reinfection was observed, patients were again considered at risk for another HCV reinfection from the first HCV RNA-negative visit following the previous reinfection (if confirmed by at least two HCV RNA-negative visits following the previous reinfection). Cumulative incidence curves were estimated for reinfection and death within a competing risks framework. The R language and environment for statistical computing, v2.8 [25], and SPSS v19.0 (SPSS Inc., Chicago, Illinois, USA) were used for data analysis.

RESULTS
General characteristics
Between April 2005 and November 2010, 69 patients were treated for HCV infection. SVR was achieved in 42 out of 69 patients (61%). Their characteristics at the start of treatment, during treatment and following EOT are presented in table 1. During follow-up, two patients with an SVR died. One patient died from pneumonia/sepsis; the other patient’s death was classified as an undefined natural death at, respectively, 3.6 and 2.7 years after EOT. These two patients had their last HCV RNA-negative test 26 and 52 weeks before death and did not report any injection drug use during or after EOT. During treatment, five out of 42 injected drugs. This number increased to 11 out of 42 in the period after EOT. During follow-up, 41 cases remained HCV RNA-negative. One case became HCV RNA-positive and is described in more detail later. Of the 27 out of 69 patients who did not achieve an SVR, 10 were defined as relapses after being HCV RNA-negative at EOT. All 10 patients were also studied for potential reinfection by phylogenetic analysis. In all cases, pretreatment and post-treatment sequences of genotypes 1a (N=3) and 3a (N=7) clustered closely together, which supports the notion of relapse.

Reinfection
One case of reinfection was observed; the case we present (study number 15895 in Fig. 1) involved a 56-year-old man of Dutch origin. He has had a history of ongoing injection drug use since 1974 and, in fact, injected shortly before the start of treatment. Before treatment, he was found to carry HCV genotype 1a at multiple visits. At the start of treatment, the baseline qualitative HCV RNA test was positive, HCV RNA by quantitative testing was less than 1000 IU/ml and ALT was 19 U/l. He was not infected with HIV and had previously achieved
clearance of HBV infection. PEG-IFN and RBV therapy was initiated in March 2008. From week 2 onwards, qualitative HCV RNA-tests were undetectable until SVR. During treatment, he was on methadone maintenance therapy and continued injecting drug use. At 40 weeks following EOT (16 weeks after SVR), he reported a needlestick incident. The needle was from his female partner, who was HCV RNA-positive with genotype 1a and who had remained untreated. The qualitative HCV RNA-test was positive; however, the viral load level was less than 1000 IU/ml. Unfortunately, we could not characterize this reinfection because of the low viral load. Six weeks after the first positive test at reinfection, HCV RNA was undetectable by qualitative testing and remained undetectable (145 weeks after EOT and 107 weeks after reinfection).

The overall incidence of HCV reinfection was 0.76/100 person-years (95% CI: 0.04-3.73). On restricting our analysis to those reporting injecting drug use, the incidence was 3.42/100 person-years (95% CI: 0.17-16.90). To examine the likelihood of mortality and reinfection following EOT, we constructed cumulative incidence curves, as shown in Fig. 2. Reinfection occurred earlier in time following EOT than all-cause mortality. At 4 years after EOT, 2.4% (95% CI: 0.0-6.9) of DU were expected to have acquired an HCV reinfection and 8.6% (95% CI: 0.0-19.8) were expected to have died (all-cause mortality). Hence, 89.2% were expected to be alive and reinfection free at 4 years after EOT.

DISCUSSION

The observed incidence rate of HCV reinfection was 0.76-3.42 cases/100 person-years. These results are comparable with the reinfection rates observed in other prospective studies performed in Germany, Norway, Canada and the USA [17-20]. These studies did not include follow-up time after the clearance of a reinfection. In addition, the occurrence of possible reinfection in cases initially defined as relapses was not studied. If reinfections were present among the relapses, the reinfection rates in these studies might have been higher. Our observed reinfection rate is close to the average yearly incidence rate of primary HCV infection among ever-injecting DU in the ACS since 2005 (i.e., 0.35 cases/100 person-years). The HCV reinfection rate we found among injecting DU is considerably lower than the current incidence of HCV reinfection following the treatment of acute HCV infection in HIV-coinfected men who have sex with men (MSM) in Amsterdam (i.e., 15.2 cases/100 person-years) [26].

We describe a case of spontaneous clearance of HCV reinfection following treatment and SVR for a chronic HCV infection. Grebely et al. [20] described the first case of spontaneous clearance after reinfection with HCV. Their patient had been treated for HCV genotype 3a and was reinfected with HCV genotype 1a, followed by spontaneous clearance [20]. Unfortunately, we could not genotype the reinfection of our case; however, as RNA became detectable again after SVR, this case is a reinfection by definition [27]. On the basis of the reported behaviour,
the patient we described was probably reinfected with the HCV genotype 1a from his female partner. This observation of spontaneous clearance after reinfection of presumably genotype 1a following an SVR, when treated for a previous chronic infection with genotype 1a, may suggest that an enhanced immune response, as a result of therapy, allowed the patient to achieve clearance of the reinfection without treatment. Following the spontaneous clearance of a primary HCV infection, strong and broad specific T-cell responses have been reported in the spontaneous clearance of HCV reinfection [28,29]. However, our patient did not achieve spontaneous resolution of his primary HCV infection, which suggests that spontaneous clearance is multifactorial.

Follow-up time was calculated from EOT instead of the date of SVR; therefore, our follow-up time increased with 24 weeks per individual. However, two recent studies have shown that HCV reinfections can occur in the window phase between EOT and SVR in HCV-HIV-coinfected MSM treated for acute HCV [30]. Importantly, these studies showed that patients originally diagnosed as late relapses should be re-evaluated for reinfections. The distinction between relapse and reinfection has important clinical consequences and should therefore be considered prospectively by clinicians. The definition of relapse or reinfection in the first 6 months after EOT should, in a population with ongoing HCV risk behaviour (e.g., injecting drug use), always be based on phylogenetic analysis. In this study, we used part of the NS5B gene for this analysis, because the phylogenetic signal in this population was sufficient to distinguish between relapse and reinfection, as pretreatment sequences were unique for each patient. In populations where highly similar viruses are circulating, for example the recent epidemic of acute HCV in HIV-coinfected MSM, the genomic region analysed in the study may not be appropriate. Formally, we cannot completely rule out that the relapse patients with clustering of pretreatment and post-treatment samples were reinfected by the same source. However, given the minor genetic distances between pretreatment and post-treatment samples and the high evolutionary rate of HCV, this seems highly unlikely.

One of the limitations of this study is that spontaneously resolved reinfections may have been missed because of 6–12-monthly testing for HCV RNA. This would lead to an underestimation of the observed HCV reinfection rate, but reinfections resulting in chronic infection would have been noticed. The results from this study are based on treatment in DU in a multidisciplinary setting with access to low-threshold comprehensive harm reduction programmes and might not be applicable for treatment in a different setting. Another limitation is that variables on drug use behaviours were self-reported and may be subjective according to socially desirable responses.

**Conclusion**

We found a low reinfection rate after the successful treatment of HCV infection in active DU participating in a multidisciplinary HCV treatment programme in a city with comprehensive
harm reduction programmes for DU. Active drug use, including injecting, during and after treatment, was relatively common; however, it should not preclude access to treatment for HCV.

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Table 1 Characteristics of patients with sustained virological response: at start of treatment, during treatment and following end of treatment (N=42). *Patients can report on both injecting and non-injecting drug use. HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At start of treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>51 (47-56)</td>
</tr>
<tr>
<td>Male Sex (n, %)</td>
<td>31 (73.8)</td>
</tr>
<tr>
<td>Dutch Nationality (n, %)</td>
<td>35 (83.3)</td>
</tr>
<tr>
<td>HCV genotype (n, %)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>2</td>
<td>12 (28.6)</td>
</tr>
<tr>
<td>3</td>
<td>22 (52.3)</td>
</tr>
<tr>
<td>4</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>HIV coinfection (n,%)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>HBV status</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc-negative, HBsAg-negative (n,%)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td>Anti-HBc-positive, HBsAg-negative (n,%)</td>
<td>25 (59.5)</td>
</tr>
<tr>
<td>Anti-HBc-positive, HBsAg-positive (n,%)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vaccinated (n, %)</td>
<td>3 (7.1)</td>
</tr>
<tr>
<td>Methadone use on prescription (n, %)</td>
<td>39 (92.8)</td>
</tr>
<tr>
<td>Alcohol, current intake &gt;5 units/day (n, %)</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Ever injected drugs (n, %)</td>
<td>41 (97.6)</td>
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<tr>
<td><strong>Active drug use during treatment</strong></td>
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</tr>
<tr>
<td>Injecting (n)</td>
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<tr>
<td>Non injecting drug use (n)</td>
<td>40</td>
</tr>
<tr>
<td><strong>Follow-up after end of treatment</strong></td>
<td></td>
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<tr>
<td>Active drug use after treatment*</td>
<td></td>
</tr>
<tr>
<td>Injecting (n)</td>
<td>11</td>
</tr>
<tr>
<td>Non-injecting drug use (n)</td>
<td>41</td>
</tr>
<tr>
<td>Median follow-up (years, IQR)</td>
<td>2.5 (1.7-3.7)</td>
</tr>
<tr>
<td>Median HCV RNA test interval (weeks, IQR)</td>
<td>28 (24-36)</td>
</tr>
</tbody>
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Figure 1 Phylogenetic tree of HCV NS5B sequences of one reinfection case and relapses (N=10) of DU treated for HCV in Amsterdam. One reinfection (15895, ■) was determined by TMA but could not be genotyped. This patient reported a needlestick incident from his HCV-positive partner (15895 partner, ■). Among relapses (N=10), samples taken before (pre) and after (post) treatment clustered closely together, supporting the notion of relapse rather than reinfection. HCV, hepatitis C virus; TMA, transcription-mediated amplification.
Figure 2 Cumulative incidence for HCV reinfection and survival among DU at risk for reinfection (N=42) since end of successful HCV treatment, within a competing risk framework. HCV, hepatitis C virus; DU, drug users; SVR, sustained virological response.
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INCIDENCE OF ACUTE HCV INFECTION
STABILIZING INCIDENCE OF HEPATITIS C VIRUS INFECTION AMONG MEN WHO HAVE SEX WITH MEN IN AMSTERDAM

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Joost W. Vanhommerig, Ineke G. Stolte, Femke A.E. Lambers, Ronald B. Geskus, Thijs J.W. van de Laar, Sylvia M. Bruisten, Janke Schinkel, Maria Prins

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INTRODUCTION

Since 2000, there has been an unexpected and substantial increase in the incidence of hepatitis C virus (HCV) among HIV-1-infected men who have sex with men (MSM).\textsuperscript{1–3} Prevalence of HCV among HIV-uninfected MSM has remained low.\textsuperscript{1} HIV may enhance sexual transmission of HCV through increased infectiousness and increased susceptibility.\textsuperscript{1,3} In combination with increased high-risk sexual behavior, these factors most likely allowed HCV to spread sexually.\textsuperscript{1,3} The Multicenter AIDS Cohort Study (MACS) in the United States reported significantly higher HCV incidence rates (IRs) among MSM recruited in 2001-2003 compared with earlier periods.\textsuperscript{4} Studies from Switzerland, Spain, and Japan showed an ongoing increase in new HCV infections among HIV-infected MSM from 2008 till 2011/2012.\textsuperscript{5–7} In contrast, data collected during biannual surveys at the sexually transmitted infection clinic in Amsterdam, the Netherlands, suggested that the prevalence of HCV among HIV-infected MSM has stabilized in recent years.\textsuperscript{8} We therefore updated our previous analysis in the Amsterdam Cohort Study (ACS) among MSM\textsuperscript{9} to examine recent changes in HCV incidence.

METHODS

Participants
The ACS among MSM is an open, ongoing prospective study initiated in 1984.\textsuperscript{10} Participation is voluntary and informed consent is obtained at intake. Participants return every 3–6 months for follow-up. Since 1999, the follow-up of nearly all HIV-infected MSM has been relocated to HIV treatment centers, where collection of behavioral data is limited. We included all HIV-infected and HIV-uninfected MSM with ≥2 study visits between October 1984 and January 2012. HCV status of each participant up to January 2003 has been retrospectively determined as described previously.\textsuperscript{9} New participants since 2003, and HIV-uninfected MSM with a negative HCV status who have remained in follow-up, were (again) tested for HCV antibodies at their first visit after October 2008. To update the HCV status among HIV-infected MSM with a negative HCV status in our previous study, we obtained all available HCV screening results from the clinical records of HIV treatment centers attended by ACS participants. If no test result was available after January 2009, the last available sample before January 2012 was tested for HCV antibodies. On finding incident HCV infection, samples from earlier visits were tested to minimize width of seroconversion interval.

Laboratory methods
HCV antibody testing was performed using a commercial microparticle enzyme immunoassay (AxSYM® HCV 3.0; Abbott Laboratories, Abbott Park, IL, USA) and confirmed by immunoblot (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics, Raritan, NJ, USA) and transcription-mediated amplification (TMA Versant; Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA).
Statistical analysis

Participants contributed followup time from the date of study entry until the date of the last negative HCV antibody test, or until the estimated date of HCV infection (i.e., the midpoint between the last negative and the first positive HCV test). IRs were calculated per year, and trends over time were analyzed using Poisson regression. Restricted cubic splines allowed for smoothly varying trends in the modeled incidence.11 In a bivariate analysis for incident HCV infection among HIV-infected MSM, adjusting for calendar period of follow-up, the effects of age, CD4 cell count, nadir CD4 cell count, HIV viral load (modeled as log10 increment above 1000 copies/mL), and combination antiretroviral therapy (cART) use were evaluated using Poisson regression. Variables subject to change were treated as time-updated covariates. Statistical software packages STATA Intercooled 11.2 (StataCorp, College Station, TX, USA) and R 2.15.212 were used for analysis.

RESULTS

The ACS enrolled 2457 MSM between October 1984 and January 2012, of whom 2104 had ≥2 study visits and HCV test results. Median age of the 2104 MSM at study entry was 30.3 years (interquartile range, IQR: 25.9-37.1) and 78.8% had Dutch nationality. At study entry, 539 (25.6%) of 2104 MSM were HIV-infected; an additional 222 MSM seroconverted during follow-up. At study entry, 2080 of 2104 MSM were HCV negative and contributed to 17,310 person-years (PYs) of follow-up; median follow-up time was 7.4 years (IQR: 3.2-12.0). Of the 422 HIV-infected MSM followed up after 1996, 345 were ever on cART.

Twenty-nine incident HCV infections were documented during the observation period. All incident cases were infected with HIV before HCV infection and none of them reported injection drug use (IDU) at study entry. Based on 6422 PYs of follow-up among HIV-infected MSM, the overall observed HCV incidence was 4.52/1000 PYs [95% confidence interval (CI): 3.02-6.49]; the effect of calendar time was significant (P<0.001). Before 2000, only 3 incident HCV infections were documented, resulting in an IR varying between 0.73 and 3.60/1000 PYs (Fig. 1A). A significant increase in HCV incidence was observed after 2000 (IR2005 vs. IR2000: IRR, 3.41; 95% CI: 1.58 to 7.34; P=.002). After 2005, however, HCV incidence stabilized at around 12/1000 PYs (IR2010 vs. IR2005: IRR, 0.94; 95% AU2 CI: 0.38-2.36; P=.906).

In Poisson regression, younger age was associated with incident HCV infection (age 50 vs. 35 years: RR, 0.31; 95% CI: 0.11-0.89; P=.041; Fig. 1B). Of the other evaluated risk factors, none were significantly associated with HCV infection after adjusting for calendar year: CD4 count (800 vs. 300 cells/mL: RR, 0.96; 95% CI: 0.33 to 2.79), nadir CD4 count (300 vs. 150 cells/mL: RR, 1.09; 95% CI: 0.46 to 2.58), HIV viral load (30,000 vs. 1,000 copies/mL: RR, 2.27; 95% CI: 0.82 to 6.29), and cART use (RR: 0.80, 95% CI: 0.34 to 1.88).
Infections were mostly of genotype 1 (14/29; 48.3%), followed by genotype 4 (7/29; 24.1%), in line with our previous study. Infection with genotype 2b (N=3) was observed after 2008 only and might indicate the introduction of a new HCV genotype among HIV-infected MSM in the Netherlands. No statistically significant time trend in genotype distribution was found.

**DISCUSSION**

We describe incidence of HCV infection among MSM in Amsterdam over the course of almost 3 decades. HCV incidence rose sharply among HIV-infected MSM between the years 2000 and 2005 but seems to have stabilized at a higher level of around 12/1000 PYs thereafter, although CIs were wider in the years 2005-2011. Our observation of stabilizing incidence corresponds with findings from the Amsterdam sexually transmitted infection clinic and may partly result from increased HCV testing and treatment uptake and increased HCV awareness leading to a reduction of risk behavior and a saturation effect in the group at highest risk for HCV infection. No incident HCV infections were documented among HIV-uninfected MSM, despite more than 10,000 PYs of followup, which supports earlier findings that HCV mainly spreads among HIV-infected MSM. Sexual transmission of HCV has occurred among a few HIV-uninfected MSM.

Our findings show a leveling off in HCV incidence among HIV-infected MSM rather than an ongoing increase, in contrast to studies from Switzerland, Spain, Japan, and the United States. However, the HCV epidemic and the subsequent public health response among MSM in the Netherlands may have started earlier.

The HCV epidemic in the MACS cohort (with 4 study sites across the United States) seems to differ from the epidemic in the Netherlands. Continuous incident infections have occurred from the mid-80s onwards, with majority of infections (i.e., 67/92; 73%) during the period 1985-1995 among both HIV-infected and HIV-uninfected MSM. The incident HCV infections in these early years might be attributed to IDU (reported by 5%) and blood transfusion (reported by 3%). Besides, the majority of MSM with incident HCV infection (i.e., 67/115; 58%) reported only 1 anal sex partner, suggesting transmission routes other than sexual contact play a major part. The actual outbreak of sexually transmitted HCV may have started later on, but unfortunately, the authors do not differentiate sexually acquired and nonsexually acquired HCV infections over time.

Also study design and methods differ between the MACS and ACS and could partly explain differences in trends. The MACS is a closed cohort with 3 separate enrolment periods, whereas the ACS has inclusion throughout the study period. In contrast to the ACS, in the MACS, 22 MSM with possible incident HCV infection tested HCV antibody positive for the first time at
their last study visit were classified as HCV-free, as the MACS definition required 2 consecutive positive visits. In addition, HCV seroconverters with a wide seroconversion interval (≥4 years) were excluded.

Since the start of the HCV epidemic among HIV-infected MSM, multiple HCV genotypes have been introduced and continued to circulate in this population. Because of the relatively few incident infections in our study, we had limited power to test for trends in HCV genotype distribution over time. Genotype 2b is known to be transmitted mainly through invasive procedures, blood transfusion, and IDU, but the current spread among MSM is most likely through sexual transmission.

The initial scope of the ACS was to study the HIV epidemic; hence data on risk factors for HCV were limited. In addition, data on sexual risk behavior were limited after 1999 as described in our Methods section. Recently initiated HCV-specific cohort studies among MSM in the Netherlands, the United Kingdom, and Germany will be better able to provide insight into the role of traditional and sexual risk factors for HCV infection and reinfection among MSM.

In conclusion, the incidence of HCV infection among HIV-infected MSM in Amsterdam seems to have stabilized after an initial increase until 2005. The overall disease burden is likely to remain high because studies in the cART era show that HIV-HCV co-infection results in an increased risk of both HCV and HIV mortality. Infected individuals will benefit from the swiftly changing landscape of HCV treatment. Continued follow-up is needed to see if the HCV epidemic among HIV-infected MSM will also stabilize in other regions of the world.

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Figure 1A. Observed and fitted HCV incidence rate (per 1000 PYs of follow-up) among 761 HIV-infected MSM participating in the Amsterdam Cohort Studies, 1984-2011. The shaded area is the 95% CI.
Figure 1B Fitted HCV incidence rate (per 1000 PYs of follow-up) by age, among HIV-infected MSM participating in the Amsterdam Cohort Studies (in 2008). The shaded area is the 95% CI.
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PART II

SEXUAL TRANSMISSION OF HCV AMONG HIV-INFECTED MSM
HIV-INFECTED MEN WHO HAVE SEX WITH MEN WHO IDENTIFY THEMSELVES AS BELONGING TO SUBCULTURES ARE AT INCREASED RISK FOR HEPATITIS C INFECTION

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Amy Matser, Joost W. Vanhommerig, Maarten F. Schim van der Loeff, Ronald B. Geskus, Henry J. C. de Vries, Jan M. Prins, Maria Prins, Sylvia M. Bruisten

Presented at the 19th ISSTDR Conference, 2011, Quebec, Canada (oral abstract #O1-S10.04), and at the 14th ISVHLD Conference, 2012, Shanghai, China (poster abstract #181). The real-time PCR assay used in this study was presented at the 14th European Society for Clinical Virology (ESCV) meeting, 2011, Funchal, Portugal (poster abstract #23).
CHAPTER 4

ABSTRACT

Background
Hepatitis C virus (HCV) emerged as sexually transmitted infection among HIV-infected men who have sex with men (MSM). We studied whether HCV circulated in identifiable high-risk MSM subcultures and performed phylogenetic analysis.

Methods
HIV-infected MSM were recruited at the sexually transmitted infections (STI) outpatient clinic and a university HIV clinic in Amsterdam, the Netherlands, 2008-2009. Participants completed a detailed questionnaire and were tested for HCV antibodies and RNA, with NS5B regions sequenced for analysis of clusters.

Results
Among 786 participants, the median age was 43 (IQR 37-48) years, and 93 (11.8%) were HCV-positive. Seropositivity was associated with belonging to subcultures identified as leather (aOR 2.60; 95% CI: 1.56-4.33), rubber/lycra (aOR 2.15; 95% CI: 1.10-4.21), or jeans (aOR 2.23; 95% CI: 1.41-3.54). The two largest HCV-RNA monophyletic clusters were compared; MSM in cluster I (genotype 1a, N=13) reported more partners (P=.037) than MSM in cluster II (genotype 4d, N=14), but demographics, subculture characteristics and other risk behaviors did not differ significantly between the two clusters.

Discussion
HCV infection is associated with identifiable groups of leather/rubber/lycra/jeans subcultures among HIV-infected MSM. Separate epidemiological HCV transmission networks were not revealed. Active HCV screening and treatment within specific subcultures may reduce HCV spread among all MSM.
INTRODUCTION

During the last decade, hepatitis C virus (HCV) has emerged among HIV-infected men who have sex with men (MSM) in several industrialized countries [1-8]. It became clear that, in this population, HCV spreads mainly by sexual transmission [9]. The HCV incidence among HIV-infected MSM rose from 0.9 per 1000 person-years in 1990 to 23.4 per 1000 person-years in 2007 [10]. Epidemiological studies showed that HCV infection in HIV-infected MSM is associated with unprotected anal intercourse, multiple sexual partners, recreational non-injecting drug use, and rough sexual techniques (e.g., sharing of sex toys and brachyproctic insertion, also known as fisting) [11,12], but these studies did not link these factors to any particular MSM subculture.

Although HCV is transmitted through a large international network, isolates tend to cluster by country or region [13]. Among MSM in Amsterdam, the predominant HCV genotypes are 1a and 4d [3], and the strain of genotype 4 found in MSM is phylogenetically distinct from the strain found in injecting drug users or migrants from Egypt [14]. These findings led us to question whether, within a country or a city, HCV strains would cluster according to MSM subpopulations that arise by clustering of MSM who share the same characteristics. One of the possible ways MSM cluster together is according to lifestyle or subculture, e.g., MSM who belong to the leather scene might cluster together. Literature about the existence of subpopulations is limited. To our knowledge, only the leather scene has been described [31]. Based on the themes of gay bars, clubs and parties, it can be deduced that a number of subpopulations or subcultures exist in the gay community. By searching the internet and the agendas of venues in Amsterdam we identified several possible subpopulations or subcultures. Besides the leather scene, we identified a subpopulation of MSM who wear military or other uniforms, a group who wears rubber/lycra clothing and have their own websites and parties, and a group of MSM wearing jeans who also have their own parties and partially overlap with the leather scene. We also identified a sports community of younger MSM who wear sports outfits and also have their own parties and dress code. We hypothesised that assortative mixing, or partnership formation within subgroups, is common and that this mixing pattern might result in HCV being more prevalent in certain subpopulations than in others.

Especially in the current epidemic phase, insight into the transmission network can contribute to more effective screening and treatment in the near future. Screening and treatment can, in turn, have a major impact on the prevalence by reducing transmission [15]. In the Netherlands, HCV screening currently occurs at STI clinics and at the HIV treatment centres. In addition to current practice, the initiation of outreach programs and on location screening might contribute to further identification of HCV-infected MSM. To determine what the most important target groups are for these types of interventions, it is important to determine characteristics that help identify subgroups. The objective of this study was to find such subgroups, identifiable by
lifestyle or subculture. Furthermore, we performed phylogenetic analysis to examine whether the presence of certain specific HCV strains was associated with specific MSM subpopulations.

**METHODS**

**Setting & participants**
This study is part of a larger cross-sectional study focusing on spread of sexually transmitted infections (STI) via sexual networks; its aims, population, and methods are discussed elsewhere [16]. The study population was recruited from MSM attending the STI outpatient clinic of the Public Health Service of Amsterdam and the HIV outpatient clinic of the Academic Medical Center (AMC), Amsterdam, the Netherlands. MSM were defined as men who reported any sexual contact with men during the six months preceding the clinic visit. They were eligible for participation if they were at least 18 years old, could understand written Dutch or English, and provided written informed consent. Those recruited at the STI outpatient clinic included MSM with and without STI symptoms. They could participate more than once if they revisited the clinic because of a possibly new STI episode. The recruitment period was from July 2008 to August 2009 and was briefly interrupted twice by logistical conflicts with another study.

The participants from the HIV outpatient clinic were recruited from a cohort of MSM visiting for routine 3- or 6-monthly clinic visits. They were included based on the same criteria as used in the STI clinic, but none of them had STI symptoms, having served previously (October 2007 through August 2008) in a study investigating STI prevalence in asymptomatic visitors of the HIV clinic [17].

At both locations, participants were screened for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum*, and when appropriate, they were screened for Hepatitis B and HIV, according to the standard procedures of the STI outpatient clinic. At the STI clinic, all HIV-infected MSM or MSM who opted-out for the HIV test were tested for the presence of HCV, unless the HCV seropositive status was known from previous testing at the clinic. At the HIV clinic, HCV antibody testing (AxSYM® HCV 3.0, Abbott Laboratories, Abbott Park, Illinois, USA) was done at the first visit and subsequently, when suspicious for HCV infection, e.g., when serum alanine aminotransferase (ALT) levels were elevated. The current analysis was restricted to the first visit of HIV-infected participants. The study was approved by the medical ethics committee of the AMC.

**HCV testing**
Serum samples were stored at -20°C and tested for HCV antibodies with a third-generation commercial microparticle enzyme immunoassay (MEIA), AxSYM® HCV 3.0 (Abbott Laboratories, Abbott Park, Illinois, USA). When positive for HCV antibodies, sera were qualitatively tested for HCV RNA using an in-house real-time PCR assay targeting the highly conserved 5' untranslated
region (UTR) of the HCV genome [18]. RNA was extracted from 200µl serum using the TriPure method (Roche Diagnostics, Almere, the Netherlands) and eluted in a volume of 50µl. Real-time PCR mixes (25µl total volume) contained 12.5µl of 2×Reaction Mix (Superscript One-Step RT PCR kit, Invitrogen, USA), 0.2µl of forward and reverse primers, 100ng/ml [19], and 0.1µl of FAM-labelled HCV TaqMan® probe, 100 ng/ml [18]. Real-time runs were performed on a Rotor Gene (Qiagen, Germany) using the following cycling conditions: 15 min at 45°C, 2 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 30 sec at 50°C, and 30 sec at 60°C. Samples with a threshold cycle (Ct) ≤37 and an expected S-curve were considered positive. Samples with Ct between 37 and 40 were retested and considered positive when Ct ≤40.

Sequence analysis
After detection of HCV RNA, viral genotype was determined using nested PCR targeting HCV core [20] and NS5B [18] regions. We obtained NS5B sequences using an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and created alignments with GenBank reference sequences using Mega v5.0 (GenBank Accession Nos. JQ917721-JQ917762) [21]. A phylogenetic tree was constructed by the neighbour-joining method, using the Tamura-Nei substitution model [22] with γ-distribution (α=0.40). Inferred phylogenies were tested with 1000 bootstrap replications.

Questionnaire
Participants completed a computer-assisted self-interview. The questionnaire reflected characteristics and behavior in the six months preceding the recruitment visit. It addressed demographics and sexual behaviors in up to four specified partnerships: one self-defined steady partner and the most recent three other partners. These others could be self-defined as steady, known (i.e., traceable), or anonymous (i.e., non-traceable). In the questionnaire, lifestyle was determined by asking whether the participant characterized himself by code of dressing or as belonging to a certain social stream or subculture within the gay community. Based on a knowledge from the internet and the agendas of bars, clubs and parties we provided the following options: casual, formal, alternative, drag, leather, military, sports, trendy, punk/skinhead, rubber/lycra, gothic, bear, jeans, skater and other if none of these characteristics applied. In the last case, MSM had the opportunity to give their own description. No a priori definition of lifestyle was given to allow participants to subjectively determine what subculture most applied to them. In the current study we only used subcultures that are typical subcultures in the MSM community and to which specific meeting venues or parties were linked. These included leather, rubber/lycra, military, jeans, and sports subcultures. Multiple answers were possible. Questions about sexual risk behaviors were asked about each of the specified partnerships.

Statistical analysis
To examine whether the presence of HCV antibodies was associated with characteristics
that could easily identify the subpopulation(s) most at risk for HCV we performed initial data analysis, including $\chi^2$-tests for independence for dichotomous and categorical variables and Mann-Whitney U tests for continuous variables. Fisher’s exact tests were performed when the expected value in a cell was less than one. Furthermore, we performed univariable and multivariable logistic regression analysis. In advance, we selected a set of variables for the analyses that could help identifying individuals at risk without asking questions. Multivariable analysis was performed by including all selected variables into the model and by using backward stepwise regression. The continuous variable age was modelled as restricted cubic splines with knots at the 2.5th, 25th, 50th, 75th, and 97.5th percentiles [23]. To examine whether specific sexual risk behavior was associated with the high-HCV risk subpopulation(s), we performed multivariable logistic regression analysis without a backward selection algorithm, with high-risk subcultures as outcome and various sexual risk behaviors as covariates. The results of this analysis are shown in Appendix I. Phylogenetic analysis was performed to identify monophyletic clusters (bootstrap >70%) of more than 10 individuals. The characteristics and also risk behavior within the resulting clusters were analysed and compared with each other and with the remainder, a group consisting of a smaller cluster and singletons. We used $\chi^2$-tests and Fisher’s exact tests for dichotomous and categorical variables, and Kruskal-Wallis tests for continuous variables. Analysis was done using STATA 11.1 (STATA Corp., College Station, TX, USA) and R version 2.14.2 [24].

RESULTS

Study population
Of the 2694 recruited MSM, 788 individuals (29.3%) were HIV-infected. Of these, two were excluded because their questionnaire data were incomplete, resulting in a study population of 786 HIV-infected MSM. Of these, 586 were recruited at the STI outpatient clinic and 200 at the HIV outpatient clinic. The median age of the total study population was 43 (IQR 37-48), and 71.3% of the population was Dutch (table 1). MSM recruited at the STI clinic were younger, less often Dutch, reported higher sexual risk behavior and were more often diagnosed with STI than MSM recruited at the HIV clinic (table 1). Sexual risk behavior in this HIV-infected population was high, with receptive unprotected anal intercourse (UAI) reported by 51.3%, receptive fisting by 14.5%, group sex by 38.7%, and recreational drugs, excluding poppers, by 42.3% (table 1). Two individuals reported a history of injecting drug use; one was HCV-antibody-positive (1.1% of all HCV-seropositive participants) and one was HCV-antibodynegative (0.1% of all HCV-seronegative participants). Characteristics of the population according to HCV serostatus are shown in table 2.

Determinants of HCV seropositive status
HCV antibodies were present in 93 of 786 HIV-infected MSM; the HCV prevalence was
11.8% (95% CI: 9.6-14.1%). We analyzed whether easily identifiable characteristics (i.e., age, ethnicity, lifestyle/subculture variables) were associated with HCV seropositivity. In univariable analysis, age and ethnicity were not significantly associated, whereas several subculture variables were (table 3). In multivariable analysis, men self-typed as leather (adjusted odds ratio [aOR] 2.60; 95% CI: 1.56-4.33), rubber/lycra (aOR 2.15; 95% CI: 1.10-4.21), or jeans (aOR 2.23; 95% CI: 1.41-3.54) were more likely to be HCV-seropositive than men who did not identify with those subcultures (table 3). Interactions between the three variables were tested, but did not improve the model ($P= .330$). Among 786 HIV-infected persons, 328 (41.7%) MSM belonged to one of the high-risk subcultures (leather, rubber/lycra or jeans); among HCV-negatives, 261 (37.7%) of the 693 and among HCV-infected MSM, 67 (72.0%) of the 93 belonged to a high-risk subculture. The likelihood of being HCV seropositive increased when MSM belonged to multiple subcultures. Compared to not belonging to a subculture, the OR was 1.73 (95% CI: 0.91-3.27) when MSM belonged to one subculture and increased to 4.56 (95% CI: 2.59-8.04) when they belonged to two subcultures, and to 6.70 (95% CI: 3.36-13.34) for when they belonged to three or more subcultures. Eighty percent of the MSM who belonged to one of the identified subcultures themselves reported at least one partner who also belonged to one of the subcultures, while only 26.6% of the MSM who did not belong to any of the subcultures reported a partner who belongs to a subculture.

In the total group of 786 MSM, we examined whether the high-risk subcultures were associated with particular sexual behaviors. In multivariable analysis, they were more often linked with a higher number of partners ($P<.001$), receptive fisting (aOR 2.82; 95% CI: 1.59-5.02), and use of recreational drugs (aOR 1.48; 95% CI: 1.03-2.12) (table 4).

**Phylogenetic analysis**

Serum samples from the 93 HCV-seropositive MSM were tested for the presence of HCV RNA, and HCV RNA was detected in 46 (49.5%). The RNA-positive and RNA-negative men did not differ as to demographics or STI coinfections. NS5B sequences were obtained from 42 (91.3%) of the 46 RNA-positive samples, yielding HCV genotypes 1a (57.1%), 1b (7.1%), 3a (2.4%), and 4d (33.3%). Phylogenetic analysis revealed two monophyletic clusters of N=13 and N=14 (clusters I and II, respectively), one smaller cluster of N=7 (cluster III) and 8 singletons (Figure 1).

Descriptive analysis was performed on clusters I and II and on the remainder group of 15. The men in the three groups did not differ significantly by age or ethnicity (table 5). Spread of the different HCV strains was not restricted to the specific subcultures (i.e., leather, military, rubber/lycra, or jeans), as there was no significant association between subcultures and HCV cluster. Those in the remainder group were more often sports-type MSM than men in clusters I or II: 60.0% vs. 15.4% and 21.4% ($P= .023$) (table 5). A significant difference between cluster I and II was seen for the median number of partners in the preceding six months ($P= .037$): in cluster I, it was 25 (IQR 15-50) and in cluster II, 5.5 (IQR 1-25); in the remainder group, it was
8 (IQR 5-30). In cluster analysis that included the remainder, this difference among groups had only borderline significance ($P = .059$). Insertive UAI was more often reported by men in the remainder group: 93.3% vs. 61.5% in cluster I and 35.7% in cluster II ($P = .005$), but the difference between cluster I and II was not significant ($P = .180$). Other sexual risk behavior (i.e., receptive UAI, fisting, group sex, use of poppers and other drugs), a history of syphilis infection, and the occurrence of chlamydia and gonorrhea did not differ significantly between the two monophyletic clusters and the remainder group.

**DISCUSSION**

In this study of 786 HIV-infected MSM, we showed that MSM belonging to the leather, rubber/lycra, and jeans subcultures were more likely to be HCV-seropositive than MSM who did not pursue those lifestyles. Moreover, high-risk sexual behavior was more common among these MSM than among those not belonging to these subcultures. It was remarkable that 72.0% of the men who were HCV-seropositive belonged to one of the high-risk subcultures, while only 37.7% of the HCV-negative HIV-infected population belonged to one of these subpopulations. The analysis of monophyletic clusters of men who were HCV RNA-positive did not show separate networks for HCV transmission. HCV genotypes in the remainder group were found to be associated with the sports scene, but because the remainder was a composite of a smaller cluster and singletons, this finding did not represent a transmission network. Our results did not confirm the hypothesis that, within a city, HCV strains from MSM with the same lifestyle would cluster together.

A strength of this study was the large amount of available epidemiological data. Participants completed a detailed questionnaire that was designed to study sexual networks. We therefore had the opportunity to study determinants of HCV that are useful for subgroup identification. No a priori definition of lifestyle was given to allow participants to subjectively determine what subculture most applied to them. Therefore, MSM who felt they belonged to a subculture because they visited venues associated with it or met partners who belonged to the subculture, but for example, did not use the subcultures dress code, could still be identified as being part of the subculture. There was a large agreement between self-defined subculture and subculture of the partners, supporting the robustness of the definition of subculture.

The presence of HCV RNA was tested with a real-time PCR targeting the highly conserved 5’UTR of the HCV genome, which has a detection limit similar to the nested NS5B PCR that was used to generate sequences. HCV-seropositive MSM who were RNA negative were likely to have cleared the infection, either spontaneously or by antiviral therapy, although the spontaneous clearance rate among HIV-infected individuals is low [25]. The genotype distribution found in this study was in accordance with earlier studies that included HCV-
infected MSM in Amsterdam [11,26], We may have missed MSM with acute HCV infection, because HCV status was determined by HCV antibody screening, and RNA testing was performed only in those who were antibody positive. A delay in formation of HCV antibodies after HCV infection has been described previously in HIV-coinfected men by Thomson et al. [27]. In a bi-annual anonymous survey held at the STI clinic in Amsterdam, prevalence of acute HCV infections (antibody negative, RNA positive) was 1.8% among HIV+ MSM in the period 2008-2009 [unpublished data]. A second and probably more important limitation was that the monophyletic clusters were relatively small. We therefore performed univariable analysis only on the two largest clusters and a remainder group consisting of a smaller cluster and singletons. Another concern is that MSM enrollment at two different facilities yielded slightly divergent groups. Those recruited at the STI clinic had all been previously involved in sexual risk behavior, as indicated by their interest in STI care. However, those recruited the HIV clinic had not necessarily engaged in risk behavior; in fact, such behavior was much lower among them. Because most participants were recruited at the STI clinic, the study population is a non-random sample of the general HIV-infected population. We do not know whether the associations between subcultures and HCV seropositivity found in the study population are similar to the associations in the general population. The reported effects of subcultures on HCV seropositivity may deviate (ie, under- or overestimation) from the true effects in the general HIV-infected population.

Finally, questionnaires reflected sexual risk behavior in the six months preceding participation. As HCV infection may have occurred before that, responses about risk behavior may not reflect behavior at actual time of infection. This time lag could also apply to responses about identification with lifestyle or subculture, although such identification tends to be more stable over time.

Our results are in line with several previous studies showing that multiple sexual partners, receptive unprotected anal intercourse, group sex, rough sexual techniques (e.g., fisting), and recreational drug use were associated with HCV infection [1,11,12]. We showed that these factors were likewise associated with the highrisk subcultures of leather, rubber/lycra and jeans-type MSM. Nevertheless, such behaviors were not the main interest of this study, because their role in subculture identification is more complicated than the role of demographic or lifestyle factors.

Little is known about subcultures within the MSM population. A few studies have described specifically the leather scene [28-31], but there is no literature on the other subcultures. The leather scene is a self-defined lifestyle characterized by leather clothing, rough sexual activities, heightened valuation of hypersexuality, and adherence to sexual control dynamics (e.g., dominance and submissiveness) [28-30], in which unprotected anal intercourse is common [31]. The fact that mixing of subpopulations occurs is supported by the notion that many
MSM reported to belong to more than one subculture and HCV spread was not restricted to just one subculture.

Due to the high risk behavior in the identified subcultures, it is possible that HCV was introduced in the MSM population by one or more of these subcultures. To test this hypothesis, temporal data of several decades on MSM subcultures is needed. To our knowledge, this data is not available. A molecular clock analysis was therefore not performed, given that the time interval between the emergence of the leather subculture and the recent HCV epidemic is relatively short, and also the sample size was limited. More extensive research is needed into the behavioral characteristics of subcultures and the extent to which subcultures mix. More information may lead to an improved understanding of risk behavior and the accompanying spread of HCV and also of HIV and other STI within and between subpopulations. We suggest that among MSM who are sexually active within such subgroups HCV screening should be intensified. In addition to the current screening practices at STI clinics and HIV treatment centres, on location screening initiatives, in specific places where such subgroups meet, could be an efficient way to achieve this goal.

Currently, the HCV outbreak is an epidemic mainly in a restricted subpopulation, but it is unknown how this epidemic will evolve. In this study we used HCV seroprevalent cases. It would be interesting for future studies to examine whether HCV reinfection, which is common among HIV-infected MSM, more often occurs in the high risk populations. While it is assumed that sexually transmitted HCV spreads only in HIV-infected MSM, there have been a few case reports of HCV infection in the absence of HIV [32]. In Amsterdam, the prevalence of HCV among HIV-infected MSM seems to be levelling off since 2011 [33], suggesting that the incidence is declining, however, the incidence of reinfection among successfully treated MSM is very high (15.2 per 100 person years) [26], demonstrating that HCV transmission occurs.

To conclude, we found that HIV-infected MSM belonging to the leather, rubber/lycra and jeans subcultures are at increased risk of acquiring HCV, compared to MSM who do not belong to these subcultures. For public health purposes, we provide a clear description of the MSM population in which most HCV infections might be detected. Implementation of active screening, followed by treatment, in the leather, rubber/lycra and jeans scenes, may reduce HCV incidence and prevalence and the future HCV disease burden among MSM.

ACKNOWLEDGMENTS

The authors would like to thank all other members of the study team, i.e., Reinier Bom, Roel Coutinho, Han Fennema, Titia Heijman, Mirjam Kretschmar, Arjen Speksnijder, and Maria Xiridou, for their contributions to the design and implementation of the study. We
would like to thank the participants, and also Martijn van Rooijen for data management, Marlies Heiligenberg and Suzanne Geerlings for organising the logistics of participants at the HIV outpatient clinic, Udi Davidovich for his scientific input, and Lucy Phillips for editing the manuscript.

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Table 1  Characteristics of 786 HIV-infected MSM, by recruitment location, who visited the STI outpatient clinic of the Public Health Service or the HIV outpatient clinic of the Academic Medical Center in Amsterdam, the Netherlands, 2008-2009.

<table>
<thead>
<tr>
<th></th>
<th>Total population (N=786)</th>
<th>GGD (N=586)</th>
<th>AMC (N=200)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age in years (IQR)</td>
<td>43 (37-48)</td>
<td>41 (36-47)</td>
<td>47 (42-53)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>560 (71.3%)</td>
<td>394 (67.2%)</td>
<td>166 (83.0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Western, non-Dutch</td>
<td>98 (12.5%)</td>
<td>84 (14.3%)</td>
<td>14 (7.0%)</td>
<td></td>
</tr>
<tr>
<td>Non-western</td>
<td>128 (16.3%)</td>
<td>108 (18.4%)</td>
<td>20 (10.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Subculture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casual</td>
<td>693 (88.2%)</td>
<td>517 (88.2%)</td>
<td>176 (88.0%)</td>
<td>.932</td>
</tr>
<tr>
<td>Leather</td>
<td>153 (19.5%)</td>
<td>123 (21.0%)</td>
<td>30 (15.0%)</td>
<td>.065</td>
</tr>
<tr>
<td>Military</td>
<td>71 (9.0%)</td>
<td>60 (10.2%)</td>
<td>11 (5.5%)</td>
<td>.044</td>
</tr>
<tr>
<td>Sport</td>
<td>171 (21.8%)</td>
<td>143 (24.4%)</td>
<td>28 (14.0%)</td>
<td>.002</td>
</tr>
<tr>
<td>Rubber/lycra</td>
<td>57 (7.3%)</td>
<td>45 (7.7%)</td>
<td>12 (6.0%)</td>
<td>.429</td>
</tr>
<tr>
<td>Jeans</td>
<td>251 (31.9%)</td>
<td>53 (26.5%)</td>
<td>198 (33.8%)</td>
<td>.056</td>
</tr>
<tr>
<td><strong>Sexual behaviour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median no. of partners in the preceding 6 months (IQR)</td>
<td>8 (3-20)</td>
<td>10 (5-25)</td>
<td>3 (1-8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Receptive UAI¹</td>
<td>400 (51.3%)</td>
<td>58 (29.9%)</td>
<td>342 (58.4%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insertive UAI¹</td>
<td>348 (44.6%)</td>
<td>303 (51.7%)</td>
<td>45 (23.2%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Receptive fisting</td>
<td>113 (14.5%)</td>
<td>100 (17.2%)</td>
<td>13 (6.7%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insertive fisting</td>
<td>126 (16.2%)</td>
<td>109 (18.7%)</td>
<td>17 (8.8%)</td>
<td>.001</td>
</tr>
<tr>
<td>Group sex</td>
<td>301 (38.7%)</td>
<td>252 (43.2%)</td>
<td>49 (25.3%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Poppers use</td>
<td>416 (53.5%)</td>
<td>347 (59.5%)</td>
<td>69 (35.6%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Other drug use²</td>
<td>329 (42.3%)</td>
<td>289 (49.6%)</td>
<td>40 (20.6%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Sexually transmitted infections diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis³</td>
<td>377 (48.0%)</td>
<td>320 (54.6%)</td>
<td>57 (28.5%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>131 (16.7%)</td>
<td>115 (19.6%)</td>
<td>16 (8.1%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>106 (13.5%)</td>
<td>104 (17.8%)</td>
<td>2 (1.0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HCV</td>
<td>93 (11.8%)</td>
<td>81 (13.8%)</td>
<td>12 (6.0%)</td>
<td>.003</td>
</tr>
</tbody>
</table>

NOTE: Numbers do not always add up to the column totals due to missing data; there was 1 missing value for the age variable, 6 missing values for receptive and insertive UAI, 9 missing for other risk behavior variables, and 2 missing for the chlamydia and gonorrhea variables.

NOTE: The subculture characteristics are not mutually exclusive. HIV = human immunodeficiency virus; STI = sexually transmitted infection; IQR = interquartile range; UAI = unprotected anal intercourse.
1 \( P \)-values were calculated for recruitment at the STI clinic (GGD) versus the HIV clinic (AMC) and considered significant when \( P<.05 \). 2 Recreational use of cocaine, XTC, gamma hydroxybutyrate (GHB), ketamines, amphetamines, or methylamphetamines before or during sexual contact. 3 Based on serological evidence.
Table 2 Characteristics of 786 HIV-infected men who have sex with men, by hepatitis C antibody status, who visited the STI outpatient clinic of the Public Health Service or the HIV outpatient clinic of the Academic Medical Center in Amsterdam, 2008-2009.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HCV-antibody-positive (%)</th>
<th>HCV-negative (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STI clinic</td>
<td>81 (87.1%)</td>
<td>505 (72.9%)</td>
<td>.003</td>
</tr>
<tr>
<td>HIV clinic</td>
<td>12 (12.9%)</td>
<td>188 (27.1%)</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age in years (IQR)</td>
<td>44 (39-49)</td>
<td>42 (37-48)</td>
<td>.062</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>71 (76.3%)</td>
<td>489 (70.6%)</td>
<td>.092</td>
</tr>
<tr>
<td>Western, non-Dutch</td>
<td>14 (15.1%)</td>
<td>84 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>Non-western</td>
<td>8 (8.6%)</td>
<td>120 (17.3%)</td>
<td></td>
</tr>
<tr>
<td>Subculture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casual</td>
<td>79 (85.0%)</td>
<td>614 (88.6%)</td>
<td>.306</td>
</tr>
<tr>
<td>Leather</td>
<td>40 (43.0%)</td>
<td>113 (16.3%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Military</td>
<td>16 (17.2%)</td>
<td>55 (7.9%)</td>
<td>.003</td>
</tr>
<tr>
<td>Sport</td>
<td>32 (34.4%)</td>
<td>139 (20.1%)</td>
<td>.002</td>
</tr>
<tr>
<td>Rubber/lycra</td>
<td>18 (19.4%)</td>
<td>39 (5.6%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Jeans</td>
<td>50 (53.8%)</td>
<td>201 (29.0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sexual behaviour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median no. of partners in the preceding 6 months (IQR)</td>
<td>10 (5-30)</td>
<td>8 (3-20)</td>
<td>.001</td>
</tr>
<tr>
<td>Receptive UAI¹</td>
<td>67 (72.0%)</td>
<td>333 (48.5%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insertive UAI¹</td>
<td>60 (64.5%)</td>
<td>288 (41.9%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Receptive fisting</td>
<td>27 (29.0%)</td>
<td>86 (12.6%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insertive fisting</td>
<td>27 (29.0%)</td>
<td>99 (14.5%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Group sex</td>
<td>59 (63.4%)</td>
<td>242 (35.4%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Poppers use</td>
<td>63 (67.7%)</td>
<td>353 (51.6%)</td>
<td>.003</td>
</tr>
<tr>
<td>Other drug use²</td>
<td>61 (65.6%)</td>
<td>268 (39.2%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sexually transmitted infections diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis³</td>
<td>64 (68.8%)</td>
<td>313 (45.2%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>18 (19.4%)</td>
<td>113 (16.4%)</td>
<td>.466</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>14 (15.1%)</td>
<td>92 (13.3%)</td>
<td>.645</td>
</tr>
</tbody>
</table>

NOTE: Percentages do not always add up to 100% due to rounding.

NOTE: Numbers do not always add up to the column totals due to missing data; there was 1 missing value for the age variable, 6 missing values for receptive and insertive UAI, 9 missing for other risk behavior variables, and 2 missing for the chlamydia and gonorrhoea variables.
NOTE: The subculture characteristics are not mutually exclusive. HIV = human immunodeficiency virus; STI = sexually transmitted infection; IQR = interquartile range; UAI = unprotected anal intercourse. \(^1\) P values were calculated for HCV antibody positives versus HCV antibody negatives and considered significant when \(p < 0.05\). \(^2\) Recreational use of cocaine, XTC, gamma hydroxybutyrate (GHB), ketamines, amphetamines, or methylamphetamines before or during sexual contact. \(^3\) Based on serological evidence.

**Table 3** Identifiable determinants of hepatitis C seropositive status among 786 HIV-infected men who have sex with men, of whom 93 were hepatitis C seropositive, in Amsterdam, 2008-2009.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>OR(^1) (95% CI)</th>
<th>p</th>
<th>aOR(^2) (95% CI)</th>
<th>p</th>
<th>aOR(^3) (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years(^4)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>35</td>
<td>0.78 (.48–1.27)</td>
<td>.082</td>
<td>0.86 (.53–1.42)</td>
<td>.310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1.26 (.90–1.75)</td>
<td>.100</td>
<td>1.26 (.79–2.01)</td>
<td>.310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1.50 (.86–2.60)</td>
<td>.100</td>
<td>1.25 (.61–2.57)</td>
<td>.310</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western, non-Dutch</td>
<td>1.15 (.62-2.13)</td>
<td>.100</td>
<td>1.13 (.59-2.16)</td>
<td>.310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-western</td>
<td>0.46 (.22-.98)</td>
<td>.100</td>
<td>0.56 (.25-1.25)</td>
<td>.310</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subculture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leather</td>
<td>3.87 (2.45-6.12)</td>
<td>&lt;.001</td>
<td>2.59 (1.50-4.47)</td>
<td>&lt;.001</td>
<td>2.60 (1.56-4.33)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Military</td>
<td>2.41 (1.32-4.41)</td>
<td>.004</td>
<td>0.74 (1.36-1.53)</td>
<td>.420</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sport</td>
<td>2.09 (1.31-3.33)</td>
<td>.002</td>
<td>1.77 (1.03-3.03)</td>
<td>.037</td>
<td></td>
<td></td>
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<tr>
<td>Rubber/lycra</td>
<td>4.02 (2.19-7.39)</td>
<td>&lt;.001</td>
<td>2.03 (1.01-4.08)</td>
<td>.046</td>
<td>2.15 (1.10-4.21)</td>
<td>.026</td>
</tr>
<tr>
<td>Jeans</td>
<td>2.85 (1.83-4.42)</td>
<td>&lt;.001</td>
<td>1.95 (1.20-3.17)</td>
<td>.007</td>
<td>2.23 (1.41-3.54)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE: There was 1 missing value in the age variable. HIV = human immunodeficiency virus; OR = odds ratio; CI = confidence interval; aOR = adjusted odds ratio.

\(^1\)Odds ratio resulting from univariable analysis. \(^2\) Odds ratio adjusted for all variables. \(^3\) Odds ratio adjusted for variables in the model after backward selection. \(^4\) Modelled as restricted cubic spline with knots at the 2.5\(^{th}\), 25\(^{th}\), 50\(^{th}\), 75\(^{th}\), and 97.5\(^{th}\) percentiles.
Table 4  Sexual behavior associated with a high-HCV-risk subculture (i.e., leather, rubber/lycra, or jeans) among 786 HIV-infected MSM, Amsterdam, 2008-2009.

<table>
<thead>
<tr>
<th></th>
<th>High-HCV-risk subculture (N = 328)</th>
<th>Other subculture (N = 458)</th>
<th>OR(^1)</th>
<th>(95% CI)</th>
<th>p</th>
<th>aOR(^2)</th>
<th>(95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of partners in the preceding 6 months(^3)</td>
<td>10 (IQR 4–30)</td>
<td>6 (IQR 3–15)</td>
<td>&lt;.001</td>
<td>.005</td>
<td>.97</td>
<td>1.80</td>
<td>(1.16–2.79)</td>
<td>.032</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.93 (.73-1.18)</td>
<td>0.84 (.60-1.17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.72 (1.16-2.56)</td>
<td>1.35 (.87-2.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2.43 (1.65-3.57)</td>
<td>1.80 (1.16-2.79)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptive UAI(^4)</td>
<td>186/400 (46.5%)</td>
<td>139/380 (36.6%)</td>
<td>1.51</td>
<td>(1.13–2.01)</td>
<td>.005</td>
<td>0.97</td>
<td>(.66–1.42)</td>
<td>.860</td>
</tr>
<tr>
<td>Insertive UAI(^4)</td>
<td>161/348 (46.3%)</td>
<td>164/432 (38.0%)</td>
<td>1.41</td>
<td>(1.06–1.87)</td>
<td>.020</td>
<td>0.88</td>
<td>(.61–1.29)</td>
<td>.520</td>
</tr>
<tr>
<td>Receptive fisting</td>
<td>79/113 (69.9%)</td>
<td>245/664 (36.9%)</td>
<td>3.97</td>
<td>(2.58–6.12)</td>
<td>&lt;.001</td>
<td>2.82</td>
<td>(1.59–5.02)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insertive fisting</td>
<td>77/126 (61.1%)</td>
<td>247/651 (37.9%)</td>
<td>2.57</td>
<td>(1.74–3.80)</td>
<td>&lt;.001</td>
<td>1.08</td>
<td>(.63–1.85)</td>
<td>.790</td>
</tr>
<tr>
<td>Group sex</td>
<td>158/301 (52.5%)</td>
<td>166/476 (34.9%)</td>
<td>2.06</td>
<td>(1.53–2.77)</td>
<td>&lt;.001</td>
<td>1.11</td>
<td>(.77–1.61)</td>
<td>.570</td>
</tr>
<tr>
<td>Poppers use</td>
<td>199/416 (47.8%)</td>
<td>125/361 (34.6%)</td>
<td>1.73</td>
<td>(1.30–2.31)</td>
<td>&lt;.001</td>
<td>1.22</td>
<td>(.87–1.70)</td>
<td>.240</td>
</tr>
<tr>
<td>Drug use(^5)</td>
<td>172/329 (52.3%)</td>
<td>152/448 (33.9%)</td>
<td>2.13</td>
<td>(1.59–2.86)</td>
<td>&lt;.001</td>
<td>1.48</td>
<td>(1.03–2.12)</td>
<td>.032</td>
</tr>
</tbody>
</table>

NOTE: Numbers do not always add up to the column totals due to missing data; there were 6 missing values in receptive and insertive UAI and 9 missing in variables for fisting, group sex, poppers, and drug use. \(^1\)Odds ratio. \(^2\)adjusted odds ratio. \(^3\)Modelled as restricted cubic spline, and thus no group size or OR could be reported; instead the median and IQR and p-values for the logistic regression are provided. \(^4\)Unprotected anal intercourse. \(^5\)Recreational use of cocaine, XTC, gamma hydroxybutyrate (GHB), ketamines, amphetamines, or methylamphetamines before or during sexual contact.
Table 5 Epidemiological characteristics of 42 men who have sex with men who tested hepatitis C RNA-positive, by phylogenetic cluster, Amsterdam, 2008-2009.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Cluster I (N=13)</th>
<th>Cluster II (N=14)</th>
<th>Other (N=15)</th>
<th>$p^1$</th>
<th>$p^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment location</td>
<td></td>
<td></td>
<td></td>
<td>.077</td>
<td>.149</td>
</tr>
<tr>
<td>STI clinic</td>
<td>13 (100.0%)</td>
<td>11 (78.6%)</td>
<td>14 (93.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV clinic</td>
<td>0</td>
<td>3 (21.4%)</td>
<td>1 (6.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age in years (IQR)</td>
<td>47 (40-49)</td>
<td>48 (44-52)</td>
<td>44 (39-46)</td>
<td>.436</td>
<td>.178</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>.995</td>
<td>.954</td>
</tr>
<tr>
<td>Dutch</td>
<td>10 (76.9%)</td>
<td>11 (78.6%)</td>
<td>10 (66.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western, non-Dutch</td>
<td>2 (15.4%)</td>
<td>2 (14.3%)</td>
<td>3 (20.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-western</td>
<td>1 (7.7%)</td>
<td>1 (7.1%)</td>
<td>2 (13.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subculture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leather</td>
<td>5 (38.5%)</td>
<td>8 (57.1%)</td>
<td>7 (46.7%)</td>
<td>.332</td>
<td>.621</td>
</tr>
<tr>
<td>Military</td>
<td>1 (7.7%)</td>
<td>4 (28.6%)</td>
<td>5 (35.7%)</td>
<td>.163</td>
<td>.248</td>
</tr>
<tr>
<td>Sport</td>
<td>2 (15.4%)</td>
<td>3 (21.4%)</td>
<td>9 (60.0%)</td>
<td>.686</td>
<td>.023</td>
</tr>
<tr>
<td>Rubber/lycra</td>
<td>3 (23.1%)</td>
<td>5 (35.7%)</td>
<td>1 (6.7%)</td>
<td>.472</td>
<td>.160</td>
</tr>
<tr>
<td>Jeans</td>
<td>6 (46.2%)</td>
<td>8 (57.1%)</td>
<td>8 (53.3%)</td>
<td>.568</td>
<td>.846</td>
</tr>
<tr>
<td>Sexual behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median no. of partners in the preceding 6 months (IQR)</td>
<td>25 (15-50)</td>
<td>5.5 (1-25)</td>
<td>8 (5-30)</td>
<td>.037</td>
<td>.059</td>
</tr>
<tr>
<td>Receptive UAI</td>
<td>11 (84.6%)</td>
<td>10 (71.4%)</td>
<td>10 (66.7%)</td>
<td>.410</td>
<td>.543</td>
</tr>
<tr>
<td>Insertive UAI</td>
<td>8 (61.5%)</td>
<td>5 (35.7%)</td>
<td>14 (93.3%)</td>
<td>.180</td>
<td>.005</td>
</tr>
<tr>
<td>Receptive fisting</td>
<td>2 (15.4%)</td>
<td>6 (42.9%)</td>
<td>3 (20.0%)</td>
<td>.118</td>
<td>.213</td>
</tr>
<tr>
<td>Insertive fisting</td>
<td>3 (23.1%)</td>
<td>5 (35.7%)</td>
<td>4 (26.7%)</td>
<td>.472</td>
<td>.752</td>
</tr>
<tr>
<td>Group sex</td>
<td>8 (61.5%)</td>
<td>8 (57.1%)</td>
<td>10 (66.7%)</td>
<td>.816</td>
<td>.870</td>
</tr>
<tr>
<td>Poppers use</td>
<td>9 (69.2%)</td>
<td>10 (71.4%)</td>
<td>9 (60.0%)</td>
<td>.901</td>
<td>.786</td>
</tr>
<tr>
<td>Drug use$^3$</td>
<td>7 (53.9%)</td>
<td>7 (50.0%)</td>
<td>12 (80.0%)</td>
<td>.842</td>
<td>.194</td>
</tr>
<tr>
<td>Sexually transmitted infections diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis$^4$</td>
<td>10 (76.9%)</td>
<td>11 (78.6%)</td>
<td>9 (60.0%)</td>
<td>.918</td>
<td>.472</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>3 (23.1%)</td>
<td>1 (7.1%)</td>
<td>4 (26.7%)</td>
<td>.244</td>
<td>.370</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>1 (7.7%)</td>
<td>3 (21.4%)</td>
<td>3 (20.0%)</td>
<td>.315</td>
<td>.576</td>
</tr>
</tbody>
</table>

NOTE: The subculture characteristics are not mutually exclusive. RNA = ribonucleic acid; STI = sexually transmitted infection; HIV = human immunodeficiency virus; IQR = interquartile range; UAI = unprotected anal intercourse; $^1$P-value for $\chi^2$-tests and Kruskal-Wallis tests of cluster I and II; $^2$P-value for $\chi^2$-tests and Kruskal-Wallis tests of cluster I, II, and the remainder group; $^3$Recreational use of cocaine, XTC, gamma hydroxybutyrate (GHB), ketamines, amphetamines, or methylamphetamines before or during sexual contact; $^4$Based on serological evidence.
Figure 1 Phylogenetic tree of 42 HCV NS5B sequences obtained from HIV-infected MSM in Amsterdam. Three clusters were identified: cluster I with genotype 1a (N=13), cluster II with genotype 4d (N=14), a smaller cluster III with genotype 1a (N=7), and 8 singletons. Self-identified subcultures are indicated as follows: leather in black; jeans in yellow; rubber/lycra in green, sports in red; no subculture in white. History of injecting drug use is indicated by a needle. More than one subculture per person is possible.
REFERENCES


RISK FACTORS FOR SEXUAL TRANSMISSION OF HEPATITIS C VIRUS AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED MEN WHO HAVE SEX WITH MEN: A CASE-CONTROL STUDY

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Joost W. Vanhommerig, Femke A.E. Lambers, Janke Schinkel, Ronald B. Geskus, Joop E. Arends, Thijs J.W. van de Laar, Fanny N. Lauw, Kees Brinkman, Luuk Gras, Bart J.A. Rijnders, Jan T.M. van der Meer, Maria Prins; on behalf of the MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) Study Group

Presented at the at the 8th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), 2014, Amsterdam, NL (oral abstract #10), the 22nd CROI, 2015, Seattle, WA, USA (poster abstract #674), the 15th ISVHD Conference, 2015, Berlin, Germany (poster abstract #125), and at the 12th International AIDS Impact Conference, 2015, Amsterdam, NL (oral abstract #2034).
ABSTRACT

Background
Since 2000, incidence of sexually acquired hepatitis C virus (HCV)-infection has increased among human immunodeficiency virus (HIV)-infected men who have sex with men (MSM). To date, few case-control and cohort studies evaluating HCV transmission risk factors were conducted in this population, and most of these studies were initially designed to study HIV-related risk behavior and characteristics.

Methods
From 2009 onwards, HIV-infected MSM with acute HCV infection and controls (HIV-monoinfected MSM) were prospectively included in the MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) study at 5 large HIV outpatient clinics in the Netherlands. Written questionnaires were administered, covering sociodemographics, bloodborne risk factors for HCV infection, sexual behavior, and drug use. Clinical data were acquired through linkage with databases from the Dutch HIV Monitoring Foundation. For this study, determinants of HCV acquisition collected at the inclusion visit were analyzed using logistic regression.

Results
Two hundred thirteen HIV-infected MSM (82 MSM with acute HCV infection and 131 MSM without) were included with a median age of 45.7 years (interquartile range [IQR], 41.0–52.2). Receptive unprotected anal intercourse (adjusted odds ratio [aOR], 5.01; 95% confidence interval [CI], 1.63-15.4), sharing sex toys (aOR, 3.62; 95% CI, 1.04-12.5), unprotected fisting (aOR, 2.57; 95% CI, 1.02-6.44), injecting drugs (aOR, 15.62; 95% CI, 1.27-192.6), sharing straws when snorting drugs (aOR, 3.40; 95% CI, 1.39-8.32), lower CD4 cell count (aOR, 1.75 per cubic root; 95% CI, 1.19-2.58), and recent diagnosis of ulcerative sexually transmitted infection (aOR, 4.82; 95% CI, 1.60-14.53) had significant effects on HCV acquisition.

Conclusions
In this study, both sexual behavior and biological factors appear to independently increase the risk of HCV acquisition among HIV-infected MSM.
INTRODUCTION

Since 2000, outbreaks of sexually transmitted hepatitis C virus (HCV) have increasingly been reported among human immunodeficiency virus (HIV)-infected men who have sex with men (MSM) in Europe, Australia, Asia, and the United States [1–4]. Although some cases have been described to have acquired HCV through sexual route in the absence of HIV [5], the HIV-uninfected MSM population remains largely unaffected by this epidemic [4, 6–9].

After the increase of HCV incidence among HIV-infected MSM, 3 case-control studies have been conducted to elucidate determinants for HCV infection [10–12]. However, the 2 studies that included participants prospectively [11, 12] comprised small numbers of cases with acute HCV infection: 34 and 22, respectively. Independent risk factors that were identified in the 3 case-control studies were as follows: receptive unprotected anal intercourse (UAI), sex while high on methamphetamines [12], rectal bleeding, frequent receptive fisting, snorting cocaine or amphetamines [11], and group sex participation [10, 11].

Determinants for acute HCV infection among HIV-infected MSM have also been investigated retrospectively, in large HIV cohort studies in the United States [13], Switzerland [14], the Netherlands [8], and Japan [15]. These cohort studies led to accurate estimates of HCV incidence. However, because the initial scope of these cohorts was to study HIV, data on HCV-specific risk factors were limited. Independent risk factors for HCV acquisition that were identified in these studies were as follows: younger age [8], positive hepatitis B surface antigen test, alcohol abuse, lower CD4 cell count [13], illicit drug use, being on social benefits [15], injecting drug use (IDU) [13, 15], receptive UAI with multiple partners, and recent syphilis infection [13, 14].

Various other studies that addressed potential risk factors for HCV infection were limited by their study design (crosssectional studies including prevalent infections and case reports) [5, 7, 16–21]. Because the majority of the studied MSM had an unknown duration of HCV infection, the reported risk behavior and clinical parameters at the time of study may differ significantly from those at the time of HCV acquisition.

The MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) cohort has been initiated to specifically study acute HCV infection among HIV-infected MSM. This cohort is one of the largest case-control studies conducted until now and therefore provides a unique opportunity to study biological and behavioral risk factors for sexual transmission of HCV.
METHODS

Study Population
The MOSAIC cohort is an open, ongoing, prospective, observational cohort, initiated to study determinants and sequelae of acute HCV infection among HIV-infected MSM [22]. The MOSAIC is a collaboration between the Public Health Service of Amsterdam, 5 large HIV outpatient clinics in the Netherlands (3 in Amsterdam, 1 in Rotterdam, and 1 in Utrecht), and the Dutch HIV Monitoring Foundation. Study subjects were HIV-infected MSM ≥18 years of age who (recently) had acquired an acute HCV infection. Acute HCV infection was defined as having an interval ≤6 months between the first positive HCV RNA test and the preceding negative HCV RNA or antibody test. To serve as controls, we aimed to include 2 HIV-infected MSM with no history of HCV, at the same hospital and in the period shortly after a case was identified. Inclusion started in 2009, and for the current study, we included all prospectively identified cases and controls who entered the study before February 2014.

Data Collection
Hepatitis C virus antibody testing was performed using either AxSYM HCV 3.0 (Abbott Laboratories, Abbott Park, IL), ARCHITECT Anti-HCV (Abbott Laboratories), or Liaison XL (DiaSorin, Saluggia, Italy). Hepatitis C virus RNA tests were performed using either the VERSANT HCV RNA Qualitative Assay (Siemens Medical Solutions Diagnostics, Tarrytown, NY), COBAS Ampliprep/COBAS TaqMan (CAP/CTM; Roche Diagnostics, Mannheim, Germany), or the Abbott m2000 sp/rt system (Abbott Laboratories). Participants were followed up every 6 months, and more often during treatment of HCV infection, at their HIV outpatient clinic. At inclusion and follow-up visits, participants completed a self-administered questionnaire regarding sociodemographics, bloodborne risk factors classically related to HCV (eg, blood transfusion, IDU), sexual behavior with steady and/or casual sex partner(s), sex-related variables (eg, number of casual sex partners, meeting location), drug use before/during sex, and quality of life. Clinical data, such as date of HIV diagnosis, CD4 cell count, HIV viral load, and use of combination antiretroviral therapy (cART), were acquired for each visit through linkage with databases from the Dutch HIV Monitoring Foundation. The HCV-negative status of controls was assured by confirming the absence of HCV antibodies at inclusion and follow-up visits. The study protocol was approved by the local ethics committee, and all participants provided written informed consent to participate in the study.

Statistical Analysis
Determinants of HCV infection that were collected using the baseline questionnaire administered at the inclusion visit were analyzed using logistic regression. In univariable analysis, Firth’s penalized likelihood method [23] was used to obtain odds ratios (ORs) and 95% confidence intervals (CIs) when a cell in the analyzed table had zero frequency. Having unprotected sex only with a steady sex partner with a confirmed negative HCV status was...
not considered to be risk behavior for HCV. It has been suggested that HCV may also be transmitted from one receptive partner to another through (1) sharing contaminated sex toys or (2) contaminated gloves during fisting [11]. Fisting without gloves and fisting with gloves in the presence of group sex are therefore defined as “unprotected fisting” throughout this study. We assumed that use of sex toys without sharing, and fisting with gloves in the absence of group sex, did not elevate the risk of HCV acquisition. The number of casual sex partners was transformed as $2 \log(N + 1)$; HIV viral load was modeled as $10 \log$-increment above 50 copies/mL (values ≤50 were set at zero); CD4 cell count was cubic root transformed, to make the relationship with the outcome (HCV acquisition) more linear.

To limit the number of risk factors included in multivariable logistic regression, we performed 2 separate analyses. The first analysis only included variables that [1] were expected to have a direct effect on HCV acquisition, ie, traditional risk factors and sexual behavior (see table 2B and 2C), and [2] were significantly associated with acute HCV infection in univariable analysis ($P < .05$). The second multivariable analysis included variables that were significantly associated in the first multivariable analysis ($P < .05$), variables related to sexual behavior that were strongly associated ($P < .001$) with acute HCV in univariable analysis, and variables that might facilitate or enhance HCV transmission (ie, recent ulcerative sexually transmitted infection [STI], lower CD4 cell count). When investigating the influence of these facilitating circumstances, we checked for the presence of interactions. We assumed that each facilitating factor had an equal interaction effect on all variables related to sexual risk behavior. When significant ($P < .05$), the interaction term was added to the final model; otherwise, the facilitating factor was included in the model without an interaction term. All analyses were performed using Stata Intercooled 13.1 (StataCorp, College Station, TX).

RESULTS

General Population Characteristics
By February 1, 2014, 82 HIV-infected MSM with acute HCV infection (cases) and 131 HIV-infected controls had entered the MOSAIC study and completed the inclusion questionnaire. Characteristics of acute HCV infection (eg, HCV subtype, HCV RNA load at first positive visit, reported symptoms of acute infection) are shown in table 1. The vast majority of participants were included in the Amsterdam region (95.3%), and most were of Western European ethnicity (79.3%). The median age at study entry was 45.7 years, which was lower among cases (43.1 years) than controls (49.4 years; $P < .001$) (table 2A).

Risk Factors for Hepatitis C Virus: Univariable Analysis
Apart from IDU, which was reported by 10 of 82 cases (12.2%) versus 2 of 131 controls (1.5%), none of the traditional bloodborne risk factors were associated with acute HCV in
univariable analysis (table 2B). Sharing of needles was relatively uncommon among MSM who reported IDU (2 of 12; 16.7%).

Sexual risk behavior was higher among MSM with acute HCV compared with HCV-negative controls, and nearly all variables related to sexual risk behavior were associated with acute HCV infection. The following variables were strongly associated (P < .001) with acute HCV infection using univariable regression: receptive UAI, sharing sex toys, unprotected fisting, group sex participation, rimming, fingering, increasing number of casual sex partners, anal rinsing, rectal bleeding during or after having sex, and meeting casual sex partner(s) at sex parties (table 2C and D).

Among 82 cases, 69 (84.1%) reported non-IDU in the 6 months preceding study entry versus 52.7% of the controls (69 of 131; OR, 2.60; 95% CI, 1.44–4.70; P = .002). Use of anally administered drugs was less common (reported by 18.3% of cases) than use of either orally administered drugs (OADs) or nasally administered drugs (NADs) reported by 78.0% and 74.4% of cases, respectively). Oral administration of methamphetamine, ecstasy/3,4-methylenedioxymethamphetamine (MDMA), γ-hydroxybutyric acid (GHB)/γ-butyrolactone (GBL), and cannabis was associated with acute HCV infection. Nasal administration of amphetamines, cocaine, ketamine, and poppers was associated with HCV acquisition (all P < .001). When analyzed by means of administration, use of orally, anally, and nasally administered drugs were more frequently reported by cases than controls; ORs increased from 1.59 for the use of OADs only to 42.9 for injecting drugs (table 2E). Sharing straws was reported by 51% of MSM who reported consumption of NADs, and it was significantly associated with HCV acquisition (OR for snorting drugs with vs without sharing straws: 2.48; 95% CI, 1.14–5.37).

Clinical variables associated with acute HCV were as follows: (1) lower CD4 cell count and higher HIV viral load at the last visit before inclusion (ie, for cases before acute HCV infection) and (2) shorter duration since HIV diagnosis (table 2F). These associations remained statistically significant in a sensitivity analysis only including those on cART at the study entry visit (N = 179; data not shown). In addition, the association between HCV acquisition and CD4 cell count remained significant in a sensitivity analysis that included only cases with a known HCV RNA negative test date preceding study entry (N = 52; OR, 1.49 per cubic root lower; 95% CI, 1.08–2.05; P = .015). Syphilis, chlamydia, and rectal gonorrhea infection in the previous 6 months were strongly associated with acute HCV infection (all P < .001). Both nonulcerative and ulcerative STIs were more often reported by MSM with acute HCV than MSM with no history of HCV (table 2F).

**Risk Factors for Hepatitis C Virus Acquisition: Multivariable Analysis**

In the first multivariable analysis that included variables that may directly cause transmission of acute HCV, receptive UAI (adjusted OR [aOR], 4.92; 95% CI, 2.00–12.10; P = .001), sharing sex toys (aOR, 6.08; 95% CI, 1.96–18.87; P = .002), unprotected fisting (aOR, 2.60; 95% CI,
Sexual transmission of HCV

1.11–6.10; P = .028), IDU (aOR, 11.26; 95% CI, 1.21–105.2; P = .034), and sharing straws when snorting drugs (aOR, 3.79; 95% CI, 1.71–8.42; P = .001) had significant effects on HCV acquisition. Group sex participation, rimming, and fingering had no significant effects on HCV acquisition (Figure 1A); these variables were therefore omitted in the second multivariable analysis.

In the second multivariable analysis that included a broader range of variables, none of the studied interactions were significant, and they were therefore omitted in the presented model. In this model, receptive UAI (aOR, 5.01; 95% CI, 1.63–15.43; P = .005), sharing sex toys (aOR, 3.62; 95% CI, 1.04–12.52; P = .042), unprotected fisting (aOR, 2.57; 95% CI, 1.02–6.44; P = .044), IDU (aOR, 15.62; 95% CI, 1.27–192.6; P = .032), sharing straws when snorting drugs (aOR, 3.40; 95% CI, 1.39–8.32; P = .007), lower CD4 cell count (aOR, 1.75 per cubic root lower; 95% CI, 1.19–2.58; P = .004), and recent ulcerative STI (aOR, 4.82; 95% CI, 1.60–14.53; P = .005) had significant effects on HCV acquisition. The number of casual sex partners had no significant effect on HCV acquisition; nor did anal rinsing, rectal bleeding, and sex parties as meeting location for casual sex partners (Figure 1B).

In an exploratory post hoc analysis, we calculated a risk score for each MSM, ranging from 0 to 6, depending on the number of the following sexual behavior acts in the 6 months preceding study entry: receptive UAI, sharing toys, unprotected fisting, group sex participation, rimming, fingering. In multivariable analysis, men with a risk score of 4 had an aOR of 8.63 (95% CI, 1.49–50.0), those with risk score of 5 had an aOR of 10.3 (95% CI, 1.54–68.4), and 12 men with a risk score of 6 were excluded from the analysis because all 12 were cases, leading to a zero cell count. Men with risk scores of 1, 2, and 3 of these sex acts had aORs of 2.61 (95% CI, .52–13.1), 2.16 (95% CI, .38–12.4), and 2.40 (95% CI, .48–12.0), respectively, compared with MSM with a zero risk score. In this analysis, the aOR for the variables that were added in the second multivariable analysis were comparable (data not shown).

DISCUSSION

We conducted a comprehensive study on risk factors for transmission of HCV among HIV-infected MSM showing that receptive UAI, sharing sex toys, unprotected fisting, IDU, sharing straws when snorting drugs, lower CD4 cell count, and recent ulcerative STI have independent effects on HCV acquisition among HIV-infected MSM. Most of these variables were not independently associated with acute HCV in previously conducted case-control studies [10–12], probably due to a lack of statistical power, or because these studies did not incorporate data on all topics mentioned. Other transmission routes that previously have been suggested (eg, rectal bleeding [11]) were measured, but they had no significant effect on HCV acquisition in our multivariable analysis.
MSM with acute HCV infection were younger than controls, concurrent with other recent studies [8, 24, 25]. In addition, cases had shorter duration of (known) HIV infection, but they had lower CD4 cell counts preceding HCV acquisition than HCV-negative controls. Although the absolute difference in median CD4 cell count was 90 cells/μL (ie, 500 for cases vs 590 for controls), the effect remained significant in multivariable analysis (also when the CD4 cell count obtained from the penultimate visit was analyzed). An effect of lower CD4 cell count on HCV acquisition has been suggested before, but studies addressing this topic are scarce. Witt et al [13] reported significantly higher HCV incidence rates among HIV-infected MSM with lower CD4 cell counts (modeled per 100 cells/μL for those with a range of 0–500). In contrast, in the Swiss HIV Cohort Study [14] and the Amsterdam Cohort Study among MSM [8], effects of CD4 cell count on HCV acquisition were marginal and not significant. The lower CD4 cell count that we observed may be a consequence of STI other than HCV [26] and thereby an indirect marker for earlier increased sexual risk behavior. The significant effect of a reduced CD4 cell count may partly explain why sexual transmission of HCV infection seems to be rare among HIV-negative MSM [1, 5]. Alternatively, lower sexual risk behavior among HIV-negative MSM might explain the absence of both HIV and HCV in this group. Another reason there may be increased HCV infection among HIV-infected MSM compared with HIV-negative MSM could be due to serosorting (ie, establishing HIV concordance in advance to practicing UAI) [27].

The associations of HCV acquisition with group sex participation, the number of casual sex partners, and meeting location of casual sex partners lost significance when corrected for sexual behavior in multivariable analysis. Hence, the sexual behavior itself (eg, having receptive UAI or not) appeared to outweigh the number of casual sex partners (either simultaneous or consecutive) in contributing to risk of acute HCV infection. In addition, the risk score analysis also showed that men who participated in 4 or more different risky sex acts in the previous 6 months were much more likely to have acquired HCV than men with less than 4 sex acts. This finding emphasizes that there are differences in the degree of sexual risk taking among MSM, and it indicates that practicing multiple risky sexual techniques may substantially increase the risk of HCV acquisition.

The majority of HCV infections in our study was of genotype 1 and 4, in line with earlier reports [7, 8, 10, 12, 14, 28]. We report a relatively high proportion of subtype 2b infections (12.2%); this subtype is likely to have been introduced more recently in the MSM population in the Netherlands [8, 29].

In contrast to recent findings in the United Kingdom [30], we did not observe a high prevalence of so-called “chem-sex” or “slamming” (ie, injection of methamphetamines or mephedrone in combination with high-risk sexual practices). Injecting drug use and, more specifically, sharing needles was relatively uncommon in our study. Still, IDU remains a major
risk factor for transmission of HCV. Sharing straws was reported by more than half of the participants that had recently consumed NADs; it had a significant effect on HCV acquisition in the multivariable analyses. Although sharing of contaminated straws could potentially increase HCV transmission [31], a systematic review regarding this topic concluded that current studies failed to show clear associations of non-IDU behavior with HCV infection [32]. Hence, whether or not sharing straws is a direct or indirect route of HCV transmission remains to be elucidated. Administration of NADs, or drug use in general, could be a marker for risky behavior that we did not measure, eg, longer sex episodes or having more rough sex. This may lead to dehydration of mucosal surfaces, which in turn may increase chances of permucosal transmission of HCV due to microtrauma or rectal bleeding [10]. A reason for not finding an association of HCV acquisition with rectal bleeding in our multivariable analysis might be underreporting, because not all bleeding is visible during or after sex [11].

This study has some limitations. The sample size still limits the number of parameters that could be estimated in multivariable analysis (including interaction terms). Diagnosis of recent STI was self-reported, and use (or sharing) of lubricant was not assessed; the latter might also facilitate HCV transmission. Various HIV-related characteristics were studied, but the precise duration of HIV infection could only be estimated for a minority of the population because for most participants, no data on HIV-negative test results were available. Because different risk behaviors might be correlated, it could be difficult to determine which is the more important one leading to HCV acquisition. However, correlation is unlikely to be a significant factor in our study, because it would have led to less significant effects of different sexual behaviors in multivariable analysis. As characteristics of local epidemics may differ (eg, the difference in the practice of chem-sex reported in this study compared with reports from the United Kingdom [30]), and the majority of participants in our study were from Amsterdam and the Netherlands, our results may not be widely generalizable to other areas.

CONCLUSIONS

This study showed significant effects of both biological and behavioral risk factors on HCV acquisition among MSM. In the ongoing HCV epidemic in which HIV-infected MSM with high-risk sexual behavior were probably infected first, MSM with lower risk profiles may become increasingly affected by acute HCV [7, 33]. Frequent testing of MSM at highest risk for (re-)infection may lead to earlier diagnosis and treatment initiation, which in turn could also limit ongoing transmission in the MSM population. In addition, tailored education and behavioral interventions are therefore needed to avoid ongoing transmission of HCV in the MSM population. Future longitudinal studies should preferably focus on temporal changes in risk behavior among HIV-infected MSM, to evaluate possible risk reduction strategies for HCV (re-)infection.
ACKNOWLEDGMENTS

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The MOSAIC collaborators are as follows: J. T. M. v. d. M., R. Molenkamp, M. Mutschelknauss, H. E. Nobel, H. W. Reesink, J. S., and M. van der Valk (Academic Medical Center, Amsterdam, the Netherlands); G. E. L. van den Berk, K. B., D. Kwa, N. van der Meche, A. Toonen, and D. Vos (OLVG Hospital, Amsterdam, the Netherlands); M. van Broekhuizen, F. N. L., and J. W. Mulder (Slotervaart Hospital, Amsterdam, the Netherlands); J. E. A., A. van Kessel, and I. de Kroon (University Medical Center, Utrecht, the Netherlands); A. Boonstra, M. E. van der Ende, S. Hullegie, and B. J. A. R. (Erasmus Medical Center, Rotterdam, the Netherlands); T. J.W. v. d. L. (Sanquin Blood Supply, Amsterdam, the Netherlands); L. G. and C. Smit (HIV Monitoring Foundation, Amsterdam, the Netherlands); F. A. E. L., M. P., J. W. V., and W. van der Veldt (Public Health Service of Amsterdam, Amsterdam, the Netherlands).
Table 1 Characteristics of acute HCV infection among 82 HIV-infected men who have sex with men

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of HCV diagnosis</td>
<td>2010.5 (2010-2011)</td>
</tr>
<tr>
<td>No. of days between last negative and first positive HCV RNA sample&lt;sup&gt;b&lt;/sup&gt;</td>
<td>148 (116-186)</td>
</tr>
<tr>
<td>No. of days between last negative and first positive anti-HCV sample</td>
<td>164 (118-218)</td>
</tr>
<tr>
<td>HCV load of first positive HCV RNA sample</td>
<td>4.5E10&lt;sup&gt;a&lt;/sup&gt; (1.2 E10&lt;sup&gt;a&lt;/sup&gt; - 3.3 E10&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Change in ALT concentration between last negative and first positive HCV sample&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99 (19–422)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak ALT between last negative HCV sample and ≤3 months after the first positive HCV sample</td>
<td>350 (164-653)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCV subtype; n (%)</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>52 (63.4)</td>
</tr>
<tr>
<td>1b</td>
<td>6 (7.3)</td>
</tr>
<tr>
<td>2b</td>
<td>10 (12.2)</td>
</tr>
<tr>
<td>4d</td>
<td>11 (13.4)</td>
</tr>
<tr>
<td>Unknown/not typable</td>
<td>3 (3.7)</td>
</tr>
<tr>
<td>Reported symptoms of acute infection; n (%)</td>
<td></td>
</tr>
<tr>
<td>Joint pain</td>
<td>7 (8.5)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3 (3.7)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>38 (46.3)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>14 (17.1)</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>23 (28.1)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>17 (20.7)</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MOSAIC, MSM Observational Study of Acute Infection with hepatitis C. <sup>a</sup>MOSAIC study, the Netherlands, 2009-2014. Numbers are median (interquartile range) unless indicated otherwise. <sup>d</sup>Data available for 52 of 82 cases. <sup>e</sup>U/mL. <sup>f</sup>Data available for 58 of 82 cases. <sup>g</sup>U/L.
Table 2 Determinants of acute HCV infection among 213 men who have sex with men, of whom 82 acquired acute hepatitis C infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subcategory</th>
<th>82 HIV+ MSM with acute HCV N (%)</th>
<th>131 HIV+ MSM without HCV N (%)</th>
<th>Odds Ratio (95% CI) P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A: SOCIO-DEMOGRAPHIC CHARACTERISTICS</td>
<td>Age (median, IQR)</td>
<td>43.1 (39.2-47.6)</td>
<td>49.4 (42.3-54.8)</td>
<td>.94 (.38-.72) per 10y increment &lt;.001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>West-European</td>
<td>65 (79.3)</td>
<td>104 (79.4)</td>
<td>1 .742</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>15 (20.7)</td>
<td>27 (20.6)</td>
<td>1.13 (56-2.27)</td>
</tr>
<tr>
<td>Living situation</td>
<td>Alone</td>
<td>32 (39.0)</td>
<td>57 (43.5)</td>
<td>1 .755</td>
</tr>
<tr>
<td></td>
<td>With steady sex partner</td>
<td>38 (46.3)</td>
<td>54 (41.2)</td>
<td>1.25 (69-2.28)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>12 (14.6)</td>
<td>20 (15.3)</td>
<td>1.07 (46-2.47)</td>
</tr>
<tr>
<td>Educational level</td>
<td>Middle &amp; low</td>
<td>27 (32.9)</td>
<td>35 (26.7)</td>
<td>1 .277</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>53 (64.6)</td>
<td>96 (73.3)</td>
<td>.72 (.39-1.31)</td>
</tr>
<tr>
<td>2B: TRADITIONAL RISK FACTORS FOR HCV 12M</td>
<td>Injecting drug use (IDU)</td>
<td>10 (12.2)</td>
<td>2 (1.5)</td>
<td>8.96 (1.91-42.01) .005</td>
</tr>
<tr>
<td></td>
<td>Tattoo</td>
<td>6 (7.3)</td>
<td>9 (6.9)</td>
<td>1.07 (37-3.12) .901</td>
</tr>
<tr>
<td></td>
<td>Blood transfusion</td>
<td>0 (0.0)</td>
<td>2 (1.5)</td>
<td>.31 (.01-6.62) .456</td>
</tr>
<tr>
<td></td>
<td>Surgery</td>
<td>7 (8.5)</td>
<td>15 (11.5)</td>
<td>.72 (.28-1.85) .498</td>
</tr>
<tr>
<td></td>
<td>Endoscopy</td>
<td>9 (11.0)</td>
<td>15 (11.5)</td>
<td>.95 (.40-2.29) .915</td>
</tr>
<tr>
<td>2C: SEXUAL BEHAVIOR 6M</td>
<td>Insertive/receptive unprotected anal intercourse (iUAI/rUAI)</td>
<td>No UAI / only with HCV-negative steady sex partner</td>
<td>10 (12.2)</td>
<td>61 (46.6)</td>
</tr>
<tr>
<td></td>
<td>Only iUAI with HCV-positive/unknown sex partner(s)</td>
<td>3 (3.7)</td>
<td>15 (11.5)</td>
<td>1.22 (30-4.99)</td>
</tr>
<tr>
<td></td>
<td>(Also) rUAI with HCV-positive/unknown sex partner(s)</td>
<td>69 (84.1)</td>
<td>55 (42.0)</td>
<td>7.65 (3.59-16.31)</td>
</tr>
</tbody>
</table>


### Sexual Transmission of HCV

#### Characteristic Subcategory

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subcategory</th>
<th>82 HIV+ MSM with acute HCV N (%)</th>
<th>131 HIV+ MSM without HCV N (%)</th>
<th>Odds Ratio (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharing of sex toys</td>
<td>No toys used / only shared toys with HCV-negative steady sex partner</td>
<td>55 (67.1)</td>
<td>126 (96.2)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Toys shared</td>
<td>27 (32.9)</td>
<td>5 (3.8)</td>
<td>12.37 (4.53-33.81)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Toys shared</td>
<td>27 (32.9)</td>
<td>5 (3.8)</td>
<td>12.37 (4.53-33.81)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Unprotected fisting</td>
<td>No fisting / gloves used and no group sex reported</td>
<td>42 (51.2)</td>
<td>113 (86.3)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>No gloves used / gloves used and group sex reported</td>
<td>40 (48.8)</td>
<td>18 (13.7)</td>
<td>5.98 (3.09-11.56)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Group sex participation</td>
<td>No group sex</td>
<td>29 (35.4)</td>
<td>84 (64.1)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>2 sex partners (i.e., only threesomes)</td>
<td>9 (11.0)</td>
<td>15 (11.5)</td>
<td>1.74 (.69-4.40)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>≥3 sex partners</td>
<td>44 (53.7)</td>
<td>29 (22.1)</td>
<td>4.39 (2.34-8.26)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Rimming</td>
<td>No rimming / only with HCV-negative steady sex partner</td>
<td>29 (35.4)</td>
<td>80 (61.1)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>(Also) with HCV-positive / unknown sex partner(s)</td>
<td>53 (64.6)</td>
<td>51 (38.9)</td>
<td>2.87 (1.62-5.08)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fingering</td>
<td>No fingering / only with HCV-negative steady sex partner</td>
<td>28 (34.1)</td>
<td>75 (57.3)</td>
<td>1</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>(Also) with HCV-positive / unknown sex partner(s)</td>
<td>54 (65.9)</td>
<td>56 (42.7)</td>
<td>2.58 (1.46-4.58)</td>
<td>.001</td>
</tr>
</tbody>
</table>

#### 2D: SEX-RELATED VARIABLES

<table>
<thead>
<tr>
<th>2D: SEX-RELATED VARIABLES</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Having a steady sex partner</td>
<td>.798</td>
</tr>
<tr>
<td>Age of steady sex partner (median, IQR)</td>
<td>.831</td>
</tr>
<tr>
<td>Number of casual sex partners</td>
<td>.831</td>
</tr>
<tr>
<td>Continuous</td>
<td>1.38 (1.18-1.62) per doubling</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Subcategory</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
</tr>
<tr>
<td>Categorical</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1-9</td>
</tr>
<tr>
<td></td>
<td>10-19</td>
</tr>
<tr>
<td></td>
<td>20-49</td>
</tr>
<tr>
<td></td>
<td>≥50</td>
</tr>
<tr>
<td>Anal rinsing</td>
<td>No anal rinsing / only with HCV-negative steady sex partner</td>
</tr>
<tr>
<td></td>
<td>Anal rinsing with HCV-positive/unknown sex partner(s)</td>
</tr>
<tr>
<td>Rectal bleeding during and/or after sex</td>
<td>No bleeding / only after sex with HCV-negative steady sex partner</td>
</tr>
<tr>
<td></td>
<td>Bleeding after sex with HCV-positive/unknown sex partner(s)</td>
</tr>
<tr>
<td>Piercing(s) in genital region</td>
<td>No piercing(s)</td>
</tr>
<tr>
<td></td>
<td>Yes, self</td>
</tr>
<tr>
<td></td>
<td>Yes, steady sex partner</td>
</tr>
<tr>
<td>Received money for sex</td>
<td></td>
</tr>
<tr>
<td>Meeting location of casual sex partner(s)</td>
<td>Leather bar / leather party</td>
</tr>
<tr>
<td></td>
<td>Gay bar</td>
</tr>
<tr>
<td></td>
<td>Internet</td>
</tr>
<tr>
<td></td>
<td>Public cruising area</td>
</tr>
<tr>
<td></td>
<td>Sex party</td>
</tr>
<tr>
<td></td>
<td>Gay sauna</td>
</tr>
<tr>
<td></td>
<td>Darkroom</td>
</tr>
<tr>
<td></td>
<td>Abroad</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>
### Characteristics

#### 2E: Drug Use Before/During Sex

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subcategory</th>
<th>82 HIV+ MSM with acute HCV N (%)</th>
<th>131 HIV+ MSM without HCV N (%)</th>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orally administered drugs (OADs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No OADs used</td>
<td></td>
<td>18 (22.0)</td>
<td>81 (61.8)</td>
<td>.18 (.09-.33)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2C-B</td>
<td></td>
<td>0 (0.0)</td>
<td>1 (0.8)</td>
<td>.52 (.02-13.10)</td>
<td>.696</td>
</tr>
<tr>
<td>Amphetamines</td>
<td></td>
<td>6 (7.3)</td>
<td>4 (3.1)</td>
<td>2.51 (0.69-9.17)</td>
<td>.165</td>
</tr>
<tr>
<td>Cannabis</td>
<td></td>
<td>31 (37.8)</td>
<td>27 (20.6)</td>
<td>2.34 (1.27-4.33)</td>
<td>.007</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td>4 (4.9)</td>
<td>2 (1.5)</td>
<td>3.31 (.59-18.48)</td>
<td>.173</td>
</tr>
<tr>
<td>Ecstasy / MDMA</td>
<td></td>
<td>57 (69.5)</td>
<td>32 (24.4)</td>
<td>7.05 (3.81-13.06)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>GHB / GBL</td>
<td></td>
<td>39 (47.6)</td>
<td>22 (16.8)</td>
<td>4.49 (2.39-8.44)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ketamines</td>
<td></td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>4.84 (0.19-12.2)</td>
<td>.336</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td></td>
<td>9 (11.0)</td>
<td>0 (0.0)</td>
<td>33.99 (1.95-592.5)</td>
<td>.016</td>
</tr>
<tr>
<td>Poppers</td>
<td></td>
<td>4 (4.9)</td>
<td>3 (2.3)</td>
<td>2.19 (.48-10.04)</td>
<td>.314</td>
</tr>
<tr>
<td><strong>Anally administered drugs (AADs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No AADs used</td>
<td></td>
<td>67 (81.7)</td>
<td>129 (98.5)</td>
<td>.07 (.02-.31)</td>
<td>.001</td>
</tr>
<tr>
<td>Amphetamines</td>
<td></td>
<td>4 (4.9)</td>
<td>2 (1.5)</td>
<td>3.31 (.59-18.48)</td>
<td>.173</td>
</tr>
<tr>
<td>Cannabis</td>
<td></td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>4.84 (0.19-12.2)</td>
<td>.336</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td>8 (9.8)</td>
<td>1 (0.8)</td>
<td>14.05 (1.72-114.6)</td>
<td>.014</td>
</tr>
<tr>
<td>GHB / GBL</td>
<td></td>
<td>1 (1.2)</td>
<td>1 (0.8)</td>
<td>1.60 (.10-26.02)</td>
<td>.739</td>
</tr>
<tr>
<td>Ketamines</td>
<td></td>
<td>7 (8.5)</td>
<td>2 (1.5)</td>
<td>6.02 (1.22-29.73)</td>
<td>.028</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td></td>
<td>3 (3.7)</td>
<td>1 (0.8)</td>
<td>4.94 (0.50-48.28)</td>
<td>.170</td>
</tr>
<tr>
<td>Poppers</td>
<td></td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>4.84 (0.19-12.2)</td>
<td>.336</td>
</tr>
<tr>
<td><strong>Nasally administered drugs (NADs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NADs used</td>
<td></td>
<td>21 (25.6)</td>
<td>83 (63.4)</td>
<td>.20 (.11-37)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Amphetamines</td>
<td></td>
<td>23 (28.0)</td>
<td>4 (3.1)</td>
<td>12.38 (4.10-37.40)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Subcategory</td>
<td>82 HIV+ MSM with acute HCV N (%)</td>
<td>131 HIV+ MSM without HCV N (%)</td>
<td>Odds Ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>----------------------------------</td>
<td>-------------------------------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td>38 (46.3)</td>
<td>19 (14.5)</td>
<td>5.09 (2.65-9.77)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ketamines</td>
<td></td>
<td>30 (36.6)</td>
<td>9 (6.9)</td>
<td>7.82 (3.47-17.62)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Methamphetamines</td>
<td></td>
<td>4 (4.9)</td>
<td>0 (0.0)</td>
<td>15.08 (8.0-283.8)</td>
<td>.070</td>
</tr>
<tr>
<td>Miau-miau</td>
<td></td>
<td>2 (2.4)</td>
<td>2 (1.5)</td>
<td>1.61 (.22-11.68)</td>
<td>.636</td>
</tr>
<tr>
<td>Poppers</td>
<td></td>
<td>50 (61.0)</td>
<td>42 (32.1)</td>
<td>3.31 (1.86-5.89)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Methods of administering drug(s), combined</td>
<td>No drugs used</td>
<td>13 (15.9)</td>
<td>62 (47.3)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Only OADs used</td>
<td>5 (6.1)</td>
<td>15 (11.5)</td>
<td>1.59 (.49-5.15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NADs used, no straws shared</td>
<td>22 (26.8)</td>
<td>33 (25.2)</td>
<td>3.18 (1.42-7.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NADs used, straws shared</td>
<td>33 (40.2)</td>
<td>20 (15.3)</td>
<td>7.87 (3.48-17.80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Injected drugs</td>
<td>9 (11.0)</td>
<td>1 (0.8)</td>
<td>42.92 (5.00-368.8)</td>
<td></td>
</tr>
</tbody>
</table>

2F: Clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subcategory</th>
<th>82 HIV+ MSM with acute HCV N (%)</th>
<th>131 HIV+ MSM without HCV N (%)</th>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cell count at the HCV-negative visit preceding study entry (cells/μL)</td>
<td></td>
<td>500 (400-670)</td>
<td>590 (450-760)</td>
<td>1.41 (1.08-1.85) per cubic root lower</td>
<td>.012</td>
</tr>
<tr>
<td>Nadir CD4 cell count until the HCV-negative visit preceding study entry (cells/μL)</td>
<td></td>
<td>260 (170-350)</td>
<td>210 (110-310)</td>
<td>.82 (.67-1.01) per cubic root lower</td>
<td>.057</td>
</tr>
<tr>
<td>No. of years between first HIV-positive test and study entry visit^a</td>
<td></td>
<td>6.5 (3.2-9.7)</td>
<td>9.1 (4.0-15.4)</td>
<td>.92 (.88-.97)</td>
<td>.001</td>
</tr>
<tr>
<td>HIV load at HCV-negative visit preceding study entry (copies/mL)</td>
<td>&lt;50 (&lt;40-12525)^b</td>
<td>&lt;40 (&lt;40-&lt;50)^b</td>
<td>1.59 (1.18-2.12) per ^bLog increment</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Use of cART at HCV-negative visit preceding study entry^a</td>
<td>68/81 (84.0)</td>
<td>111/122 (91.0)</td>
<td>.52 (.22-1.22)</td>
<td>.133</td>
<td></td>
</tr>
</tbody>
</table>

STIs (self-reported) ^ab

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subcategory</th>
<th>82 HIV+ MSM with acute HCV N (%)</th>
<th>131 HIV+ MSM without HCV N (%)</th>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td></td>
<td>20 (24.4)</td>
<td>7 (5.3)</td>
<td>5.71 (2.29-14.24)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td></td>
<td>29 (35.4)</td>
<td>13 (9.9)</td>
<td>4.97 (2.39-10.31)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Rectal gonorrhea</td>
<td></td>
<td>19 (23.2)</td>
<td>5 (3.8)</td>
<td>7.60 (2.71-21.30)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Herpes genitalis</td>
<td></td>
<td>1 (1.2)</td>
<td>1 (0.8)</td>
<td>1.60 (.10-26.02)</td>
<td>.739</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td></td>
<td>0 (0.0)</td>
<td>1 (0.8)</td>
<td>.53 (.02-13.10)</td>
<td>.696</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Subcategory</td>
<td>82 HIV+ MSM with acute HCV N (%)</td>
<td>131 HIV+ MSM without HCV N (%)</td>
<td>Odds Ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------</td>
<td>----------------------------------</td>
<td>--------------------------------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 (11.0)</td>
<td>2 (1.5)</td>
<td>7.95 (1.67-37.80)</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 (17.1)</td>
<td>6 (4.6)</td>
<td>4.29 (1.58-11.67)</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (2.4)</td>
<td>3 (2.3)</td>
<td>1.07 (.17-6.52)</td>
<td>.944</td>
</tr>
<tr>
<td></td>
<td>LGV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urethral gonorrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (e.g., genital warts, oral gonorrhea)</td>
<td>2 (2.4)</td>
<td>3 (2.3)</td>
<td>1.07 (.17-6.52)</td>
<td>.944</td>
</tr>
<tr>
<td></td>
<td>STIs (combined)</td>
<td>No STIs</td>
<td>34 (41.5)</td>
<td>1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1 non-ulcerative STI</td>
<td>22 (26.8)</td>
<td>5.43 (2.47-11.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1 ulcerative STI</td>
<td>26 (31.7)</td>
<td>9.26 (3.96-21.67)</td>
<td></td>
</tr>
</tbody>
</table>

Continuous variables are presented as median (interquartile range). Abbreviations: 2C-B, 2,5-dimethoxy-4-bromophenethylamine hydrochloride; 6M, up to 6 months preceding study entry; 12M, up to 12 months preceding study entry; cART, combination antiretroviral therapy; CI, confidence interval; GBL, γ-butyrolactone; GHB, γ-hydroxybutyric acid; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HIV+ MSM, HIV-infected men who have sex with men; LGV, lymphogranuloma venereum; STI, sexually transmitted infection. a Data missing for 1 case and 9 controls. b Fifty of 75 (66.7%) cases and 99 of 112 (88.4%) controls had undetectable HIV viral load. c Ulcerative STI: syphilis, herpes genitalis, LGV.
Figure 1A Cleveland dot plot showing results of a multivariable model including variables that potentially have direct effects on acquisition of acute hepatitis C virus (HCV); model 1 of 2. 6M, up to 6 months preceding study entry; NADs, nasally administered drugs; UAI, unprotected anal intercourse. Data were collected among 213 human immunodeficiency virus (HIV)-infected men who have sex with men (MSM), 82 of whom had acute HCV infection. All participated in the MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) study, the Netherlands, 2009–2014.
Figure 1B Cleveland dot plot showing (1) results of a multivariable model including variables that potentially have direct effects on acquisition of acute HCV and (2) variables that potentially facilitate transmission of acute HCV, model 2 of 2. *, modeled as $\log(N + 1)$; **, at the HCV-negative visit preceding study entry, cells/μL. 6M, up to 6 months preceding study entry; NADs, nasally administered drugs; UAI, unprotected anal intercourse; ulcerative STI, any of the following sexually transmitted infections: syphilis, herpes genitalis, lymphogranuloma venereum. Data were collected among 213 human immunodeficiency virus (HIV)-infected men who have sex with men (MSM), 82 of whom had acute HCV infection. All participated in the MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) study, the Netherlands, 2009–2014.
REFERENCES


LIMITED OVERLAP IN PHYLOGENIES OF HEPATITIS C VIRUS (HCV) AND HIV-1 AMONG HIV/HCV-COINFECTED MEN WHO HAVE SEX WITH MEN IN THE NETHERLANDS

Submitted for publication

Joost W. Vanhommerig, Daniela Bezemer, Richard Molenkamp, Ard van Sighem, Colette Smit, Joop E. Arends, Fanny N. Lauw, Kees Brinkman, Bart J. Rijnders, Astrid M. Newsum, Sylvia M. Bruisten, Maria Prins, Jan T. van der Meer, Thijs J. van de Laar*, Janke Schinkel* on behalf of the MOSAIC study and the ATHENA national observational cohort

Presented at the 23rd CROI, 2016, Boston, MA, USA (poster abstract #542).

* Both authors contributed equally to the writing of this article.
ABSTRACT

Background
Men who have sex with men (MSM) practicing unsafe sex are at risk of becoming infected with HIV-1 and hepatitis C virus (HCV). MSM infected with HIV/HCV-coinfection may represent high risk core groups and could be drivers of the HIV-epidemic among MSM.

Methods
For MSM in the ATHENA observational cohort with an HIV pol sequence available, transmission clusters were selected in the HIV subtype B phylogenetic tree. Results were compared between MSM with or without evidence of HCV-coinfection. In addition, HIV and HCV phylogenies of HIV/HCV-coinfected MSM were compared for men that had an HCV NS5B sequence available within the MOSAIC study.

Results
We included 5,038 HIV-infected MSM with HIV pol sequences available, 563 (11.2%) of whom were (ever) co-infected with HCV. In total, 118 HIV clusters of >10 sequences included 3,084/5,038 (61.2%) HIV pol sequences. 97 out of 118 (82.2%) HIV clusters contained ≥1 HCV infection. HCV sequences were obtained from 150 HCV infections among 126 MSM from the MOSAIC study, of whom 21 had ≥1 reinfection. Ultimately, 19/150 (12.7%) HCV infections showed overlap in HCV and HIV phylogenetic tree topologies.

Conclusions
Our results indicate a generalised HIV epidemic with no evidence for high risk core groups of HIV-infected MSM with elevated risk of HCV infection suggesting that HIV/HCV-coinfected MSM are not preferentially driving the HIV epidemic.
INTRODUCTION

Since 2000, hepatitis C virus (HCV) infection has emerged as a sexually transmitted infection (STI) among human immunodeficiency virus (HIV)-1-infected men who have sex with men (MSM) [1–3]. Incident HCV infection occurs predominantly among MSM that report condomless anal sex, a higher numbers of lifetime sex partners, recent (ulcerative) STI, group sex, use of sex toys, fisting, and/or the use of recreational drugs before or during sex [4–8]. Not only HCV but also HIV continues to affect MSM in the Netherlands. Despite the availability of successful combination antiretroviral therapy (cART), the number of newly diagnosed HIV infections remains high [9–11] and HIV incidence does not decrease in Dutch MSM [11]. Early HIV infections (up to 1.5 years after seroconversion) account for an estimated 50% of all onward HIV transmissions [12–16]. Especially MSM who practice high risk sexual behavior and are (yet) unaware of their positive HIV status [17] may play an important role in driving the HIV-epidemic. As HIV infection precedes HCV infection in >95% of co-infected MSM [18], and the acquisition of HCV indicates ongoing or recurrent risk behavior following HIV infection, co-infected MSM in particular may contribute to the onward transmission of HIV.

A recent phylogenetic study among HIV-infected individuals in the Netherlands indicated the presence of MSM-specific transmission clusters. Over time, the number of these networks has steadily increased, and onward HIV transmission is ongoing in most networks, independent of their initiation date [19]. To increase the efficacy of prevention programs with regards to both HIV and HCV, more insight is needed in the combined HCV and HIV transmission dynamics among MSM. Building further on the data derived from the study on MSM-specific transmission clusters in the Netherlands, we investigated whether HCV transmission among HIV-infected MSM is restricted to specific HIV transmission networks, which would suggest that a core group of high-risk MSM is driving the ongoing transmission of both HIV and HCV. To obtain more insight into the HCV transmission dynamics of HIV-infected MSM in the Netherlands, we initiated a molecular epidemiological study including 5,038 MSM-derived HIV subtype B sequences, and 183 HCV sequences obtained from 155 HIV-infected MSM with acute HCV, using established phylogenetic tools. We compared the HCV prevalence between distinct HIV transmission networks and examined the overlap between the HCV and the HIV epidemic among MSM by comparing the HIV and HCV phylogenetic tree topologies.
METHODS

MSM with newly diagnosed HIV infection: the ATHENA cohort
The ATHENA (AIDS Therapy Evaluation in the Netherlands) cohort is a national observational study that includes anonymized data from (nearly) all HIV-infected patients, followed longitudinally in one of the 27 Dutch HIV treatment centers since January 1996. The cohort was initiated in 1998 [20]. Demographic data were collected at entry in the cohort, e.g. sex, age, country of birth and route of transmission (self-reported). At each follow-up visit, clinical, virological and immunological data were collected, as well as data on the use of cART. HIV-1 pol sequences were obtained as part of the screening for resistance to antiretroviral drugs, before and during treatment with cART. This was routinely done following HIV diagnosis. By July 2014, ATHENA had enrolled 12,900 HIV-infected MSM; HIV-pol sequencing sequences were available for 5,435 (42.1%) HIV-infected MSM, of whom 5,038 (92.3%) were infected with HIV subtype B.

HCV status of MSM participating in ATHENA
HCV status of MSM participating in ATHENA was determined based on HCV antibody and RNA test results. Participants with a positive HCV antibody and/or HCV RNA test result during follow-up were defined as ‘confirmed HCV positive’. MSM without a positive HCV test result, and a documented anti-HCV and/or HCV RNA negative visit in the 1.5 years prior to the last ATHENA visit, were defined as ‘confirmed HCV negative’. In the absence of these HCV test results, alanine aminotransferase (ALT) levels were used to allocate assumed HCV status. Participants with ALT levels within the normal range (i.e., <45 U/L) in the year prior to the last ATHENA visit were ‘assumed HCV negative’. The remainder: (i) MSM with only elevated ALT levels and (ii) MSM without ALT measurements in the year prior to the last ATHENA visit were defined as ‘HCV status unknown’.

MSM with acute HCV infection: the MOSAIC study
The MOSAIC study (MSM Observational Study of Acute Infection with hepatitis C) is an open, ongoing, prospective, observational cohort initiated in 2008 to study the determinants and sequelae of acute HCV infection in HIV-infected MSM [21]. Inclusion takes place at five HIV outpatient clinics located in Amsterdam, Rotterdam and Utrecht, the Netherlands. All cases were ≥18 years of age, MSM, HIV-positive and had acute HCV infection. Acute infection was defined as having an interval of ≤6 months between the first positive HCV RNA test and the preceding negative HCV RNA or antibody test. For the current study, data were used from all cases included up to January 2015. All MOSAIC participants were also participants of ATHENA.

Phylogenetic analysis: identification of HIV clusters and potential HIV transmission pairs
Population sequencing of the HIV-1 protease and reverse transcriptase coding regions (i.e.,
partial pol sequences) was performed as described previously [22]. Sequences were aligned using Clustal Omega v1.2 [23] and manually checked and adjusted. Major resistance conferring mutations [24], including alternative substitutions at position 215 were excluded, which resulted in an alignment of 1140 nucleotides. HIV subtypes were identified by phylogenetic analysis of ATHENA sequences along with established HIV-1 reference sequences from the Los Alamos database [25]. To enable the identification of HIV-1 transmission clusters, a phylogenetic tree of subtype B pol sequences was constructed in FastTree v2.1.7 using a general time reversible (GTR) with gamma model [26]. PhyloPart v2.0 [27] was used to identify HIV transmission clusters within the HIV-1 subtype B phylogeny. HIV transmission clusters were defined as phylogenetic clades with a posterior probability value ≥90% and a median value of all pairwise distances of that clade (i.e., sub-tree) below the 5th percentile of the distribution of all pairwise distances of the whole tree, which corresponded to 0.08 mutations per site [19]. An ML phylogenetic tree of HIV pol sequences was constructed in MEGA using a GTR with gamma model to visualize HIV phylogeny of HIV clusters that showed overlap with HCV clusters.

In addition to HIV transmission networks based on tree topology, HIV transmission pairs were defined as two MSM with HIV sequences with a pairwise nucleotide distance of ≤1.5% [28,29].

**Phylogenetic analysis: identification of HCV clusters**

From all MOSAIC participants, the first available HCV RNA positive sample plus the first available HCV RNA positive sample of each subsequent HCV reinfection were collected. Part of the HCV NS5B gene was amplified and sequenced using the primers and conditions as previously described [30]. The viral genotype was determined after phylogenetic analysis of NS5B sequences along with established reference sequences [31]. Phylogenetic trees were constructed with MOSAIC case sequences plus a set of unrelated HCV reference sequences. This reference set included Dutch HCV NS5B sequences from risk groups other than MSM, available in (i) the Los Alamos HCV sequence Database, and (ii) the locally available HCV sequence database of the AMC virology laboratory from 2009-2014. Phylogenetic trees were constructed by ML-HKY method with gamma distribution in MEGA v6.0 [32]. Bootstrapping (n=500) was used to assess the stability of the tree topology. Phylogenetic trees were visualized in FigTree v1.4.2 [33]. New HCV sequences were submitted to GenBank (KU563369-KU563536).

**Sensitivity analysis**

Our study design might have biased the observed degree of overlap between HIV and HCV transmission networks among Dutch MSM. First, the majority of MOSAIC participants were from Amsterdam, while ATHENA is a national cohort. Second, the vast majority of sexually acquired HCV infections in MSM was acquired after 2000, while the HIV-epidemic started 20 years earlier. Third, for 48% of ATHENA participants with HIV sequences available, no confirmed
HCV status was obtained; testing for HCV may have been influenced by specific characteristics (e.g., HCV related symptoms, disclosure of high risk behavior to their physician). Fourth, as a result of our definition of HIV transmission clusters, the size of HIV transmission networks in this study may have been overestimated. To investigate whether these limitations had impact on the outcome of this study, four sensitivity analyses were performed, including subsets of only (i) HIV- and HCV sequences of MSM from Amsterdam, (ii) MSM who were diagnosed with HIV after 1999, and (iii) HIV-positive MSM with a confirmed positive or negative HCV status. The effect of HIV transmission cluster definition (iv) was investigated using a much stricter definition of HIV transmission networks: phylogenetic clades with a bootstrap value ≥95% and a median value of all pairwise distances below the 2.5th percentile threshold. Sensitivity analyses i, ii and iii were also performed following the original HIV transmission pairs analysis.

Statistical analysis
Chi-square and Wilcoxon’s signed-rank test were used to evaluate differences in proportions and continuous variables, respectively. Time from HIV diagnosis until HCV diagnosis was estimated using the Kaplan-Meier method among those newly diagnosed with HIV. Follow-up time was calculated from HIV diagnosis until the date of last contact or, for MSM with HCV co-infection, the first HCV positive visit. Univariable Cox proportional hazards analysis was used to evaluate the association between the calendar years of HIV and subsequent HCV infection; we assumed a linear relation. *P*-values <.05 were considered statistically significant. All analyses were performed using STATA v13.1 (STATA Corp, College Station, TX, USA).

RESULTS

MSM with newly diagnosed HIV infection: the ATHENA cohort
Demographic characteristics of N=5,038 MSM infected with an HIV-1 subtype B pol sequence participating in ATHENA are shown in table 1. They were more often diagnosed with HIV in recent years than those with no HIV sequence available. Overall, 91.4% of the 5,038 MSM had (ever) initiated antiretroviral therapy and 51.3% of HIV pol sequences were collected from MSM that visited hospitals located in the Amsterdam area. HCV status was ‘confirmed positive’ for 563 (11.2%), ‘confirmed negative’ for 2,022 (40.1%), ‘assumed negative’ for 2,089 (41.5%) and ‘unknown’ for 364 (7.2%) of HIV-positive MSM. The median age at HIV diagnosis was 37.1 years (IQR: 30.2-44.1), and was lower among MSM with a ‘confirmed positive’ HIV status (35.7 years, IQR 30.1-42.3) compared to MSM with a ‘confirmed negative’ HCV status (37.3 years, IQR: 30.2-44.1; *P*=.001). Overall, the median time between HIV and HCV diagnoses was 3.3 years (IQR: 1.0-7.5) among the 563 HIV/HCV-coinfected MSM in the study; the time between HIV and HCV diagnosis decreased over time (not shown). For MSM diagnosed with HIV after 1999 the hazard ratio for HCV infection increased steadily with 4.5% per calendar year of HIV diagnosis (95% CI: 0.8-8.3; *P*=.015).
MSM with acute HCV infection: the MOSAIC study
The study population consisted of 183 HIV-infected MSM who had 211 documented acute HCV infections (24 MSM had ≥1 HCV reinfection, resulting in an additional 28 new HCV infections). By January 2015, the MOSAIC study had enrolled 150 of these patients. The remainder (n=33) were HIV/HCV-coinfected MSM that attended the HIV treatment clinic of the Academic Medical Center in Amsterdam, the Netherlands. Participants had a median age of 44.2 years (IQR: 39.5-49.6) at time of HIV diagnosis, 73.8% visited hospitals in Amsterdam, 24.0% had been diagnosed with acute HIV infection in the past, and 92.9% had (ever) initiated cART. HCV NS5B sequences were available for 183/211 (86.7%) of acute HCV (re)infections. General characteristics of MOSAIC participants and the HCV genotype distribution are shown in table 1. The majority of acute HCV infections were of HCV genotype 1a (59.0%) and 4d (20.2%). HCV NS5B sequences were available for 126/143 (88.1%) participants that were infected with HCV and also had an HIV subtype B pol sequence available.

Phylogenetic analysis: identification of HIV clusters
A total of 118 HIV transmission clusters containing ≥10 sequences were identified among 5,038 HIV-infected MSM. These 118 clusters contained 3,084/5,038 (61.2%) pol sequences. Median cluster size was 52 (IQR: 23-98; range: 10-181); 97/118 (82.2%) large HIV clusters contained 352/563 (62.5%) HIV sequences from MSM with a ‘confirmed positive’ HCV status. The remaining 211 MSM with a ‘confirmed positive’ HCV status were identified among the 1,141 MSM in small HIV clusters (i.e., clusters containing <10 sequences; n=136) and 813 MSM with singleton HIV sequences (n=75). The HCV prevalence among MSM in large HIV clusters (11.4%) was comparable to the HCV prevalence among MSM in small HIV clusters (11.9%) and MSM with singleton HIV sequences (9.2%). We identified 67 large HIV clusters with ≥2 HCV infections, the median number of HCV infections in these HIV clusters was 4 (IQR: 2-5), with a maximum of 16. In 5/97 (5.2%) HIV clusters harboring MSM with a ‘confirmed positive’ HCV status, the proportion of HCV-infected individuals exceeded 25%; these HIV clusters were relatively small with 10-22 patients, of whom 4-6 were HCV co-infected.

Phylogenetic analysis: identification of potential HIV transmission pairs
Among the 5,038 HIV-infected MSM, 2,619 MSM (52.0%) with at least one putative HIV transmission partner were identified. The median number of putative transmission partners was 3 (IQR: 1-7; range: 1-50), resulting in a total of 15,538 potential transmission pairs. The number of potential transmission partners did not significantly differ between MSM with a ‘confirmed positive’ HCV status (median: 3, IQR: 1-7) and MSM with ‘confirmed negative’ HCV status (median: 3, IQR: 1-8; P=.457). The proportion of MSM with a ‘confirmed positive’ HCV status among HIV-infected MSM with a potential transmission partner (314/2,619; 12.0%) was significantly higher than in MSM who did not pair with anyone (248/2,419; 10.3%; P=.05).
Phylogenetic analysis: identification of HCV clusters

Phylogenetic trees were constructed for each HCV genotype separately, and included the 183 HCV sequences of the MOSAIC study population plus 195 unrelated reference sequences of concordant genotypes. Phylogenetic analysis revealed 7 clusters of HCV-1a (varying in size from 3 to 34 sequences, figure 1A), one HCV-4d cluster (n=39, figure 1B), one HCV-2b cluster (n=13, figure 1C), one HCV-1b cluster (n=7), two homologous pairs infected with HCV-1a (figure 1A) and HCV-3a, and 15 ‘singleton’ sequences. Phylogenetic trees of HCV-1b and HCV-3a sequences were not shown as they showed no overlap within HIV clusters (next paragraph).

Comparison of HIV and HCV phylogenies in HIV/HCV-coinfected MSM

HCV NS5B and HIV pol sequences were available for 126 HIV/HCV-coinfected MSM: 126 HIV sequences and 150 HCV sequences (126 primary HCV infections and 24 HCV reinfections). The acute HCV infections with sequences available were identified in 79/126 MSM (62.7%) that were part of 50 large HIV transmission clusters (≥10 HIV sequences), the remainder was part of 26 small HIV transmission clusters (n=27), or were singletons (n=20). Out of 50 large HIV networks with acute HCV infections, 47 HIV clusters harbored ≥2 acute HCV infections (varying between 2 to 16 acute HCV infections). Fourteen out of 47 (29.8%) large HIV transmission clusters with ≥2 HCV strains harbored HCV strains of the same genotype (table 2). Figure 2 shows a phylogenetic tree based of the 456 HIV pol sequences of MSM in these 8 HIV large transmission clusters; HCV-coinfected MSM are marked based on the corresponding HCV cluster. The combined analysis of HIV transmission networks and HCV clusters shows that 19/150 (12.7%; 95% CI: 7.8-19.1) of acute HCV infections were linked in both HIV and HCV clusters. These MSM (n=18; 1 MSM had a primary and secondary HCV-1a infection belonging to cluster H; table 2) were part of 8 large HIV transmission clusters, and did not necessarily cluster closely within the HCV and/or HIV cluster (figure 2, table 2). Ultimately, 6/150 MSM (4.0%; 95% CI: 1.5-8.5) were also linked using the HIV transmission pairs analysis.

Sensitivity analyses

When only MSM from the Amsterdam region were included in the analysis, the proportion of infections with overlapping HIV and HCV phylogenies increased from 19/150 (12.7%) to 19/132 (14.4%). When our analysis was constrained to MSM who were diagnosed with HIV after 1999, 12/86 (14.0%) acute HCV infections were linked in both the HIV and HCV phylogenies. When HIV clusters were defined using our stricter cluster definition, 13/150 (8.7%) HCV infections were closely linked in both the HIV and HCV phylogenies. The proportions obtained from the sensitivity analyses were all within the 95% CI (i.e., 7.8-19.1%) of the original analysis.

The results from the analyses including potential HIV transmission pairs (with a pairwise nucleotide distance ≤1.5%) were comparable when we restricted our analysis to (i) MSM from Amsterdam, (ii) MSM diagnosed with HIV after the year 1999, or (iii) MSM with confirmed HCV status.
DISCUSSION

Since the start of the subtype B HIV epidemic in the MSM population in the eighties, HIV subtype B has spread in the Netherlands through a large number of persistent transmission networks [19]. In the late nineties, HCV entered the same risk group with sexual transmission as the main transmission route [6], with measured HCV incidence and prevalence among MSM in Amsterdam peaking in 2005 and 2008, respectively [34,35]. As HIV and HCV share the same transmission route in MSM, and HCV acquisition indicates engagement in high-risk sex in a population most likely already HIV-infected, we investigated whether HIV/HCV-coinfection transmission networks exist. Such networks might indicate that co-infected MSM drive the ongoing spread of HIV. Using HIV and HCV sequences, these questions were addressed by identification of putative HIV transmission pairs, based on genetic distance, and by cluster analysis using phylogenetic reconstruction of both HIV and HCV sequences. Our main finding is that there are no specific HIV/HCV transmission core groups that drive the HIV epidemic as HCV infection was not confined to specific HIV clusters and overlap in phylogenetic tree topology between HIV and HCV was limited (i.e., 12.7%). Restricting the analysis to MSM diagnosed after 1999 and to those living in the Amsterdam area did not significantly change these findings.

Very few studies have addressed the overlap of these two epidemics, although sexual transmission of HCV in HIV-infected MSM has been reported from many different parts of the world [36,8,37,4]. Matser et al. [38] showed previously that MSM in Amsterdam that were at increased risk for HCV infection had acquired diverse HCV strains while reporting to belong to the same gay subculture(s). A recent study from Switzerland reported 39/99 HIV transmission pairs with concordant HCV genotype [39], suggesting that substantial overlap may exist between the two epidemics. However, the reported genotype concordance may be partly explained by the overall high prevalence of HCV genotype 1a infection [4, 18]. Importantly, we showed that approximately half of the HCV infections that have a concordant genotype, could not be linked using phylogenetic analysis. Another recently published paper found no overlap at all in HIV and HCV phylogenetic tree topologies among MSM in Hong Kong, but this could be due to the relatively small study size (n=22) [40].

A small amount of HIV transmission clusters in our study had a relatively high proportion of HCV-positivity (>25%). From the 25 HIV/HCV-coinfected MSM in these clusters, 18 (72.0%) were also linked in the HIV transmission pairs analysis. While this could indicate a core group for ongoing HIV and/or HCV transmission, we found no support for this using the HCV phylogenetic analysis: only 6/17 (35.3%) HIV/HCV-coinfected MSM that showed overlap in HIV and HCV phylogenies were also linked in the HIV transmission pairs analysis. In addition, the limited overlap we report may, to some extent, be partly explained by steady partnerships.
The limited overlap between the HIV and HCV epidemic among MSM in the Netherlands may be explained by altered sexual risk behavior following HIV diagnosis. It has been demonstrated that sexual risk behavior often decreases after HIV infection is diagnosed, although in the cART era it may rise within two years to the level that was reported before HIV diagnosis [41]. In addition, change of sexual partner and network, and also sero-adaptive behavior may play a role. Indeed self-reported data on serosorting from the MOSAIC study indicated that condomless anal sex with partners of the same HIV status was reported significantly more often by HIV-infected MSM that had recently acquired HCV infection (91.1%) than by control patients (HIV-infected MSM with no history of HCV infection; 65.8%; \( P = 0.002 \); data not shown), which may provide one explanation for the fact that HCV infection is mostly spreading among HIV-infected MSM.

Our study has some limitations. First, our choice for HIV \textit{pol} and HCV \textit{NS5B} sequences for comparing phylogenies may have influenced our outcomes [42]. Previously conducted studies that used HIV \textit{pol} sequences suggested that inferred phylogenetic clusters indeed represent actual transmission networks [29]. \textit{NS5B} sequences, as used in our study, are commonly used for genotyping, but may be too short for in-depth phylogenetic analysis. In addition, not all HCV-infected MSM in the ATHENA cohort had an HCV sequence available (i.e. 126/563; 22.4%). We also may have missed HCV reinfections. Second, for HIV we assumed that the number of closely related sequences is an indication of the level of transmission from that person, but the true transmission partners were not identified. Third, HCV status of nearly half of the HIV-infected MSM in our study could not be confirmed by HCV antibody and/or RNA test results. This also indicates that implementation of the Dutch HCV testing guidelines among HIV-infected individuals deserve more attention. The addition of ALT test results from the year prior to the last visit drastically decreased the proportion of MSM with missing HCV status. In an exploratory post-hoc analysis, the number of HIV transmission clusters with HCV, as well as the number of HCV infections per HIV transmission cluster, did not increase disproportionally when MSM with elevated ALT in the year prior to the last visit (i.e., 264 out of 364 MSM with ‘unknown’ HCV status) were considered HCV positive (data not shown). Although ongoing transmission of HIV particularly by HIV/HCV-coinfected MSM is unlikely as the majority of MSM in our study had initiated cART by the time they were infected with HCV, we assumed this group of men had elevated risk throughout their infectious time span. Finally, HIV/HCV-coinfected MSM not (yet) in care were not included in our study, but may significantly contribute to ongoing HIV transmission.

From the high number of HIV networks and the low overlap between transmission clusters of HIV and HCV we can conclude that there are no high-risk core groups of HCV transmission among MSM with specific HIV strains in the Netherlands. The results of our study do not support the hypothesis that the ongoing HIV epidemic among MSM might be attributed to HIV-infected MSM that acquired HCV infection. Prevention efforts for HIV and HCV should therefore be widespread targeting all sexually active MSM.
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CLINICAL CENTRES

* denotes site coordinating physician


**Emma Kinderziekenhuis:** **HIV nurse consultants:** A. van der Plas, A.M. Weijsenfeld. **Erasmus Medisch Centrum, Rotterdam:** **HIV treating physicians:** M.E. van der Ende*, T.E.M.S. de Vries-Sluijs, E.C.M. van Gorp, C.A.M. Schurink, J.L. Nouwen, A. Verbon, B.J.A. Rijnders, H.I. Bax, M. van der Feltz. **HIV nurse consultants:** N. Basset, J.E.A. van Beek, M. Vriesde, L.M. van


COORDINATING CENTRE

Table 1. Characteristics of participants of the ATHENA and MOSAIC observational cohorts.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MOSAIC participants with HCV infection (n=183)</th>
<th>ATHENA participants with HIV subtype B pol sequence (n=5,038)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at HIV diagnosis (IQR)</td>
<td>35.4 (30.9-41.5)</td>
<td>37 (30-44)</td>
</tr>
<tr>
<td>Diagnosed as an acute HIV infection, n (%)</td>
<td>44 (27.9)</td>
<td>898 (17.8)</td>
</tr>
<tr>
<td>Initiated ART, n (%)</td>
<td>170 (92.9)</td>
<td>4,590 (91.1)</td>
</tr>
<tr>
<td>Median year of HCV diagnosis (IQR)</td>
<td>2009 (2007-2011)</td>
<td>--</td>
</tr>
<tr>
<td>Hospital region, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsterdam</td>
<td>145 (79.2)</td>
<td>2,583 (51.3)</td>
</tr>
<tr>
<td>West NL</td>
<td>37 (20.2)</td>
<td>1,436 (28.5)</td>
</tr>
<tr>
<td>North NL</td>
<td>--</td>
<td>428 (8.5)</td>
</tr>
<tr>
<td>East NL</td>
<td>--</td>
<td>297 (5.9)</td>
</tr>
<tr>
<td>South NL</td>
<td>1 (0.6)</td>
<td>294 (5.8)</td>
</tr>
<tr>
<td>HCV status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever HCV positive¹</td>
<td>183 (100)</td>
<td>563 (11.2)</td>
</tr>
<tr>
<td>Confirmed negative²</td>
<td>--</td>
<td>2,022 (40.1)</td>
</tr>
<tr>
<td>Assumed negative³</td>
<td>--</td>
<td>2,089 (41.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>--</td>
<td>364 (7.2)</td>
</tr>
<tr>
<td>Genotype of primary HCV infection, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>108 (59.0)</td>
<td>--</td>
</tr>
<tr>
<td>4d</td>
<td>37 (20.2)</td>
<td>--</td>
</tr>
<tr>
<td>2b</td>
<td>13 (7.1)</td>
<td>--</td>
</tr>
<tr>
<td>1b</td>
<td>9 (4.9)</td>
<td>--</td>
</tr>
<tr>
<td>3a</td>
<td>4 (2.2)</td>
<td>--</td>
</tr>
<tr>
<td>Not typed</td>
<td>12 (6.6)</td>
<td>--</td>
</tr>
</tbody>
</table>

Note. ¹ based on HCV antibody status; ² based on negative HCV antibody or RNA test in the 1.5 years prior to the last documented visit; ³ based on normal levels of alanine transaminase, measured in the year prior to the last documented visit.
Table 2. HCV and HIV phylogenetic cluster characteristics of HIV/HCV-coinfected men who have sex with men in the Netherlands. Only HIV phylogenetic clusters are shown with ≥2 HCV infections of matching genotype, and only participants with known HCV genotype.

<table>
<thead>
<tr>
<th>patient</th>
<th>hospital region</th>
<th>HIV infection</th>
<th>HCV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>year of HIV dx</td>
<td>HCV genotype</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCV cluster size</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P035</td>
<td>Amsterdam</td>
<td>1996</td>
<td>1a</td>
</tr>
<tr>
<td>P049</td>
<td>Amsterdam</td>
<td>1997</td>
<td>1a</td>
</tr>
<tr>
<td>P180</td>
<td>Amsterdam</td>
<td>2003</td>
<td>1a</td>
</tr>
<tr>
<td>P145</td>
<td>Amsterdam</td>
<td>2008</td>
<td>1a</td>
</tr>
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<td>2002</td>
<td>1a</td>
</tr>
<tr>
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<td>Amsterdam</td>
<td>2005</td>
<td>1a</td>
</tr>
<tr>
<td>P006</td>
<td>Amsterdam</td>
<td>2005</td>
<td>1a</td>
</tr>
<tr>
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<td>Amsterdam</td>
<td>2006</td>
<td>1a</td>
</tr>
<tr>
<td>P184</td>
<td>Amsterdam</td>
<td>1993</td>
<td>1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1a</td>
</tr>
<tr>
<td>P087</td>
<td>Amsterdam</td>
<td>1997</td>
<td>1b</td>
</tr>
<tr>
<td>P064</td>
<td>Amsterdam</td>
<td>1997</td>
<td>4d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1a</td>
</tr>
<tr>
<td>P036</td>
<td>Amsterdam</td>
<td>1997</td>
<td>4d</td>
</tr>
<tr>
<td>P014</td>
<td>Amsterdam</td>
<td>1996</td>
<td>4d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1a</td>
</tr>
<tr>
<td>P034</td>
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<td>1992</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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</tr>
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<td>2006</td>
<td>1a</td>
</tr>
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</tr>
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</tr>
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<td>Amsterdam</td>
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<tr>
<td>P115</td>
<td>Amsterdam</td>
<td>2001</td>
<td>2b</td>
</tr>
<tr>
<td>P198</td>
<td>West NL</td>
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<td>1a</td>
</tr>
<tr>
<td>P174</td>
<td>Amsterdam</td>
<td>2009</td>
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<td>P065</td>
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<td>1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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Table 2. (continued)

<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Year</th>
<th>Cluster</th>
<th>Serum No.</th>
<th>Value</th>
<th>Type</th>
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<td>2003</td>
<td>IV</td>
<td>86</td>
<td>1b</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4d</td>
<td>A</td>
</tr>
<tr>
<td>P015</td>
<td>Amsterdam</td>
<td>2004</td>
<td></td>
<td></td>
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<tr>
<td>P031</td>
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<td></td>
<td>4d</td>
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<tr>
<td>P063</td>
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<td>25</td>
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<td>B</td>
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<tr>
<td>P076</td>
<td>Amsterdam</td>
<td>2008</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P113</td>
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<td>2009</td>
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<tr>
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<td>IX</td>
<td>30</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2b</td>
<td>E</td>
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<tr>
<td>P202</td>
<td>Amsterdam</td>
<td>2006</td>
<td></td>
<td></td>
<td>1a</td>
<td>B</td>
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<tr>
<td>P109</td>
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<td>1993</td>
<td>II</td>
<td>172</td>
<td>1a</td>
<td>C</td>
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<tr>
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<td>2003</td>
<td></td>
<td></td>
<td>1a</td>
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<tr>
<td>P024</td>
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<td>23</td>
<td>1a</td>
<td>D</td>
</tr>
<tr>
<td>P166</td>
<td>Amsterdam</td>
<td>2006</td>
<td></td>
<td></td>
<td>1a</td>
<td>D</td>
</tr>
</tbody>
</table>

Note. The background of corresponding HCV clusters (A-K, named according to cluster size) has been highlighted. Dx: diagnosis.
Figure 1. Phylogenetic trees of HCV genotypes 1a (fig. 1A), 4d (fig. 1B), and 2b (fig. 1C). NS5B sequences were collected among 126 HIV/HCV-coinfected MSM. The identified HCV transmission clusters are shaded grey and were named A-K according to cluster size (also see table 2). Study sequences are shown for MSM with and without HIV pol sequences available in pink and blue, respectively. Reference sequences are shown in black. Relative bootstrap values are visualized by the size of the dot placed at each node.
Figure 2. Phylogenetic tree of HIV pol sequences of 456 HIV-infected MSM in the Netherlands. In each of these 8 identified transmission clusters (named I-XIV according to cluster size, also see table 2), at least 2 MSM showed overlap with HCV NS5B tree topology. The corresponding HIV and HCV transmission clusters are mentioned in the caption of each patient. One patient (i.e., p034) had a primary and secondary HCV-1a infection belonging to HCV cluster H.
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43. FEDERA. Human tissue and medical research: code of conduct for responsible use. Rotterdam: Stichting FMVV. 2011.
FOOTNOTE PAGE

Conflicts of interest
The authors declare there are no conflicts of interest.

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Ethical approval
The MOSAIC study was approved by the Institutional Review Board of the Academic Medical Center at the University of Amsterdam and ethical committees of each institute recruiting participants; the assigned study number is NL48572.018.14. HCV sequences obtained from AMC patients were used in agreement with the hospital ethical guidelines and the Dutch code of conduct for responsible use of human tissue and medical research 2011 [43].
PART III

DIAGNOSIS OF ACUTE HCV INFECTION AMONG HIV-INFECTED MSM
HEPATITIS C VIRUS (HCV) ANTIBODY DYNAMICS FOLLOWING ACUTE HCV INFECTION AND REINFECTION AMONG HIV-INFECTED MEN WHO HAVE SEX WITH MEN

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Joost W. Vanhommerig, Xiomara V. Thomas, Jan T.M. van der Meer, Ronald B. Geskus, Sylvia M. Bruisten, Richard Molenkamp, Maria Prins, Janke Schinkel -- on behalf of the MOSAIC (MSM Observational Study of Acute Infection with hepatitis C) study group

Presented at the 48th International Liver Conference (EASL), 2013, Amsterdam, NL (poster abstract #667), the 20th International Symposium on Hepatitis C Virus and Related Viruses, 2013, Melbourne, Australia, and at the 64th AASLD, 2013, Washington, DC, USA (poster abstract #312).
ABSTRACT

Background
A decline of hepatitis C virus (HCV) antibody titers (anti-HCV), ultimately resulting in seroreversion, has been reported in both acute and chronic HCV infection. However, frequency of seroreversion remains unknown in HIV/HCV-coinfected patients. We describe anti-HCV dynamics among HIV-infected men who have sex with men (MSM) following acute HCV infection and reinfection.

Methods
Primary HCV infection was defined as ≤6 months between the last negative and the first positive HCV-RNA test. Among 63 subjects, anti-HCV was measured annually during a median follow-up of 4.0 years (IQR: 2.5-5.7). Time from HCV acquisition to seroconversion, and from seroconversion to seroreversion, were estimated using Kaplan-Meier methods. Longitudinal anti-HCV patterns were studied using a random effects model to adjust for repeated measures.

Results
Cumulative incidence of seroconversion was 98% (95% CI: 93-100) within a year after primary HCV infection. Median time from HCV infection to seroconversion was 74 days (IQR: 47-125). Subjects who cleared HCV (N=36) showed a significant decrease of anti-HCV following RNA clearance (P<.001). Among 31 subjects with SVR, 8 became anti-HCV undetectable during follow-up; cumulative incidence of seroreversion was 37% (95% CI: 18-66) within three years after seroconversion. Eighteen MSM became reinfected during follow-up. Peak anti-HCV levels following HCV reinfection were significantly higher than during primary infection (P=.014).

Conclusions
A decline of anti-HCV was associated with RNA clearance, and seroreversion was common following SVR. Upon reinfection, anti-HCV levels were elevated, compared to primary infection. Monitoring anti-HCV might be an effective alternative for evaluation and diagnosis of reinfection.
INTRODUCTION

Hepatitis C virus (HCV) is a major cause of liver disease. Globally an estimated 2%-3% of people are infected [1, 2]. Following acute infection, the majority of HCV-infected individuals will develop chronic HCV infection and are at risk for long-term sequelae, including liver cirrhosis and hepatocellular carcinoma [3]. Major risk factors for contracting HCV infection are injecting drug use (IDU), blood transfusions from unscreened donors, and unsafe medical procedures [2]. The risk of sexual transmission of HCV in monogamous heterosexual couples is considered negligible [4]. Since the mid-1990s, an epidemic of HCV infection has emerged among HIV-infected men who have sex with men (MSM) in high-income countries [5-8]. These men denied IDU, and phylogenetic analyses of circulating HCV strains have revealed the presence of multiple MSM-specific clusters, thereby demonstrating that sex may be an alternative transmission route [9-11].

The window period between HCV infection and detectable anti-HCV has been estimated to range from 34 to 70 days in studies among HIV-negative blood product recipients [12-15] and HIV-negative IDU [16, 17]. A delayed anti-HCV response was reported among HIV-infected MSM with acute HCV, suggesting an important role for coinfection with HIV [18]. The reported median time to seroconversion in that study was 91 days, and 158 days in a subset of eight men sampled more frequently.

A decline in anti-HCV reactivity, ultimately followed by seroreversion, has been reported following spontaneous or treatment-induced clearance, mostly in the absence of HIV infection and only after long-term follow-up [19-27]. The available literature seems to agree that seroreversion may occur in cases of profound immunodepression [28]. Only few reports have described seroreversion among HIV-coinfected patients [27, 29-31]. Therefore, the frequency of HCV seroreversion among HIV-coinfected patients remains unknown. The objectives of the current study were to examine dynamics of anti-HCV reactivity following acute HCV infection among HIV-infected MSM. The high incidence of HCV reinfection in this population [32, 33] also allowed us to study anti-HCV dynamics following reinfection.

METHODS

Participants
Participants eligible for this study included HIV-1-infected MSM, aged ≥18 years, diagnosed with acute HCV infection at the Academic Medical Center HIV outpatient clinic in Amsterdam. The majority of study subjects (42/63; 66.7%) participated in MOSAIC (MSM Observational Study for Acute Infection with hepatitis C); a multi-center open prospective cohort study in the Netherlands that was initiated in 2009 [32]. For the present study, strict inclusion criteria
were applied with respect to the maximum interval between HCV-RNA-negative and -positive visits (i.e., max. 6 months). Primary HCV infection was assumed when a subject was anti-HCV-negative prior to the first positive HCV-RNA test. Sociodemographic, clinical, and virological data, including age, use of combination antiretroviral therapy (cART), HIV viral load, CD4 cell count, concentrations of alanine aminotransferase (ALAT), HCV viral load, and HCV treatment data were retrieved from medical files.

**Laboratory methods**

To determine the interval between the last negative and the first positive HCV-RNA test, blood samples collected at earlier visits were tested retrospectively. HCV-RNA tests were performed using either transcription-mediated amplification (TMA; Versant, Siemens, limit of detection, LoD: 5-10 IU/ml) or COBAS Ampliprep/COBAS TaqMan (CAP/CTM, Roche Diagnostics, LoD: 15 IU/ml). Anti-HCV reactivity was tested at least every six months in the first year following infection (and reinfection), followed by annual testing. Anti-HCV testing was performed using a commercial microparticle enzyme immunoassay (MEIA) AxSYM HCV 3.0 (Abbott Laboratories). A positive anti-HCV test was defined as having a sample-to-cut-off (S/CO) value of ≥1.00. When a subsequent negative anti-HCV test was recorded (i.e., S/CO <1.00), this was considered seroreversion. Date of seroreversion was estimated as the midpoint between the first negative anti-HCV test after seroconversion, and the preceding sample. HCV genotype was determined by sequencing a 340 bp fragment of the NS5B region [34].

**Definition of HCV reinfection**

Reinfection was defined as the presence of a different genotype compared with primary infection. To investigate the possibility of reinfection with the same genotype in patients without a genotype switch, consecutive E2/HVR1 sequences were analyzed as previously described [32]. Relapse was defined as a positive HCV-RNA after a negative HCV-RNA at the end of treatment with the same viral strain.

**Statistical analysis**

The midpoint between the last negative and the first positive RNA test, and the midpoint between the last anti-HCV-negative and the first anti-HCV-positive test was estimated to be the dates of infection and seroconversion, respectively. When the last negative HCV-RNA coincided with the last negative anti-HCV test, and the first positive HCV-RNA coincided with the first positive anti-HCV test, the estimated dates of infection and seroconversion were estimated at the 1/3 and 2/3 time point between the last negative HCV and first positive HCV test, respectively.

First, we estimated the cumulative incidence and median time (1) from acute HCV infection to seroconversion and (2) from seroconversion to seroreversion, through Kaplan-Meier survival estimates. Univariable Cox proportional hazards analysis was used to evaluate associations
Diagnosis of acute HCV infection of age, HCV genotype, CD4 cell count before infection, and nadir CD4 cell count before infection, on time to seroconversion. Associations of these variables were also evaluated on time to seroreversion. In addition, peak level of anti-HCV was examined in analysis of the latter. Second, differences in peak levels of ALAT concentration and anti-HCV reactivity between primary HCV infection and reinfection were compared using the nonparametric Wilcoxon’s matched-pairs signed-rank test. Third, anti-HCV signal patterns were estimated from the time of primary infection until end of follow-up. Sequential anti-HCV measurements were corrected for within-subject correlation using a random effects model with random intercept. A random slope was added to the model 6 months after estimated infection; restricted cubic splines allowed for smoothly varying trends. In the analyses of primary HCV infection, measurements during treatment were included, but were censored at HCV reinfection. Statistical software STATA Intercooled 13.1 (STATA Corp, College Station, TX, USA) and R 3.0.1 [35] were used for analysis.

RESULTS

General characteristics
Sixty-three HIV-infected MSM were diagnosed with acute HCV infection and included in this study (table 1). Median age at the estimated date of infection was 42 years (IQR: 35-47), and the majority had Dutch nationality (87.3%). Genotype of primary HCV infection was most frequently genotype 1a (39/63; 61.9%) or 4d (15/63; 23.8%); other genotypes were 1b (N=5), 2b (N=3), and 3a (N=1). Anti-HCV reactivity was measured during a median observation time of 4.0 years following acute infection (IQR: 2.6-5.8), with a median test interval of 0.5 years (IQR: 0.2-1.0). Median time between the estimated date of infection and initiation of treatment was 6.7 months (IQR: 3.7-8.7), or 4.4 months (IQR: 1.8-7.5) after the first RNA-positive visit. Sampling intervals around HCV infection were wider in the group that did not clear HCV, compared to those who did (123 vs. 89 days; \( P = .015 \)). During follow-up 18/63 subjects became reinfected; 16 were reinfected once, one was reinfected twice, and one was reinfected three times. Two out of 21 reinfections (9.5%) were cleared spontaneously.Remarkably, these two patients also spontaneously cleared their primary HCV infection. Treatment outcomes during follow-up of all primary infections and reinfections are shown in Figure 1.

Seroconversion window
All subjects (63/63; 100%) seroconverted during the observation period. Median time from infection to seroconversion was 74 days (IQR: 47-125; Figure 2). The cumulative incidence of seroconversion was 59% (95% CI: 47-71) at 3 months, 73% (95% CI: 62-83) at 4 months, and 98% (95% CI: 93-100) at 12 months. In univariable Cox regression, time to seroconversion was not significantly associated with age (hazard ratio, HR, per 10 year increment: 1.15;
95% CI: 0.82-1.59), genotype (1 versus non-1, HR: 1.06, 95% CI: 0.62-1.83), CD4 cell count (HR per 100 cells/μL increment: 1.06, 95% CI: 0.92-1.22), nor nadir CD4 cell count before infection (HR per 100 cells/μL increment: 1.01, 95% CI: 0.86-1.18).

Dynamics of anti-HCV reactivity following primary HCV infection
Upon seroconversion, anti-HCV reactivity increased to peak levels well above the detection limit with a median S/CO ratio of 89.4 (IQR: 58.6-115.3). Two distinct patterns of anti-HCV dynamics emerged 6 months post-infection; Figure 3 shows the modeled estimates for anti-HCV reactivity. Of 27 subjects that were persistently viremic (i.e., untreated subjects, non-responders, and relapsers), all but one showed a stable serological profile; 80% of all anti-HCV measurements in this group were above S/CO ratio 50. After HCV clearance, either spontaneously (N=5) or following treatment (N=31), anti-HCV reactivity decreased significantly (P<.001). The median peak and subsequent nadir S/CO ratios were 89.4 (IQR: 58.6-115.3) and 5.4 (IQR: 1.3-50.8), respectively.

Incidence of seroreversion
Full seroreversion was observed in 8/31 subjects with SVR following primary HCV infection. Among those who spontaneously cleared HCV, partial seroreversion (i.e., a decrease, but not complete loss of anti-HCV signal) was observed. The cumulative incidence of seroreversion was 37% (95% CI: 18-66) within three years after seroconversion (Figure 4), or 51% (95% CI: 27-81) within three years after reaching SVR. The CD4 cell count at the visit before seroreversion was 490 cells/μL (IQR: 440-775) and the nadir CD4 cell count at that visit was 305 cells/μL (IQR: 140-380). In univariable Cox regression, seroreversion was significantly associated with lower peak anti-HCV levels during primary infection (HR, per 10 S/CO lower: 1.6, 95% CI: 1.1-2.3; P=.014). None of the other studied risk factors were significantly associated with seroreversion; age (HR, per 10 year increment: 0.88, 95% CI: 0.38-1.07), genotype 1 versus non-1 (HR: 2.62, 95% CI: 0.59-11.8), CD4 cell count before HCV infection (HR, per 100 increment: 1.25; 95% CI: 0.79-1.97), and nadir CD4 cell count before HCV infection (HR, per 100 increment: 1.18; 95% CI: 0.81-1.70).

Anti-HCV and ALAT during HCV reinfection versus primary acute infection
During follow-up, 21 reinfections were observed among 18 subjects. Reinfection occurred either following SVR (13 reinfections), before SVR was reached (three reinfections), or following spontaneous clearance (four reinfections), or without intermittent negativity (one reinfection; possible superinfection). Table 2 shows that peak anti-HCV reactivity levels (S/CO) were significantly higher during reinfection (median: 119.6, IQR: 103.4-146.6) compared to primary infection (median: 72.9, IQR: 57.1-105.5; P=.014). Anti-HCV reactivity of the subject who had three reinfections is shown in Figure 5 to illustrate what may be observed during a course of multiple infections.
At the first RNA-positive date during primary infection, ALAT concentrations were elevated (i.e., more than two times the upper limit of normal; \( \geq 80 \text{ U/L} \)) in 13/18 (72.2%) of cases, with a median of 119 U/L (IQR: 56-470). ALAT levels were less pronounced upon reinfection with a median of 66 U/L (IQR: 26-222). Moreover, ALAT concentrations were elevated in only 8/18 (44.4%) cases at the first RNA-positive date of reinfection (table 2).

**DISCUSSION**

In this study, dynamics of HCV-specific antibodies were studied among HIV-infected MSM with acute HCV infection. Our main findings were that (1) the seroconversion window in this population was comparable to the seroconversion window reported among HIV-uninfected subjects, (2) seroreversion was very common following successful antiviral treatment, and (3) after an initial decrease in anti-HCV levels following SVR, levels increased following reinfection to levels reached during primary infection (or higher).

The median time to seroconversion in our study was 74 days and comparable to the HCV seroconversion window reported for HIV-uninfected subjects [12-17]. However, in contrast to our study, in a group of HIV-infected MSM a delayed or even absent antibody response against HCV following acute infection was reported by Thomson et al. [18]. In our study, in a sensitivity analysis among subjects with narrower testing intervals around primary infection, estimates were comparable to the seroconversion window we obtained in the full dataset (data not shown). Also, in our study, all men seroconverted within the observation period, whereas in the Thomson study paper no anti-HCV antibodies were detected in 4/43 subjects at the end of follow-up [18]. As two of these subjects had spontaneously cleared HCV, seroreversion may partly explain these findings, as the authors do not give test intervals for these specific cases. In addition, differences in cART use (53% vs. 74% in our study) may partly explain the differences found with respect to seroconversion.

Among 17/63 subjects (27%) in our study, no anti-HCV antibodies could be detected four months after the estimated date of HCV infection. Screening for acute HCV is therefore preferably performed using nucleic acid testing instead of anti-HCV testing. Periodical testing of ALAT concentration levels may contribute to increased case finding, but ALAT levels can normalize within the serodiagnostic window. As a result, acute infections in patients with normal ALAT levels and an HCV-seronegative status may still be missed. ALAT levels may not always be elevated following HCV (re)infection, and when elevated, do not always indicate recent HCV infection [32]. Indeed, in our study 72.2% of reinfected subjects had elevated ALAT levels at the first RNA-positive date of primary infection, whereas only 44.4% of them had elevated ALAT at the first RNA-positive date of reinfection. This further emphasizes the need for HCV-RNA testing in patients at risk for reinfection.
Seroreversion (i.e., loss of antibodies) following HCV clearance was relatively common in our study, being 37% at three years after seroconversion. To our knowledge, we are the first to have systematically addressed the occurrence of seroreversion among HIV-infected patients with acute HCV infection. The observed incidence of seroreversion in our study is very high, especially when compared to the frequency of seroreversion reported after treatment of chronic HCV [19, 20, 22, 23, 25, 26, 36]. One explanation for the high seroreversion rate may be that in our study, treatment was initiated early in the course of infection, resulting in lower peak anti-HCV levels, as a loss of HCV-RNA coincided with decline in anti-HCV levels.

Seroreversion was observed only among those who cleared HCV following treatment; after spontaneous HCV clearance only partial seroreversion was observed, most likely because 4 out of 5 subjects that had spontaneously cleared their primary infection became reinfected during follow-up. We expect that seroreversion is also likely to occur after spontaneous clearance of HCV, as the observed slope of decline in anti-HCV was comparable to the slope in those who cleared following antiviral treatment. For the same reason, peak anti-HCV reactivity probably influenced the time to seroreversion.

The rapid decrease of anti-HCV reactivity, indicating loss of specific anti-HCV producing plasma cells in this population, may be partly due to the presence of HIV-coinfection, although most men were on cART and had relatively high CD4 cell counts. During HIV infection, the total B-cell number and number of memory B-cells may be significantly reduced [37]. While use of cART is associated with a normalization of the absolute number of B-cells, the memory B-cell subset is unlikely to be restored [37]. Also plasma cell disorders are reported more frequently among HIV-infected patients, but the exact mechanisms that drive this are still unclear [38]. An additional explanation for the rapid decline in anti-HCV reactivity following HCV clearance may be that humoral responses during antiviral therapy in patients with acute HCV infection differ from patients with chronic infection; the rapid decrease in viral antigen, required for stimulation of B-cells, and interferon therapy itself, may inhibit B-cell proliferation. Indeed, loss of anti-HCV reactivity has also been reported after treatment of acute HCV among HIV-negative individuals by Wiegand et al. [36].

Interestingly, following initial decrease in anti-HCV reactivity after HCV clearance, a subsequent increase in anti-HCV reactivity was observed in all reinfection cases. To our knowledge, this finding of ‘re-seroconversion’ is unique and may supplement current screening strategies for HCV reinfection in this population. This may be especially helpful because ALAT levels are not always elevated during reinfection.

The level of anti-HCV reactivity might be a marker for the presence of neutralizing antibodies (nAbs) after infection, as has been proposed by Mizukoshi et al. [39]. In addition, nAbs generally are thought to develop only after initial control of viremia [40]. If this is indeed the case, nAbs
titers may remain low when treatment is initiated early after primary infection. To some extent, this could even explain the high rates of reinfection reported among HIV-infected MSM [32, 33].

Our study has a number of limitations. All subjects were identified at an HIV outpatient clinic; this may have led to selection bias because symptomatic patients may have a higher frequency of visit. Average testing intervals around infection were wider among subjects not treated for HCV compared to those who were, suggesting that patients who are willing to undergo HCV treatment are more compliant to clinical visits. We did not correct for the uncertainty of the estimated dates of infection, seroconversion, and seroreversion in our analyses. Instead, we applied a strict inclusion criterion of max. 6 months between the last negative and the first positive HCV-RNA test. Another limitation is that our study did not incorporate subjects with chronic HCV infection. However, literature suggest that decline of anti-HCV occurs rarely following treatment of chronic HCV infection [19, 20, 22, 23, 25, 26, 36]. Finally, the results of this study may only apply for the antibody assay used, an assay with sufficient linear range. Our results may be less applicable when assays with a more narrow linear range are used.

In conclusion, we have shown that the seroconversion window among HIV-infected individuals is comparable to HIV-uninfected individuals. Still, the median time to seroconversion was 74 days. Screening for acute HCV infection is thus still ideally performed using nucleic acid testing. Seroreversion was common following HCV clearance, and may cause misclassification of a reinfection as an initial infection in clinical practice. Finally, anti-HCV levels increased again following HCV reinfection to levels reached during primary infection. Although the antibody assay used is not a quantitative assay, a clear association existed between anti-HCV reactivity and viremia within subjects following acute HCV infection. Monitoring antibody dynamics following SVR could thus be a useful and inexpensive alternative and additional tool for evaluation and diagnosis of HCV reinfection in the HIV-infected MSM population.

ACKNOWLEDGMENTS

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**Table 1** Characteristics of 63 HIV-infected MSM by HCV status after primary infection.

<table>
<thead>
<tr>
<th></th>
<th>All (N=63)</th>
<th>Persistent viremia (N=27)</th>
<th>Viral clearance (N=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at primary HCV infection</td>
<td>42 (35-47)</td>
<td>41 (36-45)</td>
<td>42 (35-49)</td>
</tr>
<tr>
<td>Dutch nationality, n/N (%)</td>
<td>55/63 (87)</td>
<td>22/27 (81)</td>
<td>33/36 (92)</td>
</tr>
<tr>
<td>Genotype of primary infection, n/N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>39 (62)</td>
<td>17 (63)</td>
<td>22 (61)</td>
</tr>
<tr>
<td>1b</td>
<td>5 (8)</td>
<td>3 (11)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>2b</td>
<td>3 (5)</td>
<td>1 (4)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>3a</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>CD4 cell count before primary infection (cells/μL)</td>
<td>495 (350-660)</td>
<td>475 (320-680)</td>
<td>505 (380-660)</td>
</tr>
<tr>
<td>Nadir CD4 cell count before primary infection (cells/μL)</td>
<td>260 (130-410)</td>
<td>275 (190-445)</td>
<td>230 (80-370)</td>
</tr>
<tr>
<td>On cART at first HCV-positive visit, n/N (%)</td>
<td>41/63 (65)</td>
<td>15/27 (56)</td>
<td>26/36 (72)</td>
</tr>
<tr>
<td>HIV RNA load at first HCV-positive visit (copies/ml)</td>
<td>&lt;50 (40-17590)</td>
<td>99 (&lt;50-33,680)</td>
<td>&lt;50 (&lt;40-9,916)</td>
</tr>
<tr>
<td>Anti-HCV reactivity at first anti-HCV-positive visit (S/CO)</td>
<td>60.5 (12.9-85.1)</td>
<td>61.5 (21.0-85.2)</td>
<td>50.6 (12.5-84.2)</td>
</tr>
<tr>
<td>Days between last negative and first positive HCV-RNA test</td>
<td>107 (80-133)</td>
<td>123 (98-140)</td>
<td>89 (71-119)</td>
</tr>
</tbody>
</table>

Reported values are median (interquartile range), unless indicated otherwise. Abbreviations: anti-HCV, hepatitis C virus antibody; cART, combination antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; S/CO, sample-to-cutoff value. a 27 had persistent viremia; 36 cleared HCV (5 spontaneously cleared, and 31 cleared after treatment).

**Table 2** Maximum observed values for anti-HCV and ALAT concentrations during primary infection and subsequent reinfection for 18 HIV-infected MSM who were reinfected during follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Anti-HCV</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive/elevated at first HCV-RNA+ visit during primary infection, No. (%)</td>
<td>6/15 (40)</td>
<td>13/18 (72)</td>
</tr>
<tr>
<td>Reactive/elevated at first HCV-RNA+ visit during reinfection, No. (%)</td>
<td>13/14 (93)</td>
<td>8/18 (44)</td>
</tr>
<tr>
<td>Peak following primary HCV infection, median (IQR)</td>
<td>72.9 (57.1-105.5)a</td>
<td>470 (336-840)b</td>
</tr>
<tr>
<td>Peak following HCV reinfection, median (IQR)</td>
<td>119.6 (103.4-146.6)a</td>
<td>223 (164-482)b</td>
</tr>
</tbody>
</table>

Measurements are shown from the first HCV-RNA positive (+) visit following primary infection, and reinfection. Note: during primary and reinfection, no anti-HCV result was available from the first HCV-RNA positive sample for 3 of 18 and 4 of 18, respectively. Abbreviations: ALT, alanine aminotransferase; anti-HCV, hepatitis C virus antibody; HCV, hepatitis C virus; IQR, interquartile range. a Sample-to-cutoff value. b U/L.
Diagnosis of acute HCV infection

Primary HCV infection among 63 HIV-infected MSM

- 43 Treated infections
- 20 Untreated infections
- 5 Cleared infections
- 15 Chronic infections

31 SVR
8 NR/R
4 Reinfections before SVR
21 Reinfections *

11 Treated reinfections
3 Outcome pending
6 SVR
1 NR/R
1 Reinfection before SVR
2 Cleared infections
8 Chronic infections

10 Untreated reinfections

Figure 1 Flowchart showing treatment status and outcome during follow-up of 63 HIV-infected MSM with acute HCV infection. *: 12 subjects became reinfected after reaching SVR, 4 before reaching SVR, and 2 following spontaneous clearance. Two subjects became reinfected again after treatment of their (first) reinfection episode. One reached SVR; the other became reinfected again before reaching SVR (this subject is also shown in Figure 5). SVR: sustained virological response; NR: non-response; R: relapse.

Figure 2 Kaplan-Meier estimate of the probability of seroconversion following acute HCV infection. All 63 HIV-infected MSM seroconverted within the observation period. Shaded grey: 95% CI.
Figure 3 Longitudinal anti-HCV measurements following acute HCV infection, among 63 HIV-infected MSM. Distinct patterns were apparent 6 months post infection for 36 subjects with viral clearance (N=5 cleared spontaneously; N=31 after treatment), and 27 subjects with persistent viremia (N=15 untreated; N=8 relapse; N=4 non-response). Measurements during treatment were included in the random effects model. Subjects were censored at HCV reinfection. Dots: observed values; bold solid lines: modeled estimate; shaded grey: 95% CI.
**Figure 4** Cumulative incidence of HCV seroreversion (i.e., loss of detectable HCV antibodies after HCV seroconversion), among N=31 HIV-infected MSM who cleared their primary HCV infection following treatment. Shaded grey: 95% CI.

**Figure 5** HCV antibody S/CO levels and qualitative RNA measurements in one HIV-infected MSM. After resolving a primary HCV-4d infection after treatment, this subject became reinfeected three times. All reinfections were of genotype 1a, and occurred at approximately 3, 4, and 5 years after primary infection. Notice that peak anti-HCV levels increase with every infection and that partial seroreversion is demonstrated after each RNA clearance. Connected dots: anti-HCV reactivity; +/-: RNA status (positive/negative); shaded grey: HCV treatment period.
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Diagnosis of acute HCV infection
SEVEN YEARS OF CHRONIC HCV INFECTION IN AN HIV-INFECTED MAN WITHOUT DETECTABLE ANTIBODIES

Published in: AIDS, 2015; Jan 29(3): 389-90.
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Joost W. Vanhommerig, Janke Schinkel, Marc van der Valk

Presented at the Mid-winter Meeting of the Dutch Association of HIV-Treating Physicians (NVHB), 2015, Rotterdam, NL.
After infection with hepatitis C virus (HCV), in 50% of cases anti-HCV antibodies will be detectable within 5–10 weeks [1–3]. The development of antibodies may be somewhat prolonged in HIV-coinfected individuals: median time from HCV infection to seroconversion has been estimated to be 10–13 weeks among HIV-infected men who have sex with men (MSM) [4,5]. Anecdotal evidence suggests that seroconversion in HIV-infected individuals may be severely delayed: Thomson et al. [4] reported an interval of 3.3 years between the first positive HCV-RNA test and the first positive antibody test in one patient, although testing frequency was not reported. Waning of HCV-specific antibodies (i.e. seroreversion) has been reported among HIV-positive and HIV-negative patients, after spontaneous or treatment-induced clearance of acute [5,6] or chronic [7,8] infection. In an HIV-infected patient with progressive immunosuppression, seroreversion was reported despite continuous HCV replication. However, HCV antibodies became detectable again after antiretroviral therapy (ART) was initiated [9]. Here, we report a case without documented seroconversion and/or hepatitis (reflected by elevated transaminases) despite being infected with HCV for almost 7 years. A 61-year-old bisexual man, known to use noninjecting drugs (amphetamines and cocaine), was diagnosed with HIV in 1997. Possibly related to his drug use, he infrequently visited our outpatient clinic, and was poorly adherent to ART prescribed. In 2010, he presented with onycholysis and blistering of the skin suspect for porphyria cutanea tarda (PCT) but, because of incompliance, no definite diagnosis could be made. After his third clinical admission because of a candida esophagitis in July 2013, he decided to improve his lifestyle, and started taking ART, which resulted in an undetectable HIV viral load. Between 2008 and the last recorded visit in 2014, his CD4 cell count varied between 30–190 cells per mL. Urine analysis showed that he indeed had PCT, which is highly associated with HCV infection [10]. Concurrently, his alanine transaminase (ALT) level was slightly elevated for the first time in years (i.e. 66 U/l), and the patient was tested positive for HCV RNA and negative for HCV antibodies, which is suggestive of an acute infection. Presence of antibodies was tested with the DiaSorin LIAISON XL HCV Ab assay. He denied injecting drug use and did not have sexual intercourse for the past 4 years. Sequence analysis showed infection with HCV genotype 1a. Stored samples were tested and demonstrated that HCV RNA was already detectable from January 2008 onwards. The last HCV RNA negative sample dated from August 2007. All samples were antibody-negative. The slight ALT elevation (from 18 U/l in August 2007 to 41 U/l in January 2008) fits the time frame of infection (Fig. 1). The fact that during 7 years of chronic HCV infection there was never any sign of HCV antibodies (or hepatitis) is remarkable. Several reports already showed that acute HCV infections can easily be missed by using antibody testing only, due to the seroconversion window period [1–5]. Seronegative HCV infection has been reported earlier among immunocompromised patients [11,12], but at least part of these patients probably had an acute infection and antibodies would have been detectable later on. In addition, patients in these studies were tested with second-generation anti-HCV assays, thereby increasing chances of false-negativity [13]. The case we describe shows that chronic HCV infection may also be missed when only third-generation serologic assays are used. Among HIV-infected
MSM, testing for acute HCV is thus ideally performed using HCV RNA testing when there is suspicion of infection, especially since subtle ALT elevations are easily missed when monitored infrequently.

**Figure 1** Graph showing hepatitis C virus (HCV)-RNA status, HIV viral load (solid line), and alanine transaminase (ALT) concentration (dashed line) of a 61-year-old, HIV-infected man with a 7-year documented seronegative chronic HCV infection (from 2005 till present). Poor adherence to antiretroviral therapy until July 2013 is indicated by the fluctuating HIV viral load. The dotted horizontal line shows the upper limit of normal (ULN) ALT concentration (45 U/L).
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EVALUATION OF A HEPATITIS C VIRUS (HCV) ANTIGEN ASSAY FOR ROUTINE HCV SCREENING AMONG MEN WHO HAVE SEX WITH MEN INFECTED WITH HIV

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Joost W. Vanhommerig, Thijs J.W. van de Laar, Maarten Koot, Martijn S. van Rooijen, Janke Schinkel, Arjen G.C.L. Speksnijder, Maria Prins, Henry J.C. de Vries, Sylvia M. Bruisten

Presented at the 49th International Liver Conference (EASL), 2014, London, UK (poster abstract #728).
Background
For detection of early HCV infection and reinfection, commercial HCV-RNA tests are available. However, these tests are relatively time-consuming and expensive. A commercially available test that may supplement current screening methods, targets the HCV core protein.

Methods
During five waves of anonymous surveys at the Amsterdam STI clinic between 2009-2012, all HIV-infected MSM (N = 439) were tested for HCV-antibodies (AxSYM HCV 3.0, Abbott), and HCV-RNA (TMA Versant, Siemens). To evaluate the potential value of the ARCHITECT HCV antigen (HCV-Ag) assay (Abbott), all HCV-RNA-positive sera (N = 31) were tested with this assay, as well as two HIV-infected HCV-RNA-negative controls. In addition, all included samples were tested for alanine aminotransferase (ALT).

Results
Among 439 HIV-infected MSM, 31 (7.1%) tested positive for HCV-RNA; the HCV-Ag assay showed concordant positive results for 31/31 (100%). A substantial number of MSM, i.e., 5/31 (16.1%), had detectable HCV-RNA but were HCV-seronegative at the time of screening and were presumed to have been recently infected. Concordant HCV-RNA-negative results were obtained in 57/60 control-samples. Specificity was 95.0% (95% CI: 86.1-99.0). The detection limit was between 3.0 and 3.7 Log10 IU/mL, irrespective of HCV genotype/subtype. ALT concentrations were elevated (i.e., >40 U/L) in 9/31 (29.0%) HCV-RNA positive MSM, including 1/5 (20.0%) MSM with recent HCV-infection.

Conclusions
The HCV-Ag assay proved a valuable screening tool for detection of active HCV infection among HIV-infected MSM with and without anti-HCV. Adding ALT to current screening methods would improve case finding marginally. We therefore recommend implementation of routine HCV-Ag screening for populations at risk for HCV-(re)infection.
Since the mid-1990s, hepatitis C virus (HCV) has emerged as a sexually transmitted infection (STI) among men who have sex with men infected with HIV [1,2]. In 2007 the prevalence of HCV among men who have sex with men infected with HIV attending a large STI clinic in Amsterdam reached 17.8% [3]. Since September 2007, men who have sex with men who attended the Amsterdam STI clinic and were infected with HIV, or unaware of their HIV-status, were offered routine testing for HCV antibodies (anti-HCV).

Anti-HCV is detectable several weeks after infection; two studies showed that over 33% of men who have sex with men infected with HIV were still anti-HCV negative three months after the first positive HCV-RNA test [4,5]. The serodiagnostic window, the time between HCV-infection and the detection of anti-HCV, may be prolonged for HIV-infected individuals compared to individuals without HIV-coinfection; (10-13 weeks versus 5-10 weeks, respectively) [6,7]. Due to this serodiagnostic window, a significant proportion of recently acquired HCV infections may be missed when screening for anti-HCV only. Moreover, high rates of HCV reinfection have been reported among men who have sex with men infected with HIV [8,9]. Commercial HCV-RNA assays are available to diagnose such infections, but these are time-consuming and costly. Therefore, there is room for improvement of currently used routine screening methods to detect recently acquired HCV infections, either primary or recurrent.

In many clinical settings, HCV diagnostic tests are performed when alanine aminotransferase (ALT) levels are elevated, or when specific HCV-related risk behaviour is reported. However, risk behaviour is not always disclosed, and ALT levels can remain normal or rapidly normalize even within the serodiagnostic window [8,10]. Conversely, ALT may be elevated as a result of various other reasons, including cART induced hepatotoxicity, alcohol and/or steroid use, and other viral infections that affect the liver [11].

The ARCHITECT HCV antigen (HCV-Ag) assay (Abbott Laboratories, Abbott Park, IL, USA) is a commercially available immunoassay using chemiluminescent microparticle technology for quantitative measurement of HCV core antigen; a structural protein with a highly conserved sequence across all HCV genotypes [12]. Evaluated was whether the HCV-Ag assay could supplement current routine HCV screening methods, using detectable HCV-RNA as the reference test for sensitivity and specificity.

A technical validation was performed to assess sensitivity. The detection limit of the HCV-Ag assay was determined using a set of 16 HCV-RNA positive samples obtained from clinical patients. Samples were selected based on HCV viral load (≥5.0 Log10 IU/mL; COBAS AmpliPrep/COBAS Taqman HCV assay v2.0, Roche Diagnostics, Pleasanton, CA, USA) and HCV genotype diversity. This set reflects the HCV genotypes/subtypes that are the most prevalent in the Netherlands, and consisted of HCV genotype 1a (n=3), 1b (n=3), 2a (n=1), 2k (n=1), 2b (n=2), 3a (n=3), and 4a (n=3). HCV genotyping had been performed by sequencing part of the NS5B
region [13]. Subsequently, each sample was diluted with HCV-negative plasma (final HCV RNA concentrations: 100,000, 10,000, 5,000, 1,000 and 500 IU/ml) and tested for HCV-Ag. All 16 undiluted samples including their 100,000 IU/ml dilutions showed strong HCV-Ag reactivity. Based on the dilution series, the lower limit of detection of the HCV-Ag assay was estimated to be between 3.0 Log10 and 3.7 Log10 IU/mL, irrespective of genotype/subtype. This lower limit of detection is in agreement with the range reported in other studies [14–16]. The HCV-Ag results of the HCV-RNA dilution experiments are shown in table 1.

Between 2009 and 2012, a total of 1,432 men who have sex with men participated in a series of cross-sectional surveys performed at the Amsterdam STI-clinic [17]. Of them, 439 (30.7%) were infected with HIV, of whom 31 (7.1%) were coinfected with HCV, indicated by a positive HCV-RNA test (TMA VERSANT HCV RNA Qualitative Assay; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). For each of the 31 men with HIV-HCV co-infection, two HIV-infected HCV-RNA negative controls were included from the same survey year. All samples had been screened for anti-HCV (AxSYM HCV 3.0; Abbott Laboratories, Abbott Park, IL, USA) with immunoblot confirmation (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics, Raritan, NJ, USA). Sensitivity and specificity were calculated for each assay and for several clinical test combinations. In accordance with the manufacturer's instructions, specimens with a HCV-Ag concentration level <3.00 fmol/L were considered nonreactive, values 3.00-10.00 fmol/L were considered weakly reactive and values ≥10.00 fmol/L were considered reactive. Statistical software package STATA Intercooled v13.1 was used for data analysis. Confidence intervals were calculated using exact binomial methods.

The HCV-Ag assay showed fully concordant positive results in all 31 HCV-RNA positive sera (range: 10.4 - >20,000 fmol/L; table 2); sensitivity was 100% (95% CI: 88.8-100). Concordant HCV-Ag negative results were obtained in 57/60 HCV-RNA negative controls; 3/60 sera showed weak false HCV-Ag reactivity, resulting in a specificity of detecting HCV viremia of 95.0% (95% CI: 86.1-99.0). For all 6/60 HCV-RNA negative sera from men with resolved HCV infections (i.e., HCV-RNA negative, anti-HCV positive), concordant HCV-Ag negative results were obtained (table 2).

ALT levels were elevated (i.e., >40 U/L) in 9/31 (29.0%) HCV-RNA positive sera, and in 3/61 (4.9%) negative sera (table 2). Recent -primary- HCV infection was presumed when HCV-RNA was detected without the (confirmed) presence of anti-HCV. Recent HCV was observed in 5/31 (16.1%) subjects, only one of whom had mildly elevated ALT (i.e., 63 U/L). So, 4/5 (80.0%) recent HCV infections would have been missed with a test algorithm consisting of anti-HCV and ALT only.

The HCV-Ag assay proved to be a valuable screening tool for HCV infection among men who have sex with men infected with HIV. All 31 HCV infections were detected, including 5 recently
acquired anti-HCV negative HCV infections. Combined anti-HCV and ALT testing, as currently performed in clinical practice, identified all 26 chronic HCV-infections but missed 4/5 (80%) recent HCV-infections, and was thereby clearly inferior to HCV-Ag testing.

As a result of indeterminate HCV-Ag reactivity in men without HCV infection (n=3), the estimated specificity of HCV-Ag detection was 95%. Depending on the background prevalence this may result in a relatively low positive predictive value. The manufacturer advises re-testing of specimens with an indeterminate HCV-Ag test result, but unfortunately no additional serum was available for re-testing in this study. In addition, the sensitivity and specificity for recently acquired infections was hard to determine due to the small sample-size.

A limitation of the present study is that only qualitative but no quantitative HCV-RNA viral loads were determined for our study samples. Based on the range of HCV-Ag concentrations (i.e., 10.4 - >20.000 fmol/L) of the 31 HCV-RNA positive samples, HCV-RNA loads varied between 3.0 Log10 and >6.0 Log10 IU/mL (calculated using Vermehren et al. [18]). The detection limit of the assay used in the present study was estimated to be between 3.0 Log10 and 3.7 Log10 IU/mL. Most evaluations that were previously performed were among HIV-negative subjects, with two exceptions [19,20]. However, these studies did not evaluate the added value of implementing routine HCV-Ag or ALT screening in a high-risk population, such as men who have sex with men infected with HIV. As several studies have reported on the benefits of HCV-Ag testing in the detection of chronic HCV infection, future studies should focus on the added value of HCV-Ag detection in recently acquired HCV-infections and reinfections [15,18,21,22].

Routine screening for HCV using anti-HCV and ALT testing has been recommended among men who have sex with men infected with HIV [23]. Due to prolonged HCV seroconversion intervals in patients infected with HIV and the low specificity of ALT testing with regards to recent HCV infection, a large proportion of cases with recent HCV infections will be missed (4/5 in our study). Detection of recent HCV infection is important, not only to prevent further transmission, but also to improve treatment success by starting treatment early after infection [24]. Therefore, implementation of routine HCV-Ag screening for populations at risk for HCV-infection is recommended. Screening for HCV-Ag could be used as a cost-saving approach for the detection of recently acquired HCV-infections among risk groups. Anti-HCV testing can be performed to confirm seroconversion in cases with no history of HCV, or to differentiate between acute versus chronic HCV infection. This assay may be of particular benefit to identify HCV-reinfections, which is of ongoing concern in this population, especially in the upcoming era of effective direct acting antiviral therapy.
ACKNOWLEDGMENTS

The authors would like to thank all participants of the study; T. Heijman and A. Urbanus for data management; S. Rebers for performing HCV-RNA and HCV genotyping tests.

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The medical ethics committee of the Academic Medical Center (MEC AMC) approved the parent study. No further ethical approval was needed as blinded laboratory samples were used for this study.
Table 1 HCV antigenaemia among 93 HIV-infected MSM attending a large STI outpatient clinic in Amsterdam, 2009-2012.

<table>
<thead>
<tr>
<th></th>
<th>HCV-RNA detectable (N=31)</th>
<th>HCV-RNA not detectable (N=62)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recent HCV (n=5)</td>
<td>Chronic HCV (n=26)</td>
<td>Resolved HCV (n=6)</td>
<td>No HCV (n=56)</td>
</tr>
<tr>
<td>HCV-Ag ≥10.00 fmoVL (reactive)</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>0 (0)</td>
<td>0/54 (0.0)*</td>
</tr>
<tr>
<td>HCV-Ag ≥3.00 fmoVL (at least weakly reactive)</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>0 (0)</td>
<td>3/54 (5.6)*</td>
</tr>
<tr>
<td>ALT elevated (&gt;40 U/L)</td>
<td>1 (20.0)</td>
<td>8 (30.7)</td>
<td>1 (16.7)</td>
<td>2/55 (3.6)**</td>
</tr>
<tr>
<td>HCV-Ab positive</td>
<td>0 (0)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>0/56 (0)</td>
</tr>
<tr>
<td>HCV-Ab positive and ALT elevated, combined</td>
<td>1 (20.0)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>2/55 (3.6)**</td>
</tr>
<tr>
<td>HCV-Ab positive and HCV-Ag weakly reactive, combined</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>3/54 (5.6)*</td>
</tr>
</tbody>
</table>

Numbers are n (%); recent HCV: HCV-Ab negative & HCV-RNA positive; chronic HCV: HCV-Ab positive & HCV-RNA positive; resolved HCV: HCV-Ab positive & HCV-RNA negative. *two samples gave an internal error in HCV-Ag test and were left out of the calculation; **one of these two samples also gave an internal error in the ALT test and was left out of the calculation.
Table 2: HCV-Ag assay results of 16 samples in a dilution experiment. Samples were derived from HCV-positive blood donors in the Amsterdam area, 2013.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HCV genotype</th>
<th>HCV viral load (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.0 Log₁₀</td>
</tr>
<tr>
<td>1</td>
<td>1a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1a</td>
<td></td>
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<tr>
<td>4</td>
<td>1b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1b</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1b</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2b</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2b</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3a</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3a</td>
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<tr>
<td>13</td>
<td>3a</td>
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<td>14</td>
<td>4a</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4a</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>4a</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: HCV genotyping/subtyping was performed by sequencing part of the NS5B region. Black=reactive, grey=weakly reactive, white=non-reactive.
REFERENCES


Diagnosis of acute HCV infection
GENERAL DISCUSSION

In this thesis, the epidemiology of and tools for diagnosis of acute hepatitis C virus (HCV) infection were addressed among two groups at high risk for infection in high income countries: people who inject drugs (PWID) and HIV-infected men who have sex with men (MSM). We investigated incidence trends of acute HCV infection and reinfection among PWID and MSM, risk factors for sexual transmission of HCV among HIV-infected MSM, and different assays used for detection of HCV infection among HIV-infected MSM. Increased knowledge on these topics is likely to enhance prevention, testing, detection and management of acute HCV, in particular in the groups mentioned above.

Epidemiology of HCV infection among PWID

Since the discovery of HCV in 1989, the incidence of HCV among PWID decreased significantly in Amsterdam; during the late 1980s the incidence was >25 per 100 person-years (PY), during the 1990s a steep decline was observed to an incidence less than 5 per 100 PY, and since 2004 no incident HCV infections have been observed among ever injecting drug users in the Amsterdam Cohort Studies (ACS) [1,2]. One of the major contributors to this decrease in incidence is a high competing mortality rate due to HIV infection in HCV-infected PWID [3]. Other factors that are likely to have contributed to the observed decrease in HCV incidence among PWID are: the wide availability of comprehensive harm reduction programs including needle exchange and opiate substitution therapy; a steep decrease in the number of new injectors since the 1980s; ageing of the injecting population (often accompanied by a decrease in risk behavior); and the availability of HCV treatment. The latter is probably responsible for a minor part of the decrease, as treatment was introduced relatively recently and uptake was limited among PWID, because physicians had concerns about the patient’s adherence to therapy and their risk for psychiatric decompensation, premature mortality, and reinfection after successful treatment (because of ongoing risk behavior) [4–9]. However, we know from many studies that PWID can actually be successfully treated for HCV [10–13], thereby decreasing the time of infectiousness for several decades [14].

Our study, as described in chapter 2 of this thesis, showed that the incidence of reinfection was low (i.e., 0.76-3.42 per 100 PY) among PWID that were treated for chronic HCV infection in Amsterdam [15]. The results of our study were in line with other studies that evaluated HCV reinfection following SVR among PWID in Europe [9,16–18], the USA [19], Canada [20], and Australia [21]. Our study differed in several aspects: (1) follow-up time after clearance of a (primary) reinfection was included (as a person is at risk for reinfection as soon as the previous infection is cleared) whereas others (except [21]) calculated follow-up time from SVR, and (2) phylogenetic analysis was performed using pre- and post-treatment HCV sequences obtained from cases that were assumed to have a viral relapse following treatment. Although no reinfections were discovered among subjects that had a viral relapse (by definition) in our
study, previous study among MSM showed that reinfections of the same genotype do occur in the first months after treatment completion, and can be distinguished using phylogenetic analysis (discussed later on in this chapter) [22]. Studies that assessed incidence of reinfection among PWID who cleared acute HCV infection, following either spontaneous clearance or treatment, reported reinfection rates of 1.8-46.8 per 100 PY [21,23,24]. Compared to the reinfection rate mentioned earlier in this paragraph this may seem relatively high, however the incidence of primary HCV infection among PWID is estimated to vary between 6.1-27.2 per 100 PY [23]. It may be possible that predominantly PWID with a low risk profile were treated in these studies. As the disease burden is expected to decline when both acute and chronic HCV infections are diagnosed and treated on a community-wide level, treatment should not be withheld PWID with HCV infection [14]. When scaling up treatment among PWID, prevention measures and education among PWID should be intensified in order to establish a substantial reduction of HCV infection risk. Intensified monitoring of PWID for HCV reinfection will be important.

Epidemiology of acute HCV infection among MSM

Although the HCV epidemic among MSM is relatively new, patterns differ between studies and regions. While one study claimed that the incidence of HCV has not changed over time [25], others agree that the incidence of HCV infection among HIV-infected MSM has increased notably after 2000 [26–32]. A recently published meta-analysis leaves no doubt that the incidence of HCV has increased globally (figure 1). In the ACS among MSM, the incidence of primary infection has reached a plateau phase with an incidence of around 12 per 1,000 PY [33]. In a study among 19 HCV treatment centers in the Netherlands, the HCV incidence in 2014 was estimated to be 11 per 1,000 PY [34]; these results were comparable to the incidence we found in the ACS in 2009-2011 and therefore strengthen the hypothesis of a stabilizing epidemic in the Netherlands. In addition, the overall (RNA and/or AB) prevalence of HCV among HIV-infected MSM attending a large STI clinic in Amsterdam seems to have peaked in 2008 at 20.9% and leveled off thereafter [35]. Unpublished updated data from these bi-annual surveys at the Amsterdam STI clinic conducted in 2011 and 2012 showed an overall HCV prevalence (HCV antibody and/or HCV RNA positive) in HIV-infected MSM of 9.4% and 8.9% respectively (T. Heijman, personal communication), again strengthening our hypothesis of a stabilizing epidemic among HIV-infected MSM. In addition, the ‘Hepatitis C in the UK 2015 report’ stated that between 2009-2013 the incidence of HCV infection among HIV-infected MSM in the UK had declined significantly to 2.3 per 1,000 PY [36]. However, this observation was solely based on case notifications. A recent study in an international cohort of HIV seroconverters also showed a stabilizing incidence in Western Europe. The incidence in Europe as a whole did not decline, mostly because of the ongoing increase in HCV incidence among MSM elsewhere in Europe [37].

While HCV infections with genotype 1 and 4 are most common among HIV-infected MSM,
our study in chapter 3 reported the emergence of HCV genotype 2b infections among HIV-infected MSM in Amsterdam. This genotype was not seen in this population before 2008, and the spread of genotype 2b among MSM in the Netherlands currently seems to be restricted to the Amsterdam area [34]. Our study also reaffirmed that the incidence of HCV infection among HIV-negative MSM is very low; no infections were detected during more than 10,000 PY of follow-up, leading to an incidence estimate of 0 with an upper 95% confidence bound of 0.3 per 1,000 PY in the period 1984-2012 [33].

Figure 1 Pooled HCV incidence rate among HIV-infected men who have sex with men between 1984 and 2015. Line: meta-regression with polynomial fit. The 95% confidence interval is shaded grey. Each dot represents an incidence estimate per calendar year, derived from the 17 studies included in the meta-analysis [38]. Copyright © 2015 Wolters Kluwer Health, Inc., reprinted with permission of the author (prof. H. Hagan) and the publisher.

Several hypotheses exist as of why the HCV epidemic is still largely restricted to HIV-infected MSM. One is that HIV-negative MSM are simply not screened. However, in one of the studies that argued this [39] the HCV prevalence among HIV-negative MSM was in fact comparable to the general population: 44 cases of acute HCV infection were detected among an estimated
34,657 HIV-negative MSM that visited this STI clinic in the UK, leading to a prevalence estimate of 0.13% (95%CI: 0.09-0.17). Similar observations of low prevalence among HIV-uninfected MSM have been made during bi-annual HIV surveys performed at the STI outpatient clinic in Amsterdam between 1995-2010 [35]. Because predominantly HIV-negative MSM with increased risk behavior (i.e., STI clinic attendees) were screened in this study, the prevalence of HCV infection is likely to be lower in the total HIV-negative MSM population. As cases of sexually acquired HCV infection have been reported in the absence of HIV in several studies [35,39–43], it is of importance to continue to monitor the prevalence of HCV infection among HIV-uninfected MSM. Moreover, antiretroviral pre-exposure prophylaxis (PrEP) is the latest addition to HIV prevention packages, although it is not yet registered in the Netherlands. PrEP has proven to be very effective in randomized controlled trials performed among serodiscordant heterosexual couples, as well as among MSM who either continuously or intermittently used PrEP [44–48]. However, uptake of PrEP might impact the prevalence of HCV infection among HIV-negative MSM. Two PrEP studies [47,49] indeed reported incident HCV infections among MSM using PrEP. The incidence of HCV infection among PrEP users in a clinical practice setting in San Francisco was 6.6 per 1,000 PY; two out of 485 MSM were infected with HCV while on PrEP, and the only reported risk factor for infection was condomless anal sex [49]. In the PROUD study, an open-label randomized trial performed in the UK, six incident cases of HCV were reported among 512 participants [47]. Although risk compensation (i.e., an increase in risk behavior or a decrease in other prevention methods) has not been reported by trials so far [49], HIV-uninfected MSM on PrEP should preferably be monitored on a regular basis, for HCV as well as other STI [45].

In chapter 4 of this thesis, men that self-identified with MSM subcultures (leather, rubber/lycra, and jeans subcultures) were more often anti-HCV positive than MSM belonging to other subcultures [50]. This study is unique in that it included lifestyle characteristics of MSM that participated, and of their recent sex partner(s). In addition to the analysis of questionnaires, HCV sequences of viremic MSM were analyzed to investigate whether MSM of specific subcultures were infected with similar HCV strains. We found no evidence to support this hypothesis. A large part of the higher anti-HCV prevalence found among MSM that identified themselves with these subcultures is probably explained by increased sexual behavior within these subcultures. However, because HCV infection was not restricted to one or two subcultures, HCV preventive strategies are best directed to all HIV-infected MSM.

In chapter 5 of this thesis we studied risk factors for acute HCV infection among HIV-infected MSM and found that several sexual risk behaviors were associated with sexual transmission of HCV [51]. To our best knowledge, our study was the largest and most detailed case-control study identifying risk factors for sexual transmission in MSM. In addition, and unlike other case-control studies, we explored interactions of biological risk factors with specific sexual behaviors and sex-related risk factors (e.g., anal douching). We confirmed risk factors that
were identified in other studies, including unprotected anal sex, fisting, use of sex toys, injecting/snorting drugs, and concomitant or recent other STI (predominantly syphilis) [52–54]. In addition to these risk factors, we found an effect of lower CD4 cell count on acute HCV infection. Although the median CD4 cell count of both cases and controls was ≥500 cells mL⁻¹ and the absolute difference was not large, we found that the CD4 cell count was significantly lower well before co-infection with HCV. This finding excluded the possibility that the lower CD4 cell count we observed was in fact a result of HCV infection itself. A significant effect of lower CD4 cell count on acquisition of HCV infection has been reported by one other study, especially for those with a CD4 cell count <500 [25]. Other risk factors that have been reported by case-control studies, e.g., group sex, rectal bleeding and anal douching [52–55], were strongly associated in our univariable logistic regression analysis but lost significance in our multivariable analyses [51]. As especially MSM that report a combination of different sexual behaviors seem to be at increased risk for HCV infection [51,56], risk estimation for acute HCV infection may benefit from the use of an individual risk-score based on the measurement of sexual behavior acts. Ideally, the patient’s sexual behaviors are discussed during medical consultation, in order to decide on HCV testing. Pre-screening risk assessments in other forms have shown to improve screening efficacy [57]. However, because sexual risk behavior may not always asked by or disclosed to the treating physician, the use of a short self-administered questionnaire may assist in estimating the risk of HCV infection and advising testing.

Recently, an increase in the number of MSM that report having sex under the influence of drugs has been reported in the UK (since 2015 this is often referred to as chemsex) [58]. It is unclear whether there is an increase of crystal meth use among MSM in the Netherlands, as figures over time are lacking [58]. Recent reports from London, UK, raised concern because chemsex was described as a “perfect storm” for transmission of HIV and HCV [59–63]. This trend may spread to gay scenes in other cities like Berlin [64], as well as Amsterdam. Crystal methamphetamine (meth) is most frequently mentioned in relation to chemsex; in ‘The Chemsex Study’, a study performed in three London boroughs, 10.4% of MSM reported to have used crystal meth in the past 6 months [65]. In our study on sexual risk factors for HCV described in chapter 5, the proportion of MSM that had recently used crystal meth before or during sex was also significantly higher among MSM with acute HCV infection (19.5%) than among HIV mono-infected MSM (0.8%), however crystal meth before or during sex was not entered separately in the presented multivariable models; we chose to evaluate variables with either a potential direct or a facilitating effect on HCV acquisition [51]. A study among MSM in New York City between 2005-2010 also showed that having sex while high on crystal meth was a major risk factor for acquiring HCV infection [52]. GHB/GBL, mephedrone, ketamine and cocaine are also commonly associated with chemsex. Sex-related drug use has been associated with increased sexual risk behavior, and increased prevalence of chlamydia, gonorrhea, or syphilis among MSM and women [66]. Chemsex is likely to contribute to the spread of these STI including HIV and HCV, as the drugs can lead to a long-lasting high during
which unprotected sex with multiple partners can occur. In addition, an increased risk of potentially toxic drug-drug interaction between antiretroviral and recreational drugs has been described [64]. HCV transmission is also likely to be enhanced because some of the drugs used during sex parties (e.g., crystal meth, ketamine) can be injected (known as ‘slamming’) and needles may be shared. In a retrospective study performed by a sexual health clinic in the UK between 2006 and 2014, 20% of cases that acquired acute HCV infection through a sexual route reported to have injected drugs in the preceding months [67]. In our study on risk factors for HCV infection among HIV-infected MSM, injecting was reported by 12% of cases with acute HCV infection, but sharing needles was uncommon [51]. It is important to educate MSM about the risks of injecting drugs, even when needles are not shared. Sharing straws when snorting drugs was significantly associated with HCV acquisition in our study, while this might not necessarily be a direct transmission route. Further studies are therefore needed to clarify the relation we found between snorting drugs and HCV acquisition.

Diagnosis of acute HCV infection
As successful HCV antiviral therapies are becoming increasingly available, effective screening tools need to be implemented to find people that are unaware of their HCV infection. Because the detection of acute HCV infection is hampered by its asymptomatic nature, testing is ideally performed on a regular basis among those at highest risk for infection, even in the absence of reported symptoms. Apart from knowing who should be tested, guidelines on frequency of testing, and which tests should be used are of importance. The European AIDS Treatment Network (NEAT) [68] and Infectious Diseases Society of America (IDSA) [69] guidelines state that HIV-infected patients should be tested for anti-HCV at time of HIV diagnosis and monitored yearly thereafter. In addition, serum alanine transaminase (ALT) levels should be regularly assessed during visits to the HIV clinic (ideally 4-6 months apart) to evaluate the liver function. Dutch guidelines recommend yearly anti-HCV testing among HIV-infected MSM with risk behavior, and ALT levels should be evaluated at each visit for all. Follow-up HCV testing is then advised when ALT elevations are found. However, none of the proposed guidelines are currently fully implemented [70]. As a result, a substantial proportion of those infected with HCV might remain undiagnosed and untreated [71,72]. Moreover, as risk behavior is not defined it is important that in future revisions of the guidelines ‘risk behavior’ is specified more clearly. ALT values above the upper limit of normal (i.e., >40U/L) may indicate viral hepatitis when there is no alternate cause [73,74]. However, acute HCV infection does not necessarily lead to elevated ALT values, and ALT concentrations usually normalize within several weeks after acquiring HCV infection. Moreover, ALT elevations following HCV reinfection have been shown to be lower than during initial infection in recent studies among MSM [75,76], but also already in the early 1990s in chimpanzee studies [77]. The median time between infection and detectable anti-HCV levels is estimated to be 5-10 weeks and may be slightly prolonged among HIV-coinfected patients when compared to HIV-negative patients [78,79]. Therefore, when there is a clear suspicion of recent HCV infection, preferably an HCV RNA test should be
performed especially since major, or even subtle, elevations in ALT may be missed when this is monitored infrequently. In chapter 8 of this thesis we described a unique case with absence of detectable anti-HCV and normal ALT concentrations at clinic visits for more than 7 years [80]. This patient has now been successfully treated for his HCV infection and never had significant liver damage (M. van der Valk, personal communication).

Anti-HCV testing became available for HIV-infected MSM and MSM that opted out for HIV testing at the STI outpatient clinic of the Public Health Service of Amsterdam in November 2007, in response to the increased HCV prevalence found among HIV-infected MSM [81]. Because of financial constraints this service was stopped in May 2014. As a result, HIV-infected MSM were advised to visit their general practitioner (GP) or HIV treatment centre to get tested for HCV infection. However, a GP may be less approachable than an STI clinic that offers free and anonymous testing, and partner referral. Also, GPs may be less familiar with HCV infection because the prevalence in the general population in the Netherlands is low [82,83]. From the HIV clinician’s perspective, testing for HCV infection in patients without clinical signs or symptoms may not be indicated. Reinstatement of HCV testing for HIV-infected MSM at public health services is therefore advised.

As described in chapter 9, we have assessed sensitivity and specificity of the ARCHITECT HCV Ag assay (Abbott Laboratories, Abbott Park, IL, USA) for screening among HIV-infected MSM, as have other research groups [84–86]. The cost per test of this assay is significantly lower than of most assays that are developed for the detection of HCV RNA. Despite a lower sensitivity compared to commercial RNA assays [87], the clinical sensitivity of the HCV antigen test was 100% in all three studies. In other words, the yield was equal, while the window phase of detection was considerably shorter compared to testing for anti-HCV, due to the relatively long HCV seroconversion interval. The specificity of the HCV antigen test was >95% in two studies that also tested HCV-negative individuals [84,85]. Another promising and much needed addition to the current options for HCV testing is a point-of-care test. Several point-of-care tests have been developed that can detect anti-HCV, with sensitivity and specificity of up to 92.7% and 100%, respectively [88]. In addition, detection of anti-HCV, HCV core antigen, and HCV RNA, as well as determination of genotype was reported to be possible via collection of dried blood spots (DBS) [89]. Use of the DBS technique may improve uptake of testing and may simplify and expand the monitoring of hepatitis C. This has already been shown to be effective among PWID in non-clinical settings in the UK [90,91] and was considered cost-effective [92]. Among MSM, DBS and/or point-of-care could be used for testing at home, or even at gay venues (e.g., saunas, clubs). This way, thresholds for HCV testing can be lowered and case-finding may be increased. Future studies should investigate the potential of DBS/point-of-care tests for use among HIV-infected MSM. Cost-effectiveness should be investigated as well, as outreach projects can be costly.
While currently available antiretroviral therapies for treatment of HIV infection are well tolerated, a reduction in the number of contact visits among people with HIV infection would mean that patients are seen less than twice yearly. A decrease in the number of clinical visits by HIV-infected MSM also leads to a decrease in the sensitivity and specificity to detect HCV infection by using the relatively cheap ALT test. When used infrequently, the ALT test is not suitable as a monitoring tool, as has also been shown in chapter 9 of this thesis [85]. Frequent testing remains necessary to be able to diagnose patients early after (re-)infection with HCV. However, opinions on how often to test differ, while concise data or models to make an informed decision are lacking. Future research on this topic is therefore urgently needed to improve testing protocols for MSM.

HCV reinfection among MSM
Few studies so far reported on the incidence of HCV reinfection among HIV-infected MSM. Results from the MOSAIC study [32] and a research group from the UK [93] showed high reinfection rates following SVR of 15.2 and 9.6 per 100 PY respectively; more than 20 times higher than the incidence of primary HCV infection [38]. The rate of HCV reinfection was lower among men that spontaneously cleared a primary infection: 4.2 per 100 PY [93]. Anti-HCV testing is not suitable for detection of HCV reinfection, although we found a clear decrease in serum anti-HCV levels among MSM that were successfully treated for primary or reinfection with HCV. In some cases this resulted in seroreversion (i.e., a complete loss of anti-HCV), followed by an increase in anti-HCV after reinfection [79]. Reinfection occurred up to three times during follow-up, and sometimes shortly after completing HCV treatment of the previous infection. HCV-RNA testing is however more suitable for diagnosis of HCV reinfection. The frequency of testing for HCV reinfection is likely to influence the number of reinfections that are diagnosed, as model estimates among PWID suggested [94]. A recently published paper that reported results of DAA treatment among HIV/HCV-coinfected MSM with acute HCV infection in the Netherlands showed that as much as 25/99 (25.3%) men with acute HCV genotype 1 infection were treated for a reinfection [34]. The overall burden of HCV is likely to remain high if the incidence of primary HCV infection does not decline (as discussed in chapter 3 of this thesis), and the incidence of HCV reinfection is not reduced. Increased efforts to improve case finding and subsequent treatment of those with acute or chronic HCV infection are needed to bring the HCV epidemic to a standstill. In addition, behavioral interventions may contribute to a reduction of the risk of HCV reinfection.

Molecular epidemiology of HCV
Multiple studies in this thesis combined the fields of epidemiology and phylogeny. The combination of both disciplines has led to numerous new insights in studies investigating bacterial and viral infections. In this thesis two attempts were made to discover HCV transmission networks among HIV-infected MSM (chapters 4 and 6), but we found no convincing evidence that specific transmission networks with increased risk for HCV existed within the MSM
population; neither by using MSM subculture identity [50, chapter 4], nor by examining one’s HIV phylogenetic profile (chapter 6). In the latter study we investigated to what extent the topologies of HIV and HCV phylogenetic trees of HIV/HCV-coinfected MSM overlapped. This has been done in very few studies before, most likely as it requires the availability of a large number of sequences for analyses [95,96]. We performed this study combining data from HCV-infected MSM enrolled in the MOSAIC study with nationally collected data on HIV-infected individuals (the ATHENA cohort). A large number of persisting HIV transmission networks have been identified in the Netherlands [97]. The majority of the >100 separate transmission networks consisted mainly of MSM and some of these networks originated already early in the HIV epidemic and are still ongoing. Particularly the newer clusters were found to have higher HIV reproduction numbers [97]. However, MSM with evidence of HCV infection were not confined to specific, hypothetically “high-risk”, transmission networks. It might be that the time between HIV diagnosis and HCV infection was too long (the median duration was 3.3 years in our study) to observe overlap among the transmission networks of both epidemics. However, the time from HIV diagnosis to HCV infection decreased over calendar time in our study. As a shorter time between both infections increases the likelihood that one acquires both infections within the same transmission network, future studies may find overlap between the topologies of HIV and HCV phylogenies. If this decrease in duration between HIV and HCV infection persists, increased education about HCV infection and prevention measures should also target HIV-negative MSM, especially those at increased risk of contracting HIV.

The level of resolution in phylogenetic analysis is determined by the gene of choice, and is preferably based on the type or state of the epidemic. The highly variable E2 gene gives sufficient phylogenetic signal when diversification is limited, as is the case in the ‘young’ HCV epidemic among HIV-infected MSM [22,98]. In a more endemic state, such as among PWID in western Europe, less variable genes like Core or NS5B suffice to discriminate between relapse and reinfection, because the strains that circulate are more phylogenetically distinct [18]. A recent publication argued that sequence analysis of the Core-E2 region, a fragment covering 1,350bp, would be the most suitable for cluster analysis of HCV [99]. Finally, and although probably not needed for the majority of reinfections, next-generation sequencing of E2 sequences (or even full genome sequences) may be used to distinguish between relapse and reinfection by considering the possibility that a minority strain was already present during primary HCV infection and expanded during or after antiviral treatment [100–102]. This would especially be of interest when the period of viral suppression is short (or absent). However, study from our group demonstrated that multiple HCV strains rarely co-exist for longer periods of time, probably due to competition and/or superinfection exclusion [98]. Use of molecular epidemiological studies will remain important to see whether new HCV networks arise, and existing networks change. The recently initiated typing network Netherlands, known as Type-Ned, aims to improve monitoring of infectious diseases on a nation-wide level [103]. Currently, national surveillance databases exist for enterovirus, parechovirus and norovirus, and for the
methicillin-resistant *Staphylococcus aureus* (MRSA) bacterium. It would be recommendable to add hepatitis viruses, especially HCV, to this list.

**Treatment of HCV infection**

Peg-interferon and ribavirin are likely to become obsolete drugs in the treatment of HCV infection because other, shorter, and better tolerated therapies consisting of direct acting antivirals (DAAs) are increasingly becoming available. For the short term, all-oral DAA regimens have excellent efficacy and safety profiles, albeit these agents are still relatively expensive. Even HCV treatment of the historically ‘difficult-to-treat’ HIV-coinfected population seems to be as successful as in the HCV mono-infected population [104,105]. Treatment as prevention (TasP) has shown to potentially contain and slow the spread of HIV, apart from primary prevention measures [106]. In turn, HCV TasP may also be considered as an approach to reduce the number of new HCV infections among HIV-infected MSM, depending on the prevalence and uptake. Model projections by two research groups showed that a widespread implementation of HCV TasP, combined with integrated HCV testing, counselling and care has the potential to dramatically decrease HCV prevalence among PWID in Vancouver (Canada), Melbourne (Australia), Edinburgh (Scotland) [107], and in France [108]. However, the cost of DAA treatment is a major barrier in expanding treatment to the needed coverage. A recent study evaluated the possibility of HCV TasP as a strategy among MSM in the UK [109]. Model projections showed that if in the coming years 80% of MSM would be treated within a year of acquiring HCV infection, and 20% of those with chronic infection would be treated each year, HCV incidence may decline to less than 5 per 1,000 PY and prevalence would be reduced to less than 3% by 2025. In line with these findings, a recent modeling study from Switzerland suggested that if the levels of high risk sexual behavior among HIV-infected MSM do not increase further, treatment uptake and efficacy of DAAs will be able to significantly decrease the incidence of HCV infection over the next decade [110]. Observational studies are needed to measure the effect of TasP in the real world.

**HCV elimination**

Elimination of an infectious disease (i.e., reduction to zero of the incidence in a defined geographical area as a result of deliberate efforts) and eradication (i.e., a permanent reduction to zero of the worldwide incidence of infection) are the ultimate goals of infectious diseases control [111]. Eradication has been accomplished only once before: the last case of smallpox was seen in Somalia in 1977 [112] and therefore prevention efforts are no longer necessary. Another infectious disease that is now closer than ever to eradication is poliomyelitis (polio), for which a great worldwide effort has led to a 99% decrease; poliovirus type 2 has been declared eradicated in September 2015 after its last detection in 1999, and infections with type 3 have not been seen since 2012. Up to September 2015, Afghanistan and Pakistan were the only countries that reported active cases of polio in that year [113]. However, a major difference between smallpox, polio, and HCV is the availability of a vaccine. For HCV, no prophylactic...
vaccine currently exists. Despite the availability of successful treatment, a vaccine is likely to be essential in eradication of HCV [114,115]. The search for an effective vaccine should therefore continue. Advances made in molecular vaccinology will enable to increase progress in the coming years [116]. Until then, prevention measures and treatment will form the larger part of the control of infection and disease. In resource-limited geographical areas, HCV treatment and especially the expensive DAA therapeutics, are not yet widely available. The western world should first focus on reaching a controllable state in which incidence, prevalence, morbidity and mortality are at a locally acceptable (low) level. Vigilance is important in maintaining this state. Eventually, elimination of HCV may be the next target to aim for.
REFERENCES


APPENDIX

SUMMARY
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SUMMARY

“EPIDEMIOLOGY AND DIAGNOSIS OF ACUTE HEPATITIS C VIRUS INFECTION”

This thesis focuses on acute HCV infection among risk groups in the Netherlands and consists of three main subjects: epidemiology, risk factors for sexual transmission and diagnosis of acute HCV infection. Chapter 1 is an introduction on the topics addressed in this thesis, and provides an overview of the history, and epidemiological and virological aspects of HCV infection. Recent incidence estimates among people who inject drugs (PWID) and men who have sex with men (MSM) are discussed in part I; part II addresses risk factors for sexual transmission of HCV; natural history, and tools for diagnosis of acute infection are discussed in part III of this thesis.

Chapter 2 focuses on the incidence of recurrent HCV infection among PWID successfully treated for chronic HCV infection in a multidisciplinary setting. An earlier study (the DUTCH-C project) showed that HCV testing and treatment uptake and response among HCV mono-infected PWID was high. With this follow-up study we showed that during a median follow-up of 2.5 years following successful treatment, the incidence of HCV reinfection was low at 0.8/100 person years of follow-up. HCV treatment should therefore be accessible for active drug users, including PWID. In chapter 3, the incidence of primary HCV infection was estimated among MSM between 1984 and 2012, based on serological evidence. No HCV seroconversions were documented among HIV-uninfected MSM. Among HIV-infected MSM, the incidence of primary HCV increased significantly between 2000 and 2005. After 2005, the incidence appears to have stabilized at around 1.2/100 person years of follow-up. The HCV incidence was highest among MSM that were 35 years of age.

In chapter 4, results from the baseline questionnaires of the MOSAIC study are presented. Risk factors for HCV infection were compared between HIV-infected MSM with acute HCV infection (cases) and HIV-infected MSM without HCV infection (controls). Receptive unprotected anal intercourse, sharing sex toys, unprotected fisting, injecting drugs, sharing straws when snorting drugs, lower CD4 cell count, and recent diagnosis of ulcerative sexually transmitted infection had significant effects on acquisition of HCV infection. Chapter 5 presents data from the MSM network study, in which we investigated whether HCV circulated in identifiable high-risk MSM subcultures. Seropositivity was associated with leather, rubber/lycra, and jeans subcultures. Phylogenetic analysis did not reveal separate epidemiological transmission networks. In chapter 6 we investigated whether HCV transmission among HIV-infected MSM is restricted to specific HIV transmission networks, which would suggest that a core group of high-risk MSM is driving the ongoing transmission of both HIV and HCV. However, using phylogenetic methods of both HIV and HCV, we did not find evidence that HCV predominately circulates among specific HIV transmission clusters, which suggests that
HIV/HCV-coinfected MSM are not the sole drivers of the current HIV-epidemic.

**Chapter 7** evaluates longitudinal anti-HCV measurements among MSM with acute HCV infection. The median time from HCV infection to seroconversion interval was 74 days. Furthermore, we found that loss of antibodies, i.e., ‘seroreversion’, is quite common among HIV-infected MSM that were treated for HCV infection (cumulative incidence: 37% within 3 years after seroconversion). Moreover, while this assay is commonly used as a qualitative test, partial seroreversion was present among all that cleared a previous infection (including reinfection). In **chapter 8**, an HIV-infected MSM was found to have a long lasting HCV infection without developing IgG antibodies, and no signs of hepatic inflammation (indicated by elevated ALT concentrations), stressing the need to use RNA testing when suspicion of an HCV infection exists. In **chapter 9**, an assay that targets the HCV core protein was evaluated as a screening tool for diagnosis of HCV infection at a large STI clinic in Amsterdam. Despite a somewhat lower sensitivity compared to commercial RNA assays, all acute and chronic HCV infections were detected. Addition of ALT testing to standard (anti-HCV) screening did not improve sensitivity.

An overview of the results of the studies included in this thesis and how this work adds to current knowledge is given in **chapter 10**. Also, recently published literature and new developments in the field are discussed in order to give recommendations for future directions for research and prevention.
Het hepatitis C virus (HCV) is in 1989 ontdekt als de veroorzaker van leverontsteking (Lat. hepatitis) die niet veroorzaakt werd door hepatitis A of B, of andere (toen) bekende virussen. Hepatitis kan ontstaan door hepatitis virussen, maar ook door bijvoorbeeld druggebruik, alcoholmisbruik, of andere virale infecties zoals het Epstein-Barr virus of het gele koorts virus. Twee a drie procent van de wereldbevolking is geïnfecteerd met HCV. Dit komt neer op 169-202 miljoen mensen. Nederland heeft een relatief lage prevalentie: de meest recente schatting is dat er ongeveer 0.2% van de Nederlandse bevolking geïnfecteerd is met HCV, wat overeenkomt met 28.000 mensen. HCV infectie komt in Nederland het meest voor bij migranten uit endemische landen (d.w.z. landen waar een HCV relatief veel voorkomt), injectorende druggebruikers, en mannen die seks hebben met mannen (MSM). Bij de laatste groep komt HCV infectie vooral voor bij mannen die éérst geïnfecteerd zijn met het humaan immuundeficiëntie virus (hiv). Bij hiv-negatieve MSM lijkt HCV infectie (vooralsnog) niet méér voor te komen dan bij de algemene populatie.

De overdracht van HCV verloopt het meest efficiënt via bloed-bloed contact. Voordat het virus bekend was en er op getest kon worden, konden daarom bijvoorbeeld bij bloedtransfusies mensen geïnfecteerd raken. Een tragisch voorbeeld is de vaccinatie campagne tegen bilharzia (een infectie veroorzaakt door de parasiet Schistosoma mansoni) in Egypte die tot de jaren ’80 duurde. Hierbij werden veel mensen geïnfecteerd met HCV vanwege hergebruik van naalden tijdens de vaccinatie. Als gevolg hiervan zijn er gebieden in Egypte waar meer dan 20% van de bevolking geïnfecteerd is met HCV.

In de jaren ’60 nam in Nederland het (injecterend) druggebruik toe. Door het delen van naalden en andere spuitattributen circuleerde HCV binnen deze groep. Het percentage injectorend druggebruikers dat (ooit) een HCV infectie had gehad was ongeveer 85% in 1985. Wereldwijd zijn injectorende druggebruikers de grootste risicogroep. In Nederland neemt het injectorend druggebruik en risicogedrag echter al jaren af, waardoor de huidige HCV incidentie (het aantal nieuwe infecties per tijdseenheid) fors is gedaald.

Sinds het jaar 2000 is door verschillende landen een toename van HCV infectie gezien bij hiv-geïnfecteerde MSM. HCV kan dus seksueel overgedragen worden, iets wat nauwelijks voorkwam in studies bij heteroseksuele koppels waarvan één van de partners HCV-positief was. Vrijwel alle studies die volgden toonden aan dat een HCV infectie geassocieerd is met het hebben van onbeschermde anale seks, vuistseks (fisting), groepsseks en het gebruik van seksspeeltjes. Ook het gebruik van harddrugs en het hebben gehad van één of meer (andere) seksueel overdraagbare aandoeningen (SOA) zijn markers voor een verhoogde
kans op het hebben van een HCV infectie. De toename in de incidentie van HCV infectie bij hiv-geïnfecteerde MSM hangt waarschijnlijk samen met de introductie van succesvolle hiv-medicatie in 1996. Er is sinds deze introductie ook een toename gezien in risicogedrag, SOA en hiv infecties bij MSM.

HCV infectie kan gediagnosticeerd worden met behulp van verschillende testen. Meestal wordt getest of er antistoffen tegen HCV aanwezig zijn in het bloed. Om bij een positief testresultaat vast te stellen of het om een chronische infectie gaat, wordt een HCV RNA test gedaan. Als er geen HCV RNA aanwezig is in het bloed van een persoon die nooit behandeld is voor HCV is de infectie hoogstwaarschijnlijk door de persoon zelf geklaard (dit komt in ongeveer 25% van de gevallen voor). Het kan ook zo zijn dat er wel HCV RNA aanwezig is, maar geen antistoffen tegen HCV. In dat geval is er waarschijnlijk sprake van een acute of recente infectie. De duur van het ontwikkelen van HCV antistoffen (seroconversie) verschilt per individu, en duurt ongeveer 10 weken. Er zijn echter ook uitschieters tot een jaar, of langer. Een andere test die gebruikt wordt om HCV infectie vast te stellen is de HCV antigeen test. Deze kan de aanwezigheid van bepaalde eiwitten van het virus zelf aantonen. Bij mensen die onder behandeling zijn voor een hiv infectie wordt regelmatig de concentratie van het enzym alanine-aminotransferase (ALT) gemeten in het bloed. Wanneer de ALT concentratie verhoogd is, kan dit indicatief zijn voor een ontsteking van de lever, mogelijk veroorzaakt door HCV.

De meerderheid (>70%) van de personen die een infectie oplopen rapporteert geen symptomen. Dit maakt het erg lastig om de ziekte vroegtijdig op te sporen. Wanneer een HCV infectie onbehandeld blijft, kan in 10-20% van de personen met een chronische infectie en over een periode van 20-30 jaar, fibrose van de lever uiteindelijk leiden tot cirrose. De structuur van de lever is dan zodanig veranderd dat deze zijn functie niet meer goed kan uitoefenen. Bovendien is de kans op leverdecompensatie en leverkanker verhoogd bij een levercirrose: respectievelijk 3-6% en 1-5% per jaar.

Er bestaat geen vaccin tegen HCV infectie. Een HCV infectie is wel goed te behandelen. Tot voor kort werd een HCV infectie behandeld met een wekelijkse injectie gepegyleerd interferon en dagelijks ribavirine. De duur van de behandeling en het percentage dat succesvol behandeld werd, was afhankelijk van o.a. het HCV genotype (de behandeling van genotype 1 en 4 duurt 24 tot 48 weken, van genotype 2 en 3 duurt deze 12 tot 24 weken) en de duur tussen infectie en start behandeling, en de aanwezigheid van een hiv coinfectie. Een acute infectie is maximaal 6 maanden geleden opgelopen en wordt in de regel korter behandeld dan een chronische infectie. Behandelmethoel die sinds kort beschikbaar zijn (zgn. direct-acting antivirals) hebben een kortere duur (8 tot 12 weken), minder bijwerkingen en een hogere kans van slagen (>90%) in vrijwel alle patiënten met HCV infectie.
Dit proefschrift is ingedeeld in drie delen die betrekking hebben op acute HCV infectie in risicogroepen in Nederland, te weten: de epidemiologie, risicofactoren voor seksuele transmissie en diagnose van HCV infectie.

Deel I

_De incidentie van acute HCV infectie in Nederland_

Hooftstuk 2 bespreekt de incidentie van HCV herinfectie bij druggebruikers die in een multidisciplinaire setting succesvol behandeld zijn voor chronische infectie met HCV. Een eerdere studie (het DUTCH-C project) liet al zien dat de uptake van HCV testen en behandeling hoog was in deze groep. Met deze follow-up studie hebben we laten zien dat gedurende een mediane follow-up van 2,5 jaar na succesvolle behandeling de incidentie van HCV herinfectie laag was, 0,8 per 100 persoonsjaren follow-up. HCV behandeling zou daarom toegankelijk moeten zijn voor actieve (en ook injectorende) druggebruikers. In hoofdstuk 3 is de incidentie van primaire HCV infectie geschat bij MSM tussen 1984 en 2012, op basis van serologie (HCV antistoffen). Bij hiv-negatieve MSM werden geen HCV seroconversies gedocumenteerd. Bij hiv-geïnfecteerde MSM nam de incidentie van primaire HCV significant toe in de periode 2000-2005. Na 2005 leek de incidentie te stabiliseren rond de 1.2 per 100 persoonsjaren follow-up. MSM van rond de 35 jaar hadden de hoogste incidentie.

Deel II

_Risicofactoren voor seksuele transmissie van HCV_

In hoofdstuk 4 zijn resultaten van de inclusievragenlijsten van de MOSAIC studie gepresenteerd. Risicofactoren voor HCV infectie werden vergeleken tussen hiv-geïnfecteerde MSM met acute HCV infectie (cases) en hiv-geïnfecteerde MSM zonder HCV (controles). Het hebben van onbeschermde receptieve anale seks, delen van seksspeeltjes, onbeschermd vuistneuken (fisten), het injectoren van drugs, een lagere concentratie CD4 cellen in het bloed, en een recente diagnose met een ulceratieve seksueel overdraagbare aandoening zoals bijv. syfilis of LGV, hadden een significant effect op het oplopen van HCV infectie. In hoofdstuk 5 wordt data van de MSM netwerksstudie gepresenteerd, waarin werd bestudeerd of HCV circuleerde in identificeerbare hoog-risico MSM subcultures. Anti-HCV positief zijn was geassocieerd met een zelf gerapporteerde affiniteit met de leer, rubber/lycra, en/of jeans subcultures. Analyse van gedeeltelijke HCV sequenties maakte duidelijk dat er geen afzonderlijke epidemiologische transmissienetwerken bestonden. In hoofdstuk 6 hebben we onderzocht of HCV transmissie bij hiv-geïnfecteerde MSM zich beperkt tot specifieke hiv transmissienetwerken. Indien dit het geval is, zou dat suggereren dat de aanhoudende transmissie van hiv en HCV wordt gevoed door een groep MSM met verhoogd risico op infectie. Echter, na analyse van zowel hiv als HCV sequenties hebben we geen bewijs gevonden voor transmissie van HCV binnen specifieke hiv transmissieclusters. Dit suggereert dat MSM met hiv en HCV infectie niet de enige bijdragers zijn aan de huidige hiv epidemie.
Deel III

*Diagnose van acute HCV infectie bij hiv-geïnfecteerde MSM*

**Hoofdstuk 7** bespreekt longitudinale anti-HCV metingen bij hiv-geïnfecteerde MSM met een acute HCV infectie. Het mediane interval tussen infectie en seroconversie werd geschat op 74 dagen. Ook vonden we dat het verliezen van antistoffen tegen HCV (zgn. seroreversie), relatief vaak voorkwam bij hiv-geïnfecteerde MSM die succesvol behandeld waren voor hun HCV infectie. Bovendien bleek dat ondanks het feit dat deze assay voornamelijk gebruikt wordt als een kwalitatieve test, ook gedeeltelijke seroreversie zichtbaar was bij alle MSM die een eerdere (her-)infectie hadden geklaard.

In **hoofdstuk 8** wordt een casus besproken van een hiv-geïnfecteerde MSM die een langdurige HCV infectie bleek te hebben, zonder dat hij ooit anti-HCV positief was getest. Bovendien waren er in die tijd ook de ALT concentraties, een indicatie voor het testen op HCV, nooit verhoogd. In **hoofdstuk 9** werd een assay geëvalueerd die de concentratie van HCV core antigeen meet in het bloed. Ondanks dat de sensitiviteit (dit is een maat voor de gevoeligheid van een test) van deze test enigszins lager is dan die van commerciële RNA assays, werden alle acute en chronische HCV infectie gedetecteerd in deze studie. Deze assay zou daarom een bruikbare toevoeging zijn aan het beschikbare pakket screeningsmethoden. Het toevoegen van ALT testen aan de standaard (anti-HCV) screening verbeterde de sensitiviteit van deze screeningsmethode niet.

In **hoofdstuk 10** worden de bevindingen in dit proefschrift besproken in het licht van recente literatuur en ontwikkelingen in het veld. Ook worden aanbevelingen gegeven voor toekomstig onderzoek en preventie.
Het afronden van dit proefschrift was voor mij de stip die ik ongeveer vijf jaar geleden op de horizon heb gezet. Dat die dag nu aangebroken is, maakt mij gelukkig en trots. Gedurende mijn promotietraject heb ik bij de GGD Amsterdam en het AMC gewerkt. Op beide plekken heb ik met veel enthousiaste en gedreven collega’s samengewerkt. Zonder hen was dit proefschrift er zeker niet geweest. Wat heb ik veel geleerd en ondertussen een leuke tijd gehad!

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Ook wil ik mijn copromotoren bedanken. Sylvia, bedankt voor je intensieve begeleiding, met name gedurende het eerste jaar. Het heeft de basis gevormd voor mijn verdere promotietraject. Je reactie via mail was altijd snel en adequaat. Janke, bedankt voor de prettige en productieve samenwerking. Na een kop koffie met jou op het voetenplein kon ik altijd weer (minstens) een week vooruit. Bedankt voor al je advies en sturing.


Thijs, als ik drie copromotoren had gehad, was jij de derde geweest. Waar ik eerst nog voorgesteld werd als ‘de nieuwe Thijs’, hadden we steeds vaker contact en uiteindelijk heb je bijgedragen aan maar liefst de helft (!) van de studies in dit proefschrift. Bedankt voor je hulp, inzet en feedback.

Dank aan alle medewerkers dankzij wie de MOSAIC studie loopt in de volgende centra: het AMC (Jan, Marc, Hans-Erik, Michelle), het OLVG (Kees, David, Narda, Angelique), het Slotervaartziekenhuis (Fanny, Jan-Willem, Marjolein), het UMC Utrecht (Joop, Inge) en het Erasmus MC (Bart, Ineke, Anne). Bas, jij bedankt voor het zo succesvol uitrollen van de MOSAIC studie in 010. Veel succes met de afronding van jouw proefschrift en hopelijk zien we elkaar nog eens in de WdW! Luuk, Ard, Colette, dank voor de succesvolle samenwerking met de SHM. Daniela, jij bedankt voor de spontane overleggen op de HvA (en de lekkere koffie).

Dank aan alle collega’s van het streeklaboratorium van de GGD Amsterdam: Reiner, Nadia, Raissa, Deeqa, Debby, Sahare, Meriem, Mirjam, Esther, René, Monique, Martine, Caspar, Gerard, Douwe, de lijst is eindeloos (en bovendien niet compleet). Dank voor de gezellige 11-uurtjes, de tafels vol met lekkers rond kerst, en de leuke borrels met uiteindelijk altijd weer
die waterpijp (?). Arjen (wat was het koud bij Frigo), Paul, Ineke, Maarten, Alje, dank voor jullie input en discussies tijdens de presentaties van het streeklaboratorium. Simei, dank voor het werk dat je tijdens je stage op het streeklaboratorium hebt verricht.

Dank aan mijn collega’s van de afdeling infectieziekten onderzoek van de GGD Amsterdam: Dani (I.C.A., P.I.M.P.), Martijn (de zon is jouw vriend!), Christiaan (uitjes en zuur?), Amy, Astrid, Janneke, Jannie, Rosa, Nienke, Carolien, Roel, Elske, Camiel, Freke, Anouk, Linda, Gerben Rienk, Rik, Bart-Jan, Marjolein, Marc. Bart, wat was het leuk om met jou (naast de mannenkamer) een kamer op congressen in Brussel en Shanghai te delen. Wijnand (jih!) en Titia, jullie zijn er (ook) bijna. Succes nog even. Femke, speciale dank aan jou voor de samenwerking aan twee hoofdstukken van mijn (en ongetwijfeld ook jouw) proefschrift. Ik kom nog een keer verse eitjes rapen bij je. Udi, Maarten, Ineke, Ronald, Henry, bedankt voor de discussies die we gehad hebben tijdens de OTO overleggen, als ik binnenwandde, of tijdens de lunch. Will en Nora, bedankt voor alle ondersteuning en vooral ook voor het vinden van gaatjes in de agenda van Maria.

Dan mijn collega’s op het AMC. Sylvie, dank voor het vele sequensen en wat hebben we gelachen. Xiomara, je naam zal voor altijd verbonden zijn met de HCV research op het AMC nu er een ‘Xio-ID’ is opgenomen in de database. Sjoerd, Cynthia, Gaby, Sabrina, Tim, Richard, dank voor de discussies (en bijles in de immunologie) tijdens de HCV werkbesprekingen. Brenda, er was altijd wel een reden om even op en neer te lopen naar het voetenplein (bijv. geen zin?). Ook Nienke, Lonneke, Sabine, dank voor de gezellige werkplek. Margreet, Jos, bedankt voor de logistieke afhandeling van de vele HCV-testen die ik aanvroeg.

A word of thanks to my colleagues from overseas. Dear Daniel, thank you for the discussions about (sexual) transmission of HCV (either live or through WhatsApp), and of course the dungeness crab. Dear Margaret, thank you for your Melbourne travel tips, I hope we can add a sequel to my 2011 trip (maybe this time we will go and see the penguins). Joe, I’ve enjoyed our discussions, over drinks or over lunch, during conferences around the globe. Hope to see you again. Jason, together with Bart we had a blast in Shanghai (that cocktail however, was awful). I hope we will enjoy some hot pot again in the future.

Omdat ontspanning minstens zo belangrijk is als inspanning wil ik ook mijn familie en vrienden bedanken voor de tijd die overbleef naast het werken. Zo is elk optreden met mijn soul/R&B/rock ‘n roll band ‘the Willies’ een waar genot (maar dat is dan weer ontspanning door inspanning; we zijn ook te boeken voor promotiefeesten trouwens). Kaarten op de maandagavond met kaartclub ‘hoei’n bin de beste’, ik hoop dat we het nog lang mogen blijven doen (tot in het bejaardenhuis aan toe!). Sabrina, bedankt voor het ontwerpen van de prachtige omslag en de leuke dagen die we hebben gehad als gevolg van je enthousiaste reactie toen ik je vroeg. Wendy, onzettend bedankt voor al je inspanningen, opbeurende gesprekken en berichtjes,
lunches, borrels en koffiemomentjes. Bedankt dat je mijn paranimf wil zijn op deze bijzondere dag.

Martijn, opgewekt broertje, ik hoop dat jij ook een leuke promotieplek gaat vinden. Bedankt dat je mijn paranimf wil zijn. Je moet dan wel best lang (i.e., drie kwartier) je mond houden hè (#sahweh).

Pap, mam, bedankt voor alles wat jullie me geleerd hebben en de vrijheid die jullie me altijd hebben gegeven. Bart, broeder, en Ilse, wat een leuke neefjes heb ik dankzij jullie. Ome Joost komt snel weer op de ‘koggie’.

Hans en Carla, Nienke en Roy, dank voor jullie warmte en gezelligheid. Een fijnere schoonfamilie kan ik me niet wensen. En hoe leuk is het dat we weer een reden hebben voor bubbels?

De laatste zinnen zijn voor jou, lieve Sanne. Jij, die me zo goed aanvoelt, die het beste in me naar boven haalt en die er af en toe voor zorgt dat ik weer weet waar het écht om draait in het leven. Dank voor je begrip en steun tijdens mijn promotie. Nu telt alleen nu!
ABOUT THE AUTHOR

Joost Vanhommerig was born on December 23rd, 1985 in Goes. There he grew up and completed VWO secondary school at the Sint Willibrordcollege in 2003. He then moved to Maastricht to study Health Sciences at Maastricht University and received his Bachelor’s degree in 2007. To obtain his Master’s degree, he continued to study nutrition and metabolism at Maastricht University. During his internship at the department of Human Biology at the Nutrition and Toxicology Research Institute, the existence of brown adipose tissue was demonstrated during cold exposure among >95% of the lean men in this study. This led to his first publication as co-author, in the renowned New England Journal of Medicine. After finishing this research master in 2008, he moved to Amsterdam to study infectious diseases and public health at the VU University. During this study he performed an internship under supervision of the KNCV Tuberculosis Foundation, investigating the incidence of tuberculosis infection among people living with HIV in Tembisa, a township near Johannesburg, South Africa. This internship was performed in collaboration with the Aurum Institute in Johannesburg, South Africa. Following this internship, he was determined to pursue a career in infectious diseases control. He received his Master’s degree in 2009, and started his PhD research in 2011 at the department of Infectious Diseases Research and Prevention at the Public Health Service of Amsterdam, and continued in 2013 at the department of Medical Microbiology at the Academic Medical Center in Amsterdam. His research mainly focused on acute hepatitis C virus infection, and combined the fields of virology and epidemiology. The results are presented in this dissertation. During the last year of his PhD research, Joost worked as a data manager for the HELIUS study: a large multi-ethnic population-based cohort study in Amsterdam to unravel the impact of ethnicity on communicable and non-communicable diseases, in particular cardiovascular diseases, infectious diseases, and mental health.
LIST OF PUBLICATIONS


<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation(s)</th>
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<tbody>
<tr>
<td>Dr. J.E. Arends</td>
<td>Department of Internal Medicine and Infectious Diseases, University Medical Center, Utrecht</td>
</tr>
<tr>
<td>Dr. D.O. Bezemer</td>
<td>Stichting HIV Monitoring, Amsterdam</td>
</tr>
<tr>
<td>Prof. dr. K. Brinkman</td>
<td>Department of Internal Medicine, OLVG Hospital, Amsterdam</td>
</tr>
<tr>
<td>Dr. S.M. Bruisten</td>
<td>Public Health Laboratory, Public Health Service of Amsterdam, Amsterdam</td>
</tr>
<tr>
<td>Dr. R.B. Geskus</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam; Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam</td>
</tr>
<tr>
<td>Dr. B.P.X. Grady</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam</td>
</tr>
<tr>
<td>Drs. L.A.J. Gras</td>
<td>Stichting HIV Monitoring, Amsterdam</td>
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<tr>
<td>Dr. M. Koot</td>
<td>Department of Blood-Borne Infections, Sanquin Blood Supply Foundation, Amsterdam</td>
</tr>
<tr>
<td>Dr. D. Kwa</td>
<td>Department of Medical Microbiology, OLVG Hospital, Amsterdam</td>
</tr>
<tr>
<td>Dr. T.J.W. van de Laar</td>
<td>Department of Blood-Borne Infections, Sanquin Blood Supply Foundation, Amsterdam</td>
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<tr>
<td>Drs. F.A.E. Lambers</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam</td>
</tr>
<tr>
<td>Dr. F.N. Lauw</td>
<td>Department of Internal Medicine, Slotervaart Hospital, Amsterdam</td>
</tr>
<tr>
<td>Drs. C.E.A. Lindenburg</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam</td>
</tr>
<tr>
<td>Dr. A.A. Matser</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam</td>
</tr>
<tr>
<td>Dr. J.T.M. van der Meer</td>
<td>Department of Internal Medicine, Center for Infectious Diseases and Immunology Amsterdam (CINIMA), Academic Medical Center, Amsterdam</td>
</tr>
<tr>
<td>Dr. R. Molenkamp</td>
<td>Academic Medical Center, Department of Medical Microbiology, Section of Clinical Virology, Amsterdam</td>
</tr>
<tr>
<td>Drs. A.M. Newsum</td>
<td>Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam; Center for Infection and Immunology Amsterdam (CINIMA), Academic Medical Center, Amsterdam</td>
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<tr>
<td>Prof. dr. J.M. Prins</td>
<td>Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center, Amsterdam</td>
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</table>
Prof. dr. M. Prins Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam; Center for Infection and Immunology Amsterdam (CINIMA), Academic Medical Center, Amsterdam

Dr. B.J.A. Rijnders Department of Internal Medicine and Infectious Diseases, Erasmus University Medical Center, Rotterdam

Drs. M.S. van Rooijen STI clinic, Public Health Service of Amsterdam, Amsterdam

Dr. M.F. Schim van der Loeff Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam

Dr. C.J. Schinkel Department of Medical Microbiology, Section of Clinical Virology, Academic Medical Center, Amsterdam

Dr. A.I. van Sighem Stichting HIV Monitoring, Amsterdam

Dr. C. Smit Stichting HIV Monitoring, Amsterdam

Dr. A.G.C.L. Speksnijder Public Health Laboratory, Public Health Service of Amsterdam, Amsterdam

Dr. I.G. Stolte Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam

Dr. X.V. Thomas Academic Medical Center, Department of Medical Microbiology, Section of Clinical Virology, Amsterdam

Dr. M. van der Valk Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center, Amsterdam; Center for Infection and Immunology Amsterdam (CINIMA), Academic Medical Center, Amsterdam

Prof. dr. H.J.C. de Vries STI clinic, Public Health Service of Amsterdam, Amsterdam; Department of Dermatology, Academic Medical Center, Amsterdam; Centre for Infectious Disease Control, National Institute of Public Health and the Environment (RIVM), Bilthoven; Center for Infection and Immunology Amsterdam (CINIMA), Academic Medical Center, Amsterdam

Dr. C.J. Weegink Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam
## CONTRIBUTION OF THE AUTHORS PER ARTICLE

### Chapter 2: Low incidence of reinfection with the hepatitis C virus following treatment in active drug users in Amsterdam

<table>
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<tr>
<th>Name(s)</th>
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<tr>
<td>B.P.X. Grady; J.W. Vanhommerig*</td>
<td>Substantial contributions to the conception and design of the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content and approval of the version to be published. * Corresponding author.</td>
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### Chapter 3: Stabilizing incidence of hepatitis C virus infection among men who have sex with men in Amsterdam

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<td>I.G. Stolte; F.A.E. Lambers; R.B. Geskus; T.J.W. van de Laar; S.M. Bruisten; J. Schinkel</td>
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### Chapter 4: HIV-infected men who have sex with men who identify themselves as belonging to subcultures are at increased risk for hepatitis C infection.

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### APPENDIX

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<tr>
<td>J.W. Vanhommerig; M.F. Schim van der Loeff; R.B. Geskus; H.J.C. de Vries; J.M. Prins</td>
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**Chapter 5: Risk factors for sexual transmission of hepatitis C virus among HIV-infected men who have sex with men: a case-control study**

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**Chapter 6: Limited Overlap in Phylogenies of Hepatitis C Virus (HCV) and HIV-1 Among HIV/HCV-coinfected Men Who Have Sex With Men in the Netherlands**

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<td>D.O. Bezember; A.I. van Sighem; C. Smit; F.N. Lauw; K. Brinkman; J.E. Arends; B.J.A. Rijnders; D. Kwa; A.M. Newsum; S.M. Bruisten; M. Prins; J.T.M. van der Meer</td>
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### Chapter 7: Hepatitis C virus (HCV) antibody dynamics following acute HCV infection and reinfection among HIV-infected men who have sex with men

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<td>X.V. Thomas; J.T.M. van der Meer; R.B. Geskus; S.M. Bruisten; R. Molenkamp; M. Prins</td>
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### Chapter 8: Seven years of chronic HCV infection in an HIV-infected man without detectable antibodies

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### Chapter 9: Evaluation of a hepatitis C virus (HCV) antigen assay for routine HCV screening among men who have sex with men infected with HIV

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<td>T.J.W. van de Laar; M. Koot; M.S. van Rooijen; J. Schinkel; A.G.C.L. Speksnijder; M. Prins; H.J.C. de Vries</td>
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**PHD PORTFOLIO**

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<tr>
<td><strong>General courses</strong></td>
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<tr>
<td>- Practical Biostatistics, AMC, Amsterdam, NL</td>
<td>2011</td>
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<td>- The AMC World of Science, Amsterdam, NL</td>
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<td>- Infectious Diseases, AMC, Amsterdam, NL</td>
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<td>- Career development, AMC, Amsterdam, NL</td>
<td>2014</td>
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<td><strong>Specific courses</strong></td>
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<tr>
<td>- Molecular Typing Methods, University Medical Center, Utrecht, NL</td>
<td>2011</td>
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<tr>
<td>- Advanced Topics in Biostatistics, AMC, Amsterdam, NL</td>
<td>2012</td>
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<td>- Computing in R, AMC, Amsterdam, NL</td>
<td>2012</td>
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<tr>
<td>- NIHES course: repeated measurements, Erasmus MC, Rotterdam, NL</td>
<td>2013</td>
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<tr>
<td><strong>Seminars, workshops, master classes</strong></td>
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<tr>
<td>- Hepatitis C: Future Treatment and Prevention, AMC, Amsterdam, NL</td>
<td>2012</td>
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<td>- HCV Tissue Tropism Seminar, AMC, Amsterdam, NL</td>
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<tr>
<td>- Latest developments around viral hepatitis B and C (4e Lagerhuisdebat), Utrecht, NL</td>
<td>2013</td>
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<tr>
<td>- Triple I Retreat, Kamerik, NL</td>
<td>2013</td>
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<tr>
<td><strong>Oral abstract presentations</strong></td>
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<tr>
<td>- Fall meeting of the Dutch Workgroup Clinical Virology (NWKV), ‘s-Hertogenbosch, NL</td>
<td>2011</td>
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<tr>
<td>- Spring meeting of the Dutch Workgroup Clinical Virology (NWKV), Leiden, NL</td>
<td>2013</td>
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<tr>
<td>- 49th International Liver Conference, European Association for the Study of the Liver (EASL), London, UK</td>
<td>2014</td>
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<tr>
<td>- 8th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, NL</td>
<td>2014</td>
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<tr>
<td>- Mid-winter Meeting of the Dutch Association of HIV-Treating Physicians (NVHB), Rotterdam, NL</td>
<td>2015</td>
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<tr>
<td>- 12th International AIDS Impact Conference, Amsterdam, NL</td>
<td>2015</td>
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<td><strong>Poster abstract presentations</strong></td>
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<tr>
<td>- 14th European Society for Clinical Virology (ESCV) meeting, Funchal, Portugal</td>
<td>2011</td>
</tr>
<tr>
<td>- 6th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, NL</td>
<td>2012</td>
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</tbody>
</table>
(Inter)national conferences visited
- Dutch Annual Virology Symposium (DAVS), Amsterdam, NL 2011-2012
- 2nd International Symposium on Hepatitis Care in Substance Users (INHSU), Brussels, Belgium 2011
- 5th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, NL 2011
- 1st National HCV Symposium, Amsterdam, NL 2013
- National Hepatitis Day, Amsterdam, NL 2014-2015

Member of the organizing committees of
- 7th Annual PhD Student Retreat on Infectious Diseases, Haarlem, NL 2011
- Annual GGD Research Day, Amsterdam, NL 2013
- HCV workshop at the annual STD*HIV*SEX congress, Amsterdam, NL 2013

Coordinating tasks
- The MOSAIC study 2011-2014
- Journal club / peer education at PhD meetings (GGD Amsterdam) 2012-2013

Teaching
Supervising
- Bachelor student (VU University), Simei Go, “Development of a realtime reverse-transcriptase polymerase-chain reaction for the subtyping of (mixed) hepatitis C virus infections” 2013
Lecturing
- Guest lecture during a course in the Health Sciences curriculum at VU University, Amsterdam, NL 2015-2016

Parameters of esteem

Grants awarded
- 48th International Liver Congress (EASL) 2013: Young Investigator (YI) Registration Bursary
- 49th International Liver Congress (EASL) 2014: YI Full Bursary
- 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014: YI Scholarship
- 22nd Conference on Retroviruses and Opportunistic Infections (CROI) 2015: YI Scholarship
- 23rd Conference on Retroviruses and Opportunistic Infections (CROI) 2016: YI Scholarship

Publications/reports about my research
- “Acute hepatitis C virus infection in HIV-infected men who have sex with men: should we change our screening practice?” Editorial by Thomas Reiberger, MD, in Clinical Infectious Diseases 59(12):1694-5. 2014
