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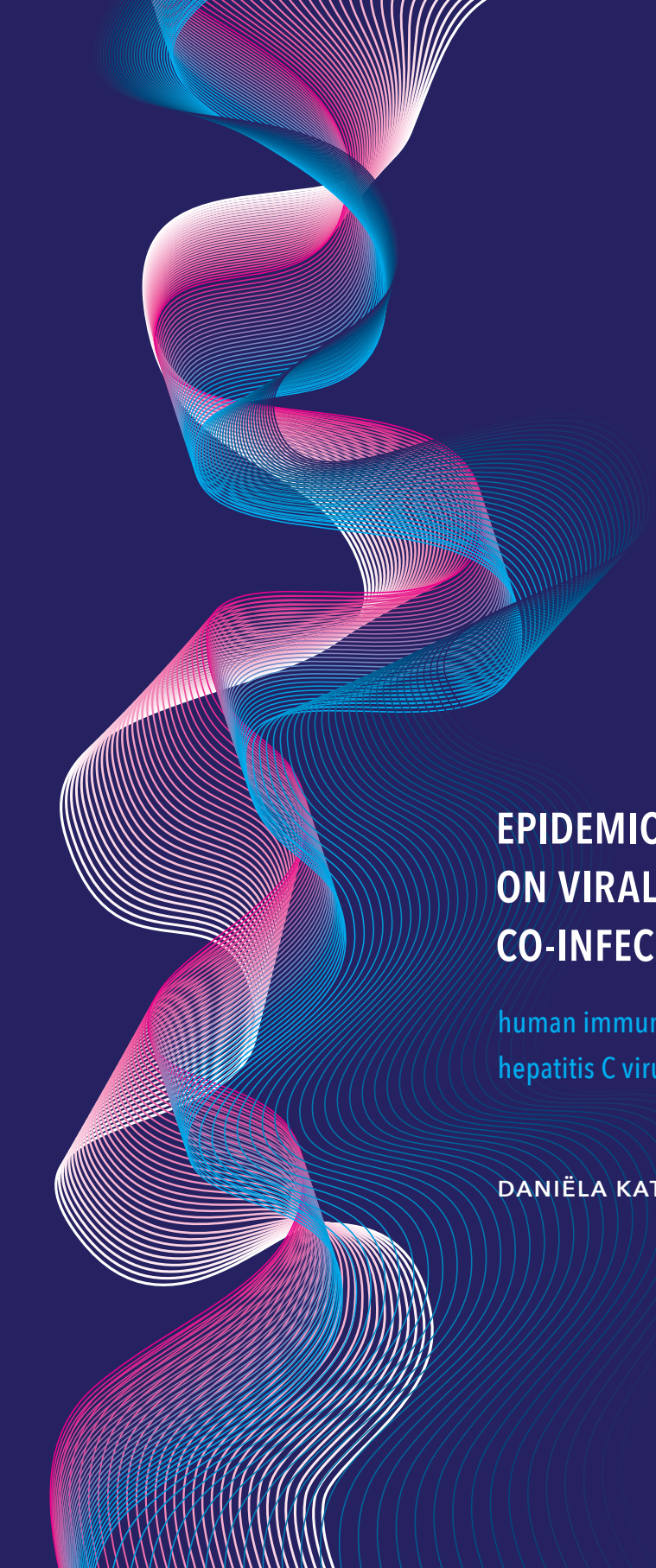

EPIDEMIOLOGICAL STUDIES ON VIRAL INFECTIONS AND CO-INFECTIONS

human immunodeficiency virus, hepatitis C virus and human papillomavirus

DANIËLA KATINKA VAN SANTEN

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aan de Universiteit van Amsterdam
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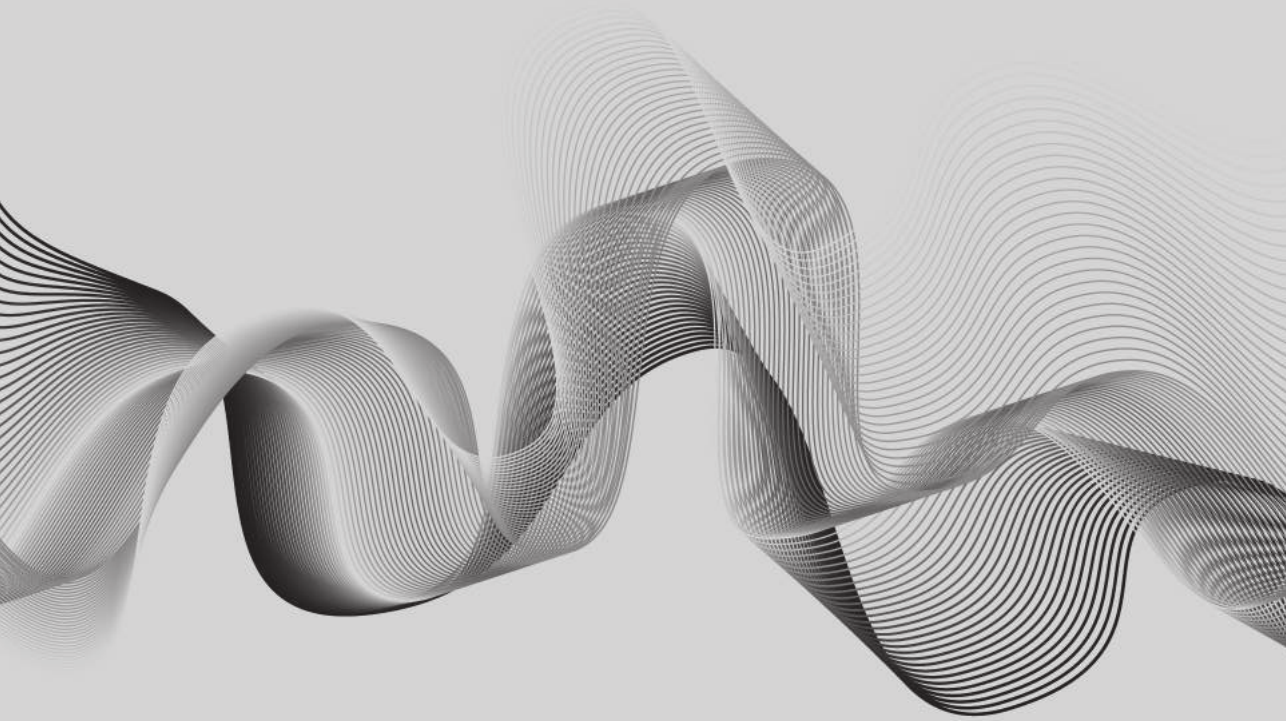
Chapter 1

General Introduction



In this thesis we aimed to increase our understanding of the incidence, disease progression, and treatment of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human papillomavirus (HPV) (co-)infections in key populations. In section 1.1 follows a description of each of the three viral infections studied in this thesis. For each of these viruses a brief description is given of the global and Dutch epidemiological situation, and the disease course of each infection. A brief summary of HIV co-infections epidemiology and how two concurrent infections influence each other is presented as well in section 1.1. Later on, in section 1.2, a brief overview of trends and preventive approaches taken over time related to these viral epidemics are described, focusing mainly on the Dutch epidemics among men who have sex with men (MSM) and people who use hard drugs (PWUD). Drug use was defined as the regular injecting or non-injecting use of predominantly heroin, cocaine, amphetamines and/or methadone. The type of study design used throughout this thesis is introduced in section 1.3. Lastly, the aims of this thesis are outlined in section 1.4.

1.1 HIV, HCV and HPV

1.1.1 Human immunodeficiency virus

HIV is a blood-borne infection that attacks the immune system, specifically CD4 T cells [1]. There are two types of HIV viruses (1 and 2). In this thesis, when we mention HIV, we are referring to an HIV-1 infection. Nowadays, in the majority of cases worldwide, HIV is transmitted through sexual contact, contact with contaminated needles (i.e. injecting drug use or occupationally acquired HIV due to needle stick injury), and mother-to-child transmission [2]. However, the risk to acquire HIV varies per transmission route and type of sexual act [3-5]. It is lowest through female-to-male vaginal sex (0.04%), and highest through blood transfusion with contaminated blood (92.5%) [3-5]. However, as blood products have been screened for HIV shortly after its discovery in most countries, this in no longer a major mode of HIV transmission [2].

The HIV epidemic and main HIV-risk groups vary considerably across geographical regions. In Sub-Saharan Africa, the hardest hit region by the HIV epidemic, women are at highest risk to acquire HIV [6], whereas in high-income countries MSM, people who inject drugs (PWID), and migrants originating from high-endemic countries are the main risk groups [7,8]. In 2016, it was estimated that a total of 36.7 million people (95% uncertainty interval (95%UI): 30.8-42.9 million) were living with HIV worldwide [9]. The global number of new HIV cases was estimated at 1.8 million. In the Netherlands, by the end of 2016, it was estimated that around 19,035 individuals were living with HIV. Based on modelling, the number of new infections in 2016 was estimated at 500 (95% confidence interval (CI): 300-700) in the Netherlands, with the majority of new infections occurring

in MSM (400, 80%) [7]. HIV incidence differs considerably across key populations in the Netherlands in recent years; in the Amsterdam Cohort Studies, incidence was estimated at around 0.5 per 100 person-years (py) in 2016 among MSM, whereas among PWUD, no new HIV infections have been observed since 2012 [7].

To date, neither a cure nor a vaccine exists for HIV, but since 1996, very effective treatment to supress the virus, known as combination antiretroviral therapy (cART), has been available [10]. If HIV is left untreated, it can lead to the development of acquired immunodeficiency syndrome (AIDS) over a median of 10 years following HIV infection [11]. AIDS is a group of diseases occurring at advances stages of HIV infection such as opportunistic infections or HIV-related cancers. Progression to AIDS and survival time varies considerably by age of HIV acquisition, ranging from 7.7 and 7.9 years post-HIV infection in those aged 45-54 years to 11 and 12.5 years post-HIV infection in those aged 15-24, respectively [12]. In 2016 it was estimated that 1 million people died due to HIV-related illnesses worldwide [9]. HIV-related deaths are no longer in the top-10 causes of death worldwide, however, in low-income countries, it was still the 5th highest cause of death in 2015 [13]. All-cause mortality among HIV-positive individuals in care in the Netherlands was estimated at 1.0 (95%CI: 0.8-1.2) per 100 py in 2016 [7]. Nowadays, individuals from high-income countries who acquire HIV through sexual contact and are on cART have similar mortality rates in the first 5 years following HIV seroconversion as the general population, but mortality rates are higher afterwards [14]. Moreover, those initiating cART during primary or early HIV infection, hence at higher CD4 T-cell counts, have a lower chance to die or to develop an HIV-related event compared to those that start later (i.e. at lower CD4 T-cell counts) [15,16]. Since the advent of cART, HIV-positive individuals are living longer, but now HIV has been associated with markers of ageing [17], and increased risk of non-HIV related morbidity and mortality when compared to HIV-uninfected individuals [18].

1.1.2 Hepatitis C virus

HCV is a blood-borne infection with human hepatocytes being the main target for infection [19]. To date, 7 HCV genotypes haven been described [20]. Around 25% of individuals who acquire HCV clear the virus spontaneously [21]. The main transmission mode is parenteral exposure to blood or blood products, through sharing of injecting equipment or blood transfusion, and by nosocomial transmission (i.e. acquired at a hospital/health facility) [22]. HCV can also be transmitted from mother-to-child and since the beginning of this millennium, it has emerged as a sexually transmitted infection, predominantly in HIV-positive MSM [22,23]. However, these transmission routes are less common [22].

Worldwide, it is estimated that 71.1 million (95%UI: 62.5–79.4) individuals are chronically infected with HCV [24]. Globally, there were 1.8 million new HCV infections (23.7 new HCV infections per 100,000 people) in 2015 [22]. Similar to the HIV epidemic, the HCV epidemic varies considerably by geographical region. Based on model projections, at the end of 2015, the 5 countries with the highest chronic HCV prevalence in the general population were: Gabon (7.0%), Mongolia (6.4%), Egypt (6.3%), Uzbekistan (4.3%), and Pakistan (3.8%) [24]. However, the quality and availability of prevalence data differs considerably across countries. In the Netherlands, HCV-antibody prevalence was estimated at 0.22% (min% 0.07- max% 0.37%) in 2009 in the general population aged 15 to 79 years [25]. Slightly lower HCV-antibody prevalence was estimated for 2016, namely 0.16% (min% 0.06- max% 0.27) [26], but the range of the 2009 and 2016 estimates overlap. Based on a Dutch modelling study, the estimated number of individuals with chronic HCV infection in the Netherlands was 19,200 in 2014 [27]. Similar to HIV, HCV incidence differs considerably across key populations in the Netherlands in recent years; in the Amsterdam Cohort Studies, incidence was estimated at 1.2 per 100 py in 2012 among HIV-positive MSM [28], whereas among PWUD, no new HCV infections have been observed since 2004 [29].

HCV can lead to liver-related disease such as liver fibrosis and cirrhosis. Cirrhosis usually develops in 15-35% of chronically HCV infected individuals after 20 to 30 years of HCV infection; with cirrhosis being the leading cause of HCV-related decompensated cirrhosis and hepatocellular carcinoma [30]. Globally, it was estimated that per year 399,000 deaths occur due to HCV-related disease [22], with around 350 deaths per year in the Netherlands since 2002 [31]. In 2013, viral hepatitis was the seventh to eight leading cause of death in the world; with HCV accounting for 48% of viral-hepatitis related deaths [32]. Although no vaccine is available yet, direct-acting antivirals are available for the treatment of chronic HCV, with cures rates of up to 95% [33].

1.1.3 Human papillomavirus

HPV is one of the most common sexually transmitted infections (STI) worldwide [23]. HPV encompasses more than 150 different types that can infect humans [34], with 40 of these able to infect the ano-genital area and upper-digestive tract [23]. Reported risk factors associated with genital HPV acquisition in men are older age, (history of) smoking, lifetime number of sex partners, and concurrent STI [35]. Women and MSM have a higher anal HPV prevalence than heterosexual men [36].

It has been estimated that around 80% of the adult population (e.g. 20 to 79 years old) has ever been infected with HPV [37], and around 90% of these infections are resolved spontaneously within 2 years [38,39]. There are low-risk HPV types that mainly cause

genital warts, and high-risk HPV types (hrHPV) that can lead to HPV-related precancerous lesions and cancers (e.g. cervical and anal cancer) [23]. Although the burden op HPV-related disease is higher among women than in men [36], in this thesis we focus on HPV in men. A study from the Netherlands reported a prevalence of penile and anal hrHPV of 32% and 65% in HIV-positive MSM and 16% and 45% in HIV-negative MSM, respectively [40]. Of all cancers, 4.5% have been attributed to HPV infection, and 6% of these are anal cancers [41]. While in women the median time from a precancerous lesion (cervical intraepithelial neoplasia (CIN) 2/3) to cervical cancer has been estimated at 23.5 years (95%CI: 20.8-26.6) [42], a similar estimated progression rate is currently lacking for anal cancer in men [43]. In HIV-negative MSM, anal cancer incidence is about 5 times higher than in the general population, at around 5 per 100,000 py [43,44]. Among HIV-positive MSM, anal cancer incidence is even higher, estimated at 46 per 100,000 py [43]. Since the advent of cART, anal cancer incidence has significantly increased over time in HIV-positive MSM [45,46], but seems to be decreasing in the Netherlands since 2006 [46].

There is no cure for HPV infection, but successful screening and treatment of cervical dysplasia can significantly reduce the risk to develop cancer [47]. For anal dysplasia there is no standard treatment available, and there is no evidence whether these treatments may prevent anal cancer [44]. Three types of vaccine are available that protect against HPV-16 and HPV-18 [48,49], which are the types that cause 81% and 4% of all anal cancers, respectively [50]. The quadrivalent and nonavalent vaccine also protects against other HPV types that cause either ano-genital warts (HPV-6 and HPV-11) or other hrHPV types that may cause HPV-related cancers.

1.1.4 HIV co-infections

The term HIV co-infection is used when an individual has an HIV infection and another viral or bacterial infection at the same time. As HIV shares transmission routes (e.g. injecting drug use) with other pathogens (e.g. HCV), HIV co-infections are common in key populations studied in this thesis [51]. Furthermore, some viral or bacterial infections can heighten the susceptibility to acquire HIV or have a detrimental effect on disease progression. For example, syphilis infection has been associated with a greater risk to acquire HIV [52], and HIV itself has been associated with a greater risk to develop active tuberculosis in HIV-positive contacts of individuals with smear-positive tuberculosis [53].

1.1.4.1 HIV/HCV co-infection

The World Health Organization (WHO) estimated that a total of 2.3 million HIV-positive individuals are co-infected with HCV globally, with 59% of these co-infections in PWID [54]. The odds to have HCV are 6 times higher among HIV-positive individuals compared

to the HIV-negative general population [55]. In the Netherlands, among 23,141 HIV-positive individuals in care who were screened for HCV between 1998 and 2017, 2,693 (12%) were HCV-antibody positive [7]. Several studies have reported a detrimental effect of HCV on HIV disease progression [56,57], and others have also reported faster HCV disease progression (e.g. faster fibrosis progression) in the presence of HIV co-infection [58]. Importantly, as HCV transmission is more than 10 times more likely to occur due to blood-blood exposure than HIV [59], HCV generally precedes an HIV infection among PWID, whereas in MSM, HIV generally precedes an HCV infection as sexual transmission of HIV is more likely than of HCV [4,5,60].

1.1.4.2 HIV/HPV co-infection

HIV has also been associated with HPV infection persistence and HPV-related cancers in numerous studies [43,61,62]. A systematic review reported that individuals infected with HPV were more susceptible for HIV acquisition [63]. To date, no data are available on the role of HPV co-infection on HIV disease progression [64].

1.2 Historical overview of the HIV, HCV and HPV epidemics

To understand current estimates, it is important to look back at epidemiological trends and developments in prevention and treatment over time. Here follows a brief overview of major events and trends from the 1960s onwards related to the HIV, HCV and HPV epidemics.

1960-1970s: The heroin epidemic and the sexual revolution

Nowadays, when we look back at the 1960s and 1970s, we think of sex, drugs and rock and roll.

Problematic drug use started in the early 1960s when the heroin epidemic hit the US and a few years later it was introduced in Europe [65]. In the Netherlands, heroin was introduced in 1972 and was primarily used by former American soldiers at the beginning of this epidemic [66]. Subsequently, in 1973/1974, young Surinamese individuals immigrated to the Netherlands shortly before the independence of Suriname from the Netherlands – this group of heroin users preferred smoking heroin [66,67]. Then, in 1976-1977, the popularity of heroin started to rise among native Dutch young individuals [68]. Also, a large group of German heroin dependant individuals immigrated to the Netherlands during the 1970s [68]. As a response of this heroin epidemic, on the 1st of June 1979, the 'methadone by bus' project was initiated in Amsterdam [69]. Before this, other governmental and non-governmental facilities provided drug-related care but many opioid dependant individuals were not reached. Therefore to facilitate access to all PWUD, the mobile clinic distributed methadone, an opioid substitution therapy

(OST), around Amsterdam [69]. This program is regarded as one of the first 'low-threshold' programs based on the principle of 'harm reduction', that is to minimize the harm people who use drugs inflict upon themselves and society at large [70]. Methadone-maintenance treatment in Europe was first introduced in Sweden during the 1960s, followed by the Netherlands, United Kingdom (UK) and Denmark, although prescription of opioids for heroin dependant individuals was allowed in the UK since the 1920s [71].

These were also decades where sexual norms were challenged. In 1960, birth control by means of 'the pill' became available in the US [72]. Additionally, in 1969, the renowned Stonewall riots (resulting from a police raid on a gay bar in New York) led to the start of the gay rights movement [73]. In 1973, homosexuality was finally removed from the Diagnostic and Statistical Manual of Mental Disorders [73,74]. Therefore, the 'typical' sex education, mainly based on abstinence, was revisited. In the 1970s, mainly due to the spread and sequelae of hepatitis B virus (HBV) infection, the terminology 'safe sex' was introduced [75]. It was defined in terms of risk reduction with a positive outlook on sex itself [75].

The 1970s were very significant in relation to HPV research. In the early 1970s, a landmark study on the link between HPV and cervical cancer was published by Harald zur Hausen, who later on was awarded the Nobel Prize [76]. Furthermore, in 1974 Valerie Beral published a landmark paper on the role of STI in cervical cancer [77].

1980-1990s: The beginning of the HIV epidemic and the Amsterdam Cohort Studies
In 1981, AIDS was first described in five homosexual men in the US [78]. At the beginning of the HIV epidemic, it was known as GRID – gay related immunodeficiency syndrome [79]. Later on, cases of AIDS were reported among other risk groups such as PWID and haemophiliacs [80]. But it was not until 1983 that HIV was discovered [80]. By the end of 1985, every region in the world had documented at least one case of AIDS [80]. Also, in 1985 the first diagnostic test for HIV became available [10]. In 1987, zidovudine/AZT, the first agent against HIV, was approved in the US [81]. In response to the HIV outbreak, the Amsterdam Cohort studies among MSM were initiated in 1984, and in 1985 among PWUD [82,83].

Importantly, another harm-reduction program, i.e. needle and syringe exchange program (NSP), was introduced in Amsterdam in 1984 (originally to prevent HBV) by the Amsterdam drug user organization known as the 'Junkiebond' [84]. This is regarded as the oldest low-threshold NSP worldwide [80,84].

Based on phylogenetic analysis, the onset of the exponential growth of the HCV-infected PWID population in high-income countries was in the 1940s [85], but it was not until 1989 that HCV was discovered and the epidemic could be recognized [86]. In Amsterdam, more than 80% of an estimated 6,275 PWID were HCV-antibody positive in the 1980s [87].

The 1990s: ART and cART era

1990-1995: ART era and the decreasing population of PWID

During the early 1990s, global HIV incidence and HIV-related mortality were on the rise [88]. Drugs such as didanosine were approved for the treatment of HIV during this period [10]. However, these drugs had major toxicities and the prognosis after HIV infection remained poor [10]. None of these drugs are widely used nowadays in high-income countries [10].

During this period, injecting drug use started to decline and few new individuals started injecting drugs in Amsterdam [84]. Furthermore, OST dosing for PWID improved in this decade. Since methadone programs were available, the average dosing was low (30 mg/day) [68]. In 1990, largely as a response to the HIV epidemic, methadone-dosing policy changed permitting higher dosages [68]. This is important as the optimal average dosing for OST maintenance is considered to be between 50 and 100 mg/day [89].

In 1991 the first commercial diagnostic test for HCV was developed. From that year onwards, screening of donor blood started in high-income countries [90,91]. Therefore, individuals who received blood products after 1991/1992 are no longer considered an HCV risk group. However, in 2013 the WHO reported that 13 of 179 reporting countries were not able to screen blood donations for all recommended blood-borne pathogens (e.g. HCV) [92].

In 1993, the Center for Disease Control and Prevention (CDC) added cervical cancer to the list of AIDS-defining conditions. This was quite controversial at the time as limited evidence was available on the role of HIV on HPV disease progression [93]. In 1996, the national cervical cancer screening program started in the Netherlands and other countries [94]. It is likely that this substantially contributed to the decline in HPV-related cancer among women in high-income countries [95].

1996-2000: The cART era: from death sentence to chronic infection

The single most significant event at the beginning of this period was the introduction of combination antiretroviral therapy (cART) in 1996, based on a combination of different

drugs to treat HIV [10,80]. This dramatically changed the treatment landscape, and led to a dramatic decrease in morbidity and mortality in HIV-infected individuals who initiated cART [96]. It is believed that as a result of this increased survival among HIV-positive MSM, and thus prolonged exposure to HPV, anal cancer incidence increased during the cART era [45,46]. Globally, the peak in HIV incidence occurred in 1997, with an estimated 3.3 million new HIV infections [88].

In 1998, the Dutch randomized controlled trial on heroin co-prescription with methadone was initiated [67]. Authors of that study reported a significant improvement in the physical, mental and social health of PWUD compared to methadone prescription alone [67]. Afterwards, heroin co-prescription could be prescribed to heroin dependant individuals who do not benefit from methadone alone [67]. In 2016, 142 clients of the Public Mental Health Service of Amsterdam were receiving heroin co-prescription (Marcel Buster, personal communication, January 2018).

2000-2010: The shift in the HCV epidemic in the Netherlands and the introduction of HPV vaccination

During this decade, while HCV prevalence and incidence were significantly decreasing among PWID in the Netherlands, outbreaks of HCV among HIV-positive MSM were noticed during the early 2000s in several Western countries [97-101]. This was also the period when HIV-related mortality reached its global peak. In 2005, it was estimated that 1.7 million individuals died due to HIV [88].

In 2006 the term 'Treatment as Prevention' (TasP) was introduced [102]. TasP refers to the use of ART to prevent onward HIV transmission. Shortly afterwards, in 2008, the renowned 'Swiss Statement' was published. Authors stated that the risk of transmitting HIV is very unlikely when virally suppressed [103].

In 2006, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved the first HPV vaccine (i.e. Gardasil) [48]. In the Netherlands, HPV vaccination (with the bivalent vaccine) was introduced in 2009 for girls 13 years of age. However, to date, neither free vaccination nor routine HPV screening is offered or available to men in the Netherlands. In contrast, the US and Australia have introduced HPV vaccination for young boys since 2011 and 2013, respectively [104,105]. In 2007 the WHO recognized HPV as a causative agent for several cancers in men [36].

2010-2017: The most awaited cure for HCV and the introduction of HIV pre-exposure prophylaxis

In 2013, the FDA approved sofosbuvir, a direct-acting antiviral (DAA) against HCV [106]. Before, other drugs such as pegylated-interferon in combination with ribavirin were available to treat HCV infection with lower cure rates than DAA, longer treatment duration and severe side effects [100]. Also, difficult to treat populations such as HIV/HCV co-infected individuals (35%) and those with genotype 1 (±40-50%) had a low probability of being cured [107-109]. Since sofosbuvir's approval, other DAA have been developed with outstanding cure rates over 95% [110]. Therefore, this is a paramount decade in HCV history. In November 2014, sofosbuvir became available in the Netherlands, although only reimbursed for those with advanced liver fibrosis. Since late 2015, DAA is reimbursed to all patients irrespective of their fibrosis stage [111]. This is in contrast to many countries where DAA are either unavailable or not reimbursed for all individuals with chronic HCV [112].

Since the publication of the 'Swiss Statement' in 2008 [103], two studies (HPTN 052 and the PARTNER study) have confirmed that the risk of transmitting HIV while virally suppressed is unlikely [113,114]. Also new biomedical preventive approaches for HIV became available during this period. In 2012, the FDA approved pre-exposure prophylaxis (PrEP) to prevent individuals from contracting HIV [115]. Additionally, in the summer of 2015, a landmark study got published, namely the START trial [15]. This randomized controlled trial showed that immediate cART initiation versus deferring the start of cART when CD4 T-cell count has dropped below 350 cells/µl led to a lower risk of AIDS and/or death. PreP, TasP and the START trial have changed the landscape of HIV treatment and prevention.

1.3 Observational Cohort Studies

In research the randomized clinical trial (RCT), an experimental study design, is considered to be the highest form of evidence to assess cause and effect. However, these studies are usually not representative for either clinical practice or real life as many exclusion criteria are applied (e.g. only including those with less severe disease). In addition this type of study can be costly, their follow-up time is usually short and in some cases an RCT is not feasible due to ethical concerns. For example, an RCT examining the effect of OST among PWID for the prevention of new incident HIV and/or HCV cases has not been conducted. An RCT would be able to give an answer to the causal effect of OST on infection risk, however, as the effects of OST for heroin treatment addiction are well recognized, one cannot ethically allocate an individual to a group that could not receive OST. Therefore other study design are used when an RCT is not feasible or when non-

experimental settings are preferred (e.g. to track the course of an epidemic or assess the natural history of an infection).

In this thesis, all studies were conducted using data from observational cohorts. Observational studies can be divided into cohort studies, case-control and cross-sectional studies [116]. One disadvantage of case-control and cross-sectional studies is that ascertainment of the exposure before the occurrence of disease can be challenging, and often one cannot disentangle which one came first. In cohort studies the temporal sequence is often better established as individuals are selected based on exposure and then followed up over time [117]. Therefore, this type of study design is considered the highest quality evidence after experimental study designs. However, there are several challenges when analysing cohort study data. Some of the approaches used in this thesis to tackle some of the inherent disadvantages when using cohort data are described below.

1. Confounding: one of the most important issues in observation studies, including cohort studies, is what is known as 'confounding', i.e. when the distribution of a particular characteristic (e.g. smoking) differs between exposed and unexposed, this characteristic has an effect on the outcome and exposure of interest and it does not lie in the causal pathway [118]. Many statistical techniques have been developed to adjust for confounding (e.g. multivariable models as used throughout this thesis in **chapter 2 and 3** or marginal structural models). But when confounders are unmeasured, unknown or in some instances when continuous variables are categorized, we speak of residual confounding. One way to reduce residual confounding is to avoid categorizing continuous variables (e.g. age) [119]. Hence in **chapter 2 and 3** we modelled continuous variables using restricted cubic splines which allowed the use of these variables even when a linear relationship between the exposure and outcome was not present.

2. Missing data: another well-recognized challenge is missing data. Missing data can arise due to incomplete measurements (e.g. HIV RNA is only measured at certain clinic visits or participants do not complete a questionnaire) or loss to follow up. This always leads to loss of power. The bias related to missing data due to incomplete measurements is dependent on its type and how this is dealt with in the statistical analysis. Missing data can be: Missing Completely at Random (MCAR), Missing at Random (MAR) and Missing Not at Random (MNAR) [120]. We speak about MCAR when the probability of a variable being missing does not depend on the actual value of the missing data and is not related to other observed or unobserved variables. In MAR, the probability of being missing is independent of the actual value given the observed data in the model. In both cases, one could impute the missing data [120]. The problem arises when data is MNAR. Then the probability of being missing depends on unobserved data and the

missing value cannot be imputed. However, by definition one cannot show whether the missing data are not MNAR. In **chapter 2.3** we believe it to be reasonable to assume that missing data in our study are at least MAR as we had a sufficient number of variables that could explain the missingness. Hence, in **chapter 2.3** we imputed missing data using a technique called multiple imputation by chained equations (MICE).

3. Selection bias: although cohorts studies are usually more representative than RCT, this design is prone to selection bias. Selection bias refers to the selection of the sample from the population (1), or loss to follow up (2) [121]. Selection bias occurs if the effect of exposure on outcome differs among those included and the eligible population.

3.1 Selection of the population: selection bias occurs when individuals included in a study differ from the entire population of interest [122]. Within this type of bias a specific term has been coined for when the 'survivors' of a specific disease are more likely to enter the study, namely 'survival bias'. Hence subjects that die shortly after becoming exposed are often not included. If the time origin is known (e.g. HIV seroconversion), one can correct for late entry and thus adjust for survival bias. This is one of the major advantages of the CASCADE Collaboration (**chapter 2.1 and 2.2**) that gathered data from HIV seroconversion onwards, while in 'prevalent' cohorts the time origin is often unknown. For example, a previous study from the CASCADE Collaboration concluded that using data from prevalent cohorts exaggerated the estimates of HIV survival improvements over time compared to an HIV seroconverter cohort, as follow-up time from individuals from the later calendar years is more likely to be from recent HIV seroconverters [123]. Authors of that study also reported that estimates from prevalent cohorts were biased even when correcting for CD4 T-cell count at entry as a proxy for HIV infection duration [123].

3.2 Differential loss to follow up: the second type of selection bias, which is also a type of missing data, is a concern when there is differential loss to follow up, which is known as informative censoring or drop out. For example, differential loss to follow up is present if participants dropping out of the study have a higher likelihood to acquire the disease or to die as a result of the exposure of interest than those individuals who remain in follow up in the study. An individual could be censored due to a competing event (e.g. death). In that case the event of interest cannot be observed among those censored. When we are interested in obtaining results in the presence of competing events, statistical methods to deal with competing risks are available [124] as used in **chapter 2.4**. When the outcome is the development of a marker over time (e.g. CD4 T cell count trajectories) and the drop out process is caused by an unobserved value of the longitudinal marker in the data, we may correct for it using a joint model that combines the marker process

and the relation between the (unobserved) marker value and dropout. We used this in the sensitivity analyses in **chapter 2.2**.

1.4 Outline of this thesis

In this thesis we aimed to increase our understanding of the incidence, disease progression, and treatment of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human papillomavirus (HPV) (co-)infections in key populations. This thesis is divided in two chapters. One with studies focusing on HIV/HCV and HIV/HPV co-infections in MSM, and the second focusing on HIV/HCV co-infection and HCV and HIV treatment among PWUD. Here follows a brief outline of the chapters, subchapters and aims. For additional information on data sources see Table 1.

1.4.1 Chapter 2. HIV co-infections in MSM: incidence and disease progression

In chapter 2 of this thesis, three studies are outlined. In **chapter 2.1 "Lack of decline in hepatitis C virus incidence among HIV-positive men who have sex with men during 1990–2014",** using data among MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration we 1) updated trends in HCV incidence; overall and by geographical region, 2) assessed the associations between HCV incidence and HIV-related measurements, geographical region, age and calendar year, and 3) assessed whether the time interval between HIV seroconversion and HCV infection has changed over calendar time. In **chapter 2.2 "Effect of incident hepatitis C virus infection and its timing following HIV seroconversion on CD4 T-cell count and HIV RNA trajectories"** we assessed the effect of incident HCV infection, and its timing relative to HIV seroconversion, on subsequent CD4 T-cell count and HIV-RNA viral load trajectories in HIV-positive MSM. Lastly, in **chapter 1.3 "The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM",** we compared the anal and penile hrHPV incidence and clearance between HIV-positive and HIV-negative MSM over two years of follow-up, and assessed the effect of HIV-related immunosuppression on HPV incidence and clearance.

1.4.2 Chapter 3. HIV and HCV in PWUD: disease progression and treatment

In chapter 3 of this thesis, four studies are outlined. In **chapter 3.1** "**Temporal trends in mortality among people who use drugs compared with the general Dutch population differ by hepatitis C virus and HIV infection status",** we identified temporal trends in all-cause and cause-specific mortality rates among PWUD compared with the general Dutch population; and determined whether excess mortality trends differed by HCV/HIV status. In **chapter 3.2 "High proportions of moderate to severe fibrosis and cirrhosis in an ageing population of people who use drugs in Amsterdam, the Netherlands"** we assessed the proportion of PWUD with moderate to severe liver fibrosis or cirrhosis

and its determinants. In **chapter 3.3** "**Cost-effectiveness of hepatitis C treatment for people who inject drugs and the impact of the type of epidemic; extrapolating from Amsterdam, the Netherlands",** we assessed the cost-effectiveness of four HCV treatment strategies among PWID in combination with HCV-treatment scale-up. Furthermore, we explored the impact of the type of epidemic on the cost-effectiveness of DAA and on the chronic HCV prevalence over time. In **chapter 3.4 "HIV and hepatitis C treatment uptake among people who use drugs participating in the Amsterdam Cohort Studies, 1985-2015**", we assessed trends in HIV and HCV treatment uptake among PWUD and, particularly, the uptake of DAA during the first full year (i.e. 2015) of its availability in the Netherlands. Furthermore, we estimated the cumulative probability of ART initiation from HIV seroconversion onwards, stratified by ART period (pre-cART and cART eras), with all-cause mortality as a competing risk.

Table 1: Data sources used in this thesis

Data source	Countries	Study population	Study design	N	Study period [a]	Chapter
			Chapter 1			
CASCADE Collaboration [b]	Canada, Australia, France, Spain, Greece, Norway, United Kingdom, Norway, Germany, the Netherlands, Austria and Kenya	HIV-positive MSM	Collaborative study pooling observational data from 28 cohorts	17,429	1990-2014	**2.1** **2.2**
HIV and HPV in MSM (H2M) [c]	The Netherlands	MSM	Observational cohort study	750	2010-2013	**2.3**
			Chapter 2			
Amsterdam Cohort Studies on HIV	The Netherlands	PWUD	Observational cohort study	Ch 3.1 : 1,254 Ch 3.2 : 140 Ch 3.3 : [d] Ch 3.4 : 1,305	1985-2015	**3.1** **3.2** **3.3** **3.4**
Drug users treatment for chronic hepatitis C unit (DUTCH-C)	The Netherlands	PWUD	Demonstration project offering HCV treatment	110 (61 also ACS participants)	2004-2013	**3.2**

Abbreviations: Ch= chapter; HIV= human immunodeficiency virus; HCV= hepatitis C virus; ACS= Amsterdam Cohort Studies; H2M= HIV and HPV in MSM; HPV= human papillomavirus; MSM= men who have sex with men; PWUD= people who use drugs.

[a] Calendar time period used in the study chapter(s) of this thesis.

[b] Including both men who have sex with men and people who use drugs participating in the Amsterdam Cohort Studies.

[c] HIV-negative men who have sex with men participating in the H2M cohort were recruited from the Amsterdam Cohort Studies.

[d] The study presented in chapter 3.3 modelled the HCV epidemic among PWID in Amsterdam. The estimated number of past and present PWID decreased over time from 5,847 in 1985 to 3,397 in 2015.

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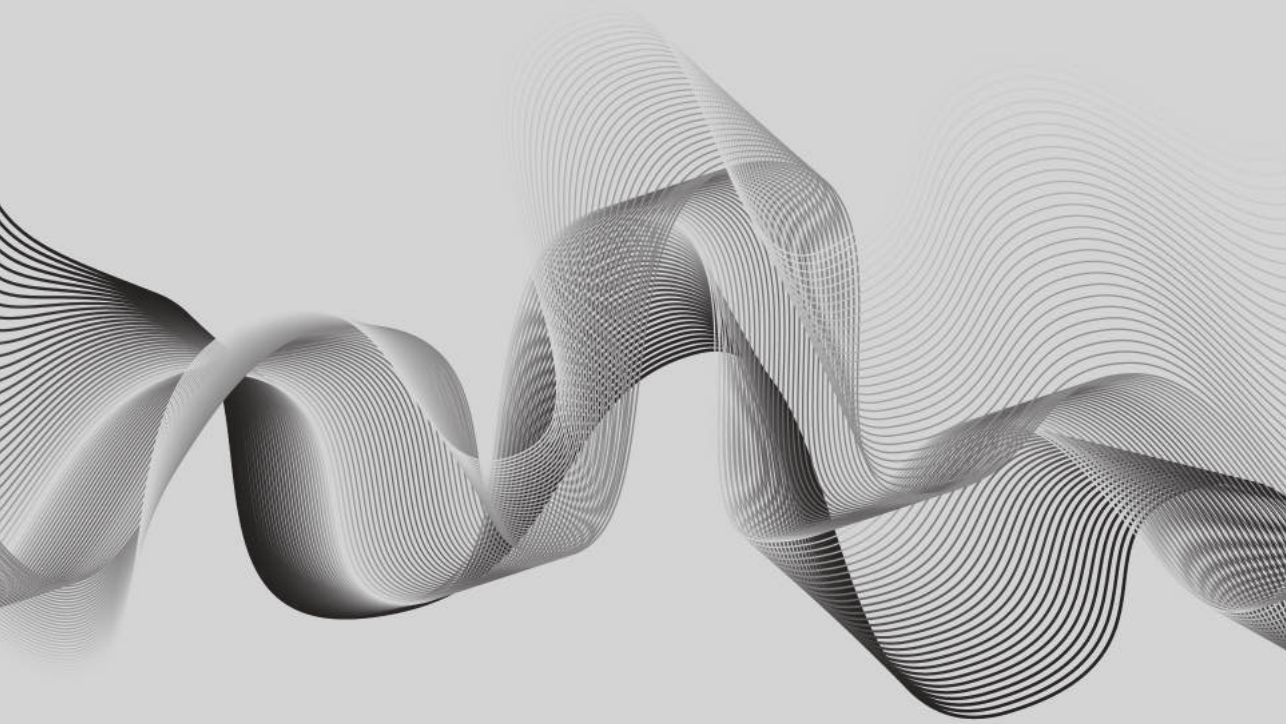
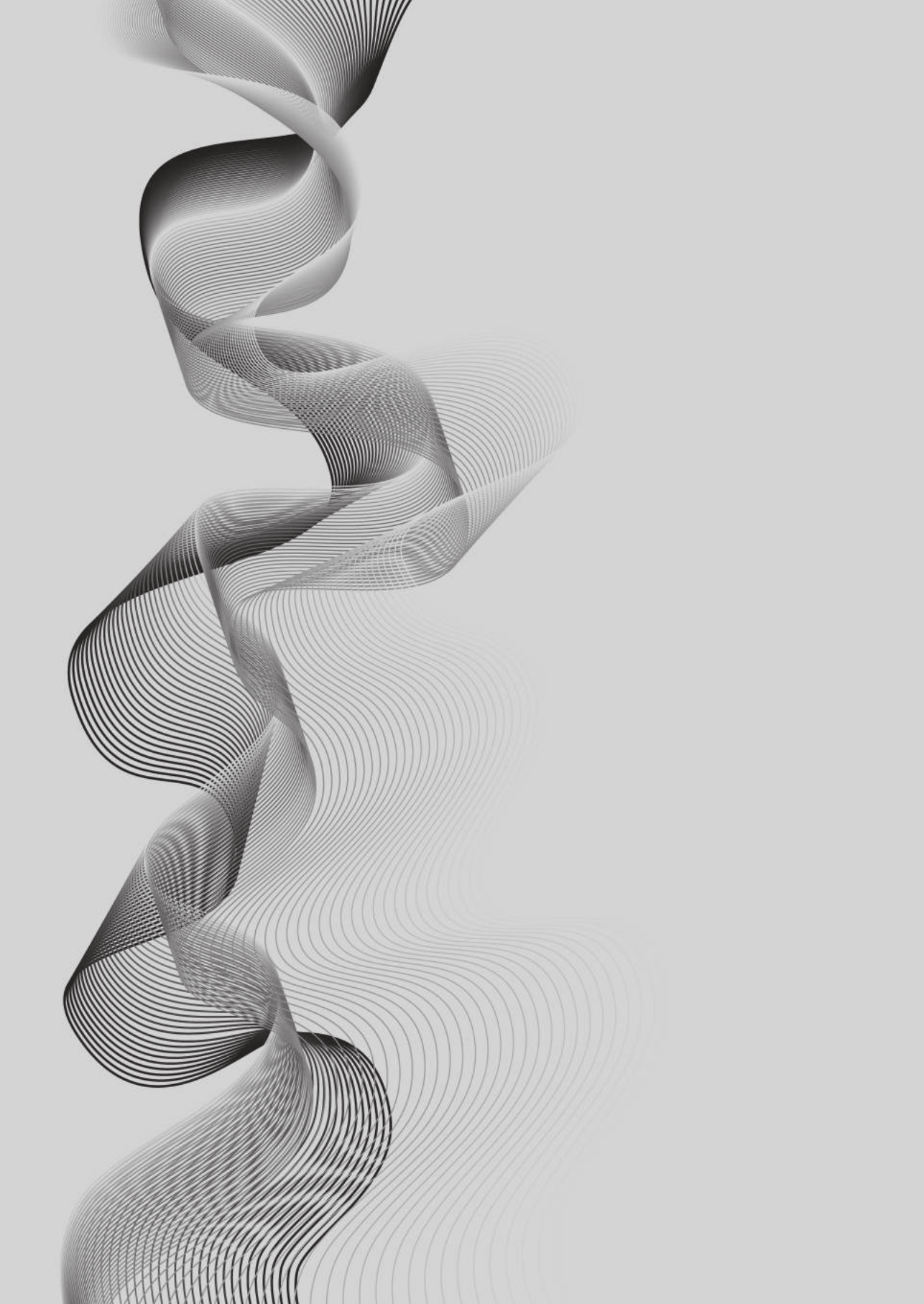
Chapter 2

HIV co-infections in men who have sex with men: incidence and disease progression





Chapter 2.1

Lack of decline in hepatitis C virus incidence among HIV-positive men who have sex with men during 1990–2014

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ABSTRACT

Background: Hepatitis C virus (HCV) incidence among HIV-positive men who have sex with men (MSM) has increased since 2000, although there are regional differences. We aimed to 1) estimate trends in HCV incidence among HIV-positive MSM, 2) assess the association between incidence and geographical region, age and HIV-related measurements and, 3) assess temporal changes in time from HIV seroconversion to HCV infection.

Methods: Data was used from MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration (1990-2014). Smoothly varying trends in HCV incidence over time were allowed, using restricted cubic splines. The association of calendar year, age, CD4 count (lagged), HIV RNA (lagged), geographical region and HIV infection stage (recent vs. chronic) with HCV incidence were assessed using Poisson regression.

Results: Of 5,941 MSM, 337 acquired HCV during follow-up. HCV incidence significantly increased from 0.7/1000 person-years in 1990 to 18/1000 person-years in 2014. Recent calendar years, younger age, recent HIV infection and higher HIV RNA levels were significantly associated with HCV incidence, while CD4 count was not. Trends differed by geographical region; while incidence appeared to have stabilized in Western Europe and remained stable in Southern Europe, it continued to increase in Northern Europe in recent years. Time from HIV to HCV infection significantly decreased over time ($p < 0.001$).

Conclusions: HCV has continued to spread among HIV-positive MSM in recent years, but trends differ by geographical region. Interventions to decrease the risk of HCV acquisition and increase early diagnosis are warranted.

Lay summary: Hepatitis C virus infection continues to spread among HIV-positive men who have sex with men, especially among younger individuals. However, trends seem to differ by European region in recent years. Furthermore, men who have sex with men with a higher HIV RNA load were more likely to get infected with the hepatitis C virus. During recent HIV infection, MSM appear to be at higher risk of acquiring hepatitis C.

INTRODUCTION

Since 2000, hepatitis C virus (HCV) incidence has increased among HIV-positive men who have sex with men (MSM) [1,2]. Using data from the CASCADE Collaboration (Concerted Action on SeroConversion to AIDS and Death in Europe) in EuroCoord, we previously showed that HCV incidence increased in MSM with well-estimated HIV seroconversion dates after 1990, but the main expansion of the HCV epidemic was observed from 2002 until 2007, the censoring date of the analysis [1]. A recent meta-analysis showed that HCV incidence has continued to increase, with an estimated pooled incidence of 13/1000 person-years (py) in 2010 to an extrapolated incidence estimate of 19/1000 py in 2015 [2]. However, other studies have shown varying trends in HCV incidence among MSM over the past years [3,4]. In Amsterdam, the Netherlands, HCV incidence seems to be stabilizing [3], whereas in Switzerland an increasing incidence among MSM has been observed [4].

A number of factors such as fisting, the presence of sexually transmitted infections (STIs), use of recreational drugs, and condomless anal intercourse have been shown to be significantly associated with acute HCV infection [4-10]. In addition, one study from the US reported that older age was independently associated with an acquired HCV infection [10], whereas another study from the Netherlands reported that younger MSM had a higher risk [3]. As acute HCV infections are predominantly found among HIV-positive MSM, it has been suggested that HIV facilitates sexual transmission of HCV [11]. However, contrasting results on the association between $CD4^+$ T-cell count (CD4 count) and HCV incidence have been reported [4,9,10,12]. Additionally, few studies have investigated the association with HIV RNA and, those that have, either dichotomized HIV RNA and/or could only assess the association in univariable analyses [4,9,12]. The role that HIV-related factors play in the spread of HCV among HIV-positive MSM is currently still being debated.

Using data among MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration we aimed to 1) update trends in HCV incidence; overall and by geographical region, 2) assess the associations between HCV incidence and HIV-related measurements, geographical region, age and calendar year, and 3) assess whether the time interval between HIV seroconversion and HCV infection has changed over calendar time.

METHODS

We used data from 16 out of 28 cohorts from the CASCADE Collaboration across Europe, Australia and Canada. Of the excluded cohorts, five were non-MSM cohorts and six cohorts had tested less than 50% of MSM for HCV and could not provide stored samples for HCV testing (missing HCV status data from 57.2% to 96.2%) (Fig. 1). The Kenyan cohort (IAVI; n=92) was also excluded as we believe that the HCV epidemic among MSM in Kenya differs from that in high-income countries (no incident HCV infections were observed). All cohorts include data from HIV-positive individuals with dates of HIV seroconversion that could be reliably estimated based on the midpoint between the last HIV-negative and first HIV-positive test (at most 36 months apart) or, evidence of acute HIV infection. Details of CASCADE have been previously described [13]. We only included men from the 16 cohorts who were recorded as having acquired HIV through sex between men and whose potential HIV transmission route excluded injecting drug use. For all cohorts, we used all available data, except for MSM from the French PRIMO cohort who were censored at the 31st of December 2005 as routine HCV testing was only recorded until that year. All collaborating cohorts received approval from their regulatory or national ethic boards (see Appendix 1 in chapter 2.2) and informed consent was obtained for all participants.

HCV negative status throughout follow-up was based on at least one HCV-negative test result and never testing HCV positive. HCV infection was based on any positive HCV test (RNA, antibodies and/or antigen). Among MSM who acquired HCV during follow-up, the date of HCV infection was estimated as the midpoint between the last HCV-negative and first HCV-positive test. To optimize testing frequency, we performed additional HCV testing in cohorts that had stored specimens (8 cohorts). Stored samples from HCV-negative MSM were tested using a sample closest to the date of their last clinic visit if more than 2 years had elapsed since their last HCV-negative test date. For HCV-positive MSM without a previous HCV-negative test date, the sample closest to HIV seroconversion but up to one year of it was tested to assess whether they had become HCV infected during follow-up; if HCV negative, midpoint samples were tested until the HCV seroconversion interval was a maximum of 2 years. For MSM with a recorded HCV infection during follow-up but with an HCV test interval >2 years, samples with dates which fell in the interval between their last HCV-negative and first HCV-positive test date were tested. All cohorts provided a date of start of routine HCV testing (defined by testing of all MSM for HCV according to prevailing guidelines or practices) and details on HCV testing strategies (e.g. retrospective testing).

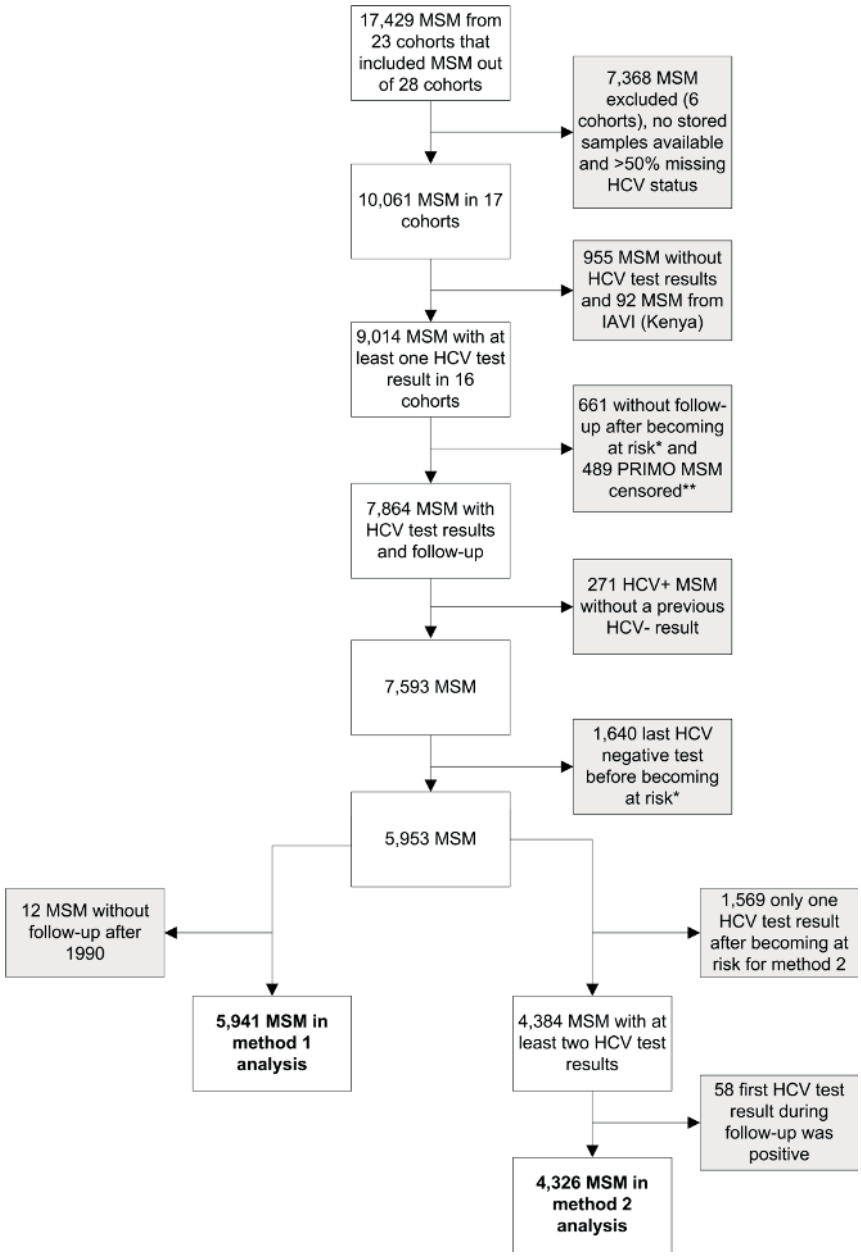

Fig. 1: Flow diagram of the study population selection for method 1 and 2 of the HCV incidence analyses.

* Becoming at risk being the latest of: enrolment in the cohort, routine HCV testing date per cohort or HIV seroconversion.

** MSM from the French Primo cohort were censored at the 31st of December 2005 as HCV testing was only systematically recorded until that year.

The grey boxes depict MSM whose data were excluded from the analyses.

HCV incidence

We estimated overall HCV incidence trends between 1990 and 2014 and stratified by European geographical region between 1997 until 2013 as not all regions have available data for the total study period. Geographical region was defined based on the United Nations (UN) classification criteria [14], namely Western (the Netherlands, Switzerland, France, Austria and Germany), Northern (United Kingdom and Norway), and Southern Europe (Italy, Spain and Greece), North America (Canada) and Australia and New Zealand (Australia) (Table 1). We only illustrate HCV incidence by geographic region for the three European regions as Canada and Australia had relatively small numbers of MSM and few HCV infections were observed. MSM were considered at risk from the latest of: HIV seroconversion, routine HCV testing date per cohort or enrolment in the cohort (Table 1). We used two methods to calculate follow-up time as previously described [1]. In both methods, MSM with one or more HCV-positive tests but without a previous HCV-negative test were excluded (Fig. 1). In method 1, follow-up time began from the moment MSM were considered at risk and will likely underestimate HCV incidence as some of the excluded MSM, who only had HCV-positive tests under active follow-up, could have become infected between the moment they were considered at risk and their first HCV test. Recognizing this possible underestimation, we applied another method (method 2) where follow-up began from the first HCV-negative test after becoming at risk (i.e. left truncation). This approach, however, leads to a shorter follow-up time for MSM who remained HCV-negative throughout follow-up as they are less likely to have been tested retrospectively compared to MSM who became HCV-positive. Consequently, this method is likely to overestimate HCV incidence. In both methods follow-up was calculated until the last HCV-negative date or, in case of HCV infection, the midpoint date. Only the first observed HCV infection during follow-up within an individual was included in the analyses. We used Poisson regression models where HCV incidence was allowed to vary smoothly over calendar time using restricted cubic splines for the overall and the stratified analyses (i.e. by geographical region). We performed a sensitivity analysis using an interval-censored approach as previously described [1] (Please see the Supplementary material (Text 1) for further details).

HCV risk factor analyses

We used three Poisson regression models that included calendar year using the method 1 approach to calculate follow-up. We assessed variation of HCV incidence by geographical region (model 1) and the associations with age (model 2), and HIV-related measurements: CD4 count, HIV RNA and HIV infection stage (model 3). All continuous variables were included as restricted cubic splines (calendar year, current age, $\log_{10}$ HIV RNA and cube root CD4 count). The knots were chosen based on the 2.5, 25, 50, 75 and 97.5 percentiles.

Model 1

We compared the fit of three submodels by means of the Akaike information criterion (AIC): model 1.1, calendar year only; model 1.2, calendar year and region as main effects; model 1.3, calendar year, region, and their interaction.

Model 2

We then added age to the best fitting model 1. In this model, we tested the interaction between age and both region and calendar year. Significant interactions were included in this model.

Model 3

This multivariable model included: age, calendar year, region, HIV RNA and CD4 count. The CD4 count and HIV RNA value from the previous visit were used, but had to be no more than one year before. Missing HIV RNA and CD4 count data were imputed based on individual predicted values from random-effects models adjusted for age and stratified by combination antiretroviral therapy (cART) use: treatment naïve, on cART, and during cART interruption among cART-experienced (Supplementary text 2). For this model we defined a treatment interruption as a stop of cART for >1 week. When a person had no CD4/HIV RNA values throughout follow-up, we used the predicted values based on the fixed effects. We defined cART as a 3 drug ART regimen containing two different classes, or three nucleoside reverse transcriptase inhibitors, provided tenofovir or abacavir were included in the regimen. In additional analyses we assessed whether a recent HIV infection (defined as the period from estimated HIV seroconversion to less than 0.7 years hereafter) was associated with HCV incidence using model 3. We also tested the interaction between HIV RNA and HIV infection stage (recent vs. chronic). We used the likelihood ratio test to test significance in model 2 and 3. Instead of reporting incidence ratios, we illustrate the association between age, CD4 count and HIV RNA and incidence by plotting the absolute incidence with 95% confidence intervals, choosing representative values (e.g. median values) for the other covariates.

Sensitivity analyses

We performed four sensitivity analyses. As we imputed missing CD4 and HIV RNA values, first, we performed the analyses using predicted values instead of using a combination of predicted and observed values. Second, we performed a complete case analyses in which only observed values were included. Third, an analysis was performed where the antepenultimate CD4 count and HIV RNA value were used. The reason for the third analysis is that antibody development might be delayed in HIV-positive individuals [15,16] and in our study 83.4% (n=281) of HCV infections were based on HCV antibody seroconversion and 15.7% (n=53) were based on a positive HCV RNA test and an HCV-

antibody negative test result. Lastly, although additional HCV testing was performed in the Italian cohort (ICoNA), we performed the overall HCV incidence analyses without this cohort as currently there is no routine HCV testing in place.

Time from HIV to HCV

Kaplan-Meier curves were constructed applying the method 1 follow-up calculation to compare cumulative HCV incidences by calendar period of HIV seroconversion. We modelled whether HCV incidence depended on calendar year using a Cox proportional hazards model, including calendar year of HIV seroconversion as a continuous variable using restricted cubic splines.

Statistical analyses were performed using R [17] and Stata [18] software.

RESULTS

Of 17,429 HIV-positive MSM, 7,368 MSM were excluded from six cohorts with more than 50% missing HCV status data and that could not provide stored samples for HCV testing (Fig. 1). Of the remaining 10,061 MSM, 9,014 had at least one HCV test result of whom 8,311 tested only HCV negative and 703 had at least one HCV-positive test result. MSM with HCV test results did not differ by ethnicity from MSM without test results, but were more likely to have a post-secondary education (37% vs. 32%). The median and mean number of HCV tests during follow-up among cohorts that routinely and prospectively collected HCV data (n=13) was 3.0 (Interquartile range (IQR)=2-6) and 4.1 (Standard deviation=3.6), respectively. A total of 7,864 MSM had follow-up and at least one HCV test result (Table 1). Among these MSM, 57.0% were white and median age was 34 years (IQR=28-41) at inclusion. The median year of HIV seroconversion was 2004 (IQR=1999-2008). Over the total study period, the median observed CD4 count was 509 cells/µl (IQR=367-684), median observed HIV RNA was 70 copies/ml (IQR=50-15522) and 70.3% started or were on cART.

A total of 5,941 and 4,326 MSM were eligible according to method 1 and 2, respectively (Fig. 1;Table 1). These MSM accounted for a total of 28,600 and 19,480 py and 337 and 279 HCV infections in method 1 and 2, respectively. The median follow-up time was 4.0 (IQR=1.7-7.2) and 3.9 (IQR=2.0-6.3) years in method 1 and 2, respectively. Of the 337 incident HCV infections observed during follow-up, 25 (7.4%) occurred during recent HIV infection.

Table 1: Number of MSM per cohort with and without HCV test results in the CASCADE Collaboration

Cohorts	At least one HCV test result	MSM with follow-up[a] & at least one HCV test result			At-risk set[b]				Start routine testing date
			HCV+[d]	HCV-[e]	Method 1		Method 2		
N[c]	n (%)	Total, n (%)	n (%)	n (%)	n	HCVsc - Pys	n	HCVsc - Pys	
Southern Europe									
AMA; n=177	172 (97.2%)	167 (94.4%)	2 (1.2%)	165 (98.8%)	128	0 - 526.3	87	0 - 347.2	1-1-1991
COR; n=365	353 (97.7%)	310 (84.9%)	5 (1.6%)	302 (97.4%)	184	3 - 246.2	68	3 - 87.7	1-1-2005[f]
ICO; n=1018	914 (89.8%)	848 (83.3%)	49 (5.8%)	770 (90.8%)	497	29 - 1926.3	411	29 - 1705.7	AT
MAD; n=342	308 (90.1%)	293 (85.7%)	16 (5.5%)	274 (93.5%)	213	3 - 1047.9	30	3 - 56.8	1-1-1993
VAL; n=165	89 (53.9%)	85 (51.5%)	13 (15.3%)	71 (83.5%)	65	1 - 66.9	2	1 - 2.9	1-1-1998
Total; n=2067	**1,836 (88.8%)**	**1,703 (82.4%)**	**85 (5.0%)**	**1,582 (92.9%)**	**1,087**	**36 - 3813.6**	**598**	**36 - 2200.4**	
Western Europe									
AQU; n=788	730 (92.6%)	707 (89.7%)	29 (4.1%)	657 (92.9%)	486	21 - 3053.1	360	19 - 2389.3	1-1-1991
AUS; n=212	206 (97.2%)	201 (94.8%)	3 (1.5%)	193 (96.0%)	181	5 - 682.3	150	4 - 575.7	1-1-2006
GER; n=1912	1,848 (96.7%)	1,543 (80.7%)	63 (4.1%)	1,393 (90.3%)	1,025	87 - 4557.0	764	51 - 2665.5	RT
LYO; n=62	60 (96.8%)	59 (95.2%)	1 (1.7%)	57 (96.6%)	11	1 - 40.2	0	0 - 0	1-1-1999[f]
NEM; n=239	239 (100%)	239 (100%)	2 (0.8%)	215 (90.0%)	224	22 - 1841.6	144	21 - 1098.1	RT
PRI; n=966	894 (92.5%)	401 (41.5%)	15 (3.7%)	381 (95.0%)	211	6 - 791.8	190	5 - 748.5	1-1-1996[f]
SWI; n=343	338 (98.5%)	320 (93.3%)	4 (1.3%)	294 (91.9%)	274	22 - 1532.6	236	17 - 1210.2	1-1-2000
Total; n=4522	**4,315 (95.4%)**	**3,470 (76.7%)**	**117 (3.1%)**	**3,190 (91.9%)**	**2,412**	**164 - 12498.5**	**1,844**	**117 - 8687.4**	
Northern Europe									
NOR; n=383	378 (98.7%)	349 (91.1%)	10 (2.9%)	328 (94.0%)	305	11 - 2165.9	258	11 - 1489.6	1-1-1995
UKR; n=2714	2,209 (81.4%)	2,073 (76.4%)	50 (2.4%)	1,903 (91.8%)	1,937	120 - 9395.2	1,582	110 - 6871.6	1-1-2004
Total; n=3097	**2,587 (83.5%)**	**2,422 (78.2%)**	**60 (2.5%)**	**2,231 (92.1%)**	**2,242**	**131 - 11561.1**	**1,840**	**121 - 8361.2**	

Table 1: Number of MSM per cohort with and without HCV test results in the CASCADE Collaboration (*continued*)

Cohorts	At least one HCV test result	MSM with follow-up[a] & at least one HCV test result			At-risk set[b]				Start routine testing date
				North America					
SAL; n=138	**138 (100%)**	**131 (94.9%)**	**4 (3.1%)**	**122 (93.1%)**	**67**	**5 - 327.2**	**43**	**4 - 230.0**	1-1-2000
				Australia					
PHA; n=145	**138 (95.2%)**	**138 (95.2%)**	**5 (3.6%)**	**132 (95.7%)**	**133**	**1 - 399.4**	**1**	**1- 0.8**	1-1-2002
Total; n=9,969	**9,014 (90.4%)**	**7,864 (78.9%)**	**271 (3.4%)**	**7,257 (92.3%)**	**5,941**	**337- 28,599.9**	**4,326**	**279 – 19,479.8**	

Abbreviations: N=number; n=number; HCVsc=HCV seroconverters; PYs=person years of observation; HCV+=HCV-positive; HCV-=HCV-negative; RT=retrospective testing; AT=additional testing only (no routine testing); AMA= AMACS cohort, Greece; AQU: Aquitaine cohort, France; AUS: Austrian HIV cohort study, Austria; COR=CoRis cohort, Spain; GER=German cohort, Germany; IAV=IAVI, Kenya; ICO=ICONA cohort, Italy; LYO= Lyon cohort, France; MAD=Madrid cohort, Spain; NEM=Amsterdam Cohort Study among MSM, the Netherlands; NOR=Oslo and Ulleval hospital cohorts, Norway; PHA=PHAEDRA cohort, Australia; PRI=PRIMO cohort, France; SAL=Southern Alberta Clinic, Canada; SWI=Swiss HIV cohort, Switzerland; UKR=UK Register of HIV seroconverters, UK; VAL=Valencia cohort, Spain; NA=not applicable.

[a] MSM with a clinic visit, and thus follow-up, after becoming at risk, being the latest of: enrolment in the cohort, HIV seroconversion or routine testing per cohort. HCV test results irrespective of the moment of becoming at risk.

[b] MSM included in the analyses from 1990 until 2014.

[c] Number of MSM per cohort irrespective of the moment of becoming at risk, HCV test, year and length of follow-up.

[d] HCV-positive MSM without a previous HCV negative test result (i.e. excluding HCV seroconverters).

[e] MSM who remained HCV negative throughout follow-up (from becoming at risk until last clinic visit).

[f] Start of routine testing date before individuals were enrolled in the cohort.

[d,e] Out of all MSM with follow-up & at least one HCV test result (third column).

HCV incidence

HCV incidence significantly increased from 1990 onwards ($p_{method1}<0.001$;$p_{method2}=0.04$); with an estimated incidence ranging from: 0.7/1000 py (95% confidence interval (CI)=0.1-5) in 1990 to 18/1000 py (95%CI=9-37) in 2014 in method 1 and from 3/1000 py (95%CI=0.4-18) in 1990 to 21/1000 py (95%CI=10-42) in 2014 in method 2 (Fig. 2). The interval-censored method showed a similar increasing trend (Supplementary Fig. 1). Excluding one cohort (ICoNA) from the overall analyses, led to similar statistically significant increasing trends by both methods, although the estimations were slightly lower (Supplementary Fig. 2). The stratified analyses by geographical region showed that in recent years HCV incidence seems to have increased in Northern Europe, but calendar year was only statistically significant in method 2 (p=0.02) (Fig. 3). In Southern Europe, a stable trend was observed and calendar year was not significant. In Western Europe the trend was significant in both methods ($p_{method1}=0.001$;$p_{method2}=0.005$); based on method 1, HCV incidence increased sharply from 14/1000 py (95%CI=10-20) in 2006 to 23/1000 py (95%CI=17-31) in 2009, but declined thereafter to 9/1000 py (95%CI=3-27) in 2013 (Fig. 3).

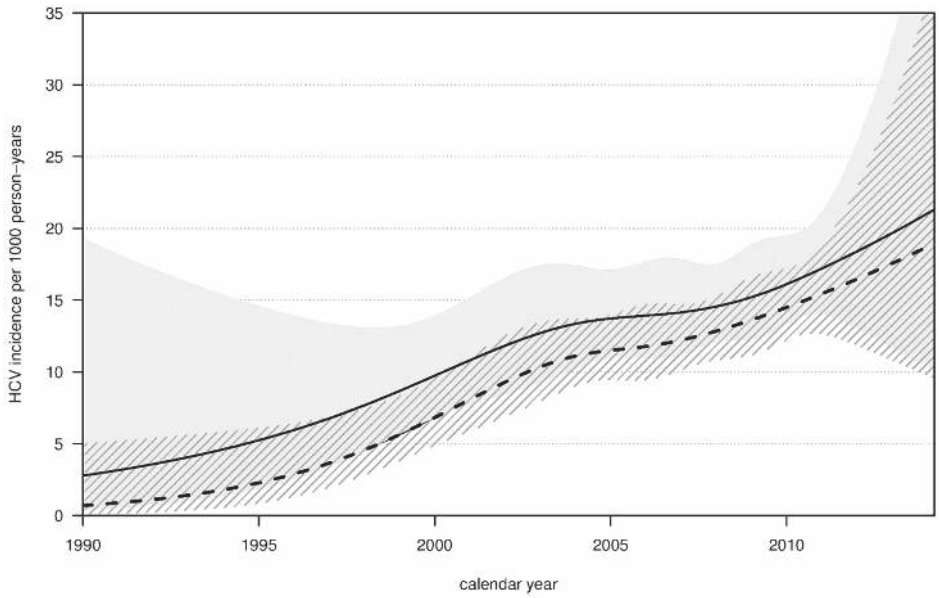

Fig. 2: HCV incidence among HIV-positive MSM using two methods to estimate follow-up in the CASCADE Collaboration; 1990-2014.

Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.

Poisson regression was used to test the overall effect of calendar year on HCV incidence between 1990 and 2014.

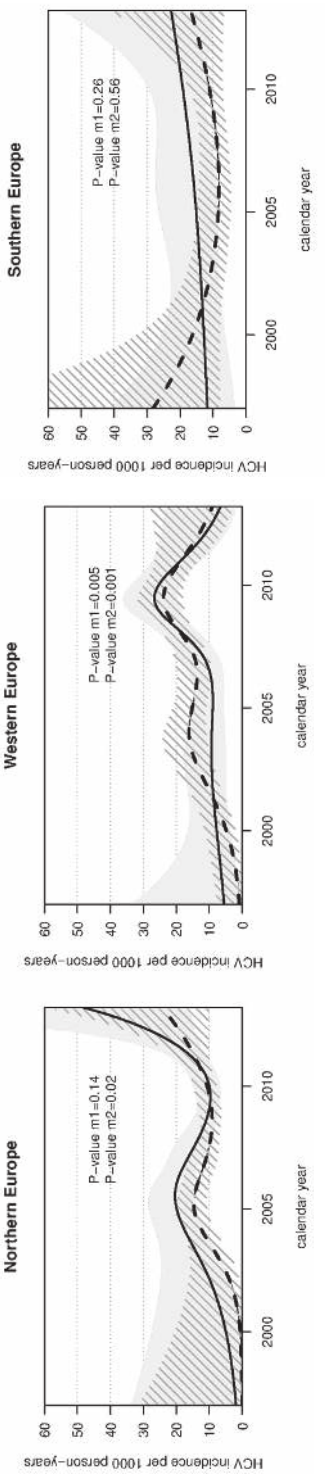

Fig. 3: HCV incidence among HIV-positive MSM by European UN geographical region in the CASCADE Collaboration; 1997-2013

Abbreviations: m1= method 1; m2= method 2

Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.

P-values: overall effect of calendar year on HCV incidence between 1997 and 2013 obtained from Poisson regression models.

HCV risk factor analyses

The first analysis showed that the model with region and calendar year as main effects only (model 1.2) had the lowest AIC of the three sub-models, thus the best fit.

The second model showed that younger HIV-positive MSM had a higher risk of HCV infection (p=0.005) (Fig. 4A). The interaction term between age and region was borderline significant (p=0.05). Based on the model with the interaction term, in Western Europe, HCV incidence remained highest and stable until around age 35 and declined thereafter (Supplementary Fig. 3). In Northern and Southern Europe, HCV incidence increased until age 35, and declined thereafter.

In the third model, a higher HIV RNA was associated with higher HCV incidence (p=0.001) (Fig. 4C), especially when $\log_{10}$ HIV RNA was ≥5 copies/ml, whereas CD4 count (Fig. 4B) was not (p=0.53). When we added "HIV infection stage" to the model, the association between HIV RNA and HCV incidence was attenuated (p=0.01) (Fig. 4D). HCV incidence was higher during recent HIV infection than during chronic HIV infection (Incidence Rate $\text{Ratio}_{\text{recent vs. chronic}}$=1.8, 95%CI=1.1-2.7, p=0.02). The interaction term between HIV infection stage and HIV RNA was not significant (p=0.60), and was left out of the model. The association with CD4 count remained non-significant (p=0.53).

Sensitivity analyses

All sensitivity analyses showed comparable associations of HIV RNA, CD4 count and calendar year with HCV incidence and the conclusions were not altered. However, in the complete case analyses, HIV RNA was non-significant (p=0.25) (Supplementary Fig. 4).

In the model that included HIV infection stage, two sensitivity analyses (i.e. antepenultimate and predicted values) showed comparable associations between HIV RNA and HCV incidence, but when antepenultimate HIV RNA values were used, the association was no longer statistically significant (p=0.09). In the complete case analyses, there was no association (p=0.40).

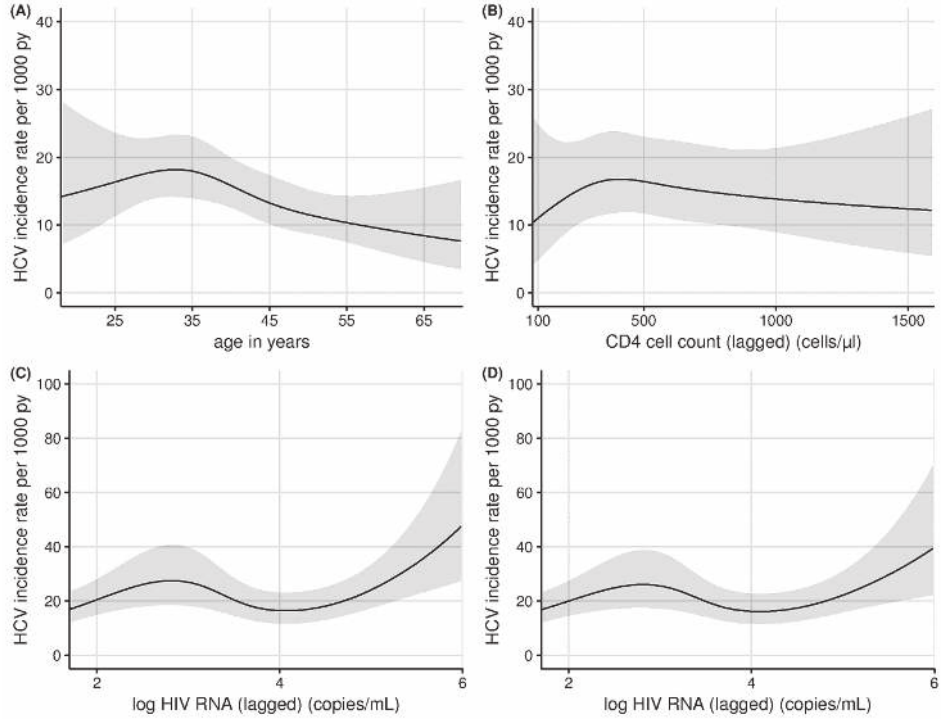

Fig. 4. HCV incidence by age, CD4 cell count and HIV RNA among HIV-positive MSM from the CASCADE Collaboration, in year 2007 in Western Europe[a]

4(A). Incidence by age in years (model 2)[b]

4(B). Incidence by CD4 count for an individual with a HIV RNA = 1000, aged 35 (model 3)

4(C). Incidence by HIV RNA for an individual with a CD4 cell count = 500, aged 35 (model 3)

4(D). Incidence by HIV RNA for an individual with a HIV RNA = 1000, aged 35, in the chronic HIV infection stage (model 3, including " HIV infection stage")

[a] The relative hazards obtained from the regression models were translated into the predicted incidence and this is illustrated for certain values of the covariates (e.g. only for Western Europe).

[b] Obtained from model 2 without the interaction term between age and region.

Time from HIV to HCV

Among 5,680 MSM who seroconverted for HIV at or after 1990, median time from HIV seroconversion to HCV infection was 5.2 years. The time from HIV seroconversion until HCV infection significantly decreased over calendar periods ($p_{log\text{-}rank}<0.001$). At 3 years after HIV seroconversion, the cumulative HCV incidence was 5.9% (95%CI=3.8-9.2%) in 2010-2014 compared to 2.0% (95%CI=0.5-7.8%) in 1990-1994 (Fig. 5). The Cox model showed that MSM who seroconverted for HIV in 2010, had a 6.1 (95%CI=2.8-13.3) times higher hazard of acquiring HCV than MSM who seroconverted in 1990 ($p<0.001$) (Supplementary Fig. 5).

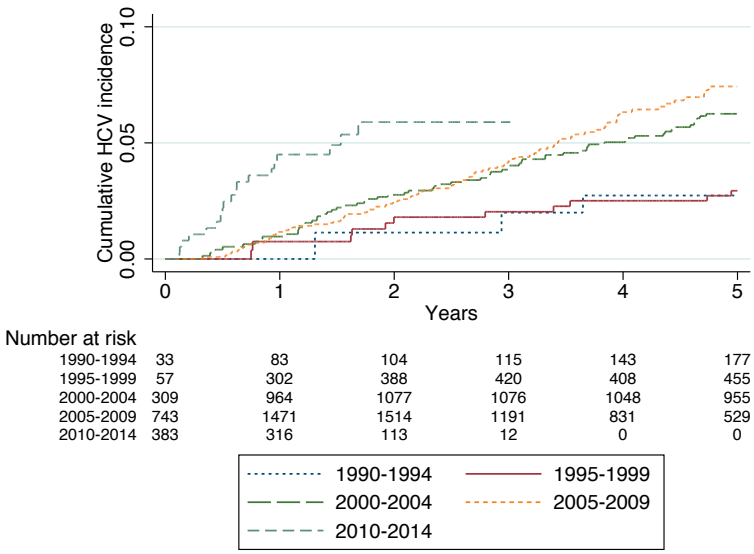

Fig. 5: Time from HIV seroconversion until HCV infection over time: Kaplan-Meier curves by calendar period of HIV seroconversion in the CASCADE Collaboration (1990-2014)[a]

[a] Curves were truncated when less than 10 individuals were at risk for HCV infection.
The log-rank test was used to assess changes in the time from HIV seroconversion to HCV infection among calendar periods.

DISCUSSION

Using data from the CASCADE Collaboration among HIV-positive MSM with well-estimated dates of HIV seroconversion, we showed that HCV incidence significantly increased from 1990 onwards and no decline was observed in recent years. This suggests on-going transmission of HCV among HIV-positive MSM. However, trends seem to differ by geographical region. While HCV incidence appears to have stabilized in Western Europe and remained stable in Southern Europe, a recent increase in HCV incidence was observed in Northern Europe. Interestingly, higher HIV RNA levels, recent HIV infection and younger age were associated with higher HCV incidence. The time from HIV seroconversion to HCV infection has significantly shortened in recent years. Hence, routine and continued surveillance following HIV diagnosis is needed.

The increasing trend in HCV incidence over time is comparable with the trend observed in a recent meta-analysis [2]. We estimated that in 2014 HCV incidence was between 18 and 21/1000 py and in the meta-analysis the extrapolated estimate was 19/1000 py in 2015 [2]. A recent study from EuroSida, not restricted to HIV seroconverters, also reported

that HCV incidence differed by European geographical region; Eastern, Northern and Southern Europe had higher odds for HCV seroconversion than Western Europe [19]. Interestingly, no HCV infections were observed among MSM from the Kenyan cohort, while another Kenyan study reported that 10% (30/300) of HIV-positive male and female patients were HCV-coinfected [20]. This might suggest that HCV has not yet been introduced in the Kenyan MSM population. The decline in HCV incidence that we observed after 2009 in Western Europe might be ascribed to earlier introduction or recognition of HCV. Consequently, as previously suggested [3], this might have led to a saturation effect among MSM at higher risk for HCV infection and/or increased HCV awareness, leading to more HCV testing and treatment, as well as safer-sex practices. Conversely, since the introduction of cART, condom use has declined among MSM [21,22], which probably led to the increase in syphilis incidence across European countries in recent years, especially among HIV-positive MSM [23]. In Northern Europe (UK and Norway) HCV incidence seems to have increased in recent years, although the overall effect of calendar year was only significant when method 2 was used. A European survey among MSM in 2010 showed that the prevalence of drug use associated with 'chemsex', i.e. drug use to enhance sexual arousal [24] – was highest in three UK cities [25]; as injecting and non-injecting drug use have been associated with acute HCV among HIV-positive MSM [5,7-9], differences in HCV trends might be partly explained by differences in drug use across European countries. However, we cannot discern whether that study is representative for MSM across Europe. Given the overall continued rise of HCV incidence, HCV-treatment guidelines should consider recommending direct-acting antivirals during acute HCV infection – when registered [26] – to prevent on-going transmission. As suggested by modelling studies, the greatest population benefit among HIV-positive MSM can be achieved when HCV treatment is provided within 1 year of HCV diagnosis, together with behavioural interventions [27,28].

Furthermore, we found that younger MSM, peaking at around age 35, are at higher risk for HCV infection, in line with findings from the Netherlands [3] but in contrast to a study in the USA, where older MSM had a higher risk of HCV infection [10]. Regional differences in the HCV epidemic among HIV-positive MSM could explain this discrepancy, in line with our finding of a borderline significant interaction between age and region.

HIV RNA was significantly associated with HCV incidence, especially when $\log_{10}$ HIV RNA was ≥5 copies/ml. Few studies have assessed the association between HIV RNA and HCV incidence [4,9,12] and, to the best of our knowledge, this is the only study to have modelled HIV RNA as a continuous variable in multivariable analysis. In univariable analyses, two observational cohort studies [4,9] found a significant association between HIV RNA with HCV incidence, whereas a clinical HIV cohort did not [1]. Although, in the Swiss

Cohort study, this association was no longer significant in multivariable analysis [4]; but ART use was included in that multivariable model which may mask the effect of HIV RNA as it may lie on the causal pathway. However, in our study, the association between HIV RNA and HCV incidence was attenuated when HIV infection stage was included in the model. The overlap in risk behaviour between HIV and HCV might result in the acquisition of both viruses simultaneously. We found that HCV infection is more likely during recent HIV infection and this is a period characterized by high HIV RNA levels, which might explain the stronger association between HCV incidence and HIV RNA when HIV infection stage is not included in the model. Additionally, until recently, these individuals might not be on cART. Our finding underscores the importance of monitoring HCV incidence and risk factors among HIV seroconverters.

Yet HIV RNA remained statistically significant. HIV RNA might partly explain why HIV-positive MSM have a higher risk of HCV infection than HIV-negative MSM [11]. The biological mechanism behind the association with HIV RNA may be through the activation of Langerhans cells (LCs) that results in the facilitation of HCV transmission, as immature LCs capture but do not transmit HCV, while activated LCs (due to HIV replication) are able to transmit the virus [29]. Alternatively, having an STI, a risk factor for HCV infection [4,6,9,10,12] leads to an increase in HIV RNA levels [30]. In that case, HIV RNA would be merely a proxy for having an STI. Also, higher HIV RNA levels might be surrogate for poor adherence to cART. Unfortunately, we could not assess the effect of STIs and cART adherence on HCV incidence, as most cohorts do not collect these data.

We found no association between HCV incidence and CD4 count, which is in line with most studies [3,4,6,12]. However, one showed that HIV-positive MSM with lower CD4 counts had a higher risk of acquiring HCV [9] while another study only found an association with CD4 counts below 500 cells/μl [10]. However, both studies did not exclusively include HIV seroconverters and did not account for time since HIV infection.

A previous study using data from the CASCADE Collaboration and the same estimating procedures, reported a similar increasing trend in HCV incidence until 2007 [1]. However, HCV incidence in 2007 using method 2 was considerably higher than our estimation (51/1000 vs. 21/1000 py); although confidence intervals were wide after 2005 and our estimates fall within this confidence intervals previously estimated [1]. The present study provides a more accurate estimate of HCV incidence after 2000 as additional HCV testing was performed to minimize bias related to selective testing and more MSM were included.

Our study has some limitations. First, as HCV infection was based on any kind of HCV test, an observed HCV infection might be a re-infection; although 99.1% (334/337) of HCV infections in our study were based on HCV-antibody seroconversion or evidence of acute/recent primary HCV infection. Also, since we lacked data on the mode of HCV transmission, we could not assess whether all HCV infections were sexually transmitted and whether changes in risk behaviour over time (e.g. increase in injecting drug use (IDU)) are driving the HCV epidemic. However, studies have reported HCV acquisition in the absence of traditional HCV risk factors, such as IDU, in the majority of MSM [4,5,7-10,12]. Hence, the increase in sexual risk behaviour among MSM (e.g. condomless anal intercourse [21]) is likely to partly explain the observed trends. Furthermore, although recreational drug use is common among MSM [25], recent studies have reported a low percentage of IDU among HIV-positive MSM with acute HCV infection (5.8% and 12.2%) [6,9]. To the best of our knowledge, evidence of an increase in IDU is scarce as only one study assessed trends in recent years; an increase in IDU, from 45.1% in 2005 to 53.8% in 2014, was observed among HIV-positive MSM reporting methamphetamine use in Australia [31]. Further research is needed to assess changes over time in HCV-related risk factors and the proportion of HCV acquisition attributable to sexual practices and drug use among MSM. Despite the lack of behavioural data, the main focus of our study was to assess temporal trends in HCV incidence, irrespective of the mode of HCV transmission. Furthermore, it is important to bear in mind that clinicians may have monitored patients at risk for HCV infection better over time, leading to more HCV-positive test results in recent years. To account for this possible bias we performed additional HCV testing and we only included data from the date of routine testing onwards. However, the median and mean number of tests was 3 and 4, respectively, over a median follow-up time of 4 years, suggesting that current guidelines [32] might not be followed consistently. This is in line with results from EuroSida where only a median of three tests were performed per patient between 2002 and 2013 [19]. In addition, due to a lack of country specific HCV testing guidelines (e.g. Italy), HCV testing practices may not be systematic.

The strengths of our study are that we had data from HIV seroconversion onwards for a large group of MSM, and extensive follow-up that enabled us to assess temporal changes in time from HIV seroconversion to HCV infection and the association between HCV incidence and HIV infection stage. We also applied different estimating methods to calculate HCV incidence and various sensitivity analyses. All methods showed comparable results suggesting that our results are robust.

To conclude, no decline in HCV incidence was observed in recent years, although trends seem to differ by geographical region. Hence, HCV screening among HIV-positive MSM should be continued and routinely and frequently offered. Furthermore, targeted pre-

ventive measures should be implemented and/or scaled-up to decrease the risk of HCV acquisition. Other than recent calendar year, younger age, recent HIV infection and high HIV RNA levels were all associated with HCV incidence.

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See **appendix 1** in **chapter 2.2** for the full list of CASCADE Collaborators.

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SUPPLEMENT CHAPTER 2.1

Supplementary text

1. Interval-censored data method

The likelihood is maximized based on the interval-censored HCV status information. As the current available version of the software cannot correct for left-truncated data, follow-up was divided in 5 calendar periods of 5 years each, from 1990 to 2014. This method has the advantage that we can also include data from MSM with only HCV-positive test results who have a left censored infection time. MSM were included in a calendar period if they were considered at risk and had been (at least partly) followed during that period, irrespective of whether or not they had HCV results in that particular calendar period. Follow-up in the interval censored method was calculated from the moment MSM became at risk (as previously described in the main text of the manuscript) until the last clinic visit. Some MSM only had HCV test results before becoming at risk. For these MSM, if the last result was positive, they were categorized HCV positive at the moment of becoming at risk. MSM with only HCV-negative test results, but with the last negative results at most two years before becoming at risk, were assumed to remain HCV negative until that moment. When the last HCV negative test took place more than 2 years before becoming at risk, the individual was excluded (n=77). The likelihood maximizes the cumulative incidence. This estimate was transformed into the hazard using kernel smoothing.

A total of 7,787 MSM were included of whom 336 acquired HCV during follow-up (i.e. had HCV negative and positive test results between being considered at risk and last clinic visit), 271 had HCV-positive results without HCV-negative test results and 7,180 MSM had only HCV-negative test results. Among these 7,180 HCV-negative MSM, 809 had their last HCV negative test within two years before becoming at risk. The median time between the last HCV-negative test and becoming at risk among the 809 MSM was 0.08 years (IQR=0.03-0.23). The number of MSM included in each of the five calendar periods was 231 in 1990–1994, 1,005 in 1995–1999, 3,517 in 2000-2004, 5,691 in 2005-2009 and 5,760 in 2010-2014.

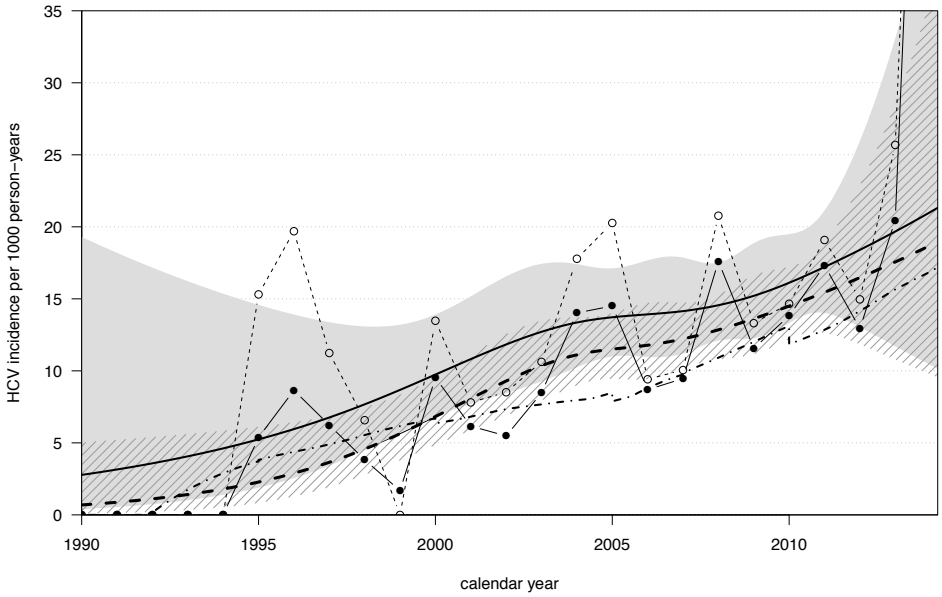
2. Random-effect models

Missing data:

In model 3, if individuals had a clinic visit, but a missing value for CD4 and/or HIV RNA, we imputed missing values using a separate random-effects model for each marker, stratified by cART usage (as described in the main text). In each model, we allowed for a

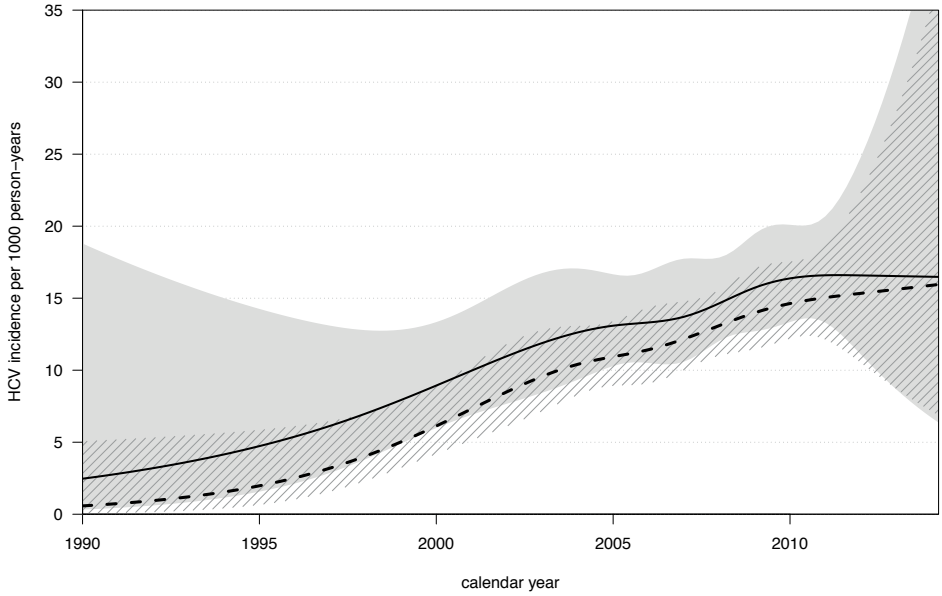
random intercept and a random slope per individual. When an individual did not have a clinic visits in a calendar year and hence CD4 and HIV RNA were not measured, we created an additional record in between when consecutive visits were less than 2 years apart; this additional record was also imputed.

Supplementary Figures

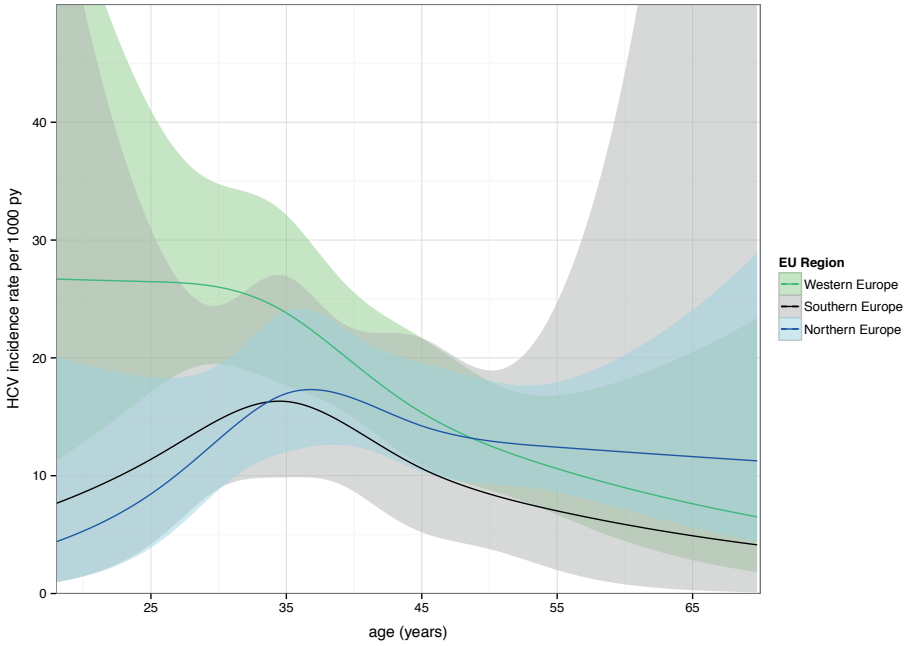


Supplementary fig. 1: HCV incidence among HIV-positive MSM using three methods to estimate follow-up in the CASCADE Collaboration; 1990-2014

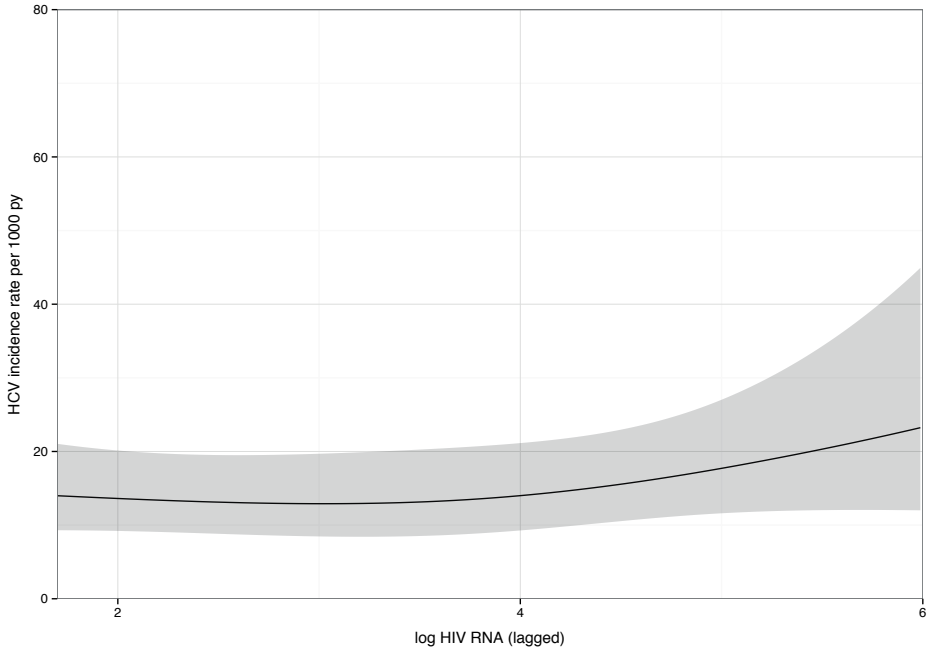
Method 1: dashed line; 95%CI: dashed area. Method 2: solid line; 95%CI: grey area. Interval censored data method (IC): point-dash line. Black points are the observed incidence per year in method 1 and white points are the observed incidence per year in method 2.



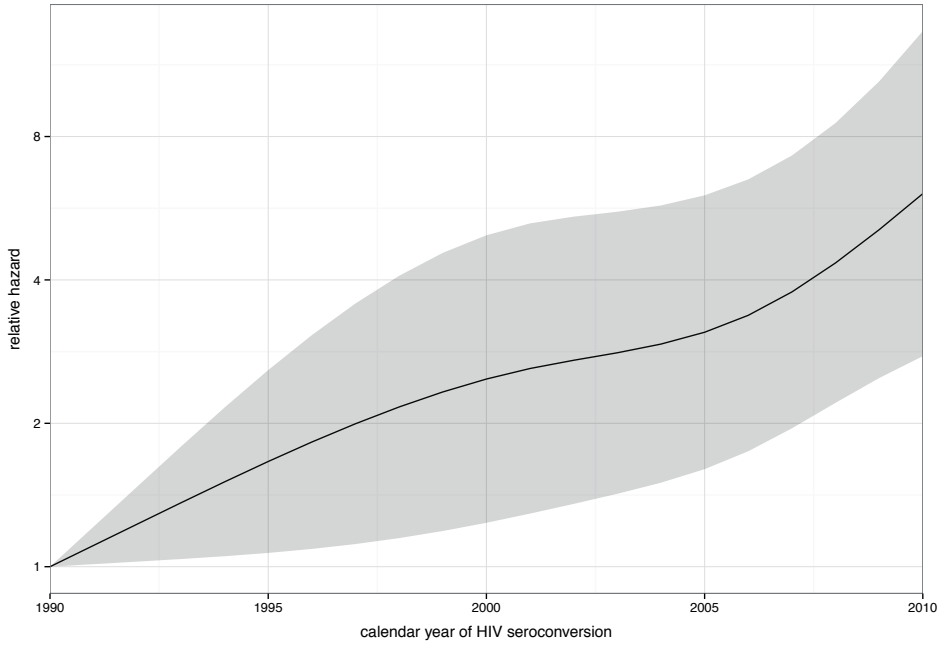
Supplementary Fig. 2: Sensitivity analyses: HCV incidence among HIV-positive MSM excluding the Italian cohort (ICoNA) in the CASCADE Collaboration; 1990-2014
Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.



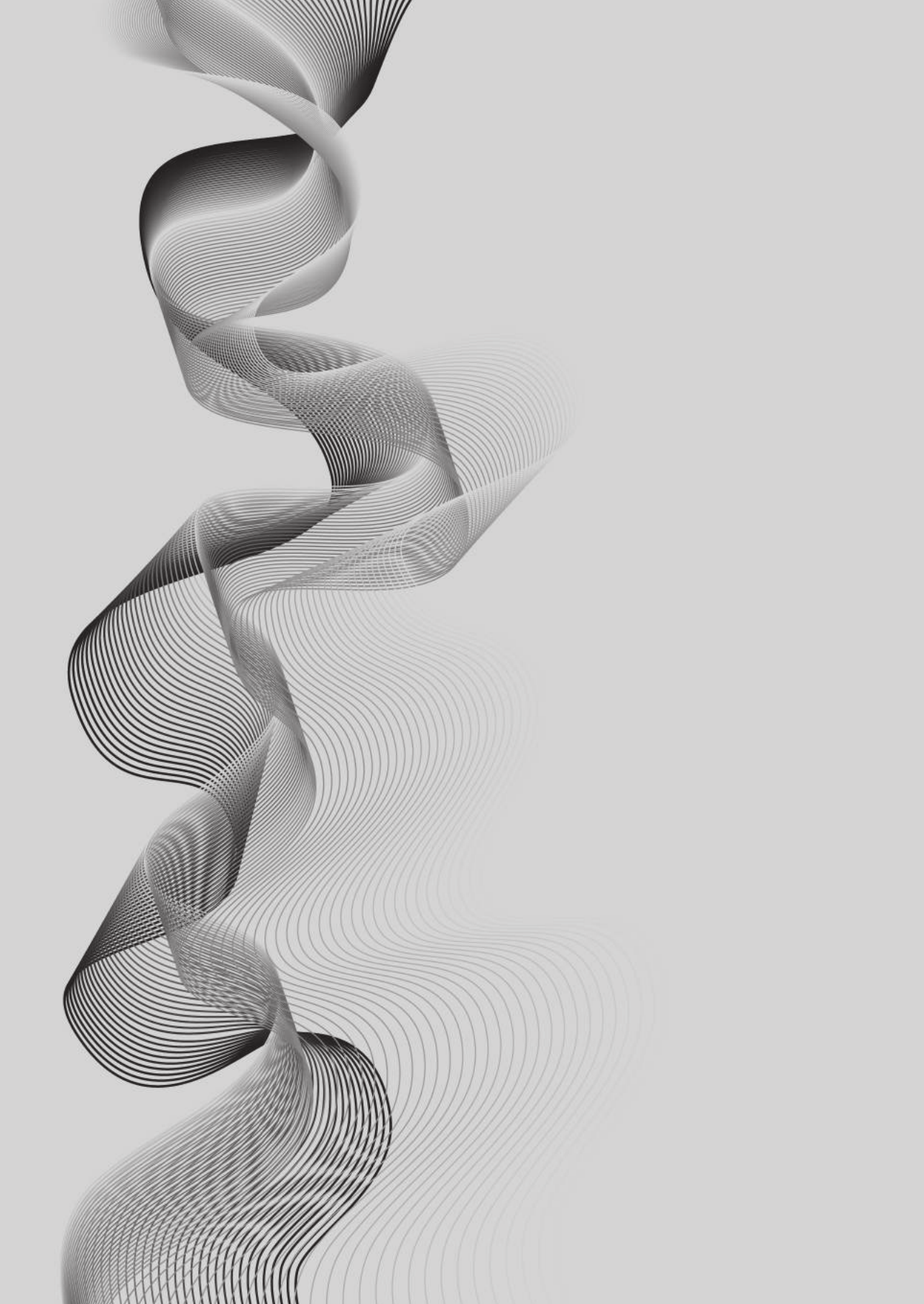
Supplementary Fig. 3: HCV incidence by age and European geographical region among HIV-positive MSM from the CASCADE Collaboration; 1990-2014[a]
[a]Canada and Australia are included in the model but are not depicted in this graph.
EU = European



Supplementary Fig. 4 (Complete case analyses): HCV incidence by HIV RNA value among HIV-positive MSM from the CASCADE Collaboration; 1990-2014
Grey solid areas depict the 95% confidence interval.



Supplementary Fig. 5: Relative hazard of HCV infection by calendar year of HIV seroconversion among MSM from the CASCADE Collaboration; 1990-2014
Grey solid areas depict the 95% confidence interval.



Chapter 2.2

Effect of incident hepatitis C virus infection and its timing following HIV seroconversion on CD4 T-cell count and HIV RNA trajectories

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* Maria Prins and Ronald Geskus contributed equally as senior co-authors.

Submitted for publication

ABSTRACT

Background: Most studies examining the effect of hepatitis C virus (HCV) co-infection on HIV disease progression do not account for the order of these two infections. We aimed to assess the effect of incident HCV infection, and its timing relative to HIV seroconversion (HIVsc) in HIV-positive men who have sex with men (MSM) on their subsequent CD4 T-cell count (CD4) and HIV-RNA viral load (VL) trajectories.

Methods: We included HIV-positive MSM with well-estimated dates of HIVsc from 17 cohorts within the CASCADE Collaboration. HCV co-infected MSM were matched to as many HIV mono-infected MSM as possible by HIV-infection duration and cART use. We used multilevel random-effects models stratified by cART use to assess differences in CD4 and VL trajectories by HCV co-infection status.

Results: We matched 214 (ART-naïve) and 147 (on cART) HCV co-infected MSM to 5,384 and 3,954 respectively matched controls. The timing of HCVsc relative to HIVsc had no demonstrable effect on VL or CD4 trajectories. In the first 2-3 years following HCVsc CD4 counts were lower among HCV co-infected compared to HIV mono-infected MSM, but became comparable thereafter (both ART-naïve and MSM on cART). In ART-naïve MSM, during the first two years after HCVsc, VL levels were lower or comparable to HIV mono-infected, tending to be higher thereafter. In MSM on cART, HCV had no significant effect on having a detectable VL.

Conclusions: Among HIV-positive MSM, the timing of HCVsc relative to HIVsc did not impact CD4 or VL trajectories. CD4 counts were temporarily lower following HCVsc irrespective of cART use. ART-naïve HCV co-infected MSM had a different VL trajectory than HIV mono-infected. The clinical short- and long-term implications of our findings remain to be elucidated.

INTRODUCTION

At the beginning of the millennium, a hepatitis C virus (HCV) epidemic emerged in HIV-positive men who have sex with men (MSM) [1]. In recent years, HCV has continued to spread in this group [2,3]. HIV infection often precedes HCV infection in MSM. This differs from the main risk groups in early studies of HIV/HCV co-infection, people who inject drugs (PWID) and haemophiliacs, in whom HCV was generally acquired before HIV infection [4,5]. It has been suggested that the order of HIV and HCV acquisition may influence the effect of HCV co-infection on disease progression [6]. Moreover, given that the extent of excessive alcohol use and other factors associated with HCV and HIV disease progression differ between groups at risk of HCV infection, HIV/HCV co-infected individuals are not a homogeneous population.

One recent meta-analysis concluded that among ART-naïve HIV-positive individuals, those co-infected with HCV had similar HIV RNA viral loads (VL) to HIV mono-infected individuals [5], while other studies have reported that they do have faster CD4 T-cell count (CD4) decline [7, 8]. Among individuals on cART, another meta-analysis reported that HCV co-infection leads to significantly lower CD4 counts shortly after initiating cART, but that HCV co-infection has no effect on achieving viral suppression [4]. However, most studies in both of these meta-analyses included a heterogeneous risk group population (e.g. PWID and haemophiliacs), assessed the difference in VL using a single measurement in ART-naïve individuals, and included individuals with a prevalent HIV and/or HCV infection [4, 5]. They were therefore unable to distinguish the sequence or duration of the two viral infections. Consequently, little is known about the effect of incident HCV infection and its timing relative to duration of HIV infection on subsequent HIV disease progression among MSM. Using data from the CASCADE Collaboration with a large number of MSM with well-estimated dates of HIV seroconversion (HIVsc), we are uniquely positioned to study HIV/HCV co-infection in this group. In this study, we aimed to assess the effect of HCV seroconversion (HCVsc) and its timing, relative to HIVsc, on the VL and CD4 trajectories following HCVsc among MSM with newly-acquired HCV while ART-naïve and while on cART.

METHODS

Study population

We used data from the CASCADE Collaboration within EuroCoord (www.EuroCoord.net) that includes cohorts across Europe, Australia, Canada and Sub-Saharan Africa. Details of CASCADE have been previously described [9]. All cohorts include data from

HIV-positive individuals with dates of HIVsc that could be reliably estimated based on the midpoint between the last HIV-negative and first HIV-positive test dates (at most 36 months apart) or, with evidence of acute HIV infection. We included 17 of the 28 participating cohorts. Five cohorts were excluded as these were non-MSM cohorts, and 6 cohorts because they had tested less than 50% of MSM for HCV (missing HCV status ranging from 57.2%-96.2%) and could not provide stored samples for additional HCV testing (Figure 1). To optimize testing frequency, we performed additional HCV testing in cohorts that had stored specimens (9 cohorts), as previously described [3]. We included only men who were self-reported as having acquired HIV through sex between men and whose potential HIV transmission route excluded injection drug use.

Definitions and exclusion criteria

HCV negative status throughout follow-up was based on having at least one HCV-negative test result and never being tested HCV positive. HCV-positive status was based on any positive HCV test (RNA, antibodies and/or antigen). For MSM who acquired HCV during follow-up, we assumed that HCV seroconversion (HCVsc) occurred at the mid-point date between the last HCV-negative and first HCV-positive test. In ART-naïve MSM, the date of HCVsc was based on HCV-antibody seroconversion in 74.7% of cases, and two RNA tests in 6%. Among MSM on cART, these percentages were 80.0% and 3.6%, respectively. The remainder was based on a combination of antibody and RNA test results. Among MSM on cART, individuals who were HCV-positive before cART initiation were excluded from the cART analyses (n=207), and thus our analyses do not consider the effect of incident HCV while ART-naïve on CD4 and VL trajectories after the start of cART (Figure 1). In order to determine the timing of HCVsc as precisely as possible, we also excluded MSM with an HCVsc interval of more than two years (n=69). Furthermore, men with only HCV-positive test results throughout follow-up were excluded if the first HCV-positive test result was more than one year after HIVsc (n=119). For MSM with a positive HCV test within one year of HIVsc but without a recorded HCV negative one (n=127), the date of HCVsc was estimated as the midpoint date between HIVsc and first HCV-positive test date, as HCV infection is not common among HIV-negative MSM [10,11]. Alternative methods for estimating the date of HCVsc were considered as described in sensitivity analyses. MSM with an HCV-positive test before HIVsc (n=28) were excluded from the main analyses.

Timing of HCVsc relative to HIVsc (hereafter referred to as "timing") was calculated as the interval between the estimated dates of HIVsc and HCVsc. In those who acquired HCV while on cART, we calculated the cumulative time on cART, excluding time off cART due to a treatment interruption (hereafter referred to as "cumulative cART exposure"). We

defined cART as a 3 drug ART regimen containing 2 different classes, or 3 nucleoside reverse transcriptase inhibitors (NRTIs), provided tenofovir or abacavir were included in the regimen.

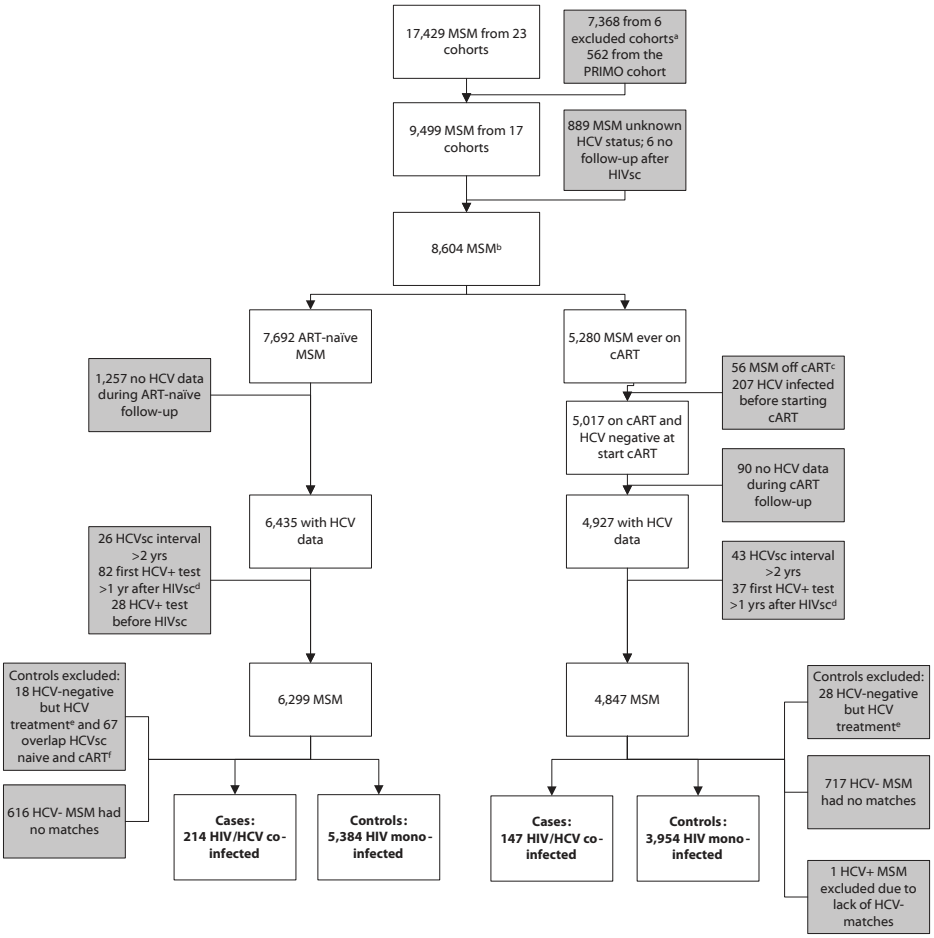

Figure 1: Flow diagram of the study population selection for ART-naïve MSM and MSM on cART from the CASCADE Collaboration

Abbreviations: yr(s), year(s), HIVsc, HIV seroconversion; HCV, Hepatitis C virus; HCVsc, HCV seroconversion; cART, combination antiretroviral therapy; HCV+, HCV-positive MSM; HCV-, HCV-negative MSM. The grey boxes depict MSM who were excluded from the analyses.

[a] Excluded cohorts: cohorts of which > 50% of MSM had a missing HCV status.

[b] Of 8,604 MSM, 4,502 (53.2%) MSM contributed data as ART-naïve as well as when on cART.

[c] 56 MSM had ever been on cART, but were off cART during follow-up.

[d] MSM without a recorded HCV-negative test results.

[e] Excluded due to possible HCV treatment, defined as having ever received pegylated-interferon and/or ribavirin, and never having an HCV-positive test result.

[f] Excluded as the interval between HCVsc while on cART and last visit while ART-naïve was less than two.

Statistical analyses

Follow-up data

Individuals could contribute data from the first clinic/cohort visit after the estimated date of HIVsc from 1983 until 2014. Furthermore, for all cohorts, we used all available follow-up data, except for MSM from the French PRIMO cohort who were censored at the 31st December 2005 as routine HCV test results were only recorded until that year. ART-naïve MSM were censored at start of (c)ART, or last study visit if they remained (c)ART naïve. MSM on cART were censored at the moment of a treatment interruption (if off cART for more than a week) or last study visit.

Matching

We performed separate analyses for ART-naïve MSM and MSM on cART. To assess the effect of incident HCV infection and its timing, each HCV-infected individual (the 'case') was matched to all HCV-negative MSM (the 'controls') who fulfilled the matching criteria by HIV infection duration (For details on matching criteria see supplementary text 1). Hence, we could compare CD4 and VL trajectories following the estimated date of HCVsc of an HCV co-infected MSM to that of an HIV mono-infected MSM with a similar duration of HIV infection. Hereafter we refer to 'matched time' of the control as the matched duration since HIVsc. The duration since HIVsc used to matched cases and controls was determined by the moment of HCVsc relative to HIVsc of the case.

Statistical models

The time origin of our analyses is the estimated date of HCVsc of each case, and their control's matched time. From this time origin onwards, we modelled trends in CD4 and VL over time using multilevel random-effects models which included a random intercept and slope. Based on the scatterplot, we decided to use the 8th root transformation of VL, which gave a more symmetric distribution than the $\log_{10}$ transformation. For CD4 we used the cube root transformation. Given the small numbers of records with a detectable VL among MSM on cART (8.9% of all VL measurements), we assessed the effect of HCV on having a detectable VL (defined as: VL >400 copies/mL) using a multilevel random-effects logistic regression model. In the multilevel model structure, measurements were nested within individuals (second level) and individuals were nested within case-control groups (first level).

The multivariable models included duration from HIVsc to HCVsc (i.e. 'timing') and the following co-variables as potential confounders and/or effect modifiers: age and calendar year at matched time. For the ART-naïve model we also included method of HIVsc determination (i.e. midpoint or (laboratory) evidence of acute infection). For those

on cART, we also included cumulative cART exposure. We used restricted cubic splines to model the effect of continuous variables on the outcome, with four knots based on 5th, 33rd, 66th, 95th percentiles. We included interaction terms between time since HCVsc/matched time, HCV co-infection status and timing of HCVsc relative to HIVsc to assess whether HCV co-infection or its timing influenced CD4 and VL trajectories. Furthermore, we included interaction terms to assess whether the effect of HCV co-infection and its timing on the CD4 and VL trajectory differed by age or calendar year, and for those on cART, cumulative cART exposure (for model details see supplementary text 2). We graphically illustrate the VL and CD4 trajectories by HCV-infection status at the median value for the other continuous co-variables (e.g. age) and the most frequent category for the dichotomous variables (i.e. method of HIVsc determination).

Sensitivity analyses

First, for ART-naïve MSM with an HCV-positive test result but without a recorded HCV-negative test result, we applied two alternative strategies to estimate the moment of HCVsc. In the first strategy, we assumed that risk behaviour led to simultaneous infection with both HCV and HIV. In the second strategy, we assumed they became HCV infected at the time of their first HCV-positive test result. Second, we repeated the analyses restricting our population to ART-naïve cases with both an HCV-negative and an HCV-positive test during follow-up and their matched controls.

Additionally, we examined the effect of HCV co-infection on CD4 and VL trajectories using joint models [12] (except for the analysis with detectable VL as outcome in MSM on cART) in order to correct for informative censoring (due to cART initiation among ART-naïve, and cART interruption among MSM on cART).

RESULTS

Of 17,429 MSM included in CASCADE, 8,604 MSM from 17 cohorts were eligible after applying the exclusion criteria described above (Figure 1). Of these individuals, 7,692 (89.4%) were ART-naïve during the first visit after HIVsc and 5,224 (60.7%) had available data while on cART. A total of 214 HCV co-infected ART-naïve MSM and 147 HCV co-infected MSM on cART were included in the study, of whom 95 and 139 had well-estimated dates of HCVsc, respectively. HCV co-infected MSM were successfully matched at random to 5,384 and 3,954 HIV mono-infected ART-naïve MSM and MSM on cART, respectively. Among HCV co-infected MSM, median age at matched time and follow-up since the time origin were respectively, 35 years [Interquartile range (IQR):29-41] and 1.1 years [0.3-2.6] in ART-naïve MSM, and 40 years [IQR:35-47] and 2.1 years [0.9-3.9] in MSM

on cART (Table 1). Median time from HIVsc to HCVsc was 0.4 years [IQR=0.1-1.0] among ART-naïve and 6.2 years [IQR=3.3-10.7] among MSM on cART. Among HCV co-infected MSM on cART, median cumulative cART exposure at HCVsc was 3.2 years [IQR=1.0-6.1] and 75.5% of these MSM were on their first cART regimen when they acquired HCV.

Table 1: General and clinical characteristics of HIV-positive MSM with and without HCV infection from the CASCADE Collaboration by cART use

	ART-naïve MSM		MSM on cART	
	HIV/HCV co-infected	**HIV mono-infected**	**HIV/HCV co-infected**	**HIV mono-infected**
N	214	5,384	147	3,954
Age, m (IQR)[a,b]	35 (29-41)	34 (28-40)	40 (35-47)	38 (32-45)
HIVsc estimation method				
Midpoint, n (%)	154 (72.3%)	3,998 (75.2%)	121 (82.3%)	2,752 (69.6%)
Acute HIV, n (%)	59 (27.7%)	1,321 (24.8%)	26 (17.7%)	1,202 (30.4%)
HCV+ and HCV- test result[c]	95	*NA*	139	*NA*
Width HCV infection interval, m (IQR)[b,c]	0.7 (0.4-1.2)	*NA*	0.8 (0.5-1.1)	*NA*
Matches per case, m (IQR)	21 (15-30)	*NA*	19 (10-38)	*NA*
Time from HIVsc to matched time[b,d]	0.4 (0.1-1.0)	0.5 (0.1-1.4)	6.2 (3.3-10.7)	3.7 (1.9-6.3)
Follow-up, m (IQR)[b,e]	1.1 (0.3-2.6)	0.7 (0.04-2.2)	2.1 (0.9-3.9)	1.5 (0.5-3.6)
Calendar year of HIVsc, m (IQR)	2005 (2002-2008)	2006 (2002-2009)	2001 (1996-2004)	2003 (1998-2007)
Calendar year, m (IQR)[a]	2007 (2004-2009)	2007 (2003-2010)	2008 (2005-2011)	2008 (2004-2011)
CD4 cell count (cells/µl), m (IQR)[a]	483 (358-660)	494 (363-663)	508 (367-710)	535 (398-700)
VL (copies/ml), m (IQR)[a]	46,175 (15,950-150,037)	40,900 (10,455-130,612)	50 (40-50)	50 (40-104)
Detectable VL, %[f]	93.7%	95.3%	3.6%	9.2%
Cumulative cART exposure, m (IQR)[a,b]	*NA*	*NA*	3.2 (1.0-6.1)	1.0 (0.3-2.7)

Abbreviations: N, number; n, median; IQR, interquartile range; VL, HIV RNA viral load; HIVsc, HIV seroconversion; NA, not applicable; HCV+, HCV positive; HCV-, HCV negative.

[a] At matched time[d]

[b] Represented in years.

[c] MSM with an HCV-negative and HCV-positive test result during follow-up.

[d] Matched time: HCV seroconversion among HCV co-infected MSM and matched time among HIV mono-infected.

[e] From matched time onwards; i.e. time origin.

[f] Percentage of detectable VL records during follow-up since matched time onwards.

CD4 and VL trajectories

ART-naïve MSM

Fitted VL trajectories are shown in Figure 2A for three different values of the timing of HCVsc relative to HIVsc. At the time origin (i.e. HCVsc or matched time), VL was not significantly different between cases (i.e. HCV co-infected) and controls (i.e. HIV mono-infected) (p=0.32). The difference in VL trajectory between cases and control was statistically significant (p=0.03). VL trajectories by HCV co-infection status, although not statically significant (p=0.24), differed by the timing of HCVsc. If HCV and HIV seroconversion occurred around the same time, both cases and controls showed a strong downward trend in VL during the first year following HIV and HCV seroconversion (Figure 2A, first left panel). However, in MSM who seroconverted for HCV at one year after HIVsc or later, we observed a downward trend in VL for about one year following HCVsc, which was not observed in the controls. After two years from HCVsc, HCV co-infected MSM appeared to have a faster increase in VL, and some suggestion of a higher VL later on compared to HIV mono-infected MSM (Figure 2A, second and third panel). However differences in actual VL values at any time point were small. The effect of HCV co-infection on VL trajectory did not significantly differ by age (p=0.21).

At the time origin, CD4 did not significantly differ between cases and controls (p=0.90) (Figure 2B). The difference in CD4 trajectory between cases and controls was highly significant (p<0.001), but this difference did not depend on HCVsc to HIVsc timing (p=0.78). CD4 decreased more rapidly during the first years following HCVsc in HCV co-infected MSM, but after three years following HCVsc values became comparable to those of HIV mono-infected MSM. For example, when comparing MSM who seroconverted for HIV and HCV simultaneously and their controls, the difference in CD4 at one year following HCVsc/matched time was 43 CD4 cells/µl (figure 2B, left panel). The effect of HCV co-infection on CD4 trajectory did not significantly differ by age (p=0.50).

MSM on cART

Fitted probabilities of having detectable VL are shown in Figure 3A for three different values of the timing. For an "average" individual, the probability of having a detectable VL was below 2% for both cases and controls, and did not significantly differ by HCV co-infection status over time following HCVsc/matched time (p=0.17). However, controls had a borderline higher probability of having a detectable VL at the time origin (p=0.05). The timing of HCVsc relative to HIVsc had no effect on VL (p=0.35). The effect of HCV co-infection on VL trajectory did not significantly differ by age (p=0.76) nor by cumulative cART exposure (p=0.60).

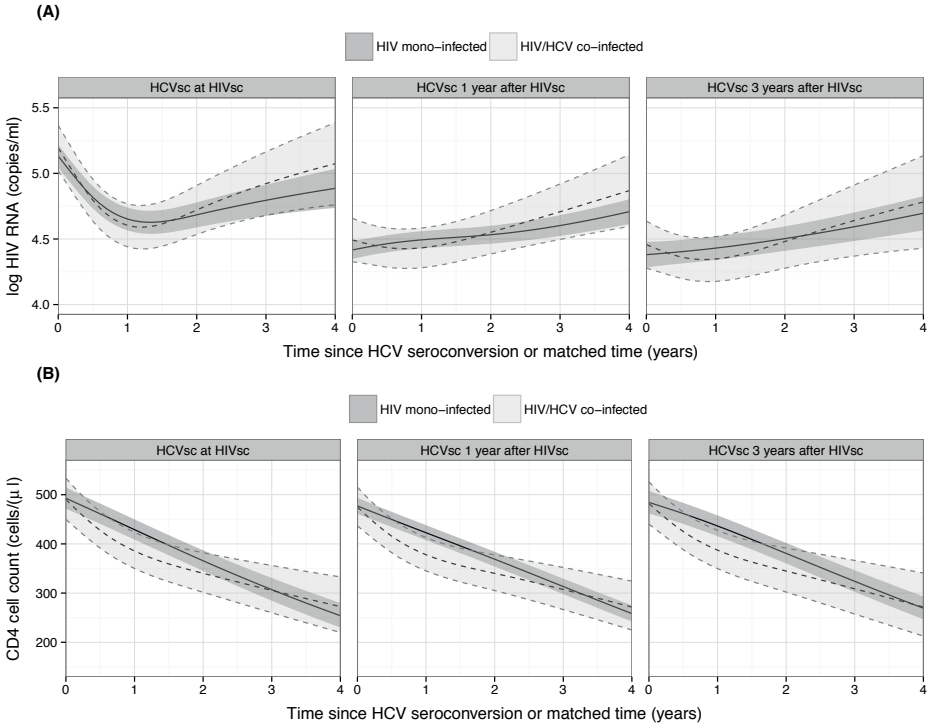

Figure 2: CD4 counts and HIV RNA viral load trajectories from HCV seroconversion or matched time onwards per timing of HCV seroconversion relative to HIV seroconversion, among ART-naive MSM from the CASCADE Collaboration. Figure 2A: HIV RNA viral load trajectories; Figure 2B: CD4 cell count trajectories

Abbreviations: HCVsc, HCV seroconversion; HIVsc, HIV seroconversion. The solid lines represent median HIV RNA viral load (VL) and CD4 counts trajectories for HIV mono-infected MSM, with 95%CI illustrated in gray. Dashed lines represent median VL and CD4 counts trajectories for HIV/HCV co-infected MSM, with 95%CI illustrated with light gray dashed lines. VL and CD4 counts were back-transformed from 8th root of VL to 10-log VL copies/ml and cube root CD4 counts to CD4 counts cells/µl. The first (left) panel (i.e. 'HCVsc at HIVsc', timing=0) represents VL or CD4 counts trajectory for those individuals who acquired HCV concurrently with HIV. The second (middle) panel represents MSM who seroconverted for HCV 1 year following HIVsc, and the third (last) panel represents MSM whose HCV seroconversion took place 3 years after HIVsc. All graphs are illustrated for an individual aged 35 years whose HIV seroconversion was estimated based on the midpoint date of a negative and a positive antibody test date, and seroconverted for HCV in 2005 (or matched calendar year for HIV mono-infected).

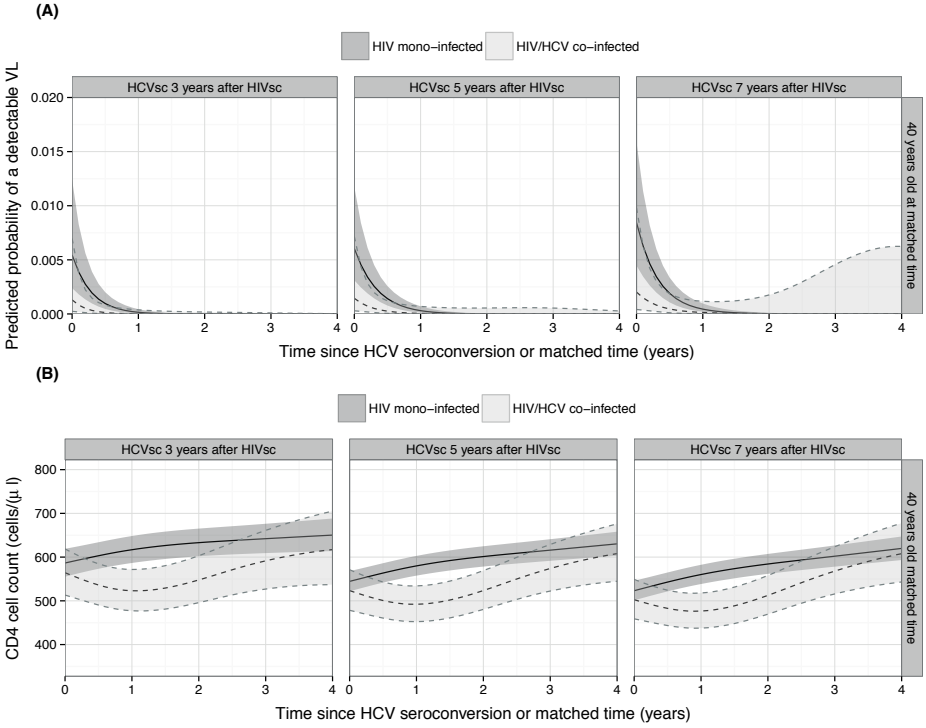

Figure 3: CD4 counts trajectories and predicted probabilities of having a detectable HIV RNA viral load from HCV seroconversion or matched time onwards per timing of HCV seroconversion relative to HIV seroconversion, among MSM on cART from the CASCADE Collaboration. Figure 3A: Predicted probabilities of having detectable HIV RNA viral load; Figure 3B: CD4 cell count trajectories

Abbreviations: HCVsc, HCV seroconversion; VL,HIV RNA viral load; HIVsc, HIV seroconversion. The solid lines represent predicted probabilities of having a detectable HIV RNA viral load (VL) and median CD4 counts trajectories for HIV mono-infected MSM, with 95%CI illustrated in gray. Dashed lines represent the predicted probabilities and median CD4 counts trajectories for HIV/HCV co-infected MSM, with 95%CI illustrated with light gray dashed lines. Cube root CD4 counts were back-transformed to CD4 counts cells/µl. First (left), second (middle) and third (right) panel represent MSM who seroconverted for HCV 3, 5 and 7 years after HIVsc, respectively. All graphs are illustrated for an individual aged 40 years who had been on cART for 3 years at the matched visit and seroconverted for HCV in 2008 (or matched calendar year for HIV mono-infected).

At the time origin, CD4 did not significantly differ between cases and controls (p=0.33) (Figure 3B). Similar to ART-naïve MSM, CD4 trajectories were significantly different between cases and controls (p<0.001), and did not depend on the timing of HCVsc (p=0.69). During the first two to three years after HCVsc, CD4 were significantly lower among HCV co-infected MSM compared to HIV mono-infected MSM, and became comparable to HIV mono-infected MSM thereafter. For example, when comparing MSM who seroconverted for HCV three years after HIVsc (Figure 3B, lest panel) to their controls, the difference in CD4 count at one year following HCVsc/matched time was 83 CD4 cells/µl. The effect of HCV co-infection on CD4 trajectory did not significantly differ by age (p=0.38) nor by cumulative cART exposure (p=0.99).

Sensitivity analyses

When we assumed that HCVsc took place simultaneously with HIV or at the time of the first HCV-positive test among HCV co-infected ART-naïve MSM without HCV-negative results, comparable results to the main analyses were obtained and conclusions were not altered. When analyses were restricted to ART-naïve MSM with a documented HCVsc during follow-up, the difference in CD4 and VL trajectory was still statistically significant (p_{CD4}<0.001; p_{VL}=0.04) (Supplementary Figure 2). However, a lower VL in cases than controls was no longer observable, while differences in VL trajectory were more pronounced after two years from HCVsc, especially when HCVsc was closer to HIVsc (p_{timing}=0.09). Similarly, the effect of timing was not significant for the CD4 model. Furthermore, joint models yielded similar results to the main analysis, although the effect of HCV co-infection on VL trajectory became borderline non-significant (p=0.05).

DISCUSSION

We investigated in MSM with pre-existing HIV infection the effect of newly-acquired HCV infection, and its timing relative to HIV seroconversion, on subsequent HIV-RNA viral load and CD4 T-cell count trajectories. We did not find a significant effect of the timing of HCV acquisition relative to HIV seroconversion on these trajectories. In HCV co-infected MSM, CD4 cell counts were however temporarily lower during the first two to three years following HCVsc compared to HIV mono-infected MSM, in both ART-naïve MSM and MSM on cART. Furthermore, we found that HCV co-infection had an effect on the VL trajectories in ART-naïve MSM, but we did not find a change in the probability of having a detectable VL following HCVsc in MSM on cART.

Few studies have been able to assess the effect of HCV co-infection on CD4 trajectories among ART-naïve HIV-positive individuals. Two studies, with relatively small sample sizes

and with an unknown sequence of HIV and HCV acquisition [7,8], also reported a steeper CD4 decline in HCV co-infected individuals when compared to HIV mono-infected individuals [8] or individuals who spontaneous cleared HCV as the control group [7]. However, the effect of HCV co-infection was not found to be statistically significant in the latter study [7]. To the best of our knowledge, only one study from the UK among ART-naïve patients (i.e. PWID, MSM and heterosexuals), with known HIV seroconversion dates, measured the effect of the HCV co-infection on CD4 trajectories. This study also found a temporary effect of HCV co-infection: during the first 13 months after HCV infection there was borderline evidence of a faster CD4 decline among HCV co-infected individuals, and afterwards, values became comparable to HIV mono-infected individuals [13].

Similar to our findings among MSM on cART, a meta-analysis and an original study among HIV-positive MSM with acute HCV also found an initial decline in CD4 among HCV co-infected individuals, but the difference between the two groups attenuated or disappeared after 2 years [4,14]. The primary outcome in the meta-analysis was difference in CD4 increase 3 to 12 months after cART initiation whereas our study examined CD4 trajectories after HCV seroconversion. In addition, they did not account for the sequence and duration of both infections. These factors might explain why some of the individual studies in this meta-analysis did not find an effect of HCV on CD4 trajectories [4]. The temporary effect of HCV co-infection on CD4 might be mediated through a heightened state of chronic inflammation, leading to enhanced CD4 apoptosis [15,16]. Interestingly, in our study the negative effect of HCV and convergence of CD4 trajectories between cases and controls occurred irrespective of cART use. Hence, the attenuation of the effect of HCV co-infection is probably not affected by cART use only.

The clinical short- and long-term implications of the temporary CD4 decline warrant further research. It is unknown whether the observed temporary CD4 decline attributes to the faster liver fibrosis progression observed in HIV/HCV co-infected individuals when compared to HCV mono-infected individuals [17]. Among HIV-positive MSM with acute HCV, recent studies have suggested an accelerated liver fibrosis progression compared to the classical HCV risk groups caused by the underlying degree of immune-compromise due to HIV infection when HCV is acquired [18-20], although others could not confirm this finding [21]. In our study though, the timing of HCVsc after HIVsc did not seem to affect CD4 nor VL trajectories, irrespective of cART use. Moreover, a previous study using data from the CASCADE Collaboration showed that in the cART era (>1996), HCV co-infected MSM have a higher HIV/AIDS mortality than HIV mono-infected MSM [22]. Whether the temporary CD4 decline contributes to a faster HIV disease progression still needs to be elucidated. Furthermore, ART response may be affected by HCV infection if

ART initiation takes place within the first three years following an HCV infection as CD4 will be temporarily lower, which in turn is associated with HIV disease progression [23]. The effect of the temporary lowering of CD4 in relation to HCV treatment effectiveness is also unknown, although cure rates with direct-acting antivirals (DAA) among HIV co-infected patients are similar to those in HCV mono-infected patients [24,25]. One could argue that HCV treatment shortly after an HCV infection is justifiable to prevent accelerated liver disease progression and a CD4 decline. Notwithstanding, continued follow-up after HCV infection is warranted to assess the long-term effects of HCV on liver fibrosis and HIV disease progression.

Our results on the effect of HCV on VL in ART-naïve individuals are not in agreement with a meta-analysis reporting no difference in VL by HCV co-infection status [5]. However, the primary outcome in this meta-analysis was based on the mean VL difference from a single VL measurement and most of the included studies could or did not account for duration of HIV and HCV infection [5]. Interestingly, 4 of the 15 individual studies in the meta-analysis reported a significantly higher VL among HIV mono-infected individuals, which was also observed in our main analysis during the first year following HCVsc. However, when our analyses were restricted to those with a documented HCVsc during follow-up, we did not observe a lower VL in HCV co-infected MSM. Importantly, although VL differences by HCV co-infection status in our study were small, it has been demonstrated that even small increments in VL among ART-naïve individuals are associated with a higher risk of heterosexual transmission and AIDS-defining event or death [26].

Another explanation for the statistically significant difference in VL trajectory among ART-naïve MSM by HCV co-infection status is that we may have observed a decreasing VL trend following an unobserved peak in VL at the actual moment of HCV infection. However, the investigators of a previous study among HIV-positive individuals with acute HCV only observed such a temporary increase in VL around HCV infection among those on cART [14]. In our study, we did not observe such a peak among MSM on cART either. We may have missed this VL peak as our time origin was HCV seroconversion in the majority of cases, whereas that study estimated the date of HCV infection based on the midpoint date between HCV RNA tests with a narrower test interval (i.e. 12 months). Our results of VL among MSM on cART are in line with the previously described meta-analysis where authors also reported that virological control of HIV infection after cART initiation remains unaffected by the presence of HCV [4].

There are some limitations in our study. Due to a lack of systematic data on HCV treatment, we could not account for it. A study among HIV/HCV co-infected patients comparing CD4 changes before and after pegylated-interferon (and ribavirin) treatment

reported that CD4 decreased during the first 12 weeks of treatment, increasing thereafter and stabilizing from week 24 onwards [27]. However, HCV treatment alone could not explain the temporarily lower CD4 among HCV co-infected MSM as we observed an effect of HCV on CD4 trajectories for at least two to three years following HCVsc. Furthermore, we also did not account for spontaneous HCV clearance. This may have led to an underestimation of the effect of chronic HCV co-infection on CD4 and VL trajectories. However, only around 15% of HIV-positive individuals have been reported to clear HCV spontaneously [28]. Also, we did not account for other factors that could influence CD4 and VL trajectories such as ART adherence, HIV super-infection and co-infection with sexually-transmitted infections other than HCV [29,30]. However, if these factors play an important role in the observed differences, we cannot explain why CD4 converged after three years after HCVsc.

One of the major strengths in our study is our relatively large group of MSM with well-estimated dates of both HIV and HCV seroconversion, hence, we could account for infection duration, and study the effect of the timing of HCVsc relative to HIVsc. Additionally, unlike most studies, we used all available CD4 and VL measurement to assess differences in trajectories by HCV co-infection status.

In conclusion, we found no difference in CD4 and VL trajectories following HCV seroconversion by its timing relative to HIV seroconversion. Importantly, CD4 counts are temporarily lower during the first two to three years following HCV seroconversion among HIV-positive MSM. Even though it is expected that more MSM will start cART earlier in the coming years, reflecting changing guidelines [31], CD4 counts are temporarily negatively affected following HCV seroconversion despite use of cART. Our findings would point to a consideration by clinicians to test for HCV if their HIV-positive patient's CD4 count drops while on cART. HCV co-infected ART-naïve MSM appear to have a higher VL trajectory two years after HCVsc than HIV mono-infected MSM, whereas we did not observe an effect of HCV on the probability of having a detectable VL among MSM on cART. Continued HCV prevention, testing and treatment are warranted in this group. The short- and long-term clinical implications of our findings still need to be further elucidated.

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SUPPLEMENT CHAPTER 2.2

Supplementary text

1. Matching criteria

The following criteria were applied to match cases and controls: 1) during the first year following HIV seroconversion (HIVsc), the matched follow-up visit of the control had to be at most 36 days apart (i.e. 0.1 years) from the first visit of the case at or following the estimated date of HCV seroconversion (HCVsc) – as acute HIV infection is a phase characterized by fluctuating and dynamic CD4 T-cell counts (CD4) and HIV RNA viral load (VL) values [1] – and 2) after one year following HIVsc, the matched visits had to be ≤0.5 years apart – as changes in CD4 and VL are more gradual during the chronic HIV infection phase [1]. Additionally, for ART-naïve MSM, we matched by country of the cohort, to adjust for unmeasured differences (e.g. HIV-related care guidelines) that may influence CD4 and VL trajectories. MSM on cART were matched additionally for cumulative cART exposure. Although we intended to also match MSM on cART by country of cohort, due to difficulties in finding matches for some MSM, we omitted this matching criterion. Each case was matched to as many HIV mono-infected MSM as possible who fulfilled all matching criteria. Every control could only be matched once to an HCV co-infected MSM when ART-naïve or when on cART, but an individual (i.e. controls or cases who acquired HCV while on cART) could contribute data to both the ART-naïve and on cART analyses. Furthermore, as illustrated in Supplementary Figure 1, follow-up time following the matched time and the reason for censoring could differ between cases and controls.

2. Multivariable models

2.1 Covariates and interaction terms

In the multivariable models, calendar year at matched time was included as a proxy for unmeasured HCV treatment, as this increased over time among men who have sex with men (MSM) [2,3]. Also, better cART regimes over time may have influenced CD4 and VL trajectories. The method of HIVsc determination was included because those MSM whose HIVsc was determined through evidence of acute (symptomatic) HIV infection might have a more accurate estimate of the date of HIV seroconversion. In addition, CD4 and VL trajectories might differ between MSM with and without symptomatic acute HIV infection [4]. In the interaction terms, all continuous variables were modelled linearly, except for the interaction between time since HCVsc or matched time (i.e. time origin) and timing of HCVsc relative to HIVsc, which was modelled using restricted cubic splines. For MSM on cART, the interaction term between time since HCVsc or matched time and cumulative cART exposure was also modelled via splines. We graphically illustrate the

VL and CD4 trajectories (back-transformed to 10-log VL copies/ml and CD4 cells/µl) by HCV-infection status at the median value for the other continuous co-variables (e.g. age) and the most frequent category for the dichotomous variables (i.e. method of HIVsc determination). Similarly, we illustrate the effect of HCV co-infection on having a detectable VL while on cART by translating the results from the random-effects logistic regression models into predicted probabilities for an average individual, i.e. an individual with values zero for the random effect terms.

2.2 Code used in R

Example of the model used to model VL trajectories among ART-naïve MSM.

Variable	Description:
VL	HIV RNA viral load
Case [1]	HIV/HCV (co)-infection status (case/control)
Time (in years)	Time from HCV seroconversion or matched time onwards (i.e. time origin)
Age (in years)	Age at matched time
Calendaryr	Calendar year at matched time
Timing (in years)	Timing of HCV seroconversion relative to HIV seroconversion
Serohow [1]	HIV seroconversion determination method
Groupmatch	Case and controls matched group

[1] *Dichotomous variables. The remainder are continuous variables.*

Code:	**Explanation:**
lme((HIV RNA)^(1/8)	*VL at the time origin[a] (baseline) and*
~ns(time,kn=c(0.5,2),	*VL trajectory (over follow-up time)*
Bo=c(0,7))*case	*can differ by HCV co-infection status*
+ns(age,kn=c(32,40),Bo=c(24,52))	*VL at the time origin can depend on age in a non-linear way*
+time:age	*VL trajectory can depend on age*
+time:case:age	*Effect of age on VL trajectory can differ by HCV co-infection status*
Effect of the timing:	
+ns(timing,kn=c(0.3,1.1),Bo=c(0,4))	*VL at the time origin can depend on timing in a non-linear way*
+ns(time,kn=c(0.5,2),Bo=c(0,7)): ns(timing,kn= c(0.3,1.1),Bo=c(0,4))	*VL trajectory can depend on timing in* a *non-linear way*

+timing:age	*Effect of timing on VL at time origin can differ by age*
+time:case:timing	*Effect of timing on VL trajectory can differ by HCV co-infection status*
+time:timing:age	*Effect of the timing on VL trajectory can depend on age*
+time:case:timing:age	*Effect of the timing on VL trajectory can depend on age, and this effect can differ by HIV/HCV co-infection status*

Effect of calendar year:

+ns(calendaryr,kn=c(2002,2007), Bo=c(1991,2011))	*VL at the time origin can depend on calendar year*
+calendaryr:time	*VL trajectory can depend on calendar year*
+calendaryr:time:case	*Effect of calendar year on VL trajectory can differ by HIV/HCV co-infection status.*

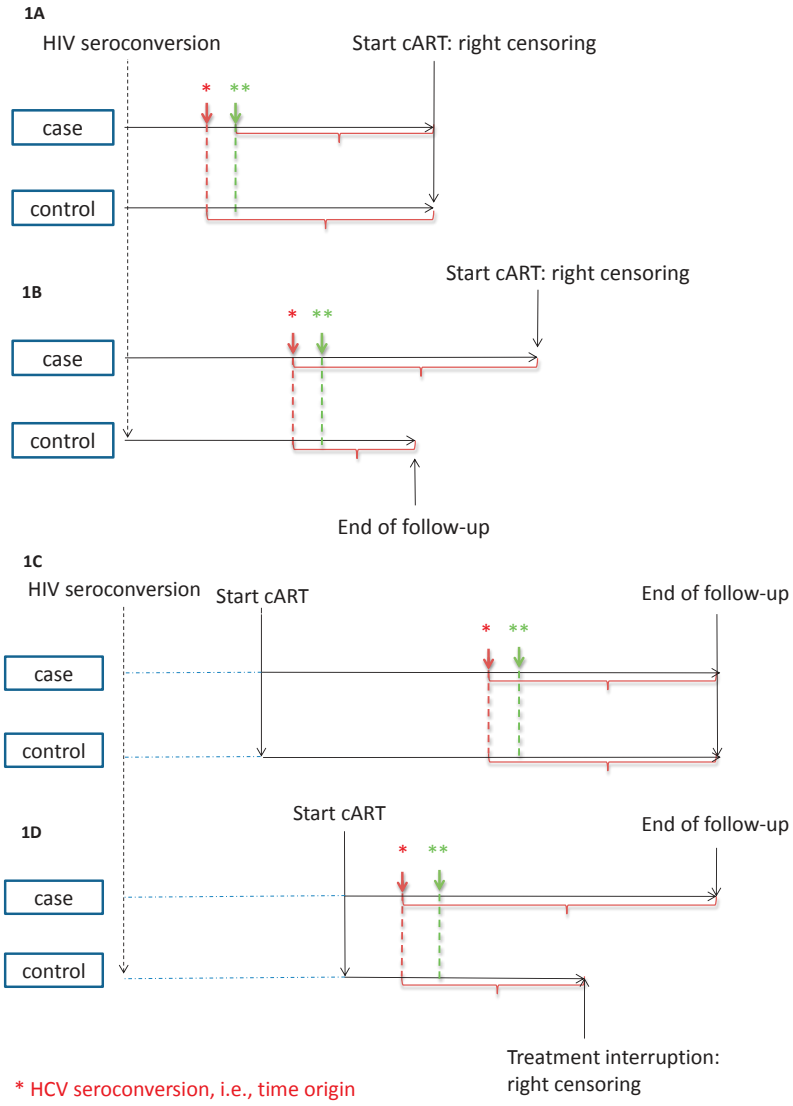
Effect of HIV seroconversion method of estimation:

+serohow	*VL at the time origin can depend on method of HIVsc determination*
+serohow:case	*Effect of the method of HIVsc determination on the overall VL can depend on HIV/HCV co-infection status*
+serohow:time	*VL trajectory can depend on method of HIVsc determination*
random=list(groupmatch= ~1,patient=~time))	*Multilevel model with random intercept & slope per individual, nested within matched groups*

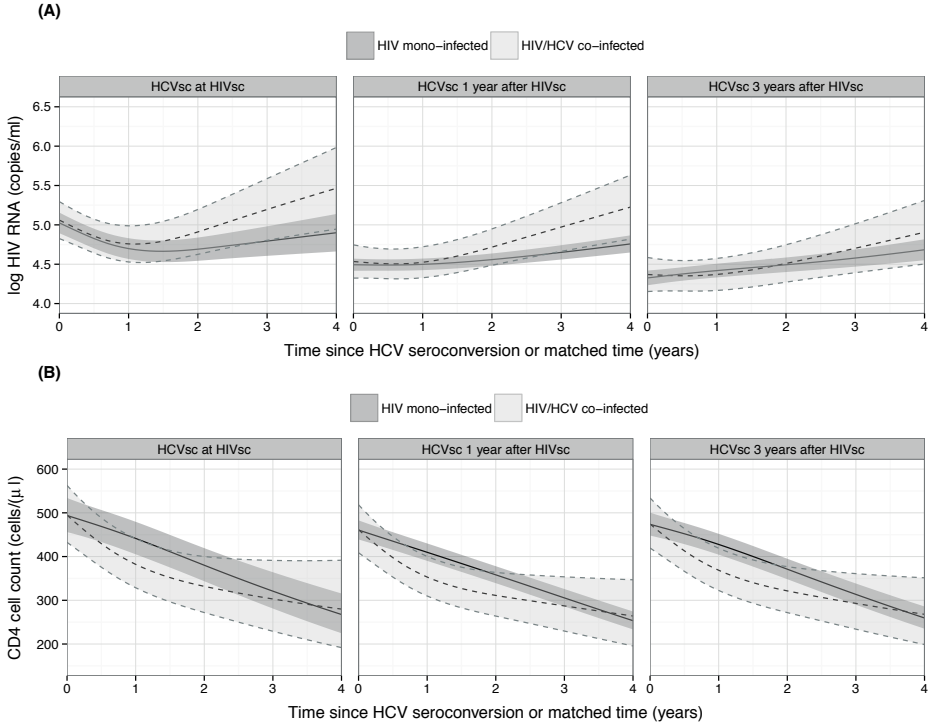
Abbreviations: ns,natural spline function; kn, Knots; Bo, Boundary.Knots; HIVsc, HIV seroconversion

[a]*Time origin:* The time origin of our analyses is the estimated date of HCV seroconversion of each case, and their control's matched time.
In the cART model we added "*cumulative time on cART*" in the same way as the variable "timing". Also, a 4-way interaction including cumulative time on cART "timing:timingcART:case:time" was included.

Supplementary Figures



Supplementary Figure 1. Graphical illustration of the matching process and follow-up among ART-naïve MSM (1A & 1B) and MSM on cART (1C & 1D) using four examples.
Red arrows represent the timing of HCV seroconversion relative to HIV seroconversion and the red horizontal brackets represent the included follow-up in our study. Green arrows represent the first visit after HCVsc in cases and the matched visit of the controls. Blue dotted lines among MSM on cART represent follow-up while ART-naïve. Example 1A and 1C, represent matched cases and controls with the same follow-up period following HCV seroconversion, whereas in example 1B and 1D, follow-up after HIV seroconversion is shorter among controls. However, in other case-control groups follow-up may be longer in controls. Among HCV co-infected MSM, the median time from HCVsc to the first matched visit was 0.1 years for both ART-naïve MSM and for MSM on cART.



Supplementary Figure 2: CD4 counts and HIV viral load trajectories from HCV seroconversion or matched time onwards per timing of HCV seroconversion relative to HIV seroconversion, among ART-naive MSM with a recorded HCV-negative and a HCV-positive test date from the CASCADE Collaboration. Figure 2A: HIV RNA viral load trajectories; Figure 2B: CD4 cell count trajectories

Abbreviations: HCVsc, HCV seroconversion; HIVsc, HIV seroconversion. The solid lines represent median HIV RNA viral load (VL) and CD4 counts trajectories for HIV mono-infected MSM, with 95%CI illustrated in gray. Dashed lines represent median VL and CD4 counts trajectories for HIV/HCV co-infected MSM, with 95%CI illustrated with light gray dashed lines. VL and CD4 counts were back-transformed from 8th root of VL to 10-log VL copies/ml and cube root CD4 counts to CD4 counts cells/µl. The first (left) panel (i.e. 'HCVsc at HIVsc', timing=0) represents VL or CD4 counts trajectory for those individuals who acquired HCV concurrently with HIV. The second (middle) panel represents MSM who seroconverted for HCV 1 year following HIVsc, and the third (last) panel represents MSM whose HCV seroconversion took place 3 years after HIVsc. All graphs are illustrated for an individual aged 35 years whose HIV seroconversion was estimated based on the midpoint date of a negative and a positive antibody test date, and seroconverted for HCV in 2005 (or matched calendar year for HIV mono-infected).

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Ethics committees: Ethics approval has been granted by the following committees: Austrian HIV Cohort Study: Ethik-Kommission der Medizinischen Universität Wien, Medizinische Universität Graz – Ethikkommission, Ethikkommission der Medizinischen Universität Innsbruck, Ethikkommission des Landes Oberösterreich, Ethikkommission für das Bundesland Salzburg; PHAEDRA cohort: St Vincent's Hospital, Human Research Ethics Committee; Southern Alberta Clinic Cohort: Conjoint Health Research Ethics Board of the Faculties of Medicine, Nursing and Kinesiology, University of Calgary; Aquitaine Cohort: Commission Nationale de l'Informatique et des Libertés; French PRIMO Cohort: Comite Consultatif de Protection des Personnes dans la Recherché Biomedicale; German HIV-1 Seroconverter Study: Charité, University Medicine Berlin; AMACS: Bioethics & Deontology Committee of Athens University Medical School and the National Organization of

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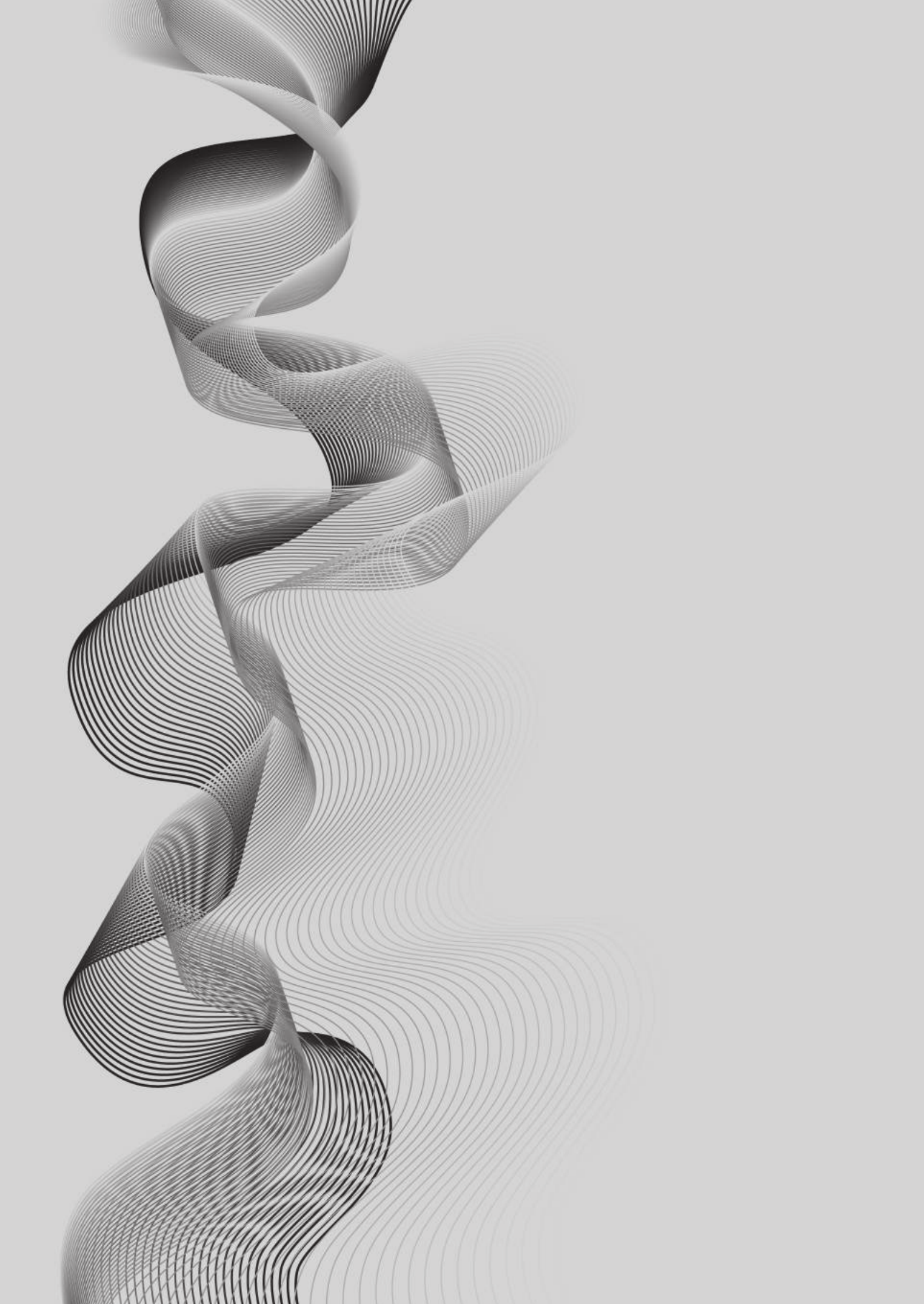

Chapter 2.3

The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM

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ABSTRACT

Objectives: A large portion of anogenital cancers is caused by high-risk human papillomavirus (hrHPV) infections, which are especially common in HIV-infected men. We aimed to compare the incidence and clearance of anal and penile hrHPV infection between HIV-infected and HIV-negative MSM.

Design: Analyses of longitudinal data from a prospective cohort study.

Methods: MSM aged 18 years of older were recruited in Amsterdam, the Netherlands, and followed-up semi-annually for 24 months. At each visit, participants completed risk-factor questionnaires. Anal and penile self-samples were tested for HPV DNA using the SPF_{10}-PCR DEIA/$LiPA_{25}$ system. Effects on incidence and clearance rates were quantified via Poisson regression, using generalized estimating equations to correct for multiple hrHPV types.

Results: Seven hundred and fifty MSM with a median age of 40 years (interquartile range= 35-48) were included in the analyses, of whom 302 (40%) were HIV-infected. The incidence rates of hrHPV were significantly higher in HIV-infected compared with HIV-negative MSM (adjusted incidence rate ratio (aIRR) 1.6; 95% confidence interval (CI) 1.3-2.1 for anal and aIRR 1.4; 95%CI 1.0-2.1 for penile infection). The clearance rate of hrHPV was significantly lower for anal (adjusted clearance rate ratio (aCRR) 0.7; 95%CI 0.6-0.9), but not for penile infection (aCRR 1.3; 95%CI 1.0-1.7). HrHPV incidence or clearance did not differ significantly by nadir $CD4^+$ cell count.

Conclusion: Increased anal and penile hrHPV incidence rates and decreased anal hrHPV clearance rates were found in HIV-infected compared with HIV-negative MSM, after adjusting for sexual behavior. Our findings suggest an independent effect of HIV infection on anal hrHPV infections.

INTRODUCTION

Infection with human papillomavirus (HPV) is highly prevalent throughout the world, and is associated with several types of cancer [1,2]. Persistent HPV infection with a high-risk (hr)HPV type (mainly HPV-16 or -18) is the likely cause of around 80% of anal cancers and 40-50% of penile cancers [3-5]. Anal cancer is relatively rare in the general population, but men who have sex with men (MSM), and especially HIV-infected MSM, are at much higher risk [6]. Moreover, the incidence of anal cancer has shown an increasing trend in recent decades [7,8].

Prevalence of anogenital HPV infection in men varies widely across studies, with estimates up to 93% in HIV-infected MSM [9-11]. However, there is little insight in the anal and penile HPV incidence and clearance among MSM. In addition, although it is known that HIV-infected individuals are at increased risk of HPV infection and HPV-related cancer [6,12], the effect of HIV infection on the natural history of HPV infection is not well understood. In particular, it is not clear whether the increased HPV infection risk among HIV-infected individuals can be mainly explained by biological (i.e. HIV-related immunosuppression) or behavioral factors (i.e. shared routes of transmission).

The present study aimed to compare the anal and penile hrHPV incidence and clearance between HIV-infected and HIV-negative MSM over two years of follow-up, and to assess the effect of HIV-related immunosuppression on HPV incidence and clearance.

MATERIALS AND METHODS

Study participants

Study methods have been described in detail previously [13]. In short, HIV-negative and HIV-infected MSM were invited to participate in the HIV and HPV in MSM (H2M) cohort study from July 2010 to July 2011 at three sites in Amsterdam, the Netherlands: the Amsterdam Cohort Study (ACS) among MSM (Public Health Service Amsterdam), a sexually transmitted infections clinic (Public Health Service Amsterdam), and an infectious disease outpatient clinic (Jan van Goyen Medical Center). Men were eligible for participation if they were aged 18 years or older and conversant in Dutch and/or English. The Medical Ethics Committee of the Academic Medical Center Amsterdam approved this study and all participants provided written informed consent prior to enrolment.

Data collection

Data were collected at baseline and at 6-month intervals during an intended follow-up time of 24 months, aiming at five visits per participant. At each visit, participants completed detailed self-administered questionnaires and collected an anal and a penile self-swab (regular flocked swab with 1 ml Universal Transport Medium, Copan, Brescia, Italy). For the anal swab, participants inserted the swab 3 cm into the anal canal and turned it around for 5-10 seconds. For the penile swab, participants rubbed the swab firmly over the skin of the penile shaft, including the outside of the foreskin, for 20 seconds (i.e. 5 seconds per side). HIV-related data were obtained from the Dutch HIV Monitoring Foundation's national HIV patients database.

Human papillomavirus DNA detection and genotyping

The anal and penile samples were stored at '20° Celsius and analyzed as previously described [13]. DNA extraction was performed using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany). DNA amplification, testing for HPV DNA, and HPV genotyping was performed using the highly sensitive SPF_{10}-PCR DEIA/$LiPA_{25}$ system (version 1) [14]. $LiPA_{25}$ allows simultaneous detection of 25 specific mucosal HPV genotypes, of which HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 were classified as high-risk [1].

Statistical analyses

Baseline characteristics were compared between groups by X^2-squared test for categorical data; for continuous variables, the Student's t-test was used for normally distributed data and the rank sum test when the data were not normally distributed. To deal with missing questionnaire data (Supplementary Text 1) we imputed missing values using the MICE (multivariate imputation by chained equations) technique [15]. Before imputing missing data, the number of recent (i.e. in the last 6 months) anal sex partners, lifetime male sex partners, and current and nadir CD4 cell count were transformed to the logarithmic scale given their skewed distribution. In addition, complete-case analyses were performed, using only the available data per participant.

The outcomes of interest were anal and penile hrHPV incidence and clearance. In our main analyses (assumption I), incidence was defined as one positive test result for a specific hrHPV type at a given visit preceded by two consecutive negative test results for that HPV type. Clearance was defined as one positive test result for a specific hrHPV type followed by two consecutive negative visits. All analyses were performed on an HPV type-specific level and restricted to the 12 hrHPV types. Persons were at risk for a specific HPV type until their first event of that type, but remained at risk for other types.

Type-specific anal and penile hrHPV incidence and clearance rates were calculated separately by HIV status, with corresponding incidence rate ratios (IRR) and clearance rate ratios (CRR). Kaplan-Meier curves were constructed to explore the cumulative incidence and clearance of HPV-16 and HPV-18 by HIV status. To further assess the effect of HIV infection, crude and adjusted IRR and CRR for HIV-infected versus HIV-negative MSM were calculated for HPV-16 and -18 separately using Poisson regression. We also assessed the effect for the 12 hrHPV types combined via Poisson regression, using generalized estimating equations with an exchangeable correlation structure to account for multiple HPV types per person [16].

Variables that were a priori included in the multivariable models for anal HPV incidence and clearance – based on literature [17] and our previous analyses [13] – were: age, number of lifetime male sex partners (both collected at baseline), number of recent anal sex partners, current smoking, recent cannabis and/or poppers use, anal sex position, having been rimmed (i.e. anal-oral contact), receptive fisting (all time-updated and asked over the previous 6 months), anal STI diagnosis at time of visit (chlamydia and/or gonorrhea), and HPV type. The same variables were a priori included in the multivariable models for penile HPV incidence and clearance, with the exception of having been rimmed, receptive fisting and anal STI, and the addition of circumcision status at baseline and urethral STI diagnosis at time of visit (chlamydia and/or gonorrhea). All continuous variables (i.e. age, number of lifetime and recent sex partners) were allowed to vary smoothly using natural cubic splines [18].

We also assessed crude and adjusted anal and penile IRR and CRR according to immune status (categorized into HIV-negative; HIV-infected with nadir CD4 cell count >350 cells/µl; HIV-infected with nadir CD4 cell count between 200 and 350 cells/µl; and HIV-infected with nadir CD4 cell count <200 cells/µl) using the same models and outcomes as described above. Furthermore, we assessed anal and penile CRR for incident (i.e. infections that were not detected at baseline) versus prevalent (i.e. infections that were detected at baseline) hrHPV infections using the same models and outcomes as described above.

Sensitivity analyses were performed using alternative definitions of incidence and clearance. In sensitivity analyses with assumption II, incidence was defined as one positive test result preceded by one negative visit, while clearance was defined as one positive test result followed by one negative visit. In sensitivity analyses with assumption III, incidence was defined as two consecutive positive test results preceded by one negative visit, while clearance was defined as two consecutive positive test results followed by one negative visit.

Statistical analyses were performed using Stata software package version 13.1 (Stata Intercooled, College Station, TX, USA) and the R statistical computing environment version 3.0.2. [19].

Role of the funding source

The funders had no role in study design; the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

RESULTS

Characteristics of the study population

Seven hundred and ninety-five MSM were enrolled in the H2M study, of which 750 provided sufficient baseline data and attended at least one follow-up visit. The 45 excluded MSM did not significantly differ with respect to HIV status or age compared with the 750 included MSM.

Of the 750 MSM, 302 (40%) were HIV-infected at baseline. Median follow-up time was 25 months (IQR 24-26) in HIV-negative and 24 months (IQR 23-25) in HIV-infected MSM; 83% had five study visits. HIV-infected MSM were significantly older, with a median age of 46 years (IQR 40-53) compared with 38 years (IQR 34-42) in HIV-negative MSM ($P<0.001$). The median number of lifetime male sex partners was 300 (IQR 100-1000) in HIV-infected versus 100 (50-400) in HIV-negative MSM ($P<0.001$). In HIV-infected MSM, the median nadir CD4 cell count was 230 cells/µl (IQR 170-320), 87% was using combination antiretroviral therapy and 79% had undetectable HIV viral load at baseline.

At baseline, anal hrHPV infection was detected in 46% (95% confidence interval (CI) 41-50%) of HIV-negative and 63% (95% CI 58-69%) of HIV-infected MSM ($P<0.001$). Penile hrHPV infection was detected in 17% (95% CI 13-20%) and 31% (95% CI 26-37%) of MSM, respectively ($P<0.001$). A detailed description of the study population with anal and penile HPV prevalence at baseline has been published previously [13].

Anal and penile high-risk HPV incidence

In total, 705 MSM attended at least two follow-up visits, and were included in our main analyses. Among these 705 MSM, 652 incident anal and 419 incident penile hrHPV infections were observed. Type-specific anal and penile hrHPV incidence rates, stratified by HIV status, are shown in Table 1A. Anal incidence rates were higher in HIV-infected compared with HIV-negative MSM (except for HPV-51), with statistically significant differences for HPV types 16, 31, 35, 52, and 56. Anal HPV-16 incidence rate was 4.7/1000

Table 1A: Incidence rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM (H2M study, Amsterdam 2010-2013).

Anal incidence												
	HIV-negative MSM					**HIV-infected MSM**						
HPV type	**Number at risk**	**Incident events**	**PMO**	**IR**	**95% CI**	**Number at risk**	**Incident events**	**PMO**	**IR**	**95% CI**	**IRR**	**95% CI**
16	377	30	6430.3	4.7	(3.3-6.7)	230	31	3415.0	9.1	(6.4-12.9)	2.0	(1.2-3.2)
18	404	26	7051.2	3.7	(2.5-5.4)	249	22	3920.6	5.6	(3.7-8.5)	1.5	(0.9-2.7)
31	394	35	6635.2	5.3	(3.8-7.3)	225	40	3223.6	12.4	(9.1-16.9)	2.4	(1.5-3.7)
33	401	23	7014.5	3.3	(2.2-4.9)	252	18	4033.9	4.5	(2.8-7.1)	1.4	(0.7-2.5)
35	412	21	7270.8	2.9	(1.9-4.4)	255	26	3878.2	6.7	(4.6-9.8)	2.3	(1.3-4.1)
39	406	32	7043.0	4.5	(3.2-6.4)	261	24	4152.7	5.8	(3.9-8.6)	1.3	(0.8-2.2)
45	410	31	6913.2	4.5	(3.2-6.4)	258	26	4031.1	6.4	(4.4-9.5)	1.4	(0.9-2.4)
51	378	52	6276.2	8.3	(6.3-10.9)	233	29	3508.9	8.3	(5.7-11.9)	1.0	(0.6-1.6)
52	402	42	6763.6	6.2	(4.6-8.4)	225	34	3286.8	10.3	(7.4-14.5)	1.7	(1.1-2.6)
56	404	25	7017.6	3.6	(2.4-5.3)	255	33	3849.4	8.6	(6.1-12.1)	2.4	(1.4-4.1)
58	414	12	7464.4	1.6	(0.9-2.8)	269	14	4297.5	3.3	(1.9-5.5)	2.0	(0.9-4.4)
59	418	14	7494.9	1.9	(1.1-3.2)	279	12	4575.2	2.6	(1.5-4.6)	1.4	(0.7-3.0)
Total		343					309					
Penile incidence												
	HIV-negative MSM					**HIV-infected MSM**						
HPV type	**Number at risk**	**Incident events**	**PMO**	**IR**	**95% CI**	**Number at risk**	**Incident events**	**PMO**	**IR**	**95% CI**	**IRR**	**95% CI**
16	411	30	7197.7	4.2	(2.9-6.0)	271	24	4240.5	5.7	(3.8-8.4)	1.4	(0.8-2.3)
18	418	22	7410.8	3.0	(2.0-4.5)	278	14	4532.7	3.1	(1.8-5.2)	1.0	(0.5-2.0)
31	420	19	7506.6	2.5	(1.6-4.0)	272	22	4279.2	5.1	(3.4-7.8)	2.0	(1.1-3.8)
33	419	16	7551.8	2.1	(1.3-3.5)	277	11	4475.8	2.5	(1.4-4.4)	1.2	(0.5-2.5)
35	420	14	7550.3	1.9	(1.1-3.1)	277	21	4409.3	4.8	(3.1-7.3)	2.6	(1.3-5.1)
39	419	19	7417.9	2.6	(1.6-4.0)	274	10	4497.6	2.2	(1.2-4.1)	0.9	(0.4-1.9)
45	419	20	7471.4	2.7	(1.7-4.1)	274	15	4452.2	3.7	(2.0-5.6)	1.3	(0.6-2.5)
51	418	33	7274.0	4.5	(3.2-6.4)	269	28	4265.7	6.6	(4.5-9.5)	1.5	(0.9-2.4)
52	416	17	7456.9	2.3	(1.4-3.7)	269	18	4222.3	4.3	(2.7-6.8)	1.9	(1.0-3.6)
56	421	21	7561.9	2.8	(1.8-4.3)	273	21	4304.3	4.9	(3.2-7.5)	1.8	(1.0-3.2)
58	423	8	7707.2	1.0	(0.5-2.1)	281	6	4711.9	1.3	(0.6-2.8)	1.2	(0.4-3.5)
59	421	5	7741.4	0.6	(0.3-1.6)	281	5	4692.3	1.1	(0.4-2.6)	1.7	(0.5-5.7)
Total		224					195					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; IR=incidence rate per 1000 person-months of observation at risk; IRR=incidence rate ratio; PMO=person-months of observation at risk.

person-months of observation (PMO) in HIV-negative and 9.1/1000 PMO in HIV-infected MSM (IRR 2.0; 95% CI 1.2-3.2). Penile incidence rates were also higher in HIV-infected compared with HIV-negative MSM (except for HPV-39), with statistically significant differences for HPV types 31 and 35. Penile HPV-16 incidence rate was 4.2/1000 PMO in HIV-negative and 5.7/1000 PMO in HIV-infected MSM (IRR 1.4; 95% CI 0.8-2.3). Figure 1 shows Kaplan-Meier curves of the cumulative incidence of anal and penile HPV-16 and -18 infections, respectively, stratified by HIV status. Similar patterns were observed in sensitivity analyses with assumption II and III (Supplementary Tables 1 and 2, respectively), with most IRRs above 1.0 for both anal and penile HPV infection.

Effect of HIV infection on anal and penile high-risk HPV incidence

In univariable Poisson analyses, the anal incidence rates among HIV-infected MSM were significantly higher than among HIV-negative MSM for HPV-16 and for the 12 hrHPV types combined, although not for HPV-18 (Table 1B). In multivariable analyses, the associations for HPV-16 and the hrHPV types combined remained significant, with an adjusted IRR (aIRR) of 1.9 (95% CI 1.1-3.2) and 1.6 (95%CI 1.3-2.1), respectively. In univariable analyses for incident penile HPV infection, a significantly increased IRR was observed for the 12 hrHPV types combined, which remained borderline significant in multivariable analyses with an aIRR of 1.4 (95% CI 1.0-2.1). In sensitivity analyses with assumptions II and III similar results were obtained (Table 1B). Complete-case analyses showed largely similar results (data not shown).

Anal and penile high-risk HPV clearance

In total, 744 events of cleared anal hrHPV infection were observed in 519 MSM; 397 cleared penile infections were observed in 262 MSM. Type-specific anal and penile hrHPV clearance rates, stratified by HIV status, are shown in Table 2A. Anal clearance rates were lower in HIV-infected compared with HIV-negative MSM (except for HPV-58 and HPV-59), with statistically significant differences for HPV types 31, 35, 45, and 52. Anal HPV-16 clearance rate was 46.9/1000 PMO in HIV-negative and 43.7/1000 PMO in HIV-infected MSM (CRR 0.9; 95% CI 0.6-1.4). In contrast, penile clearance rates were comparable to or higher among HIV-infected compared with HIV-negative MSM. Penile HPV-16 clearance rate was 71.4/1000 PMO in HIV-negative and 132.8/1000 PMO in HIV-infected MSM (CRR 1.9; 95% CI 1.0-3.3). Figure 2 shows Kaplan-Meier curves of the cumulative clearance of anal and penile HPV-16 and -18 infections stratified by HIV status. Comparable patterns were observed in sensitivity analyses with assumptions II and III (Supplementary Tables 3 and 4, respectively), with most CRR below 1.0 for anal infection and above 1.0 for penile infection.

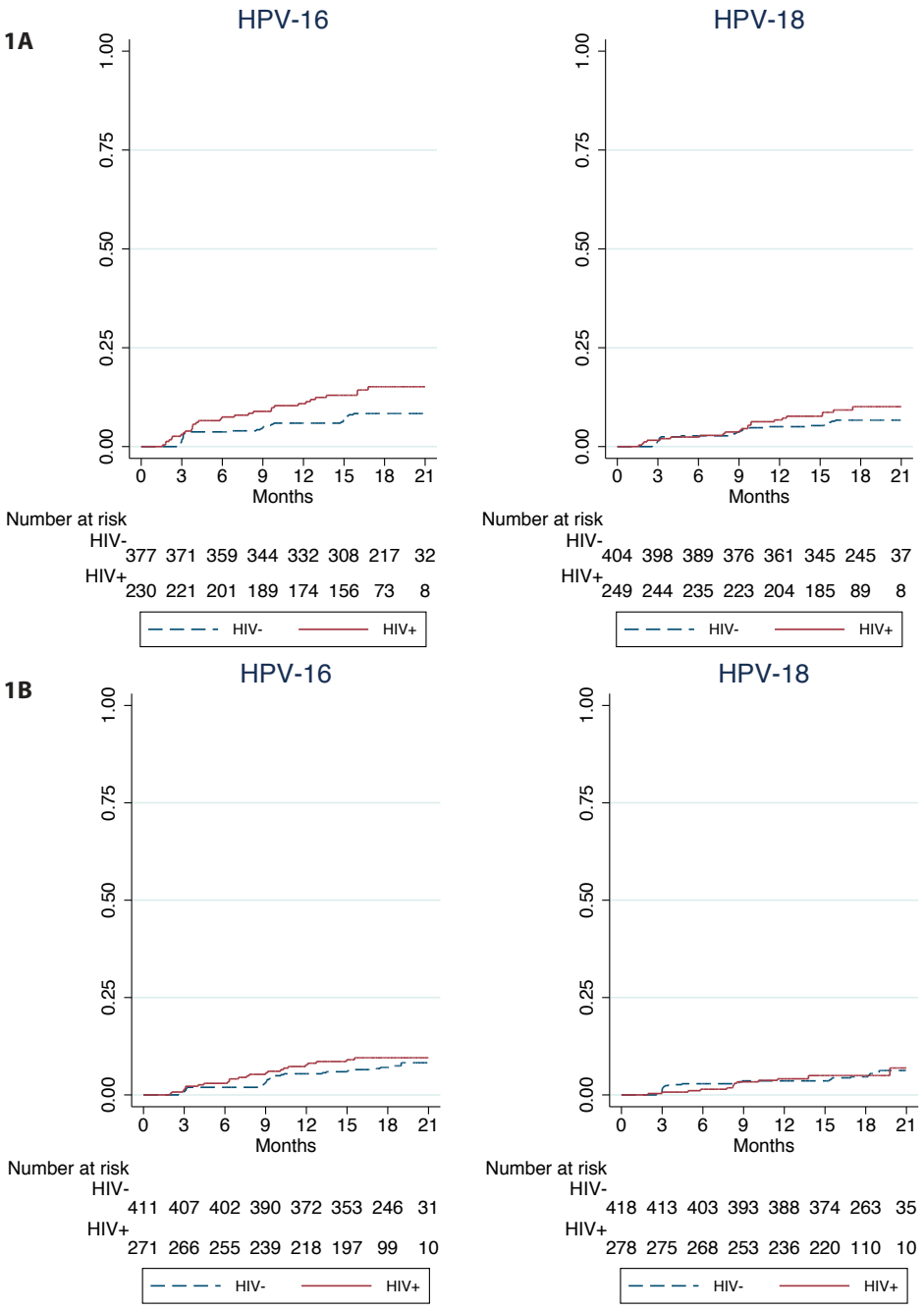

Figure 1: Cumulative incidence of anal and penile HPV-16 and -18.

The cumulative incidence of anal (Figure 1A) and penile (Figure 1B) HPV-16 and HPV-18 infections in HIV-negative (blue dotted lines) and HIV-infected (red continuous lines) MSM (H2M study, Amsterdam 2010-2013).

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; HIV-=HIV-negative MSM; HIV+=HIV-infected MSM.

Table 1B: Crude and adjusted incidence rate ratios for anal and penile high-risk HPV infections among HIV-infected versus HIV-negative MSM (H2M study, Amsterdam 2010-2013).

	Anal incidence									
		HPV-16[b]			**HPV-18**[b]			**12 hrHPV types**[d]		
Outcome assumption[a]		**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
I	Crude	1.96	(1.19-3.24)	0.008	1.54	(0.87-2.71)	0.14	1.76	(1.40-2.20)	<0.001
	Adjusted	1.87	(1.08-3.23)	0.02	1.90	(1.01-3.58)	0.05	1.63	(1.29-2.06)	<0.001
II	Crude	1.77	(1.25-2.51)	0.001	1.55	(1.02-2.36)	0.04	1.78	(1.42-2.24)	<0.001
	Adjusted	1.66	(1.14-2.43)	0.008	1.72	(1.08-2.76)	0.02	1.59	(1.27-2.00)	<0.001
III	Crude	1.78	(1.05-3.00)	0.03	1.43	(0.67-3.02)	0.35	1.95	(1.41-2.69)	<0.001
	Adjusted	1.92	(1.08-3.41)	0.03	[f]			1.86	(1.34-2.58)	<0.001
	Penile incidence									
		HPV-16[c]			**HPV-18**[c]			**12 hrHPV types**[e]		
Outcome assumption[a]		**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
I	Crude	1.37	(0.80-2.34)	0.26	1.05	(0.54-2.05)	0.89	1.42	(1.04-1.93)	0.03
	Adjusted	1.53	(0.85-2.78)	0.17	1.27	(0.61-2.63)	0.52	1.43	(1.00-2.07)	0.06
II	Crude	1.24	(0.78-1.98)	0.36	1.06	(0.60-1.86)	0.84	1.74	(1.29-2.34)	<0.001
	Adjusted	1.37	(0.82-2.28)	0.31	1.39	(0.75-2.58)	0.29	1.65	(1.14-2.39)	0.008
III	Crude	0.97	(0.36-2.63)	0.96	1.41	(0.38-5.23)	0.61	1.76	(1.10-2.81)	0.02
	Adjusted	[f]			[f]			1.66[g]	(1.00-2.75)	0.05

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; IRR=incidence rate ratio; CI=confidence interval; hrHPV=high-risk HPV.

[a] Outcome assumption I=0-0-1 (main analyses: one positive visit preceded by two consecutive negative visits); assumption II=0-1 (sensitivity analyses: one positive visit preceded by one negative visit); assumption III=0-1-1 (sensitivity analyses: two consecutive positive visits preceded by one negative visit).

[b] Anal HPV-16 and HPV-18 multivariable models were a priori adjusted for age, smoking, and number of recent anal sex partners.

[c] Penile HPV-16 and HPV-18 multivariable models were a priori adjusted for age only.

[d] Multivariable models for anal hrHPV incidence were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, having been rimmed, receptive fisting, anal STI (chlamydia and/or gonorrhea), and HPV type.

[e] Multivariable models for penile hrHPV incidence were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, circumcision status, urethral STI (chlamydia and/or gonorrhea), and HPV type.

[f] Because of limited number of events no multivariable model was made.

[g] Because of limited number of events only a priori adjusted for age, smoking, and number of recent anal sex partners.

Table 2A: Clearance rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM (H2M study, Amsterdam 2010-2013).

Anal clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	Person months at risk	CR	95% CI	Number at risk	Cleared events	Person months at risk	CR	95% CI	CRR	95% CI
16	86	43	917.5	46.9	(34.8-63.2)	86	38	870.5	43.7	(31.8-60.0)	0.9	(0.6-1.4)
18	46	28	350.2	80.0	(55.2-115.8)	56	27	564.4	47.8	(32.8-69.8)	0.6	(0.4-1.0)
31	70	50	516.3	96.8	(73.4-127.8)	90	43	937.7	45.9	(34.0-61.8)	0.5	(0.3-0.7)
33	47	31	428.9	72.3	(50.8-102.8)	46	22	464.0	47.4	(31.2-72.0)	0.7	(0.4-1.1)
35	33	25	241.2	103.7	(70.0-153.4)	56	30	491.9	61.0	(42.6-87.2)	0.6	(0.4-1.0)
39	44	31	323.2	95.9	(67.4-136.4)	43	25	347.1	72.0	(48.7-106.6)	0.8	(0.4-1.3)
45	62	45	394.6	114.0	(85.1-152.7)	47	26	390.8	66.5	(45.3-97.7)	0.6	(0.4-1.0)
51	81	38	821.8	46.2	(33.6-63.5)	86	38	858.4	44.3	(32.2-60.8)	1.0	(0.6-1.5)
52	63	42	485.9	86.4	(63.9-117.0)	97	49	913.6	53.6	(40.5-71.0)	0.6	(0.4-0.9)
56	49	30	413.7	72.5	(50.7-103.7)	57	28	484.8	57.8	(39.9-83.7)	0.8	(0.5-1.3)
58	19	12	167.2	71.8	(40.8-126.4)	32	19	251.2	75.6	(48.2-118.6)	1.1	(0.5-2.2)
59	17	14	119.9	116.8	(69.2-197.1)	15	10	76.9	130.0	(69.9-241.5)	1.1	(0.5-2.5)
Total		389					355					
Penile clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	Person months at risk	CR	95% CI	Number at risk	Cleared events	Person months at risk	CR	95% CI	CRR	95% CI
16	32	20	280.1	71.4	(46.1-110.7)	34	27	203.3	132.8	(91.1-193.7)	1.9	(1.0-3.3)
18	23	17	146.5	116.1	(72.2-186.7)	17	15	64.2	233.5	(140.8-387.4)	2.0	(1.0-4.0)
31	15	11	113.1	97.3	(53.9-175.6)	32	25	157.7	158.5	(107.1-234.6)	1.6	(0.8-3.3)
33	13	9	111.0	81.1	(42.2-155.8)	23	20	97.4	205.4	(132.5-318.4)	2.5	(1.2-5.6)
35	14	11	85.3	128.9	(71.4-232.8)	21	19	110.7	171.6	(109.4-269.0)	1.3	(0.6-2.8)
39	25	20	144.4	138.5	(89.3-214.6)	19	17	102.8	165.4	(102.8-266.0)	1.2	(0.6-2.3)
45	18	16	105.4	151.8	(93.0-247.8)	22	17	112.2	151.5	(94.2-243.7)	1.0	(0.5-2.0)
51	34	29	192.4	150.7	(104.7-216.9)	32	24	188.7	127.2	(85.2-189.7)	0.8	(0.5-1.5)
52	20	15	147.7	101.6	(61.2-168.5)	41	31	240.1	129.1	(90.8-183.6)	1.3	(0.7-2.4)
56	12	11	60.4	182.3	(100.9-329.1)	30	25	143.8	173.9	(117.5-257.4)	1.0	(0.5-1.9)
58	4	4	15.9	252.1	(94.6-671.6)	5	5	11.7	425.7	(177.2-1022.7)	1.7	(0.5-6.3)
59	3	2	39.5	50.6	(12.7-202.4)	7	7	25.8	270.9	(129.1-568.2)	5.4	(1.1-25.8)
Total		165					232					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; CR=clearance rate per 1000 person-months of observation at risk; CRR=clearance rate ratio; PMO=person-months of observation at risk.

Effect of HIV infection on anal and penile high-risk HPV clearance

In univariable Poisson analyses, the anal CRR for HIV-infected versus HIV-negative MSM was 0.7 (95% CI 0.6-0.8) for the 12 hrHPV types combined; the CRR remained significantly below 1.0 in multivariable analyses (adjusted CRR (aCRR) 0.7; 95%CI 0.6-0.9) (Table 2B). The penile CRR in univariable analyses was 1.3 (95% CI 1.0-1.7) for the hrHPV types combined, and was non-significantly increased in multivariable analyses (aCRR 1.3; 95% CI 1.0-1.7). In sensitivity analyses with assumptions II and III comparable results were obtained (Table 2B). Complete-case analyses showed similar results (data not shown).

Effect of immune status on anal and penile human papillomavirus incidence and clearance

No effect of immune status on anal or penile HPV incidence or clearance rates was observed; 95% confidence intervals for different categories of nadir CD4 cell count in HIV-infected MSM overlapped in all analyses, without any clear trends (Table 3).

Clearance among incident versus prevalent human papillomavirus infections

The clearance rates of both anal and penile hrHPV infections were significantly higher for incident compared with prevalent hrHPV infection (aCRR for anal HPV 1.4 (95% CI 1.2-1.6); aCRR for penile HPV 1.3 (95% CI 1.1-1.6)) (Supplementary Table 5).

Table 2B: Crude and adjusted clearance rate ratios for anal and penile high-risk HPV infections among HIV-infected versus HIV-negative MSM (H2M study, Amsterdam 2010-2013).

	Anal clearance									
		HPV-16[b]			**HPV-18[b]**			**12 hrHPV types[d]**		
Outcome assumption[a]		**CRR**	**95% CI**	***P*-value**	**CRR**	**95% CI**	***P*-value**	**CRR**	**95% CI**	***P*-value**
I	Crude	0.94	(0.61-1.46)	0.80	0.62	(0.36-1.04)	0.07	0.67	(0.55-0.82)	<0.001
	Adjusted	0.94	(0.56-1.59)	0.83	0.63[h]	(0.36-1.09)	0.10	0.72	(0.58-0.89)	0.003
II	Crude	1.02	(0.74-1.41)	0.88	0.53	(0.36-0.79)	0.001	0.71	(0.61-0.82)	<0.001
	Adjusted	1.05	(0.70-1.56)	0.81	0.58	(0.38-0.88)	0.01	0.74	(0.63-0.87)	<0.001
III	Crude	1.17	(0.72-1.90)	0.52	0.58	(0.29-1.15)	0.12	0.70	(0.56-0.88)	0.002
	Adjusted	1.11[h]	(0.60-2.08)	0.73	0.57[h]	(0.28-1.18)	0.13	0.76	(0.59-0.98)	0.04
	Penile clearance									
		HPV-16[c]			**HPV-18[c]**			**12 hrHPV types[e]**		
Outcome assumption[a]		**CRR**	**95% CI**	***P*-value**	**CRR**	**95% CI**	***P*-value**	**CRR**	**95% CI**	***P*-value**
I	Crude	1.86	(1.04-3.32)	0.04	2.01	(1.00-4.03)	0.05	1.33	(1.02-1.73)	0.04
	Adjusted	2.25	(1.12-4.56)	0.02	1.87	(0.86-4.05)	0.11	1.28	(0.96-1.71)	0.10
II	Crude	1.44	(0.94-2.23)	0.10	2.35	(1.36-4.15)	0.003	1.07	(0.89-1.29)	0.45
	Adjusted	1.36	(0.81-2.31)	0.25	2.24	(1.18-4.28)	0.01	1.03	(0.85-1.25)	0.74
III	Crude	2.05	(0.77-5.47)	0.15	[f]			1.09	(0.76-1.56)	0.64
	Adjusted	3.18[h]	(0.90-1.13)	0.07	[f]			1.09[g]	(0.72-1.67)	0.68

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CRR=clearance rate ratio; CI=confidence interval; hrHPV=high-risk HPV.

[a] Outcome assumption I=1-0-0 (main analyses: one positive visit followed by two consecutive negative visits); assumption II=1-0 (sensitivity analyses: one positive visit followed by one negative visit); assumption III=1-1-0 (sensitivity analyses: two consecutive positive visits followed by one negative visit).

[b] Anal HPV-16 and HPV-18 multivariable models were a priori adjusted for age, smoking, and number of recent anal sex partners, unless indicated otherwise.

[c] Penile HPV-16 and HPV-18 multivariable models were a priori adjusted for age only.

[d] Multivariable models for anal hrHPV clearance were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, having been rimmed, receptive fisting, anal STI (chlamydia and/or gonorrhea), and HPV type.

[e] Multivariable models for penile hrHPV clearance were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, circumcision status, urethral STI (chlamydia and/or gonorrhea), and HPV type.

[f] Because of an empty cell no univariable or multivariable model was made.

[g] Because of limited number of events only a priori adjusted for age, smoking, and number of recent anal sex partners.

[h] Because of limited number of events only a priori adjusted for age.

Table 3: Crude and adjusted incidence rate ratios and clearance rate ratios for anal and penile high-risk HPV infections according to immune status (H2M study, Amsterdam 2010-2013).

		Anal incidence by immune status									Anal clearance by immune status								
		HPV-16[a]			HPV-18[a]			12 hrHPV types[c]			HPV-16[a]			HPV-18[a]			12 hrHPV types[c]		
		IRR	95% CI	*P*	IRR	95% CI	*P*	IRR	95% CI	*P*	CRR	95% CI	*P*	CRR	95% CI	*P*	CRR	95% CI	*P*
C	HIV-	1		0.05	1		0.48	1		<0.001	1		0.99	1		0.15	1		0.001
	HIV+; CD4 >350	1.58	(0.62-4.05)		1.52	(0.55-4.23)		2.34	(1.55-3.53)		0.98	(0.44-2.18)		0.62	(0.25-1.56)		0.69	(0.46-1.05)	
	HIV+; CD4 200-350	2.27	(1.21-4.26)		1.36	(0.62-3.00)		1.76	(1.33-2.34)		0.91	(0.53-1.57)		0.44	(0.21-0.95)		0.66	(0.51-0.84)	
	HIV+; CD4 <200	1.82	(0.87-3.79)		1.76	(0.80-3.87)		1.36	(0.95-1.95)		0.98	(0.49-1.96)		0.96	(0.46-1.99)		0.69	(0.50-0.95)	
A	HIV-	1		0.11	1[e]		0.18	1		<0.001	1[e]		1.00	1[e]		0.22	1		0.03
	HIV+; CD4 >350	1.32	(0.54-3.43)		1.70	(0.61-4.77)		1.79	(1.20-2.68)		0.93	(0.39-2.21)		0.68	(0.27-1.74)		0.76	(0.49-1.17)	
	HIV+; CD4 200-350	2.13	(1.10-4.12)		1.68	(0.74-3.80)		1.64	(1.26-2.15)		0.98	(0.53-1.83)		0.43	(0.19-0.97)		0.70	(0.53-0.92)	
	HIV+; CD4 <200	2.03	(0.90-4.57)		2.53	(1.04-6.16)		1.43	(1.00-2.06)		1.02	(0.48-2.21)		0.80	(0.42-1.74)		0.72	(0.51-1.01)	
		Penile incidence by immune status									**Penile clearance by immune status**								
		HPV-16[b]			HPV-18[b]			12 hrHPV types[d]			HPV-16			HPV-18			12 hrHPV types[d]		
		IRR	95% CI	*P*	IRR	95% CI	*P*	IRR	95% CI	*P*	CRR	95% CI	*P*	CRR	95% CI	*P*	CRR	95% CI	*P*
C	HIV-	1		0.77	1		0.25	1		0.15	1		0.49	[g]			1		0.07
	HIV+; CD4 >350	1.43	(0.53-3.80)		2.02	(0.76-5.34)		1.65	(0.85-3.26)		1.58	(0.58-4.29)					1.04	(0.66-1.64)	
	HIV+; CD4 200-350	1.37	(0.68-2.79)		1.07	(0.43-2.65)		1.41	(0.89-2.24)		1.44	(0.72-2.85)					1.49	(1.09-2.04)	
	HIV+; CD4 <200	1.14	(0.50-2.62)		0.25	(0.03-1.87)		1.26	(0.77-2.06)		1.76	(0.75-4.15)					1.33	(0.92-1.93)	
A	HIV-	[f]			[f]			1		0.30	[f]			[g]			1		0.10
	HIV+; CD4 >350							1.59	(0.74-3.38)								0.99	(0.63-1.56)	
	HIV+; CD4 200-350							1.43	(0.85-2.42)								1.49	(1.07-2.08)	
	HIV+; CD4 <200							1.28	(0.75-2.28)								1.34	(0.90-2.02)	

Abbreviations: H2M=HIV and HPV in MSM; CI=confidence interval; IRR=incidence rate ratio; CRR: clearance rate ratio; HIV-=HIV-negative MSM; HIV+=HIV-infected MSM; CD4=nadir CD4 cell count in cells/µl; C=crude analyses; A=adjusted analyses; *P*=*P*-value.

[a] Anal HPV-16 and HPV-18 multivariable models were a priori adjusted for age, smoking, and number of recent anal sex partners, unless indicated otherwise.

[b] Penile HPV-16 and HPV-18 multivariable models were a priori adjusted for age only.

[c] Multivariable models for anal hrHPV incidence or clearance were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, having been rimmed, receptive fisting, anal STI (chlamydia and/or gonorrhea), and HPV type.

[d] Multivariable models for penile hrHPV incidence or clearance were a priori adjusted for age, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, circumcision status, urethral STI (chlamydia and/or gonorrhea), and HPV type.

[e] Because of limited number of events only a priori adjusted for age.

[f] Because of limited number of events no multivariable model was made.

[g] Because of an empty cell no univariable or multivariable model was made.

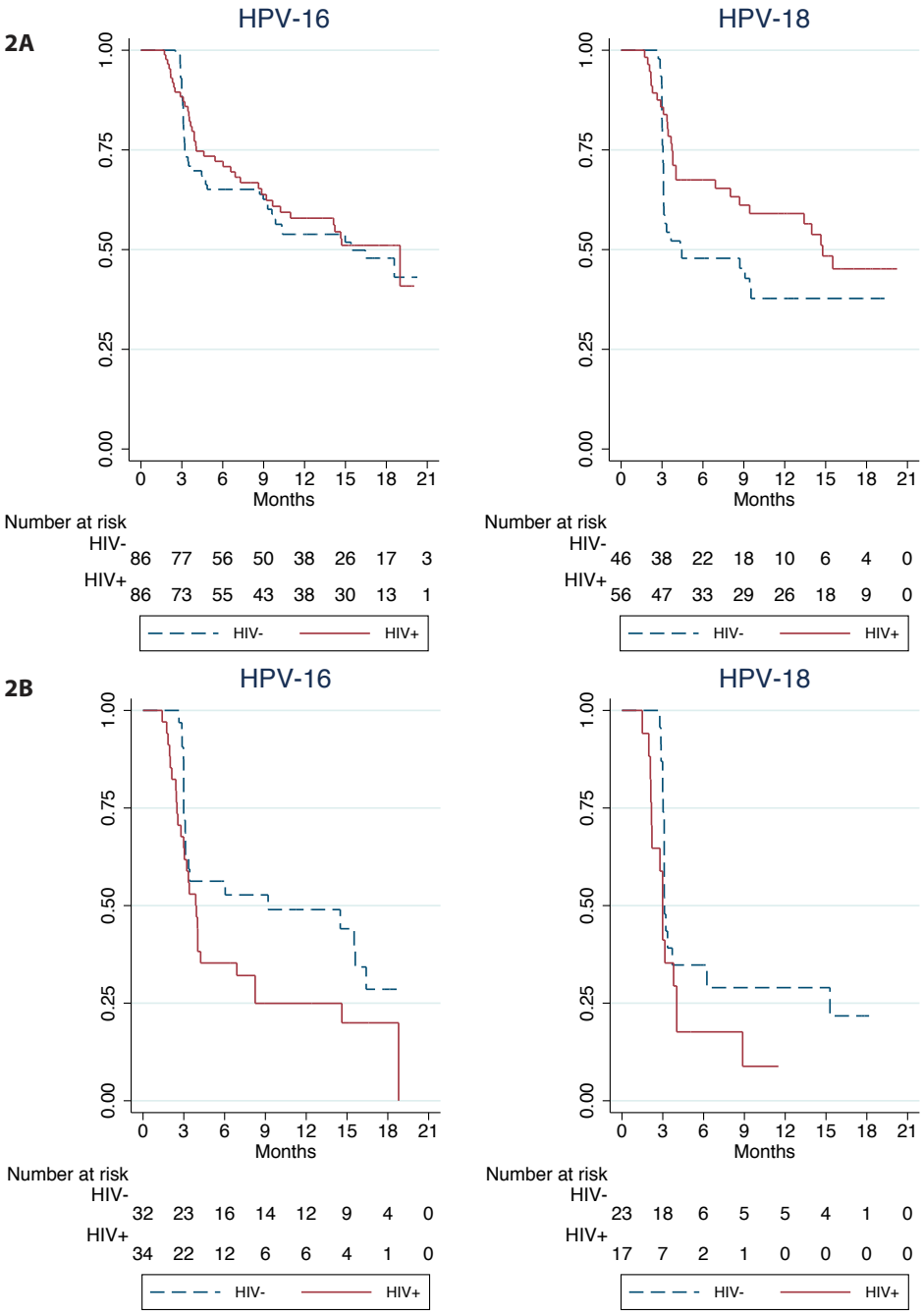

Figure 2: Cumulative clearance of anal and penile HPV-16 and -18.

The cumulative clearance of anal (Figure 2A) and penile (Figure 2B) HPV-16 and HPV-18 infections in HIV-negative (blue dotted lines) and HIV-infected (red continuous lines) MSM (H2M study, Amsterdam 2010-2013).

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; HIV-=HIV-negative MSM; HIV+=HIV-infected MSM.

DISCUSSION

HIV-infected MSM had an increased incidence of anal and penile hrHPV infection, including anal HPV-16, the main causative agent of anal cancer. In addition, HIV-infected MSM had a decreased clearance of anal hrHPV. No significant independent effect of HIV infection was observed for penile hrHPV clearance. Anal and penile HPV incidence or clearance did not significantly differ by level of nadir CD4 cell count among HIV-infected MSM.

Anal HPV incidence rates were largely comparable to those found in other studies among HIV-negative [20,21] and HIV-infected [21-24] MSM, although comparisons across studies are hampered by differences in study populations, laboratory methods, and statistical methods used (e.g. definitions of incidence and clearance). Anal HPV clearance rates observed in our study were mostly higher than reported for HIV-infected MSM so far [22,23]. Interestingly, the clearance rate of HPV-16 was low compared with other HPV types, which is in line with previous studies [22,23], and may partly explain the high oncogenic potential of HPV-16.

Penile HPV incidence and clearance rates were considerably higher than previously reported in HIV-infected MSM [22]. Our observed incidence rates among HIV-negative MSM were comparable to rates of genital HPV infection reported in a large multinational sample of HIV-negative, mostly heterosexual men [25]. The high penile HPV clearance rates observed in our study are striking. Although we have no clear explanation for the difference in clearance between anal and penile HPV infection, it may be conceivable that infections in keratinized epithelium (such as at the penile shaft, where penile samples were taken) clear faster than mucosal infections (such as at the anal canal, where anal samples were taken), or that HPV viral latency differs between both anatomical sites

HIV infection showed a significant effect on anal and penile HPV incidence, and anal HPV clearance, independent of reported sexual behavior or other potential confounders. These findings are in line with the few data available regarding anogenital HPV infection in men [21,26] and cervical infection in women [27]. However, even in our study where we adjusted for multiple sexual behavior variables, residual confounding cannot be ruled out. For example, assortative mixing (whereby HIV-infected men preferentially have sex with other HIV-infected men) may lead to increased HPV incidence among HIV-infected MSM. Nevertheless, our data suggest a biological effect of HIV infection on HPV acquisition and clearance, implying more persistent hrHPV infections independent of sexual behavior among HIV-infected individuals, which presumably contributes to their increased risk of HPV-related cancer. The fact that we could not demonstrate an effect of

immune status as measured by nadir CD4 count may be due to a relatively low number of participants with severe immunosuppression, or due to HIV-related immunological effects not captured by CD4 values.

As persistent hrHPV infection poses a risk of becoming malignant, understanding which infections are transient versus persistent is crucial. Our analyses confirm previous data [28] that incident HPV infections clear faster than prevalent infections. This seems logical, as prevalent infections are a combination of recently acquired infections and long-standing infections that are less likely to clear.

Strengths of this study include the large and well-characterized cohort consisting of HIV-negative and HIV-infected MSM (enabling us to assess the effect of HIV infection while adjusting for multiple potential confounders), the longitudinal analyses of both anal and penile hrHPV infection, and the sensitive laboratory methods used. In addition, by conducting various sensitivity analyses, we were able to show the major impact of different methodological definitions on reported incidence and clearance rates, while the independent effect of HIV infection on incidence and clearance was not affected by assumptions.

This study has also some limitations. First, we may have had limited power to observe an effect of immune status on HPV incidence or clearance. Second, in this kind of epidemiologic studies it is impossible to distinguish true HPV clearance from viral latency, and to distinguish true incident infection from re-activation of latent infection. As viral latency may be more common in HIV-infected individuals [29], this may partly explain the observed increased HPV incidence rates. Third, no clinical examinations were performed, and therefore we could not link virological endpoints to clinical lesions. Fourth, our cohort consists of highly sexually active, adult MSM. Therefore, our results may not be generalizable to all MSM.

In conclusion, anal and penile hrHPV incidence rates were higher among HIV-infected compared with HIV-negative MSM, and anal hrHPV clearance rates were lower, independent of sexual behavior. This suggests a biological effect of HIV infection on anogenital hrHPV infection. Further studies are needed into the mechanisms by which HIV exerts its effect on HPV infection and sequelae.

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SUPPLEMENT CHAPTER 2.3

Supplementary Text

Supplementary Text 1: Imputation

The following variables included in the analyses contained missing values, and these values were imputed: number of lifetime male sex partners at baseline (56 missing values); circumcision status at baseline (14 missing values); CD4 cell count at baseline (56 missing values); nadir CD4 cell count as known at baseline (38 missing values); undetectable HIV viral load at baseline (50 missing values); recent use of poppers (139 missing values in total); recent use of cannabis (122 missing values in total); current smoking (104 missing values in total); number of recent anal sex partners (135 missing values in total); recent anal sex position (121 missing values in total); recently having been rimmed (116 missing values in total); recent receptive fisting (94 missing values in total); anal chlamydia or gonorrhea diagnosis (78 missing values in total); penile chlamydia or gonorrhea diagnosis (52 missing values in total).

Imputation was done separately for HIV-positive and HIV-negative MSM. When a variable was not normally distributed we transformed it to the logarithm before including the variable in the imputation model. The following variables were transformed: number of lifetime male sex partners (at baseline), CD4 count (at baseline), nadir CD4 cell count (at baseline) and recent number of anal sex partners (time updated). We created 20 imputed dataset for HIV-positive and HIV-negative with 25 iterations per imputed dataset, which resulted in a total of 40 imputed datasets.

For both HIV-positive and HIV-negative MSM, the following variables were used to impute the missing data: HPV test results for 14 HPV-types (12 high-risk and HPV 6 and 11), number of lifetime male sex partners at baseline, circumcision status at baseline, recent use of poppers, recent use of cannabis, current smoking, number of recent anal sex partners, recent anal sex position, recently having been rimmed (passive rimming) and having rimmed someone (active rimming), recent receptive fisting, anal chlamydia or gonorrhea diagnosis, penile chlamydia or gonorrhea diagnosis, follow-up time in years, recent alcohol use, country of birth, income status at baseline and anal and genital warts. For HIV-positive only: CD4 cell count at baseline, nadir CD4 cell count as known at baseline and undetectable HIV viral load at baseline.

Supplementary Table 1: Incidence rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM; sensitivity analyses using assumption IIa (H2M study, Amsterdam 2010-2013).

Anal incidence												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Incident events	PMO	IR	95% CI	Number at risk	Incident events	PMO	IR	95% CI	IRR	95% CI
16	420	64	8963.2	7.1	(5.6-9.1)	286	64	5101.1	12.5	(9.8-16.0)	1.8	(1.2-2.5)
18	431	46	9713.1	4.7	(3.5-6.3)	290	41	5659.5	7.2	(5.3-9.8)	1.5	(1.0-2.3)
31	432	68	9231.7	7.4	(5.8-9.3)	285	78	4860.3	16.0	(12.9-20.0)	2.2	(1.6-3.0)
33	432	43	9717.9	4.4	(3.3-6.0)	293	40	5778.4	6.9	(5.1-9.4)	1.6	(1.0-2.4)
35	435	32	9999.3	3.2	(2.3-4.5)	297	49	5646.0	8.7	(6.6-11.5)	2.7	(1.7-4.2)
39	431	46	9743.4	4.7	(3.5-6.3)	299	38	5966.5	6.4	(4.6-8.8)	1.4	(0.9-2.1)
45	434	59	9490.4	6.2	(4.8-8.0)	298	44	5814.1	7.6	(5.6-10.2)	1.2	(0.8-1.8)
51	424	96	8776.6	10.9	(9.0-13.4)	282	57	5147.2	11.1	(8.5-14.4)	1.0	(0.7-1.4)
52	432	66	9375.6	7.0	(5.5-9.0)	286	71	4948.0	14.3	(11.4-18.1)	2.0	(1.5-2.9)
56	430	46	9636.6	4.8	(3.6-6.4)	297	54	5637.5	9.6	(7.3-12.5)	2.0	(1.4-3.0)
58	436	23	10219.8	2.3	(1.5-3.4)	303	30	6133.1	4.9	(3.4-7.0)	2.2	(1.3-3.7)
59	436	26	10201.8	2.5	(1.7-3.7)	307	18	6480.7	2.8	(1.7-4.4)	1.1	(0.6-2.0)
Total		615					584					

Penile incidence												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Incident events	PMO	IR	95% CI	Number at risk	Incident events	PMO	IR	95% CI	IRR	95% CI
16	434	41	9951.3	4.1	(3.0-5.6)	303	31	6100.8	5.1	(3.6-7.2)	1.2	(0.8-2.0)
18	433	30	10126.7	3.0	(2.1-4.2)	307	20	6447.5	3.1	(2.0-4.8)	1.1	(0.6-1.8)
31	436	22	10287.3	2.1	(1.4-3.2)	303	34	6128.5	5.5	(4.0-7.8)	2.6	(1.5-4.4)
33	436	20	10337.3	1.9	(1.2-3.0)	303	19	6326.9	3.0	(1.9-4.7)	1.6	(0.8-2.9)
35	434	17	10316.8	1.6	(1.0-2.7)	307	27	6321.8	4.3	(2.9-6.2)	2.6	(1.4-4.8)
39	436	29	10143.3	2.9	(2.0-4.1)	305	18	6389.9	2.8	(1.8-4.5)	1.0	(0.6-1.8)
45	436	24	10249.4	2.3	(1.6-3.5)	305	27	6303.8	4.3	(2.9-6.2)	1.8	(1.1-3.2)
51	434	43	9996.6	4.3	(3.2-5.8)	304	43	6085.7	7.1	(5.2-9.5)	1.6	(1.1-2.5)
52	435	26	10206.6	2.5	(1.7-3.7)	303	35	6026.5	5.8	(4.2-8.1)	2.3	(1.4-3.8)
56	434	22	10338.0	2.1	(1.4-3.2)	305	36	6150.6	5.9	(4.2-8.1)	2.8	(1.6-4.7)
58	437	10	10500.3	1.0	(0.5-1.8)	306	6	6627.7	0.9	(0.4-2.0)	1.0	(0.4-2.6)
59	436	6	10530.8	0.6	(0.2-1.3)	308	9	6603.3	1.4	(0.7-2.6)	2.4	(0.9-6.7)
Total		290					305					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; IR=incidence rate per 1000 person-months of observation at risk; IRR=incidence rate ratio; PMO=person-months of observation at risk.

[a] Assumption II: incidence was defined as one positive visit preceded by one negative visit.

Supplementary Table 2: Incidence rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM; sensitivity analyses using assumption IIIa (H2M study, Amsterdam 2010-2013).

Anal incidence												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Incident events	PMO	IR	95% CI	Number at risk	Incident events	PMO	IR	95% CI	IRR	95% CI
16	408	29	6904.9	4.2	(2.9-6.0)	251	27	3614.5	7.5	(5.1-10.9)	1.8	(1.1-3.0)
18	417	16	7473.1	2.1	(1.3-3.5)	256	12	3938.9	3.0	(1.7-5.4)	1.4	(0.7-3.0)
31	419	17	7359.1	2.3	(1.4-3.7)	245	24	3595.0	6.7	(4.5-10.0)	2.9	(1.6-5.4)
33	419	13	7453.7	1.7	(1.0-3.0)	264	13	4075.8	3.2	(1.9-5.5)	1.8	(0.9-3.9)
35	424	10	7604.8	1.3	(0.7-2.4)	265	17	4042.9	4.2	(2.6-6.8)	3.2	(1.5-7.0)
39	420	13	7474.3	1.7	(1.0-3.0)	266	9	4168.8	2.2	(1.1-4.1)	1.2	(0.5-2.9)
45	421	12	7489.7	1.6	(0.9-2.8)	269	15	4133.4	3.6	(2.2-6.0)	2.3	(1.1-4.8)
51	414	27	7119.5	3.8	(2.6-5.5)	248	23	3618.0	6.4	(4.2-9.6)	1.7	(1.0-2.9)
52	421	23	7340.5	3.1	(2.1-4.7)	249	27	3580.9	7.5	(5.2-11.0)	2.4	(1.4-4.2)
56	419	13	7429.6	1.7	(1.0-3.0)	263	14	4056.3	3.5	(2.0-5.8)	2.0	(0.9-4.2)
58	425	11	7642.9	1.4	(0.8-2.6)	277	8	4335.6	1.8	(0.9-3.7)	1.3	(0.5-3.2)
59	425	1	7752.5	0.1	(0.0-0.9)	279	6	4438.2	1.4	(0.6-3.0)	10.5	(1.3-87.1)
Total		185					195					
Penile incidence												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Incident events	PMO	IR	95% CI	Number at risk	Incident events	PMO	IR	95% CI	IRR	95% CI
16	420	11	7570.4	1.5	(0.8-2.6)	274	6	4269.7	1.4	(0.6-3.1)	1.0	(0.4-2.6)
18	422	5	7739.4	0.6	(0.3-1.6)	279	4	4425.8	0.9	(0.3-2.4)	1.4	(0.4-5.2)
31	424	3	7746.3	0.4	(0.1-1.2)	277	7	4361.6	1.6	(0.8-3.4)	4.1	(1.1-16.0)
33	425	5	7756.8	0.6	(0.3-1.5)	277	2	4438.1	0.5	(0.1-1.8)	0.7	(0.1-3.6)
35	424	1	7798.6	0.1	(0.0-0.9)	277	2	4408.9	0.5	(0.1-1.8)	3.5	(0.3-39.0)
39	425	3	7768.3	0.4	(0.1-1.2)	276	3	4390.7	0.7	(0.2-2.1)	1.8	(0.4-8.8)
45	425	3	7769.9	0.4	(0.1-1.2)	277	4	4419.8	0.9	(0.3-2.4)	2.3	(0.5-10.5)
51	424	3	7674.6	0.4	(0.1-1.2)	272	6	4330.6	1.4	(0.6-3.1)	3.5	(0.9-14.2)
52	425	4	7740.2	0.5	(0.2-1.4)	275	5	4287.5	1.2	(0.5-2.8)	2.3	(0.6-8.4)
56	425	4	7800.1	0.5	(0.2-1.4)	278	3	4421.8	0.7	(0.2-2.1)	1.3	(0.3-5.9)
58	426	0	7852.7	0.0	NA	279	0	4516.5	0.0	NA	NA	NA
59	425	1	7822.8	0.1	(0.0-0.9)	278	1	4487.4	0.2	(0.0-1.6)	1.7	(0.1-27.9)
Total		43					43					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; IR=incidence rate per 1000 person-months of observation at risk; IRR=incidence rate ratio; PMO=person-months of observation at risk; NA=not applicable.

[a] Assumption III: incidence was defined as two consecutive positive visits preceded by one negative visit.

Supplementary Table 3: Clearance rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM; sensitivity analyses using assumption IIa (H2M study, Amsterdam 2010-2013).

Anal clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	PMO	CR	95% CI	Number at risk	Cleared events	PMO	CR	95% CI	CRR	95% CI
16	95	76	932.8	81.5	(65.1-102.0)	98	75	911.6	82.3	(65.6-103.2)	1.0	(0.7-1.4)
18	57	51	386.3	132.0	(100.3-173.7)	67	47	686.2	68.5	(51.1-91.2)	0.5	(0.4-0.8)
31	82	75	534.2	140.4	(112.0-176.0)	106	83	994.1	83.5	(67.3-103.5)	0.6	(0.4-0.8)
33	53	49	356.0	137.7	(104.0-182.1)	58	45	512.6	87.8	(65.5-117.6)	0.6	(0.4-1.0)
35	37	34	226.6	150.1	(107.2-210.0)	68	57	513.5	111.0	(85.6-143.9)	0.7	(0.5-1.1)
39	55	43	409.7	105.0	(77.8-141.5)	53	42	392.9	106.9	(79.0-144.7)	1.0	(0.7-1.6)
45	68	60	402.2	149.2	(115.8-192.1)	54	43	423.5	101.5	(75.3-136.9)	0.7	(0.5-1.0)
51	104	86	781.1	110.1	(89.1-136.0)	95	67	973.0	68.9	(54.2-87.5)	0.6	(0.5-0.9)
52	72	64	487.5	131.3	(102.7-167.7)	116	94	942.3	99.8	(81.5-122.1)	0.8	(0.6-1.0)
56	59	52	409.7	126.9	(96.7-166.5)	68	56	572.4	97.8	(75.3-127.1)	0.8	(0.5-1.1)
58	24	21	151.3	138.8	(90.5-212.8)	37	31	222.0	139.6	(98.2-198.5)	1.0	(0.6-1.8)
59	21	21	104.0	202.0	(131.7-309.8)	18	18	100.0	180.0	(113.4-285.7)	0.9	(0.5-1.7)
Total		632					658					
Penile clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	PMO	CR	95% CI	Number at risk	Cleared events	PMO	CR	95% CI	CRR	95% CI
16	47	37	330.9	111.8	(81.0-154.3)	51	45	278.6	161.5	(120.6-216.3)	1.4	(0.9-2.2)
18	29	23	192.9	119.3	(79.3-179.5)	26	25	89.0	280.8	(189.8-415.6)	2.4	(1.3-4.2)
31	24	23	141.3	162.7	(108.1-244.9)	45	38	223.2	170.3	(123.9-234.0)	1.1	(0.6-1.8)
33	18	16	102.2	156.6	(95.9-255.6)	28	26	130.8	198.8	(135.4-292.0)	1.3	(0.7-2.4)
35	19	18	100.2	179.7	(113.2-285.2)	31	30	144.0	208.4	(145.7-298.0)	1.2	(0.7-2.1)
39	26	25	127.8	195.6	(132.2-289.5)	25	22	145.8	150.9	(99.3-229.1)	0.8	(0.4-1.4)
45	27	25	115.4	216.7	(146.4-320.7)	29	29	117.1	247.6	(172.1-356.3)	1.1	(0.7-2.0)
51	40	40	189.7	210.9	(154.7-287.5)	45	42	239.8	175.2	(129.5-237.0)	0.8	(0.5-1.3)
52	27	25	130.0	192.3	(129.9-284.6)	49	43	277.6	154.9	(114.9-208.9)	0.8	(0.5-1.3)
56	19	17	99.2	171.3	(106.5-275.6)	41	38	165.7	229.3	(166.9-315.2)	1.3	(0.8-2.4)
58	8	8	27.8	287.8	(143.9-575.5)	8	8	27.9	286.6	(143.3-573.2)	1.0	(0.4-2.7)
59	4	4	28.6	139.9	(52.5-372.9)	10	9	41.4	217.5	(113.2-418.0)	1.6	(0.5-5.1)
Total		261					355					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; CR=clearance rate per 1000 person-months of observation at risk; CRR=clearance rate ratio; PMO=person-months of observation at risk.

[a] Assumption II: clearance was defined as one positive visit followed by one negative visit.

Supplementary Table 4: Clearance rates of anal and penile high-risk HPV infections among HIV-negative and HIV-infected MSM; sensitivity analyses using assumption IIIa (H2M study, Amsterdam 2010-2013).

Anal clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	PMO	CR	95% CI	Number at risk	Cleared events	PMO	CR	95% CI	CRR	Lower
16	49	30	488.4	61.4	(43.0-87.9)	58	35	486.1	72.0	(51.7-100.3)	1.2	(0.7-1.9)
18	19	14	155.0	90.3	(53.5-152.5)	34	19	365.4	52.0	(33.2-81.5)	0.6	(0.3-1.2)
31	25	19	199.1	95.4	(60.9-149.6)	53	36	527.6	68.2	(49.2-94.6)	0.7	(0.4-1.3)
33	19	15	144.5	103.8	(62.6-172.2)	26	17	262.2	64.8	(40.3-104.3)	0.6	(0.3-1.3)
35	13	12	66.0	181.8	(103.3-320.2)	27	20	225.8	88.6	(57.2-137.3)	0.5	(0.2-1.0)
39	18	11	164.4	66.9	(37.1-120.8)	21	14	149.7	93.5	(55.4-158.0)	1.4	(0.6-3.1)
45	23	12	121.6	98.7	(56.1-173.8)	42	14	202.0	69.3	(41.0-117.0)	0.7	(0.3-1.5)
51	54	29	326.5	88.8	(61.7-127.8)	27	25	542.2	46.1	(31.2-68.2)	0.5	(0.3-0.9)
52	27	19	188.3	100.9	(64.4-158.2)	56	38	451.7	84.1	(61.2-115.6)	0.8	(0.5-1.5)
56	21	16	153.0	104.6	(64.1-170.7)	31	21	277.3	75.7	(49.4-116.1)	0.7	(0.4-1.4)
58	11	9	46.4	193.9	(100.9-372.6)	13	7	90.7	77.2	(36.8-161.9)	0.4	(0.2-1.1)
59	5	5	22.4	223.1	(92.9-536.1)	7	7	26.8	260.9	(124.4-547.4)	1.2	(0.4-3.7)
Total		191					253					
Penile clearance												
	HIV-negative MSM					HIV-infected MSM						
HPV type	Number at risk	Cleared events	PMO	CR	95% CI	Number at risk	Cleared events	PMO	CR	95% CI	CRR	Lower
16	15	8	137.8	58.1	(29.0-116.1)	10	8	67.1	119.2	(59.6-238.4)	2.1	(0.8-5.5)
18	6	2	75.0	26.7	(6.7-106.6)	2	2	6.5	305.9	(76.5-1223.1)	11.5	(1.6-81.4)
31	6	6	41.2	145.7	(65.5-324.3)	6	2	48.5	41.3	(10.3-165.0)	0.3	(0.1-1.4)
33	4	4	26.6	150.2	(56.4-400.2)	3	3	36.4	82.5	(26.6-255.8)	0.6	(0.1-2.5)
35	2	1	33.4	29.9	(4.2-212.3)	4	4	29.2	136.9	(51.4-364.7)	4.6	(0.5-41.0)
39	5	4	31.3	127.8	(48.0-340.6)	6	5	38.3	130.7	(54.4-314.0)	1.0	(0.3-3.8)
45	2	2	17.6	113.5	(28.4-453.7)	4	3	30.7	97.7	(31.5-303.0)	0.9	(0.1-5.2)
51	6	6	36.7	163.6	(73.5-364.1)	10	8	53.4	149.7	(74.9-299.4)	0.9	(0.3-2.6)
52	5	2	41.8	47.9	(12.0-191.4)	10	6	91.8	65.4	(29.4-145.5)	1.4	(0.3-6.8)
56	4	4	11.7	343.0	(128.7-913.8)	4	3	24.9	120.6	(38.9-374.0)	0.4	(0.1-1.6)
58	0	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA
59	1	1	9.2	109.1	(15.4-774.5)	1	1	3.0	329.0	(46.4-2335.9)	3.0	(0.2-48.2)
Total		40					45					

Abbreviations: MSM=men who have sex with men; H2M=HIV and HPV in MSM; CI=confidence interval; CR=clearance rate per 1000 person-months of observation at risk; CRR=clearance rate ratio; PMO=person-months of observation at risk; NA=not applicable.

[a] Assumption III: clearance was defined as two consecutive positive visits followed by one negative visit.

Supplementary Table 5: Crude and adjusted clearance rate ratios for anal and penile incident versus prevalent high-risk HPV infections (H2M study, Amsterdam 2010-2013).

	Anal clearance[a]							Penile clearance[b]					
	Outcome assumption I							Outcome assumption I					
	CRR	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**		**CRR**	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**
Prevalent	1			1			Prevalent	1			1		
Incident	1.40	(1.19-1.65)	<0.001	1.36	(1.15-1.62)	<0.001	Incident	1.33	(1.09-1.63)	0.004	1.34	(1.10-1.64)	0.004
	Outcome assumption II							Outcome assumption II					
	CRR	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**		**CRR**	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**
Prevalent	1			1			Prevalent	1			1		
Incident	1.64	(1.45-1.86)	<0.001	1.60	(1.40-1.82)	<0.001	Incident	1.35	(1.14-1.59)	0.04	1.34	(1.15-1.58)	<0.001
	Outcome assumption III							Outcome assumption III					
	CRR	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**		**CRR**	**95% CI**	***P*-value**	**aCRR**	**95% CI**	***P*-value**
Prevalent	1			1			Prevalent	1			1[c]		
Incident	1.24	(1.01-1.52)	0.04	1.17	(0.96-1.44)	0.13	Incident	1.36	(0.89-2.08)	0.16	1.46	(0.95-2.24)	0.09

Abbreviations: H2M=HIV and HPV in MSM; CRR=clearance rate ratio; aCRR=adjusted clearance rate ratio; CI=confidence interval.

Incident versus prevalent infections were defined as infections that were not detected at baseline versus infections that were detected at baseline.

Outcome assumption I=1-0-0 (main analyses: one positive visit followed by two consecutive negative visits); assumption II=1-0 (sensitivity analyses: one positive visit followed by one negative visit); assumption III=1-1-0 (sensitivity analyses: two consecutive positive visits followed by one negative visit).

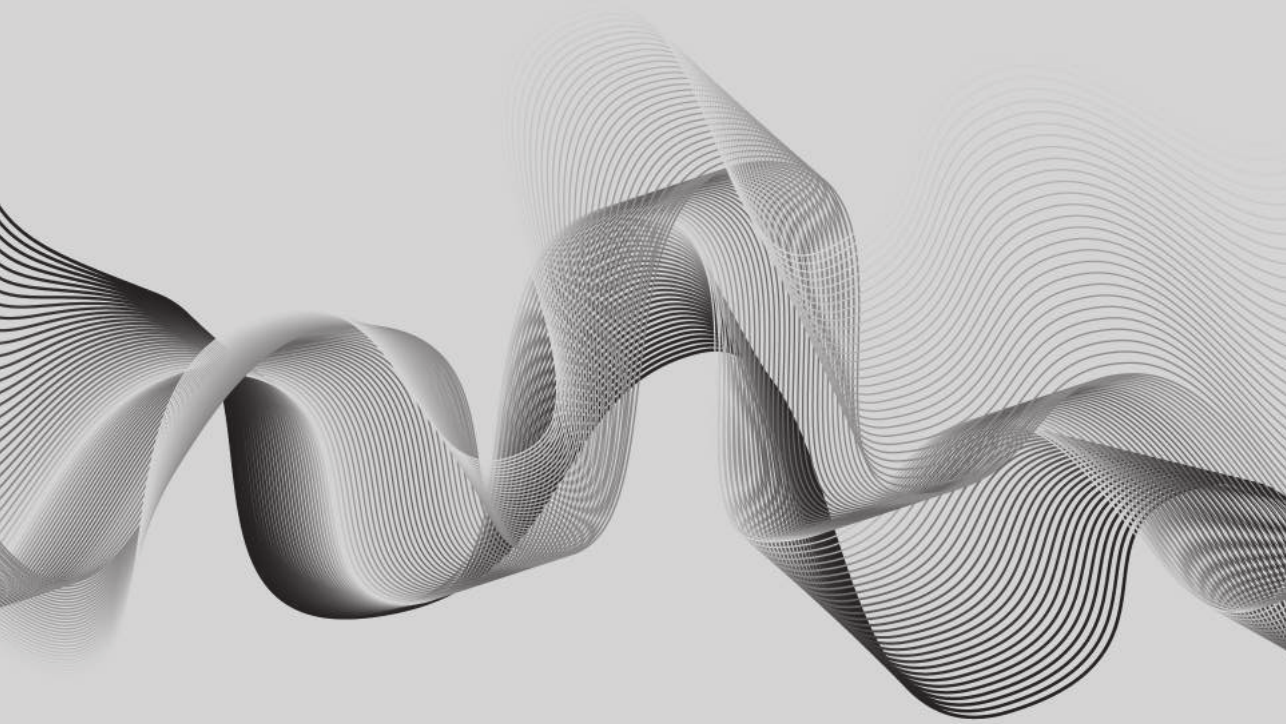
[a] Multivariable models for anal high-risk HPV clearance were a priori adjusted for age, HIV infection, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, having been rimmed, receptive fisting, anal STI (chlamydia and/or gonorrhea), and HPV type.

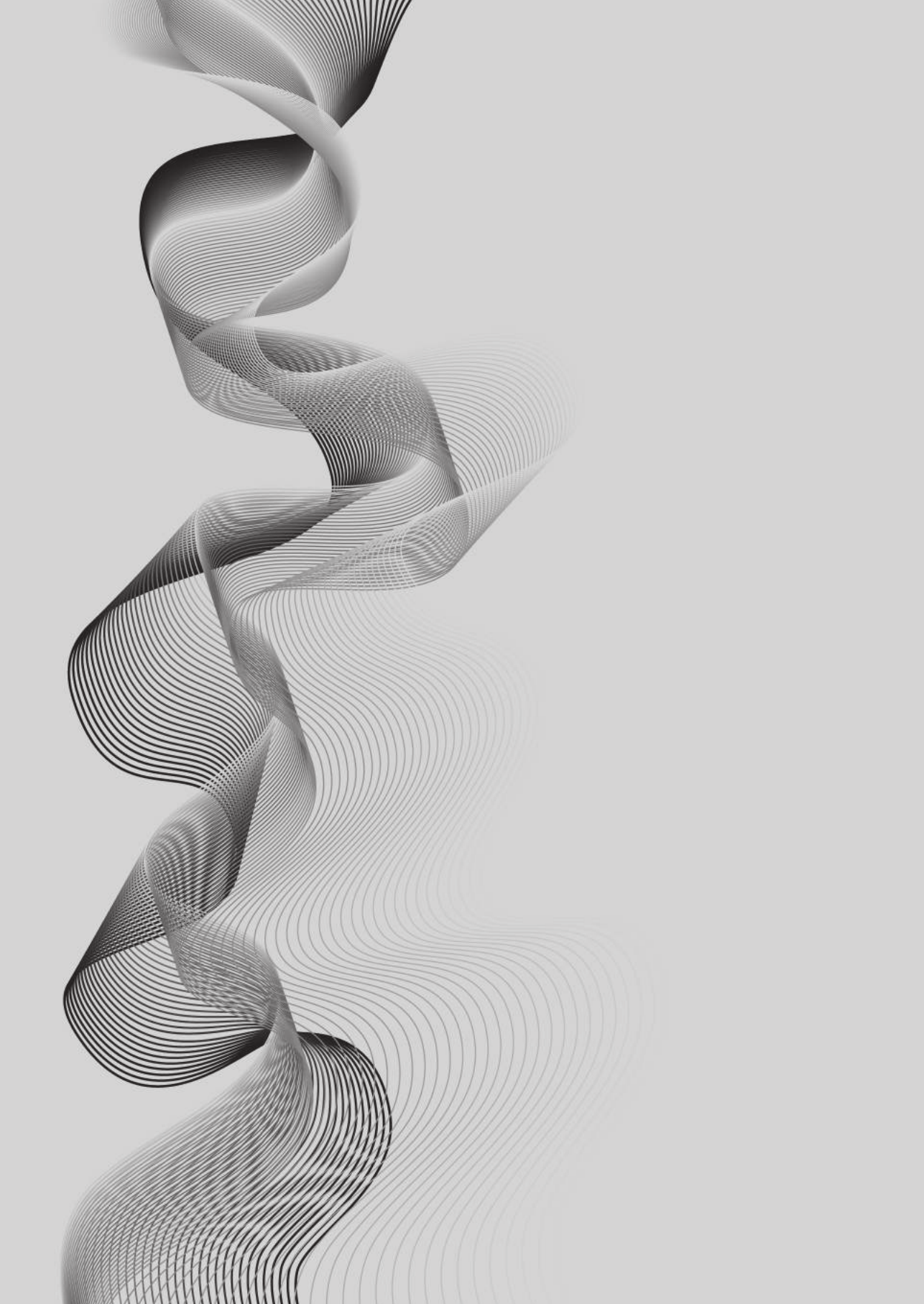
[b] Multivariable models for penile high-risk HPV clearance were a priori adjusted for age, HIV infection, smoking, number of recent anal sex partners, number of lifetime male sex partners, recent cannabis and/or poppers use, anal sex position, circumcision status, urethral STI (chlamydia and/or gonorrhea), and HPV type, unless indicated otherwise.

[c] Because of limited number of events only a priori adjusted for age, smoking, and number of recent anal sex partners.

Chapter 3

HIV and HCV in people who use drugs: disease progression and treatment





Chapter 3.1

Temporal trends in mortality among people who use drugs compared with the general Dutch population differ by hepatitis C virus and HIV infection status

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ABSTRACT

Objectives: We aimed to identify temporal trends in all-cause and cause-specific mortality rates among people who use drugs (PWUD) compared with the general Dutch population and to determine whether mortality trends differed by hepatitis C virus (HCV)/HIV status.

Design: Longitudinal cohort study.

Methods: Using data from the Amsterdam Cohort Studies among 1,254 PWUD (1985–2012), all-cause and cause-specific standardized mortality ratios (SMRs) were calculated; SMRs were stratified by serological group (HCV/HIV-uninfected, HCV-monoinfected, and HCV/HIV-coinfected) and calendar period. Temporal trends were estimated using Poisson regression.

Results: The overall all-cause SMR was 13.9 (95% confidence interval 12.6–15.3). The SMR significantly declined after 1996, especially due to a decline among women ($P<0.001$). The highest SMR was observed among HCV/HIV-coinfected individuals during 1990–1996 (SMR 61.9, 95% confidence interval 50.4–76.0), which significantly declined after this period among women ($P=0.001$). In contrast, SMR for HCV-monoinfected, and HCV/HIV-uninfected PWUD did not significantly change over time. The SMR for non-natural deaths significantly declined ($P=0.007$), whereas the SMR for HIV-related deaths was the highest during all calendar periods.

Conclusions: We found evidence for declining all-cause mortality among PWUD compared with the general population rates. Those with HCV/HIV-coinfection showed the highest SMR. The decline in the SMR seems to be attributable to the decline in mortality among women. Mortality rates due to non-natural deaths came closer to those of the general population over time. However, HIV-related deaths remain an important cause of mortality among PWUD when compared with the general Dutch population. This study reinforces the importance of harm-reduction interventions and HCV/HIV treatment to reduce mortality among PWUD.

INTRODUCTION

People who use drugs (PWUD) are at an increased risk of premature mortality compared with the general population. In the Netherlands, the start of a major heroin epidemic occurred during the 1970s [1,2]. The prevalence of hepatitis C virus (HCV) peaked during the 1980s, when more than 85% of ever-injectors tested HCV-positive [3,4]. The HIV epidemic commenced during the 1980s [5,6]. In 1985, the prospective Amsterdam Cohort Studies (ACS) among PWUD were initiated to track the HIV epidemic [5].

To minimize the damage PWUD inflict upon themselves and society, harm-reduction interventions were implemented in Amsterdam, the Netherlands, from 1979, making it one of the first countries to do so [7,8]. These included methadone programs and needle exchange programs (NEPs) [7,8]. Especially, the combination of methadone programs and NEP uptake has been associated with a lower risk of HIV and HCV acquisition among people who inject drugs (PWID) [9]. For HIV-infected individuals, combination antiretroviral therapy (cART) became widely available from 1996, which has been shown to substantially reduce HIV-related mortality [10–12]. However, HIV-infected PWID tend to initiate cART at a later stage than other risk groups [13], leading to a diminished probability of survival [14–16]. For HCV-infected PWUD, HCV treatment became relatively easy to access in Amsterdam from 2005, when a special unit was launched to treat HCV-infected PWUD [17], and might have reduced liver-related mortality, which generally requires two to three decades of chronic HCV infection [18].

Previous studies have shown that PWUD have a 4.4–47.6 higher risk of dying than the general population [19–24]. This increased risk of dying differs between studies and countries, which could be ascribed to differences in calendar period of follow-up, study population characteristics, drug-use practices, HIV/HCV incidence and background prevalence, and availability and uptake of harm-reduction interventions [20]. As the ACS have reached 27 years of follow-up, which is one of longest worldwide, we had the unique opportunity to identify changes in mortality rates over different calendar periods. Trends in mortality rates can serve as a proxy for changing health patterns and the effectiveness of health-related interventions that have been implemented.

We hypothesized that because of the introduction and availability of harm-reduction interventions and HCV and HIV therapy, PWUD have reached mortality rates closer to those of the general Dutch population in recent calendar periods. Our study aimed to identify temporal trends in all-cause and cause-specific mortality rates among PWUD compared with the general Dutch population; and to determine whether excess mortality trends differed by HCV/HIV status.

METHODS

Study population

The ACS among (injecting and non-injecting) PWUD is an open, prospective cohort study initiated in 1985, aiming to investigate the epidemiology, natural history, and pathogenesis of HIV, and to evaluate the effect of interventions. Participation in the ACS is voluntary and written informed consent is obtained at intake [25]. Recruitment is by means of local methadone outposts, a sexually transmitted diseases clinic, and word of mouth. PWUD visit the Public Health Service of Amsterdam every 4–6 months; they give blood and complete a standardized questionnaire about their health and sexual and drug use behavior during each cohort visit. To encourage continued participation, 12 Euros were paid per follow-up visit. Participants, aged between 20 and 64 years with at least two cohort visits and with both longitudinal HCV and HIV test results (1,254/1,661), were included in our study. This study was approved by the Medical Ethical Committee of the Amsterdam Medical Centre, the Netherlands.

Laboratory methods

At entry, the ACS participants were tested for HIV antibodies by ELISA and at every follow-up visit if previously negative. In 2005, participants with at least two cohort visits between December 1985 and January 2005 were retrospectively tested for HCV antibodies with a third-generation ELISA (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). Individuals who were anti-HCV-negative at entry were tested for HCV antibodies at the most recent visit. On finding HCV seroconversion (defined as the presence of HCV antibodies in a previously HCV-seronegative individual), we tested samples between the first and the most recent visit to obtain the most exact seroconversion interval. From 2005 onwards, HCV testing occurred prospectively.

Definitions and statistics

Information about vital status was obtained by matching the ACS data at regular intervals against the municipal and national population registries in the Netherlands. Causes of death (CODs) were systematically obtained from hospital records, general practitioners, the national HIV Monitoring Foundation, or coroners. The CODs were divided into four categories: HIV-related deaths; liver-related deaths; natural causes other than HIV or liver-related deaths (e.g. cardiovascular-related deaths, non-AIDS-related cancers); and non-natural causes (e.g. overdose, accidents, homicide, and suicide).

Follow-up time was calculated from the first cohort visit until: loss to follow-up, last known date to be alive, death, or cohort censoring date (i.e. 31 December 2012). Calendar periods were defined as follows: period 1 (1985–1989) – pre-cART and before

adequate methadone dose (≥60 mg/day) was generally available in Amsterdam; period 2 (1990–1996) – pre-cART with adequate methadone substitution therapy; period 3 (1997–2000) – early cART era; period 4 (2001–2005) – late cART era; period 5 (2006–2012) – late cART and HCV treatment era.

Four HCV/HIV serological groups were defined: HCV/HIV-uninfected, HIV-monoinfected, HCV-monoinfected, and HCV/HIV-coinfected. HIV and HCV status were treated as time-dependent variables. For HIV and HCV seroconverters, the midpoint between the last negative and the first positive antibody test was used to estimate the moment of seroconversion. After the last cohort visit, the serological group was carried forward until the end of follow-up. In a sensitivity analysis, a more stringent censoring strategy was applied (i.e. censoring took place at the end of the calendar period of the last cohort visit) to check whether results were robust.

Crude mortality rates (CMRs) per 1000 person-years, including 95% confidence intervals (CIs), were calculated. Standardized mortality ratios (SMRs) were used to compare the mortality rate among the PWUD with the mortality rate in the general Dutch population. SMR is the ratio of the observed deaths among PWUD from the ACS and the expected number of deaths. Expected deaths were calculated by multiplying the person-years accrued from our study population by the mortality rate of the general Dutch population matched by age group, sex, and calendar period.

Mortality rates of the general Dutch population were obtained from the Human Mortality Database (HMD) (www.mortality.org), and the numbers of deaths per COD were obtained from Statistics Netherlands (www.cbs.nl). To calculate the mortality rate of the general Dutch population per calendar period, age group, and sex, the following formula was used:

$$MR = \frac{Total\ \ deathsGP - Total\ \ deathsACS}{\mu PopulationGP - PopulationACS}$$

where MR is the mortality rate; GP the general population; and μ the average.

The SMRs for each calendar period, serological group, and COD were calculated using univariable Poisson regression models with the natural logarithm of the expected deaths as offset term; the exponential of the coefficient of the Poisson model is the SMR. Multivariable Poisson models, with the same offset term, were used to obtain the effect of calendar period and serological group on the SMR and are expressed in SMR ratios; SMR ratios can be interpreted as a relative SMR. *P* values for trends were obtained from the multivariable models. Multivariable models were corrected for age group, sex and serological group. We additionally checked for interactions between calendar period

and the other covariates. For the calculation of the SMR per cause of death, PWUD were included in the analysis irrespective of their HCV/HIV status. Stata version 11.2 was used (Stata Statistical Software: Release 11. College Station, Texas, USA: StataCorp LP).

RESULTS

Out of 1,254 PWUD, at entry, 63.9% (n=801) were men, their median age was 30 years [interquartile range (IQR) 26–36], and 72.4% (n=908) had ever injected drugs (Table 1). The median follow-up time was 15.0 years (IQR=9.5–20.9). At the study entry, the proportion of those who ever injected was similar among those included (i.e. at least two cohort visits) and excluded in this study. However, individuals included in our study were older, more often of Dutch nationality, less often homeless and a higher proportion of them were men compared to the excluded PWUD.

Table 1: General characteristics of 1,254 people who use drugs on active follow-up from the Amsterdam Cohort Studies with at least two follow-up visits by calendar period (1985- 2012).

Calendar period	Total	1985-1989	1990-1996	1997-2000	2001-2005	2006-2012
N = 1,254	**n %**	541	853	823	699	498
Age median (IQR) [a]	30 (26-36)	29 (25-33)	32 (28-36)	36 (31-41)	39 (32-44)	44 (39-49)
Age group in years n (%) [a]						
20-34	886 (70.65)	446 (82.44)	554 (64.95)	331 (40.22)	209 (29.90)	59 (11.85)
35-49	356 (28.39)	93 (17.19)	291 (34.11)	470 (57.11)	441 (63.09)	332 (66.67)
50-64	12 (0.96)	2 (0.37)	8 (0.94)	22 (2.67)	49 (7.01)	107 (21.49)
Sex n (%)						
Men	801 (63.88)	293 (54.16)	512 (60.09)	559 (67.92)	489 (69.96)	338 (67.87)
Women	453 (36.12)	248 (45.84)	341 (39.98)	264 (32.08)	210 (30.04)	160 (32.13)
Nationality n (%)						
Dutch	934 (74.48)	401 (74.12)	628 (73.62)	655 (79.59)	567 (81.12)	408 (81.93)
Non-Dutch	320 (25.52)	140 (25.88)	225 (26.38)	168 (20.41)	132 (18.88)	90 (18.07)
Homeless n (%) [a]						
Yes	116 (9.34)	9 (1.66)	71 (8.38)	62 (7.60)	68 (9.91)	33 (7.02)
No	1,126 (90.66)	532 (98.34)	776 (91.62)	754 (92.40)	618 (90.09)	437 (92.98)

Table 1: General characteristics of 1,254 people who use drugs on active follow-up from the Amsterdam Cohort Studies with at least two follow-up visits by calendar period (1985- 2012). (*continued*)

Calendar period	Total	1985-1989	1990-1996	1997-2000	2001-2005	2006-2012
Ever injected drugs n (%)						
Yes	908 (72.41)	450 (83.18)	695 (81.48)	586 (71.20)	465 (66.52)	338 (67.87)
No	346 (27.59)	91 (16.82)	158 (18.52)	237 (28.80)	234 (33.48)	160 (32.13)
Injected drugs in the preceding 6 months n (%) [a,b]						
Yes	668 (73.98)	349 (77.56)	496 (72.20)	292 (50.52)	179 (38.91)	86 (26.88)
No	235 (25.88)	101 (22.44)	191 (27.80)	286 (49.48)	281 (61.09)	226 (72.44)
Borrowed needles n (%) [a,c]						
Yes	234 (35.03)	157 (44.99)	99 (19.96)	45 (15.41)	14 (7.82)	3 (3.49)
No	434 (64.97)	192 (55.01)	397 (80.04)	247 (84.59)	165 (92.18)	83 (96.51)
Methadone dosage in mg n (%) [a]						
0	208 (17.33)	20 (4.05)	159 (18.84)	226 (27.80)	222 (32.13)	137 (29.72)
1-60 mg	310 (25.83)	40 (8.10)	414 (49.05)	260 (31.98)	166 (24.02)	103 (22.34)
>60 mg	253 (21.08)	21 (4.25)	249 (29.50)	297 (36.53)	302 (43.70)	219 (47.51)
Unknown[d]	429 (35.75)	413 (83.60)	22 (2.61)	30 (3.69)	1 (0.14)	2 (0.43)
HCV/HIV serogroup n (%) [a]						
HCV/HIV-uninfected	428 (34.13)	109 (20.15)	199 (23.33)	294 (35.72)	291 (41.63)	193 (38.76)
HIV-monoinfected	16 (1.28)	7 (1.29)	9 (1.06)	9 (1.09)	10 (1.43)	9 (1.81)
HCV-monoinfected	564 (44.98)	283 (52.31)	421 (49.36)	356 (43.26)	292 (41.77)	227 (45.58)
HCV/HIV-coinfected	246 (19.62)	142 (26.25)	224 (26.26)	164 (19.93)	106 (15.16)	69 (13.86)
Chronic hepatitis B [e]						
Yes	72 (7.00)	46 (9.78)	63 (8.55)	36 (5.27)	22 (3.95)	14 (3.75)
No	956 (93.00)	424 (90.21)	674 (91.45)	646 (94.72)	535 (96.05)	359 (96.24)
Seroconversion during follow up n						
HIV seroconversion	96	23	56	9	5	3
HCV seroconversion	54	19	25	4	6	0

Abbreviations: IQR: inter-quartile range; HCV: hepatitis C virus; HBV: hepatitis B virus; HIV: human immunodeficiency virus; mg: milligrams. Missing values at study entry for: "methadone dosage"=54; "Chronic hepatitis B"=173; "Homeless"=12; "Injected drugs in the preceding sex months"=5; Missing values are not included in the percentages.

[a] At study entry in the cohort (total) and at the first visit during the calendar period.

[b] Out of those who ever injected drugs.

[c] Out of those who injected drugs in the last 6 months preceding the first visit during that calendar period.

[d] Unknown dosage among those enrolled in a methadone program.

The characteristics and demographical distribution of PWUD changed across the calendar periods (Table 1). During the first calendar period, 82.4% of PWUD were aged between 20 and 34 years compared with only 11.9% in the most recent calendar period. The percentage of participants who ever injected drugs or injected drugs during the calendar period decreased over time. During the first calendar period, 20.2% were HCV/HIV-uninfected, 1.3% HIV-monoinfected, 52.3% HCV-monoinfected, and 26.3% HCV/HIV-coinfected. During the latest calendar period, 38.8% were HCV/HIV-uninfected, 1.8% HIV-monoinfected, 45.6% HCV-monoinfected, and 13.9% HCV/HIV-coinfected. During 18,575 person-years of total follow-up time, 96 PWUD seroconverted for HIV and 54 for HCV.

Overall mortality rates

Among 1,254 PWUD, 406 deaths were observed during the study period. The overall CMR for all-cause mortality was 21.9 per 1000 person-years (95% CI 19.8–24.1). The overall effect of calendar period on the CMR was significant (*P*=0.003). The highest CMR was observed between 1990 and 1996 (27.5 per 1000 person-years; 95% CI 23.1–32.6) (Table 2). The CMR declined from 27.5 in 1990–1996 to 17.4 in 2001–2005, and was followed by a slight increase to 23.7 in the period 2006–2012 (Fig. 1a).

The overall SMR was 13.9 (95% CI 12.6–15.3), meaning that drug users from the ACS had a 13.9 times higher mortality rate compared with the age, sex, and calender-matched general Dutch population. During 1985–1990, the SMR was 15.8 (95% CI 9.7–25.9), it increased to 25.5 (95% CI 21.5–30.3) in 1990–1996, followed by a decline to 14.1 (95% CI 11.2–17.8) in 1997–2000, 9.9 (95% CI 7.9–12.3) in 2001–2005, and 10.8 (95% CI 9.0–13.1) in 2006–2012 (Fig. 1b, Table 3). The SMR decreased with increasing age ($SMR_{20–34}$=22.9, 95% CI 18.7–28.2; $SMR_{35–49}$=15.5, 95% CI 13.7–17.5; $SMR_{50–64}$=7.3, 95% CI 5.8–9.3) and was higher among women (SMR_{men}=12.8, 95% CI 11.3–14.4; SMR_{women}=16.6, 95% CI 14.0–19.6).

In multivariable analysis, a significant interaction between calendar period and sex (*P*=0.03) was observed. The SMR significantly decreased in later calendar periods among women [adjusted SMR (aSMR)$r_{2006–2012}$=0.6, 95% CI 0.4–0.9, compared to 1990–1996; *P*<0.001] (Table 3), while remaining stable among men. HCV-monoinfected (aSMRr=1.9, 95% CI 1.4–2.8) and HCV/HIV-coinfected (aSMRr=7.0, 95% CI 5.0–9.8) PWUD had a significantly higher SMR compared with HCV/HIV-uninfected PWUD (*P*<0.001) (Table 3).

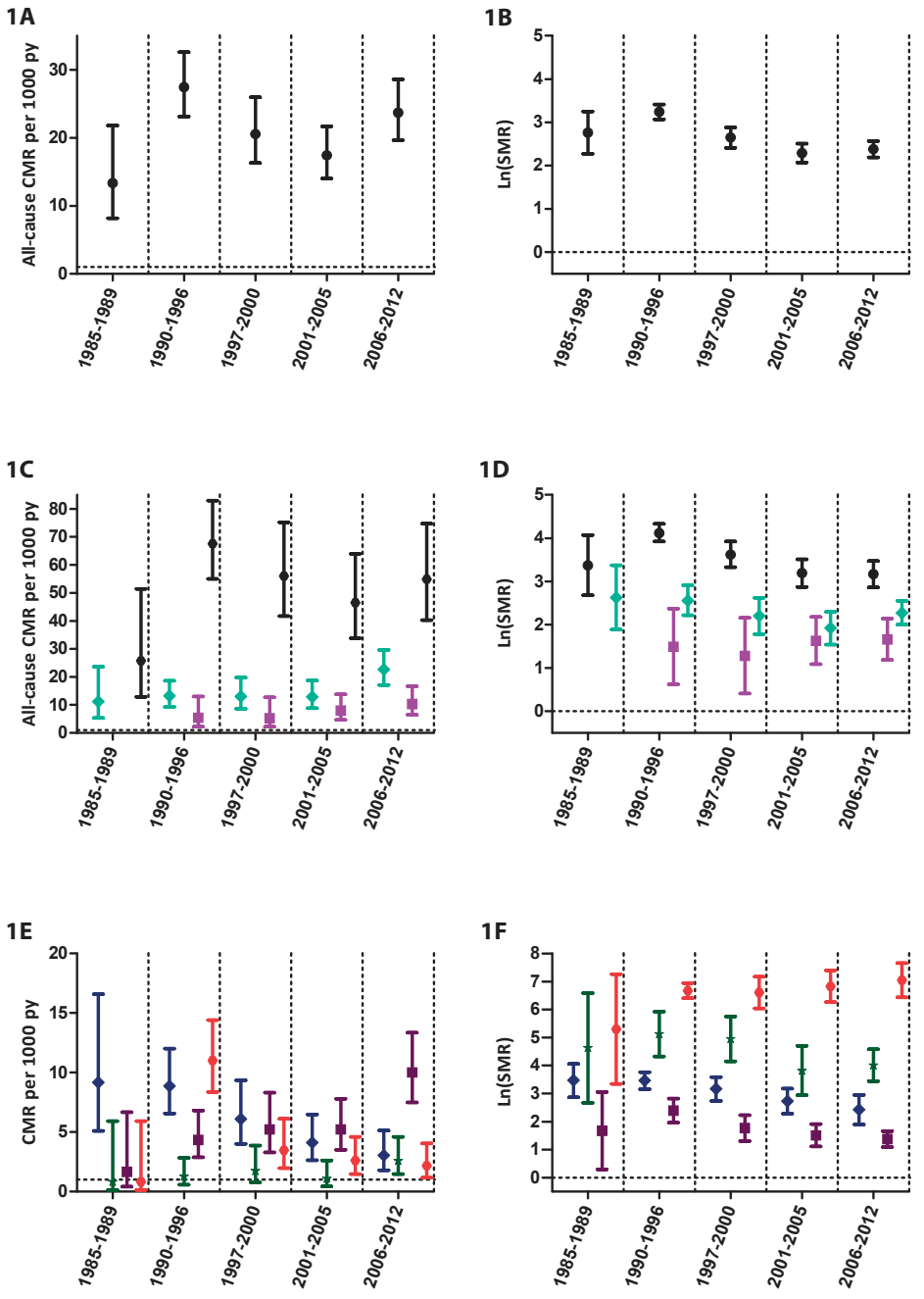

Figure 1 (figures 1a-1f): Crude mortality rates (CMRs) 1a) overall, 1c) per serological group and 1e) per cause of death stratified by calendar period; and standardized mortality ratios (SMRs) 1b) overall, 1d) per serological group, and 1f) per cause of death stratified by calendar period among people who use drugs from the Amsterdam Cohort Studies (1985-2012).

Figure 1 (figures 1a-1f): ***(continued)***
On the left side, all graphs show the CMRs and on the right side all graphs show the natural logarithm of the SMRs. The SMRs depicted on the graphs are transformed to the natural logarithm in order to fit either all serological groups or causes of death into one graph. Note: Most of the graphs have different scales in order to fit each of the CMR or SMR into one graph. (a) All-cause CMR per 1000 py per calendar period among 1,254 PWUD; (b) all-cause SMR per calendar period among 1,254 PWUD; (c) all-cause CMR per 1000 py per calendar period and serological group; (d) all-cause SMR per calendar period and serological group; (e) cause-specific CMR per 1000 py per calendar period; (f) cause-specific SMR per calendar period. CMRs and SMRs for HIV-monoinfected are not shown given the small number of individuals and few endpoints. Ln, natural logarithm; py, person-years.

All-cause mortality per serological group

Hepatitis C virus/HIV-uninfected people who use drugs

Although the overall effect of calendar period was not significant (*P*=0.16), the CMR for HCV/HIV-uninfected PWUD slightly increased from 2001 to 2005 onwards (Fig. 1c, Table 2). The overall SMR was 4.7 (95% CI 3.5–6.5) (Table 3). As illustrated in Fig. 1d, the SMR slightly increased after 2000, although the overall effect of calendar period was not statistically significant in multivariable analysis (*P*=0.54) (Table 3).

HIV-monoinfected people who use drugs

The overall CMR for HIV-monoinfected PWUD was 37.9 (95% CI 19.0–75.9) (Table 2) and the overall SMR was 26.3 (95% CI 13.2–52.6). Given the small number of HIV-monoinfected PWUD and deaths among them, the SMRs per calendar period were not calculated for them.

Hepatitis C virus-monoinfected people who use drugs

The CMR for HCV-monoinfected PWUD was borderline significantly different across the calendar periods (*P*=0.05) and was the highest between 2006 and 2012 (CMR=22.7, 95% CI 17.1–30.0) (Fig. 1c, Table 2). The overall SMR was 9.5 (95% CI 8.1–11.3) (Table 3). As illustrated in Fig. 1d, the SMR decreased until 2001–2005 and was followed by a slight increase in 2006–2012, although the overall effect of calendar period was not statistically significant in the multivariable analysis (*P*=0.23) (Table 3).

Hepatitis C virus/HIV-coinfected people who use drugs

The CMR for HCV/HIV-coinfected PWUD significantly differed across the calendar periods (*P*=0.03) and was the highest between 1990 and 1996 (CMR=67.5, 95% CI 55.0–82.9), which is also the highest CMR observed in this study (Fig. 1c, Table 2). The CMR for HCV/HIV-coinfected PWUD was more than twice that of the CMR for all PWUD and had a pattern similar to the CMR for all PWUD over time (Fig. 1a and c, Table 2). The overall SMR was 35.9 (95% CI 31.5–41.0) (Table 3). HCV/HIV-coinfected PWUD had the highest SMR during all calendar periods compared with the other serological groups and especially

Table 2: All-cause crude mortality rates (overall and by HCV/HIV serological group) and cause-specific crude mortality rates among 1,254 people who use drugs from the Amsterdam Cohort Studies by calendar period (1985- 2012).

	Total	1985-1989	1990-1996	1997-2000	2001-2005	2006-2012	*P* value
Overall CMR by calendar period							
Person years	18,575	1,198	4,736	3,454	4,594	4,598	
Number of deaths (n)	406	16	130	71	80	109	
Number of PWUD[a]	1,254	541	923	1,031	1,029	899	
CMR (95% CI)	21.86 (19.83-24.09)	13.36 (8.18-21.80)	27.45 (23.11-32.60)	20.59 (16.31-25.98)	17.42 (13.99-21.68)	23.70 (19.65-28.60)	0.003
CMR by serological group							
HCV/HIV-uninfected n (%)	40 (100)	0	5 (12.50)	5 (12.50)	13 (32.50)	17 (42.50)	
CMR (95%CI)	7.44 (5.46-10.14)	0 (0-15.23)[b]	5.40 (2.25-12.97)	5.30 (2.21-12.74)	7.99 (4.64-13.76)	10.38 (6.45-16.69)	0.160
HIV-monoinfected n (%)	8 (100)	1 (12.50)	2 (25.00)	0	2 (25.00)	3 (37.50)	
CMR (95%CI)	37.96 (18.98-75.90)	60.22 (8.48-427.52)	50.46 (12.62-201.74)	0 (0-99.96)[b]	40.18 (10.04-160.65)	44.25 (14.27-137.21)	0.523
HCV-monoinfected n (%)	137 (100)	7 (5.11)	32 (23.36)	22 (16.06)	27 (19.71)	49	
CMR (95%CI)	15.23 (12.88-18.00)	11.14 (5.31-23.37)	13.21 (9.34-18.68)	13.07 (8.60-19.85)	12.86 (8.82-18.75)	22.65 (17.12-29.97)	0.047
HCV/HIV-coinfected n (%)	221 (100)	8 (3.62)	91 (41.18)	44 (19.91)	38 (17.19)	40 (18.10)	
CMR (95%CI)	55.37 (48.53-63.18)	25.73 (12.87-51.45)	67.52 (54.98-82.92)	55.97 (41.67-75.22)	46.98 (33.83-63.90)	54.86 (40.24-74.79)	0.029
CMR by cause of death							
HIV-related n (%)	87 (21.43)	1 (6.25)	52 (40.00)	12 (16.90)	12 (15.00)	10 (9.17)	
CMR (95%CI)	4.68 (3.79-5.78)	0.83 (0.12-5.92)	10.98 (8.37-14.41)	3.47 (1.97-6.12)	2.61 (1.48-4.60)	2.17 (1.17-4.04)	<0.001
Liver-related n (%)	30 (7.39)	1 (6.25)	6 (4.62)	6 (8.45)	5 (6.25)	12 (11.01)	
CMR (95%CI)	1.61 (1.13-2.31)	0.83 (0.12-5.92)	1.27 (0.56-2.82)	1.74 (0.78-3.88)	1.09 (0.45-2.61)	2.60 (1.48-4.60)	0.376
Natural n (%)	111 (27.34)	2 (12.50)	21 (16.15)	18 (25.35)	24 (30.00)	46 (42.20)	
CMR (95%CI)	5.97 (4.96-7.20)	1.67 (0.42-6.68)	4.43 (2.89-6.80)	5.22 (3.29-8.30)	5.22 (3.50-7.79)	10.00 (7.49-13.36)	0.001
Non-Natural n (%)	107 (26.35)	11 (68.75)	42 (32.31)	21 (29.78)	19 (23.75)	14 (12.84)	
CMR (95%CI)	5.76 (4.76-6.96)	9.18 (5.08-16.58)	8.87 (6.55-12.00)	6.09 (4.00-9.34)	4.14 (2.64-6.48)	3.04 (1.80-5.14)	0.001
Unknown	71 (17.49)	1 (6.25)	9 (6.92)	14 (19.72)	20 (25.00)	27 (24.77)	

Abbreviations = CMR: crude mortality rate; HCV: hepatitis C virus; HIV: human immunodeficiency virus; n: number of deaths; 95% CI: confidence intervals; PWUD: People who use drugs.

[a] The number of PWUD per calendar period also includes those PWUD who were not on active follow-up but contributed follow-up time until censoring.

[b] Calculation based on exact binomial methods.

Table 3: Multivariable Poisson models for all-cause standardized mortality ratios (overall and stratified per HCV/HIV serological group) among 1,254 people who use drugs from the Amsterdam Cohort Studies (1985-2012).

	All-cause mortality [a]			Per serological group			
				HCV/HIV-uninfected	HCV-monoinfected	HCV/HIV-coinfected [a]	
	SMR (95%CI)	aSMRr(95%CI) *P*	aSMRr(95%CI) *P*	aSMRr (95%CI) *P*	aSMRr (95%CI) *P*	aSMRr (95%CI) *P*	aSMRr (95%CI) *P*
		Women	Men			Women	Men
Calendar period		<0.001	0.569	0.543	0.227	<0.001	0.382
1985-1989	15.84 (9.70-25.85)	0.54 (0.23-1.26)	0.59 (0.29-1.18)	NE	0.92 (0.40-2.09)	0.28 (0.07-1.16)	0.62 (0.26-1.44)
1990-1996	25.52 (21.49-30.31)	1	1	1	1	1	1
1997-2000	14.12 (11.19-17.82)	0.46 (0.28-0.77)	0.87 (0.61-1.26)	0.86 (0.25-3.01)	0.88 (0.50-1.55)	0.33 (0.17-0.64)	0.93 (0.59-1.46)
2001-2005	9.87 (7.93-12.29)	0.28 (0.16-0.49)	0.82 (0.58-1.18)	1.23 (0.43-3.52)	0.80 (0.46-1.41)	0.29 (0.15-0.56)	0.63 (0.38-1.05)
2006-2012	10.83 (8.98-13.07)	0.60 (0.39-0.94)	0.92 (0.64-1.33)	1.30 (0.45-3.71)	1.38 (0.81-2.37)	0.30 (0.15-0.58)	0.77 (0.46-1.32)
Serological group			<0.001	NA	NA	NA	NA
HCV/HIV-uninfected	4.73 (3.47-6.45)	1					
HCV-monoinfected	9.52 (8.06-11.26)	1.93 (1.36-2.75)					
HCV/HIV-coinfected	35.91 (31.47-40.97)	6.97 (4.96-9.80)					

Abbreviations= SMR: Standardized mortality ratio; aSMRr= Adjusted SMR ratio; HCV: hepatitis C virus; HIV: human immunodeficiency virus; NA: not applicable; 95% CI: confidence interval; P: p-value; NE: no events

Serological group, age group and sex were included as covariates in all multivariable models. Statistically significant interaction terms were also included as covariates in the model.

HIV-monoinfected people were not included in the multivariable model due to limited numbers.

[a] Significant interaction between calendar period and sex.

during the period 1990–1996 (SMR=61.9, 95% CI 50.4–76.0) (Fig. 1d). As illustrated in Fig. 1d, the SMR declined after 1990–1996 and remained stable in the two most recent periods. In multivariable analysis, the effect of calendar period was significantly different among women and men (*P*=0.03). Among women, the SMR was significantly lower in the last three calendar periods compared with 1990–1996 (*P*<0.001), whereas the SMR remained relatively stable over time among men (Table 3).

Mortality per cause of death

The most common CODs were natural deaths (n=111), followed by non-natural (n=107), HIV-related (n=87), and liver-related deaths (n=30).

Natural causes

The CMR for natural deaths significantly increased over time (*P*=0.001) – from 1.7 (95% CI 0.4–6.7) between 1985 and 1989 to 10.0 (95% CI 7.5–13.4) between 2006 and 2012 (Fig. 1e, Table 2). The overall SMR was 5.0 (95% CI 4.1–6.0) and was the lowest of the cause-specific SMR. As illustrated in Fig. 1f, the SMR for natural causes decreased after 1990–1996 (Fig. 1f), although the overall effect of calendar period was not significant (*P*=0.38) (Table 4).

Non-natural causes

In contrast to the CMR for natural causes, the CMR for non-natural deaths significantly decreased over time (*P*=0.001) – from 9.2 (95% CI 5.1–16.6) in 1985–1989 to 3.0 (95% CI 1.8–5.1) in 2006–2012 (Fig. 1e, Table 2). The overall SMR for non-natural deaths was 21.3 (95% CI 17.6–25.7), and we observed a steady decline of the SMR over time (*P*=0.007) (Fig. 1f, Table 4).

HIV-related causes

The CMR for HIV-related deaths reached its peak in the period 1990–1996 (CMR=11.0, 95% CI 8.4–14.4), followed by a statistically significant decline after 1996 (*P*<0.001) (Fig. 1e, Table 2). The overall SMR was 798.2 (95% CI 647.0–984.9). As illustrated in Fig. 1f, we observed a higher HIV-related SMR after 1990, although the overall effect of calendar period was not significant in the multivariable analysis for men (*P*=0.30) and bordeline signficant for women (*P*=0.06) (Table 4).

Liver-related causes

The overall CMR for liver-related deaths was 1.6 (95% CI 1.1–2.3) and did not significantly differ across the calendar periods (*P*=0.38) (Table 2). The overall SMR for liver-related deaths was 72.4 (95% CI 50.6–103.5). As illustrated in Fig. 1f, the SMR for liver-related deaths decreased after 2000, although the overall effect of calendar period was not significant in the multivariable analysis (*P*=0.11) (Table 4).

Table 4: Multivariable Poisson models for cause-specific Standardized Mortality Ratios among 1,254 people who use drugs from the Amsterdam Cohort Studies (1985-2012).

	SMR-ratios by cause of death									
	Natural		Non-Natural		HIV-related [c]				Liver-related [b]	
	aSMRr(95%CI)	*P*	aSMRr(95%CI)	*P*	aSMRr(95%CI)	*P*	aSMRr(95%CI)	*P*	aSMRr(95%CI)	*P*
					Women		Men			
Calendar period		0.383		0.007		0.055		0.298		0.105
1985-1989	1 [a]		1		1 [a]		1 [a]		1 [a]	
1990-1996			0.94 (0.48-1.84)							
1997-2000	0.73 (0.39-1.36)		0.84 (0.40-1.80)		0.53 (0.20-1.38)		1.11 (0.48-2.53)		0.89 (0.30-2.5)	
2001-2005	0.67 (0.37-1.23)		0.47 (0.21-1.06)		0.26 (0.08-0.88)		2.08 (0.97-4.46)		0.33 (0.10-1.06)	
2006-2012	0.60 (0.34-1.06)		0.31 (0.13-0.74)		0.50 (0.16-1.58)		1.76 (0.67-4.60)		0.38 (0.15-0.97)	

Abbreviations= SMR: Standardized mortality ratio; aSMRr= Adjusted SMR ratio HCV: hepatitis C virus; HIV: human immunodeficiency virus; 95% CI: confidence interval; P: p-value.

Serological group, age group and sex were included as covariates in all multivariable models. Statistically significant interaction terms were also included as covariates in the model.

No multivariable model was made for HIV-monoinfected due to limited numbers.

[a] The first and the second calendar period are grouped together due to low number of deaths.

[b] Only sex was included as a covariate given the limited number of events (number of deaths=30).

[c] Significant interaction between calendar period and sex

Finally, in a sensitivity analysis with a more stringent censoring strategy, comparable results were observed.

DISCUSSION

We investigated whether mortality among PWUD from the ACS has come closer to that of the general Dutch population in recent calendar periods. As hypothesized, we observed a decline in mortality among PWUD compared with the general Dutch population after 1996. However, despite this decline, mortality rates among PWUD are still 11 times higher than those of the general population in the most recent calendar period. Of interest, mortality due to non-natural deaths came closer to the general Dutch population over time.

The decline in the SMR among PWUD seems to be mainly attributable to the decline in mortality after 1996 among HCV/HIV-coinfected women; this is in line with a study among PWID in Norway, which showed that compared to men, women had a lower risk of mortality in the long term, although in the short term – within 3 years of inclusion in the study –, women had a higher risk of mortality [26]. One explanation for the different mortality trends among men and women might be that women sought HIV and/or drug treatment earlier than men and were less likely to be imprisoned [26]. Furthermore, the decline among HCV/HIV-coinfected PWUD might be explained by the availability of cART from 1996 onwards. In line with our findings, a study among HIV seroconverters showed that overall and cause-specific mortality rates decreased after the introduction of cART [27]. However, the benefits of cART appeared to be less pronounced for PWUD than for MSM [27]. Nonetheless, cART availability alone cannot explain the decrease of the SMR observed, as a more constant SMR would be expected because the positive effects of cART have also reduced mortality among HIV-infected individuals in the general Dutch population. Hence, the decline of the SMR after 1996 is also likely to be attributable to the significant decline in non-natural deaths over time. This is in contrast to the findings from the 2008 Annual Report by the European Monitoring Centre for Drugs and Drug Addiction, in which a rebound of overdose mortality was observed in many European countries from 2003 to 2005 [28]. The observed decline in non-natural deaths in our study could be explained by a decrease in the popularity of injecting drug use in Amsterdam over time [29,30]. During 2006–2012, only 26.9% of the ACS participants injected drugs compared with 77.6% during 1985–1989. Also, PWID might have become safer injectors over time, reducing the risk of overdose. These changes in injecting risk behavior could be ascribed to harm-reduction interventions and demographical changes, as recently demonstrated in our modeling study [31]. To summarize, a combination

of factors such as availability of comprehensive harm-reduction interventions, HIV and HCV therapy and changing drug patterns among PWUD probably led to the reduction in mortality observed in the present study, especially among HCV/HIV-coinfected women. In addition, HIV-infected PWUD who survived the pre-cART period might have been the PWUD exhibiting less risk behavior and the lowest risk of dying.

Of interest, the HIV-related CMR among PWUD significantly and substantially decreased after 1996, whereas the HIV-related SMR did not significantly change over time. HIV-related mortality is dependent on the incidence and prevalence of HIV in a population. Therefore, given our higher proportion of HIV-infected PWUD compared with the general Dutch population, as expected, we observed a high HIV-related SMR. However, even though the proportion of HIV-infected PWUD in our study population decreased over calendar time, no decrease was observed in the HIV-related SMR. Therefore, the HIV-related mortality in the general Dutch population probably decreased at a faster rate than it did for PWUD from the ACS. HCV-coinfection, which is common among our study population, might play a role as it has been shown that HCV/HIV-coinfected individuals have a higher risk of death from HIV/AIDS than HIV-monoinfected individuals [12,32]. Also, even though the benefits of cART have been observed in all risk groups [33], the HIV-positive individuals from exposure groups other than PWUD might have easier access to care, be more adherent to cART, have a better socioeconomic status, and a healthier lifestyle.

In line with the two studies [22,34] we found that HCV/HIV-coinfected PWUD have a higher SMR than HIV-monoinfected and HCV-monoinfected PWUD. Our overall SMR (35.9) for HCV/HIV coinfection was higher than the SMR (12.8) described by Hernando *et al.* [22], but was very similar to the findings by McDonald *et al.* (34.0) [34]. The difference in SMR between the studies might be explained by differences in the study period. Hernando et al.'s study comprises data from the cART era (1997–2010), whereas both our and McDonald et al.'s study had follow-up both during the pre-cART and the cART era [22,34].

The SMR for liver-related deaths did not significantly change over time. This could be attributed to an increase in liver-related deaths in the general Dutch population [35]. Furthermore, HCV/HIV-coinfected PWUD might have died of HIV-related causes before experiencing the consequences of HCV [4]. However, the burden of HCV-related disease among PWUD in our study was made visible by the increased liver-related CMR during the latest calendar period.

We found that the CMR for natural causes significantly increased over time. This can be partly explained by ageing of our cohort participants and the lower risk of HIV-related mortality among PWUD in the cART era. However, if CODs are misclassified (i.e. underlying HCV and HIV-related cause of death is not recognized), this might contribute to the increasing CMR for natural deaths. Nevertheless, the SMR for natural causes did not significantly change over time.

Several limitations of our study should be mentioned. First, even though we adjusted for age group, sex, calendar period, and serological group, other determinants, such as active drug use, alcohol consumption, hepatitis B, or smoking, were not taken into account. Second, PWUD who were ever positive for HCV antibodies were considered HCV-positive for the whole study period, thus spontaneous or treatment-induced HCV clearance was not taken into account. Although the minority, especially in HCV/HIV-coinfected individuals, clears the virus spontaneously [35], this definition of HCV positivity might have led to an underestimation of the SMR for PWUD with a chronic HCV infection. However, active PWID who clear the virus are at risk of HCV re-infection. Third, although coverage and components of harm-reduction interventions changed during the study period, they were already implemented before the ACS started; therefore we do not have a proper comparison with a period without such interventions. Furthermore, our sample of PWUD might not be representative for the general PWUD Dutch population.

In conclusion, in line with our hypothesis, significant declines in all-cause and non-natural morality rates were observed among PWUD compared with the general Dutch population. Women with an HCV/HIV coinfection contributed to the decline in the all-cause SMR over time. However, PWUD are still at an increased risk of dying even when uninfected with HCV and HIV. Our results also suggest that, despite the availability of cART, HIV-related deaths remain an important cause of mortality among PWUD when compared with the general Dutch population. This study reinforces the importance of a high coverage of comprehensive harm-reduction interventions combined with timely HIV and HCV treatment uptake to reduce excess mortality among PWUD.

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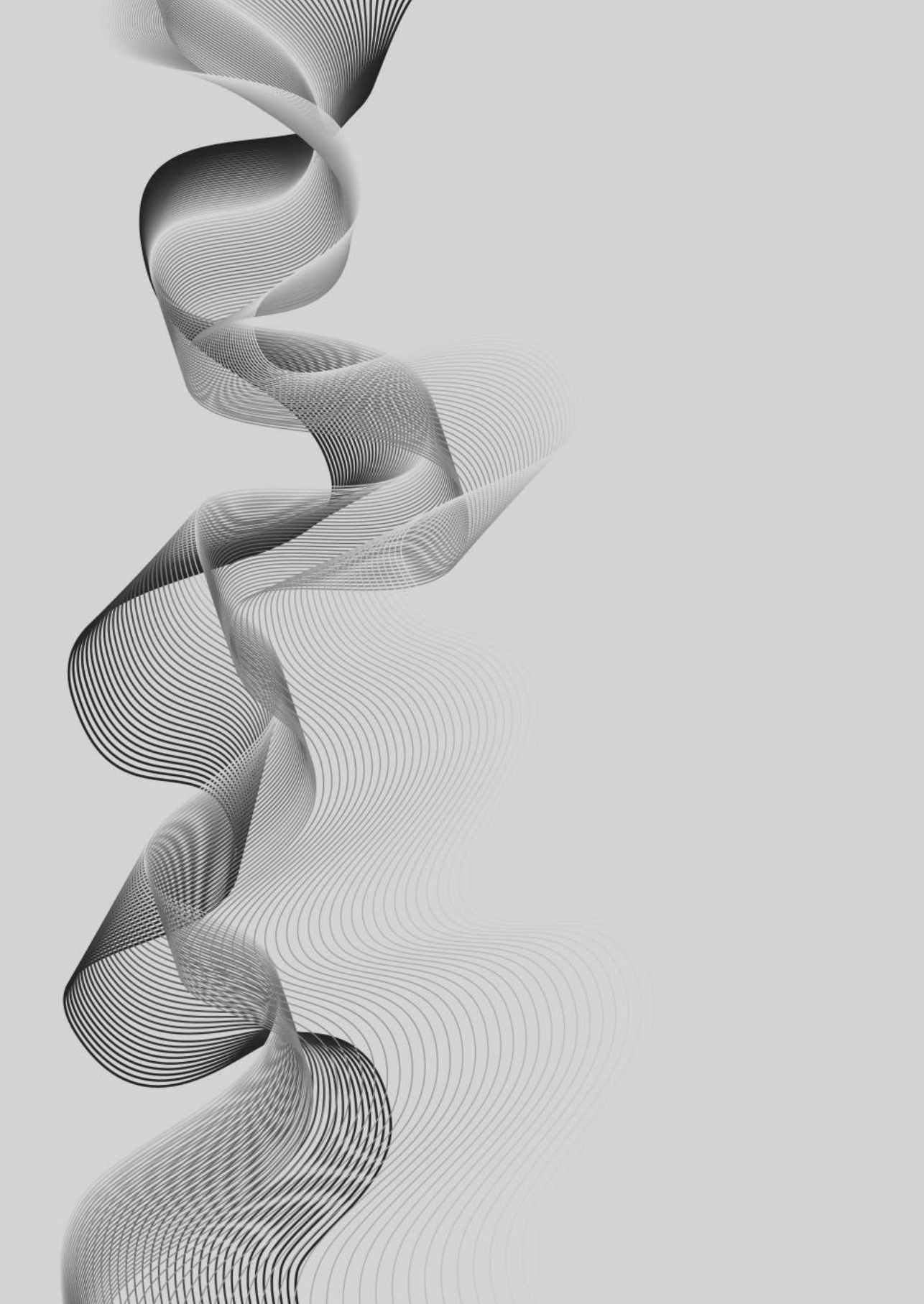

Chapter 3.2

High proportions of moderate to severe fibrosis and cirrhosis in an ageing population of people who use drugs in Amsterdam, the Netherlands

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ABSTRACT

Background: the incidence and prevalence of hepatitis C virus (HCV) infection among people who use drugs (PWUD) peaked in the 1980s in Amsterdam. As liver cirrhosis develops several decades after HCV-infection and PWUD bear other risk factors for liver fibrosis, we hypothesized that significant liver fibrosis or cirrhosis is now common among PWUD in Amsterdam.

Methods: PWUD were recruited from the Amsterdam Cohort Studies, methadone programs, and addiction clinics during 2009-2016. Transient elastography was performed to assess liver stiffness. We estimated METAVIR fibrosis levels based on the following liver stiffness measurements (LSM) cut-offs: F0-F2 (no/mild) <7.65 kPa; F2-F3 (moderate/severe) ≥7.65-<13 kPa; F4 (cirrhosis) ≥13 kPa. Using linear regression models, we assessed the association between LSM and socio-demographic, clinical, and behavioural determinants in (1) all PWUD and (2) chronic HCV (cHCV)-infected PWUD.

Results: for 140 PWUD, median LSM was 7.6 kPa (interquartile range=4.9-12.0); 26.4% had moderate/severe fibrosis, and 22.9% had cirrhosis. Of 104 chronically infected PWUD, 57.7% had evidence of significant fibrosis (≥F2). In multivariable analysis including all PWUD, increased LSM was significantly associated with chronic HCV mono-infection and HIV/HCV co-infection. In cHCV-infected PWUD, older age was significantly associated with increased LSM. In all groups, longer duration of heavy alcohol drinking was associated with increased LSM.

Conclusion: A high proportion of PWUD had significant fibrosis or cirrhosis which were associated with chronic HCV infection, HIV/HCV co-infection, and duration of heavy alcohol drinking. Increased uptake of HCV-treatment and interventions to reduce alcohol use are needed to decrease the liver disease burden in this population.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection may lead to progressive hepatic fibrosis, cirrhosis, and hepatocellular carcinoma [1]. A few studies have evaluated the burden of liver disease among people who use drugs (PWUD) in high-income countries [2-9], reporting prevalence of cirrhosis ranging from 1% to 47% [2-9]. However, different methods to define cirrhosis and HCV infection were employed in these evaluations, making comparisons difficult. In addition, the known duration since start of injecting drugs differed among study populations, suggesting wide variation in the duration of HCV infection and the resulting time to liver fibrosis development [2-9].

In the Netherlands, heroin use was introduced in the 1970s [10]. The heroin epidemic resulted in an increased risk for blood-borne infections due to shared injecting equipment. Injecting drug use (IDU) became more common and the prevalence and incidence of HCV infection increased, peaking in the 1980s, with more than 80% of an estimated 6,275 people who inject drugs (PWID) testing anti-HCV positive in the capital city of Amsterdam [11,12]. A burgeoning HIV-epidemic occurred during the same decade among PWID [13], spurring the local municipality and non-profit organizations to implement comprehensive low-threshold harm-reduction programs (HRP) [14]. These programs are associated with a decreased risk of HCV and HIV acquisition among PWID [15] and probably contributed to a decrease in their non-natural deaths over time [16]. Nowadays, IDU is uncommon in the Netherlands, and HIV and HCV incidence among PWUD have dropped to nearly zero [15,17]. Hence, the remaining PWUD in Amsterdam represent an ageing population, in which many have been HCV-infected for more than 20 years [18], are largely engaged in HRP, and most no longer inject drugs [16]. Understanding the burden of liver disease in this particular PWUD population helps to identify those at greatest need for HCV treatment and/or liver care.

Cirrhosis has the potential to occur up to 46 years after HCV infection [19], and other risk factors related to liver fibrosis progression, such as heavy alcohol drinking, are common among PWUD. Hence we hypothesized that the presence of severe liver fibrosis and/or cirrhosis among PWUD in Amsterdam is considerable. In the present study, we aimed to assess the proportion of PWUD with moderate to severe liver fibrosis or cirrhosis and its determinants.

METHODS

Study population

We included PWUD attending the Public Health Service of Amsterdam (PHSA) who: 1) participated in the Amsterdam Cohort Studies (ACS) and/or 2) attended the Drug Users Treatment of Chronic Hepatitis-C (DUTCH-C) unit (Figure 1).

Study population 1 – PWUD participating in the ACS

ACS is a prospective cohort study of HIV that began in 1985 [20]. This cohort included people who used hard drugs (injecting or non-injecting), which are defined as the regular use of heroin, cocaine, amphetamines and/or methadone. The cohort remained open for enrolment until 2014. Details of the ACS have been previously described [20,21]. In brief, enrolment was voluntary, and written informed consent was obtained beforehand. Participants made follow-up visits to the PHSA every 4 to 6 months. At each visit, trained research nurses interviewed them about their health, drug use and sexual behaviour, using a standardised questionnaire. Blood was drawn for HIV-antibody testing and storage. HCV and hepatitis B virus (HBV) testing was performed retrospectively on samples collected before 2005, as previously described [15,22], and HCV testing was performed prospectively thereafter. Additional clinical data were collected by matching ACS data against data from the Dutch HIV Monitoring Foundation, hospital records, and municipal population registries.

Study population 2 – non-ACS PWUD attending the DUTCH-C treatment unit

The DUTCH-C project was initiated at the PHSA in December 2004, as detailed elsewhere [23]. Initially for PWUD participating in the ACS, the project aimed to offer HCV screening and treatment outside a hospital setting, with a protocol adapted to the specific needs of the PWUD population. In 2007, due to the successful uptake of testing and treatment, DUTCH-C services were extended to non-ACS participants (herein referred to as "non-ACS PWUD"). These individuals were either HCV-positive or considered to have a high likelihood of being infected. They were referred from methadone and other addiction clinics in Amsterdam until the DUTCH-C unit closed late in 2013 due to reorganisation.

Liver fibrosis assessment

The degree of liver fibrosis was assessed by transient elastography (TE) using the FibroScan® (Model F402, Echosens, Paris, France), which yields liver stiffness measurements (LSM) expressed in kilopascals (kPa). Increased liver stiffness correlates with increased liver fibrosis [24,25]. After ten successful measurements are performed per individual, the median value is calculated. We considered this median value to be valid based on the manufacturer's validity criteria: 1) at least 60% of measurements being suc-

cessful and 2) width of the interquartile range (IQR) being less than 30% of the median LSM value.

Study design and inclusion criteria

Individuals were included based on the following criteria:1) self-reported PWUD, 2) an available TE result, 3) having an ACS or DUTCH-C visit after 2004. We collected TE data from PWUD from three data sources (Figure 1), including 1) DUTCH-C records, 2) ACS routine visits records, and 3) HIV clinic visits records collected by the HIV Monitoring Foundation.

The first data source contained TE assessment for both study populations (i.e. ACS and non-ACS PWUD) performed as part of liver-fibrosis screening in subjects from the DUTCH-C project (2011-2012). The second data source contained information from a 4-month cross-sectional liver-screening research project among participants attending an ACS routine study visit (2015-2016). Additional TE data for study population one (i.e. ACS PWUD) were obtained from data source 3. The latter contained data obtained through matching of HIV-positive ACS participants against data from the HIV Monitoring Foundation, which collects liver screening results from routine HIV clinic visits (2009-2015). If PWUD had more than one valid TE (n=7), the measurement collected during an ACS study visit was preferred, and otherwise the first available result was used.

Data collection: study determinants

Socio-demographic and clinical determinants

The following socio-demographic and clinical variables were assessed as potential determinants of liver stiffness: age and body mass index (BMI) at TE assessment, ethnicity, and gender. We separately assessed HIV, HCV (chronic and cleared), and HBV (chronic and cleared) infection status. Chronic HCV infection (cHCV) was based on the HCV-RNA test result at or closest to the visit of the TE assessment. Chronic HBV infection was based on hepatitis B-surface antigen serology. HCV clearance was defined as spontaneous or treatment-induced clearance. In addition, we assessed HCV and HIV co-infection status by combining HIV (antibody) and HCV infection status (antibody and RNA) in five categories: 1) HIV/HCV uninfected, 2) HCV cleared (HCV antibody-positive and HCV-RNA negative), 3) HIV mono-infected, 4) cHCV mono-infected (positive for both HCV-antibody and HCV-RNA), and 5) HIV/cHCV co-infected. Among cHCV-infected PWUD, we also assessed the association with HCV genotype.

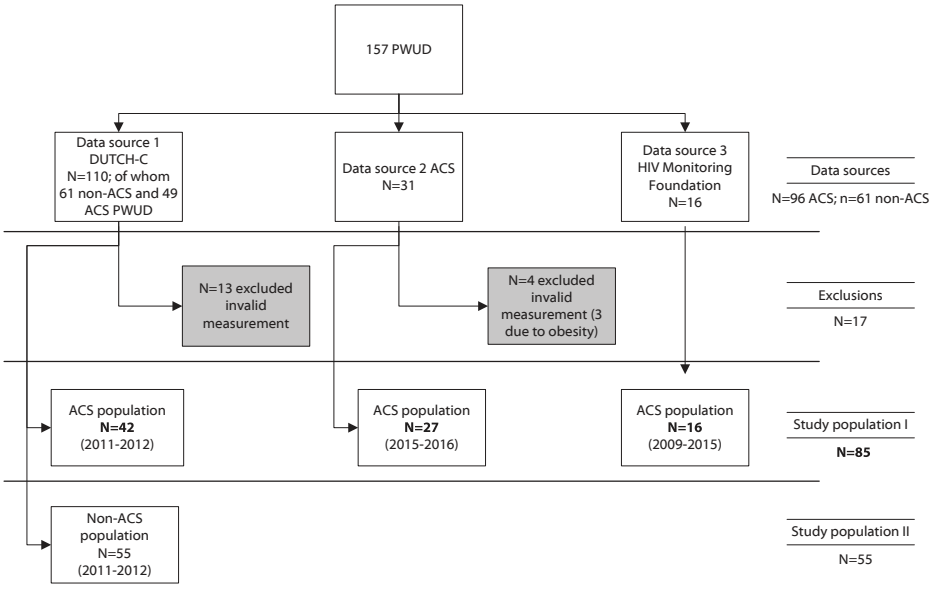


Figure 1: Flow diagram of study participants selection and data sources of transient elastrography measurements [1,2].

[1]Participants highlighted in bold were included in univariable and multivariable analyses.

[2]Grey boxes represent excluded individuals due to invalid liver stiffness measurement.

Abbreviations: PWUD: people who use drugs; DUTCH-C: Drug Users Treatment of Chronic Hepatitis-C unit; ACS: Amsterdam Cohort Studies; TE: transient elastography.

Behavioural determinants

Based on cumulative self-reported data at the time of TE assessment, the following behavioural variables were assessed: duration of heavy alcohol drinking (≥5 glasses per day), duration of regular use (≥3 times per week) of heroin, methadone or stimulants (i.e. crack cocaine, amphetamines, or heroine/cocaine cocktail), and number of years since start of IDU.

Statistical analyses

We calculated median LSM values for the whole study population and by socio-demographic, clinical, and behavioural characteristics in ACS participants. To determine the degree of METAVIR liver fibrosis, we used cut-off values obtained from a meta-analysis [26]: for no to mild fibrosis (F0-F1), LSM <7.65 kPa; for moderate to severe fibrosis (F2-F3), LSM ≥7.65-<13 kPa, and for cirrhosis (F4), LSM ≥13 kPa. Among ACS participants, we used chi-square tests, t-test, or rank-sum tests to assess whether those with and without a TE assessment differed in socio-demographic, clinical, and behavioural characteristics at the first ACS study visit after 2004.

To assess factors associated with LSM as a continuous variable, we performed univariable and multivariable linear regression. In this analysis, we included only ACS participants, as behavioural data was lacking for non-ACS participants (study population 2). To obtain normally distributed residuals, we subtracted 2 from LSM values (LSM-2) and then $\log_{10}$ transformed the results. We assessed linearity for all continuous covariables, and when a non-linear relationship was observed between the independent and dependent variable, we modelled the variable using restricted cubic splines, with knots at the 10^{th}, 50^{th} and 90^{th} percentile. Multivariable models included variables that were associated in univariable analyses at $p<0.10$. Site of TE assessment was not included in the multivariable model, as this is not a risk factor for liver stiffness. A correlation matrix was computed to assess for collinearity. Two multivariable models were constructed: one for the total included ACS population and another for cHCV-infected PWUD only.

RESULTS

Study populations and TE results

Total study population: ACS and non-ACS PWUD

Of 157 PWUD who underwent a TE assessment, 143 (91.1%) were HCV-antibody positive and 117 (74.5%) were chronically infected with HCV at the time of assessment. Of the 143 HCV-antibody positive PWUD, 27 (18.9%) had ever been treated for HCV at TE assessment. A total of 140 (89.2%, 85 ACS and 55 non-ACS) PWUD had a valid LSM result (Figure 1).

For this population (n=140), median LSM was 7.6 kPa [interquartile range (IQR)=4.9-12.0], and 50.7% had no to mild fibrosis; 26.4% moderate to severe fibrosis, and 22.9% had cirrhosis. Among those chronically infected with HCV (n=104), 57.7% had moderate to severe fibrosis or cirrhosis, of whom 26.9% had cirrhosis.

Study population 1: ACS PWUD

In comparing ACS participants with (n=96) and without (n=483) a TE assessment, no differences were detected with respect to ethnicity or gender, and both groups had a similar duration of drug, alcohol and methadone use at the first ACS visit after 2004. However, the ACS participants in our study were slightly older (45 vs. 43), were more often HIV positive (23.4% vs. 12.3%) or HCV-antibody positive (86.5% vs. 53.4%) and more often had a history of IDU (86.5% vs. 62.5%) than those without a TE assessment.

Of the 85 ACS participants with at least one valid measurement, 42 (49.4%) had their assessment at the DUTCH-C unit, 27 (31.8%) were assessed during a routine ACS study visit, and 16 (18.8%) at a HIV clinic, collected by the HIV Monitoring Foundation. A total of 73 out of 85 (85.9%) PWUD were HCV-antibody-positive at TE assessment, and of these, 58 (79.5%) had a cHCV infection and 15 (20.5%) had cleared HCV, of whom 3 cleared due to HCV treatment. Among the 22 HIV-positive PWUD, the median CD4 count was 412 cells/µl [IQR=310-583], and all had an undetectable HIV viral load around the time of TE assessment. Socio-demographic, clinical, and behavioural characteristics of ACS participants with a valid LSM result are shown in Table 1.

Table 1: Socio-demographic, clinical, and behavioural characteristics, and proportions with liver fibrosis and cirrhosis of people who use drugs participating in the Amsterdam Cohort Studies with a valid liver stiffness measurement (N=85) at transient elastography assessment.

			LSM in kPa		Moderate/severe fibrosis	cirrhosis
			m	**[IQR]**	%	%
Total, N	85		6.8	[4.6-11.1]	21.2%	20.0%
Age in years, m [IQR]	53	[47-56]				
Gender	*n*	%				
Female	27	31.8	6.1	[3.7-8.2]	7.4%	18.5%
Male	58	68.2	7.6	[4.8-11.8]	27.6%	20.7%
Ethnicity						
Western	71	83.5	6.8	[4.6-11.1]	18.3%	22.5%
Non-western	14	16.5	6.3	[4.5-10.2]	35.7%	7.2%
Injecting drug use (IDU)						
Never IDU	12	14.1%	5.0	[3.4-7.6]	25.0%	0%
Ever IDU	73	85.9%	7.1	[4.8-11.8]	20.6%	23.3%
Duration in years, m [IQR][a,b]	29.6	[24.0-36.4]				
Drug use, duration in years of, m [IQR] [c]						
Heroin use	18.5	[11.0-26.0]				
Stimulants use [d]	18.1	[12.4-23.2]				
Methadone use	22.3	[13.7-28.2]				
Heavy alcohol drinking						
Never heavy drinker	31	36.5%	6.3	[4.2-7.7]	19.4%	9.7%
Ever heavy drinker	54	63.5%	7.6	[4.9-14.4]	22.2%	25.9%
Duration in years, m [IQR] [e]	6.2	[2.2-14.7]				
TE assessment						
DUTCH-C unit	42	49.4%	9.4	[6.1-15.7]	23.8%	31.0%
ACS visit	27	31.8%	4.6	[3.6-6.3]	11.1%	3.7%
HIV clinic	16	18.8%	7.6	[5.9-10.2]	31.3%	18.8%

Table 1: Socio-demographic, clinical, and behavioural characteristics, and proportions with liver fibrosis and cirrhosis of people who use drugs participating in the Amsterdam Cohort Studies with a valid liver stiffness measurement (N=85) at transient elastography assessment. (*continued*)

			LSM in kPa		Moderate/severe fibrosis	cirrhosis
BMI (kg/m^2), m [IQR]	21.9	[20.2-25.2]				
Underweight (BMI<18.5)	7	8.2%	4.9	[4.1-8.1]	14.3%	14.3%
Normal (BMI 18.5-<25)	46	54.1%	7	[4.3-11.8]	19.6%	21.4%
Overweight (BMI >=25)	20	23.5%	6.6	[5.3-11.1]	20.0%	20.0%
Missing	12	14.1%	7.2	[5.0-11.6]	33.3%	16.7%
HBV infection status[f]						
HBV negative	34	40.0%	6.2	[4.3-8.8]	26.5%	11.8%
HBV cleared	42	49.4%	7.1	[5.3-12.0]	19.1%	23.8%
HBV chronic	6	7.1%	11.3	[7.6-14.8]	16.7%	50.0%
HCV infection status						
HCV negative	12	14.1%	4.0	[3.4-5.0]	0%	0%
HCV cleared[g]	15	17.7%	6.1	[4.1-8.1]	13.3%	13.3%
HCV chronic	58	68.2%	8.1	[5.5-14.4]	27.6%	25.9%
HIV infection status						
HIV negative	63	74.1%	6.3	[4.5-12.0]	19.1%	22.2%
HIV positive	22	25.9%	7.1	[5.4-8.8]	27.3%	13.6%
HIV/HCV/HBV co-infection status[h]						
HIV/HCV co-infected	18	85.7%	7.3	[6.2-9.2]	27.8%	16.7%
HCV/HBV co-infected	2	9.5%	17.9	[14.4-21.3]	0%	100.0%
HIV/HBV co-infected	1	4.7	8.2	[8.2-8.2]	100.%	0%
HCV genotype						
1	36	62.1%	7.6	[6.3-11.6]	29.0%	18.4%
2	4	6.9%	6.6	[5.1-27.1]	25.0%	25.0%
3	13	22.4%	11.0	[7.3-30.5]	25.0%	43.8%
4	3	5.2%	5.5	[3.8-9.2]	33.3%	0%

Abbreviations: LSM=liver stiffness measurement; kPa=kilopascal; n=number; m= median; IQR=interquartile range; IDU=injecting drug use; yrs= years; BMI=body mass index; HCV=hepatitis C virus; HBV= hepatitis B virus; HIV=human immunodeficiency virus; g/d=glasses per day; TE=transient elastography; ACS=Amsterdam Cohort Studies. The calculation of the percentages may include missing values and therefore do not always add up to 100%. Missing values: HBV status n=3; HCV genotype n=2.

[a] Years since start of injecting drug use at the time of the transient elastography assessment.

[b] Among those with a history of injecting drug use.

[c] Among those that ever used heroin, methadone, or stimulants.

[d] (Crack) cocaine, amphetamines, or heroine/cocaine cocktail (injecting or non-injecting use).

[e] Among those with a history of heavy alcohol drinking.

[f] Cleared HBV based on positive HBV core antibodies and negative surface antigen (HbsAg) serology. Chronic Hepatitis B based on HbsAg positive serology.

[g] PWUD with a positive HCV antibody test and a negative HCV-RNA test result. The median number of HCV-RNA-negative tests among those who had cleared the virus was 6 [IQR=2-9]. For 4 of the 15 PWUD who cleared HCV, only one HCV-RNA-negative test was available to establish clearance.

[h] Among individuals with a co-infection with at least two of the following infections: chronic HBV, chronic HCV and/or HIV.

Among ACS participants, median LSM was 6.8 kPa [IQR=4.6-11.1], and 20.0% had evidence of cirrhosis (Table 1). Higher LSM values were found in males and individuals with Western ethnicity, ever IDU, a history of heavy alcohol drinking, TE assessment at the DUTCH-C unit, overweight, HIV/HCV/HBV (co-)infection, or HCV-infection with genotype 3 (Table 1). Of the 58 ACS participants with a cHCV infection, 53.5% had evidence of moderate to severe fibrosis or cirrhosis, compared to 26.6% of those who had cleared HCV. Of the 17 individuals with cirrhosis, 5 (29.4%) had started injecting 20-24 years ago, 3 (17.6%) 25-34 years ago, and 9 (52.9%) 35-45 years ago.

Study population 2: non-ACS PWUD

The remaining 55 PWUD with a valid LSM value belonged to the non-ACS population (Figure 1), of whom 54 were HCV-antibody-positive. Of these 54 PWUD, 46 (85.2%) had a cHCV infection and 8 (14.8%) had cleared HCV, of whom 3 due to HCV treatment. Among non-ACS PWUD with available socio-demographic and clinical data, 82.2% were male, median age was 51 years [IQR=47-57], 80.0% had ever injected drugs and 72.7% were of Western ethnicity (Table 2). In this study population, median LSM was 8.8 kPa [IQR=5.9-13.9], 34.6% had moderate to severe fibrosis and 27.3% had cirrhosis (Table 2).

Determinants of liver stiffness

In univariable analyses (among all ACS participants), variables significantly associated with increased liver stiffness were older age, longer duration since start of IDU, longer duration of methadone use, longer duration of heavy alcohol drinking, TE assessment at the DUTCH-C unit, cleared and chronic HCV infection, HIV/cHCV co-infection status, and HCV genotype 3 (Table 3 & Figure 2). Each 10-year increase in the duration of IDU was associated with a 0.13 $\log_{10}$ kPa (95%CI= 0.06,0.20) increase in LSM. Among cHCV-infected individuals, those infected with HCV genotype 3 had a 0.35 $\log_{10}$ kPa (95%CI= 0.11,0.59) higher LSM than those infected with genotype 1 (Table 3).

In the multivariable model, duration of methadone use was not included, as it was moderately correlated with IDU duration (r=0.51). Age and IDU duration were weakly correlated (r=0.39) and could thus be included in the multivariable models. In the multivariable model, duration of heavy alcohol drinking and HIV/cHCV co-infection status remained statistically significant ($p<0.001$) (Table 3 & Figure 3). Compared to HIV/HCV-uninfected PWUD, cHCV-mono-infected PWUD had a 0.45 $\log_{10}$ kPa (95%CI= 0.15,0.75) higher LSM, and for HIV/cHCV-co-infected LSM was 0.39 $\log_{10}$ kPa (95%CI= 0.05,0.73) higher. LSM increased with longer duration of heavy alcohol drinking up to 7 to 8 years of heavy drinking, followed by a stabilization of LSM values (Figure 3).

Table 2: Socio-demographic, clinical, and behavioural characteristics and proportions with liver fibrosis and cirrhosis of non-ACS people who use drugs with a valid liver stiffness measurement (n=55) attending the DUTCH-C unit at the transient elastography assessment.

	n[a]	m [IQR]
Age in years		51 [47-57]
		%[b]
Male	37	82.2%
Western ethnicity	32	72.7%
Ever IDU	32	80.0%
HBV infection status		
HBV uninfected	44	95.7%
HBV cleared	0	0%
HBV chronic	2	4.4%
HCV infection status		
HCV uninfected	1	1.8%
HCV cleared	8	14.6%
HCV chronic	46	83.6%
HIV infection status		
HIV negative	26	100%
HIV positive	0	0%
HCV genotype		
1	29	76.3%
2	0	0%
3	7	18.4%
4	2	5.3%
Fibrosis stage		
Moderate/severe	19	34.6%
Cirrhosis	15	27.3%

Abbreviations: n=number; m=median; IQR=interquartile range; HCV=hepatitis C virus; HBV=hepatitis B virus; HIV=human immunodeficiency virus; IDU=injecting drug use.

[a] Limited data were available for non-ACS participants. The following numbers refer to the number of individuals with available (non-missing) data per socio-demographic, clinical or behavioural characteristic. Age n=52, gender n=45, ethnicity n=44, ever injected drugs n=40, HCV-antibody/HBV-surface antigen infection status n=55, HIV status n=26, HCV genotype n=38, fibrosis stage n=55.

[b] Percentage out of those with available data (see footnote a).

In the second multivariable analysis, restricted to cHCV-infected individuals only, longer duration of heavy alcohol drinking was also significantly associated with LSM and the direction of the association was comparable to that observed in the multivariable analysis including all ACS PWUD. In this group (cHCV infected), LSM increased with increasing age until about 55 years of age, similar to the association observed in univariable analysis including all ACS PWUD.

Table 3: Univariable and multivariable associations with liver stiffness using linear regression models among people who use drugs participating in the Amsterdam Cohort Studies.

	Univariable		Multivariable[a]			
			Model 1: all PWUD		Model 2: cHCV-infected PWUD	
	Coef[b] (95%CI)	p-value	Coef (95%CI)	p-value	Coef (95%CI)	p-value
Age in years	#	0.02	#	0.09	#	0.04
Gender		0.15				
Female	1					
Male	0.14 (-0.05,0.33)					
Ethnicity		0.47				
Western	1					
Non-western	-0.09 (-0.33,0.15)					
Duration since first IDU[b]	0.13 (0.06,0.20)	<0.001	0.02 (-0.06,0.11)	0.55	0.01 (-0.11,0.12)	0.92
Duration of:						
Heroin use	#	0.96				
Stimulant use[6]	-0.06 (-0.16,0.04)	0.26				
Methadone use	#	<0.001				
Duration heavy alcohol drinking	#	0.03	#	<0.001	#	0.01
BMI	0.01 (-0.01,0.04)	0.22				
Site of TE assessment		<0.001				
DUTCH-C unit	1					
ACS visit	-0.46 (-0.64,-0.28)					
HIV centre	-0.08 (-0.29,0.14)					
HBV infection status		0.19				
HBV negative	1					
HBV cleared	0.16 (-0.05,0.37)					
HBV chronic	0.29 (-0.12,0.70)					
HCV infection status		<0.001				
HCV negative	1					
HCV cleared	0.31 (0.01,0.62)					
HCV chronic	0.58 (0.34,0.83)					
HIV infection status		0.85				
HIV negative	1					
HIV positive	-0.02 (-0.23,0.19)					
HIV/HCV (co-)infection status[d]		<0.001		<0.001		
HIV/HIV uninfected	1		1			
HCV cleared[e]	0.25 (-0.05,0.55)		0.16 (-0.16,0.49)			
HIV mono-infected	-0.47 (-1.03,0.10)		-0.52 (-1.05,0.003)			

Table 3: Univariable and multivariable associations with liver stiffness using linear regression models among people who use drugs participating in the Amsterdam Cohort Studies. (*continued*)

	Univariable		Multivariable[a]	
cHCV mono-infected	0.52 (0.26,0.78)		0.45 (0.15,0.75)	
HIV/cHCV co-infected	0.44 (0.16,0.73)		0.39 (0.05,0.73)	
HCV genotype[f]		0.02		0.38
1	1		1	
2	0.06 (-0.33,0.45)		0.14 (-0.23,0.51)	
3	0.35 (0.11,0.59)		0.21 (-0.10,0.51)	
4	-0.23 (-0.67,0.22)		-0.15 (-0.58,0.27)	

Abbreviations: coef=linear regression coefficient; 95%CI= 95% confidence interval; IDU=injecting drug use; BMI=body mass index (kg/m^2); HCV=hepatitis C virus; HBV=hepatitis B virus; HIV=human immunodeficiency virus; cHCV=chronic HCV; LSM=liver stiffness measurement; ACS=Amsterdam Cohort Studies; cHCV=chronic HCV infection. To obtain normally distributed residuals, we subtracted 2 from LSM values. Hence, the regression coefficient represents a one unit increase in the $\log_{10}$ kPa of LSM-2 values. # Modelled using restricted cubic splines. A graphical representation of the univariable associations with LSM are shown in Figure 2 for variables with a p-value <0.10, and in Figure 3 for the multivariable model 1.

[a] Included PWUD in model 1: n=83 (2 excluded due to missing values), and model 2: n=54 (4 excluded due to missing values)

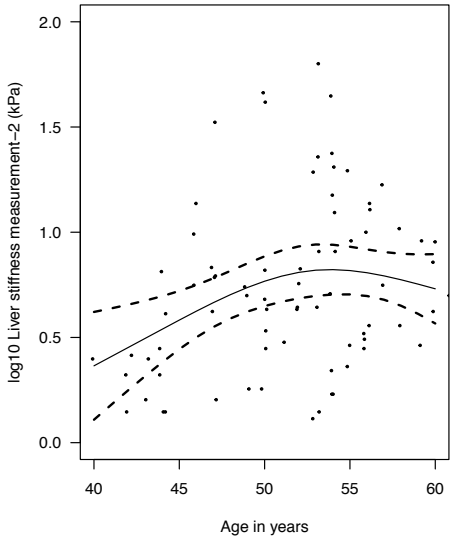
[b] Coefficient of duration of since first IDU presented per 10-year increase.

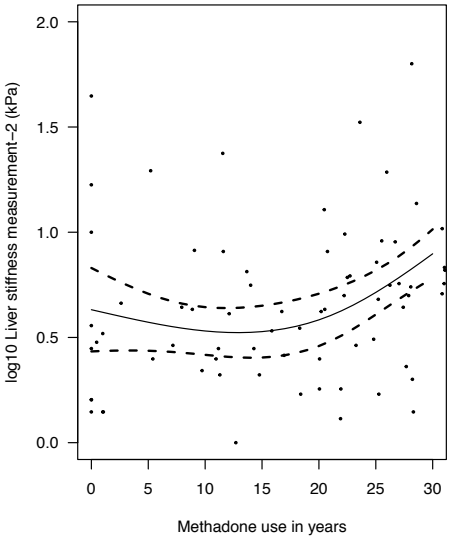
[c] Cocaine, amphetamines, and heroine/cocaine cocktail (injecting or non-injecting use). Coefficient of duration of stimulant use presented per 10-year increase.

[d] chronic HBV infection was not included due to the low number of cases (n=6).

[e] HCV-cleared: HCV-antibody-positive while HCV RNA-negative.

[f] Among HCV-RNA-positive PWUD.





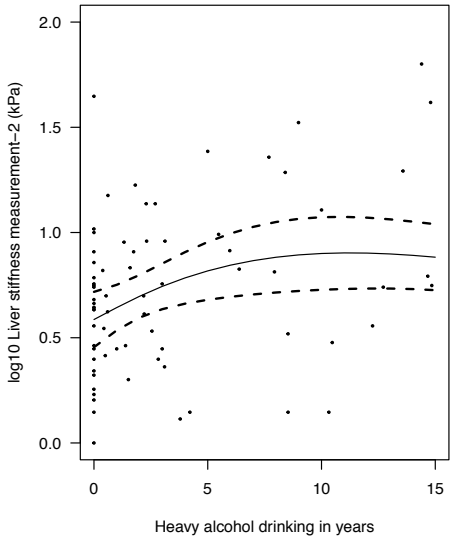


Figure 2: Univariable associations with liver stiffness among people who use drugs participating in the Amsterdam Cohort Studies[1].

[1]Dots represent observed values. Dashed lines represent 95% confidence intervals. y-axis were truncated around the 90th percentile.

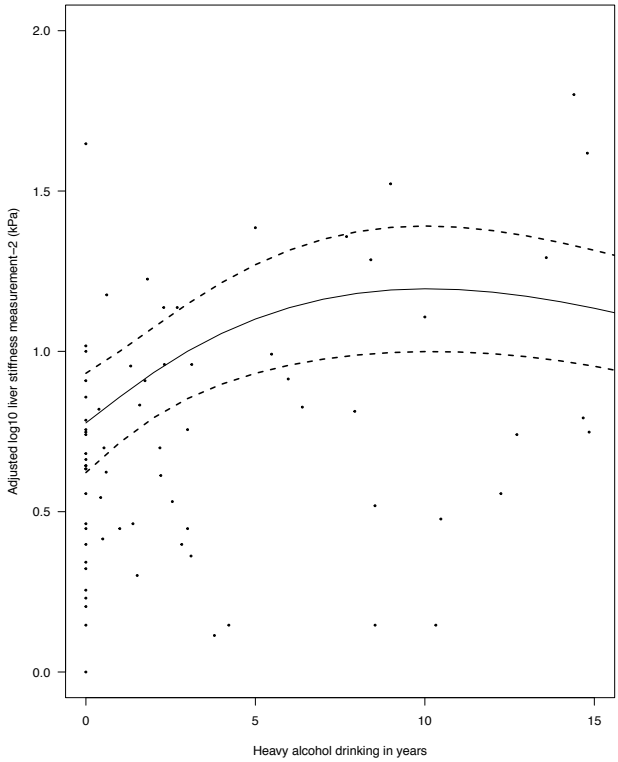


Figure 3: Adjusted association of duration of heavy alcohol drinking (≥5 glasses per day) and liver stiffness[1,2]

[1]Dots represent observed values. Dashed lines represent 95% confidence intervals.

[2]The multivariable model includes: duration since start of injecting drug use, HIV/cHCV infection status and age at the transient elastography assessment (n=83).

y-axis were truncated around the 90th percentile.

DISCUSSION

In an ageing population of PWUD in Amsterdam, we found high proportions of moderate to severe fibrosis and cirrhosis. Among chronically HCV-infected individuals, 58% had evidence of moderate to severe fibrosis or cirrhosis. Longer duration of heavy alcohol drinking, age at TE assessment, cHCV mono-infection, and HIV/cHCV co-infection were significantly associated with increased LSM in multivariable analyses. Individuals infected with genotype 3 and those with a longer duration of IDU and methadone use had higher LSM values in univariable analyses. This study emphasizes the importance of assessing liver fibrosis in this key population, followed by adequate linkage to liver care, as individuals with cirrhosis require specialised surveillance (for hepatocellular carcinoma, oesophageal varices and liver function), irrespective of HCV infection. In addition, next to testing for HIV, HCV, and HBV, routine liver fibrosis assessments of ageing PWUD populations should strongly be considered.

Most studies assessing the burden of liver disease among PWUD have reported a lower prevalence of cirrhosis ranging from 1% to 14% [2-9]. Their study populations included mainly HCV-antibody- or cHCV-infected PWUD, comparable to ours, but differed in ways that help explain the higher proportion of PWUD with cirrhosis we observed. First, the age distribution differs: while the median age in our study was 53 years, previous studies were conducted among PWUD with a median age ranging from 32 to 49 years [2-9]. This is important, as the association of older age with liver stiffness/fibrosis is one of the most consistent findings across studies [3,4,6-8]. Second, the median time since start of IDU reported by previous studies is shorter than ours, and duration of IDU is usually a proxy for duration of HCV infection. For example, in a US-based study among HCV-antibody-positive PWID, median duration since first injection was 19.7 years, and prevalence of cirrhosis was 1% at the first biopsy [8]. A median of 4.2 years later, the proportion increased to 7% [27]. Our median duration of IDU was longer, at almost 30 years, and 71% of HCV-antibody-positive PWUD with cirrhosis had started injecting more than 24 years before the TE assessment. Third, the method to assess cirrhosis varied across studies. Although biopsy remains the gold standard for assessing the presence of cirrhosis, TE is accurate in diagnosing cirrhosis, but less accurate for the diagnosis of significant fibrosis [24,28].

Treatment with direct-acting antivirals (DAA) has the potential to reverse liver fibrosis among those with chronic HCV infection [29,30]. But in 2015, the first full year of DAA availability in the Netherlands, few treatment-naïve HCV-infected PWUD (3.0%) from the ACS initiated HCV treatment [21]. This is worrisome, as we report high proportions of moderate to severe fibrosis and cirrhosis in this population. Hence other models of HCV treatment delivery, such as the DUTCH-C unit (a specialized HCV treatment unit for PWUD), should be considered to increase treatment uptake in the DAA era [31].

In line with most studies assessing risk factors for liver fibrosis [2-4,6-9], heavy alcohol drinking, older age at TE assessment, cHCV infection, and HIV/cHCV co-infection status were significantly associated with increased liver stiffness. Although not statistically significant in multivariable analysis, our participants infected with genotype 3 had higher LSM values, and 44% had cirrhosis. This agrees with other studies reporting a statistically signification association [4,32], perhaps due to an increased prevalence of liver steatosis and/or insulin resistance associated with genotype 3 infections. Unfortunately, we did not have data on steatosis or diabetes mellitus available for our study population. Although liver fibrosis progression is accelerated in HIV/cHCV-co-infected individuals [33], we found that they had slightly lower LSM values than cHCV mono-infected individuals. This may be partly explained by a survival effect: HIV-infected PWUD exhibiting the highest risk behaviour had probably died before the start of our study period [16]. Also,

the included HIV-positive PWUD were well engaged in care, as demonstrated by their median CD4 count of 412 cells/µl and undetectable HIV viral load at TE assessment.

While the proportion of PWUD with cirrhosis was highest among those with a cHCV infection and a history of heavy alcohol use, 13% of those who had cleared HCV had evidence of cirrhosis. In a population-based study of individuals aged 45 years and older in Rotterdam, the Netherlands, the prevalence of cirrhosis (defined by LSM ≥13 kPA) was 27 times lower than was found in our study population of HCV-antibody positive PWUD with a resolved HCV infection [34]. Routine liver assessment among cHCV-infected PWUD linked to specialized care is recommended in the Netherlands. Further research should assess whether adding TE screening to harm-reduction or outreach programs could identify PWUD in need of liver-related care while uninfected, a population that might otherwise be missed.

There are some limitations in our study. First, we did not include a representative sample of PWUD and the reasons for TE assessment among those screened at HIV clinics and the DUTCH-C unit were unknown and may have resulted in selection bias. However, including site of TE assessment in the multivariable model did not alter our conclusions (data not shown). Moreover, during the 4-month liver screening research project at the ACS during 2015-2016 (data source 2), we intended to assess liver fibrosis in a group more representative of our ACS population. Importantly, still 15% of PWUD screened in that period were categorized as having moderate to severe liver fibrosis or cirrhosis. Third, our sample of PWUD was relatively small, which may have underpowered our analyses. Fourth, different health professionals performed the TE assessments. However, inter- and intra-observer agreement for TE assessment of >85% has been reported [35]. Lastly, in our study, we used the same cut-off values for the whole study population. It has been reported that they should differ depending on the underlying liver condition [28], but data is lacking on validated cut-off values for some HIV/HCV/HBV serological groups.

There are also several strengths. First, the ACS is a well-characterized cohort collecting detailed longitudinal clinical and behavioural data since 1985. This allowed us to assess important risk factors accurately, such as the duration of heavy alcohol drinking. Second, we included HCV-uninfected individuals, allowing us to assess the association of liver stiffness with HCV and other co-infections. This is the first study reporting the burden of liver disease, defined by moderate to severe fibrosis or cirrhosis, among a population of PWUD of Amsterdam, the Netherlands, a city with an increasingly ageing PWUD population. Although almost zero new HCV and HIV infections have been observed in this group over the last decade [17], the prevalence of long-standing infections is considerable, comparable to the epidemiological situation in some Western European

countries [36,37]. Therefore, our recommendations for TE assessment in a broader PWUD population is likely to be applicable outside of the Netherlands.

In conclusion, a high proportion of PWUD from Amsterdam had evidence of moderate to severe fibrosis or cirrhosis and, as expected, particularly PWUD with a chronic HCV infection and those with a history of heavy alcohol drinking. Increased uptake of HCV treatment and interventions that tackle modifiable factors such as heavy alcohol drinking are needed. Routine assessment of liver fibrosis among a broader PWUD population may be justifiable, but future research is needed to corroborate our findings.

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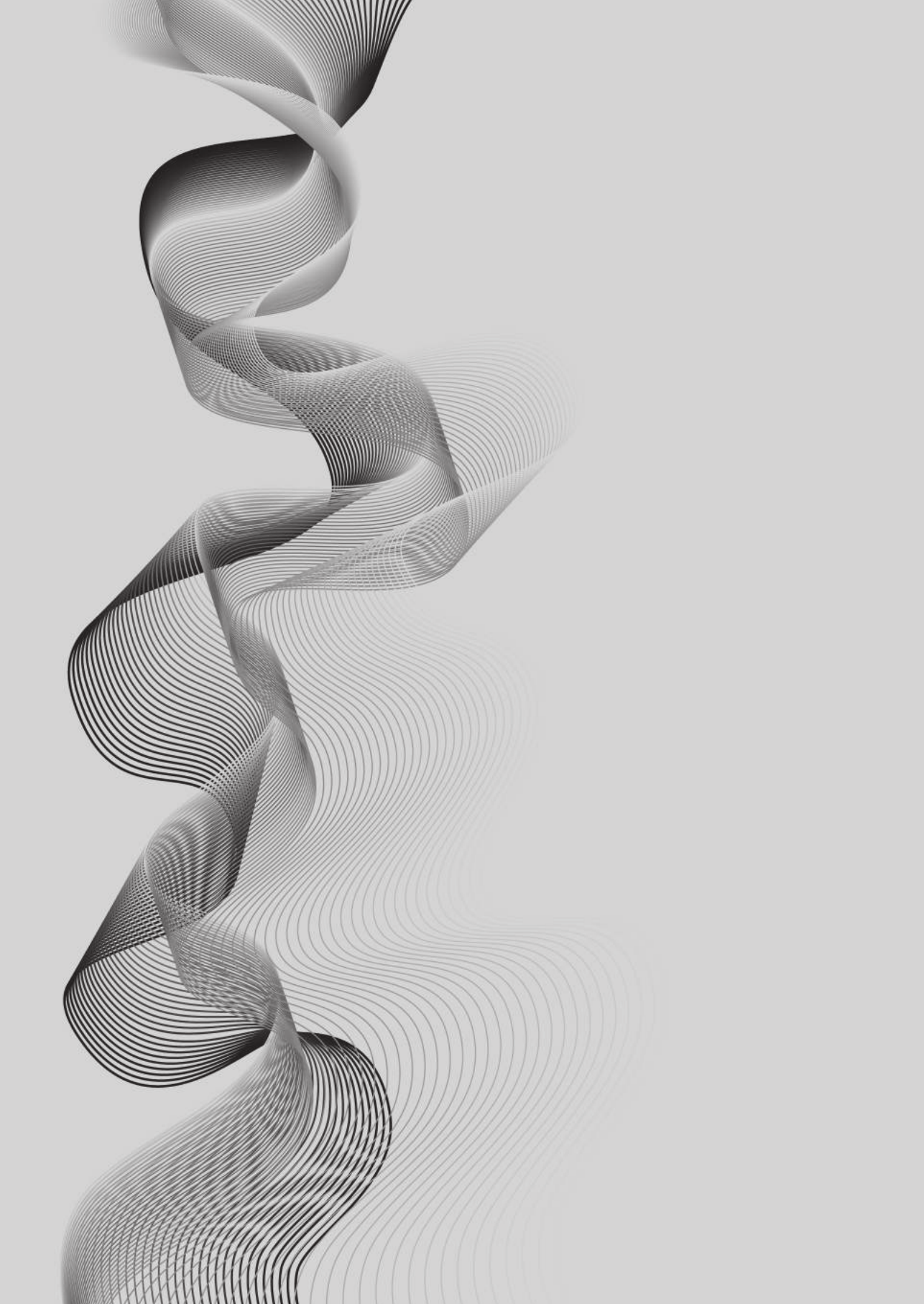

Chapter 3.3

Cost-effectiveness of hepatitis C treatment for people who inject drugs and the impact of the type of epidemic; extrapolating from Amsterdam, the Netherlands

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ABSTRACT

Background: People who inject drugs (PWID) are disproportionally affected by the hepatitis C virus (HCV) infection. The efficacy of HCV treatment has significantly improved in recent years with the introduction of direct-acting antivirals (DAAs). However, DAAs are more costly than pegylated-interferon and ribavirin (PegIFN/RBV). We aimed to assess the cost-effectiveness of four HCV treatment strategies among PWID and treatment scale-up.

Methods: An individual-based model was used describing HIV and HCV transmission and disease progression among PWID. We considered two epidemiological situations. A declining epidemic, based on the situation in Amsterdam, the Netherlands, and a stable HCV epidemic, as observed in other settings. Data on HCV incidence, prevalence, treatment setting and uptake were derived from observed data among PWID in Amsterdam. We assessed the incremental cost-effectiveness ratio (ICER, costs in €/quality-adjusted life year (QALY)) of four treatment strategies: 1) PegIFN/RBV; 2) sofosbuvir/RBV for genotype 2-3 and dual DAA for genotype 1-4; 3) Dual DAA for all genotypes; 4) Dual DAA with 3x treatment uptake.

Results: In both types of epidemic, dual DAA therapy was most the cost-effective strategy. In the declining epidemic, dual DAA yielded an ICER of 344 €/QALY while in the stable epidemic dual DAA led to cost-savings. Scaling-up treatment was also highly cost-effective. Our results were robust over a range of sensitivity analyses.

Conclusion: HCV treatment with DAA-containing regimens is a highly cost-effective intervention among PWID. Based on the economic and population benefits of scaling-up treatment, stronger efforts are needed to achieve higher uptake rates among PWID.

INTRODUCTION

People who inject drugs (PWID) are disproportionally affected by the hepatitis C virus (HCV) infection [1] as sharing unsafe injecting equipment poses a high risk for HCV transmission [2]. The global anti-HCV prevalence among PWID is estimated to be 67% [3], though the HCV epidemic among PWID differs by geographical region. In Eastern European countries such as Poland, [4] a continued spread of HCV has been documented among PWID whereas a declining HCV epidemic has been observed in Western European countries such as the Netherlands [5]. HCV infection is one of the leading causes of liver-related disease and cirrhosis usually develops around 20 to 30 years after infection in 16% to 41% of HCV-infected individuals [6]. A modeling study extrapolating from the Amsterdam Cohort Study (ACS) among PWID reported that the burden of HCV infection is expected to rise in in the next decade in the absence of HCV treatment and/or treatment scale-up [7].

Treatment with pegylated-interferon and ribavirin (PegINF/RBV) became available after 2001 with an overall sustained virological response (SVR) ranging from 33-79% [8]. Among PWID, similar SVR have been documented [9] and PegINF/RBV has been shown to be cost-effective among current and former PWID [10]. However, PegIFN is known to cause many side effects and the burdensome treatment may take up to 48 weeks [9]. Furthermore, as active drug used to be a contraindication for HCV treatment [11], and other barriers, such as limited access to care and lack of a social support system among PWID [12], a relatively low HCV-treatment uptake among PWID has been reported [13]. Additionally, especially with this type of HCV treatment, PWID require a flexible and permissive setting with extensive follow-up and extra healthcare support due to psychiatric comorbidities and the lifestyle of PWID [14].

New all-oral treatment regimens with direct-acting antivirals (DAAs) are highly effective, with SVR rates reported up to 95-100% [15]. A cost-effectiveness study among PWID in Australia showed that DAA treatment can be cost-effective [16]. In the Netherlands, DAAs are reimbursed for all HCV-infected individuals, irrespective of their fibrosis stage, since November 2015 [17]. However, despite the availability of DAAs in the Netherlands, the high costs of DAAs and the lifestyle of PWID may still pose barriers to provide treatment to PWID.

Beyond the costs, the cost-effectiveness of HCV treatment may depend on several factors such as HCV-screening uptake and whether PWID with a re-infection after successful HCV treatment are re-treated. The type of HCV epidemic may also influence the cost-effectiveness. In a stable epidemic, with a stable PWID population inflow and HCV

incidence, transmission is on-going and treatment may prevent new infections (treatment as prevention). In contrast, in a declining epidemic as observed among PWID in the Netherlands [5], small population prevention effects can be expected, thus limiting the impact of treatment to the treated population only. To date, few studies have assessed the cost-effectiveness of DAAs among PWID and whether the cost-effectiveness of HCV treatment depends on the type of HCV epidemic. In this study we aim to assess the cost-effectiveness of four HCV treatment strategies among PWID and HCV-treatment scale-up. Furthermore, we aim to explore the impact of the type of epidemic on the cost-effectiveness of DAAs and on the chronic HCV prevalence over time.

METHODS

Model

An individual-based model describing demographic changes and infection dynamics of HIV and HCV was employed. This model was used previously to study the effects of harm reduction policy on the spread of HIV and HCV in Amsterdam [18], as well as the potential of treatment as prevention for HIV among PWID [19] . Since detailed features have been described before [18], below we describe only the main features of this model and how it was adapted. In summary, PWID entered the model at the beginning of their injecting career; subsequently they could cease injecting, relapse, acquire HCV and/or HIV or die and leave the model. Cycle length in the model was one month. PWID population inflow was based on back calculations from the number of participants in methadone programs in Amsterdam [7]. The probability of PWID participants having acquired HIV or HCV depended on the syringe-sharing rate and the probability that a borrowed syringe came from an infected PWID. PWID who cleared an HCV infection, either spontaneously or after successful treatment, were at risk of re-infection. For the purpose of this study, the model was expanded with information on HCV genotype (grouped into: genotype 1-4 (G1-4) and genotype 2-3 (G2-3)) (Text A in Supplementary (S) File), HCV disease progression, HCV screening, and HCV treatment. Based on data from the Amsterdam Cohort Study, 70% of HCV-positive PWID were infected with G1-4 [20].

We considered two epidemiological scenarios. For the first epidemiological scenario, the declining HCV epidemic, demographic parameters were estimated from the ACS [21]. In Amsterdam, HCV prevalence was 60% during 2006-2012 among ACS participants [22]. The number of PWID and HCV and HIV incidence have declined over time [5, 7]. HCV and HIV incidence were estimated to be 27.5 and 8.5 per 100 person years in the late 1980s, respectively, and declined to almost 0 for both infections in 2013 [5].

As the Dutch epidemiological situation is not representative for the majority of countries, we made a counter-factual scenario to explore the impact of the type of epidemic on the cost-effectiveness of DAAs in a more general setting. The model demographics were adapted to obtain a stable HCV and HIV epidemic. The number of new PWID entering the population was set at 4 per month; resulting in a stable population size of approximately 1,500 PWID (this number is comparable to the peak of the Amsterdam PWID population size as in 1985). Risk behavior of PWID in this scenario was similar to that at the start of the Amsterdam epidemic. In contrast to the declining epidemic, syringe sharing frequency did not decline over time. Prior to the introduction of HCV treatment, we ran the model until all variables reached their equilibrium distributions.

Base case parameters

Natural history of HCV

In Figure (Fig) 1, a schematic overview of the model is given. The model simulates PWID through each fibrosis stage (F0-F4) by age group, HIV status, and sex (Table 1 and Text B in S file). HIV/HCV-coinfected PWID had a two-fold risk to progress to the following fibrosis stage [23] and had a lower spontaneous clearance rate than HCV-monoinfected PWID [24,25]. Cirrhotic individuals could develop decompensated cirrhosis (DC) or hepatocellular carcinoma (HCC) and consequently, die as a result of liver-related causes. Liver transplantation was not incorporated in the model as active alcohol and drug use is a contra-indication [26]. Age-dependent all-cause mortality among HIV-uninfected PWID was based on data from the ACS [18]. Mortality estimates for HIV-infected PWID were based on data from the CASCADE Collaboration among HIV seroconverters [27].

HCV screening

HCV screening in both epidemics was based on the situation in Amsterdam, where PWID attending harm-reduction programs (HRP) have been routinely offered screening for HCV and HIV since 2001. Around 75% of PWID living in Amsterdam received or had received methadone substitution therapy [43] and, based on a pilot study in Amsterdam, around 80% of PWID in HRP were screened for HCV in methadone programs (Epidemiology, Health Promotion and Innovation Department, Public Health Service of Amsterdam (PHSA) – personal communication). PWID who test HCV-antibody negative are screened every two years for HCV antibodies. PWID who test HCV-antibody positive are tested for HCV RNA and HCV genotyping is performed. In the model, we assumed that 20% of the total PWID population would never be screened for HCV reflecting the Amsterdam estimated percentage of PWID that abstain from screening and/or HRP.

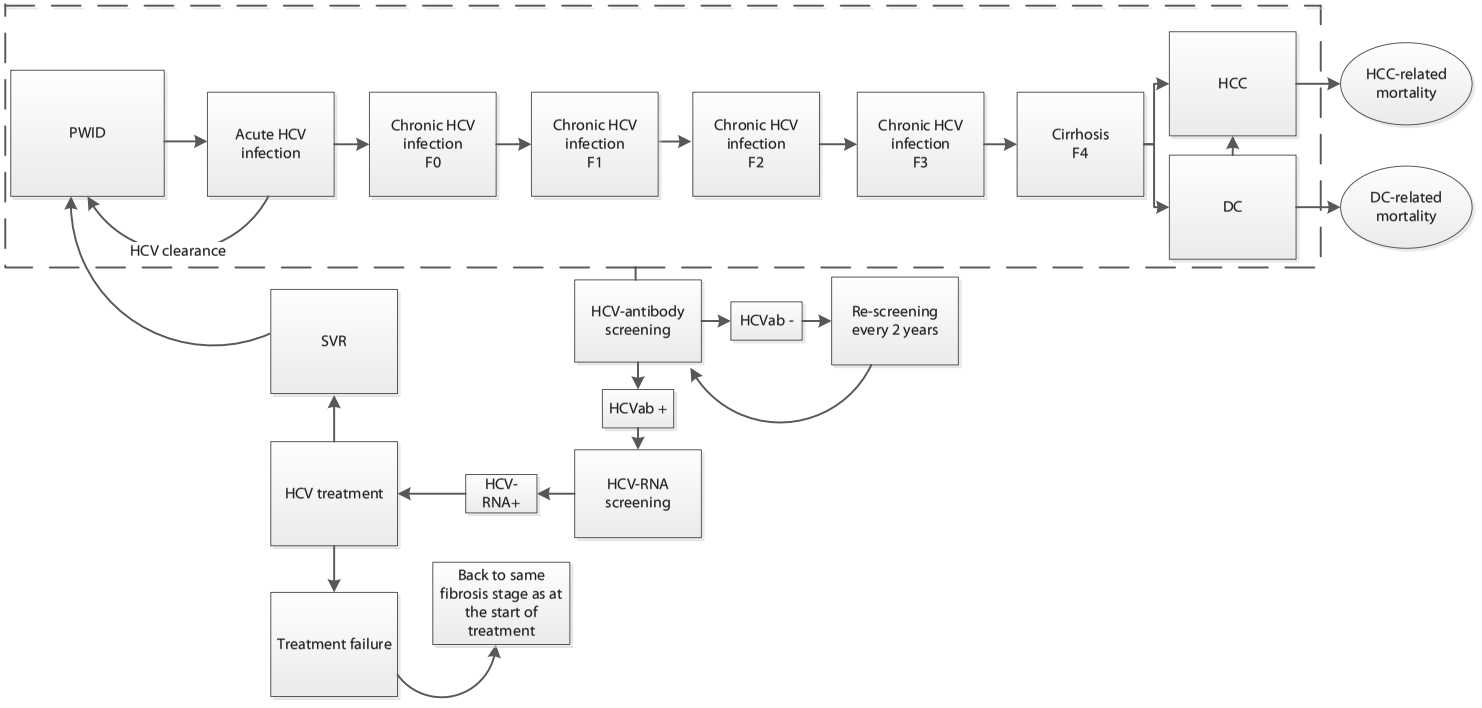

Figure 1: Natural history of HCV, screening, and treatment among PWID [a]

[a] PWID enter the model uninfected with HCV and HIV and may follow different health state trajectories as shown in the flow diagram. At any point, PWID may acquire HIV or exit the model due to background mortality or HIV-related mortality among HIV-infected PWID. Dashed lines depict the health states where PWID can be screened for HCV. Arrowed lines depict annual transition probabilities with the exception of HCV-antibody screening (which takes place every two years).

Treatment setting and uptake

In 2005, a special unit to treat HCV-RNA positive PWID with PegIFN/RBV was launched outside a hospital setting in Amsterdam (DUTCH-C project); details of this project have been previously described [9]. In summary, treatment was coordinated by a multidisciplinary team consisting of a physician and a nurse from the PHSA, and a hepatologist and a virologist from the Amsterdam Medical Center (AMC). The nurse gave counseling, provided PegINF injections every week, and contacted family members or friends to provide health counseling and/or support. In our analyses, based on this past experience in Amsterdam and expert opinion, 15 PWID (out of an estimated 1,783 HCV-RNA-positive PWID in Amsterdam in 2015), irrespective of their fibrosis stage, were treated at the PHSA annually; corresponding to a 1% treatment uptake rate in 2015 (Text C in S File). Chronically infected PWID were assumed to complete the course of treatment. However, SVRs in our study were based on intention to treat analyses where those lost to follow-up or who stopped treatment were included as treatment failures in the SVR calculation. After achieving SVR, PWID could get re-infected. Treatment-experienced PWID were not eligible for re-treatment.

Treatment strategies

Dutch treatment guidelines currently recommend a combination of two DAAs (dual DAA) for genotype 1 and 4 and either dual DAA therapy or sofosbuvir (SOF)/RBV for genotype 2 and 3 [17]. The following four HCV-treatment strategies for treatment-naïve PWID were evaluated (schematic representation: Table A in S File):

1. Treatment with PegIFN/RBV (24 weeks G2-3 and 48 weeks G1-4): standard treatment in the Netherlands until November 2014. This strategy is used as a comparator strategy to calculate the cost-effectiveness of DAAs.
2. SOF/RBV for G2-3 (weighted average 22 weeks) and dual DAA for G1-4 (12 weeks).
3. Dual DAA therapy for all genotypes (12 weeks).
4. Dual DAA therapy for all genotypes with a three times higher treatment uptake.

HCV-treatment uptake in our study is based on PegIFN/RBV treatment among PWID within the DUTCH-C project which was limited by the eligibility for PegIFN/RBV treatment and the manpower available given the long treatment durations. Based on reduced treatment duration and fewer side effects with DAA-containing regimens, we believe that with the same health-service capacity, a higher treatment uptake could be attained. Therefore a strategy with a higher uptake with dual DAAs was also assessed incrementally. SVRs for DAAs are mainly based on clinical trial data as limited real-world results were available (Table 1). We assumed no difference in SVR rates with DAAs by HIV status based on the PHOTON-2 and ALLY-2 trial [37, 44]. The backbone for all DAA treatment regimens is SOF. Dual DAA treatment SVR is based on the high SVR rates observed in trials with sofosbuvir combined with daclatasvir [45, 46] (Table 1 and Text D in S File).

Table 1: Base case demographics, annual transition probabilities, and SVR probabilities per treatment scenario.

Demographics	**%**		**Source**
Sex distribution			[22]
Men	0.64		
Genotype distribution			[28]
1-4	0.70		
2-3	0.30		
Annual transition probabilities			
	HIV-negative	**HIV-positive**	
Acute HCV to chronic HCV			[29]
Women	0.58	0.64 [a]	
Men	0.80	0.89 [a]	
Fibrosis progression in METAVIR staging (yearly transition is a METAVIR transition) [b]			
Women			[30, 31]
<49	0.05	0.10	
50 to 59	0.12	0.25	
60 to 69	0.22	0.44	
>=70	0.30	0.60	
Men			[30, 31]
<49	0.03	0.06	
50 to 59	0.07	0.13	
60 to 69	0.11	0.23	
70 -79	0.15	0.31	
>=80	0.21	0.42	
Disease progression after cirrhosis (F4)			
To DC or HCC [c]			
F4 to DC	0.039	0.059	[32]
DC to HCC	0.068	0.102	[33]
F4 to HCC	0.021	0.032	[30]
Death			[30]
DC to death	0.31	[d]	
HCC to death	0.43	[d]	
Treatment scenarios – SVR probabilities			
	HIV-negative	**HIV-positive**	
Scenario 1: PegIFN/RBV			
Genotype 1-4 (48 weeks)			[34, 35]
F0-F2	0.47	0.28	
F3-F4	0.33	0.20	
Genotype 2-3 (24 weeks)			[35, 36]
F0-F2	0.76	0.71	
F3-F4	0.52	0.47	

Scenario 2: DAA/RBV & Dual DAA			
Dual DAA therapy			[15, 37]
Genotype 1-4 (12 weeks)			
F0-F4	0.95	0.95	
DAA/RBV			[38-40]
Genotype 2-3 (22 weeks [e,f])			
F0-F4	0.90	0.90	
Scenario 3&4: Dual DAA therapy			[15, 37]
All genotypes (12 weeks)			
F0-F4	0.95	0.95	

Abbreviations: PegIFN: pegylated-interferon; RBV: Ribivarin; No: Number; SVR: sustained virological response; DAA: direct-acting antiviral.

[a] Clearance rate reported to be 15 and 20% among HIV/HCV-coinfected individuals [25, 41]; therefore we assumed an overall clearance rate of 17% among them. Clearance rate by sex among HIV/HCV-coinfected was proportional to that among HIV-negative individuals.

[b] 2 times the fibrosis progression rate of HIV-negative individuals [31].

[c] 1.5 times the progression rate among HIV-negative individuals [42].

[d] Not related to HIV status.

[e] We calculated a weighted average for the number of treatment weeks for those with genotype 2-3 as those with genotype 2 should be treated for 12 weeks while those with genotype 3 should be treated for 24 weeks with SOF/RBV. We assumed that a maximum of 20% of PWID [28] in this genotype group are infected with genotype 2, therefore the weighted number of weeks of treatment is 21.6.

[f] At the time the model was built, data for 24 weeks treatment for genotype 3 was scarce and the Positron trial showed similar SVR among cirrhotic and non-cirrhotic patients with genotype 2. In post-hoc sensitivity analyses SVR probability for F3-F4 was set at 0.70.

Utilities

Utilities were based on an UK Health Technology Assessment Report [47]. We multiplied all utilities by 0.85 to reflect the lower base-case quality of life among PWID [10]. Treatment with PegIFN/RBV leads to a 0.11 decrement in quality of life [47]. As a recent study [48] suggests a limited impact of DAA regimens on the quality of life, we conservatively assumed half the loss in quality of life during treatment with DAAs compared with PegIFN/RBV (Table 2). As no utilities were available during treatment among cirrhotic patients, we assumed a proportional decrement in utility similar to the difference between mild (F0-F1) and moderate fibrosis (F2-F3) (Table 2).

Healthcare utilization and costs

We adopted a healthcare perspective in our study. Healthcare utilization and costs associated with HCV disease state were subdivided based on treatment outcome: no SVR (untreated or unsuccessfully treated) or SVR after treatment (Table 2). Healthcare utilization (e.g. diagnostic tests) before, during, and after treatment were based on Dutch HCV guidelines [50] and standard treatment at the AMC determined by an hepatologist (Table B in S File). Healthcare costs were obtained from the Dutch Health Authority, Academic Medical Center (AMC), and the PHSA, and included overhead costs. For DAA-treatment regimens, the number of diag-

Table 2: Costs and utilities used in the cost-effectiveness analysis.

Costs of treatment			**Distribution**	**Source**
Treatment strategy		**Costs in euro 2014**		
PegIFN/RBV G1-4 (48 weeks)		29,712	Gamma(k=29,712, θ=1)	Own cost calculation [a]
PegIFN/RBV G2-3 (24 weeks)		19,298	Gamma(k=19,298, θ=1)	Own cost calculation [a]
DAA/RBV G2-3 (22 weeks)		106,476	Gamma(k=106,476, θ=1)	Own cost calculation [a]
Dual DAA therapy [b] (12 weeks)		84,216	Gamma(k=84,216, θ=1)	Own cost calculation [a]
Annual costs per health state before or after treatment				
Chronic HCV	F0-F2	130	Gamma(k=130, θ=1)	AMC/PHSA
	F3	289	Gamma(k=289, θ=1)	AMC/PHSA
	F4	433	Gamma(k=433, θ=1)	AMC/PHSA
	DC	27,905	Gamma(k=27,905, θ=1)	[49]
	HCC	21,389	Gamma(k=21,389, θ=1)	[49]
After SVR	F0-F2[c]	179	Gamma(k=179, θ=1)	AMC/PHSA
	F3	227	Gamma(k=227, θ=1)	AMC/PHSA
	F4	496	Gamma(k=496, θ=1)	AMC/PHSA
Utilities [d,h,i]				
		Utility value		
SVR	F0-F1	0.82	Beta(α=29.6, β=12.87)	[47]
	F2-F3	0.72	Beta(α=38.19, β=24.21)	[47]
	F4	0.62	Beta(α=46.77, β=41.98)	[e]
Chronic HCV	F0-F1	0.77	Beta(α=33.90, β=17.89)	[47]
	F2-F3	0.66	Beta(α=43.34, β=33.91)	[47]
	F4	0.55	Beta(α=52.78, β=60.12)	[47]
DC		0.45	Beta(α=61.37, β=99.07)	[47]
HCC		0.45	Beta(α=61.37, β=99.07)	[47]
PegIFN treatment	F0-F1	0.66	Beta(α= 43.34, β= 33.91)	[47]
	F2-F3	0.55	Beta(α= 52.78, β= 60.12)	[47]
	F4	0.45	Beta(α= 61.37, β= 99.07)	[e]
PegIFN-free treatment	F0-F1	0.72	Beta(α= 38.19, β= 24.21)	[f]
	F2-F3	0.61	Beta(α= 47.63, β= 44.23)	[f]
	F4	0.50	Beta(α= 57.08, β= 77.22)	[f]

Abbreviations: AMC: Amsterdam Medical Center; PHSA: Public Health Service of Amsterdam; G: genotype; SVR: sustained virological response

[a] For more detailed information, see Table B in S file.

[b] Costs of sofosbuvir and daclastavir in the Netherlands as in 2016.

[c] Healthcare utilization only once after achieving SVR.

[d] Utilities were multiplied by 0.85 in the analyses to account for drug dependency.

[e] Similar utility decrement assumed as the decrement from F0-F1 to F2-F3 in the chronic HCV health state.

[f] During IFN-free treatment, we assumed a lower utility decrement (-0.05 decrement instead of -0.11 decrement during treatment with PegIFN) than the decrement during PegIFN treatment.

[h] In order to use the utilities by Shepherd et al. we assumed that F0-F1 = mild disease, F2-F3 = moderate disease, and F4 = severe disease based on expert medical opinion.

[i] parameters from the beta distribution of the utilities based on the utilities accounted for drug dependency (see d).

nostic tests and consultations was adapted to reflect the shorter treatment duration. Costs for PegIFN/RBV were based on the mean costs of treatment, including side effects, in the

Netherlands [51]. Weekly sofosbuvir and daclatasvir costs in the Netherlands were 3,621 and 2,252 euros, respectively [52]; daclatasvir combined with sofosbuvir costs was used for the dual DAA therapy scenario as both medications are pan-genotypic. It is important to note that the Dutch ministry negotiated prices with pharmaceutical companies and actual DAA costs have not been made public, hence DAA prices might be lower at present. All costs were indexed to 2014 prices. We included specific costs for treating PWID based on the healthcare utilization accrued from the DUTCH-C project, determined in consultation with the medical coordinator (Table 2). As DAA regimens have been shown to have fewer side effects than PegIFN/RBV [15, 37-39, 53, 54], we conservatively assumed the costs of side effects with DAAs to be half of that with PegIFN/RBV (Table A in S1 File). Table 2 displays "treatment costs" which are the sum of medication and healthcare-related cost (e.g. HCV RNA monitoring).

Analyses

Total costs and effects (quality adjusted life-years (QALYs)) were calculated by adding up all costs and QALYs over a 15-year time horizon (from 2015 onwards), among PWID with a chronic HCV infection and PWID who were screened and treated during the modeled period. The ICER was calculated by dividing the difference in costs between two strategies by the difference in QALYs and represents the incremental cost associated with an additional QALY gained following a strategy that is more effective than the comparator strategy [55]. We applied a 4.0% discount rate for the costs and 1.5% for the effects based on Dutch guidelines for health economic evaluation [56].

Strategies were compared incrementally (among each other) to identify the most cost-effective strategy. According to WHO guidelines, a strategy can be considered highly cost-effective when the ICER is ≤1 times the Gross Domestic Product (GDP) per capita and as cost-effective when the ICER ≤3 times GDP/capita [57]. The Dutch GDP/capita was €38,255 in 2013 [58], implying that ICERs below 38,255 €/QALY would be considered highly cost-effective in the Netherlands. Graphically, all strategies are depicted on the cost-effectiveness frontier (Text E in S File). We also assessed the effect of treatment on the HCV-RNA prevalence over time.

Sensitivity and uncertainty analyses

We performed one-way deterministic sensitivity analyses to assess the impact of certain model parameters on the ICER in the declining epidemic. The following sensitivity analyses were done: fibrosis progression 2x the base transition probability, 0.70 SVR probability for F3-F4 in the SOF/RBV strategy (instead of 0.90), 40% never screened for HCV (instead of 20%), higher and lower utility values (+0,1, +0,2 and -0,1, -0,2), 20% and 50% lower DAA costs, excluding costs specific for PWID care, and a 0% discount rate for the costs and effects. A probabilistic sensitivity analysis using 1,000 bootstraps was also performed to reflect uncertainty in the costs and utilities parameter values

Scenario analysis

We calculated the cost-effectiveness of increased treatment uptake, by treating 100 PWID per year with dual DAA in Amsterdam (the declining epidemic), corresponding to an estimated 6% treatment uptake rate among HCV-RNA positive PWID in 2015. PWID specific treatment costs were doubled in this scenario analysis.

RESULTS

Table 3 shows the cumulative discounted QALYs and costs, ICERs, and averted HCV infections. Fig 2, the cost-effectiveness frontier, illustrates the point-estimates of QALYs gained and additional costs for all treatment strategies compared to PegIFN/RBV.

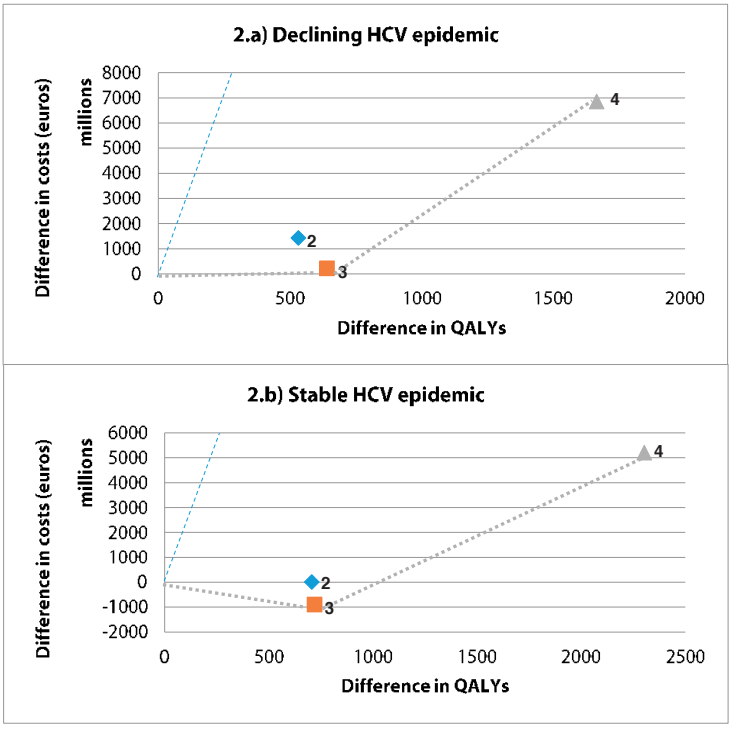

Fig 2: Cost-effectiveness frontier of DAA-treatment strategies among PWID compared to PegIFN/RBV[a]. Strategy 2: DAA/RBV (G2-3) & dual DAA (g1-4) ; 3: Dual DAA for all genotypes; 4: Dual DAA with a 3x higher treatment uptake.

[a] The strategies that fall below the dashed blue line are strategies that fall bellow a willingness to pay threshold reflecting 1 GDP per head of the population, i.e. €38,255 for the Netherlands, and are considered highly cost-effective compared to PegIFN/RBV. However, scenarios are compared incrementally to identify the *most* cost-effective strategy. The most cost-effective strategy is shown on the "cost-effectiveness frontier", the line that is closest to the X-axis.

Table 3: Cumulative discounted costs, effects, and incremental cost-effectiveness ratios of four hepatitis C treatment strategies in a declining epidemic and a stable HCV epidemic.

	Total screening costs (thousand €)	Total health state costs (million €)	Total treatment costs (million €)	Total costs in euro (million €)	Total number of new infections averted	Total no. of QALYs	Comparing	ICER[b] €/QALY
Declining epidemic								
1. PegIFN/RBV	3.30	23.66	2.93	26.92	2.5	16,659		
2. DAA/RBV & dual DAA[a]	3.31	23.35	4.66	28.35	2.5	17,192	2 vs. 1	**Ext.Dominated**
3. Dual DAA	3.29	23.50	3.32	27.14	1.7	17,300	3 vs. 1	**344**
4. Dual DAA HU	3.31	22.34	11.11	33.78	4.0	18,324	4 vs. 3	**4,115**
Stable epidemic								
1. PegIFN/RBV	4.78	8.24	2.77	11.49	6.7	9,802		
3. DAA/RBV & dual DAA[a]	4.73	7.12	3.90	11.49	10.0	10,508	2 vs. 1	**Dominated**
4. Dual DAA	4.75	7.10	3.01	10.59	11.2	10,522	3 vs. 1	**Dominant**
4. Dual DAA HU	4.72	6.59	9.62	16.69	30.7	12,104	4 vs. 3	**2,258**

Abbreviations: No: number; PegIFN: pegylated-interferon; RBV: ribavirin; DAA: direct-acting antiviral; ICER: incremental cost-effectiveness ratio; QALYs: quality-adjusted life years; Ext= extendedly; HU: higher uptake (3x); €: euros.

[a] Dual DAA therapy only for genotype 1 and 4.

[b] A strategy is ext. (extendedly) dominated when another treatment strategy is more attractive (i.e. yielding better outcomes (more QALYs)), even if that ICER falls below the willingness to pay threshold. A strategy is dominated when the costs are higher and effects are lower than the comparator strategy. A dominant strategy is better (yields more QALYs) and cheaper than the comparator strategy.

Base case, declining epidemic

Treatment with dual DAAs was the most cost-effective strategy (strategy 3) and is highly cost-effective (ICER=344 €/QALY) (Table 3). As illustrated in Fig 2a, all DAA strategies fall below the willingness to pay threshold (WTP) of a highly cost-effective strategy (i.e. 38,255 €/QALY). This means that when assessed on their own as compared to PegIFN/RBV, all strategies would be highly cost-effective; however when compared with each other, dual DAA therapy is most cost-effective. A small number of new infections were averted in all treatment scenarios (i.e. 2 in scenario 3) (Table 3). Over a 15-year period, dual DAA led to a 13% and 5% reduction in chronic HCV prevalence for G1-4 and G2-3 while treating 45 PWID annually (i.e. 3x higher uptake) with dual DAAs (strategy 4) led to a 19% and 8% reduction, respectively (Figure A in S File).

Impact of the type of epidemic

Treatment with dual DAA in a stable epidemic was the dominant strategy and led to cost-savings (Table 3 and Fig 2b). Eleven new infections were averted with dual DAA over the modeled period. Over a 15-year period, dual DAA led to a 5% and 3% reduction in chronic HCV prevalence for G1-4 and G2-3 while treating 45 PWID annually with dual DAAs led to a 17% and 7% reduction, respectively (Figure A in S File).

Sensitivity and uncertainty analyses

Dual DAA therapy remained highly cost-effective throughout the deterministic sensitivity analyses and ICERs were comparable to the main analysis. Although when DAA costs are 20% or 50% lower, and when fibrosis progression is twice that of the baseline scenario, this strategy becomes cost-saving (Fig 3). On the other hand, when specific PWID treatment costs were excluded from the analysis, the ICER for dual DAA increased to 2,150 €/QALY. The probability of dual DAA therapy being cost-effective was 100% at a WTP of one time GDP/capita. Therefore, no cost-effectiveness acceptability curves were plotted.

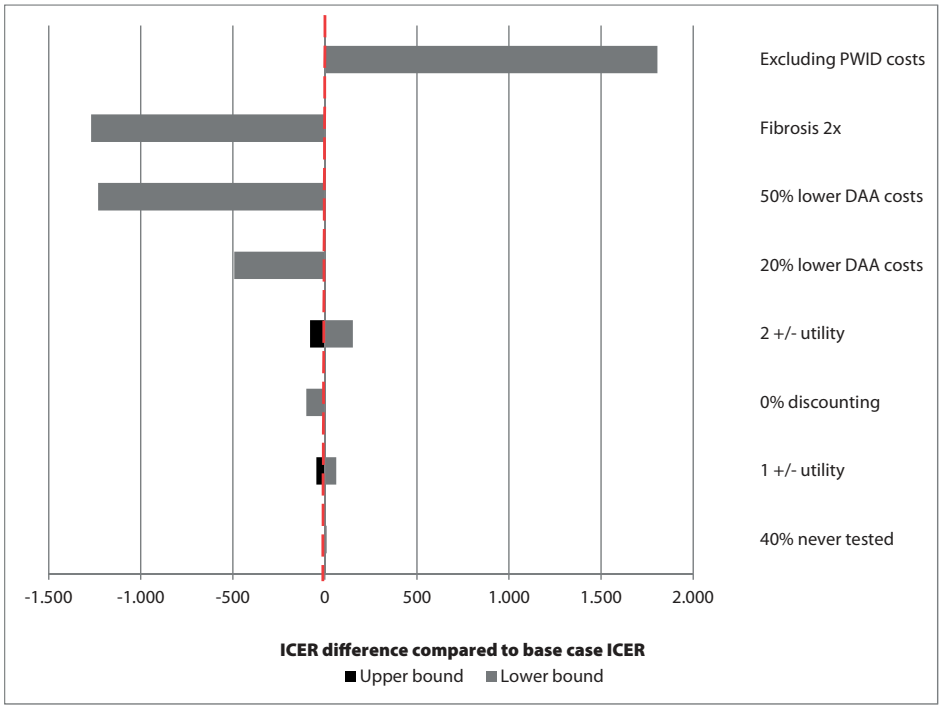

Fig 3: Tornado diagrams illustrating deterministic sensitivity analyses of dual DAA compared to PegIFN/RBV in the declining epidemic (Amsterdam, The Netherlands).
Abbreviations: DAA: direct-acting antiviral.
The red dashed line represents the base case ICER (ICER=344 €/QALY). For the deterministic sensitivity analyses of the utilities: the grey bars represent a lower utility than the base case; the black bars represent a higher utility than the base case. Fibrosis 2x: means a fibrosis progression two times that of the base case scenario.

Scenario analysis

When 100 PWID were treated annually in the declining epidemic, dual DAA remained highly cost-effective when compared to PegIFN/RBV (ICER=4,192 €/QALY). This scenario led to a 23% and 6%, reduction of chronic HCV prevalence over a 15-year period for G1-4 and G2-3, respectively; though in 2029, chronic HCV prevalence was still estimated at 18% for G1-4.

DISCUSSION

We determined the cost-effectiveness of four HCV treatment strategies among PWID in a stable and a declining HCV epidemic. This study showed that treatment with DAA-containing regimens for PWID is a highly cost-effective intervention, irrespective of the

type of epidemic. Although dual DAA therapy was most cost-effective, the two other DAA-treatment strategies fell also below the WTP. Sensitivity analyses showed that our ICERs were very robust. These analyses provide economic support for the treatment of PWID with DAAs.

Our results are in line with other cost-effectiveness studies with sofosbuvir-containing regimens among chronically HCV-infected individuals [59-62]. Hagan et al. reported that dual DAA (SOF/simeprevir (SOF/SMR)) resulted in both better outcomes and less costs compared to SOF/RBV [60]. Similarly, a cost-effectiveness study in Germany showed that SOF/SMV is both more effective and cheaper than SOF/RBV [63]. The lower costs and higher QALYs accrued in these studies and our present study with dual DAA can be explained by the shorter treatment durations and higher efficacy, which result in lower healthcare utilization costs and more prevention of liver-related morbidity (e.g. HCC). In a cost-effectiveness study of DAAs among PWID, Hellard et al. showed that in Australia, early treatment (from F0) and late treatment (from F2) yielded an ICER of 10,272 and 5,078 Australian dollars, respectively, compared to no treatment [16]. However, this study did not capture the benefits of reduced transmission (treatment as prevention) and therefore, ICERs might be even more favorable in reality than those reported. Another cost-effectiveness study also showed that treating PWID is cost effective in the UK; especially among PWID with moderate fibrosis at a 40% baseline chronic HCV prevalence [64]. Our low ICERs might be a result of incorporating a healthcare model specific for PWID in our analysis, as sensitivity analysis without these costs resulted in a higher ICER. Also, we included fibrosis progression and a clearance rate specific for HIV/HCV-coinfected which is usually not explicitly incorporated in cost-effectiveness analyses for HCV treatment [16, 63-65]. As HIV/HCV-coinfection leads to faster HCV disease progression [31] and our sensitivity analysis showed that this parameter (faster fibrosis progression) had the biggest impact on the ICER, excluding these HIV-related parameters in the model would result in less favorable ICERs. Furthermore, in the stable epidemic dual DAA led to cost-savings. This might be a result of preventing HCV transmission (population benefit) compared to the declining epidemic with few new infections among PWID. Healthcare costs were also higher in the declining than the stable epidemic, which is probably a result of the younger PWID population in the stable epidemic that has not progressed to advance fibrosis stages (which incur higher costs than early fibrosis stages). Hence more liver-related morbidity can be prevented in the stable epidemic compared to the declining epidemic.

Although DAA-containing treatments are cost-effective, treating only 15 PWID annually will probably not contain the epidemic in an on-going transmission setting (stable epidemic) as only a slight decrease in chronic HCV prevalence was observed with dual DAA in this epidemiological setting. When treatment is scaled-up (i.e. 45 PWID treated annu-

ally) more new infections were averted (31 vs. 11 with baseline uptake) and HCV RNA prevalence decreased 17% for G1-4. Martin et al. also showed that current treatment uptake in England was unlikely to achieve observable reductions in HCV prevalence, while scaling up treatment (to 26/1000 annually) could lead to a substantial reduction [66]. However, the absolute number needed to treat in other settings may differ from our analysis and may depend on the population size. In the declining epidemic, a higher reduction of chronic HCV prevalence is observed even when treating 15 PWID annually compared to the stable epidemic (13% vs. 5% reduction for G1-4 in the declining and stable epidemic, respectively). This is probably a result of HCV-treatment and mortality in this ageing PWID population. Although treating 100 PWID annually led to a substantial reduction of HCV RNA prevalence (23% for G1-4) and was also highly cost-effective. Nevertheless, still after 15 years, HCV RNA prevalence was 18% for G1-4, suggesting that to eliminate HCV among PWID in Amsterdam, treatment should be scaled-up even further and those who abstain from screening or HRP should be identified and actively approached.

Our analyses have several limitations. First, SVR for DAAs are mainly based on clinical trials with relatively small sample sizes. However, a recent real-life study showed comparable SVR rates as those observed in clinical trials [67]. Second, we assumed that there was no difference in SVR for SOF/RBV for G2-3 with F3-F4 as only limited trial data was available at the time the model was built, although sensitivity analysis showed similar results. If SVRs were overestimated in our study, this could have led to more favorable ICERs compared to PegIFN/RBV. On the other hand, we might have overestimated the costs of (pre- and post-)DAA treatment monitoring as we made conservative assumptions on frequency of laboratory/diagnostic tests and clinical visits. In real life, less healthcare utilization might be feasible when no PegIFN and/or RBV are given, as these medications cause significantly more side effects than DAAs [8]. Costs due to adverse events for DAAs were assumed to be half of cost accrued with PegIFN/RBV, but we believe those costs might be even lower in a real-life setting. Also, our analysis used list prices for DAAs, while actual costs may be lower after price negotiations. Lower costs of DAA treatment would make DAAs even more favorable than PegIFN/RBV as shown in our sensitivity analyses. Furthermore, for simplicity, we did not account for the percentage of those ineligible for or intolerant to PegIFN. Also, we assumed that HRP, such as low-threshold methadone programs where PWID could be screened for HCV, were in place. Although this is true for Amsterdam, our results from the stable epidemic might not be generalizable to countries without wide coverage of such programs, as only screening costs were taken into account. Furthermore, for the stable HCV epidemic analysis, key parameters from the model may not be appropriate for countries with a stable epidemic (e.g. screening coverage). Therefore, caution must be taken when extrapolating these

results. Last but not least, real-life DAA studies among PWID with large sample sizes are necessary to confirm our assumptions on SVR in this population. Furthermore, other treatment models, such as HCV treatment fully integrated into methadone maintenance programs or supervised injecting facilities [68], might reduce treatment costs compared to our integrated treatment setting. Lower healthcare utilization costs is likely to be the case with DAAs as we believe that less health-provider support might be necessary as fewer significant side effects can be expected. Future research should evaluate the cost-effectiveness of different treatment models for PWID with DAA-containing regimens.

There are also several strengths in this study. First, we used an individual-based transmission model which took re-infections into account and thus the population benefit of HCV treatment; particularly important for countries with on-going HCV transmission. If re-infections are not taken into account, the burden of HCV might be underestimated. Second, our study is mostly based on observed data from Amsterdam on incidence, prevalence, mortality, HCV treatment uptake, and real-world DAA prices. Also, we took screening and a treatment setting specific for PWID into account, which is usually not included in recent cost-effectiveness studies for DAA-containing regimens [16, 59-62, 64]. Third, we analyzed different HCV treatment scenarios among PWID to depict the possible choices of treatment based on current Dutch guidelines.

In conclusion, DAA-containing regimens are highly cost-effective among PWID, irrespective of the type of HCV epidemic. Given the current evidence, dual DAA therapy should be considered the standard recommended HCV treatment, not only because of its higher efficacy but also the lower net costs compared to other DAA regimens [60, 63]. Also, based on the economic and population benefits of scaling-up treatment, stronger efforts are needed to achieve higher uptake rates among PWID

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SUPPLEMENT CHAPTER 3.3

Supplementary text methods

A Model expansion

Genotype: we categorized genotype into two groups, genotype 1-4 or genotype 2-3. Studies have shown evidence to suggest at least partial HCV-related immunity [1, 2] and although the strength of this immunity effect remains unclear, we assumed that PWID who cleared the virus had a 20% lower probability to become re-infected with HCV with the same genotype, but were equally susceptible to get infected with another genotype group.

B Natural history

Transition probabilities are based on original research or previous modeling studies. HCV clearance has been shown to be dependent on HIV-coinfection status [3].Therefore, in our model, HCV/HIV-coinfected PWID had a lower HCV clearance rate (17% vs. 25% among HCV-monoinfected [4]); this percentage was based on two prospective longitudinal studies reporting a clearance rate of 15% and 20% [3, 5]. Furthermore, based on a systematic review of longitudinal studies, females have a higher probability to clear HCV [6]. Fibrosis progression based on the METAVIR scoring system was dependent on sex and age group [7] based on an empirically-calibrated model from the US, and assumed to be twice as fast among HCV/HIV-coinfection status based on a meta-analyses [8]. An individual with cirrhosis (METAVIR: F4) could develop decompensated cirrhosis (DC) or hepatocellular carcinoma (HCC), which is dependent on HIV-coinfection status [9] (by a factor of 1.5 [9]). The HCC- and DC-related mortality rates do not depend on HIV status. For simplicity, we assumed that during treatment, individuals could not transmit HCV to other PWID. After achieving SVR individuals remained in their fibrosis stage, without progressing to other fibrosis stages [10, 11]. After a re-infection, individuals could progress to another fibrosis health state.

C Treatment uptake

We assumed that 15 PWID were treated annually at the PHSA irrespective of their fibrosis stage. This was based on data from the DUTCH-C project where on average 10 PWID were treated per year until 2013; of whom around 7% were HCV/HIV-coinfected. At the beginning, only HCV-monoinfected PWID were treated. Later, treatment was extended to HCV/HIV-coinfected PWID. Based on expert medical opinion, few PWID, and usually more stable (ex-)PWID, were treated outside the DUTCH-C project from 2005 until 2013, therefore we assumed that an additional 5 PWID could be treated. Though, in our model, all 15 PWID were treated at the PHSA as we included a model of care specific for PWID.

We assumed the same treatment uptake in the stable epidemic scenario; however, in contrast to the declining epidemic, the proportion of HCV/HIV-coinfected who were treated was proportional to the HIV prevalence among HCV-infected PWID as we assume this to be a more realistic scenario given that DAAs have been shown to be well tolerated by HCV/HIV-coinfected individuals [12]. Furthermore, treatment with PegIFN/RBV started in 2005 in the declining epidemic until 2014 (unless the strategy from 2015 onwards was PegIFN/RBV), based on the situation in Amsterdam at that time. Starting date of HCV treatment in the stable epidemic was 2015.

D Treatment options SVR sources

1. Treatment with PegIFN with RBV (PegIFN/RBV): SVR rates were dependent on HIV status and fibrosis stage [13-15].
2. SOF/RBV for genotype 2-3 and dual DAA for genotype 1-4: SVR rates for genotypes 2 are based on averaged results from the FISSION, POSITRON, and VALENCE trial [16-18]. The latter trial was used to obtain the SVR for genotype 3. Dual DAA therapy SVRs are based on averaged results from trials with daclatasvir combined with sofosbuvir [19, 20].
3. Dual DAA therapy for all genotypes: SVRs were the same as in scenario 3 for all genotypes.

For simplicity, no fibrosis to moderate fibrosis (F0-F2) and severe fibrosis and cirrhosis are grouped together (F3-F4). Therefore, treatment duration with SOF/RBV for individuals with cirrhosis and genotype 2 is underestimated (12 weeks instead of 16) [21], although very few PWID are infected with this genotype. Only 4% of HCV-infected participants from the Amsterdam Cohort Study had a genotype 2 infection during 2000-2004 [22].

E Analyses & model output

A strategy can be (highly) cost-effective, dominant, dominated, or extendedly dominated. Strategies are ruled out by either dominance or extended dominance. Strategies with higher costs and lower effects than a comparator strategy are considered to be dominated and are no longer used as a comparator strategy for the next strategies. Strategies are extendedly dominated when another strategy has lower costs and more QALYs; even if the extendedly dominated strategy has an ICER that falls below the willingness to pay threshold. A strategy that is more effective (more QALYs) is compared to a previous strategy that has not been previously dominated or extendedly dominated. We used a 15 year time horizon as we believe that it is sufficient to capture the effects of treatment on healthcare-related costs in this ageing PWID population in the Netherlands [23].

Model output:

From 2015 onwards, the model annually estimated the following for each strategy:

1. The number of PWID screened, the number of HCV-antibody-positive (HCVab+) among those screened, and HCV-RNA-positive among HCVab+;
2. Number of person months during treatment per genotype group and fibrosis stage;
3. The number of person months per fibrosis stage and genotype group: during treatment among HCV-RNA-positive PWID (treatment-naïve), unsuccessfully treated, and among those who achieved SVR;
4. The number of person months with HCC or DC and the number of deceased PWID;
5. HCV-antibody and -RNA incidence and prevalence by genotype group.

Supplementary Table A: Overview of HCV treatment scenarios in the stable and the declining HCV epidemic among PWID.

Scenario:	Genotype group	Weeks	Genotype group	Weeks
	1-4		2-3	
1	PegIFN/RBV	48	PegIFN/RBV	24
2	Dual DAA	12	DAA/RBV	22*
3 & 4	Dual DAA	12	Dual DAA	12

* weighted average = 24 weeks of treatment for genotype 3 and 12 weeks for genotype 2; As only 20% [22] in this genotype group is infected with genotype 2, the weighted number of weeks of treatment is in between 20 and 24 weeks. We averaged the latter two, and rounded it to 22 weeks of treatment.

Supplementary Table B: Costs of HCV treatment & screening

	Costs	year	source [g]	Costs in 2014 [j]	PegIFN		SOF/RBV Dual DAA	
					G1-4	G2-3	All genotypes	
Staff costs (includes overhead costs)								
Medical specialist consultation (hour) and medical coordinator	103	2009	[24]	113.2	1,188.7	849.1	792.5	792.5
Nurse consultation (hour)		2014	PHSA	62.2	11,944.3	5,972.2	2,986.1	1,493.0
Psychiatric consultation (hour)	103	2009	[24]	113.2	113.2	113.2	113.2	113.2
Cardiologist/Pulmonologist (hour) [a]	103	2009		56.6	56.6	56.6	56.6	56.6
HIV specialist (hour) [b]	103	2009		7.9	15.8	15.8	15.8	15.8
HCV screening [c]								
HCV antibodies		2014	PHSA	11.4				
HCV RNA + genotype		2014	AMC	222.8				
Routine laboratory tests							194.7	97.35
Hemoglobin		2014	70702	1.7				
Leucocyte		2014	70741	1.7				
Leucocyte differential		2014	AMC	2.3				
Thrombocytes		2014	70715	1.7				
ALAT		2014	74891	2.1				
ASAT		2014	74489	1.9				
Alkaline phosphatase		2014	74896	2				
Gamma - GT		2014	72417	1.9				
Glucose		2014	70402	1.8				
HbA1c (if glucose is high) [d]		2014	74065	2.4				

Supplementary Table B: Costs of HCV treatment & screening (*continued*)

	Costs	year	source [g]	Costs in 2014 [j]	PegIFN		SOF/RBV Dual DAA	
					G1-4	G2-3	All genotypes	
Screening diagnostic tests							32.7	32.7
PTT		2014	70707	4.1				
APTT		2014	77371	3.3				
AT III		2014	AMC	13.5				
Albumin		2014	74802	1.6				
Creatine		2014	70128	1.8				
ANA		2014	70693	8.4				
RNA concentration								
Qualitative & quantitative		2014	AMC	126.8			760.7	760.7
Endocrinology								
TSH		2014	72573	6.7			20.1	20.1
Radiology							966.3	966.3
Echo		2014	AMC	58.7				
X ray thorax		2014	85070	44.2				
FibroScan	120	2012	AMC	126.1				
MRI liver [e]	118.5	2014	AMC					
Endoscopy		2014	34620	210.8				
ECG [f]		2014	39757	43.4			43.4	43.4
Medication costs [h,i]			[25,26]		16,393	12,291	99,485	79,195
Side effects DAA			[26]	1009.3				
TOTAL TREATMENT COSTS					**29,712**	**19,298**	**106,476**	**84,216**

Abbreviations: PHSA: Public Health Service of Amsterdam; AMC: Amsterdam Medical Center; PTT: partial thromboplastic time; APTT: activated PTT; AT: antithrombin; ANA: antinuclear antibody; TSH: thyroid stimulating hormone; ECG: electrocardiogram; G: genotype; SOF: sofosbuvir.

[a] For 50% of PWID based on expert opinion (incorporated in total costs in 2014).

[b] 7% of PWID (based on the declining epidemic model). Total HIV specialist costs based on two visits (pre- and post-HCV treatment).

[c] Included in screening costs only.

[d] Assumed 30% high glucose level ; 30% * 7,9 = 2.37 euros.

[e] Not included in the treatment costs.

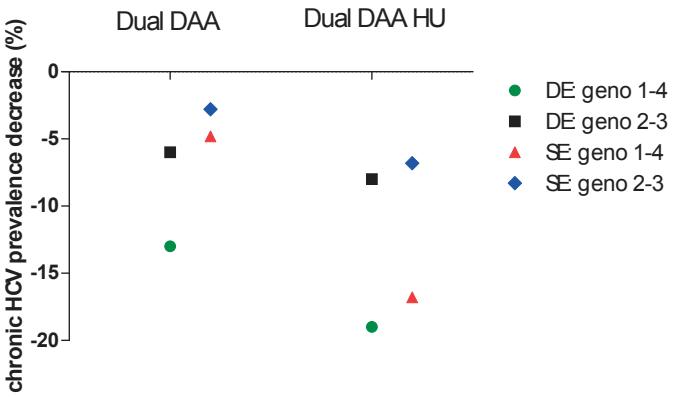
[f] Standard for PWID.

[g] Numbers shown in this column are derived from the Dutch Health Authority (NZA).

[h] PegIFN/RBV side effects costs already included in the mean total costs; costs for PegIFN-containing treatment were indexed to 2014 euros.

[i] RBV included in total costs when applicable.

[J] The column "costs in 2014" denote the cost per test or medical consultation while costs depicted under each treatment strategy incorporate total costs of these services/ tests during the whole (pre-, during and post-HCV) treatment period, when applicable.



Supplementary figure A: Chronic HCV prevalence decrease in the declining and the stable epidemic over a 15-year period.

Abbreviations: DAA: direct-acting antiviral; HU: higher uptake (3x higher uptake than the base case); geno: genotype; DE= declining epidemic; SE= stable epidemic.

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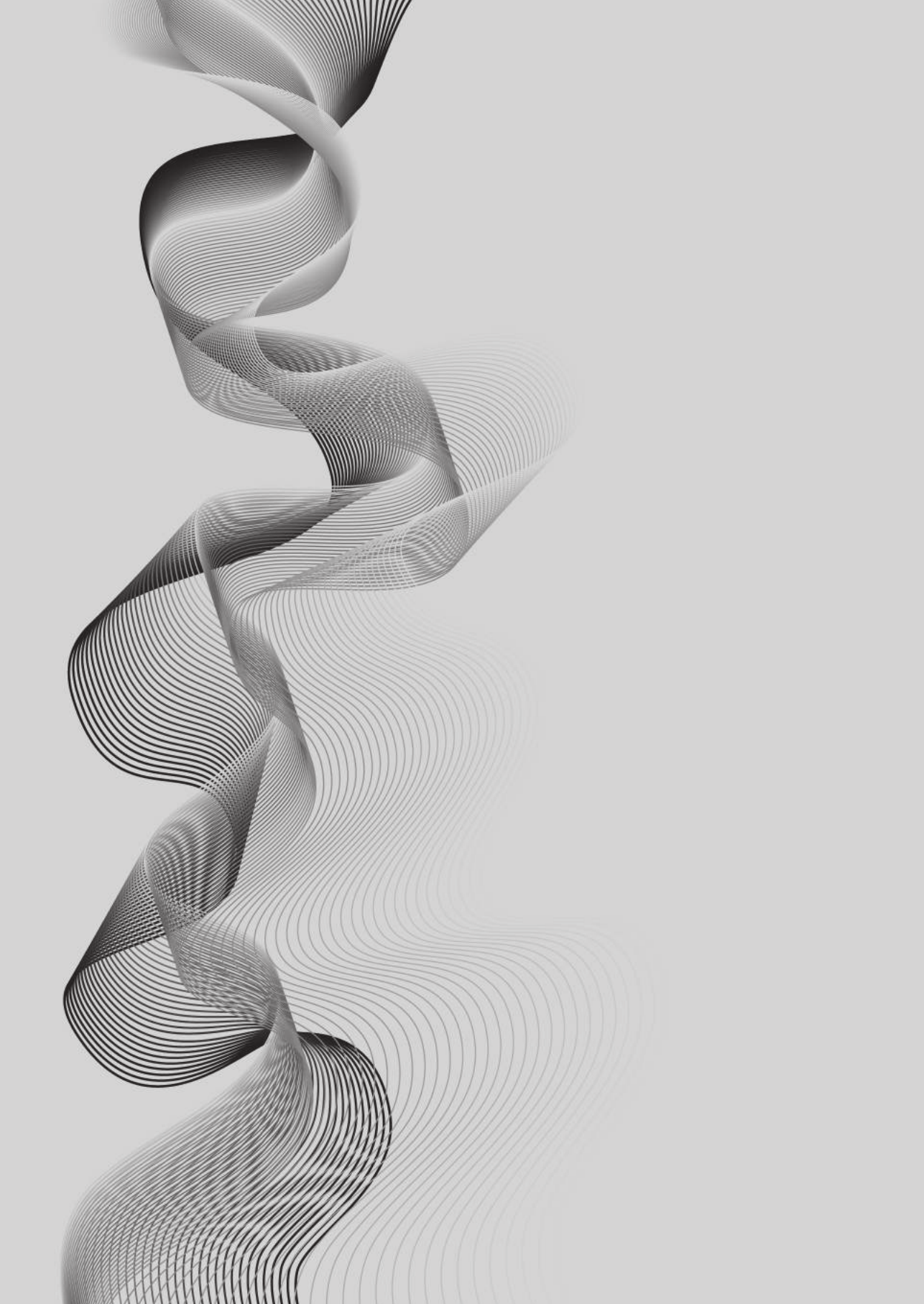

Chapter 3.4

HIV and hepatitis C treatment uptake among people who use drugs participating in the Amsterdam Cohort Studies, 1985–2015

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ABSTRACT

Background: HIV-positive people who use drugs (PWUD) start antiretroviral therapy (ART) later than other risk groups, and among HCV-positive PWUD, HCV treatment uptake is low. Nowadays, HCV direct-acting antivirals (DAAs) are available and reimbursed in the Netherlands (since 2014). The Amsterdam Cohort Studies (ACS), initiated in 1985, provides us the opportunity to describe temporal trends in ART and HCV-treatment uptake among PWUD through 2015.

Methods: We analyzed data from PWUD participating in the ACS between 1985 and 2015. ART and HCV-treatment data were obtained from ACS questionnaires and medical records. Treatment uptake was defined by: treatment initiation (the proportion initiating any kind of ART/HCV treatment when treatment-naïve) and coverage (the proportion ever treated for HIV/HCV) among all HIV-/HCV-RNA-positive PWUD. Each was calculated per calendar year. We estimated the cumulative probability of ART uptake in the pre-cART (<1996) and cART era (January 1, 1996) among HIV seroconverters, with all-cause mortality as a competing risk.

Results: Of 1,305 PWUD, 263 (20.2%) were HIV-antibody positive and 810 (62.1%) were HCV-antibody positive, at study entry. ART coverage increased over time, from 5.7% in 1990 and 42.2% in 1996 to 91.7% in 2015. The proportion initiating ART ranged from 4.8% in 1990 to 33.3% in 2011. At 8 years after HIV seroconversion, cumulative probability of ART uptake was 42.5% in the pre-cART era and 61.5% in the cART era. HCV treatment initiation peaked in 2006 (9.7%). HCV-treatment coverage was 43.9% in 2015 but lower among HIV-coinfected (23.5%) than HCV-monoinfected PWUD (52.5%). In 2015, 3.0% initiated HCV treatment with DAAs.

Conclusion: We observed an increase in ART and HCV-treatment coverage among PWUD over time. As expected, ART uptake was higher in the cART era than the pre-cART era. Although in 2015 HCV treatment coverage was relatively high, DAA uptake was still low.

INTRODUCTION

People who use drugs (PWUD) account for a large burden of HIV and hepatitis C virus (HCV) infections worldwide [1, 2]. Although studies in high-income countries report an increasing uptake of antiretroviral therapy (ART) over time [3, 4], PWUD who are HIV-positive are more likely to begin ART later than other HIV-risk groups [5-8], even in the combination-ART (cART) era [9]. As for PWUD infected with HCV, a systematic review in Europe reported that the percentage of those chronically infected who had ever initiated interferon-based HCV treatment, ranged from 0 to 57%, with the higher uptake observed predominantly in hospital settings [1]. Community-based studies in Canada and Australia have reported HCV-treatment uptake among PWUD to be 6% [10, 11]; however, uptake estimation methods differ between studies.

The advent of direct-acting antivirals (DAAs) has led to worldwide optimism that HCV can be eliminated [12]. In November 2014, sofosbuvir became available in the Netherlands for patients with advanced liver disease (i.e. with a fibrosis level at or above F3 on the Metavir scoring system). Since November 2015, several DAAs (e.g. sofosbuvir/ledipasvir) are reimbursed for all patients with a chronic HCV infection irrespective of fibrosis stadium [13]. As HCV-treatment effectiveness has increased and DAAs have fewer contraindication and a shorter treatment duration than interferon-based therapy [14], we expect more PWUD to be treated with DAAs in the coming years. Similarly, we expect that ART uptake has increased over time among PWUD, as ART initiation guidelines have changed and more efficient types of ART have been developed, with easier regimens and fewer tablets [15].

Few studies have been able to assess temporal changes in ART and HCV treatment uptake among PWUD over a long period within a community-based cohort. The Amsterdam Cohort Studies (ACS), a long-standing community-based cohort started in 1985, gave us the opportunity to assess these trends among PWUD through 2015 and, particularly, the uptake of DAA during the first full year of its availability in the Netherlands. Furthermore, we estimated the cumulative probability of ART initiation from HIV seroconversion onwards, stratified by ART period (pre-cART and cART eras), with all-cause mortality as a competing risk.

METHODS

We analysed data from PWUD participating in the ACS between 1985 and 2015. Including injecting and non-injecting PWUD, ACS is a prospective cohort study on HIV that

began in 1985 [16] and remained open for new enrolments until 2014. Participants were recruited at methadone outposts, a special sexually transmitted infections outpatient clinic for sex workers who used drugs (until 1997), and by word of mouth. After 2000, recruitment was directed in particular towards young PWUD. Drug use was defined as the use of 'hard' drugs (i.e. heroin, cocaine, amphetamines and/or methadone) for at least three times a week. Participation in the ACS was voluntary, and written informed consent was obtained before enrolment. Over time, an estimated 15% of the Amsterdam population who injected drugs participated in the ACS [17]. Participants made follow-up visits to the Public Health Service of Amsterdam (PHSA) every 4 months until 2003 and thereafter every 6 months. At each visit, trained research nurses interviewed them about their health and sexual and drug-use behavior using a standardized questionnaire. Blood was drawn for storage and HIV-antibody testing (Ag/Ab Combo test, Axsym; Abbott Laboratories and bioMerieux, France). Reactive samples were confirmed by immunoblot (Line Immuno Assay, Inno-Lia HIV I/II Score; Innogenetics NV, Ghent, Belgium). Before 2004, reactive samples were confirmed by Western blot. In 2006-2007, all participants with at least two cohort visits by January 2005 were retrospectively tested for HCV antibodies using a third-generation ELISA (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). Individuals who were anti-HCV-negative at entry were tested for HCV antibodies at the most recent visit. On finding HCV seroconversion (defined as the presence of HCV antibodies in a previously HCV-seronegative individual), we tested samples taken between the first and the most recent visit to obtain the most exact seroconversion interval. After 2005, HCV-antibody testing was performed prospectively, and those that tested positive were also tested for HCV RNA. For participants who did not return for follow-up, information about vital status was obtained by matching against local population registries.

ART and HCV treatment

ART data since 1990 and HCV-treatment data since 2004 were obtained from ACS questionnaires in which PWUD were asked about initiation of ART and HCV treatment since their last cohort visit. As ART was not routinely administrated at the PHSA, self-reported ART uptake was confirmed and completed by checking clinical data from medical records received after hospital admission, and by matching against the national HIV register from the Dutch HIV Monitoring Foundation since 1998. We used HCV treatment data from the DUTCH-C HCV-treatment unit, which was integrated within the PHSA between 2005 and 2013. The DUTCH-C unit facilitated HCV treatment for PWUD by actively offering HCV testing and treatment using a protocol adapted to specific needs of PWUD outside a hospital setting [18]. In the first years of the DUTCH-C unit, HCV treatment was only accessible for HCV-monoinfected PWUD, and from 2007 onwards, it was extended to HCV/HIV-coinfected PWUD. Since 2014, HCV treatment is administered only at hospital-based

clinics and is coordinated and assisted by nurses from the Public Mental Health Service (PMHS) within the PHSA, where harm-reduction services (i.e. opioid substitution therapy (OST) and needle-exchange programs (NEP)) are provided. Additionally, we obtained HCV-treatment data for all HIV-positive PWUD collected by the Dutch HIV Monitoring Foundation, and we reviewed PMHS clinical records for HCV-RNA-positive PWUD.

Definitions & statistical analyses

Uptake of ART and HCV treatment was defined by two distinct concepts, namely: treatment initiation and coverage. ART initiation was defined as the proportion of PWUD who for the first time initiated any kind of ART (i.e. monotherapy, dual or combination ART) among all ART-naïve HIV-positive PWUD with at least one cohort visit per calendar year. ART coverage was defined as the proportion of PWUD ever having received ART among all HIV-positive PWUD with at least one cohort visit per calendar year. ART initiation and coverage were calculated per calendar year from 1985 until December 31, 2015. HCV-treatment initiation was defined as the proportion of PWUD who for the first time initiated HCV treatment among all treatment-naïve HCV-RNA-positive PWUD with at least one cohort visit per calendar year. HCV-RNA-positive status was based on at least one HCV-RNA-positive test result. HCV-treatment coverage was defined as the proportion of PWUD ever treated for HCV among all HCV-RNA-positive and successfully HCV-treated PWUD with at least one cohort visit per calendar year. HCV-treatment coverage was also stratified by HIV-coinfection status. HCV-treatment initiation and coverage were calculated per calendar year from January 1, 2005 until December 31, 2015. Our analysis starts in 2005, as data on HCV treatment were systematically collected from that year onwards. HCV retreatment was not considered in our analyses.

We estimated the cumulative probability of ART uptake during the pre-cART era (<1996) and the cART era (January 1, 1996) among PWUD who seroconverted for HIV during follow-up. We took into account all-cause mortality as a competing risk, since we believe censoring due to mortality to be informative. PWUD who died were probably a select group of individuals less likely to be engaged in care and with higher risk behaviour. As mortality is common among PWUD, we cannot ignore the informative censoring. Estimated date of HIV seroconversion was calculated as the midpoint date between the last negative and first positive HIV test. Follow-up was calculated from the participant's estimated date of HIV seroconversion to the earliest of date of ART initiation, date of death, or to the last date the participant was known to be alive and ART-naïve. Individuals could contribute data to two calendar periods (pre-cART and cART-eras), and for the second period (cART era), their observation time was left-truncated at January 1, 1996.

RESULTS

Of 1,305 PWUD with at least two cohort visits, median follow-up time was 9.4 years (interquartile range [IQR]= 3.7-16.0). At study entry, 69.4% injected or had a history of injecting drug use, 64% were male, median age was 30 years (IQR= 26-36), and 76% were of west-European ethnicity. At study entry, 263 (20.2%) were HIV-positive, and 810 (62.1%) were HCV-antibody positive. Of the latter, 9.4% did not have an available, stored blood sample at study entry and were tested for HCV during follow-up. During follow-up, 99 and 60 PWUD seroconverted for HIV and HCV - of whom 93 (93.4%) and 56 (93.3%) had a history of injecting drug use at the estimated date of seroconversion - raising totals to 362 and 870, respectively. The percentage of PWUD engaged in harm-reduction programs during 1985 to 2015, varied between 64.1% and 92.8% (Table 1). By the end of the study period (December 31, 2015), a total of 470 deaths had been recorded, and 104 PWUD had emigrated from the Netherlands.

ART uptake

Of 362 HIV-positive PWUD, 175 initiated treatment during follow-up. Sixty-four percent of the HIV treatment initiation data in our study were obtained from clinical records collected by the Dutch HIV Monitoring Foundation, and before 1998, the majority of the self-reported data was confirmed with hospital records. In 1989, ART usage was first documented in the ACS. From 1989 until 1993, ART initiation increased steadily and thereafter fluctuated, from 8.0% in 1994 to 25.0% in 2007 and 33.3% in 2011 (Figure 1A). Thereafter, ART initiation went down to 0% as a result of previous high ART coverage and the small number of PWUD who remained ART-naïve (n= 2 in 2015). The proportion of HIV-positive PWUD ever treated for HIV (coverage) increased over time from 5.7% in 1990, 42.2% in 1996, and 59.2% in 2000, to 91.7% in 2015 (Figure 1B).

ART uptake and mortality among HIV seroconverters

Among 98 PWUD who seroconverted for HIV during follow-up, the median seroconversion year was 1992 (IQR= 1989-1995). Of these 98, 75 seroconverted during the pre-cART era and 23 during the cART era. Excluded from the analysis was one participant whose last HIV-negative test was recorded in 1986, and the first HIV-positive test in 2010, hampering a reliable estimate of the HIV seroconversion date. During the pre-cART and cART era, respectively, 19 and 45 initiated ART whereas 13 and 14 died as ART-naïve. At 5 after HIV seroconversion the cumulative probability of having initiated ART in the pre-cART era was 21.8% (95%CI= 9.8-32.2%) whereas the probability for all-cause mortality was 18.2% (95%CI= 7.1-28.0%) (Figure 2). In the cART era, at 5 years after HIV seroconversion, 45.7% (95%CI= 28.9-58.6%) had initiated ART whereas 18.2% (95%CI= 5.6-29.2%) had died (Figure 2). Furthermore, at 5 years after HIV seroconversion, 60.0%

Table 1: Demographic characteristics and HIV and HCV treatment initiation among 1,305 PWUD with at least two cohort visits participating in the Amsterdam Cohort Studies - 1985-2015

Calendar year	Total risk-set	IDU[b]	HRP[c]	Deaths	HIV+[d]	Initiated HIV treatment[a]	HCV-RNA +[d]	Initiated HCV treatment[a]
	n	%	%	n	n	n		n
1985	13	92.3	92.3	0	4	0	*NA*	
1986	205	82.4	92.7	1	66	0		
1987	278	87.4	92.8	4	96	0		
1988	323	88.9	88.5	5	113	0		
1989	372	89.0	82.0	6	138	2		
1990	445	87.8	83.2	17	157	8		
1991	434	88.0	81.3	12	140	12		
1992	468	86.7	85.0	20	130	16		
1993	529	86.0	84.5	29	126	22		
1994	561	84.6	82.0	20	127	11		
1995	579	81.8	83.6	19	114	14		
1996	579	80.4	82.2	18	97	18		
1997	572	78.9	78.7	17	83	9		
1998	629	74.2	74.9	23	76	15		
1999	629	74.1	72.7	15	66	8		
2000	659	70.1	72.2	20	49	8		
2001	661	66.9	70.4	24	42	6		
2002	601	67.4	70.7	15	39	3		
2003	550	66.6	68.0	22	29	1		
2004	507	66.3	68.8	8	22	5		
2005	473	66.4	67.0	12	19	3	148	10
2006	453	66.7	66.2	12	17	3	154	15
2007	421	67.9	66.0	16	12	4	134	13
2008	388	66.5	66.8	18	7	1	109	5
2009	368	66.8	64.4	18	7	2	100	1
2010	347	66.1	65.1	16	7	2	92	3
2011	329	66.9	64.1	20	4	2	84	4
2012	288	66.3	69.4	24	4	0	68	1
2013	254	64.5	70.9	18	2	0	54	1
2014	218	64.0	71.6	12	2	0	42	0
2015	187	63.0	71.7	9	2	0	33	1
Total	**1,305**			**470**	**362**	**175**	**209**	**54**

Abbreviations: n, number; IDU, injecting drug use; HRP, harm-reduction program; NA, not applicable

[a] Among HIV or HCV treatment-naïve PWUD with a cohort visit in that year.

[b] Percentage of PWUD who ever injecting drugs during a calendar year.

[c] Percentage of PWUD engaged in a harm-reduction program (HRP) during a calendar year.

[d] An individual could contribute to more than one calendar year if she/he remained HIV or HCV treatment naïve.

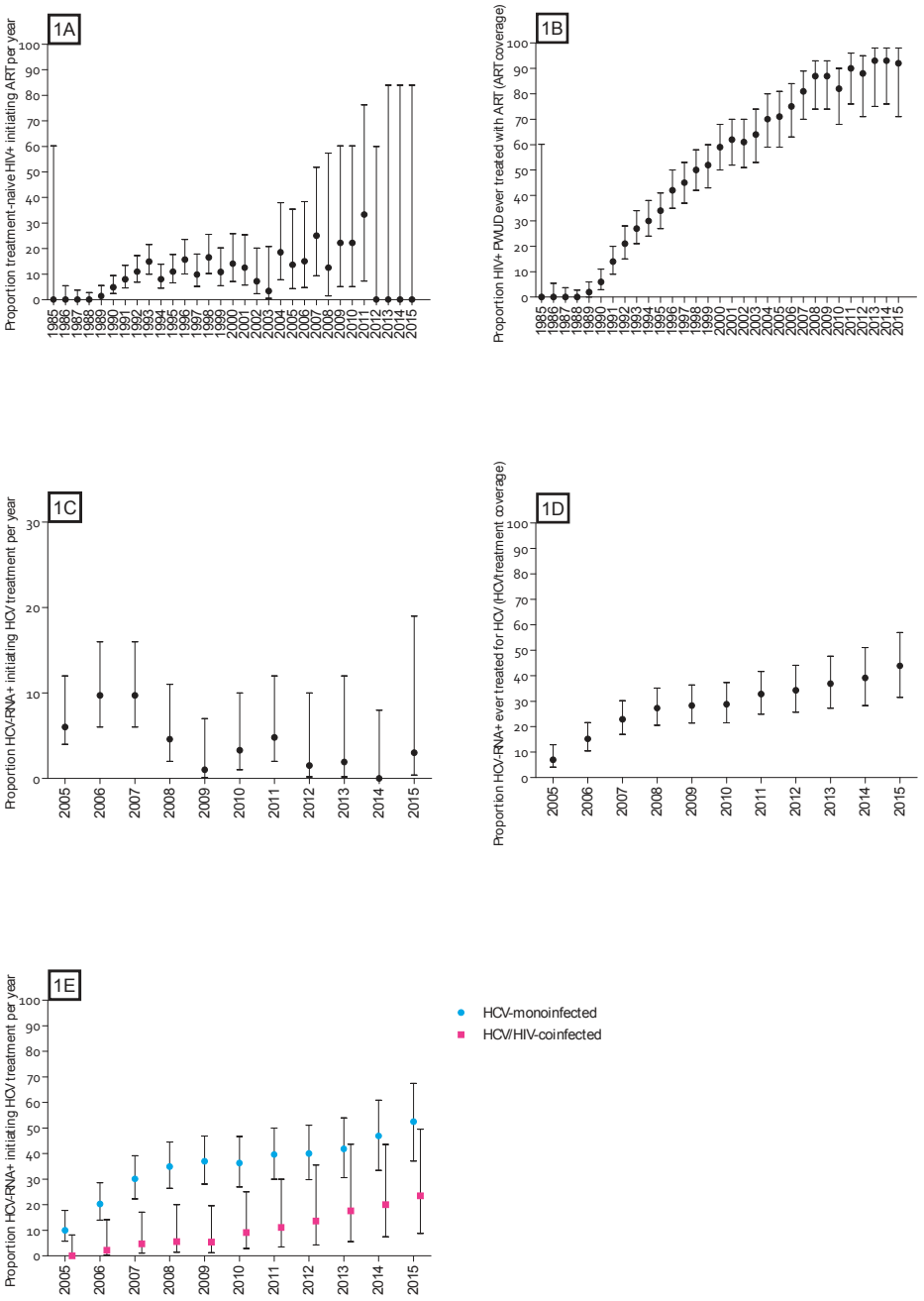

Figure 1: ART and HCV-treatment uptake among people who use drugs (PWUD) participating in the Amsterdam Cohort Studies (HIV: 1985-2015; HCV: 2005-2015). (1A) ART initiation among HIV-positive ART-naïve PWUD; (1B) ART coverage among HIV-positive PWUD; (1C) HCV-treatment initiation among HCV-RNA-positive treatment-naïve PWUD; (1D) HCV-treatment coverage among HCV-RNA-positive PWUD; (1E) HCV treatment coverage among HCV-RNA-positive PWUD by HCV/HIV-(co)infection status. Lines depict 95% confidence intervals.

(95%CI= 54.1-66.5%) and 36.1% (95%CI= 24.4-53.1%) of PWUD were alive and ART-naïve (see 'Event-free' area in Figure 2) in the pre-cART and cART eras, respectively.

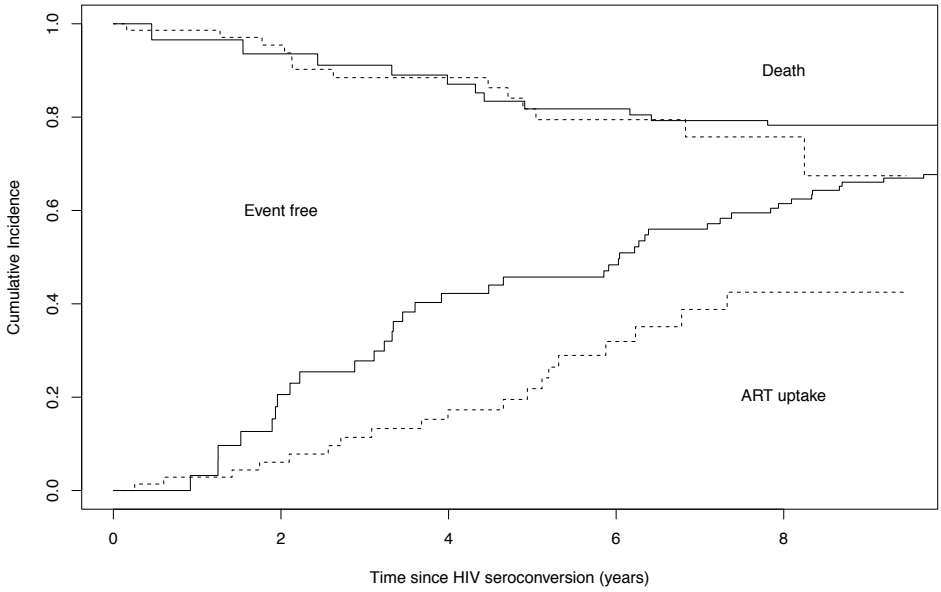

Figure 2: Cumulative incidence of ART initiation (on event scale) and all-cause mortality (on survival scale) among people who use drugs who seroconverted for HIV during follow-up (n=98) participating in the Amsterdam Cohort Studies (1985-2015)

Pre-cART era: dotted lines; cART-era: solid lines. Lines starting at 1 on the y-axis refer to all-cause mortality, while lines starting at 0 on the y-axis refer to ART uptake. Curves were truncated after 10 years since HIV seroconversion. One PWUD who seroconverted for HIV during follow-up was excluded from the analysis as the HIV seroconversion interval was 26 years.

HCV-treatment uptake

Of 566 PWUD with a cohort visit after 2004, 323 (57.3%) were HCV-antibody positive. Of these 323, 95.0% (n=307) had ever been tested for HCV RNA, of whom 217 (70.7%) PWUD were HCV-RNA positive. Of these 217, 8 (3.7%) had been treated for HCV before 2005 and were excluded from the analyses. From 2005 onwards, 54 of the 209 started HCV treatment, of whom 49 were HCV-monoinfected and 5 HCV/HIV-coinfected. Of these 54 recorded HCV treatments, 85.2% were obtained from clinical records. Forty-three PWUD were treated at the integrated HCV-treatment unit within the PHSA and 11 in hospital-based clinics. The majority of included HCV RNA-positive PWUD (198/209; 97.5%) after 2004, had a previous positive HCV test results before the DUTCH-C unit commenced. At the first cohort visit after 2004, the median duration since the first HCV (antibody and/or HCV RNA)-positive test result was 13 years [IQR=8-18].

The highest proportion of PWUD initiating HCV treatment (9.7%) was observed in 2006, but this had decreased to 1.9% in 2013 (Figure 1C). During the first full year of DAA availability (2015), HCV treatment initiation remained low: of 33 HCV-RNA-positive PWUD with a cohort visit during that year, only one individual (3.0%) initiated treatment. We had FibroScan results data for 19 of these 33 PWUD (57.5%), of whom 11 (57.9%) had no or mild fibrosis (F0-F1), 3 (15.8%) moderate fibrosis (F2), and 5 (26.3%) severe fibrosis or cirrhosis (F3-F4). HCV-treatment coverage increased from 7.0% in 2005 to 43.9% in 2015 (Figure 1D) and was higher in HCV-monoinfected PWUD than in HIV/HCV-coinfected PWUD (Figure 1E). From 2005 to 2015, it increased from 10.0% to 52.5% in those with HCV monoinfection and from 0.0% to 23.5% in those with HCV/HIV coinfection.

DISCUSSION

ART and HCV-treatment coverage increased among PWUD from the ACS between 1985-2015 and 2005-2015, respectively. Among HIV-positive PWUD still alive in 2015, HIV treatment coverage was high (91.7%). HCV treatment initiation peaked in 2006 (9.7%), but decreased to 1.9% in 2013, most likely because physicians and patients awaited the availability of DAAs. HCV treatment coverage was 43.9% at the end of our study period. During the first full year of DAA availability in the Netherlands (2015), HCV treatment uptake was low (3.0%). HCV/HIV-coinfected PWUD seem to be lagging behind in HCV treatment coverage compared to HCV-monoinfected PWUD.

Our results are in line with two studies from Canada and the US [3, 4] regarding increasing ART uptake among PWUD over time. A community-based cohort study in Vancouver, Canada, reported that <70% were ART-exposed in 2006, whereas in 2014 this had increased to >95% [3]. Changes over time in ART guidelines (initiation at higher CD4 counts; fewer tablets and side effects [15]) probably played a role in the higher ART uptake during the cART era compared to the pre-cART era. Moreover, the high HIV-treatment coverage and decreasing number of ART-naïve PWUD in Amsterdam over time might be ascribed to the availability of low-threshold harm-reduction programs since early in the HIV epidemic [19]. Through these programs, the majority of PWUD in Amsterdam were reached, facilitating linkage to testing and HIV care. A study in Vancouver found that use of OST was independently associated with more rapid ART uptake [20], which suggests that OST lowers barriers to ART access. In line with this study, a former study among ACS HIV-positive participants reported that individuals who had never received cART were more likely to have joined an incomplete harm-reduction program (e.g. no or irregular NEP use and/or low methadone dosage) and received methadone less often compared to those reporting cART use [21]. Importantly, in this study, ART non-adherence was

only reported in 11.9% of all study visit, which challenges the negative believes of physicians regarding ART adherence among PWUD. Finally, Amsterdam's high ART coverage can be partly explained by a survival effect: those who did not initiate ART are more likely to have died from HIV- or drug-related causes. In addition, given the decreasing HIV incidence in Amsterdam [22, 23] and the low number of new individuals that start injecting drugs [17], there are only a few HIV-positive ART-naïve PWUD in recent years.

The proportion of HCV-treated PWUD at the end of follow-up was higher in our study (28% in 2009 and 43.9% in 2015) than in a study in Vancouver, Canada, where only 5.7% of HCV-antibody-positive PWUD, without evidence of spontaneous clearance, had been treated in 2009 [10]. Such differences might be partly ascribed to the ageing PWUD population in Amsterdam, where many PWUD have ceased injecting [24] and few new individuals have started injecting in recent years [17, 25]. In addition, a high proportion in Vancouver also used crack cocaine or methamphetamines, which posed a barrier to HCV treatment: many practitioners would only treat PWUD who were on OST, but no analogous treatment like methadone is available for these drugs [10]. Similar to findings from Vancouver, in Australia, in a cohort of HCV-RNA-positive PWUD on OST, only 5.3% ever commenced treatment between 2009 and 2011 [10, 11]. In Norway, in a cohort of PWUD admitted for residential drug dependency treatment between 1970-1984, HCV-treatment uptake among those still alive in 2012 was 19% [26]. However, as suggested by Keats et al. (2015) [11], many without advanced fibrosis may have awaited new therapies, suggesting that the low uptake before DAAs introduction might not reflect a low interest in HCV treatment. It is important to note that methods to estimate HCV uptake differ among studies, making comparison difficult. For example, if we had calculated the proportion of HCV-RNA-positive PWUD ever treated during follow-up without accounting for calendar time, as did Keats et al. (2015) [11], our finding would be 26% (54/209) instead of the 43.9% HCV coverage among PWUD still alive in 2015. Nevertheless, 26% is still higher than percentages found in previous studies in Canada, Australia and Norway. The integrated multidisciplinary approach of HCV treatment within the PHSA (DUTCH-C unit) and use of harm-reduction programs [18] may have led to the high uptake observed. It might be explained, as previously suggested [18], by the extensive counseling and the involvement of family members and shelter personnel. Also, peg-interferon injections were provided weekly to each participant at the DUTCH-C unit, and ribavirin was distributed at methadone clinics, shelters, or the DUTCH-C unit, and matched with the individual's methadone distribution if applicable. Furthermore, ACS participants came for 4-6 monthly cohort visits where testing and counseling were provided, which may have additionally contributed to our high HCV and HIV treatment coverage. Importantly, HCV-monoinfected PWUD who received treatment at the DUTCH-C unit reached sustained virological responses (overall SVR= 65% with pegylated-interferon/ribavirin

treatment) comparable to non-PWUD populations, despite active drug and/or alcohol use and psychiatric comorbidities [18].

During the first year of DAAs availability in the Netherlands, only 3.0% (1 of 33) of HCV-treatment-naïve HCV-RNA-positive PWUD initiated treatment. Until November 2015, access to HCV treatment was restricted, based on fibrosis stage (Metavir ≥F3), so physicians may have awaited the dismissal of such restriction. This was the case for 11 of the 19 (57.9%) with FibroScan results who were HCV-RNA-positive in 2015; although 5 of the 19 (26.3%) were eligible for HCV treatment since late 2014. However, we did not have FibroScan results for all 33 participants, and it might be that some of these 33 PWUD were not engaged in HCV care or harm-reduction programs. On the other hand, DAA treatment for PWUD still requires additional support, as many of them have psychiatric co-morbidities and may lack social support. In any case, the process to engage PWUD into care may take longer that for non-PWUD patients. Furthermore, DAA treatment in Amsterdam has been limited to hospital-based clinics since the DUTCH-C unit closed at the end of 2013, and there is increasing evidence of the benefit of community-based treatment compared to care at tertiary hospitals [27-29], as was observed during the peak of treatment initiation at the PHSA in 2006. Further research on DAA-based treatment models (e.g. integrated in harm-reduction programs or permanent homeless shelters) on HCV treatment uptake is needed.

Our study has some potential limitations. First, our not accounting for HCV treatment provided before 2005 may have led us to underestimate HCV-treatment coverage. However, uptake among PWUD was very low before 2005, and this was the motive to initiate the DUTCH-C HCV treatment unit [18]. Second, our cohort participants may not be representative of the whole PWUD population in Amsterdam. Their ACS participation and easier access to HCV care at the PHSA may have led to higher coverage and uptake than in the larger PWUD population. However, PWUD not participating in the ACS (e.g. from de PMHS) were actively approached to access HCV treatment at the PHSA. Conversely, as the Dutch epidemiological situation, model of care and coverage of HRP differ from other high-income countries, our findings may not be generalizable to PWUD populations outside the Netherlands. Lastly, we defined chronic HCV infection by at least one positive HCV RNA test result and did not account for possible HCV clearance. However, very few HCV seroconversions occurred after 2000 [30] among ACS participants, and the majority of them were tested for HCV RNA after 2004. In addition, at least two HCV RNA-positive test results were available for 48.8% of all HCV-RNA positive PWUD included in our analyses. Therefore, we believe that of those included in our analyses, most if not all had a chronic HCV infection.

Our study also has several strengths. The majority of HIV and HCV treatment initiation data were confirmed with clinical records. Even though some of the data was based on self-reports, an earlier study among ACS participants demonstrated that our study population was able to give valid self-reports on methadone treatment modalities [31]. Furthermore, we have a well-characterized cohort with extensive follow-up since early in the HIV epidemic, allowing us to study HIV and HCV uptake trends over time.

In conclusion, ART and HCV-treatment uptake have increased over time among PWUD from the Amsterdam Cohort Studies, although DAA uptake during the first full year of its availability was still low. ART coverage was very high in recent years. As DAAs are currently available for all chronically HCV-infected individuals in the Netherlands, HCV treatment uptake monitoring should be continued to assure that PWUD receive adequate HCV treatment in the coming years. Additional strategies targeted to the specific needs of PWUD may be needed to achieve higher DAA treatment uptake rates in Amsterdam.

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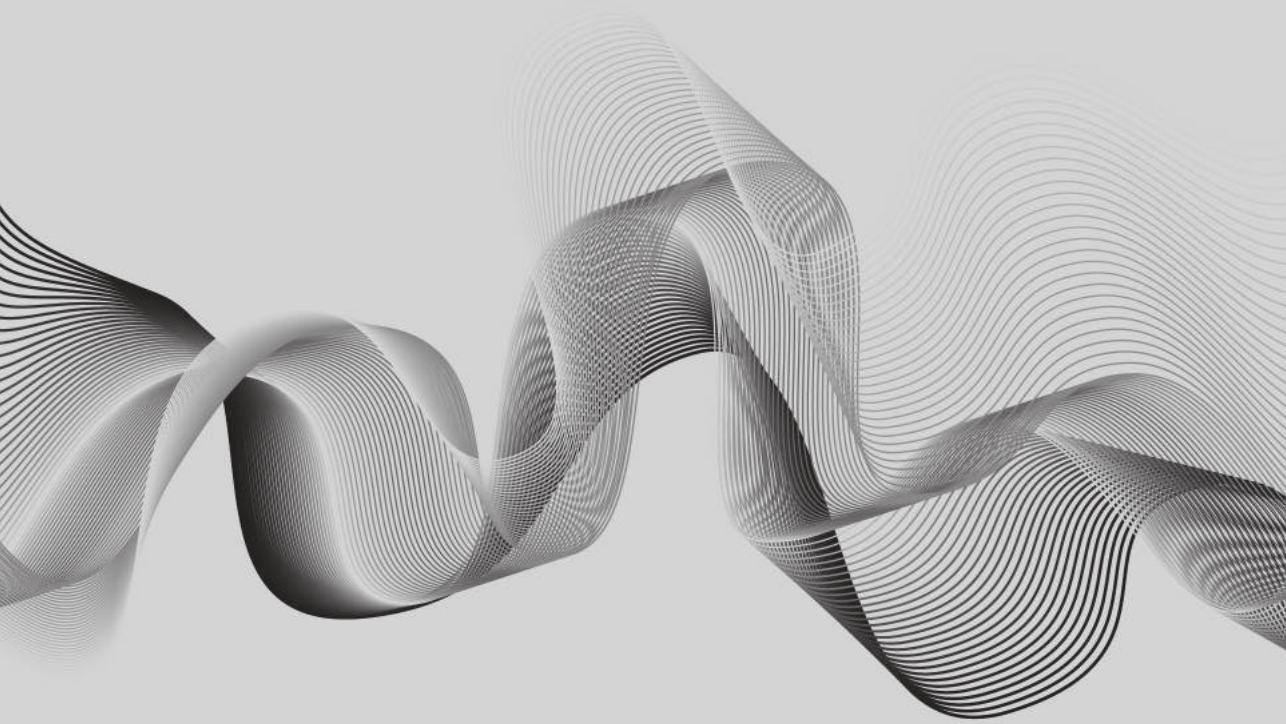
Chapter 4

General Discussion



In this thesis we aimed to increase our understanding of the incidence, disease progression, and treatment of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human papillomavirus (HPV) (co-)infections in key populations. This chapter will focus on the challenges and opportunities, from 2018 onwards, for research, care, and prevention strategies related to these viral epidemics. This section will mainly focus on the situation in the Netherlands and Western Europe among men who have sex with men (MSM) and people who use drugs (PWUD).

1. The current context

The World Health Organisation (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) established specific targets to halt the HIV and HCV epidemics worldwide [1,2]. These are the "90-90-90" strategy for HIV and the "elimination of viral hepatitis as a public health threat by 2030", respectively [1,2].

1.1 90-90-90: a target to end the AIDS epidemic

In 2014, UNAIDS established targets to end the AIDS epidemic based on the *cascade of care* [1]: a well-recognised tool consisting of key stages of care of a particular disease to identify gaps in the continuum of care [3]. These targets entail that by 2020, 90% of people living with HIV are aware of their HIV status, 90% of diagnosed HIV-positive individuals receive sustained antiretroviral therapy (ART), and 90% of those on ART have an undetectable HIV viral load. This means that when this three-part target is achieved, 73% of all HIV-positive individuals worldwide will be virally suppressed [1]. Sweden was the first country to achieve these goals and Amsterdam was amongst the first cities to achieve them [4,5]. In the capital city of Amsterdam, a city harbouring more than a quarter of all HIV-positive individuals in the Netherlands, the following percentages were achieved in 2015: 94-90-94 [5]. In the Netherlands, these target have almost been met, with estimates at 89-92-95 by the end of 2016 [6]. Modelling studies have suggested that reaching these targets can have a significant impact on controlling the HIV epidemic [1], suggesting it may be feasible to end the AIDS epidemic in the Netherlands by 2030. However, the last mile to zero new HIV infections poses a big challenge [7]. This is supported by a recent study modelling the HIV epidemic in Australia – a country with similar high HIV care coverage levels as the Netherlands – reporting that when the 90-90-90 targets are achieved, only a small reduction in HIV incidence (10% reduction in 10 years) can be expected [8]. Hence innovative strategies to halt on-going HIV transmission are warranted. In 2016, the WHO embraced the UNAIDS targets, while also establishing other targets to address the needs beyond viral suppression as well as reducing HIV incidence and HIV-related stigma [9].

1.2 WHO viral hepatitis targets

In 2015, in response to the optimism around direct-acting antivirals (DAA) HCV therapy, and increasing viral hepatitis morbidity and mortality, the WHO set specific targets for viral hepatitis elimination as a public health threat by 2030, with a special focus on hepatitis B and C [2]. Compared to baseline incidence and mortality in 2015, these targets are 1) to reduce incidence of new infections by 90%, and 2) to reduce viral hepatitis-related mortality by 65% [2]. These WHO targets may seem ambitious, but a study modelling the HCV epidemic in the European Union (EU) reported that these targets could be achieved if HCV treatment is scaled up, access is unrestricted to the patient's fibrosis stage and HCV screening, and thus HCV diagnosis increases compared to the 2015 baseline scenario [10]. Importantly, the term "elimination" that has been used throughout the WHO targets does not correspond to the epidemiological definition. Epidemiological elimination of an infectious agent is actually a reduction of incidence to zero in a defined geographical area as a result of deliberate efforts, in which continued surveillance to prevent the reoccurrence of transmission is required [11]. Therefore the term "elimination as a public health threat" seems to correspond with the term "control" in the epidemiological sense (i.e. reduction in the incidence, prevalence, morbidity, or mortality of an infectious disease to a locally acceptable level) [11].

1.3 Monitoring the state of the epidemic: surveillance and cohort studies

Both HIV and HCV targets are often assessed using surveillance data. Surveillance data can provide good estimates, depending on the quality of the collected data, on targets that correspond to stages in the cascade of care measured while individuals are engaged in care, for example the number of individuals in HIV care who are virally supressed [6]. Observational cohort studies, on the other hand, can provide incidence estimates as well as estimates on the time from infection until the outcome of interest (e.g. ART initiation), depending on the population studied. In **chapter 3.4,** for example, we estimated ART uptake in the pre-combination ART (cART) and cART era among PWUD participating in the ACS with a well-estimated date of HIV seroconversion. In this way, PWUD who may have died before being linked to HIV care could be included in the analysis, whereas they would have been missed in an analysis using surveillance data from PWUD in HIV care.

2. Men who have sex with men

In the Netherlands, the majority (63%) of diagnosed HIV-positive individuals are MSM. The UNAIDS HIV targets have been achieved among these MSM, with current estimates at 90-94-96 [6]. As mentioned in the introduction (**chapter 1**), the majority of new HIV infections in the Netherlands are found in this group [6]. In other countries from Western Europe, the HIV epidemic is also mostly concentrated in MSM [12]. In 2016

it was estimated that of all HCV-antibody-positive individuals in the Netherlands, 3% were HIV-positive MSM [13], but of all notified cases of acute HCV in 2016, 68% were found in this group [14]. As HIV and HCV continue to spread among MSM, and incidence and prevalence of sexually transmitted infections (STI), including HPV, are high [6,15], primary prevention (i.e. averting new infections) strategies are warranted.

2.1 HIV and HCV co-infection

Reducing HCV incidence

In **chapter 2.1** of this thesis we found that HCV incidence continued to rise among HIV-positive MSM until 2014, although trends seem to differ by European geographical region. In the Netherlands and Western Europe there is evidence of a stabilisation of the HCV incidence at about 1 per 100 person-years (py) ([16] and **chapter 2.1)**. Since 2014/2015, DAA have been available in many high-income countries [17] and DAA treatment uptake in the Netherlands is now high [18]. Of all HIV/HCV co-infected MSM retained in HIV care by mid 2017 in the Netherlands, 89.0% had ever been treated for HCV and 83% had achieved a sustained virological response (SVR, i.e. undetectable HCV RNA 12 weeks after treatment) [18]. We now face the question: *Is DAA going to curb the HCV epidemic among HIV-positive MSM?* The impact of DAA on the HCV epidemic will probably largely depend on high-risk behaviour trends in this group. A Swiss modelling study reported that regardless of DAA treatment scale-up, if high-risk behaviour continues to increase as observed over the past decade, HCV incidence would continue to rise in the coming years [19]. The Swiss study also reported that with a stabilisation of high-risk behaviour and considerable treatment scale-up (to 100% per year) a decrease in HCV incidence could be expected [19]. The most rapid and strongest decline in HCV incidence was projected when a decline in high-risk behaviour was modelled, irrespective of treatment scale-up [19]. This is in line with findings from a UK modelling study reporting that the biggest reduction in HCV incidence could be achieved through behavioural risk reduction combined with DAA treatment scale-up, although the authors did report a decline in HCV incidence with DAA treatment irrespective of reductions in risk behaviour [20]. It is important to note that the UK study modelled a stable HCV incidence based on findings up to 2011. In **chapter 2.1** we showed that in Northern Europe, including predominantly MSM from the UK, HCV incidence appears to have increased after 2011. One could argue that given the current trend in condomless anal sex [21] and the availability of pre-exposure prophylaxis (PrEP), a further increase in condomless anal sex can be expected, as acquiring HIV is becoming less of a concern, which has been one of the main reasons for MSM to use condoms [22]. In addition 'chemsex' (often defined as the use of certain recreational drugs like methamphetamines to prolong sexual encounters), which has been associated with condomless anal sex and HCV acquisition, is

common among MSM [23-25]. The aforementioned mathematical studies assessing the impact of reductions in high-risk behaviour on HCV incidence are based on hypothetical behavioural interventions, which raises another question: *Which interventions could lead to such a high-risk behaviour decrease or stabilisation?* One intervention for MSM currently being assessed at the STI clinic within the Public Health Service of Amsterdam (PHSA), is screening of psychosocial problems by health professionals and referral to drug and/or mental health services [26]. Another intervention available at the PHSA that has the potential to help decrease high-risk behaviour in MSM is the 'chemsex spreekuur' (i.e. chemsex consultation). This intervention provides a support system and peer-based counselling for MSM who engage in chemsex, where MSM can also be linked to mental or substance use care [27]. Although the effects of these interventions still need to be evaluated, it does draw our attention to understanding high-risk behaviour of MSM using a holistic approach. A study including a representative sample of the population in the UK living in private households reported that MSM had significantly more psychiatric disorders, such as alcohol and drug dependence [28]. Hence integrating mental and sexual care might aid in halting the HIV and HCV epidemic in this group and also improve MSM's overall (mental) health. A lot of attention today is given to biomedical HIV-preventive interventions such as HIV Treatment as Prevention (TasP) or PrEP, which although adequate to prevent HIV infection, could potentially lead to a further increase in the incidence of other STI such as HCV in the long run. Therefore future research should evaluate the implementation of interventions to stimulate condom use and other risk-reduction strategies in MSM.

Moreover, even with a substantial scale-up of DAA treatment, other challenges remain in halting the HCV epidemic among HIV-positive MSM. Re-infection following HCV treatment is substantial in this group [29,30]. This underscores the need for behavioural counselling and interventions targeted at reducing reinfection risk after treatment. Another strategy to prevent onward transmission of HCV might be through increased frequency of HCV-RNA testing for the early detection of HCV (re-)infections [31]. A modelling study in Australia reported that with increased HCV monitoring (e.g. every 4 months) and prompt treatment, major HCV incidence reductions can be achieved in HIV-positive MSM within two years [31]. In the Netherlands, guidelines recommend HCV antibody testing once a year for HIV-positive MSM who report high-risk behaviour (e.g. condomless anal sex) and HCV-RNA testing when alanine aminotransferase levels are elevated, at the discretion of the clinician [32]. Based on the limited number of HCV tests per individual reported in **chapter 2.1** and a study among HIV-positive individuals in care in Europe [33], HCV testing guidelines for HIV-positive MSM may not be followed consistently. Given the on-going spread of HCV among HIV-positive MSM and supporting evidence from mathematical models on the benefits of increased HCV-RNA testing

in this group, guidelines recommending more frequent HCV testing among HIV-positive MSM who report HCV-related risk factors [34] should be incorporated in HIV clinical practice and/or routine STI care.

HCV co-infection: role of biology vs. risk behaviour

Another issue that continues being discussed is the biological role of HIV versus risk behaviour on HCV acquisition, as HCV infections are mainly found in HIV-positive MSM [35]. A recent modelling study from the UK examined the role of HIV and sexual risk behaviour in the HCV epidemic among HIV-positive MSM. Authors of that study reported that sexual behaviour patterns largely explain why the HCV epidemic is concentrated among HIV-positive MSM [36]. However, the modelling study only accounted for an increased risk of transmitting HCV due to HIV infection (by a factor of 2.4), based on data derived from other modes of HCV transmission (e.g. vertical transmission). Thus they did not account for the possibility that HIV-positive individuals may be more susceptible to acquiring HCV. Additionally, they did not account for a different risk of HCV acquisition based on HIV RNA or CD4 count levels, as found to be associated with HCV acquisition in **chapter 2.1** and in other studies [25,37]. In **chapter 2.1** we reported that MSM with a $\log_{10}$ HIV-RNA viral load of 6 copies/ml had an HCV incidence of 5 per 100 py compared to 3 per 100 py among those with a $\log_{10}$ HIV-RNA viral load of 3 copies/ml, adjusted for age, CD4 T-cell count, calendar year, and geographical region. It is therefore possible that the UK modelling study [36] underestimated the biological effect of HIV on HCV acquisition. Yet recent findings from the AmPrEP demonstration project in Amsterdam reported an HCV incidence of about 1 per 100 py over a median follow-up of 1.3 years among HIV-negative MSM on PrEP [38], which is comparable to the HCV incidence among HIV-positive MSM in the same city [16]. However, HCV re-infections were included in the AmPrEP analysis, making comparison difficult between AmPrEP and ACS HCV incidence estimates. Nevertheless, a higher than expected incidence was still observed, which might be attributable to the increase in condomless anal sex observed in the AmPrEP project [39], that has been associated with HCV infection in HIV-positive MSM [25]. Additionally, if HCV-negative MSM on PrEP are engaging in condomless anal sex with HIV/HCV co-infected partners more often – who would have higher HCV-RNA levels than HCV mono-infected individuals – one could expect a further spread of HCV in HIV-negative MSM driven in part by the higher infectiousness of HCV due to HIV. Although Dutch PrEP guidelines include HCV testing [40], currently there are no HCV testing guidelines for the wider HIV-negative MSM population. Moreover these men do not have regular appointments with medical specialists like their HIV-positive counterparts, and thus have less contact with health professionals. Therefore, if HCV starts to spread in the same way as it has among HIV-positive MSM over the last two decades, HIV-negative MSM may form a reservoir for on-going transmission among both HIV-negative and HIV-positive MSM.

In support of the biological role, as described in the discussion section in **chapter 2.1**, activated Langerhans cells due to HIV infection have been shown to facilitate HCV acquisition [41]. Another study found that incident syphilis infection, but not incident ano-rectal chlamydia infection, was a predictor of incident HCV infection in HIV-positive MSM [42]. Authors of that study suggest a biological mechanism of HCV acquisition through syphilis infection [42], falling in line with a previous finding where ulcerative STI resulted in higher odds for HCV acquisition than non-ulcerative STI [25]. These findings would support the hypothesis that STI, such as syphilis, lead to an increase in HIV-RNA viral load [43], which then leads to increased risk of HCV acquisition (**chapter 2.1**); in which case HIV RNA would be in the causal pathway between syphilis and incident HCV. Alternatively, an increase in HIV-RNA viral load might be a result of another process lying in the causal pathway between STI and HCV or merely be a proxy for having an STI as stated in **chapter 2.1**. Findings supporting either behaviour or biology do not necessarily contradict each other. It may be that HIV facilitated the initial spread of HCV, and that sexual risk behaviour drove the HCV epidemic among HIV-positive MSM. In any case, even if HIV plays an important role, without sexual risk behaviour HCV is unlikely to be sexually transmitted among MSM. Future research could aid the discussion of the association between HIV with HCV acquisition by assessing the causal effect of HIV on HCV. Ideally to study this, both biological (e.g. HIV-RNA viral load) and behavioural factors (e.g. the practice of 'serosorting') should be analysed, using a study population with and without HIV infection at risk for HCV infection, and having data from HIV seroconversion onwards for the HIV-infected group

Incident HCV infection and HIV disease progression

In **chapter 2.2** of this thesis we reported that HCV infection in MSM with pre-existing HIV infection led to a temporary CD4 count decline in both ART-naïve MSM and those on cART. As discussed in **chapter 2.2**, one of the limitations in this study was the insufficient follow-up to study long-term effects (i.e. all-cause mortality), including the temporary decline in CD4 count after HCV seroconversion. Therefore we are not certain what the clinical implications of our findings are. One might argue that as uptake of DAA treatment is high among MSM, the detrimental effects of HCV on HIV disease progression will be small in this group. It has been previously shown that all-cause mortality among those with SVR is substantially lower than those without SVR, although that study only included HCV mono-infected individuals [44]. However, if the temporary CD4 decline does have long-term clinical consequences, DAA treatment needs to be provided very shortly after HCV infection. Our findings can help clinicians be alert when a drop in CD4 count is observed in an HIV-positive patient while on cART, as this might indicate a newly-acquired HCV infection. It has been suggested that acquisition of HCV after HIV

may lead to accelerated HCV disease progression compared to the classical HCV risk groups [45,46]. Therefore one could hypothesise that the longer the duration of HIV infection when HCV is acquired, the worse HIV-related outcomes might be expected. Based on our findings, however, incident HCV infection appears to have a similar effect on CD4 and VL trajectories irrespective of the duration of HIV infection.

2.2 HIV and HPV co-infection

In **chapter 2.3** we showed that anal and penile high-risk HPV (hrHPV) incidence was higher among HIV-positive MSM compared to HIV-negative MSM, while anal hrHPV clearance was lower. Persistent anal HPV is necessary for the development of precursor lesions of anal cancer [47]. Hence, our findings of a higher incidence and lower clearance of anal hrHPV in HIV-positive MSM helps explain the higher incidence of anal cancer among this group compared to HIV-negative MSM [48,49]. The biological mechanism behind the effect of HIV infection on anal cancer might be due to differences in the immune micro-environment in the anal canal [50]. There is currently no standard treatment for anal dysplasia, and there is no evidence whether currently used treatments of anal dysplasia may prevent anal cancer [51]. Our findings, the lack of evidence-based guidelines for the treatment of anal cancer precursors to prevent anal cancer [51], and the partial protection against HPV acquisition by consistent condom use [52] indicate that additional preventive strategies are needed to decrease the burden of HPV among MSM, especially when HIV-positive.

Decreasing the burden of HPV in men

HPV vaccination offers both primary and possibly some degree of secondary HPV prevention (i.e. preventing recurrence of anal intraepithelial neoplasia) [53,54]. In the Netherlands, HPV vaccination is only provided to young girls [55]. While heterosexual men can benefit from herd immunity (i.e. indirect protection of unvaccinated individuals against an infectious disease by vaccinating a proportion of the population at risk), MSM cannot. A study in Australia, where HPV vaccination for young girls has been available since 2007, showed decreases in genital warts in heterosexual men but not in MSM after the vaccination was introduced [56]. One hurdle in extending HPV vaccination to males is the budgetary impact of introducing a gender-neutral vaccine programme. A systematic review stated that the reported cost-effectiveness of extending HPV vaccination to males is often above the country-specific cost-effectiveness threshold [57]. However, many original studies included in the systematic review only accounted for female-related outcomes (i.e. cervical cancer). The cost-effectiveness of vaccinating males also depended on the level of HPV vaccination coverage among females [57]. This is important as female HPV vaccination coverage in the Netherlands is relatively low: 53% in 2016 [58]. The few studies assessing the cost-effectiveness of HPV vaccination

in MSM found it to be cost-effective in this group [57,59], and the incremental cost-effectiveness ratio (ICER) became more favourable when vaccination was targeted at HIV-positive MSM [59]. Additionally, a study among HIV-negative MSM found that HPV vaccination could lead to a 61% decrease in the lifetime risk of anal cancer [60]. Another factor influencing the cost-effectiveness of HPV vaccination is that wholesale prices (including value-added tax (VAT)) were modelled; hence tender negotiations of HPV vaccination prices were not accounted for [61]. A recent Dutch study including tender prices and a two-dose (bivalent) vaccine, concluded that vaccinating 40% of boys and 60% of girls was highly cost-effective, and the authors reported that variations in the ICER are mainly driven by the vaccination costs [61]. Given the population-based benefits and the fact that a gender-neutral HPV vaccination programme can be highly cost-effective, current vaccination programmes in the Netherlands and other countries with female-based vaccination should be re-evaluated to decrease the burden of HPV among men. However, cost-effective does not mean cheap as this intervention still has budgetary impact.

Future directions

One important question that remains to be answered is whether HPV increases the risk of HIV acquisition among MSM. A systematic review showed that HPV was associated with HIV acquisition in women [62], but a recent original study, with a larger sample size of women than those of the observational studies included in the systematic review, could not confirm this finding [63]. As sexual practices and risk to acquire HIV differs by mode of transmission, the effect of HPV on HIV acquisition in MSM should also be evaluated. In addition, there is some evidence pointing towards a possible interplay between HPV and HCV [64,65]. Repeated surgeries (e.g. proctosurgery), which authors hypothesize are often due to genital and anal condylomata in the MSM population, have been associated with acute HCV infection [65], and HCV has been associated with high-grade anal intraepithelial neoplasia recurrence in HIV-positive MSM [64]. Yet more studies are needed to gain insight into the potential interaction between these two viral infections.

It is noteworthy that the epidemiological and statistical methods employed in HPV research seem to be lagging behind when compared to the HIV and HCV field. For example, to estimate the moment of HIV infection, using the midpoint date between the first positive and the last negative test date is the norm, and even more sophisticated methods have been used based on the estimated trend in the incidence in the population studied [66]. In contrast, in numerous HPV studies, the date of the first positive HPV test is used, thus the moment of HPV infection is based on the diagnosis [67-71]. As the biological event time (i.e. the actual moment of infection) is most probable to have occurred before the diagnosis, the midpoint is recommended. Additionally, comparison of

overall or any HPV incidence between studies is difficult as there is no consensus on how to calculate this term. Calculating HPV incidence is not as straightforward as dividing the number of cases by person-years of follow-up (PYFU) as there are multiple types of HPV that can occur concurrently. For example, in one study two types of hrHPV incidence were separately estimated [72]. 'Any penile hrHPV incidence' was estimated at 7.1 per 100 py by dividing the sum of all incident events per person by the sum of the total PYFU per person and HPV type (i.e. multiple PYFU records per person) [72]. The other approach used to estimate 'incident HPV infection' accounted for all incident hrHPV types (numerator) but only one PYFU record per person (denominator), which resulted in a higher hrHPV incidence at 66.5 per 100 py [72]. Future research using better estimates of the infection date and forming a consensus on HPV incidence calculations is recommended to enable better comparison between studies and inform clinical practice.

In conclusion, **chapter 2** of this thesis allowed us to gain insight into the HCV epidemic among HIV-positive MSM and the consequences of HIV/HCV co-infection on HIV disease progression in this group. Considering the on-going spread of HCV among HIV-positive MSM and the temporary, negative effect of HCV on CD4 T-cell count, monitoring HCV incidence and the long-term effects after infection is warranted in this group even in the DDA era. Ideally research should account for HIV infection duration as findings in recent years from **chapter 2.1** suggest incident HCV occurs closer to HIV seroconversion, and otherwise incidence estimates may be biased. Future real-life epidemiological studies can help inform whether availability of DAA treatment can substantially prevent on-going HCV transmission. Lastly, we showed an independent effect of HIV co-infection on HPV acquisition and persistence, both being important factors for anal cancer development. Further studies are needed to unravel the biological mechanism behind the effect of HIV infection on hrHPV infection.

3. People who use drugs

Today HIV and HCV incidence have nearly dropped to zero among PWUD in Amsterdam, and few new individuals are starting to inject drugs [6,73]. A previous study reported that the incidence of HCV re-infection after successful HCV treatment was low among PWUD participating in the ACS (0.8 per 100 py) [74]. In the Netherlands, only one case of acute HCV out of 44 notified cases in 2016 was from an individual with a history of injecting drug use (IDU) [14]. Hence, epidemiological elimination of HCV infection among PWUD appears to have been almost achieved in the Netherlands. However, the proportion of PWUD with a long-standing HCV infection is considerable, as HCV incidence peaked during the 1980s [75]. In 2016 it was estimated that 15% of all HCV-antibody-positive cases in the Netherlands could be attributed to IDU [13].

As the UNAIDS HIV targets attained in Amsterdam and the Netherlands have not been stratified by all modes of transmission, we cannot say with certainty whether all targets have been achieved for PWUD. Yet 98% of HIV-positive PWID in care in the Netherlands were on cART as of December 2016 [6]. In addition, among HIV-positive ACS participants, 97% (28 out of 29) had an undetectable HIV-RNA viral load in 2014 (unpublished ACS data) and in 2015 coverage of ART was high (92%) (**chapter 3.4**). Slightly lower estimates of individuals with a history of IDU in HIV care with an undetectable viral load (86.2%) were reported by the Dutch HIV Monitoring Foundation in 2016 [6]. Based on the current prevalence and incidence of HIV and HCV in PWUD, primary prevention is not a priority, but rather secondary and tertiary prevention (i.e. preventing HIV- or HCV-related morbidity and mortality).

3.2 Defining the population

Throughout this thesis we used the term people who USE drugs (PWUD), while most studies focus on people who INJECT drugs (PWID), often including past and present IDU. *Why this difference?* This is related to the inclusion criteria in the ACS. Individuals with a history of 'hard' drug use could be included. Drug use was defined as the regular injecting or non-injecting use of predominantly heroin, cocaine, amphetamines and/or methadone. A history of IDU was not an inclusion requirement. Including former-, current-, and non-IDU in the ACS allowed the study of the incidence of IDU initiation along with the effect of needle and syringe exchange programmes (NSP) on an increase in IDU, among other research questions [76]. Therefore, in most chapters in this thesis pertaining to this group, we included and used the term 'people who use drugs'. The only exception can be found in **chapter 3.3** where we assessed the cost-effectiveness of DAA among PWID, as the underlying model was parameterised using data from individuals with a history of IDU only, including those who actively injected drugs [75].

3.3 HCV

Disease burden

In **chapter 3.1** we reported that liver-related mortality among PWUD slightly increased between 1985 and 2012, although the increase was not statistically significant. A Dutch modelling study reported that HCV-related disease, defined as decompensated cirrhosis (DC) and hepatocellular carcinoma (HCC), among PWID in Amsterdam is expected to rise by 36% in 2025 compared to the situation in 2011 in a scenario without HCV treatment [77]. Hence, in **chapter 3.1** we may have observed the start of the increasing trend of the liver disease burden in this group. In a best case scenario reported by the Dutch modelling study, it was estimated that if 95% of HCV mono-infected PWID and 65% of HIV/HCV co-infected PWID are successfully treated for HCV, the cumulative HCV-related

disease could decrease by 47% between 2011 and 2060. This result suggests that HCV treatment is essential to decrease the burden of liver disease in this population.

HCV treatment uptake

In **chapter 3.4** we showed that the reported HCV treatment coverage (39% in 2014) in Amsterdam among PWUD participating in the ACS during the pegylated-interferon era was much higher than in other countries [78,79]. Another study from the Netherlands (i.e. "*Doorbraak project",* i.e. Breakthrough project) that aimed to improve the HCV cascade of care for PWUD in addiction clinics reported that 62% of PWUD identified with a chronic HCV infection initiated treatment between 2013-2014 [80,81]. This percentage, however, is most likely an overestimate of the actual HCV treatment uptake among PWUD with a chronic HCV infection, as only 42% of PWUD identified were screened for HCV antibodies, and 69% of HCV-antibody-positive PWUD were subsequently tested for HCV RNA. In our study, the majority of individuals had been screened for antibodies and HCV RNA at routine ACS visits or during the DUTCH-C screening period. Hence our denominator covers the majority of the HCV-infected ACS population, which might explain our lower HCV coverage estimate. We also distinguished HCV uptake as treatment initiation (treatment-naïve PWUD initiating treatment per calendar year) and coverage (PWUD ever treated per calendar year). As discussed in **chapter 3.4**, the definition of HCV treatment uptake differs across studies, making comparisons difficult. Ideally studies should take into account follow-up time of individuals and differentiate between initiation and coverage.

During the first full year of DAA availability (2015) only 3% of HCV-RNA positive treatment-naïve PWUD participating in the ACS initiated HCV treatment (**chapter 3.4**). As discussed in **chapter 3.4**, from November 2014 to November 2015 DAA treatment was restricted to those with severe liver fibrosis, which may have resulted in a low DAA uptake. But still higher DAA initiation was anticipated because most HCV-positive PWUD had been HCV infected for over 20 year. Therefore high proportions of severe fibrosis and cirrhosis were expected in this group, and consequently many would have been eligible for treatment in 2015. The latter was confirmed in **chapter 3.2** where we reported that 58% of PWUD with a chronic HCV infection screened for liver fibrosis between 2009 and 2016 had evidence of significant fibrosis or cirrhosis, and among them, 27% had cirrhosis. This estimate, though, is based on a convenience sample, potentially overestimating the proportion with significant fibrosis or cirrhosis. On the other hand, some PWUD may have progressed to another fibrosis stage after the transient elastography assessment in our study.

As DAA treatment access has been unrestricted (i.e. treatment is reimbursed to all patients regardless of fibrosis stage) in the Netherlands since 2015, and HCV has stopped spreading among PWUD, we are uniquely positioned to reduce the burden of HCV-related disease in this group. However, the low HCV treatment initiation in 2015 and HCV screening reported by the Dutch '*Doorbraak project*', highlights important gaps in the cascade of HCV care [81]. To understand and improve the HCV treatment landscape for PWUD in the Netherlands the HCV cascade of care should therefore be monitored and evaluated nationwide. In this thesis only one stage of the cascade of care was evaluated in **chapter 3.4**, namely HCV treatment uptake. Another Dutch study by Boerenkamps et al. evaluating this stage among HIV/HCV co-infected individuals linked and retained in HIV care in the Netherlands reported that 35% of (former) PWID had never initiated HCV treatment between 2000 and 2016 compared to 12% of MSM [18]. Our findings from **chapter 3.4** and Boerenkamps et al.'s findings underscore that unrestricted availability of HCV treatment is not enough to successfully treat all PWUD or reach a similar uptake rate as observed among HIV-positive MSM. Hence, other models of HCV care delivery should be considered. A randomised-controlled trial concluded that integrated multidisciplinary HCV-, drug-treatment-, and mental-health care led to higher HCV treatment initiation than a model of treatment delivery without an integrated and multidisciplinary protocol [82]. We also showed in **chapter 3.4** that the DUTCH-C unit, a multidisciplinary integrated HCV treatment model, probably largely contributed to the higher HCV treatment coverage compared to rates observed in other high-income countries without such a model of care [78,79]. One barrier for easy access to DAA in the Netherlands is that unlike countries like the US and Australia, only medical specialists can prescribe HCV treatment. A recent study in the US showed that DAA treatment by community-based providers, like nurse practitioners, was as safe and effective as treatment provided by a specialist [83]. Telemedicine may also help increase HCV treatment uptake, as it has previously shown to result in equal SVR rates in prison and rural settings as in tertiary care [84]. Both our and the US-based findings provide a set of additional strategies to upscale DAA uptake. Patient and health provider knowledge, attitudes, and (negative) perceptions towards treatment should also be addressed as this has the potential to impact HCV treatment uptake [85].

Cost-effectiveness of DAA treatment

In **chapter 3.3** we reported that DAA treatment is highly cost-effective among PWID, with a lower ICER in the modelled PWID population than in studies modelling HIV-positive MSM or HCV-infected patients [86,87]. Importantly, we incorporated additional costs for tailored treatment delivery for PWID based on the DUTCH-C unit. Furthermore, we made conservative assumptions in monitoring and treatment costs based on wholesale prices. As DAA costs decrease and if less monitoring is needed, DAA treatment for PWID may

be cost-saving in the Netherlands as shown in the sensitivity analysis in **chapter 3.3**. In this study, we also showed that the state of the HCV epidemic plays a role in the cost-effectiveness of DAA, with DAA being cost-saving when HCV incidence and the PWID population are stable, whereas it is cost-effective in a declining epidemic. This can be attributed to treating individuals early in their IDU career, who therefore have a shorter duration of HCV infection, and thus more liver disease can be prevented. In addition due to HCV treatment onwards HCV transmission can be prevented (Treatment as Prevention, TasP) than in a declining epidemic. This is relevant, as the WHO targets do not account for the state of the epidemic, while our findings suggest it should be taken into account when assessing the burden of liver disease. In **chapter 3.3** we showed that with dual DAA treatment (base case), a slightly bigger decline in chronic HCV prevalence of genotype 1 and 4 is projected by 2029 in the stable compared to the declining epidemic (17% vs. 13%). Additionally, in the dual DAA treatment scenario for the stable epidemic, DC is expected to decline by 10% in 2029, whereas HCC is expected to increase by 13% resulting in a 2% increase in the burden of liver disease (defined as DC and HCC) when compared to the baseline scenario in 2015. In the declining epidemic, an increase of 124% for DC and 179% for HCC is projected in 2029, resulting in a 151% increase in the burden of liver disease (data not shown). Our findings from **chapter 3.3** would suggest that in countries with ageing HCV-infected PWID populations and a declining number of PWID, achieving the second WHO target (i.e. 65% reduction in HCV-related mortality) will probably be more challenging.

Beyond HCV infection in the burden of liver disease

While infectious diseases play an important role in PWUD prevention and care, heavy alcohol drinking might play a bigger role in the burden of liver disease in the coming years as more PWUD get treated with DAA. A recent international study showed that the population attributable fraction of alcohol use disorder on DC among individuals with a notified HCV-positive serology test ranged between 13% and 40% [88]. In **chapter 3.2** we reported that 48% of PWUD with a history of heavy alcohol drinking had significant fibrosis or cirrhosis. In **chapter 3.2** we also reported that significant liver fibrosis or cirrhosis was present in 27% of PWUD who had cleared HCV (predominantly due to spontaneous HCV clearance). Furthermore, even when HCV-infected PWUD with cirrhosis have been successfully treated, these individuals still needed liver-related screening post-SVR. This is supported by a recent modelling study reporting that to be able to achieve the WHO targets, additional health systems interventions are required to prevent HCV-related mortality after SVR among those with cirrhosis [89]. Given our and previous findings, liver screening in a broader PWUD (e.g. without HCV infection) population should strongly be considered.

Evaluating WHO targets

In light of the WHO HCV targets, further research could assess which factors are crucial to achieving the second WHO viral hepatitis target on mortality reductions in the Netherlands among PWUD. Studies usually evaluate the effect of HCV treatment on the burden of liver disease using the number needed to treat per year or the HCV treatment uptake rate [90,91]. In order to understand how the time frame in which individuals are treated affects infection spread and the burden of liver disease, we must know when a given proportion of individuals needs to be treated and whether we can de-escalate this proportion over time. Additionally, given the state of the HCV epidemic in the Netherlands, and the current burden of liver disease and HCV treatment uptake, monitoring liver- and all-cause mortality among HCV-positive PWUD (e.g. ACS participants) could help confirm whether our efforts were sufficient to significantly decrease liver-related morbidity and mortality. This finding could inform other countries on strategies to eliminate HCV as a public health threat in this key population.

3.3 HIV and HIV/HCV co-infection

As shown in **chapter 3.4** and **chapter 3.1** of this thesis, coverage of ART in PWUD is high (92% in 2015) and AIDS-related mortality has significantly decreased over time. In **chapter 3.1** we showed that the proportion of HIV/HCV co-infected PWUD among all included ACS participants in that study declined over calendar periods, which is probably attributable to high mortality during the 1990s, although we did not formally test whether this decline was statistically significant. Based on the observed mortality trends in **chapter 3.1,** causes of death other than HIV will have a bigger contribution to the (excess) mortality in the HIV/HCV co-infected group in the coming years.

In recent years, outbreaks of HIV among PWID have been documented in different countries such as Athens, Greece, and Indiana, US [92,93]. Additionally, in some European countries HIV continues to spread among PWID, mainly in Eastern European countries [94]. As the HIV epidemic in the Netherlands appears to be controlled in this group, it is without doubt that the Dutch HIV epidemiological situation among PWUD is remarkably positive compared to other countries. Nevertheless, we did report in **chapter 3.1** that HIV-related excess mortality (i.e. standardised mortality ratio (SMR)) did not significantly decline over time. This means that even though HIV-related crude mortality rates did significantly decline over time among PWUD, the proportional difference in HIV-related mortality rates between PWUD and the general Dutch population (both HIV-positive and HIV-negative) did not change over time. As previously mentioned, HIV-positive individuals from other exposure groups may have accessed HIV care faster, have a higher socioeconomic status, a lower prevalence of HCV co-infection, and a healthier lifestyle. These factors are associated with slower HIV disease progression [95], which may have

contributed to the stable HIV-related excess mortality. Additionally, we reported in **chapter 3.1** that in 2006-2012, HIV/HCV co-infected PWUD had 23 times higher all-cause mortality than the general Dutch population, whereas in HCV/HIV-uninfected PWUD this was 5 times higher. The high excess mortality and stable HIV-related SMR underscores that PWUD remain a vulnerable population, even in this era with good access to HIV and HCV care, high ART coverage, and moderate HCV treatment coverage. Therefore, if we want to end the AIDS epidemic by 2030 in the Netherlands, PWUD continue to warrant special attention.

In **chapter 3.4** we showed that HIV/HCV co-infected PWUD were lagging behind in HCV treatment uptake compared to HCV mono-infected PWUD. However, based on recent findings from the Dutch HIV Monitoring Foundation, the current percentage of HIV/HCV co-infected PWUD ever treated for HCV is now relatively high (65%), but still lower than in co-infected MSM [18]. This increase might be explained by well-tolerated DAA treatment by HIV/HCV co-infected individuals who used to be a difficult to treat population. Hence our finding of a difference in HCV treatment uptake by HIV/HCV co-infection status probably does not apply to the DAA era.

3.4 Amsterdam's approach to the HIV and HCV epidemic in PWUD

As IDU accounts for 78% and 16% of new HCV and HIV infections with a known transmission route in the EU and the European Economic Area, respectively [94,96], it is important to remember that this key population, whilst declining in the Netherlands, continues to be driving the HCV epidemic in many European countries, and to a lesser extent the HIV epidemic. The European Commission launched a joint action in 2015 engaging representatives from 18 EU member states to intensify efforts for HCV and HIV prevention and recognised PWID as a key population [85]. Amsterdam's approach to the HCV and HIV epidemic among PWUD could serve as a blueprint for other European countries. For example, in Amsterdam HCV, HIV, and HBV testing is offered free of charge and is easily accessible to all PWUD at harm-reduction programmes (HRP) within the PHSA. This is important, as we reported in **chapter 3.3** that in a setting with a stable HCV epidemic, DAA treatment can be cost-saving when 80% of PWID are screened for HCV. Also, coverage of HRP in the Netherlands is high and DAA access is not restricted to advanced liver fibrosis stages. A modelling study in eleven European countries or cities reported that if DAA treatment uptake remains stable (as in 2015) among PWID with the current country-specific HRP coverage, little or moderate difference in chronic HCV prevalence after 10 years is projected at most sites. If HRP coverage is 80%, the model projects reductions in chronic HCV prevalence at all sites with the current DAA uptake rates [97].

In conclusion, studies from **chapter 3** of this thesis improved our understanding of the HIV and HCV epidemics, as well as disease progression related to these viral infections and their interaction. We transitioned from high HIV and HCV incidence during the 1980s, to high HIV- and drug-related (excess) mortality during the 1990s, to a lower (excess) mortality in the last decade; with current mortality mainly attributable to natural deaths as the PWUD population ages. Furthermore, we also showed a high burden of HCV- and alcohol-related disease among PWUD over the last decade. Moreover, this thesis allowed us to gain insight into trends in HIV and HCV treatment uptake, as well as the cost-effectiveness of current HCV treatment schemes. In Amsterdam, HIV treatment uptake is high and HCV treatment was higher than in other countries during the pegylated-interferon era, but stronger efforts are needed to scale up DAA treatment.

4. Observational cohort studies used in this thesis: their legacy

4.1 The Amsterdam Cohort Studies among PWUD

The ACS among PWUD ended at the beginning of 2016 as the incidence of HIV and HCV had remained almost zero in recent years. This is probably attributable to the approach towards PWUD in the Netherlands. A landmark study among ACS participants reported that a combination of high uptake of NSP and opioid substitution therapy (OST) was associated with a lower risk of acquiring HIV as well as HCV [98]. ACS findings fall in line with a recent Cochrane systematic review reporting that the combination of NSP and OST uptake is associated with a 76% reduction in HCV acquisition risk [99]. As described in the introduction (**chapter 1**), previous studies, however, have only been able to assess 'associations' of HRP and HIV and/or HCV acquisition, as individuals who participate in HRP could be a select group with a lower risk for acquisition of these infections. As indicated in **chapter 3.1**, other factors probably also contributed to the decline, such as high rates of drug- and HIV-related competing mortality and the decline in the number of new injectors, leading to a decrease in the pool of susceptible and higher-risk individuals. The decline in the number of new injectors may be due to the increased availability of other (non-injecting) drugs such as ecstasy and fear of the 'junky status' [100].

The ACS has helped improve our understanding of the natural history, the epidemiology of HIV and HCV, and the effect of interventions among PWUD, amongst other topics such as HBV (molecular) epidemiology and HIV pathogenesis. The ACS has also provided a great body of work that has helped refute misconceptions such as low adherence and poor HCV treatment outcomes in this group. For example, ACS findings have shown that SVR is similar among individuals who actively use drugs to the general HCV population [101]. Such findings are important as, for example, in some US states drug abstinence of at least 6 months before DAA initiation is still required [102]. Furthermore, in some

countries, IDU is a criminal offense [103], whereas the harm-reduction approach in Amsterdam has gone hand in hand with a decrease in IDU [104]. Our hope is that findings generated from the ACS and other cohorts among PWUD will guide and strengthen drug-related policy worldwide. Furthermore, although the ACS no longer collects new data among PWUD, existing data and stored samples could contribute to vaccine development research [105] or other questions that may arise over time. Furthermore, building on the lack of causal evidence of HRP on HIV and HCV acquisition risk, existing data is being used to assess this question.

Lastly, even though the ACS among PWUD ended, some reports of new (speed) injectors have been reported in the south of the Netherlands, and of MSM injecting methamphetamines in the context of sex, known as 'slamming' [106,107]. Hence, continued monitoring of injecting trends and maintenance of HRP is essential to prevent future outbreaks.

4.2 The CASCADE Collaboration among HIV-seroconverter cohorts

The CASCADE Collaboration established in 1997 ended in December 2015. Both MSM and PWUD HIV seroconverters participating in the ACS were included in this pooled CASCADE database. This collaboration resulted in 59 scientific publications and had a major contribution to our understanding of the natural history of HIV, response to cART, HIV co-infections on disease progression, methodology (e.g. causality), among other subjects [108-110]. These findings have been incorporated in national and international treatment guidelines [111]. The single most significant feature of this collaboration is the availability of data since HIV seroconversion among a large number of individuals (over 30,000) who acquired HIV via different modes of transmission. That allowed adjusting or accounting for HIV infection duration, examining events close to HIV seroconversion, and studying the complete course of HIV infection. For example, as shown in **chapter 2.1,** HCV infections appear to occur closer to HIV seroconversion in recent years when compared to earlier calendar periods. Additionally, in **chapter 2.2** we reported that the moment of HCV acquisition relative to HIV seroconversion did not have an impact on the effect of HCV infection on CD4 T-cell counts or HIV-RNA viral load trajectories. Without such a collaboration, it would not have been possible to answer such research questions with sufficient precision. Also, rare events could be studied given the large number of HIV seroconverters. Similar to the ACS among PWUD, though the CASCADE Collaboration is no longer pooling data, existing data are still being used and continue to improve our understanding of HIV infection.

4. Concluding remarks

We are living in a historical period of the HCV and HIV epidemics. The developments in HCV treatment have brought considerable optimism to eliminate HCV as a public

health threat. Hopefully a vaccine against HCV can aid the epidemiological elimination of HCV in the long run. In the Netherlands we now face the following challenges: in MSM, newly-acquired HCV infections as well as re-infections pose serious barriers to achieving the first WHO target (i.e. reducing HCV incidence), whereas in PWUD, reducing the burden of liver disease is crucial to meeting the second WHO target (i.e. reducing HCV-related mortality). Hence HCV treatment uptake among PWUD should be scaled up, and liver screening may be warranted among a broader PWUD population. Among MSM, stronger efforts are needed to increase knowledge and promote safe sex in the context of HCV, and to increase HCV testing to provide prompt treatment to halt on-going HCV transmission. With respect to HIV, this may very well be the beginning of the end of the AIDS epidemic in the Netherlands as the WHO HIV targets have almost been met. Among PWUD, the HIV epidemic appears to be under control and PWUD seem to be well engaged in HIV care. In this group, causes of death other than HIV will probably have a bigger contribution to the excess mortality in the coming years, but to end AIDS, this group continue to warrant special attention, as HIV-related excess mortality has not declined over time. For MSM, on-going HIV transmission emphasises the need for innovative primary prevention strategies. An integrated model of care (e.g. mental and drug use care) for both MSM and PWUD could potentially remove barriers for HIV and HCV testing and treatment, as well as potentially targeting the root cause of risky behaviour. Even though global HIV and HCV targets can provide a baseline for tracking progress, guidance on how to reverse these viral epidemics, and create awareness by serving as an advocacy tool, they do not necessarily account for worldwide differences in the epidemics. The 90-90-90 targets, for example, do not explicitly incorporate on-going HIV transmission. Therefore, for the epidemiological control and elimination of HIV and HCV, it is important to act according to the state of the country-specific epidemic and set country-specific goals. Observational cohort studies, such as those used in this thesis, are considered the most powerful observational study design and can help track these viral epidemics and assess whether our efforts and the uptake of interventions have an impact on the epidemic and the burden of disease.

With respect to HPV research, more evidence is needed to unravel the mechanism behind HIV on HPV acquisition and clearance, and assess the effect of HPV on HIV acquisition among MSM. Given the high HPV-related burden among MSM, the Dutch government appears to be lagging behind when it comes to extending HPV vaccination to males.

At the beginning of the HIV and heroin epidemic, the Dutch government adopted a forward-thinking approach for PWUD by being amongst the first to implement harm-reduction programmes, and the Netherlands is now considered an example to other countries. Such an approach can provide a blueprint for other countries based on sound

epidemiological research. Lastly, we should keep evaluating the epidemiological situation in the Netherlands regarding the viral infections studied in this thesis given the major biomedical developments over the last few decades: from assessing incidence, treatment and intervention uptake, and the burden of disease, to disentangling the interaction between HIV, HPV, and HCV.

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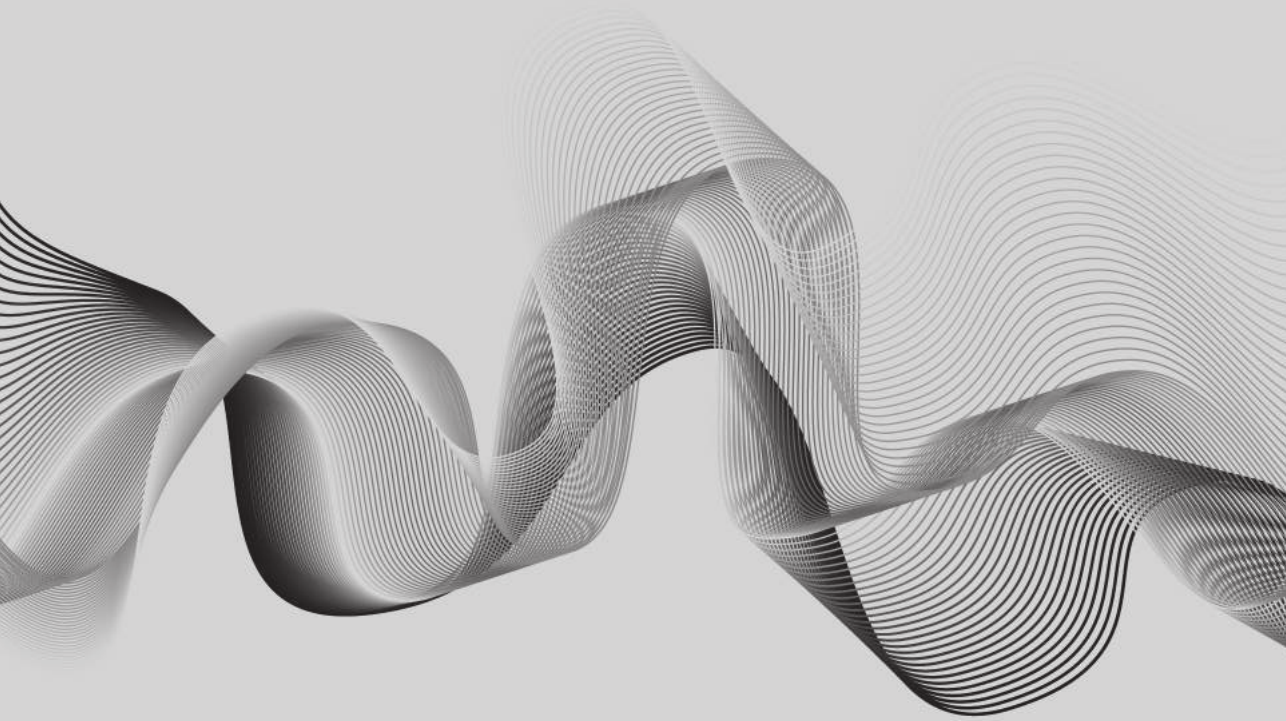
Appendices



SUMMARY

The research described in this thesis aimed to increase our understanding of the incidence, disease progression and treatment of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human papillomavirus (HPV) (co-)infections in key populations. In **chapter 1** (general introduction), an overview of the HIV, HCV, and HPV epidemics is given. This chapter also contains a special section highlighting the value of type of study design used in this thesis, and some of the major historical events related to these viral epidemics: starting with the heroine-use epidemic in the 1970s and HIV epidemic in the 1980s, which gave rise to the Amsterdam Cohort Studies (ACS), up until the availability of a vaccine against HPV infection and novel treatments for HCV.

Chapter 2 covers studies focusing on HIV in combination with HCV or HPV among men who have sex with men (MSM). At the beginning of the second millennium, outbreaks of HCV have been increasingly reported among HIV-positive MSM. While the HCV epidemic seems to be concentrated in HIV-positive MSM, the role in which HIV plays in HCV acquisition remains under debate. In **chapter 2.1** and **chapter 2.2**, we studied HIV and HCV co-infection among MSM using data from the Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) Collaboration, pooling data from 28 HIV-seroconverter cohorts across Europe, Australia, Canada and Sub-Saharan Africa.

In **chapter 2.1**, we estimated temporal trends in the occurrence of newly-acquired HCV infections between 1990 and 2014 among HIV-positive MSM at risk of acquiring HCV ('incidence'). Additionally, we assessed whether acquiring HCV was associated with age, geographical region, and HIV-related markers, that is, CD4 T-cell count and HIV-RNA viral load. We found that HCV infection continued to spread over time among HIV-positive MSM, especially among younger individuals. Trends in incidence seemed, however, to differ by European region in recent years, while incidence appeared to have stabilized in Western Europe and remained stable in Southern Europe, it continued to increase in Northern Europe in recent years. Furthermore, MSM with a higher HIV-RNA viral load were more likely to get infected with HCV. Shortly after HIV infection, MSM appeared to be at higher risk of acquiring HCV than in the chronic HIV infection stage. Lastly, the time from HIV seroconversion to HCV infection significantly decreased over calendar time.

In **chapter 2.2**, we assessed the effect of newly-acquired HCV infection, particularly in relation to HIV seroconversion, on subsequent trajectories of CD4 T-cell counts and HIV-RNA viral load. CD4 T-cell counts were temporarily lower following HCV seroconversion, irrespective of antiretroviral therapy (ART) use. We also observed that the timing of HCV seroconversion in relation to HIV seroconversion did not impact CD4 T-cell counts or

HIV-RNA viral load trajectories. The shape of HIV-RNA viral load trajectory was different in ART-naïve MSM acquiring HCV than those without HCV infection. The clinical short- and long-term implications of our findings remain to be elucidated.

HIV-positive MSM are at higher risk of developing anal cancer than their HIV-negative counterparts. A large proportion of ano-genital cancers can be attributed to high-risk HPV (hrHPV) infection. In **chapter 2.3**, data from the HIV and HPV in MSM cohort (H2M) were used to compare the incidence and clearance of anal and penile hrHPV infection between HIV-positive and HIV-negative MSM. We found that HIV-positive, compared to HIV-negative MSM, have a higher risk of acquiring anal and penile hrHPV and have a decreased risk for clearing anal hrHPV. Our findings suggest a biological effect of HIV infection on anal and penile hrHPV infections.

Chapter 3 covers studies on HCV and HIV, and their interaction, among people who use drugs (PWUD). Drug use was defined as the regular injecting or non-injecting use of predominantly heroin, cocaine, amphetamines and/or methadone. Consequent to the HIV epidemic, the ACS on HIV among PWUD was initiated in 1985. The data derived from this cohort allowed us to study both HIV and HCV epidemics over time. From early on during the HIV epidemic, comprehensive harm-reduction programs, including needle and syringe exchange and opioid substitution therapy, have been available in the Netherlands. These low-threshold programs aimed to minimize the harmful effects of drug use on the individual and societal level without being condemned or criminalized. We hypothesized that harm-reduction programs would contribute to a significant decline in mortality among PWUD, and hence mortality rates would become more comparable to the general Dutch population during recent years. In **chapter 3.1,** we therefore studied temporal trends in all-cause and cause-specific mortality rates between 1985 and 2012 among PWUD compared to the general Dutch population and determined whether excess mortality trends differed by HCV/HIV (co-)infection status. Although PWUD have higher mortality rates than the general Dutch population, specifically those with HCV/HIV co-infection, we found evidence for an overall decline in excess all-cause mortality among PWUD over time. This decline seems to be largely driven by decreases in excess mortality among women. Mortality rates due to non-natural deaths, such as suicide and overdose, converged closer to those of the general population as time progressed, yet HIV-related causes of death remained relatively important when compared with the general Dutch population. This study reinforces the importance of harm-reduction interventions and effective HIV and HCV treatment to reduce mortality among PWUD.

HCV-related mortality is often preceded by liver fibrosis and cirrhosis (i.e. accumulation of scar tissue in the liver), which develops several decades after HCV infection. As HCV

incidence peaked during the 1980s, and PWUD bear other risk factors for liver fibrosis unrelated to HCV (i.e. heavy alcohol drinking), as they become older, PWUD could commonly present with significant liver fibrosis and cirrhosis in this decade. In **chapter 3.2**, we used a convenience sample of PWUD from Amsterdam and reported that one in four PWUD screened between 2009 and 2016 had significant liver fibrosis or cirrhosis. Both chronic HCV infection and longer duration of heavy alcohol drinking were associated with an increased risk of liver fibrosis. This analysis highlights the need for increased uptake of HCV treatment and interventions to reduce excessive alcohol use in order to decrease liver disease burden in this key population.

New treatments against chronic HCV infection have recently become available and while cure rates are up to 95%, treatment remains costly. In **chapter 3.3,** we assessed the cost-effectiveness of four HCV treatment strategies among people who inject drugs (PWID) in combination with treatment scale-up (i.e. increasing the number of individuals treated for HCV), and whether it was influenced by the type of HCV epidemic (stable or declining). We found that HCV treatment with direct-acting antivirals (DAA) is a highly cost-effective intervention in this key population regardless of either a stable or declining epidemic. Although less cost-effective, increasing the number of PWID treated per year led to a bigger reduction in HCV prevalence over time than with treatment rates as in 2015. In **chapter 3.4,** using data from the ACS, we turned our focus towards temporal trends in ART and HCV-treatment uptake among PWUD, defined as treatment initiation (i.e. starting treatment among previously untreated PWUD) and treatment coverage (i.e. ever treated). Increase in ART and HCV-treatment coverage was observed over time, yet despite high HCV-treatment coverage in 2015, DAA treatment initiation was still low.

Lastly, in **chapter 4** (general discussion), the relevance of these studies is discussed together with recommendations for future research, prevention and care strategies. Considering the on-going spread of HCV among HIV-positive MSM and the temporary, negative effect of HCV on CD4 T-cell count, monitoring HCV incidence and the long-term effects on morbidity and mortality after infection is warranted in this group, even in the DDA era. To halt on-going HCV transmission, stronger efforts are needed to increase knowledge and promote safe sex in the context of HCV, and to increase HCV testing to provide prompt treatment. While our results suggest a biological effect of HIV on HPV acquisition and clearance among MSM, further research is needed to unravel the mechanisms involved in the interplay between these two viruses. Given the high HPV-related burden in MSM, extending HPV vaccination to males is recommendable. Given our findings on the economic and population benefits of HCV treatment scale-up, high burden of liver disease in the ageing PWUD population, and relatively low proportion of PWUD initiating DAA treatment, stronger efforts are needed to achieve higher

uptake rates in this key population. International targets have brought optimism to eliminate HIV and HCV as a public health threat. To achieve these targets, innovative primary prevention strategies are need to reduce HIV and HCV incidence in MSM, while in PWUD reducing the burden of disease related to these viral infections is a priority. Observational cohort studies, such as those used in this thesis, are considered the most powerful observational study design and can help track these viral epidemics and assess whether our efforts and the uptake of interventions have an impact on the epidemic and the burden of disease.

NEDERLANDSE SAMENVATTING

In dit proefschrift hebben we ons gericht op het beter begrijpen van de incidentie, ziekteprogressie en behandeling van humaan immunodeficiëntievirus (hiv), hepatitis C virus (HCV) en humaan papillomavirus (HPV) infecties en hoe deze infecties elkaar beïnvloeden. In **hoofdstuk 1** (algemene inleiding) geven we een overzicht van de hiv-, HCV- en HPV-epidemieën. In dit hoofdstuk is een speciale sectie gewijd aan de waarde van het type onderzoek dat is gebruikt in dit proefschrift. Ook zijn enkele belangrijke historische gebeurtenissen met betrekking tot deze virale epidemieën besproken: vanaf het vele gebruik van heroïne in de jaren zeventig en de daaropvolgende hiv-epidemie in de jaren tachtig, wat leidde tot de start van de Amsterdam Cohort Studies (ACS), tot de beschikbaarheid van een vaccin tegen HPV en zeer effectieve behandelingen voor HCV.

Hoofdstuk 2 behandelt studies naar hiv-infectie in combinatie met HCV- of HPV-infectie bij mannen die seks hebben met mannen (MSM). Vanaf 2000 werden steeds vaker infecties met het hepatitis C virus waargenomen bij MSM met hiv. Hoewel deze HCV-epidemie zich voornamelijk lijkt te beperken tot hiv-positieve MSM, staat de rol die het hiv-virus speelt bij het oplopen van HCV nog steeds ter discussie. In **hoofdstuk 2.1** en **hoofdstuk 2.2** hebben we onderzoek gedaan bij MSM die beide infecties hadden (ook wel hiv/HCV co-infectie genoemd). Hiervoor hebben we gebruik gemaakt van gegevens uit de CASCADE studie (Concerted Action on SeroConversion to AIDS and Death in Europe); dit is een internationale samenwerking waarin gegevens van 28 studies uit Europa, Australië, Canada en Sub-Sahara Afrika zijn samengevoegd. Deze studies volgden personen van wie bekend is wanneer ze precies hiv opliepen. Hierdoor konden we in onze analyses rekening houden met de duur van de hiv-infectie van elke persoon.

In **hoofdstuk 2.1** hebben we de trends in het aantal nieuw opgelopen HCV-infecties tussen 1990 en 2014 berekend onder hiv-positieve MSM die niet eerder een HCV-infectie hadden gehad ('incidentie'). Daarnaast hebben we onderzocht of het krijgen van HCV geassocieerd is met leeftijd, de regio waar mensen wonen, en de hoeveelheid hiv-virusdeeltjes en CD4 T-cellen in het bloed. CD4 T-cellen zijn witte bloedcellen die betrokken zijn bij het immuunsysteem en die door hiv worden aangevallen. We lieten zien dat het hepatitis C virus zich blijft verspreiden onder hiv-positieve MSM, en dan vooral onder jonge mannen. In de afgelopen jaren verschilden de trends echter per Europese regio. Terwijl de incidentie van HCV in West- en Zuid-Europa lijkt te zijn gestabiliseerd, werd een recente stijging van de HCV incidentie waargenomen in Noord-Europa. Verder wijzen onze resultaten erop dat MSM met meer hiv-virusdeeltjes in hun bloed een verhoogd risico hebben om geïnfecteerd te raken met HCV. Ook bleek kort na een hiv-infectie het risico om een HCV infectie op te lopen hoger dan in een later stadium van

de hiv-infectie. Ten slotte zagen we dat de tijd tussen hiv- en HCV-infectie steeds korter is geworden over de loop van de tijd.

In **hoofdstuk 2.2** hebben we het effect van het oplopen van een nieuwe HCV-infectie op kenmerken van hiv ziekteprogressie bestudeerd. Daartoe bekeken we het beloop van aantal CD4 T-cellen en het aantal hiv-virusdeeltjes over tijd. We lieten zien dat na een HCV infectie het aantal CD4 T-cellen tijdelijk lager is (wijzend op een slechter werkend immuunsysteem), ongeacht of een persoon wel of niet behandeld werd voor hiv. MSM die HCV hadden opgelopen en geen hiv-therapie kregen, bleken over de tijd een hogere hoeveelheid hiv-virusdeeltjes in het bloed te hebben dan MSM zonder HCV. We hebben ook gekeken of het moment van het krijgen van HCV ten opzichte van de duur van een hiv-infectie een effect had op de kenmerken van hiv ziekteprogressie. Wij lieten zien dat het moment waarop iemand een HCV-infectie oploopt gedurende zijn hiv-infectie geen invloed heeft op het beloop van het aantal CD4 T-cellen en het aantal hiv-virusdeeltjes.

Hiv-positieve MSM hebben een grotere kans om anuskanker te ontwikkelen dan hiv-negatieve MSM. Een groot deel van de gevallen van anuskanker kan worden toegeschreven aan hoog-risico HPV infecties (hrHPV). We hebben in **hoofdstuk 2.3** daarom onderzocht of de kans op het oplopen van hrHPV-infecties, in de anus en op de penis, verschilt tussen hiv-positieve en hiv-negatieve MSM. Hiervoor maakten we gebruik van gegevens van het "HIV and HPV in MSM cohort (H2M)". Daarnaast onderzochten we of hiv een effect had op het vermogen van het lichaam om zelf een hrHPV-infectie op te ruimen (ook wel 'klaring' genoemd). We vonden dat hiv-positieve MSM een grotere kans hadden om hrHPV-infecties op te lopen dan hiv-negatieve MSM, zowel in de anus als op de penis. Bovendien lieten we zien dat er bij hiv-positieve MSM een kleinere kans was dat het lichaam zelf anale hrHPV-infecties opruimde. Onze bevindingen suggereren een biologisch effect van een hiv-infectie op het oplopen en klaren van een hrHPV-infectie.

Hoofdstuk 3 behandelt studies over HCV en hiv, en hun interactie, onder mensen die drugs gebruiken. Onder drugsgebruik verstaan we het regelmatig injecterend of niet-injecterend gebruik van 'hard' drugs. Deze drugs zijn voornamelijk heroïne, cocaïne, amfetamine en/of methadon. In 1985, als gevolg van de hiv-epidemie, werden de Amsterdam Cohort Studies (ACS) onder drugsgebruikers gestart. De gegevens van de ACS stelden ons in staat om het beloop van de hiv- en HCV-epidemieën in deze groep in kaart te brengen. Sinds het begin van de hiv-epidemie zijn laagdrempelige 'harm reduction' programma's, waaronder spuitomruil en methadonverstrekking, in Nederland beschikbaar. Deze programma's zijn erop gericht de schadelijke effecten van drugsgebruik voor het individu zelf en de omgeving te beperken zonder drugsgebruik te criminaliseren. We veronderstelden dat deze programma's hebben bijgedragen aan een daling van het

aantal sterfgevallen in deze groep over de tijd. Onze hypothese was dat in recentere jaren de sterfte onder drugsgebruikers meer vergelijkbaar is met de sterfte onder de algemene Nederlandse bevolking dan dit het geval was in de jaren tachtig en negentig van de vorige eeuw.

In **hoofdstuk 3.1** hebben we trends in (oorzaak-specifieke) sterftecijfers van drugsgebruikers vergeleken met de sterfte onder de algemene bevolking in Nederland tussen 1985 en 2012. We onderzochten of de sterfte onder drugsgebruikers hoger is dan de verwachte sterfte onder een groep uit de Nederlandse bevolking die vergelijkbaar is qua leeftijd en geslacht, in dezelfde tijdsperiode. Hierbij hebben we ook gekeken of de trends in sterfte verschilden voor drugsgebruikers met en zonder een hiv- en/of HCV-infectie. Hoewel sterftecijfers onder drugsgebruikers hoger waren dan onder de algemene Nederlandse bevolking, toonden we aan dat het verschil in sterftecijfers tussen drugsgebruikers en de Nederlandse bevolking afnamen in de loop van de tijd. Deze daling lijkt voornamelijk toe te schrijven te zijn aan de afname van de sterftekans van vrouwen die drugs gebruiken. De sterftekans van drugsgebruikers aan niet-natuurlijke doodsoorzaken, zoals zelfmoord en overdosis, kwam na verloop van tijd dichter bij die van de algemene bevolking te liggen. Hiv-gerelateerde sterfte kwam echter veel vaker voor in deze groep dan in de algemene Nederlandse bevolking. Drugsgebruikers met zowel een hiv- als HCV-infectie hadden de hoogste sterftekans.

HCV-gerelateerde sterfte wordt vaak voorafgegaan door leverfibrose en -cirrose (d.w.z. ophoping van littekenweefsel in de lever, waarbij cirrose het eindstadium is van fibrose), die zich enkele decennia na een HCV-infectie kunnen ontwikkelen. Het merendeel van de drugsgebruikers in Amsterdam zijn in de jaren zeventig en tachtig geïnfecteerd met HCV. Aangezien drugsgebruikers ook nog andere risicofactoren voor leverfibrose hebben (bijvoorbeeld overmatig alcoholgebruik), stelden we als hypothese dat in recente jaren leverfibrose en cirrose vaak voorkomen onder (voormalige) drugsgebruikers in Amsterdam. In **hoofdstuk 3.2** toonden we aan dat één op de vier drugsgebruikers onderzocht tussen 2009 en 2016 leverfibrose of -cirrose had, en dan met name drugsgebruikers met een chronische HCV-infectie of met een geschiedenis van zwaar alcoholgebruik. HCV-behandeling en interventies om alcoholgebruik te verminderen zijn nodig om de lever-gerelateerde ziektelast in deze groep te verminderen.

Nieuwe behandelingen tegen een chronische HCV-infectie, 'direct-acting antivirals' (DAA) genoemd, zijn beschikbaar sinds 2014. Het genezingspercentage van deze behandelingen ligt op meer dan 95%, maar deze behandelingen zijn vooralsnog erg duur. In **hoofdstuk 3.3** hebben we daarom de kosteneffectiviteit van verschillende HCV-behandelstrategieën onderzocht bij mensen die (ooit) drugs hebben geïnjecteerd.

Daarnaast hebben we onderzocht of het behandelen van meer drugsgebruikers t.o.v. het aantal dat behandeld werd in 2015 kosteneffectief was. De kosteneffectiviteit geeft de verhouding weer tussen de effectiviteit en de kosten van een nieuwe behandeling ten opzichte van een standaard behandeling of een situatie zonder behandeling voor een bepaalde ziekte. We hebben ook onderzocht of de fase waarin een epidemie zich bevindt (d.w.z. een stabiele of dalende HCV-incidentie) de kosteneffectiviteit van de behandeling beïnvloedt. We vonden dat HCV-behandeling met DAA een zeer kosteneffectieve interventie is bij mensen die (ooit) drugs hebben geïnjecteerd, ongeacht de fase van de epidemie. Bovendien toonden we aan dat het opschalen van behandeling bij drugsgebruikers leidde tot minder chronische HCV-infectie in deze groep in de loop van de tijd. Dit scenario bleek echter minder kosteneffectief dan het niet opschalen van behandeling.

In **hoofdstuk 3.4** beschreven we**,** met behulp van ACS data, trends in het gebruik van hiv- en HCV-medicatie onder drugsgebruikers. We zagen een toename in het gebruik van hiv- en HCV-behandeling over de tijd. In 2015 was het percentage drugsgebruikers met een chronische HCV-infectie dat ooit hiervoor behandeld werd hoger in Amsterdam dan in andere westerse landen. Het percentage drugsgebruikers met een chronische HCV-infectie die voor het eerst startten met de nieuwe medicatie was echter nog steeds laag.

Ten slotte plaatsten we in **hoofdstuk 4** (algemene discussie) onze bevindingen in een breder perspectief en gaven we aanbevelingen voor toekomstig onderzoek, preventie en zorg. HCV blijft zich verspreiden onder hiv-positieve MSM en heeft een tijdelijk negatief effect op het immuunsysteem. Het is daarom belangrijk om de HCV-epidemie, en de effecten van een HCV-co-infectie op ziekte en sterfte, in deze groep in het DAA-tijdperk te blijven monitoren. Om te zorgen dat minder MSM een HCV-infectie oplopen, is het ook belangrijk om hun kennis over HCV-transmissie te vergroten en veilig vrijen te stimuleren. Daarnaast zouden MSM ook frequenter getest moeten worden op HCV. Zo kunnen recente infecties eerder worden opgespoord en tijdig worden behandeld en kan verdere verspreiding worden tegengegaan. Onze bevindingen wijzen op een direct effect van hiv op het oplopen van een HPV-infectie en de klaring ervan. Verder onderzoek is nodig om het mechanisme hierachter te ontrafelen. Gezien het hoge percentage hiv-positieve MSM die HPV oplopen en de verminderde kans die iemand met hiv heeft om HPV zelf op te ruimen, zou HPV-vaccinatie aan mannen aangeboden moeten worden. Op basis van de economische voordelen van HCV-behandeling en het belang hiervan voor het individu en de publieke gezondheid, het vaak voorkomen van leverschade bij drugsgebruikers, en het relatief lage percentage drugsgebruikers dat start met de nieuwe HCV behandeling, zijn grotere inspanningen nodig om te zorgen

dat de groep drugsgebruikers met een HCV-infectie wordt behandeld. Nieuw geformuleerde internationale doelstellingen hebben ervoor gezorgd dat het beëindigen van de hiv- en HCV-epidemie op de kaart is gezet. Om deze doelenstellingen in MSM te realiseren moet het voorkomen van nieuwe hiv- en HCV-infecties een prioriteit worden. Onder drugsgebruikers lijkt hiv en HCV niet meer in Nederland te verspreiden, maar de ziektelast van deze infecties in deze groep blijft hoog. Het is daarom van wezenlijk belang dat de schade die hiv- en HCV-infectie veroorzaken de komende jaren drastisch wordt beperkt. Verder zou de Amsterdamse aanpak op de hiv- en heroïne-epidemie onder drugsgebruikers handvatten kunnen bieden voor andere landen. Cohortstudies, zoals die werden gebruikt voor de studies in dit proefschrift en die in het algemeen worden beschouwd als een van de beste type onderzoeken, kunnen van waarde zijn bij het verder volgen van deze virale epidemieën. Daarnaast zijn we met dit soort studies in staat om erachter te komen welke interventies een succesvolle impact kunnen hebben op de epidemie en ziektelast.

PHD PORTFOLIO

PhD training		ECTs
AMC Graduate School, Amsterdam, the Netherlands, 2012-2016		
2016	Observational clinical epidemiology	0.6
2015	Project management	0.6
2014	Advanced topics in biostatistics	2.1
2013	Computing in R	0.4
2013	Scientific (English) writing	1.5
2012	Infectious diseases	1.3
2012	AMC World of Science	0.7
Johns Hopkins Summer School, Baltimore, USA, 2016		
2016	Investigation of outbreaks	1.0
2016	Longitudinal data analysis	1.0
2016	Intermediate epidemiology	1.5
2016	Epidemiology in evidence-based policy	1.0
GGD courses, 2012-2017		
2017	Appealing writing skills (*Aansprekend schrijven*)	0.3
2015	Time management	0.3
2012-2016	Weekly epidemiological PhD training	15.0
Web-based course		
2015	Introduction to Stata Programming, online course	1.4
University of Maastricht, Maastricht, the Netherlands		
2013	Cost-effectiveness modelling methods	6.0

Oral presentations

2017 *"Frequent delayed hepatitis B spontaneous clearance among high risk groups"*; 20th International Workshop on HIV and Hepatitis Observational Databases (IWHOD), Lisbon, Portugal

2015 "*No decline in Hepatitis C Virus (HCV) incidence among HIV-positive men who have sex with men (MSM) within CASCADE: 1990-2014*"; 9th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands

2015 *"Hepatitis C Treatment for People who Inject Drugs: Are Direct-Acting Antivirals Cost-Effective?";* 4th International Symposium on Hepatitis Care in Substance Users (INHSU), Sydney, Australia

2015 *"No decline in Hepatitis C Virus (HCV) incidence among HIV-positive men who have sex with men (MSM) within CASCADE: 1990-2014";* 15th European AIDS Conference (EACS), Barcelona, Spain 2015 (presented by Maria Prins) – *Best poster presentation*

2015 *"The effect of HIV infection on penile high-risk HPV incidence and clearance among MSM"; E*uropean Research Organisation on Genital Infection and Neoplasia (EUROGIN), Sevilla, Spain

2014 *"Temporal trends in mortality rates among drug users in Amsterdam compared to the general Dutch population differ by hepatitis C and HIV (co) infection status"*; 8th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands

2013 "*Temporal trends in mortality rates among drug users in Amsterdam compared to the general Dutch population differ by hepatitis C and HIV (co) infection status*"; 3rd International Symposium on Hepatitis Care in Substance Users (INHSU), Munich, Germany

2013 *"Anal high-risk HPV incidence among HIV-negative and HIV-positive men who have sex with men";* European Research Organisation on Genital Infection and Neoplasia (EUROGIN), Florence, Italy

Total ECTs for all oral presentations	4.0
Total ECTs for attending the conferences	5.0

Poster presentations

2017 *"Delayed spontaneous clearance of hepatitis B more frequent among HIV/HCV-coinfected and young men who have sex with men and people who use drugs";* 11th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands

2017 *"Frequent delayed hepatitis B spontaneous clearance among high risk groups"*; 6th International Symposium on Hepatitis Care in Substance Users (INHSU), New York, US

2017 *"Frequent delayed hepatitis B surface antigen spontaneous clearance among high risk groups";* European Association for the Study of the Liver (EASL), Amsterdam, the Netherlands

2016 *"Effect of hepatitis C virus infection and its timing relative to HIV seroconversion on CD4 T-cell and HIV RNA trends among HIV-positive MSM";* 10th Netherlands conference on HIV pathogenesis, epidemiology, prevention and treatment (NCHIV), Amsterdam, the Netherlands

2016 *"Prevalence and determinants of liver disease among people who use drugs in Amsterdam, The Netherlands";* 5th International Symposium on Hepatitis Care in Substance Users (INHSU), Oslo, Norway

2016 *"Effect of the timing of hepatitis C Virus infection, relative to HIV seroconversion on CD4 T-cell and HIV RNA evolution among HIV-positive MSM";* 21st AIDS Conference, Durban, South Africa

2015 *"Stabilizing Hepatitis C incidence among HIV-positive MSM; an update from the CASCADE Collaboration";* 19th Workshop on HIV Observational Databases (IWHOD), Sicily, Italy

2015 "*Hepatitis C treatment for people who inject drugs: are direct-acting antivirals cost-effective?*; 2de National hepatitis day, Amsterdam, the Netherlands

2015 *"HIV and Hepatitis C treatment uptake among people who use drugs from the Amsterdam Cohort Studies, 1985-2013";* 2de National hepatitis day, Amsterdam, the Netherlands

2015 *"HIV and Hepatitis C Treatment Uptake among People Who Use Drugs from the Amsterdam Cohort Studies, 1985-2013";* 4th International Symposium on Hepatitis Care in Substance Users (INHSU), Sydney, Australia

2014 *"The effect of HIV infection on anal and penile high-risk human papillomavirus incidence and clearance. A cohort study among men who have sex with men";* 8th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands

2014 *"Standardized Mortality Ratios among Drug Users in Amsterdam Differ by HCV and HIV Infection Status";* 21st Conference on Retroviruses and Opportunistic Infections (CROI), Boston, USA

2013 *"Standardized Mortality Ratios among Drug Users in Amsterdam Differ by HCV and HIV Infection Status";* Dutch Epidemiology Conference (WEON), Utrecht, the Netherlands

2012 *"Does provider-initiated HIV testing and counselling lead to higher HIV testing rate and HIV case finding in Rwandan clinics?"* 6th Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands, 2012

Total ECTs for all poster presentations	7.0
Total ECTs for attending the conferences [a]	7.7

[a] excluding double oral & poster presentation conference attendance

Seminars, workshops and masterclasses attended

2017	Masterclass "*Causal Inference*", 2nd Amsterdam Public Health Annual Meeting Amsterdam, the Netherlands	0.1
2017	"*International Viral Hepatitis Elimination Meeting*", Virology Education, Amsterdam, the Netherlands	0.3
2017	Symposium "*Banning P-values*", Academic Medical Center, Amsterdam, the Netherlands	0.1
2017	*"HCV Elimination in the Netherlands: Lessons Learned & Challenges"*, Virology Education, Amsterdam, the Netherlands	0.1
2017	*"Comorbidity & Ageing in people living with HIV (COBRA symposium)"*, Amsterdam Institute for Global Health and Development, Amsterdam, the Netherlands	0.3
2016	"*International Viral Hepatitis Elimination Meeting*", Virology Education, Amsterdam, the Netherlands	0.3
2016	AIDS pre-conference *"3rd International HIV/Viral Hepatitis Co-Infection Meeting";* 21st AIDS Conference, Durban, South Africa	0.3
2016	"*3de National hepatitis day*", Virology Education, Amsterdam, the Netherlands	0.3
2016	*"Nationale bijeenkomst Academische Werkplaatsen Infectieziektebestrijding",* Sarphati Initiatief, Utrecht, the Netherlands	0.3
2016	Masterclass *"How do you deal with biomedical journals?"*, with Prof. Steven N. Goodman, Academic Medical Center, Amsterdam, the Netherlands	0.1
2015	*"19de National Conference on STI, sexual behaviour and AIDS",* Soa Aids Nederland, Amsterdam, the Netherlands	0.3
2015	"*DAAs in de dagelijkse Hepatitis C praktijk - een klinische workshop*", Virology Education, Amsterdam, the Netherlands	0.1
2014	"*18de National Conference on STI, sexual behaviour and AIDS*", Soa Aids Nederland, Amsterdam, the Netherlands	0.3
2014	"*International AIDS conference symposium in Amsterdam*", Soa Aids Nederland, Amsterdam, the Netherlands	0.1
2013	"*Treatment As Prevention: The Key To An Aids-Free Generation*", Amsterdam Institute for Global Health and Development, Amsterdam, the Netherlands	0.1

2013	WEON pre-conference *"Causality";* Dutch Epidemiology Conference (WEON), Utrecht, The Netherlands	0.1
2012	Debate evening: "*Lagerhuisdebat Hepatitis C and B*", Virology Education, Utrecht, the Netherlands	0.1

Academic activities

Peer-Reviewer:

- AIDS (1) 0.2
- PLOS One (2) 0.4
- Value in Health (1) 0.2
- International Journal of Drug Policy (1) 0.2
- Open Forum Infectious Diseases (1) 0.2
- Abstracts conference: International Symposium on Hepatitis Care in Substance Users (INHSU) abstracts (2) 0.2

Supervision:

- Medical bachelor student: Judith Cartier van Dissel 2.0
  University of Amsterdam
  Period: 2015 (4 months)
  Project: "Prevalence and determinants of cirrhosis among people who use drugs from the Amsterdam Cohort Studies"
- Master student: Jonie P.D. Martens 3.0
  VU University Amsterdam
  Period: 2015-2016 (6 months)
  Project: "Prevalence and determinants of fibrosis among people who use drugs from the Amsterdam Cohort Study and a qualitative insight in HCV-related healthcare in the Netherlands"

Other academic activities

- Volunteer at the international AIDS Impact conference, 2015, Amsterdam, the Netherlands 0.6
- Coordinator of the weekly PhD seminar at the GGD Amsterdam (2014-2016) 1.0
- Guest lecturer at:

i. Infectious disease seminar for staff, 0.3
VU University Medical Center, 2017, Amsterdam, the Netherlands

ii. GGD Amsterdam: Danish delegation of researchers, 2017, 0.3
Amsterdam, the Netherlands

iii.	GGD Amsterdam: Indonesian delegation of health professional and policymakers, 2017, Amsterdam, the Netherlands	0.3
iv.	Epidemiology, Health promotion and Health Innovation seminar for staff, 2016, GGD Amsterdam, Amsterdam, the Netherlands	0.3
v.	CASCADE Workshop, 2014, Mallorca, Spain	0.3
vi.	Infectious disease seminar for staff, Julius Centrum, University of Utrecht, 2013, Utrecht, the Netherlands	0.3
vii.	CASCADE Workshop, 2013, Rhodes, Greece	0.3
•	Organized the annual GGD Research day for all employees from the Infectious Disease department (2014).	1.0

Scholarships and prizes

2017 **Spinoza travel award** (Amsterdams Universiteit fonds) – to attend the 6th International Symposium on Hepatitis Care in Substance Users (INHSU) – New York, USA

2017 **Amsterdam Infection and Immunity travel grant** – to attend the 20th International Workshop on HIV and Hepatitis Observational Databases (IWHOD) – Lisbon, Portugal

2017 **Young investigator registration bursary** – European Association for the Study of the Liver (EASL) – Amsterdam, the Netherlands

2016 **Full scholarship award** – 5th International Symposium on Hepatitis Care in Substance Users (INHSU) – Oslo, Norway

2016 **Young investigator scholarship award** – 21st AIDS conference – Durban, South Africa

2015 **Best poster presentation** – 15th European AIDS Conference (EACS), – Barcelona, Spain

2015 **AMC young talent fund Louise Gunning Public Health Study Fund**, specific Public Health course at Johns Hopkins University – Baltimore, USA

2015 **Full scholarship award** – 4th International Symposium on Hepatitis Care in Substance Users (INHSU) – Sydney, Australia

2014 **Young investigator scholarship award** – 21st Conference on Retroviruses and Opportunistic Infections (CROI) – Boston, USA

LIST OF PUBLICATIONS

1. Fraser H, Martin NK, Brummer-Korvenkontio H, Carrieri P, Dalgard O, Dillon J, Goldberg D, Hutchinson S, Jauffret-Roustide M, Kaberg M, Matser AA, Maticic M, Midgard H, Mravcik V, Ovrehus A, Prins M, Reimer J, Robaeys G, Schulte B, van **Santen DK**, Zimmermann R, Vickerman P, Hickman M. **Model projections on the impact of HCV treatment in the prevention of HCV transmission among people who inject drugs in Europe"**. *J Hepatol* 2017. [epub ahead of print]

2. European Union HCV Collaborators. **Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study**. *Lancet Gastroenterol Hepatol* 2017; 2(5):325-336.

3. **van Santen DK**, van der Helm JJ, Lindenburg K, Schim van der Loeff M, Prins M. **HIV and hepatitis C treatment uptake among people who use drugs participating in the Amsterdam Cohort Studies, 1985-2015**. *Int J Drug Policy* 2017; 47:95-101. Special issue for the 6th International Symposium on Hepatitis Care in Substance Users (INHSU).

4. **van Santen DK**, van der Helm JJ, del Amo J, Meyer L, D'Arminio Monforte A, Price M, Beguelin CA, Zangerle R, Sannes M, Porter K, Geskus RB, Prins M, on behalf of the CASCADE Collaboration. **Lack of decline in hepatitis C virus incidence among HIV-positive men who have sex with men during 1990-2014**. *J Hepatol* 2017; 67(2):255-262.

5. Kayigamba FR, **Van Santen D**, Bakker MI, Lammers J, Mugisha V, Bagiruwigize E, De Naeyer L, Asiimwe A, Schim Van Der Loeff MF. **Does provider-initiated HIV testing and counselling lead to higher HIV testing rate and HIV case finding in Rwandan clinics?** *BMC Infect Dis* 2016; 16:26.

6. **van Santen DK**, de Vos AS, Matser A, Willemse SB, Lindenburg K, Kretzschmar ME, Prins M, de Wit GA. **Cost-Effectiveness of Hepatitis C Treatment for People Who Inject Drugs and the Impact of the Type of Epidemic; Extrapolating from Amsterdam, the Netherlands**. *PLoS One* 2016; 11(10):e0163488.

7. Mooij SH, **van Santen DK**, Geskus RB, van der Sande MA, Coutinho RA, Stolte IG, Snijders PJ, Meijer CJ, Speksnijder AG, de Vries HJ, King AJ, van Eeden A, van der Loeff MF. **The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM**. *AIDS* 2016; 30(1):121-132.

8. Willemse SB, Razavi-Shearer D, Zuure FR, Veldhuijzen IK, Croes EA, van der Meer AJ, **van Santen DK**, de Vree JM, de Knegt RJ, Zaaijer HL, Reesink HW, Prins M, Razavi H. **The estimated future disease burden of hepatitis C virus in the Netherlands with different treatment paradigms**. *Neth J Med* 2015; 73(9):417-431.

9. Saraswat V, Norris S, de Knegt RJ, Sanchez Avila JF, Sonderup M, Zuckerman E, Arkkila P, Stedman C, Acharya S, Aho I, Anand AC, Andersson MI, Arendt V, Baatarkhuu O, Barclay K, Ben-Ari Z, Bergin C, Bessone F, Blach S, Blokhina N, Brunton CR, Choudhuri G, Chulanov V, Cisneros L, Croes EA, Dahgwahdorj YA, Dalgard O, Daruich JR, Dashdorj NR, Davaadorj D, de Vree M, Estes C, Flisiak R, Gadano AC, Gane E, Halota W, Hatzakis A, Henderson C, Hoffmann P, Hornell J, Houlihan D, Hrusovsky S, Jarcuska P, Kershenobich D, Kostrzewska K, Kristian P, Leshno M, Lurie Y, Mahomed A, Mamonova N, Mendez-Sanchez N, Mossong J, Nurmukhametova E, Nymadawa P, Oltman M, Oyunbileg J, Oyunsuren T, Papatheodoridis G, Pimenov N, Prabdial-Sing N, Prins M, Puri P, Radke S, Rakhmanova A, Razavi H, Razavi-Shearer K, Reesink HW, Ridruejo E, Safadi R, Sagalova O, Sanduijav R, Schreter I, Seguin-Devaux C, Shah SR, Shestakova I, Shevaldin A, Shibolet O, Sokolov S, Souliotis K, Spearman CW, Staub T, Strebkova EA, Struck D, Tomasiewicz K, Undram L, van der Meer AJ, **van Santen D**, Veldhuijzen I, Villamil FG, Willemse S, Zuure FR, Silva MO, Sypsa V, Gower E. **Historical epidemiology of hepatitis C virus (HCV) in select countries - volume 2**. *J Viral Hepat* 2015; 22 Suppl 1:6-25.

10. Gane E, Kershenobich D, Seguin-Devaux C, Kristian P, Aho I, Dalgard O, Shestakova I, Nymadawa P, Blach S, Acharya S, Anand AC, Andersson MI, Arendt V, Arkkila P, Baatarkhuu O, Barclay K, Ben-Ari Z, Bergin C, Bessone F, Blokhina N, Brunton CR, Choudhuri G, Chulanov V, Cisneros L, Croes EA, Dahgwahdorj YA, Daruich JR, Dashdorj NR, Davaadorj D, de Knegt RJ, de Vree M, Gadano AC, Gower E, Halota W, Hatzakis A, Henderson C, Hoffmann P, Hornell J, Houlihan D, Hrusovsky S, Jarcuska P, Kostrzewska K, Leshno M, Lurie Y, Mahomed A, Mamonova N, Mendez-Sanchez N, Mossong J, Norris S, Nurmukhametova E, Oltman M, Oyunbileg J, Oyunsuren T, Papatheodoridis G, Pimenov N, Prins M, Puri P, Radke S, Rakhmanova A, Razavi H, Razavi-Shearer K, Reesink HW, Ridruejo E, Safadi R, Sagalova O, Sanchez Avila JF, Sanduijav R, Saraswat V, Schreter I, Shah SR, Shevaldin A, Shibolet O, Silva MO, Sokolov S, Sonderup M, Souliotis K, Spearman CW, Staub T, Stedman C, Strebkova EA, Struck D, Sypsa V, Tomasiewicz K, Undram L, van der Meer AJ, **van Santen D**, Veldhuijzen I, Villamil FG, Willemse S, Zuckerman E, Zuure FR, Prabdial-Sing N, Flisiak R, Estes C. **Strategies to manage hepatitis C virus (HCV) infection disease burden - volume 2**. *J Viral Hepat* 2015; 22 Suppl 1:46-73.

11. Hatzakis A, Chulanov V, Gadano AC, Bergin C, Ben-Ari Z, Mossong J, Schreter I, Baatarkhuu O, Acharya S, Aho I, Anand AC, Andersson MI, Arendt V, Arkkila P, Barclay K, Bessone F, Blach S, Blokhina N, Brunton CR, Choudhuri G, Cisneros L, Croes EA, Dahgwah-

dorj YA, Dalgard O, Daruich JR, Dashdorj NR, Davaadorj D, de Knegt RJ, de Vree M, Estes C, Flisiak R, Gane E, Gower E, Halota W, Henderson C, Hoffmann P, Hornell J, Houlihan D, Hrusovsky S, Jarcuska P, Kershenobich D, Kostrzewska K, Kristian P, Leshno M, Lurie Y, Mahomed A, Mamonova N, Mendez-Sanchez N, Norris S, Nurmukhametova E, Nymadawa P, Oltman M, Oyunbileg J, Oyunsuren T, Papatheodoridis G, Pimenov N, Prabdial-Sing N, Prins M, Radke S, Rakhmanova A, Razavi-Shearer K, Reesink HW, Ridruejo E, Safadi R, Sagalova O, Sanchez Avila JF, Sanduijav R, Saraswat V, Seguin-Devaux C, Shah SR, Shestakova I, Shevaldin A, Shibolet O, Silva MO, Sokolov S, Sonderup M, Souliotis K, Spearman CW, Staub T, Stedman C, Strebkova EA, Struck D, Sypsa V, Tomasiewicz K, Undram L, van der Meer AJ, **van Santen D**, Veldhuijzen I, Villamil FG, Willemse S, Zuckerman E, Zuure FR, Puri P, Razavi H. **The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm - volume 2**. *J Viral Hepat* 2015; 22 Suppl 1:26-45.

12. **van Santen DK**, van der Helm JJ, Grady BP, de Vos AS, Kretzschmar ME, Stolte IG, Prins M. **Temporal trends in mortality among people who use drugs compared with the general Dutch population differ by hepatitis C virus and HIV infection status**. *AIDS* 2014; 28(17):2589-2599.

13. **van Santen DK**. **HIV prevention with Latinos: theory, research and practice**. *Aids care* 2014 [Book review]; 26(5):658.

Publications about my research

"Hepatitis C incidence remains stable among gay men living with HIV in Europe, but varies across countries" by Liz Highleyman

Weblink: http://www.aidsmap.com/page/3011384

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AUTHORS' CONTRIBUTIONS

Chapter 2.1

Authors: Daniëla K. van Santen, Jannie J. van der Helm, Julia del Amo, Laurence Meyer, Antonella D'Arminio Monforte, Matt Price, Charles A. Béguelin, Robert Zangerle, Mette Sannes, Kholoud Porter, Ronald B. Geskus & Maria Prins, on behalf of the CASCADE Collaboration in EuroCoord

Contributions: DvS performed the statistical analyses together with RG, also interpreted the data, wrote the manuscript and provided substantial contribution to the design of the study. JvdH provided substantial contributions to the design, analyses and interpretation of the data as well as the manuscript. MP and RG designed and supervised the overall study, and substantially contributed to the conception of the study, analyses, interpretation of the data and manuscript. KP obtained funding for the study. All authors contributed to the design, additional HCV testing, interpretation of the data, subsequent drafts and approved the final version of the manuscript.

Chapter 2.2

Authors: Daniëla K. van Santen, Jannie J. van der Helm, Giota Touloumi, Nikos Pantazis, Roberto Muga, Barbara Gunsenheimer-Bartmeyer, M. John Gill, Eduard Sanders, Anthony Kelleher, Robert Zangerle, Kholoud Porter, Maria Prins and Ronald B. Geskus, on behalf of the CASCADE Collaboration within EuroCoord

Contributions: DvS performed the statistical analyses together with RG, also interpreted the data, wrote the manuscript and provided substantial contribution to the design of the study. JvdH provided substantial contributions to the design, analyses and interpretation of the data as well as the manuscript. MP and RG designed and supervised the overall study, and substantially contributed to the conception of the study, analyses, interpretation of the data and manuscript. KP obtained funding for the study. All authors contributed to the design, additional HCV testing, interpretation of the data, subsequent drafts and approved the final version of the manuscript.

Chapter 2.3

Authors: Sofie H. Mooij, Daniëla K. van Santen, Ronald B. Geskus, Marianne A.B. van der Sande, Roel A. Coutinho, Ineke G. Stolte, Peter J.F. Snijders, Chris J.L.M. Meijer, Arjen G.C.L. Speksnijder, Henry J.C. de Vries, Audrey J. King, Arne Van Eeden, Maarten F. Schim van der Loeff

Contributions: MSvdL designed and leads the H2M study as principal investigator. MvdS, RC, IS, HdV, AS, PS, CM, and SM contributed to the study design. AS, HdV, AvE, and SM contributed to the implementation of the study and data collection. AS, AK, and SM were responsible for laboratory analyses. DvS performed the statistical analyses and

interpreted the data together with SM, RG, and MSvdL. SM wrote a draft manuscript in collaboration with DvS, which was reviewed by all authors for important intellectual content.

Chapter 3.1

Authors: Daniëla K. Van Santen, Jannie J. van der Helm, Bart P.X. Grady, Anneke S. de Vos, Mirjam E.E. Kretzschmar, Ineke G. Stolte, Maria Prins
Contributions: DKvS performed the statistical analyses, interpreted the data, wrote the manuscript and provided substantial contribution to the design of the study. JJvdH and IGS provided substantial contributions to the design, analyses and interpretation of the data as well as the manuscript. BPXG provided data management support and feedback on the interpretation of the data and the draft manuscript. MEEK and ASdV provided feedback on the interpretation of the data and the draft manuscript. MP designed and supervised the overall study, and substantially contributed to the conception of the study, analyses, interpretation of the data and manuscript. All authors contributed to subsequent drafts and approved the final version of the manuscript.

Chapter 3.2

Authors: Daniëla K. van Santen, Maarten F. Schim van der Loeff, Judith Cartier van Dissel, Jonie P.D. Martens, Marc van der Valk, Maria Prins
Contributions: DKvS and MP were involved in the conception of the study. DKvS MSvdL JCvD MvdV MP provided substantial contribution to the design of the study. DKvS performed the statistical analyses, acquired data, interpreted the results, and wrote the manuscript. JCvD and JPDM gathered data and provided data management support. MSvdL substantially contributed to the analyses and interpretation of the data. MP supervised the overall study, and substantially contributed to the analyses, interpretation of the data and manuscript. All authors contributed to subsequent drafts and approved the final version of the manuscript.

Chapter 3.3

Authors: Daniëla K. van Santen, Anneke S. de Vos, Amy Matser, Sophie B. Willemse, Karen Lindenburg, Mirjam E.E. Kretzschmar, Maria Prins, G. Ardine de Wit
Contributions: DKvS and AdV performed the analyses. AdV build the mathematical model under supervision of MEEK. DKvS supplied additional data for the model, performed all economic-related analyses under supervision of GAdW, interpreted the data and wrote the manuscript. DKvS AdV AM MP GAdW substantially contributed to the conceptualization of the study. All authors were involved in supplying data and interpreting the results. AM MP obtained the funding for the study. MP and GAdW supervised

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Chapter 3.4

Authors: Daniëla K. van Santen, Jannie J. van der Helm, Karen Lindenburg, Maarten Schim van der Loeff, Maria Prins

Contributions: DKvS performed the statistical analyses, interpreted the data, wrote the manuscript and provided substantial contribution to the design of the study. JJvdH and MSvdL provided substantial contributions to the analyses and interpretation of the data as well as the manuscript. KL was responsible for the DUTCH-C project and contributed to the interpretation of the data. MP designed and supervised the overall study, and substantially contributed to the conception, analyses, interpretation of the data and manuscript. All authors contributed to subsequent drafts and approved the final version of the manuscript.

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ABOUT THE AUTHOR

Daniëla Katinka van Santen was born in Maracaibo, Venezuela on the 22nd of February 1985 to a Venezuelan mother and a Dutch father. After graduation with honors at Mater Salvatoris high school in Maracaibo, she immigrated to the Netherlands to improve her Dutch, her second language. After studying life sciences at the Rotterdam University of Applied Sciences for one year, she switched to study Nursing at the Utrecht University of Applied Sciences. It was during this study that she discovered that she wanted to continue her education in medical research and decided to work as a research assistant at the same university to gain experience in the research field. In 2010 she earned her bachelor's degree and soon after completed the pre-master Health Sciences at the VU University of Amsterdam. In 2011 she enrolled in the Health Sciences masters programme at the VU University, specializing in infectious disease research. In 2012 she completed her master's internship at the Public Health Service of Amsterdam (GGD Amsterdam) and the Royal Tropical Institute (KIT) under the supervision of Dr. Maarten Schim van der Loeff and Dr. Mirjam Bakker. During this internship, she worked on two research projects. In one of the projects she investigated whether provider-initiated testing in Rwanda led to a higher HIV case finding and higher HIV testing uptake. In 2012 she obtained a cum laude master's degree in Health Sciences.

In September 2012, Daniëla commenced her PhD training at the department of Infectious Disease Research and Prevention at the Public Health Service of Amsterdam under the supervision of Prof. Maria Prins, Dr. Ronald Geskus and Dr. Jannie van der Helm. The results of this work are presented in this thesis. In 2016 she worked at the same institution as a researcher in the department of Epidemiology, Health Promotion and Care Innovation on the Amsterdam Health Monitoring study. Currently, Daniëla is working as a post-doctoral fellow at the department of Infectious Disease Research and Prevention at the Public Health Service of Amsterdam.