25 years of HIV: trends in mortality, HIV coinfections, and HIV-related risk behaviour

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General introduction

Since AIDS was diagnosed for the first time in 1981[1], an estimated 25 million people have died from the disease. In 2005, the total number of persons living with HIV infection was 40.3 million worldwide, with almost five million new infections occurring in that year [2]. Sub-Saharan Africa is hardest hit, but the HIV epidemic is rapidly growing in Eastern Europe and Central Asia [2] .
In Western countries, homosexual contact and injecting drug use remain important routes of transmission among those infected with HIV today [2]. Over the years, however the proportion of HIV-positive men and women infected through heterosexual contact has increased considerably. In the mid-1990s highly active antiretroviral therapy (HAART) became widely available in most industrialised countries. In the developing countries of Sub-Saharan Africa, Asia, and South America, access to HAART is still very limited.
In Western countries, the widespread use of HAART has led to a strong reduction in the overall mortality and morbidity among HIV-infected persons [3] [4] [5]. The number of HIV-infected persons is increasing not only because people with HIV live longer due to HAART but also because immigration is increasing from AIDS endemic areas. Moreover, since the introduction of HAART, HIV risk behaviour and HIV incidence have increased among homosexual men [6].
For this thesis, trends in mortality, certain co-infections, and HIV-related risk behaviour were studied among homosexual men and drug users in Amsterdam [7] [8]. Also, the impact of HAART availability on changes in causes of death among HIV-infected persons were studied by pooling data from 22 cohorts of HIV-infected persons with a known date of HIV seroconversion [9].

Trends in mortality

In the Netherlands, AIDS was first diagnosed in 1982 [10]. The HIV epidemic predominantly affected homosexual men, who comprise 50% of the HIV-infected patients registered at the national HIV monitoring foundation [11]. In 1990, AIDS became the leading cause of death among all men in Amsterdam aged 24- 54 years [12]. The development of effective therapy in the late 1990s changed the HIV epidemic by improving life expectancy among HIV infected persons. As they now live longer, they are more likely to die from non-HIV-related causes of death, such as the consequences of co-infection with hepatitis viruses or age related disorders like cancer and cardio-vascular disease[13] [14] [15].
The progression of liver disease associated with hepatitis C infection is accelerated in persons infected with HIV [16]. Due to the increasing HAART-related survival of HIV-infected persons, mortality from hepatitis C infection is likely to take on a greater significance among those co-infected with HIV [17].

Trends in co-infections

In HIV-infected individuals, several co-infections are common, such as sexually transmitted diseases, hepatitis B and hepatitis C infections, m. tuberculosis, human herpes virus 8 or herpes simplex virus infections [18] [19] [20] [21] [22] [23]. Some of these co-infections facilitate HIV transmission, while others influence HIV disease progression, resulting in a poorer survival [24] [17]. In the present thesis focus was on hepatitis C virus and herpes simpex virus types 1 and 2.

Hepatitis C
Hepatitis C virus (HCV) is an infection that is mainly transmitted by blood-blood contact and is very common among injecting drug users, haemophiliacs, and recipients of blood products, with prevalence as high as 85% among injecting drug users in Europe [25] [26]. HCV was identified for the first time in 1989 [25]. Up to 85% of those infected with HCV are not able to clear the virus and become chronically infected. A chronic HCV infection leads to a high risk of developing liver cirrhosis within 20 years; this eventually can progress to liver failure or hepatocellular carcinoma [27].
Because of the use of contaminated needles and injecting equipment, injecting drug users are at high risk for HIV as well as HCV. Since the beginning of the HIV epidemic, a decline in the HIV incidence has been observed due to a decrease in injecting risk behaviour [28]. However, the HCV incidence among drug users is probably less affected by the decrease in injecting risk behaviour, because HCV is more transmissible than HIV [29].

Herpes Simplex Virus Type 1&2
Herpes simplex virus types 1 and 2 (HSV1&2) are common infections, both causing a lasting infection with recurrent lesions. Generally, HSV1 has been associated with oro-pharyngeal lesions, usually transmitted during childhood, with prevalence ranging between 44% among young adults to 90% among those aged 70 years and older in the Western countries [30]. Nowadays, however, childhood transmission has decreased and genital lesions caused by HSV1 have increased [31]. HSV2 causes genital lesions and has lower prevalence in the general population compared to HSV1. HSV2 is more restricted to specific risk groups, such as homosexual men, where the prevalence is as high as 50%. Epidemiological studies show that infection with HSV2 is a risk factor for HIV infection, genital ulcerations may facilitate HIV transmission and, among HIV infected persons, co-infection with HSV2 may up-regulate HIV, increasing local HIV replication and leading to an increased risk of HIV transmission to a HIV-negative partner. Therefore, the control of HSV2 plays an important role as a HIV prevention strategy [32].

Trends in HIV related risk behaviour

The introduction of HAART in the mid-1990s has affected sexual behaviour and the transmission of sexually transmitted infections (STI) among homosexual men. Sexual risk behaviour and STI started to increase among homosexual men in the United States, Western Europe and Australia, where HAART availability may have generated optimism regarding its effectiveness [33] [34] [35] [36]. Regarding drug users, little is known about the trends in sexual and injecting risk behaviour related to HAART availability [37] [38] [39]. Compared to other HIV risk groups, HIV-infected drug users appear less likely to be treated with HAART and most studies show a lower response to treatment among drug users [40]. Delayed initiation of treatment, a sub-optimal adherence, and HCV-co-infection may be responsible for the poorer response in this group.

Data used in this thesis

Table 1 presents an overview of the different data sources used in this thesis, as detailed below.

Table 1: Data Sources that are used in this thesis.

Data Sources	Study Population	Period	Chapter
Amsterdam Cohort Study	HIV-positive and -negative homosexual men	1984- 2004	2.1 3.2 4.1
Amsterdam Cohort Study	HIV-positive and -negative drug users	1985-2004	2.3 3.1 4.1
CASCADE	HIV seroconverters	1997-2004	2.2
Amsterdam AIDS Surveillance	AIDS cases	1982-2000	2.1
STI clinic surveys	HIV-positive and -negative homosexual men	1991-2000	2.1
Hepatitis B vaccine trial	HIV-positive and -negative homosexual men	1980-1982	2.1

The Amsterdam Cohort Study on HIV seroconversion and AIDS
Most research presented in this thesis is based on the Amsterdam Cohort Studies (ACS) [8] [7], an open and prospective study among homosexual men that began in 1984 and is still ongoing. Sexually active HIV-positive and HIV-negative homosexual men participate by visiting the Health Service of Amsterdam every 3 to 6 months. In 1985, the ongoing ACS among HIV-negative and HIV-positive drug users was started. Drug users are recruited via local methadone outposts, STI

clinics and by word of mouth. They are asked to return for their follow-up visits every 4 to 6 months.
At each visit for both cohorts, data are collected by standardised questionnaires and physical examination. Blood samples are taken for virologic testing and, if HIV-positive, for further immunologic testing. All these blood samples are stored at −80 °C. Recently, the stored blood samples were retrospectively tested hepatitis B, HCV, and HSV1 and 2.
The ACS provides a large database with epidemiological and clinical data on 1847 homosexual men and 1640 drug users from 1984 up to now. Due to its longitudinal setting, the ACS provides a unique opportunity to study trends in mortality, co-infections, and HIV-related risk behaviour over a long time period among both HIV-positive and HIV-negative homosexual men and drug users.

CASCADE: Concerted Action on SeroConversion to Aids and Death in Europe
CASCADE was established in 1997 as a collaboration among 22 HIV seroconverter cohort studies in Europe, Canada and Australia (including the ACS). Only HIV-infected persons with a documented date of HIV seroconversion are included in CASCADE [9]. Seroconverters are enrolled in the individual cohorts, locally and nationally, and are typically followed for their lifetime. Data on demographic characteristics, on anti-HIV therapies, clinical status, and laboratory measurements are collected and pooled. The major advantage of the CASCADE collaboration is the size of the study population, as around 8500 seroconverters are included.

Additional data sources
To supplement data from the ACS and CASCADE, data from the AIDS surveillance system of the Health Service of Amsterdam were used [41]. In this system, no longer ongoing, epidemiological data on AIDS patients in Amsterdam were collected from beginning of the AIDS epidemic until 2000. Physicians from all hospitals in Amsterdam reported newly diagnosed AIDS patients on voluntary basis; only AIDS cases meeting the 1987 and 1993 AIDS definition of the US Centers for Disease Control and Prevention were allowed in the AIDS surveillance system.
Since 1991, cross-sectional HIV prevalence surveys have been conducted at the STI clinic of the Health Service of Amsterdam. This thesis used data from the homosexual men participating in these surveys until 2000.
The last data source used for this thesis is the Amsterdam hepatitis B vaccine trial among homosexual men, which ran from 1980 to 1982. Since many of its participants also became prospective participants of the ACS, serum collected during this trial was retrospectively tested for HIV.

Outline of this thesis

The main objectives of the present thesis were to study trends in overall mortality, specific causes of death, HIV-related behaviour, and HIV co-infections, especially HCV and HSV, among individuals followed since the start of the HIV epidemic, 25 years ago.
Chapter 2 includes three studies investigating the mortality among HIV-infected persons. The first study describes the decline in overall mortality among homosexual men in Amsterdam in terms of two contributions: declining HIV incidence among homosexual men and the introduction of HAART. The second study, evaluates the changes in the specific causes of the death after the introduction of HAART and the relationships between CD4 cell counts and HIV RNA levels and specific causes of death in a large population of HIV-infected persons with a well known date of seroconversion, who were registered in the CASCADE study. The third study compares the mortality from specific causes of death among drug users in the ACS who are HCV/HIV-co-infected, HCV-mono-infected, HIV-mono-infected, and HCV-negative and HIV-negative, before and after the introduction of HAART.
Chapter 3 covers two studies regarding trends in HIV co-infections. The first study reports the HCV incidence among injecting drug users over nearly two decades and compares that with the HIV incidence. The second study describes risk factors associated with the presence of HSV1&2 antibodies and changes in these risk factors between 1984 and 2003 among homosexual men in Amsterdam.
Chapter 4 examines the impact of HAART availability on behavioural changes among HIV-infected drug users from the ACS, as well as their response to HAART treatment.

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2.1

Mortality

Declining AIDS Mortality in Amsterdam

Contributions of Declining HIV Incidence and Effective Therapy

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Introduction: We aimed to evaluate the impact of highly active antiretroviral therapy (HAART) on AIDS mortality, taking into account earlier HIV incidence patterns.

Methods: Using AIDS Surveillance data (1982–2000), we calculated the observed course of the AIDS epidemic among homosexual men in Amsterdam, The Netherlands. We used the HIV incidence patterns (1980–2000) among homosexual men participating in the hepatitis B vaccine trial and the Amsterdam Cohort Study and those attending the Amsterdam sexual transmitted infections clinic, together with the time from seroconversion to AIDS and death in the pre-HAART era, to estimate the natural course of the AIDS epidemic if HAART had not been introduced.

Results: The estimated course of the AIDS epidemic without the benefits of HAART showed a decline in AIDS mortality, but this estimated decline was not as strong as the observed decline. Taking into account the HIV incidence over calendar time, we estimated that 331 deaths among homosexual men were prevented by HAART between 1996 and 2000 in Amsterdam.

Conclusion: The decline in AIDS mortality was the result of both HAART and a decline in the HIV incidence in the early 1980s. When evaluating the effect of HAART on mortality, changes in HIV incidence must also be considered.

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From 1995 onward, mortality resulting from AIDS has been declining in most industrialized countries,[1,2] probably as a result of the success and widespread use of highly active antiretroviral therapy (HAART). However, mortality trends are also influenced by the HIV incidence pattern in the past, which must be taken into account when evaluating the effect of HAART. Such an analysis is not feasible for surveillance data, which is often used in mortality studies, because HIV incidence data are not available.

In The Netherlands, AIDS was first diagnosed in 1982. From then until 2000, 2305 AIDS cases (39% of all cases in the country) had been reported to the AIDS Surveillance System of the Municipal Health Service of Amsterdam. The HIV epidemic predominantly affected homosexual men. In 1990, AIDS became the leading cause of death for all men age 25–54 years in Amsterdam, a city with 750,000 inhabitants; mortality resulting from AIDS exceeded mortality caused by heart diseases and malignant neoplasms.[3]

A reconstruction of the yearly incidence of HIV infection among a cohort of homosexual men in Amsterdam shows a peak in 1984, followed by a decline from 1985 onward.[4,5] Without effective treatment, the median time from HIV seroconversion to death is estimated to be 11 to 12 years.[6,7] Consequently, more than 50% of those infected with HIV in the early 1980s died in the early 1990s.[7] For this reason, a decline in deaths from AIDS is likely to have begun before the introduction of HAART in 1996.

In the present study, we differentiated the observed AIDS mortality with the estimated mortality based on the HIV incidence patterns without the benefits of HAART among homosexual men. This analysis was possible because HIV incidence data among homosexual men in the early years of the AIDS epidemic were available.

METHODS

Multiple data sources were used. We illustrated the observed course of the AIDS epidemic among homosexual men in Amsterdam with Amsterdam AIDS surveillance data.

We used the HIV incidence patterns among homosexual men participating in the Amsterdam Cohort Study and the Amsterdam Hepatitis B vaccine trial and those attending the Amsterdam sexually transmitted infections (STI) clinic, together with the time from seroconversion to AIDS and death in the pre-HAART era, to estimate the natural course of the AIDS epidemic if HAART had not been introduced.

Table 1 presents an overview of the different data sources.

AIDS Surveillance

Since the beginning of the AIDS epidemic, epidemiologic data about AIDS patients has been collected by the AIDS Surveillance System of the Municipal Health Service of Amsterdam (1982–2000). In this system, physicians from all hospitals in Amsterdam report newly diagnosed AIDS cases on a voluntary basis. As backup, the physicians are every year contacted by a public health nurse or medical doctor of the Municipal Health Service and reminded to report these cases. Only AIDS cases meeting the 1987 AIDS case definition criteria of the U.S. Centers for Disease Control and Prevention (CDC) are allowed in the AIDS Surveillance System.[8] In The Netherlands as of January 1, 1994, 3 new indicator diseases (pulmonary tuberculosis, recurrent pneumonia, and invasive cervical carcinoma) were added to CDC criteria,[9] and a CD4 count below 200×10^6 cells/L criteria is not included. For every newly diagnosed AIDS case, information on demographics and risk group was obtained. Dates of death were obtained from the municipal death records. For this study, we matched AIDS cases without a date of death against the register of the municipality of Amsterdam to make sure these cases were still alive and residing in Amsterdam. All AIDS cases who had died were checked to verify Amsterdam residence at the date of death. Only officially registered residents of Amsterdam were included in our analyses.

HIV Incidence Data

To estimate the course of the AIDS epidemic without the impact of HAART, we used HIV incidence data from 3 sources.

One of these sources was the Amsterdam Cohort Study, which is an ongoing prospective study that started in October 1984.[10] Sexually active HIV-seropositive and HIV-seronegative homosexual men participate by visiting the Municipal Health Service every 3 to 6 months. At each visit, data are collected by standardized questionnaires and physical examination. Blood is taken for virologic testing, and, if HIV-positive, for further immunologic testing.

Secondly, HIV incidence has been calculated based on retrospectively identified HIV-positive and HIV-negative samples of participants obtained in the Amsterdam Hepatitis B vaccine trial among homosexual men. This trial started in 1980 and was terminated in 1982. Subsequently, many of the participants in the hepatitis B vaccine trial consented to the retrospective testing of stored serum samples for HIV. Many of these men also became prospective participants in the Amsterdam Cohort Study.

Our third source of data was the cross-sectional HIV prevalence surveys that have been conducted at the sexually transmitted infection clinic of the Municipal Health Service of Amsterdam since 1991 (except for 1993 as a result of logistic problems). For this analysis, we used HIV incidence calculated for homosexual participants in the 1991–2000 HIV prevalence surveys.[11] Recent infections were identified using a new testing strategy to detect early HIV infections among people who were HIV-positive.[12] Homosexual men who tested HIV-positive with a sensitive test were then retested with a less sensitive test; men who were nonreactive on the less sensitive test were classified as recently infected. The incidence was calculated as the prevalence of persons with a recent infection among the susceptible population (HIV-negative men and recently infected men) divided by the mean time between seroconversion on the assays.

Statistical Analyses

Effect of the Introduction of Treatment on the Risk of Death From AIDS

We used a Poisson regression analysis to test the effect of various treatments on the risk of death after an AIDS diagnosis at the population level. We categorized calendar time as multiyear periods, taking the years 1982–1985 as the reference period. In that earlier period, no therapy was available. The other time periods were defined as 1986–1990 (when *Pneumocystis carinii* pneumonia prophylaxis and monotherapy were available), 1991–1995 (dual therapy), and 1996–2000 (HAART). Incidence, relative risk (RR), and their 95% confidence intervals (CIs) were calculated. Subjects were con-

TABLE 1. Data Sources Used in This Study

Data Sources	Study Population	Period	Person-years of Follow Up	No.
AIDS Surveillance	AIDS cases	1982–2000	5651 after AIDS diagnosis	Deaths: 1611
Hepatitis B vaccine trial	Homosexual men	1980–1982	6736 HIV-negative	New HIV infections: 580
Amsterdam Cohort Study	Homosexual men	1984–2000		
STI clinic surveys	Homosexual men	1991–2000	2082 HIV-negative	New HIV infections: 25

sidered to be at risk from the date of AIDS diagnosis until the date of death or the end of the study period, which was January 1, 2000. In addition to calendar time, the following covariates, which were associated with death from AIDS in univariate analyses, were considered in the multivariate analysis: age, nationality defined as Western (European, North American, Australian, and New Zealand combined) versus non-Western, sex, and risk group. Because sex and risk group are correlated, we used a combined variable with 11 categories: homosexual men; male and female intravenous drug users; male and female heterosexuals with multiple sexual partners; men and women originating from an AIDS endemic country; men and women with a high-risk partners (ie, bisexual, intravenous drug user, or from an AIDS endemic country). The remaining risk groups were merged as one but divided by sex. AIDS cases belonging to 2 risk groups were classified in the most important risk group, ie, homosexual or drug users.

Effect of HIV Incidence and HAART on AIDS Mortality Time Trends

To separate the course of the HIV epidemic from the effects of HAART on AIDS mortality, the incidence of AIDS cases and deaths without the influence of HAART was estimated. The number of new AIDS cases among homosexual men in a specific calendar year k was determined by multiplying the number of new HIV-infected individuals (λ) in the calendar year j before year k by the probability of developing AIDS $k–j$ years after seroconversion. The numbers thus obtained for each calendar year before year k were added to obtain the total number of cases. The number of AIDS deaths could be determined by the same method. The number of AIDS cases and deaths $Nd(k)$ is expressed by the formula:

$$Nd(k) = \sum_{j=1980}^{k-1} \lambda(j)\, f(k-j) \qquad (1)$$

We were not able to obtain accurate estimates of the absolute number of HIV seroconversions among the homosexual population of Amsterdam for each year. However, the shape of the AIDS and death curves can be reliably estimated for each year if we know the shape of the HIV seroconversion distribution over calendar time. Therefore, our estimated curves are based on the HIV incidence pattern.

The homosexual men in Amsterdam comprise a dynamic population, with new people entering the risk set over calendar time. If we assume that the total population of HIV-negative men remains fairly constant over time, it follows that the yearly HIV incidence in the Amsterdam Cohort Study represents the HIV dynamics of the Amsterdam population of homosexual men. The annual probability of seroconversion is a less accurate measurement because the probability does not take into account changes on the population size over time.

From October 1984 to April 1985, both HIV-positive and HIV-negative men entered the Amsterdam Cohort Study. A seroconversion density has been estimated for this group,[5] and we used this as the seroconversion pattern until 1985. From April 1985 until 1988, only HIV-seronegative men entered the Amsterdam Cohort Study. From 1988 until 1995, the emphasis was on recruiting HIV-positive people, and so very few seronegatives entered the study. In May 1995, a new cohort was started among young (under age 30 years) homosexual men. For 1991 onward, we have supplemented the Amsterdam Cohort Study (ACS) HIV incidence data with data from homosexual men attending the Amsterdam sexually transmitted infection clinic.[11] The estimated HIV incidence pattern in the Amsterdam population of homosexual men was derived by visually combining the incidence of the ACS and the sexually transmitted infection (STI) clinic. Because homosexual men attending the STI clinic are assumed to have more risk behavior, more weight is given to the HIV incidence in the ACS.

The density of the distribution of time from seroconversion to AIDS diagnosis and death was based on the Kaplan-Meier estimate from the ACS data in the pre-HAART era using kernel smoothing.[5] This density was summarized over yearly intervals to obtain the probability of AIDS diagnosis and death for each year after HIV seroconversion.

To evaluate the effects of HAART by comparing the number of observed deaths with the number of estimated deaths, we combined the 2 curves into one figure. Because the estimated curve does not reflect absolute numbers, its height was determined by matching the 2 curves based on the same cumulative incidence until 1996, when HAART became generally available in The Netherlands.

We are aware that the HIV incidence pattern that was used to estimate the AIDS incidence and death curves might not be representative. Therefore, we carried out a sensitivity analysis. If this pattern does not reflect the HIV incidence pattern in the Amsterdam population of homosexual men, the estimated curves will not be valid. In this analysis, the impact of changes in the HIV incidence on the shape of the AIDS and death curves was determined by moving the peak in HIV incidence to earlier and later times, and also to higher and lower incidences.

RESULTS

General Characteristics

From 1982, when the first AIDS case was diagnosed in Amsterdam, until January 1, 2000, 2305 AIDS patients living in Amsterdam had been reported to the AIDS Surveillance System. Of these, 1611 had died by the beginning of 2000;

91% of the total number of AIDS patients were male, and the median age at diagnosis was 38 years (interquartile range: 33–44). Most AIDS cases were homosexual or bisexual men (75%), whereas 12% were intravenous drug users. Heterosexual groups were those with multiple sexual partners (2%), those from AIDS-endemic countries (2%), and those with a partner who is bisexual, injecting drugs, or originating from an AIDS-endemic country (2%).

The annual number of new AIDS cases rose from 1982 until 1992. It then stabilized and began to decline in 1996 (Table 2). The yearly number of AIDS deaths increased until 1992 and started to decline in 1994.

Effect of Changing Treatment Over Time on the Risk of Death From AIDS

The total number of person-years at risk, from AIDS diagnosis until death from AIDS or censor date, was 5666.8. The incidence of death from AIDS decreased over calendar time compared with the 1982–1985 reference period (Table 2). The risk of death from AIDS was lower in 1986–1996 when *P. carinii* pneumonia prophylaxis (RR = 0.40; 95% CI = 0.29–0.57) and then dual therapy (0.39; 0.28–0.55) became available. The likelihood of AIDS death was more than 10 times lower (0.09; 0.06–0.12) after the introduction of HAART in The Netherlands in 1996. In the univariate analysis, age, western nationality, and the combined variable of gender and risk group (female intravenous drug users, men from AIDS endemic countries, and women from remaining risk groups) were associated with the risk of death from AIDS. Adjustment for these variables and their interaction with calendar time did not substantially change the relative risk for any calendar period.

HIV Incidence

In Figure 1 the HIV incidence among homosexual men participating in the ACS and attending the STI clinic are shown. The incidence from the ACS peaked in 1984 and then declined. Among homosexual men participating in HIV surveys at the STI clinic, the HIV incidence is presented from 1991 until 2000 and shows a peak in 1997. When the 2 incidence curves are combined, the shape of the resulting curve represents the HIV incidence pattern of the homosexual population of Amsterdam.

Homosexual Men: Observed versus Estimated Curves of AIDS Cases and Deaths

Figure 2A shows the observed number of AIDS cases and deaths from AIDS among homosexual men reported to the AIDS Surveillance System from the beginning of the AIDS epidemic. After a strong increase in the number of AIDS cases, numbers began to stabilize in 1988 and peaked in 1992. Thereafter, the number of homosexual men diag-

TABLE 2. Annual Death Rates After the Diagnosis of AIDS per Calendar Year and Multiyear Rate Ratios Among AIDS Cases Residing in Amsterdam, 1982–1999

Year	No. of AIDS Cases per Year	No. of Deaths per Year	Annual Person-years of Follow-Up After AIDS Diagnosis	Death Rate	(95% CI)	Relative Risk*	(95% CI)
1982	2	1	0.28	3.52	(0.49–24.93)		
1983	9	5	2.75	1.82	(0.76–4.37)		
1984	20	11	9.66	1.04	(0.56–1.92)		
1985	32	20	19.64	0.97	(0.62–1.52)	1.0	
1986	83	40	44.45	0.88	(0.64–1.20)		
1987	121	50	100.14	0.50	(0.38–0.66)		
1988	151	84	179.90	0.45	(0.36–0.56)		
1989	201	103	256.90	0.40	(0.33–0.49)		
1990	191	131	333.33	0.38	(0.32–0.45)	0.40	(0.29–0.57)
1991	195	173	374.11	0.45	(0.39–0.53)		
1992	234	203	410.76	0.49	(0.43–0.56)		
1993	209	189	431.61	0.43	(0.37–0.50)		
1994	214	196	445.36	0.43	(0.38–0.50)		
1995	222	161	483.92	0.32	(0.28–0.38)	0.39	0.28–0.55
1996	193	108	564.92	0.19	(0.15–0.23)		
1997	131	73	638.55	0.11	(0.09–0.14)		
1998	61	39	676.06	0.06	(0.04–0.08)		
1999	36	24	694.46	0.03	(0.02–0.05)	0.09	0.06–0.12

*The years 1982–1985 comprise the reference period.

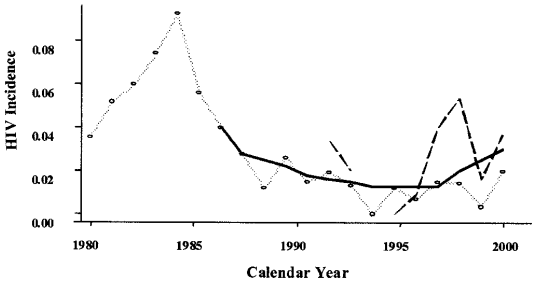

FIGURE 1. The estimated HIV seroincidence pattern in the Amsterdam population of homosexual men (thick black line). Incidence is estimated by combining the incidence from the Amsterdam Cohort Study (gray line) and the incidence from the sexual transmitted infections outpatient clinic (dashed line); however, sexual transmitted infections data for 1993 were not available.

nosed with AIDS began to decrease and continued to decrease until the end of the study period. The number of AIDS deaths increased from one in 1982 to 167 in 1992, remaining fairly stable through 1994 and then dropping to 13 in 1999. The decline in AIDS deaths was steeper and began earlier than the decline in AIDS diagnoses.

Figure 2B shows the estimated shape of the curve of new AIDS cases based on the HIV incidence data from ACS and the STI clinic, as well as the time from HIV seroconversion until AIDS diagnosis in the pre-HAART era. Comparable to the observed AIDS cases, the estimated curve of AIDS cases started to decline in 1992. The estimated curve of AIDS deaths decreased from 1993 onward, beginning 1 year earlier than for the observed cases. As expected, the decline in estimated AIDS cases and deaths was not as steep as the decline in observed cases and deaths.

We found that the sensitivity of the AIDS incidence and death curves was low. The shape of the curves were not affected by moving the peak in HIV incidence (Fig. 1) to higher and lower incidences. As expected, moving the 1984 peak in HIV incidence to earlier or later years simply shifted the curve to earlier or later.

The observed and estimated number of deaths from AIDS among homosexual men in Amsterdam are combined in Figure 2C. The observed and estimated curves cross in July 1994. By subtracting the number of observed deaths from the estimated numbers between 1996 and 2000, we estimated that 331 deaths have been prevented as a result of HAART.

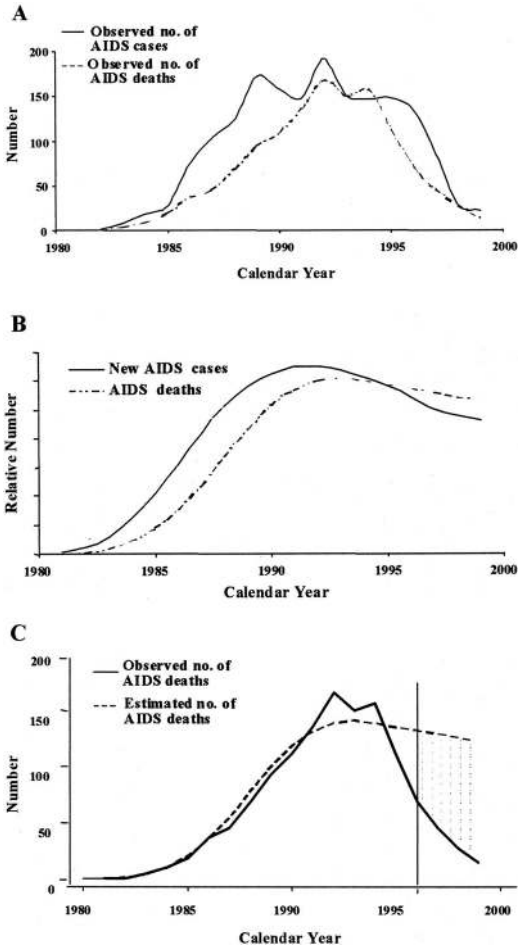

FIGURE 2. (**A**) Observed number of new AIDS cases and AIDS deaths among homosexual men in Amsterdam, reported to the AIDS surveillance system between 1982 and 1999. (**B**) Estimated AIDS incidence and death in the Amsterdam population of homosexual men. This estimate is based on an estimate of the shape of the HIV seroincidence curve and on the time to AIDS diagnosis and death after HIV seroconversion in the pre-HAART era. (**C**) The observed and estimated number of AIDS deaths among homosexual men in Amsterdam combined in one figure. The shaded area represents the number of cases that have been prevented by HAART.

DISCUSSION

We aimed to evaluate the impact of HAART on AIDS mortality, taking into account earlier HIV incidence patterns. The AIDS epidemic clearly had a major impact on mortality in Amsterdam, because AIDS was the leading cause of death among men age 25–54 years between 1989 and 1996 (data not shown).

We are not aware of any study that has taken into account HIV incidence patterns to determine whether the declines in AIDS mortality are also as a result of decreasing HIV incidence in the past. Other studies have attributed decreases in AIDS mortality to the introduction of HAART without taking into account HIV incidence patterns.[1,2,13–15] These studies on mortality primary relied on surveillance data that do not include HIV incidence data. The ACS is one of the few epidemiologic studies worldwide that provide incidence data from the beginning of the AIDS epidemic.[7]

In the ACS, the yearly HIV incidence declined from 1984 onward among homosexual men and from 1986 among drug users.[4,5,16] In the most affected group in Amsterdam, homosexual men, deaths from AIDS peaked in 1992 and then declined. Furthermore, the estimated curves of AIDS cases and deaths without the benefits of HAART in the Amsterdam population of homosexual men showed a decrease in mortality before the introduction of HAART. Therefore, we showed that even without HAART, the number of deaths would have declined because of a decreasing HIV incidence 10 years earlier.

The estimated curves of AIDS cases and deaths were based on an estimated pattern of the HIV incidence among the total population of homosexual men in Amsterdam. This pattern was derived from the HIV incidence data of the homosexual men participating in the ACS and attending the STI clinic. It could be questioned whether this HIV incidence is representative for the total population of homosexual men in Amsterdam. Sexual behavior, age, and history of syphilis have compared between a random sample of homosexual men living in Amsterdam, in 1989 and 1990, and homosexual men participating in the ACS during the same period.[17] Their age was similar, but ACS participants reported a higher number of sexual partners in the preceding year, more receptive anogenital intercourse, more lifetime partners, and, more often, a history of syphilis—indicating that the participants of the ACS are more sexually active and might be engaged in risky sexual behavior. This finding suggests that HIV incidence among the general population of homosexual men might be lower. However, we found low sensitivity of AIDS incidence and death to changes in the HIV incidence. Results will change only if the pattern from the unseen population of homosexual men is very different from those excluded in our data sources and moreover if this group has a major contribution to HIV incidence. We consider both of these conditions to be unlikely.

From 1988 until 1995, when the ACS emphasis was on recruiting HIV-positive persons, a small number of HIV-negative homosexual men entered the study. Nonetheless, we believe that the estimated curves of AIDS and death in the first years after 1988 remain valid because a sufficient number of HIV-negative persons entered the ACS before 1988. Afterward, the validity could have decreased as a result of saturation of the participants at risk and increasing age of the participants in the ACS. However, it more likely increased as a result of the 1995 start of a new cohort among young homosexual men and the use of the STI clinic data to estimate the HIV pattern.

We compared the number of AIDS deaths reported to the AIDS Surveillance System of the Amsterdam Municipal Health Service with the mortality data of Statistics Netherlands, a government bureau, that collects this data from death certificates supplied by physicians or coroners (data not shown). The annual numbers of AIDS deaths were compared between the 2 sources and only small differences were seen, except for the first and last years of study. For 1985, Statistics Netherlands showed fewer AIDS deaths, probably reflecting retrospective coding of AIDS deaths. For 1999, Statistics Netherlands shows 19 more AIDS deaths than did the AIDS Surveillance System database—which we learned is incomplete for 1999 because one of the hospitals ceased reporting AIDS cases (an indication that physicians could be less concerned about such reporting in the era of HAART). The 1999 discrepancy suggests that our mortality rate in the most recent years might be underestimated. The incomplete data of the AIDS Surveillance System is unlikely to result from reporting delay, because such delay would mostly influence the most recent years and our calculation in 2003 used data through 1999. Furthermore, there were no changes in the procedure for obtaining data, because the AIDS Surveillance System regularly contacted physicians asking them to report AIDS cases.

The decrease in the observed number of AIDS deaths among homosexual men was not preceded by a decline in the number of new AIDS cases. Also seen in New York City,[1] this finding could be explained by the availability of *P. carinii* pneumonia prophylaxis and dual therapy. In a previous study, we showed that after the introduction of *P. carinii* pneumonia prophylaxis in Amsterdam in 1985, the 1-year survival for AIDS cases diagnosed with this disease pneumonia improved in 1986 and continued to rise in the following years.[18]

This study did not encompass the total impact of the HIV epidemic on the mortality in Amsterdam, because it did not take into account the HIV-infected persons who died before an AIDS diagnosis. However, this number is relatively small,[19] and among HIV-infected individuals, living in The Netherlands and known to be using HAART, HIV-related mortality decreased and non-HIV related mortality did not change over time.[20]

In conclusion, our study shows that when evaluating the effect of HAART on AIDS mortality, changes in the HIV incidence pattern should also be taken into account. When we did so, we estimated that 331 deaths have been prevented by HAART in Amsterdam between 1996 and 2000. Although the 1996 introduction of HAART greatly contributed to the

decline in AIDS mortality, mortality rates had already begun to drop as a result of a decline in the HIV incidence in the 1980s before the introduction of HAART.

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2.2

Mortality

Effective therapy has altered the spectrum of cause-specific mortality following HIV seroconversion

CASCADE Collaboration*

Introduction: Although HAART has led to a reduction in overall mortality among HIV-infected individuals, its impact on death from specific causes is unknown.

Methods: Twenty-two cohorts of HIV-infected individuals with known dates of seroconversion are pooled in the CASCADE collaboration. Causes of death (COD) were categorized into three AIDS-related and seven non-AIDS-related causes. The unknown causes were assigned a separate category. The cumulative incidence for each COD was calculated in the presence of the other competing COD, for the pre-HAART and HAART eras. A multivariate regression analyses for the cumulative rate of progression to the different COD was performed.

Results: A total of 1938 of 7680 HIV-seroconverters died. Pre-HAART, AIDS opportunistic infections (OI) was the most common COD, followed by unknown and HIV/AIDS-unspecified. In the HAART era, the cumulative incidence for all AIDS-related COD decreased, OI remaining the most important. Large reductions in death due to other infections and organ failure were seen. Cumulative death risk decreased in the HAART era for most causes. The effect of HAART was not the same for all risk groups. The cumulative risk of death from AIDS-related malignancies, OI and non-AIDS-related malignancies decreased significantly among homosexual men (MSM), whereas the risk of dying from (un)-intentional death increased significantly among injecting drug users (IDU). A non-significant increase in hepatitis/liver-related death was seen in MSM, IDU and haemophiliacs.

Conclusion: Overall and cause specific mortality decreased following the introduction of HAART. OI remain the most common COD in the HAART era, suggesting that AIDS-related events will continue to be important in the future. Future trends in COD should be monitored using standardized guidelines.

AIDS 2006, **20**:741–749

Keywords: HIV, cohort, seroconverters, causes of death, highly active antiretroviral therapy

Introduction

The life expectancy of HIV-infected individuals in the developed world has improved substantially since highly active antiretroviral therapy (HAART) became widely available from 1997 [1,2]. As HIV-infected persons live longer, they are increasingly more likely to die from non-HIV related causes [3,4], such as the consequences of co-infection with hepatitis viruses or aging, and increased exposure to HAART may place individuals at risk of death from therapy-related toxicities, including cardiovascular side-effects [5].

For example, the progression of liver disease associated with hepatitis C (HCV) infection is known to be accelerated in HIV-infected persons [6]. Due to the increasing survival of HIV-infected persons in the HAART era, mortality from HCV may take on a greater significance among HIV-infected drug users and haemophiliacs, in whom HCV infection is common.

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Chronic hepatitis B infection has also been shown to increase mortality among HIV-infected persons [7,8].

In the pre-HAART era, particularly in injecting drug users (IDU) and homosexual men, the risk of pre-AIDS mortality increased as the CD4 cell count decreased and HIV RNA level increased [9]. Among HIV-infected persons treated with HAART, the risk of death has also been shown to be associated with the CD4 cell count [10]. However, it is not clear whether these relationships are the same for all causes of death, or whether any relationship with the CD4 cell count or HIV RNA level may simply reflect a longer duration of infection in those with more advanced disease. The CASCADE cohort collaboration, which includes a large group of HIV-infected individuals with reliable estimated dates of seroconversion from all risk groups, provides a unique opportunity to describe in detail the incidence of death from different causes over the first 20 years following the start of the HIV epidemic, adjusting for the duration of HIV-infection.

We evaluated the impact of the introduction of HAART on cause-specific mortality and investigated the relationships between CD4 cell counts and HIV RNA levels and specific causes of death in this large collaboration.

Methods

Study population

Data from a collaboration of 22 HIV seroconverter cohort studies in Europe, Canada and Australia (CASCADE: Concerted Action on SeroConversion to AIDS and Death in Europe) were used for this analysis.

The CASCADE collaboration, which includes HIV-1 infected persons where a date of seroconversion can be estimated reliably, has been described in detail elsewhere [11]. Seroconverters were either enrolled into one of the constituent cohorts while HIV negative or else when already infected but with the ability to estimate HIV seroconversion through the availability of previously stored blood samples. The majority (85%) of seroconversion dates were calculated as the mid-point between the last negative and first positive HIV antibody tests; individuals in whom this period was greater than 3 years were excluded from current analysis (91% having an interval less than 2 years). Analyses were restricted to individuals from 19 cohorts that collected information on specific causes of death (COD). Children under 15 year of age at seroconversion were excluded.

Specific causes of death

Causes of death were grouped into 11 categories. These included three AIDS-related categories [AIDS-related malignancy, AIDS opportunistic infections (OI), AIDS/HIV-related unspecified] and seven non-AIDS-related causes [hepatitis/liver-related, other infections, organ failure, non-AIDS-related malignancy, (un)-intentional death (including accidents, suicide, overdose), cardiovascular disease (CVD)/diabetes mellitus and other (including epilepsy, fits, blood disorders)]. The final category consisted of the unknown COD, which could include AIDS-related as well as non-AIDS-related deaths. Most cohorts reported the primary COD for individuals who died, while some reported multiple COD. When more than one COD was given, the most likely underlying COD was scored independently by a panel of three physicians. Causes about which the physicians disagreed were reviewed by the panel until a final consensus was reached.

Analysis

Follow-up was calculated from the estimated date of seroconversion until the earliest of: death, loss to follow-up, the cohort censoring date (each cohort had their own censoring date reflecting the processes and timelines in place for ascertaining events) or 31 December 2003. Those who were enrolled retrospectively into the constituent cohorts were included in the risk set from the date of cohort enrolment (i.e. a correction for left truncation was applied). We used calendar time as a proxy for the introduction and widespread use of HAART and defined two calendar periods, pre-1997 and 1997 onwards, to reflect the pre-HAART and HAART eras, respectively.

The cumulative incidences of all 11 categories of COD were calculated within a competing risks framework, for the pre-HAART and HAART eras separately [12]. Non-parametric estimates of cumulative transition probabilities from seroconversion to one of the competing COD were calculated using the multiple decrement model transition programme.

We also performed multivariate regression analyses for the rate of progression to the different COD [11,13] taking into account competing risks, allowing for late entry and adjusted for potential confounders. An administrative censoring time was created for each person who died of a COD that was not the COD of interest as this would be the date the person would have theoretically been alive at. Therefore, persons were censored at 1 January 1997 if they had died before that date from a COD that was not the COD of interest or at the cohort censoring date if they had died after it.

We evaluated the effect of calendar time within each risk group, as well as the interaction between risk group and calendar time for each COD. Hazards ratios were only calculated for COD with more than five deaths. To reduce the chance of a type 1 error as a result of multiple testing, we also calculated 99% confidence intervals (CI). All models were adjusted for sex, age at seroconversion,

and presentation during acute HIV infection (defined when the interval between the last HIV-negative and first HIV positive test was less than 1 month [14]).

To evaluate the impact of HAART on the relationships between the CD4 cell count, HIV RNA level and each specific COD, the cause-specific hazard ratios (CHR) were estimated using a Cox proportional hazards model for progression from HIV seroconversion to each cause. In this analysis these markers were evaluated as continuous variables (after square-root and logarithmic transformation, respectively) and were treated as time-dependent covariates. If the interval between two consecutive marker measurements or between the last measurement and either death or end of follow-up exceeded 18 months, individuals were temporarily removed from the risk set from the 18-month point until the date of their next available measurement.

Finally, a sensitivity analysis was conducted by excluding data from all cohorts in which more than 25% of the COD were unknown.

Results

The median age of the 7680 seroconverters included in the analyses was 29 years [interquartile range (IQR), 24–35] and 81% were male. Sex between men was the most frequent exposure category (49%) followed by IDU (25%), sex between men and women (15%) and haemophilia (3%). A total of 1938 of the 7680 seroconverters died during follow up (26%); a specific COD was available for 1437 of these deaths (74%, Table 1). Of these 1938 deaths, 248 patients had more than one COD and 1424 of the deaths (72%) occurred in individuals who had been diagnosed with AIDS.

Cumulative incidences

The cumulative incidence of all cause mortality was 0.10 at 5 years and 0.44 at 10 years following seroconversion in the pre-HAART era. The cumulative incidences of all cause mortality were 0.05 at 5 years and 0.15 at 10 years in the HAART era (Fig. 1). For those who became infected after the introduction of HAART, and were, therefore, potentially able to fully benefit from HAART, the 5-year cumulative incidence of all cause mortality was 0.02.

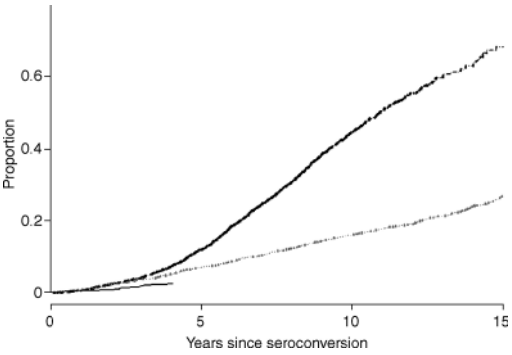

Fig. 1. Overall mortality in the pre-HAART and HAART era in the CASCADE collaboration. Black line represents the mortality in the pre-HAART era. The grey line represents the mortality in the HAART era among those who seroconverted before the introduction of HAART, dark grey line represents the mortality among those who seroconverted after HAART.

Cumulative incidences for each specific COD are shown in Fig. 2a and b. In the pre-HAART era, AIDS OI was the most common cause, followed by unknown causes, HIV/AIDS not further specified and other infections. A small proportion (3% at 15 years following seroconversion, 95% CI, 2–4) died from hepatitis/liver-related causes (Fig. 2a).

In contrast, in the HAART era (Fig. 2b), unknown deaths was the most common category. All AIDS-related COD decreased by at least 50% in the HAART era. At 15 years after seroconversion, the cumulative probability of dying from AIDS OI decreased from 20% (95% CI, 17–23) in the pre-HAART era to 5% in the HAART era (95% CI,

Table 1. Causes of death among 1938 HIV-seroconverters in CASCADE who died, in the pre-HAART and HAART eras.

	All deaths	Pre-HAART (pre-1997)	HAART (1997 onwards)
AIDS-related causes of death			
AIDS related malignancy	126 (6.5)	93 (6.5)	33 (6.4)
AIDS opportunistic infections	550 (28.3)	451 (31.7)	99 (19.3)
AIDS/HIV not further specified	155 (8.0)	142 (10.0)	13 (2.5)
Non-AIDS related causes of death			
Hepatitis/liver related death	97 (5.0)	46 (3.2)	51 (9.9)
Other infections	184 (9.5)	123 (8.6)	61 (11.9)
Organ failure	22 (1.1)	16 (1.1)	6 (1.2)
Non-AIDS related malignancy	60 (3.1)	35 (2.5)	25 (4.9)
(un)-intentional death	198 (10.2)	116 (8.1)	82 (16)
Cardiovascular disease/diabetes mellitus	40 (2.1)	18 (1.3)	22 (4.3)
Other	5 (0.3)	3 (0.2)	2 (0.4)
Unknown	501 (25.9)	381 (26.8)	120 (23.3)
Total	1938	1424	514

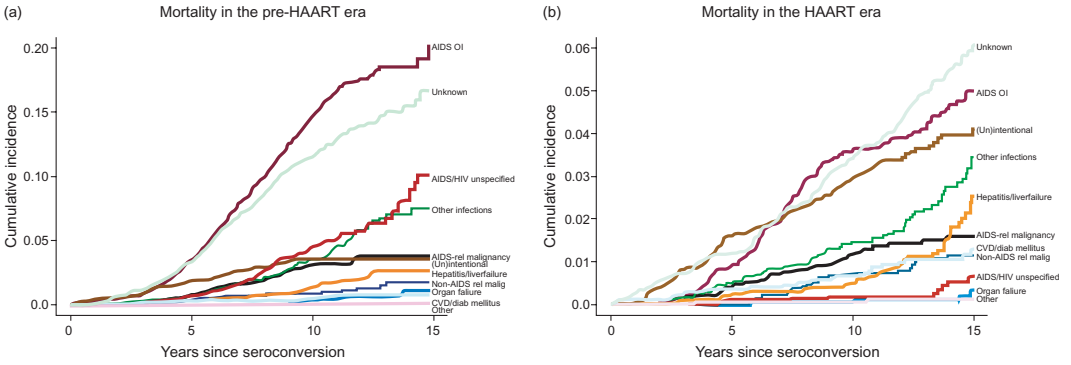

Fig. 2. (a) Cumulative incidences in the CASCADE collaboration of each specific cause of death (COD) in the pre-HAART era within a competing risks frame work, according to time from seroconversion. (b) Cumulative incidences in the CASCADE collaboration of each specific COD in the HAART era within a competing risks frame work, according to time from seroconversion. CVD, cardiovascular disease: OI, opportunistic infection.

4–6). However, AIDS OI remained the most important known COD. HIV/AIDS not further specified became a less important COD in the HAART era. Although the percentage of persons dying from AIDS-related malignancies is small, it also halved in the HAART era, in comparison with the pre-HAART era. Although the probability of dying from hepatitis or liver-related causes did not change in the era after HAART was introduced, the probability of dying from other infections decreased from 8% at 15 years following seroconversion (95% CI, 6–9) in the pre-HAART era to 3% (95% CI, 3–4) in the HAART era. Death from organ failure also showed a large reduction, from 1% in the pre-HAART era to 0.4% in the HAART era.

Cumulative risks

We compared the risk of dying from each specific COD in the pre-HAART era with the risk of dying from that COD in the HAART era. The effect of calendar time was not the same for each risk group. Therefore, the relative hazards for each risk group are shown separately in Fig. 3. Within each risk group the risk of dying in the HAART era is compared with the risk of dying from the same cause in the pre-HAART era (left column of Fig. 3). We also compared the risk of dying among IDUs, heterosexuals and haemophiliacs to that among men who have sex with men (MSM), separately for the pre-HAART and HAART eras (middle and right column).

Changes in risks of dying in the HAART era within each risk group

Among MSM, there was a reduction in the cumulative risk of dying from most COD in the HAART era in comparison with the pre-HAART era. Large cumulative risk reductions were seen for AIDS-related malignancies, AIDS OI and the unknown categories. All reductions in cumulative risks were significant at the 5% level, with the exception of organ failure. Although not significant, the cumulative risk of dying from organ failure strongly decreased. However, a small non-significant increase in the cumulative risk of dying from hepatitis/liver-related causes and CVD/diabetes mellitus was seen.

Among IDU, a significant reduction was observed in the cumulative risk of death from AIDS OI and a large significant reduction was seen in the cumulative risk of death from non-AIDS-related malignancy. The cumulative risks of dying from AIDS-related malignancies, other infections and organ failure were all reduced, but these reductions were not significant. For the organ failure category, the cumulative risk strongly reduced. However, the cumulative risk of dying from (un)-intentional death increased significantly, whereas a non-significant increase in the cumulative risk of dying from hepatitis/liver-related causes was seen.

In heterosexuals, strong reductions in the cumulative risk of dying from AIDS-related malignancies, AIDS OI, other infections, hepatitis/liver-related death and (un) intentional death were seen, although the effect was not significant for AIDS-related malignancies and hepatitis/liver-related causes. A non-significant increase was seen for the cumulative risks of dying from non-AIDS-related malignancies and CVD/diabetes mellitus.

Finally, among haemophiliacs, no significant reductions or increases in the risks of dying were seen, although there was a large increase in the cumulative risk of dying from hepatitis/liver-related causes, organ failure and non-AIDS-related malignancies.

Comparison of the risk of death between risk groups

In the pre-HAART era, we observed a significantly higher cumulative risk of death among IDUs in comparison with MSM, from AIDS OI, hepatitis or

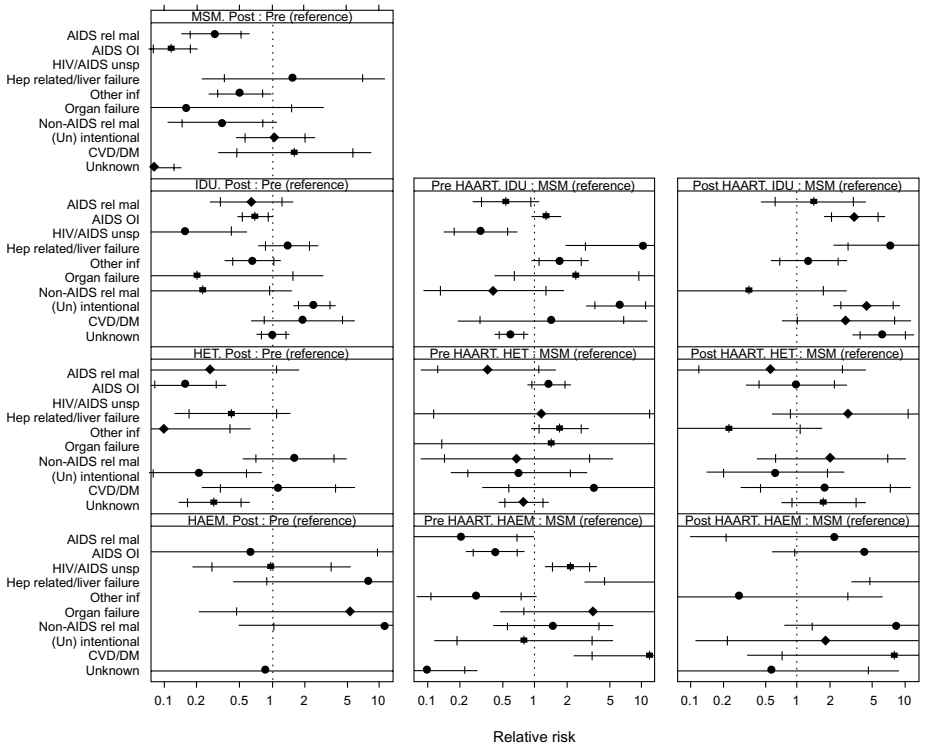

Fig. 3. The cumulative risk (CR) for each cause of death (COD). CR with their 95% (dashes) and 99% (line) confidence intervals are estimated within each risk group and in the pre-HAART and HAART eras separately, and compared with the risk of dying from each COD in injection drug users, heterosexuals and haemophiliacs to those in men who have sex with men. CVD, cardiovascular disease; DM, diabetes mellitus; HAEM, haemophiliac; HET, heterosexual; IDU, injection drug users; MSM, men who have sex with men; OI, opportunistic infection.

liver-related deaths, other infections and (un)-intentional death. A significantly cumulative lower risk was observed for AIDS and non-AIDS-related malignancies, unspecified HIV/AIDS-related deaths and unknown in IDUs. In the HAART era, IDU had a higher cumulative risk of death in comparison with MSM for all COD, except non-AIDS-related malignancies. The cumulative risks of death from AIDS OI, hepatitis/liver-related causes, (un)-intentional causes and unknown COD were significantly higher. Pre-HAART, heterosexuals had a significantly higher cumulative risk of death from other infections in comparison with MSM, whereas no significant differences were seen in the cumulative risks of death from other specific COD. In the HAART era, no significant differences were found in the cumulative risks of each specific COD between heterosexuals and MSM.

In the pre-HAART era, haemophiliacs had a significantly lower cumulative risk of death from AIDS-related malignancies, AIDS OI, other infections and unknown COD in comparison with MSM. The cumulative risk of dying from unspecified HIV/AIDS-related causes, hepatitis/liver-related causes and CVD/diabetes mellitus increased. After HAART became available, the cumulative risk of death from hepatitis/liver-related causes remained significantly higher.

Association between laboratory markers and specific causes of death

All COD showed a significant relationship with lower CD4 cell counts, except (un)-intentional death in the pre-HAART era (Table 2).

For almost all COD, the relationship with the CD4 cell count did not differ significantly in the HAART era, compared to the pre-HAART era. However, the relationships between the CD4 cell count and the risk of dying from unknown COD was significantly weaker in the HAART era.

Table 2. Cause specific relative hazards (CHR) and 95% confidence intervals (CI) for the relationship between death from each specific cause and the most recent CD4+ cell count and HIV RNA level.

	AIDS-related malignancy	AIDS opportunistic infection	HIV/AIDS unspecified	Non-AIDS-related malignancy	Other infections	Hepatitis/liver-related deaths	Organ failure	(Un) intentional	Cardiovascular disease/ diabetes mellitus	Unknown
Pre HAART: CD4 cell count[a]	0.80 (0.77–0.83)	0.76 (0.74–0.77)	0.75 (0.72–0.78)	0.89 (0.85–0.94)	0.77 (0.74–0.80)	0.89 (0.84–0.92)	0.83 (0.76–0.91)	0.98 (0.95–1.01)	0.92 (0.85–0.99)	0.81 (0.79–0.83)
Post- HAART: CD4 cell count[a]	0.81 (0.77–0.86)	0.74 (0.70–0.78)	0.67 (0.58–0.79)	0.86 (0.81–0.92)	0.81 (0.77–0.85)	0.90 (0.85–0.94)	0.83 (0.70–0.98)	0.95 (0.92–0.99)	0.89 (0.82–0.97)	0.86 (0.83–0.89)
P-value[b]	0.51	0.12	0.26	0.22	0.06	0.61	0.98	0.11	0.58	0.03
Pre-HAART: HIV RNA[c]	1.26 (0.64–2.45)	3.38 (2.28–5.01)	–[d]	1.38 (0.62–3.09)	3.24 (1.18–8.90)	2.64 (1.01–6.90)	0.33 (0.05–2.12)	–[d]	0.93 (0.26–3.32)	3.79 (1.94–7.40)
Post-HAART: HIV RNA[c]	1.70 (1.20–2.42)	4.03 (2.72–5.95)	1.18 (0.95–1.46)	1.27 (0.91–1.78)	1.82 (1.42–2.33)	1.37 (1.09–1.73)	1.85 (0.86–3.99)	1.18 (0.95–1.46)	1.08 (0.70–1.67)	1.54 (1.26-1.87)
P-value[b]	0.38	0.41		0.70	0.44	0.36	0.05		0.65	0.02

Analyses are adjusted for sex, age at seroconversion, risk group and presentation with acute infection. CD4 and HIV RNA are considered as time updated factors.
[a]Per 100 cells/μl higher).
[b]For the difference between pre-HAART and HAART era.
[c]Per $\log_{10}$ copies/ml higher. –[d]Results not presented due to small numbers.

Fewer HIV RNA data were available in the pre-HAART era, due to the lack of availability of these tests at that time. None of the relationships between the COD and higher HIV RNA levels differed between the pre-HAART and HAART eras, with the exception of the relationship between HIV RNA levels and unknown COD, which was weaker in the era of HAART (Table 2). The relationship between organ failure and HIV RNA levels became stronger in the era of HAART ($P = 0.05$).

Discussion

This study, conducted among a large group of HIV-infected individuals with well-estimated dates of seroconversion followed in the pre-HAART and HAART eras, demonstrates clearly that the probability of dying from most causes of death decreased substantially after HAART became widely available. Nevertheless, although the cumulative incidence of death from all AIDS-related causes decreased in the era of HAART, AIDS OI still remained the most common COD.

Although individuals in the pre-HAART era may have been infected with HIV for longer periods of time than those in the HAART era, it is unlikely that our findings can be explained by differences in duration of infection between those followed in the in the two time periods because we were able to adjust our analyses for the time since seroconversion.

However, the population in which mortality can be estimated in the first years following the introduction of HAART does inevitable include many individuals infected in the pre-HAART era, some of whom would have had limited opportunity to benefit from HAART, for example due to very low CD4 cell counts at HAART initiation [15] or prior use of sub-optimal treatments [16]. For those who did become infected in the HAART era, however, who had the opportunity to fully benefit from HAART, we found that mortality was even lower than the wider population at risk in the HAART era, suggesting that further reductions in mortality in HIV-infected persons may emerge over time [15].

A reduction in AIDS-related mortality at the population level following the widespread use of HAART has been shown in earlier studies [17–21]. However, our study is the first to show a general reduction in the risk of dying from specific causes in seroconverters in the era of HAART. Interestingly, the benefits of HAART appear to be less strong in IDU across the specific causes. Furthermore, the cumulative risk of dying from (un) intentional causes significantly increased among IDU in the HAART era. One reason could be temporal changes in other risk factors (e.g. injecting behaviour). However, as fewer IDU are now dying from the other specific causes

investigated in our study, more IDU remain at risk of dying from (un) intentional causes, therefore in the HAART era more IDU will die from this cause leading to an increase in the cumulative risk. In line with a study from the pre-HAART era these deaths are not associated with having lower CD4 cell counts [22]. All other COD, including non-AIDS-related COD, were associated with lower CD4 cell counts, with virtually no differences in the association with CD4 cell count between the HAART and pre-HAART eras. Hence all COD, other than (un)intentional deaths, appeared to be related in some way to the underlying immunodeficiency caused by HIV infection regardless of their putative association with AIDS itself. Similarly, the relationship between HIV RNA levels and the risk of all COD remained virtually unchanged in the HAART era, although this relationship did become slightly weaker for unknown COD, perhaps suggesting that many unknown causes in the pre-HAART era were, in fact, HIV-related.

Other studies have shown that hepatitis/liver-related deaths and malignancies are now the most common non-AIDS-related COD among HIV-infected individuals [10]. Among HIV-infected individuals who died, the proportion of deaths accounted for by these COD and CVD have increased since 1996 [23]. Although we found that the risk of dying from CVD increased, (un)-intentional death, other infections and hepatitis/liver-related deaths were the most common non-AIDS-related COD in the HAART era in our study. We believe this highlights one of the limitations of conducting studies based only on persons who have died, since these cannot account for the substantially larger number of persons surviving in the HAART era. Our study shows a trend towards a higher probability of dying from hepatitis/liver-related causes in the HAART era among most risk groups, in line with other studies [24,25], and most likely because large numbers of patients survive to experience this competing COD. However, this finding is not supported by Qurishi *et al*. [26], most likely due to survivorship bias not accounted for in their analyses [27].

Since HAART became generally available, several studies have demonstrated reductions in the incidence of AIDS-related malignancies among HIV-infected persons [28,29] and we found parallel reductions in the incidence of death from AIDS-related malignancies. However, we observed a small non-significant increase in the risk of death from non-AIDS-related malignancies in the HAART era among heterosexuals in our study. Furthermore, although cancer rates among those with HIV have not markedly increased in the HAART era [30], HIV-infected individuals do have an increased risk of non-AIDS-related cancers in comparison with the general population [31].

One limitation of our study is the lack of uniform classification of COD within cohorts that participate in the CASCADE collaboration with each cohort using their own classification. Differences in COD distribution were observed when comparing the cohorts that reported one COD with the cohorts that reported multiple causes. In particular, cohorts with one COD were more likely to report unspecified AIDS/HIV-related causes than cohorts with multiple COD, suggesting that deaths were allocated a COD according to a hierarchy. This difference in process might cause some bias and it is possible that the proportion of deaths due to AIDS/HIV-related causes may be underestimated. A recent study has shown that the lack in precise information about the COD might result in misinterpretation of results [32], so a standardized classification of COD among HIV-infected persons is needed The recent CoDe project provides such a uniform system for collecting and classifying the COD (www.cphiv.dk/CoDe) and this may help to further improve the quality of reporting in the future and allow monitoring of trends in COD. Another limitation of our study is the relatively large proportion of deaths from unknown causes. However, a sensitivity analysis restricted to cohorts in which less than 25% of the deaths were from unknown causes showed no major differences in findings, except that in the era of HAART, the cumulative incidence of hepatitis/liver-related deaths became somewhat higher. This suggests that hepatitis/liver-related deaths might become even more important as classification of deaths improves in the future.

In conclusion, both overall mortality as well as cause-specific mortality has substantially decreased following the introduction of HAART. However, AIDS OI remain the most common COD in the era of HAART, suggesting that AIDS-related events will continue to be an important cause of death in the future. Since the time after HAART became available is limited and the majority of HIV-infected individuals in our study became infected in the pre-HAART era, it might be too early to observe an increase in non-AIDS-related death due to therapy-related toxicities. Therefore monitoring mortality trends among HIV-infected individuals using standardized international guidelines for the coding of deaths among HIV-infected persons will be essential for the future. Furthermore, the development of prevention measures and treatment guidelines for non-AIDS-related conditions aimed specifically at HIV-infected individuals is warranted.

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Appendix

CASCADE collaboration

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Steering committee: Valerie Beral, Roel Coutinho, Janet Darbyshire (project leader), Julia Del Amo, Noël Gill (chairman), Christine Lee, Laurence Meyer, Giovanni Rezza.

Co-ordinating centre: Kholoud Porter (scientific co-ordinator), Abdel Babiker, A. Sarah Walker, Janet Darbyshire, Krishnan Bhaskaran.

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Hatzakis, Anastasia Karafoulidou, Olga Katsarou; Edinburgh Hospital cohort, United Kingdom: Ray Brettle; Madrid cohort, Spain: Julia Del Amo, Jorge del Romero; Amsterdam Cohort Studies among homosexual men and drug users, the Netherlands: Liselotte van Asten, Akke van der Bij, Ronald Geskus, Maria Prins, Roel Coutinho; Copenhagen cohort, Denmark: Court Pedersen; Valencia IDU cohort, Spain: Ildefonso Hernández Aguado, Santiago Pérez-Hoyos; Oslo and Ulleval Hospital cohorts, Norway: Anne Eskild, Johan N Bruun, Mette Sannes; Royal Free haemophilia cohort, United Kingdom: Caroline Sabin, Christine Lee; UK Register of HIV Seroconverters, United Kingdom: Anne M Johnson, Andrew N Phillips, Abdel Babiker, Janet H Darbyshire, Noël Gill, Kholoud Porter; Swiss HIV cohort, Switzerland: Patrick Francioli, Philippe Vanhems, Matthias Egger, Martin Rickenbach; Sydney AIDS Prospective Study, Australia: David Cooper, John Kaldor; Sydney Primary HIV Infection cohort, Australia: David Cooper, John Kaldor, Tim Ramacciotti, Don Smith; Badalona IDU hospital cohort, Spain: Roberto Muga, Jordi Tor; Lyon Primary Infection cohort, France: Philippe Vanhems; South Alberta clinic, Canada: John Gill; Barcelona IDU cohort, Spain: Joan Cayla, Patricia Garcia de Olalla.

2.3

Mortality

Risk of hepatitis related mortality increased among HCV/HIV-co-infected drug users compared to drug users infected only with HCV: a 20-year prospective study

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The Amsterdam Cohort Studies on HIV infection and AIDS, a collaboration between the Amsterdam Health Service, the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation and the University Medical Center Utrecht, are part of the Netherlands HIV Monitoring Foundation and financially supported by the Netherlands National Institute for Public Health and the Environment.

There are no competing interests to declare.

Abstract

Background: Progression of liver related disease is accelerated in individuals co-infected with HIV and hepatitis C virus (HCV). Since the life expectancy of HIV-infected drug users (DU) improved after the widespread use of highly active anti-retroviral therapy (HAART), HCV related death is likely to become more important.
To disentangle the effects of HCV and HIV, we compared the overall and cause-specific mortality between HCV/HIV-infected DU and both HCV-infected DU and DU without HCV or HIV, followed up between 1985-2006.
Methods: 1276 participants in the Amsterdam Cohort Study were included. Cause specific hazard ratios (CHR) were estimated for the eras before (<1997) and since HAART (≥1997) within and among serologic groups.
Results: The risk of dying decreased for most causes of death (COD) upon the introduction of HAART, this decrease was not the same for the different serologic groups. Among HCV/HIV co-infected DU, the risk of hepatitis/liver-related death did not substantially change over time (CHR: 0.87, 95% CI:0.21-3.58), whereas the risk of AIDS-related mortality decreased.
Compared to DU solely infected with HCV, HCV/HIV-co-infected DU were at increased risk of dying from hepatitis/liver related disease (CHR: 7.15, 95% CI: 1.98-25.8), other natural causes (CHR: 3.09, 95% CI: 1.41-6.79), and non-natural causes (CHR: 2.30, 95% CI: 1.07-4.95) in the HAART era.
Conclusion: HCV/HIV-co-infected DU remain at increased risk of dying from hepatitis/liver-related death in the HAART-era compared to HCV-mono-infected DU. This risk did not change in HCV/HIV-co-infected DU after HAART was introduced, suggesting that in the HAART era, HIV continues to accelerate HCV disease progression. Efforts should be made to establish effective treatment for HCV infection in HCV/HIV-co-infected individuals.

Introduction

The progression of liver disease associated with hepatitis C (HCV) is known to be accelerated in HIV-infected persons (1). In the mid-nineties, highly active anti-retroviral therapy (HAART) became available and improved their survival. In the HAART era, mortality from HCV is therefore likely take on a greater significance among these individuals (2), and several studies have indeed shown an increase in liver related deaths (3) (4) (5) (6). Drug users (DU) with HIV also benefit from HAART at the population level, although their improvement is less than observed for heterosexual and homosexual individuals (7) (8). Since HIV-infected DU are almost universally co-infected with HCV, HCV/HIV co-infection has a major impact on their mortality (9). Indeed, an earlier study showed that the risk of dying from hepatitis and liver related disease among HIV-infected DU is increased in the HAART era compared to the pre-HAART era (10).
The Amsterdam Cohort Study (ACS) among DU include non-HIV-infected DU and HIV-infected DU, with or without HCV-co-infection, information on causes of

death is available, providing the unique opportunity to disentangle the effects of HIV and HCV on cause-specific mortality in DU with one or both virusses. In addition, their mortality can be compared with that of uninfected DU who are nevertheless at increased risk of dying compared to the general population.
In the present study, we therefore compared the cause-specific mortality between HCV/HIV-co-infected DU, HCV-mono-infected DU and DU without HCV or HIV. They were followed between 1985 to 2006, to compare the pre-HAART and HAART-era.

Methods

Study population
The prospective ACS among drug users began in December 1985 and is still ongoing (11), with 1640 DU included as of 1 January 2006. Recruitment is via local methadone outposts, sexually transmitted diseases clinics, and word of mouth. Both injecting and non-injecting DU are invited to participate.
DU return for their ACS follow-up visit every 4-6 months at the Health Service of Amsterdam. At each visit, a standardised questionnaire is administrated by trained nurses and blood is drawn for laboratory testing and storage. HIV-positive DU undergo a clinical examination by a physician.
The ACS has been conducted in accordance with the ethical principles set out in the declaration of Helsinki and written informed consent is obtained prior to data collection.

Serological testing
Following each ACS visit, blood samples are prospectively tested for HIV antibodies by enzyme-linked immunosorbent assay (ELISA), and positive results are confirmed using Western blot (since 1995: HIV Blot version 2.2, Genelab diagnostics). In the present study, stored samples from DU with at least two cohort visits were retrospectively tested for HCV antibodies, starting with the blood samples collected at the first cohort visit in each case. A third generation ELISA assay was used to detect HCV antibodies (Axsym HCV version 3.0; Abbott, Wiesbaden, Germany). DU who were HCV-negative at their first cohort visit were tested for HCV-antibodies at their last cohort visit. If this blood sample was HCV positive, samples taken in between the first and last visit were tested to identify the approximate moment of seroconversion.

Specific Causes of death
Information about the vital status was obtained by matching the ACS data against the local and national registries.
To obtain information on the cause of death (COD), we reviewed medical records from the hospitals, methadone clinics, and general practitioners.
Causes were grouped into 5 categories: AIDS/HIV related, HCV/liver related, non-natural causes of death (including: overdose, accidents, suicide and homicide), natural causes and unknown. When more than one cause of death was recorded,

the most likely cause was scored according to the following hierarchy: non-natural as most likely, followed by AIDS/HIV related, HCV/liver related and natural.

Statistical analyses
Of the 1640 DU participating in the ACS, 1276 had at least 2 cohort visits and were included in this study. Follow-up was calculated from ACS entry until the earliest of the following: death, one year after the last visit, or censoring date 1 January 2006. Using calendar time as a proxy for the introduction and widespread use of HAART, we defined two calendar periods: pre-1997 and 1997 onwards, to reflect the pre-HAART and HAART eras, respectively.
Four serologic groups were defined: 1) HCV+/HIV+, 2) HCV+/HIV-, 3) HCV-/HIV- and 4) HCV-/HIV+. Individuals could switch between groups (time updated covariate) when they acquired an infection during follow-up.
The date of HIV or HCV seroconversion was estimated as the midpoint between the last seronegative and first seropositive ACS visit.
Using the Kaplan Meier method, we estimated the time from ACS entry to death by any cause for each serologic group. Cause-specific hazard ratios (CHR) were estimated within and between serologic groups using a Cox proportional hazards model. All analyses were adjusted for age, sex, hepatitis B status at ACS entry and duration of injecting. The confounding effect of current injecting and homelessness was also evaluated. All variables subject to change, such as age, duration of injection and current injecting, were treated as time-updated variables. The number of individuals (n=17) and deaths (n=5) for the HCV-/HIV+ serologic group was too small to estimate the CHR. Also in the HCV-/HIV- group and HCV-/HIV- group the number of deaths for some specific COD was too small to estimate the CHR. Finally, a sensitivity analysis was conducted by excluding those DU who had never injected drugs.

Results

Baseline characteristics of the DU are presented in table 1. The median age of the 1276 DU with at least two cohort visits was 30 years, and 64% were male. At baseline, 621 DU (72%) had ever injected drugs, and 31 non-injecting DU started to inject during follow up. At ACS entry, 20% had an HCV/HIV co-infection, 44% were mono-infected with HCV, 36% of the DU were not infected with HIV or HCV, and 1% were solely infected with HIV. During follow-up, 95 HIV and 59 HCV seroconversions occurred, and 272 DU died. A specific COD was available for 252 death cases.
HCV/HIV-co-infected DU and HCV-mono-infected DU were more often from Dutch origin, had higher anti-HBc prevalence rates, and had more often a history of injecting drugs.

Overall mortality

The all cause mortality was highest among DU infected with both HCV and HIV: after 10 years of follow-up, 49% [95% confidence interval (CI): 42-54] had died (figure 1). HIV-mono-infected DU show a slightly lower death rate: 10 years after ACS entry 43% [95% CI: 2-66] had died. All-cause mortality was lowest among DU without HIV or HCV infections and among those with mono HCV infection 7% [95% CI: 3-11] and 13% [95% CI: 10-16] had died, respectively, after 10 years of follow-up.

Risk of dying

We compared the risk of dying from each specific COD in the pre-HAART era with the HAART era. Overall, the risk of dying decreased in the HAART era for almost all COD, but the effect of calendar time was not the same for each serologic group. Therefore the CHR and their 95% CI are shown for the serologic groups separately in figure 2. Within each group the risk of dying in the HAART era is compared to that in the pre-HAART era (left column of figure 2). As we wanted to know the impact of HCV infection on mortality, we likewise compared separately the risk of dying from specific causes among the co-infected and uninfected DU (HCV+/HIV+ and HCV-/HIV-) with the risk among HCV-mono-infected DU (reference) for the pre-HAART era (middle column of figure 2) and HAART era (right column of figure 2).

Changes in the risk of death within serologic groups

In the HAART era compared to the pre-HAART era, in DU infected with both HCV and HIV, we observed a significant reduction in the risk of dying from AIDS-related death (CHR adjusted for age, sex, hepatitis B status at ACS entry and duration of injection: 0.37, 95% CI: 0.19-0.72) (figure 2, left column, middle panel). In this group the risk of dying from liver related death did not significantly change (CHR: 0.87, 95% CI: 0.21-3.58).
No significant reductions in the risk of dying for all COD were observed in HCV-mono-infected and the uninfected serologic groups. The risk of dying specifically from hepatitis or liver-related death could not be estimated for DU solely infected with HCV because, no hepatitis/liver-related deaths were observed in the pre-HAART era.

Comparison of the risk of death among serologic groups:

When comparing the risk of dying in HCV/HIV-co-infected DU with the risk of dying among HCV-mono-infected DU, those co-infected had a significantly higher risk of dying from non-natural COD (CHR: 3.03, 95% CI: 1.22-7.58) in the

pre-HAART era. The same was true for the HAART era (CHR: 2.30, 95% CI: 1.07-4.95).
In the HAART era, the co-infected DU had a significantly higher risk of dying from hepatitis/liver-related death than HCV-mono-infected DU (CHR: 7.15, 95% CI: 1.98-25.8) and from natural COD (CHR: 3.09, 95% CI: 1.41-6.79). No major differences were seen between DU without infections and HCV-mono-infected DU, except that in the pre-HAART era, the non-infected DU had a non-significantly lower risk of dying from natural COD (CHR: 0.58, 95% CI: 0.13-2.60).

Adjustment for homelessness and current injecting did not affect the results. When including ever-injectors only (n=952) in a sensitivity analysis, we found that the risk of dying from hepatitis/liver-related disease within the HCV/HIV-co-infected group was somewhat higher in the HAART era than in the pre-HAART era (CHR: 1.23, 95% CI: 0.18-8.26) when compared to the CHR in the total study population. However, the effect remained non-significant. In the HAART era, the increased risk of dying from hepatitis/liver-related disease was smaller than observed in the total population for co-infected DU compared to HCV-mono-infected (CHR: 3.94, 95% CI: 0.59-26.22). The other CHRs were comparable to the results in the total population.

Discussion

This study describes the cause-specific mortality in a large group of DU over a 20-year period. The risk of dying was highest among DU solely infected with HIV or co-infected with HCV/HIV. Although the risk of dying substantially decreased for almost all causes in the HAART era, the decrease was not the same for all serologic groups. The risk of dying from hepatitis/liver-related disease did not change significantly over time among HCV/HIV co-infected DU, but this study demonstrates a strongly increased risk of their dying from hepatitis/liver-related disease compared to DU solely infected with HCV in the era of HAART. This suggests that in the HAART-era HIV co-infection continues to accelerate HCV disease progression.

One might argue that DU have not benefited from HAART, but comparing the risk of dying among DU infected with HCV and HIV between the pre-HAART and HAART-era shows that the risk of dying from AIDS-related causes decreased over time. This is in line with other studies (12) (10) and indicates that DU indeed benefit from HAART, although their uptake of HAART is lower than seen in other HIV risk groups (13) (14).
Although several studies have shown an increase in liver-related mortality among HIV-infected individuals in the HAART era (15) (4) (5) (6) (10), the impact of HCV co-infection on HIV disease progression remains contradictory (16) (17) (18). When mortality was compared between DU infected with HCV and/or HIV and DU without an infection, one study found higher overall mortality rates among HCV/HIV co-infected DU versus non HCV/HIV infected DU (9), but did not

compare cause-specific mortality. In an earlier study, no liver-related deaths occurred among HCV-mono-infected individuals (19), whereas 10% of the HCV/HIV-co-infection DU developed liver decomposition. However, this study was analysed cross-sectionally. In the present longitudinal ACS study, we had the unique opportunity to evaluate the effect of the time-updated HIV and HCV status of all DU on cause-specific mortality. In addition, we were able to correct for duration of injecting drugs, which served as a proxy for duration of HCV infection since most DU get infected with HCV within 2 year after start injecting (20).

The results of this study show an increased risk of dying from hepatitis and liver-related death in the era of HAART among those infected with both HIV and HCV, compared to those solely infected with HCV. Theoretically, the increase could be explained by HBV infection, which was highest among those DU who were HCV/HIV co-infected. However, we adjusted for anti-Hbc status at study entry. This adjustment may be a limitation of the study, since an anti-Hbc positive test result is a marker for past HBV infection but not for chronic HBV infection. In the general population, 5-10% of the HBV infections become chronic, whereas a higher percentage become chronic in HIV infected individuals (21). Therefore we might have overestimated the effect anti-Hbc, but this overestimation would be smaller for those DU infected with HIV.

In this study, HCV treatment was not taken into account, but it occurred very sporadically and only recently in our cohort (1%) and would therefore only marginally affect our results.

The risk of dying from non-natural causes (i.e. overdose, suicide, homicide and accidents) was increased in HCV/HIV-co-infected DU compared to HCV-mono-infected DU in both the pre-HAART and HAART eras, whereas no differences were seen between HCV-mono-infected DU and DU without HCV and HIV. This finding suggests that HCV/HIV-co-infected DU had been taking more risk in general with respect to drug use.

Although the impact of HCV co-infection on HIV disease progression is still debated, this study shows higher all cause mortality among HCV/HIV-co-infected DU than in our other serologic groups. When they are compared to HCV-mono-infected DU, their risk of hepatitis and liver-related death remains higher in the HAART era, suggesting that HIV continues to alter HCV disease progression. Although HCV treatment among HCV/HIV-co-infected individuals is complicated, our results highlights its importance and the need to establish effective treatment for HCV in HCV/HIV-co-infected individuals. We believe that daily observed therapy for DU with HIV and HCV is likely to increase their uptake, adherence, and therapy success.

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Table 1: Baseline and follow-up characteristics among drug users with at least two cohort visits in the Amsterdam Cohort Study, 1985-2006,

	Total	HCV+/HIV+	HCV+/HIV-	HCV-/HIV-	HCV-/HIV+
Baseline:					
Number (%)	1276 (100%)	256 (20%)	565 (44%)	457 (36%)	17 (1%)
Men (%)	828 (64%)	156 (61%)	340 (60%)	318 (70%)	14 (82%)
Age (median, IQR)	30 (26-36)	32 (28-36)	31 (27-36)	29 (26-35)	28 (26-33)
Dutch nationality	973 (76%)	206 (80%)	482 (85%)	274 (60%)	11 (64%)
Anti-Hbc antibodies	663 (51%)	213 (83%)	359 (64%)	85 (19%)	6 (35%)
Ever-injecting	921(72%)	250 (98%)	535 (95%)	127 (28%)	9 (53%)
Time since first injecting (median, IQR)	8 (4-13)	10 (6-14)	8 (4-14)	3 (0-8)	7 (4-12)
Homeless	123 (10%)	16 (6%)	46 (8%)	58 (13%)	3 (18%)
Follow-up:					
Follow-up time (median, IQR)	7 (4-13)	9 (5-14)	9 (5-14)	6 (3-11)	7 (4-12)
Total deaths	272	172	70	24	6
Causes of death: (%)					
AIDS related	75(28%)	70(40%)	0	0	5(83%)
Hepatitis related	21(28%)	18(10%)	3(4%)	0	0
Non-natural	86(32%)	37(22%)	39(56%)	10(42%)	0
Natural	70(26%)	36(22%)	20(29%)	13(54%)	1(17%)
Unknown	20(7%)	11(6%)	8(11%)	1(0.4%)	0
Seroconversions:					
HCV	59	15	44		
HIV	95	89			6

Mortality according to HIV/HCV antibody status

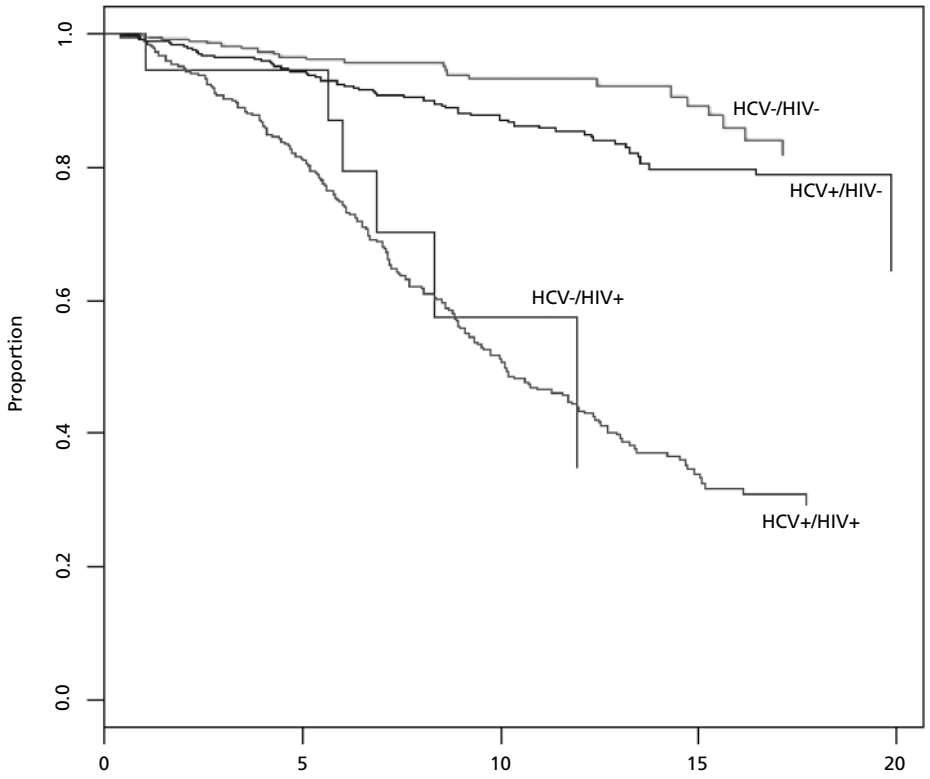

figure 1: All-cause mortality among HCV-/HIV- infected drug users, HCV-mono-infected drug users, HIV-mono-infected drug users, and those infected with both HIV and HCV.

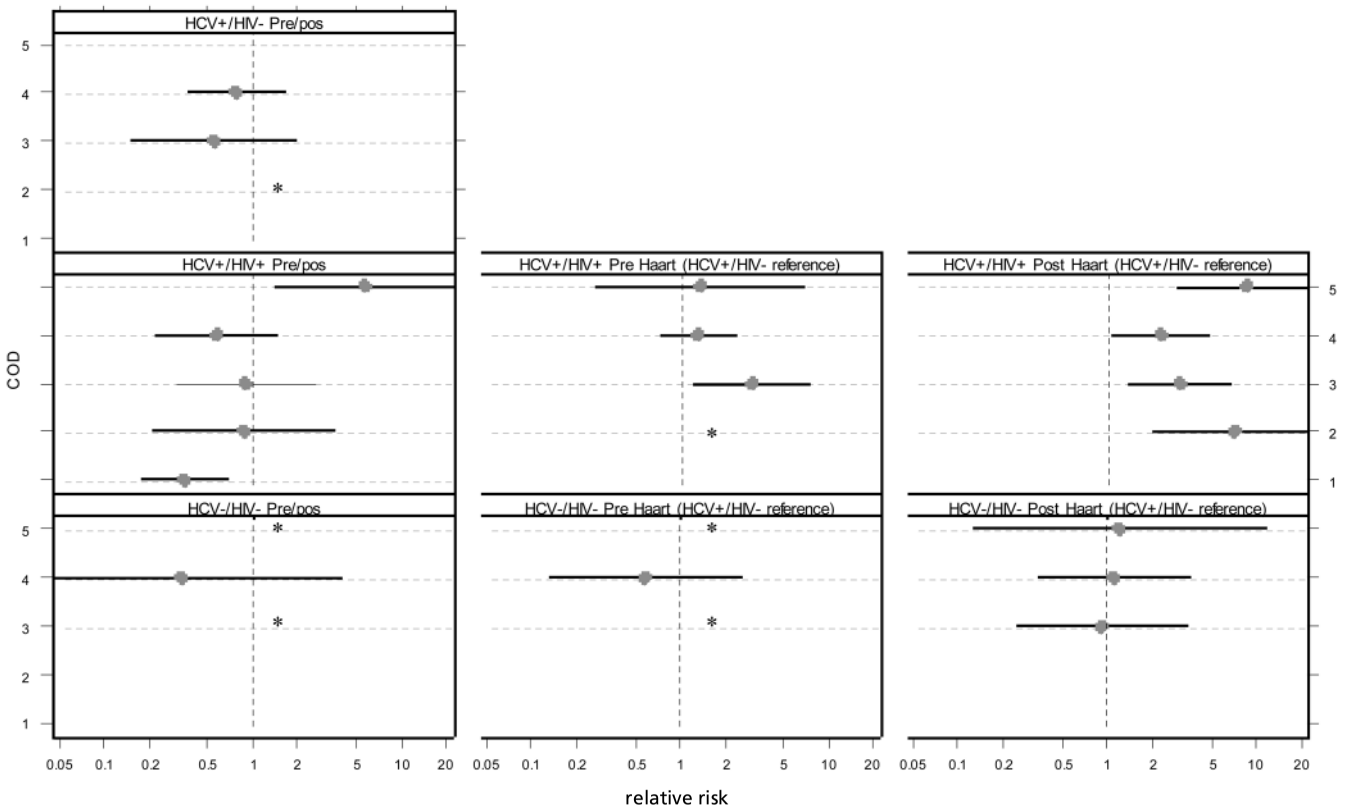

Figure 2: The adjusted cause-specific hazards (CHR) and their 95% confidence intervals for each cause of death. CHR are estimated within each serologic group (left column) and in the pre- and post HAART separately, compared to the risk of dying among HCV+/HIV- infected drug users (middle and right columns). 1: AIDS/HIV related causes, 2: Hepatitis/liver related causes, 3: non-natural causes, 4: natural causes, 5: unknown causes. *) Results not presented due to small numbers

3.1

HIV co-infections

Major decline of hepatitis C virus incidence rate over two decades in a cohort of drug users

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Abstract

Injecting drug users (DU) are at high risk for hepatitis C virus (HCV) and HIV infections. To examine the prevalence and incidence of these infections over a 20-year period (1985-2005), the authors evaluated 1276 DU from the Amsterdam Cohort Studies who had been tested prospectively for HIV infection and retrospectively for HCV infection. To compare HCV and HIV incidences, a smooth trend was assumed for both curves over calendar time. Risk factors for HCV seroconversion were determined using Poisson regression. Among ever-injecting DU, the prevalence of HCV antibodies was 84.5% at study entry, and 30.9% were co-infected with HIV. Their yearly HCV incidence dropped from 27.5/100 person years (PY) in the 1980s to 2/100 PY in recent years. In multivariate analyses, ever-injecting DU who currently injected and borrowed needles were at increased risk of HCV seroconversion (incidence rate ratio 29.9, 95% CI 12.6, 70.9) compared to ever-injecting DU who did not currently inject. The risk of HCV seroconversion decreased over calendar time. The HCV incidence in ever-injecting DU was on average 4.4 times the HIV incidence, a pattern seen over the entire study period. The simultaneous decline of both HCV and HIV incidence probably results from reduced risk behavior at the population level.

Introduction

The most important mode of hepatitis C virus (HCV) transmission is through exposure to infected blood (Memon et al 2002; van der Poel et al. 1989). Therefore injecting drug users (DU) are at high risk for HCV infection. Their main route of transmission is the sharing of needles or other injecting equipment (Hagan et al. 2001). In this population, the reported prevalences of HCV range from 40 to 85% in Europe and North America (Des Jarlais et al. 2003; Goldberg et al. 2001; Hope et al. 2001; Hutchinson et al. 2002; Lorvick et al. 2001; McCoy et al. 2004; Memon et al 2002; Smyth et al. 2003; Steffen et al. 2001).

Under the threat of AIDS, DU reduced their injecting risk behavior and consequently their incidence of HIV infection in the mid-1980s (van Beek et al. 1998; Nelson et al. 2002). However, their HCV incidence appears to be less affected by this decreased risk behavior, perhaps because HCV is more transmissible than HIV. This hypothesis is confirmed by several studies that show a high and stable prevalence of HCV antibodies in this population (Emmanuelli et al 2005; Fuller et al. 2004; Hernandez-Aguado et al. 2001; Judd et al. 2005). In recent years, we reported a high but declining HCV prevalence among young DU in Amsterdam (van de Laar et al. 2005), whereas others still report high and stable HCV incidence among young DU who have recently started injecting (Fuller et al. 2004; Hahn et al. 2002; Judd et al. 2005; Miller et al. 2002).

The open and ongoing Amsterdam Cohort Studies (ACS) among drug users started in 1985, and stored serum was retrospectively tested for HCV antibodies. Therefore, the ACS has the unique potential to present HCV incidence data for DU over two decades. The objectives of our study were to measure the HCV incidence

over this long period, to evaluate risk factors associated with HCV seroconversion, and to compare the HCV incidence to the HIV incidence in this cohort over the same period.

Materials and Methods

The ACS is an open, prospective cohort study initiated to investigate the prevalence, incidence, and risk factors of infections with HIV-1 and other blood-borne and/or sexually transmitted diseases, as well as the effects of intervention (van den Hoek et al. 1988). The DU cohort was initiated in 1985; recruitment is ongoing and in recent years has been directed in particular to young DU.
Participation in the ACS is voluntary, and informed consent is obtained for every participant at intake. ACS participants visit the Health Service of Amsterdam every 4-6 months. At every visit, they complete a standardized questionnaire about their health, risk behavior, and socio-demographic situation. Questions about current behavior refer to the period between the present and the preceding ACS visit. Questions at baseline refer to the period since 1980 or since the start of regular use of hard drugs. Blood is drawn for laboratory testing and storage.

Laboratory methods
To study HIV prevalence and incidence, all ACS participants since 1985 (n=1640) were prospectively tested for HIV antibodies by enzyme linked immunosorbent assays (ELISA), with confirmation by Western blot (since 1995: HIV Blot version 2.2, Genelab diagnostics).
To study the HCV prevalence and incidence, all participants with at least two visits between December 1985 and November 2005 (n=1276) were retrospectively tested for HCV antibodies, using the first sample available in each case. Third generation ELISA tests were used to detect HCV antibodies (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). Individuals who were HCV-negative at ACS entry were tested for HCV antibodies at their most recent ACS visit. On finding HCV seroconversion, samples taken in between these two visits were tested to identify the moment of seroconversion.

Statistical analyses
The date of HCV or HIV seroconversion was estimated as the midpoint between the last seronegative and the first seropositive ACS visit. The median duration of the HCV seroconversion interval between visits was 4.0 months, interquartile range (IQR) 3.7, 5.1 months. Using the Kaplan-Meier method, we examined the time elapsed from the start of injecting drugs to HCV seroconversion. Only HCV-negative DU were included and they were considered to be at risk from their start of injecting. Those who had started injecting before ACS enrolment entered the risk set at their date of ACS entry (i.e., left truncation). Those who did not seroconvert or who were lost to follow-up were censored at their last ACS visit or ultimately 1 November 2005. We stratified the dates of starting injection into

two decennia to investigate differences in HCV-free survival according to decade of starting injection.

Incidence rate curves were calculated by person-time methods. Poisson regression was used to test for the trend in HCV incidence over time and to determine risk factors for HCV seroconversion. All variables subject to change were treated as time-dependent variables. Due to the relatively long time-period between the point of infection and the appearance of HCV antibodies (Netski et al. 2005), the most probable moment of infection was assumed to have occurred around the last seronegative visit. Therefore, we assigned the risk behavior reported at that visit to the HCV seroconversion period. However, for nine participants who reported starting injection at the first HCV antibody-positive visit, we set back the report of injecting risk factors from this visit to the last HCV antibody-negative visit. Multivariate models were built using forward-stepwise techniques, and variables with a univariate p-value < 0.20 were considered as potential independent determinants. A p-value < 0.05 was considered statistically significant (SAS Institute 1996; SPSS Inc. 1998). Interactions in the final model were checked.

Variables related to general characteristics, drug use, and sexual risk behavior were examined as potential determinants of HCV seroconversion. General characteristics included sex, body mass index, calendar year of study visit, nationality, ethnicity, age, homelessness, hospitalization, and HIV status. The drug use variables included current injecting and the calendar period of starting injection. For current injectors, we also examined the frequency of injecting, the main type of drug injected, whether they injected mainly at home or borrowed needles, and needles obtained through a needle exchange program (NEP). Because there was a very strong association between current injecting and current borrowing of needles, we combined these two variables as follows: no current injecting; current injecting but no current borrowing of needles; current injecting and current borrowing of needles. Sexual behavior included having a steady sexual partner, injecting drug use of the steady partner, having unprotected sex (with an injecting partner), and current prostitution (women only).

To compare the HCV and HIV incidence, we assumed that the observed data (i.e., the number of new infections per year) follows a Poisson distribution. We adopted a Bayesian approach. The logarithm of the incidence over calendar time was modeled using penalized splines. In this way, the incidence of both HCV and HIV was allowed to vary smoothly and nonlinearly over time (OpenBugs 2006; Development Core Team 2005, Crainiceanu et al. 2004). If the trends have the same pattern, then the difference between the incidences on a logarithmic scale is a constant.

Results

General characteristics and HCV prevalence

In total, 1640 DU have been enrolled in the ACS since December 1985. Of these, 1259 DU met the follow-up criteria of at least two visits before November 2005 and also had enough stored serum to allow HCV testing. Of these participants,

803/1259 (63.8%) were male and 937/1259 (74.5%) had a Dutch nationality. The median age at ACS entry was 30.5 years (IQR 26.5, 35.8) (table 1).
Of the 1259, 952 participants were ever-injectors: DU who had ever injected drugs before entry (n=905) or who had started injecting drugs during follow up (n=47). The median age at start of injection was 21.7 years (IQR 17.8, 26.0).
The median ACS follow-up time for ever-injectors was 7.3 years (IQR 3.8,12.6), whereas it was 5.4 years (IQR 2.6, 10.4) for never-injectors. In ever-injectors, the main drugs recorded at ACS entry were a cocktail of heroin and cocaine (40.0%), and most participants had injected daily or more frequently in the preceding 6 months (34.0%).
Of the 1259 DU, 803 (63.8%) had HCV antibodies at entry; of these, 30.6% (246/803) were HIV-co-infected. The prevalence at entry of HCV antibodies in ever-injectors varied from 92.9% in 1986 to 69.2% in 2001. The prevalence among never-injectors was 6.5% over the total study period and varied from 0 to 22.2% per calendar year.
When evaluating HCV prevalence at entry by the time elapsed since start of injection, such prevalence was 59/99 (59.6%) for participants who had injected for less than 2 years before entry vs. 137/164 (82.5%) for participants who had injected for 3 to 5 years before entry. Among participants with >10 years of injecting drug use before ACS entry, the HCV prevalence was 327/346 (94.5%).

HCV incidence
Of the 456 DU seronegative for HCV at ACS entry, 59 seroconverted during follow-up, of whom 58 injected and 1 did not. Among ever-injectors, the incidence declined from 27.5/100 PY in the late 1980s to approximately 2/100 PY in recent years (Figure 1a). There was a significant downward trend in HCV incidence over calendar time (IRR 0.86 per calendar year; 95% CI 0.82, 0.90, p<0.001) (figure 1a).
In line with the decline of the HCV incidence, the time since starting injection until HCV seroconversion has lengthened in more recent calendar periods. In 1980-1989, the median interval was 2.27 years (IQR 1.2, 5.6 years), whereas in 1990-1999, the median was 9.10 years (IQR 2.1, ∞ years) (figure 2).
When restricting our analysis to DU who reported injecting since the preceding visit, a higher incidence but similar pattern was observed. In 1985-1990, the incidence rate in this group was extremely high, between 50–80/100 PY, but it dropped to 5-10/100 PY in 1990-1999.

Comparison of HCV and HIV incidence
Of 1276 DU, those HIV-negative at entry numbered 1013, of whom 95 (including 90 ever-injectors) seroconverted for HIV during follow-up. The HIV incidence rate among ever-injectors dropped from 8.52/100 PY in 1986 to approximately 0 since 2000, with a slight increase in 2005 (Figure 1b).
When the observed HCV and HIV incidence curves and their fitted smooth curves are plotted in one graph with two scales, the curves look similar in shape. When we plotted the differences between the logs of the fitted model, we found no convincing evidence for a difference in pattern. The mean value of the differences on a log-scale over the twenty years is 1.48; hence the scale factor is estimated to

be 4.4 (data not shown). The observed and fitted incidence patterns for both HCV and HIV with 95% confidence intervals are shown in figure 2c.

Risk factors for HCV seroconversion

Time since start injecting can be seen as a proxy for the duration of exposure time, and preliminary analysis showed a very strong association between time since start of injecting and the time point of HCV seroconversion (IRR 0.80 per year), 95% CI 0.74, 0.86) (table 2). Therefore, in bivariate analysis, to adjust for variation in time from start of injecting (and thus time of exposure), all other variables were adjusted for time from start of injecting as a time-updated variable.

After correction for time since starting injection, the following risk factors were found to be significantly associated with an increased risk of HCV seroconversion: the combined variable of current injecting and current borrowing of needles, earlier calendar year of visit, use of needle exchange programs (NEPs), type of drugs injected, frequency of injecting drug use, and earlier decennium of starting injection (table 2). Interestingly, in univariate analysis persons were more at risk for HCV if they had seroconverted for HIV (IRR 5.68; 95% CI 2.27, 14.2) or were chronically infected with HIV (IRR 3.12; 95% CI 0.76, 12.8) than if they were HIV-negative. The type of drugs injected, and frequency of injection were associated with an increased risk of HCV infection, their effect is attributable to current injecting drug use itself. In fact, when evaluating these variables among only DU injecting drugs within the past six months we found no association between NEP use, the type of drug injected, or injection frequency and HCV infection.

In multivariate analysis, we found that current injecting combined with current borrowing of needles was a major risk for HCV seroconversion; the IRR was 29.9 (95% CI 12.6, 70.9) for current injecting and borrowing compared to no injecting in the preceding period. The longer the time between start of injecting and study visit, the smaller the risk of HCV infection: IRR 0.89; 95% CI 0.83, 0.96) (table 2). Calendar year remained significantly associated with a decreased risk of HCV infection when it was evaluated continuously in the model (IRR 0.87; 95% CI 0.82, 0.93).

Discussion

This study describes the prevalence and incidence of HCV in a large group of DU in Amsterdam, the Netherlands, over two decades. Findings show that the HCV incidence dropped considerably in that period. Interestingly, when we compared the HCV incidence rate to the HIV incidence rate in the same group of DU that have ever injected the decrease was similar for the two infections. In line with the decline of the HCV incidence, the time from the start of injecting drugs until HCV seroconversion is longer at present than in the past.

To our knowledge, this is the first study to document among DU, over such a long period, a decline in HCV incidence that is not only strong but also comparable to the decline in HIV incidence. Our finding of a decline in HCV incidence contrasts with other studies that show a stable HCV incidence (van Ameijden et

al. 1993; Hahn et al. 2002). One explanation may be that those studies analyzed the HCV incidence over a shorter time interval, which might have been insufficient to show a significant decline. In Baltimore, USA, a significant decline of the HCV incidence was found in injecting DU followed between 1988 and 1996, but in contrast to our study with ongoing recruitment of participants, this decline was observed in a closed cohort study and a saturation effect probably has contributed to this decline (Villano et al. 1997).

In addition, the risk behavior of the total group of DU included in the ACS has substantially declined over time in Amsterdam (Lindenburg et al. 2006). This finding suggests that a decline in risk behavior at the population level has contributed to the simultaneous decline of HCV and HIV incidence. The decreasing HCV incidence in Amsterdam DU, as opposed to high incidences in DU elsewhere, may likewise be partly explained by a larger reduction in injecting risk behavior in Amsterdam, compared to reductions elsewhere. The impact of methadone provision and NEP on this decline of risk behavior is very important and should be a focus of future studies. Methadone and NEP were readily available throughout the study period, and the median prescribed daily methadone dose increased during this period. Murray et al. (Murray et al. 2003) demonstrated by mathematical modeling that the level of risk behavior determines whether HCV incidence decreases. They calculated that if injecting risk behavior is sufficiently decreased (through intense needle exchange programs and/or harm reduction strategies), then HCV incidence will accordingly decline.

Mathematical models have additionally shown the natural course of an epidemic might bring a decline in the incidence of infection (Anderson & May 1991). When a new infectious agent enters a population, the number of infected individuals and the incidence soon increase. Thereafter, as the number of susceptibles decreases, the chance for an infected individual to come into contact with an uninfected individual decreases as well. When the density of uninfected persons reaches a threshold below which the number of susceptibles cannot sustain an ongoing epidemic, incidence peaks and then starts to decline. In this light, the decrease in HIV incidence observed shortly after the introduction of HIV in Amsterdam in the early 1980s was due to the depletion of susceptibles, along with a reduction in risk behavior. However, such depletion is less likely to be the case for HCV, which has existed among DU since the 1960s and possibly even before (Pybus et al. 2001; Pybus et al. 2005). This implies that the decrease in injecting risk behavior might have an even greater impact on HCV than on HIV.

The contrast in study findings may be explained in part by the HCV test used. We used third-generation ELISA tests to measure HCV antibodies, whereas studies from the late 1980s/early 1990s used first- or second-generation ELISA tests, which were more inclined to give false positive test results (Erensoy 2001).

The HCV prevalence among DU at ACS entry varies between 70-90%, with lower prevalence rates in recent years. This is consistent with what was described among DU in Amsterdam in the early 1990s (Ameijden et al. 1993) and among recently starting injectors in Amsterdam and elsewhere (Des Jarlais et al. 2005; van de Laar et al. 2005). The HCV prevalence in never-injecting DU is much lower than in ever-injectors but still much higher than in low-risk populations (e.g.,

blood donors) or the general populations in Western countries (Hutchinson et al. 2004; Memon et al 2002), household transmission, rare sexual transmission, and reliability/unreliability of answers given in interviews may contribute to this finding among never-injecting DU.
Among DU in Amsterdam who have injected in the past 6 months, incidence rates were extremely high in the 1980s (50 – 80 / 100 PY). Similarly high incidence rates have been described by Smyth et al. among young DU who have recently started injecting in Ireland, in the 1990s (Smyth et al. 2003).
A possible limitation of our study is its lack of confirmatory testing for positive results of HCV antibody testing. However, such results in a high-risk population are likely to be true positives (Erensoy 2001), and 232/803 (28.9%) of the positive participants were tested at two study visits or more, all with consistent HCV-positive test results. Therefore we believe the lack of confirmatory testing did not influence our results. Furthermore, although the ACS is an open, prospective cohort study, the influx of new participants in recent years has been lower than in earlier years. Lower risk DU could be overrepresented due to the decease of high-risk DU. However, the most recent HCV seroconversions took place in young drug users who entered the cohort after 1994.
Our risk factor analysis showed that HCV seroconversion is associated not only with current injecting and borrowing needles, as expected, but also with calendar year and time since start of injecting. The majority (70%) of HCV infections could have been prevented by eliminating the borrowing of needles. This might partly reflect the effect of the use of NEP, which were always available during the study period, but individual factors also might play a role in the decision to use NEP.
In conclusion, HCV incidence in our cohort showed a sharp decline in the past two decades, similar to the decline in HIV incidence, most likely due to a decrease in injecting risk behavior. We found that those who started injecting in a recent calendar period are at lower risk of HCV infection, presumably due to prevention activities. Thus it is important to continue and enhance such activities among DU and others at risk of starting injection, especially because the HCV risk is highest just after the start of injecting, when probably injectors are inexperienced.
Although we did not find an independent effect from either participation in a methadone program or from the use of needle exchange programs, these prevention measures in combination are likely to have contributed to the decline in risk behavior related to drug use at the population level. Therefore, it is important to evaluate the possibilities for harm reduction worldwide. During the late 1980s many acute HCV infections occurred, so there might have been more DU with high HCV RNA levels associated with acute HCV infection. Therefore, in that period there may have been more and/or easier transmission of HCV. Higher HCV RNA levels have also been associated with HIV co-infection (Eyster et al., 1994). However, we believe that because the HCV prevalence remained relatively high and the pattern of the HIV and HCV incidence was comparable during the study period, on population level the HCV RNA level varied only little over time, also because treatment prescription for HCV was very limited in our cohort.

Finally, it is important to decrease the prevalence of chronic HCV carriers and thus reduce the possibility for HCV transmission. DU should therefore be systematically screened for HCV infection, and those chronically infected should be treated (Sylvestre 2005).

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Table 1: General characteristics of drug users in the Amsterdam Cohort Study (* = at entry).

	Total	Ever-injecting DU	Never-injecting DU
Total number of participants	1259	952	307
Median age * (IQR)	30.5 (26.5, 35.8)	29.84 (26.0, 36.0)	30.6 (26.8, 35.7)
% Male sex	63.8	61.3	71.3
% Dutch nationality	74.7	86.0	71.0
Median duration of follow-up (IQR)	6.95 (3.56, 12.1)	7.33 (3.84, 12.6)	5.41 (2.60, 10.4)
Median age at start of injecting drugs (IQR)	-	21.7 (17.8, 26.0)	-
Main drugs injected (%) *			
cocktail, heroin/cocaine		40	
heroin		12.2	
cocaine	-	8.9	-
Main other drugs used (%) *			
cocktail, heroin/cocaine		4.4	41.0
heroin		31.5	43.0
cocaine	-	26.7	4.2
Frequency of injecting (%)			
no current injecting		28.5	
daily		34.0	
weekly		30.7	
monthly	-	4.4	-
Number of recently borrowed needles(%) *			
0		44.9	
1-10		7.6	
>10		0.9	
unknown	-	46.4	-
% HCV-antibody positive *	63.8	82.2	6.5
HCV seroconversions during follow-up	59	58	1
% HIV-positive *	20.4	25.8	3.6
HIV seroconversions during follow-up	95	90	5

Ever-injecting DU: DU who had injected before ACS entry (n=905) or started injecting during follow up (n=47). Current/recently: in previous six months.

Table 2: Risk factors for HCV seroconversion (HCV sc).

	Univariate Analysis						Bivariate Analysis			Multivariate Analysis		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Methadone dosage												
0 mg	34	408	8.33	2.23	(0.68, 7.26)	0.18	1.07	(0.33, 3.51)	0.92			
1-59 mg methadone	20	358	5.59	1.49	(0.44, 5.02)		0.95	(0.28, 3.21)				
>60 mg	3	80	3.75	1			1					
HIV status												
HIV-negative	51	831	6.14	1		0.006	1		0.25			
HIV primary infection	5	14	35.7	5.68	(2.27, 14.2)		2.18	(0.85, 5.57)				
HIV chronically infected	2	10	20.0	3.12	(0.76, 12.8)		1.95	(0.47, 8.07)				
Decennium of starting injection												
1970-79	1	146	0.68	1		0.002	1		0.13			
1980-89	37	443	8.35	12.2	(1.67, 88.9)		1.56	(0.19, 12.6)				
1990-99	19	239	7.95	11.6	(1.55, 86.6)		1.01	(0.12, 8.37)				
2000-present	1	23	4.35	6.26	(0.39, 100.0)		0.32	(0.02, 5.74)				
Use of NEPs												
No current injecting	10	623	1.61	1		<0.001	1		<0.001			
Current injecting, no NEP	18	92	19.6	12.3	(5.66, 26.6)		7.61	(3.43, 16.8)				
Current injecting, irregular NEP	11	36	30.6	19.1	(8.09, 44.9)		8.40	(3.39, 20.8)				
Current injecting, always NEP	19	99	19.2	11.9	(5.53, 25.6)		7.87	(3.58, 17.3)				
Age (per 10 years)	58	856	6.78	0.45	(0.31, 0.65)	<0.001		0.87	(0.59, 1.26)	0.45		

	Univariate Analysis						Bivariate Analysis			Multivariate Analysis		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Type of drugs mainly injected												
No current injecting	10	623	1.61	1		<0.001	1		<0.001			
Heroin	9	46	19.8	12.2	(4.97, 30.1)		7.18	(2.86, 18.0)				
Cocaine	10	41	24.4	15.2	(6.31, 36.4)		8.69	(3.53, 21.4)				
Cocktail, heroin/cocaine	21	115	18.3	11.4	(5.37, 24.2)		6.51	(2.99, 14.2)				
Amphetamines	2	17	11.8	7.46	(1.64, 34.1)		3.81	(0.82, 17.6)				
Methadone	3	9	33.3	21.3	(5.87, 77.5)		35.5	(9.7, 129.5)				
Other/unknown	3	4	75.0	44.6	(12.3, 162.2)		25.6	(7.00, 93.5)				
Frequency of injecting												
No current injecting	10	623	1.60	1		<0.001	1		<0.001			
More times per day	17	49	34.7	21.7	(9.92, 47.3)		10.4	(4.54, 23.9)				
Once daily	1	4	25.5	15.9	(2.03, 124.3)		12.2	(1.55, 95.9)				
More times per week	19	66	28.8	18.0	(8.36, 38.6)		10.5	(4.75, 23.3)				
Once weekly	1	12	8.23	5.13	(0.66, 40.1)		5.27	(0.67, 41.2)				
More times per month	3	37	8.13	5.07	(1.40, 18.4)		2.71	(0.72, 10.1)				
Once monthly	2	13	15.2	9.46	(2.07, 43.2)		8.18	(1.79, 37.4)				
Less than once monthly	4	48	8.36	5.21	(1.63, 16.6)		4.19	(1.31, 13.4)				
Frequency of non-injecting drug use												
More times per day	21	249	8.43	0.73	(0.25, 2.14)	0.35	0.60	(0.20, 1.74)	0.39			
Once daily	2	38	5.26	0.45	(0.08, 2.48)		0.45	(0.08, 2.47)				
More times per week	16	235	6.81	0.59	(0.20, 1.77)		0.60	(0.20, 1.80)				
Once weekly	2	68	2.94	0.25	(0.05, 1.39		0.20	(0.04, 1.09)				
More times per month	2	47	4.26	0.37	(0.07, 2.03)		0.47	(0.09, 2.58)				
Less than once monthly	0	17	0.00	1			1					

	Univariate Analysis						Bivariate Analysis			Multivariate Analysis		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Type of drugs mainly used (non-injecting)												
Heroin	20	311	6.43	1		0.98	1		0.81			
Cocaine	24	332	7.23	1.12	(0.62, 2.03)		1.33	(0.73, 2.41)				
Cocktail, heroin/cocaine	2	32	6.25	0.96	(0.22, 4.11)		1.07	(0.25, 4.58)				
Amphetamines	1	13	7.69	1.16	(0.16, 8.66)		1.48	(0.20, 11.0)				
Having a steady partner												
No	36	496	7.26	1.19	(0.70, 2.02)	0.52	1.55	(0.91, 2.64)	0.10			
Yes	22	360	6.11	1				1				
Injecting drug use of the steady partner												
No	12	255	4.71	1		0.10	1		0.99			
Yes	10	105	9.52	2.04	(0.88, 4.71)		0.99	(0.42, 2.33)				
Homelessness												
No	52	800	6.50	0.61	(0.26, 1.43)	0.29	0.67	(0.29, 1.57)	0.39			
Yes	6	57	10.5	1			1					
Hospitalized in past 6 months												
No	56	817	6.85	1.34	(0.33, 5.50)	0.67	1.36	(0.33, 5.59)	0.65			
Yes	2	39	5.13	1			1					
Current prostitution (females only)												
No	24	243	9.88	1		0.19	1		0.82			
Yes	2	47	4.26	0.43	(0.10, 1.81)		1.19	(0.28, 5.07)				

	Univariate Analysis						Bivariate Analysis			Multivariate Analysis		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Current injecting and borrowing needles												
No current injecting	10	623	1.61	1	<0.001	1			<0.001	1		<0.001
Current injecting, no current borrowing of needles	25	159	15.7	9.80	(4.71, 20.4)		6.26	(2.94, 13.3)		8.70	(4.03, 18.8)	
Current injecting and current borrowing of needles	12	23	52.2	32.7	(14.1, 75.7)		21.4	(9.17, 50.1)		29.9	(12.6, 70.9)	
Time since start of injecting	58	856	6.8	0.80	(0.74, 0.86)	<0.001				0.89	(0.83, 0.96)	<0.001
Year of visit	58	856	6.8	0.86	(0.82, 0.90)	<0.001	0.94	(0.89, 0.99)	0.009	0.87	(0.82, 0.93)	<0.001
Sex												
Male	32	566	5.65	1		0.085	1		0.36			
Female	26	290	8.97	1.59	(0.95, 2.66)		1.28	(0.76, 2.16)				

Figure 1a: Figure 1 (a,b) observed HIV and HCV incidence curves among ever injecting DU in the ACS (1985-2005); (c) observed and fitted HCV (left Y-axis) and HIV (right y-axis) incidence curves among ever injecting DU in the ACS (1985-2005).

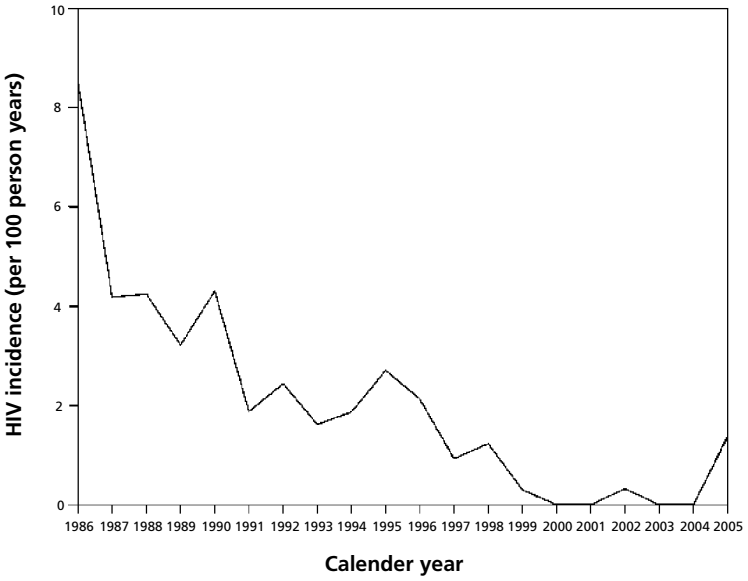


Figure 1b: Figure 1 (a,b) observed HIV and HCV incidence curves among ever injecting DU in the ACS (1985-2005); (c) observed and fitted HCV (left Y-axis) and HIV (right y-axis) incidence curves among ever injecting DU in the ACS (1985-2005).

Figure 1c: Figure 1 (a,b) observed HIV and HCV incidence curves among ever injecting DU in the ACS (1985-2005); (c) observed and fitted HCV (left Y-axis) and HIV (right y-axis) incidence curves among ever injecting DU in the ACS (1985-2005).

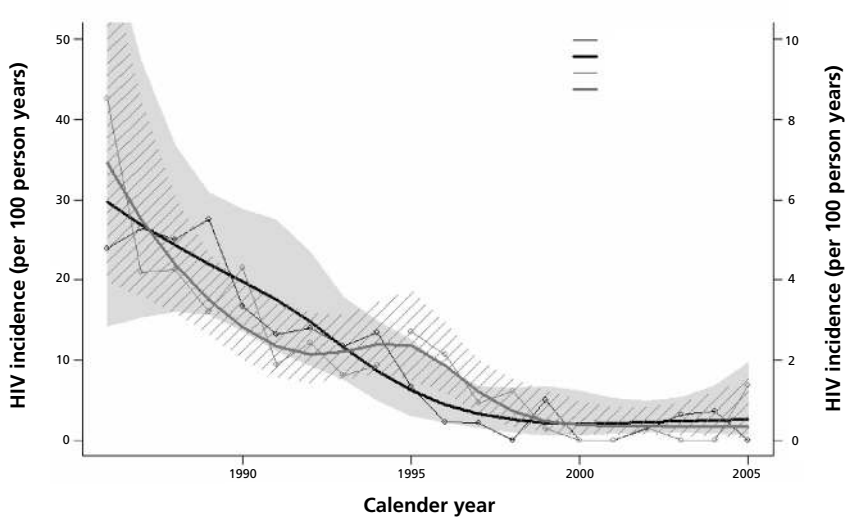
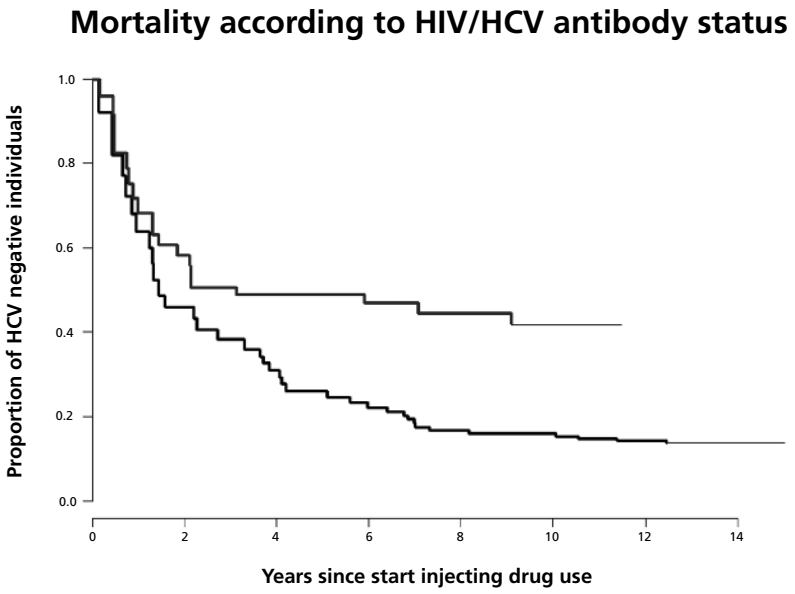


Figure 2: Kaplan-Meier estimates of the cumulative proportion of DU who remain without HCV infection since starting injection, grouped per decennium: the 1980s (black line) and 1990s (blue line). Curves were truncated when fewer than 10 persons remainded at risk for HCV (thin line). Persons who started injecting before 1980 or after 2000 are not depicted in this figure, because at any moment in those peroids, less than 10 persons were at risk.



3.2

HIV co-infections

Rise in Seroprevalence of Herpes Simplex Virus type 1 among highly sexual active homosexual men and an increasing association between Herpes Simplex Virus type 2 and HIV over time (1984-2003).

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Abstract

Objectives: Herpes Simplex virus type 1 and type 2 (HSV-1 and HSV-2) are both highly prevalent. The rate of genital HSV-1 transmission is reportedly increasing over time. HSV-2 is considered to be an important risk factor for HIV transmission. We therefore studied changes in the HSV-1 and HSV-2 prevalence in a large cohort of men who have sex with men (MSM) over a 20-year time period.
Methods: Among 1847 HIV-infected and HIV-uninfected MSM participating in the Amsterdam Cohort Studies, seroprevalence of HSV-1 and HSV-2 was determined and prevalence rate ratios (PRR) and 95 % confidence intervals were calculated.
Results: Between 1984-2003 the HSV-1 and HSV-2 prevalence decreased among HIV-uninfected MSM ($p<0.001$), but remained stable among HIV–infected MSM. HSV-1 prevalence increased among men with at least 200 sexual partners over lifetime (PRR: 1.49, $p<0.001$). The association between HIV infection and HSV-2 became stronger over time (PRR: 3.45, $p<0.001$).
Conclusions: Seroprevalence of HSV-1 and HSV-2 remained high among HIV infected MSM from 1984 to 2003. The association of HIV and HSV-2 increased during the HIV epidemic. Since the proportion of sexual transmission of HSV-1 is rising, it is important to study the potential role of HSV-1 as risk factor for HIV acquisition.

Introduction

Herpes simplex virus type 1 (HSV-1) is widespread in the general population, while herpes simplex virus type 2 (HSV-2) is more restricted to risk groups such as men who have sex with men (MSM). HSV-1 prevalence is around 70% in the general population[1] [2]. Transmission usually occurs during childhood through oral contact and normally causes oropharyngeal infection. Childhood HSV-1 transmission has declined in industrialised countries, resulting in a lower prevalence of HSV-1 and leaving a larger population of adolescents at risk for sexual transmission of HSV-1. Earlier studies have reported sexually related risk factors for HSV-1 infection in women[3]. Among those persons attending the STD clinic and blood donors, HSV-1 infection is associated with younger age of first intercourse[4]. However, less is known about sexually related risk factors for HSV-1 infection among MSM[5]. HSV-2 infection is usually transmitted sexually and is considered as a marker for sexual risk behaviour in populations[6]. In HIV-infected MSM, the prevalence of HSV-2 is as high as 61%[1], while being 15-25% in the general population[7,8]. HSV-2 is a risk factor for HIV acquisition, especially in the African setting[9] and in MSM[10]. HSV-2 infected persons are more susceptible for HIV [11,12]. Moreover, HIV-infected persons are more likely to have subclinical reactivation of HSV-2 and are therefore more likely to transmit the virus[13].
We previously demonstrated a decline in the prevalence of HSV-2 among MSM in Amsterdam between 1984-1997, which could be explained by a decrease in sexual risk behaviour[5]. However, in the second half of the 1990s their sexual risk behaviour increased after effective HIV therapy became generally available.

This may have caused an increase in the prevalence of HSV-2 and possibly also in HSV-1 since 1996.
We here studied the trend in HSV-1 and HSV-2 prevalence among homosexual men over a 20-year time period (1984-2003) and whether risk factors for infection changed during this period.

Methods

Study population
In 1984 an open and prospective cohort study on HIV seroconversion and AIDS among sexually active HIV-negative and positive homosexual men was started. The Amsterdam Cohort Study (ACS) is still ongoing, although entry criteria with respect to HIV status and age have changed over time. From 1984 until May 1985, both HIV-positive and HIV-negative men were included. From May 1985 until February 1988, only HIV-negative men were allowed in the study. From February 1988 through 1994, HIV-positive and HIV-negative men could enter the study, but since 1995, they must be ≤30 year of age.
At an ACS visit, a standardised questionnaire is administered regarding demographics, sexual behaviour, and medical history for sexually transmitted infections (STI). Blood samples are collected for immunologic and virologic testing and for storage. For this study, stored sera taken from ACS participants with at least two cohort visits (1847/2100 (88%)) were tested for HSV-1 and HSV-2.

Laboratory methods
Sensitive and specific FDA approved serological assay for HSV-1&2 was used (HerpeSelect by FOCUS technologies, USA). Its manufacturer recommends an index value>1.1 as positive. However, there is evidence that using this cut-off value in HSV-2 studies yields a high rate of false positive results in populations with multiple infections, such as those in Africa[14]. Raising the positive cut-off will increase the specificity[14]. Since the optimal cut-off for our target population has not been established, 100 samples with results in the range of 1.1 and 3.5 were re-tested with a highly specific Western blot. HSV-2 ELISA and Western blot results were concordant for 80/100 samples. The proportion of samples that were positive with both ELISA and Western blot increased with increasing index value (figure 1). Based on these results, we consider a cut-off value of ≥2.1 as being positive and an index value<2.1 is classified as negative. The HSV-2 index value< 2.1 had 36% concordance with Western blot results, while the HSV-2 index value of ≥ 2.1 showed 93% concordance. There were no differences in Western blot outcomes between HIV-infected and HIV uninfected MSM.
Blood samples were tested for HSV-1 and HSV-2 at the Public Health Laboratory of the Health Service of Amsterdam. The Western blot was conducted at the Institute for Pathology and Medical Research (ICPMR) in Sydney, Australia. Blood samples are also tested for HIV antibodies by enzyme linked immunosorbent assay (ELISA) (Abbot Laboratories, North Chicago, Illinois, USA; Vironostika, Or-

ganon, Teknika, Boxtel, the Netherlands), and when positive, are confirmed by Western blot.

Variables and statistical analyses
Variables used in this study were calendar year of ACS entry, HIV-status, age, nationality, education, age of first homosexual contact, lifetime sexual partners, and self-reported history of syphilis and gonorrhoea in the past 5 years.
Variables concerning sexual practices included orogenital, anogenital and oro-anal contact in the prior 6 months.

The prevalence of HSV-1 and 2 at the ACS entry was determined and risk factors for HSV-1 and 2 were assessed by calculating prevalence rate ratios(PRR), with their 95% confidence interval(CI). Odds ratios could not be interpret as relative risks, since the rare event assumption was not reached. Therefore PRR's were directly estimated, using a modified Poisson regression approach[15]. This approach provided a correctly estimated standard error for the estimated relative risk. Since inclusion criteria with respect to age and HIV-status changed over time, all risk factor analyses were adjusted for age and HIV-status. Variables that were statistically significant were included in the multivariate model, using a stepwise forward approach forcing age and HIV-status in the model.
We tested whether risk factors changed over time by testing for interaction between variables under investigation and calendar time in the multivariate model. Calendar time therefore was categorised as 1984-1986, 1987-1991, 1992-1996, 1997-2003.
Confounding was defined to be present when the included variable caused a change of the prevalence ratio by more than 10%. Interaction was defined to be present when the addition of an interaction term improved the original model and the p-value was less than 10%. Statistical significance was defined as a p-value<0.05. To reduce residual confounding when measuring the association between HSV and sexual practices, three variables measuring sexual practices over the prior 6 months were included in the model at the same time, together with the lifetime sexual partners. We modelled time trends in the HSV 1 and 2 prevalence with calendar time as a continuous variable using restricted cubic splines with four knots, resulting in a smoothly varying curve.
Finally, sensitivity analyses for HSV-2 were conducted by using the cut-off value of 1.1, as recommended by the manufacturer and by using the cut-off value of 3.5, excluding those with an index value in the grey area (between 0.9-1.1 and 0.9-3.5). However, time trends in prevalence and risk factors found were comparable to when 2.1 was the cut-off value (data not shown).

Results

General characteristics
Between 1984 and 2003, a total of 1847 MSM had at least two visits. General characteristics of the total study group are presented in table 1. Of the 1847, 1207

(65%) MSM were HSV-1 antibody positive, while 759/1847 (41%) of the men were HSV-2 antibody positive. Of the total group, 558 (30%) were positive for both. Participants were predominantly of Dutch nationality (86%) and had a median age of 29 years (interquartile range: 25-36).

Prevalence of HSV-1 and HSV-2 over time
There was an overall decline in the prevalence of both HSV-1 and 2 between 1984 and 2003 (tables 1 and 2). To investigate time trends in HSV seroprevalence we included an interaction term between time and HIV-status (figure 2a and 2b). Among HIV-negative MSM, the HSV-1 prevalence decreased significantly over time, $p<0.001$ (figure 2a). Among HIV-positive MSM, the HSV-1 prevalence remained stable over time, $p=0.35$ (figure 2a). The HSV-2 prevalence significantly decreased among HIV-negative men, $p<0.001$ (figure 2b).

Result from the regression models showed that the decline in HSV-2 prevalence could not be explained by changes in demographic characteristics or sexual behaviour (PRR adjusted 0.92, $p<0.001$). In contrast, the HSV-2 prevalence remained stable over time for men infected with HIV ($p=0.12$). Again, this result was observed after controlling for age, demographic characteristics and sexual behaviour.

Risk factors
Risk factors for HSV-1 and HSV-2 infection, adjusted for age and HIV-status, are presented in table 1.
In the final model calendar year, HIV-status, nationality, education, and number of lifetime sexual partners remained independent predictors for HSV-1 infection (table 2). For HSV-2 infection, earlier year of study entry, positive HIV-status, HSV-1 co-infection, lifetime sexual partners, a history of syphilis and sexual behaviour remained independent predictors (table 2).

Changing risk factors over time
It appeared that the effect of calendar year differed between HIV-infected and HIV-uninfected MSM. Its effect differed with respect to nationality, number of lifetime sexual partners, and with HSV-2 co-infection and HSV-1 and with respect to the number of lifetime sexual partners, HSV-1 co-infection and HSV-2.
The association between HIV and HSV-1 became stronger over time (adjusted PRR after 1996: 1.56, $p<0.0001$ and PRR before 1986: 1.08, $p=0.11$). This was due to the decline in HSV-1 prevalence over time among HIV-negative MSM but not among HIV-positive MSM (figure 2a). As shown by the regression model, a decrease in HSV-1 infection over time was observed only among MSM with Dutch or Northern/Central-European nationality. This resulted in a stronger association over time between HSV-1 and having non-European origin over time (adjusted PRR after 1996: 1.58, $p<0.0001$ and PRR before 1986:1.08, $p=0.3$). Also the association between HSV-1 and a higher number of lifetime sexual partners became stronger after 1996 (adjusted PRR after 1996: 1.49, $p<0.0001$ and PRR before 1986:1.01, $p=0.77$). Figure 3a shows the prevalence of HSV-1 infection over time according

to the number of sexual lifetime partners. A decrease in the HSV-1 prevalence was seen among MSM with fewer than 21 partners ($p<0.0001$), while among MSM with more than 200 partners, the HSV-1 prevalence increased between 1988 and 2003 ($p=0.01$).

For HSV-2 the association with HIV infection increased with calendar year and was highest after 1996 ($p<0.001$). A large number of lifetime partners also became more strongly associated with HSV-2 over time. However, a decrease in the HSV-2 prevalence was seen among all categories of lifetime sexual partners (figure 3b).

DISCUSSION

In the present study, we demonstrated an overall decrease in HSV-1 and HSV-2 prevalence among HIV-negative MSM, but not among HIV-positive MSM. In the 1984-2003 period, the association between HSV-2 and HIV among MSM became stronger over time, and HSV-1 prevalence increased in highly sexually active HIV-negative MSM. To our knowledge, this is the first study based on almost 20 years of HSV-1 and HSV-2 prevalence data among MSM.

The decrease seen in the seroprevalence of HSV-1 and HSV-2 over time, could not be explained by changes in demographic characteristics or sexual behaviour. The decrease in HSV-1 is likely to reflect a decrease in childhood transmission by the oropharyngeal contact of HSV-1. Since fewer individuals are infected in childhood with HSV-1, there is a growing population of persons at risk at the time they become sexually active, resulting in a larger proportion of sexual transmission of HSV-1. Two risk factors for HSV-1 infection that could be important in its sexual transmission were identified by this study. First, HIV infection is associated with HSV-1 seropositivity. Second, the prevalence of HSV-1 was higher among highly sexual active MSM (at least 200 lifetime sexual partners). An association also shown earlier by others[3].
HIV infection in this respect may reflect an epidemiological marker for sexual risk behaviour for HSV-1 transmission. HSV-1 prevalence did not decrease in those infected with HIV, and we consider that genital HSV-1 infection has a growing role in the acquisition of HIV.
Likewise, HSV-2 prevalence did not decline over time among those infected with HIV, whereas a decline was noted among HIV uninfected MSM.

The overall prevalence of HSV-2 in this study is similar to that among MSM in San Francisco in 1989, but higher than the prevalence found in more recent studies in the US[16-18]. The lower prevalence in those more recent studies probably reflects the decline in HSV-2 prevalence over time, as found in our study.
Russell et al., found high prevalence rates of HSV-2 among HIV-infected MSM in Australia[1]. The HSV-2 seroprevalence was more than twice as high as among HIV-uninfected MSM; there was no significant difference in HSV-1 prevalence between HIV-infected and HIV-uninfected MSM.

Several epidemiological studies have described an association between HIV and HSV-2[10]. HSV-2 is recognised as a risk factor for HIV acquisition in MSM. In addition, HSV-2 may up-regulate HIV and increase local HIV replication on mucosal surfaces, leading to an increased risk of HIV transmission. Our study is the first to show an increase in the association between HSV-2 and HIV since 1996, suggesting that HSV-2 may play a growing role in driving the HIV epidemic in MSM. If this is the case, prevention of HSV-2 may well contribute to the prevention of HIV among highly sexually active MSM. Although serological screening for HSV-2 among MSM is still under debate, the increasing association between HSV-2 seropositivity and HIV is an argument in its favour. Several reasons against screening have been raised, such as the lack of a reliable serological test. We are aware of the low specificity of the various HSV-2 serological assays. However, these serological assays might be useful as a screening tool, when used with an increased cut-off value less individuals will be classified as false positive. A second argument against serological screening is that HSV-2 infection is largely asymptomatic and condom use appears only partially protective against HSV transmission. These factors complicate the prevention of HSV-2 and genital HSV-1 infection. However, it has been shown that half of the patients, initially unaware of their HSV-2 infection are able to recognise symptoms after being educated to do so[19]. Also, knowledge of the HSV-2 status of a sexual partner has been associated with a reduced risk of HSV-2 transmission[20]. Antiviral drugs used as suppressive therapy will lower the frequency of recurrences by 70 to 80%[21,22]. A combined approach of offering serological screening to highly sexual active MSM together with encouraging condom use to reduce the risk of HSV transmission and using suppressive therapy among those with recurrent lesions might eventually play an effective part in controlling the HIV epidemic among MSM.

One limitation of our study is its cross-sectional design. As a consequence, we cannot reveal the relation between HIV and HSV infection, being unable to determine which occurred first. As both HIV and HSV are sexually transmitted diseases, their association may well reflect shared sexual behavioural practices leading to transmission as well as a biological relation. Longitudinal studies, in which incident HIV and HSV cases are captured are therefore needed to give more insight into the relationship between HIV and HSV as affected by changes in sexual risk behaviour.

The results of this study have two implications for HIV and HSV research among highly sexually active MSM. First, it appears that HSV-2 and HIV are now more strongly related than in the early days of the HIV epidemic. As a vaccine against HSV-2 for MSM is not yet available, a determination of the extent to which the prevention of HSV-2, specially aimed for MSM at high risk for HIV, can contribute to controlling the HIV epidemic is needed. Second, since the extent of sexual transmission of HSV-1 is rising, we need to clarify its potential role as a risk factor for HIV acquisition in longitudinal studies.

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Table 1: Demographic and sexual characteristics of 1847 homosexual men, for the total study group, and for HSV-1-infected participants and HSV-2-infected participants separately, between 1984-2003, with the prevalence ratios for HSV-1 and HSV-2 with their 95% confidence intervals.*

Characteristics	Total	HSV-1-infection	PRR (95% CI)	Overall P-value	HSV-2-Infection	PRR (95% CI)	Overall P-value
total	1847	1207 (65)			759 (41)		
Year of study entry:				<0.0001			<0.0001
1984-1986	943	675 (72)	1		461 (49)	1	
1987-1991	165	113 (68)	0.87 (0.78-0.97)		92 (56)	0.84 (0.72-0.98)	
1992-1996	222	138 (43)	0.88 (0.79-0.96)		80 (36)	0.66 (0.56-0.77)	
>1997	517	281 (54)	0.76 (0.70-0.84)		126 (24)	0.47 (0.40-0.55)	
Index value:							
<0.9	-	535			853		
0.9-1.1	-	29			32		
1.1-2.1	-	100			112		
≥2.1	-	1107			759		
missing		77			91		
Age:				<0.0001			<0.0001
<30years	1002	570	1		252	1	
≥30 years	845	637	1.20 (1.13-1.29)		507	1.98 (1.75-2.23)	
Nationality				0.006			0.62
Dutch	1586	947 (60)	1		615 (39)	1	
Northern/central Europe	116	72 (62)	1.04 (0.92-1.18)		54 (47)	1.08 (0.90-1.38)	
Non-European	145	117 (81)	1.17(1.07-1.28)		75 (52)	1.04 (0.91-1.23)	
Education:				0.005			0.007
Low	96	77 (80)	1		57 (59)	1	
Middle	669	408 (61)	0.85 (0.76-0.95)		239 (36)	0.72 (0.59-0.87)	
High	971	574 (59)	0.81 (0.73-0.81)		387 (40)	0.79 (0.66-0.94)	
Missing	115	77 (67)			54 (47)		

Characteristics	Total	HSV-1-infection	PRR (95% CI)	Overall P-value	HSV-2-Infection	PRR (95% CI)	Overall P-value
Sexual partners in lifetime							
1-20	860	497 (58)	1	<0.0001	277 (32)	1	0.51
21-200	531	360 (68)	1.12 (1.04-1.22)		212 (40)	2.30 (0.93-1.21)	
>200	443	339 (77)	1.24 (1.15-1.34)		262 (59)	1.07 (0.95-1.20)	
Age of first homosexual contact (median, IQR)	18 (15-20)	17 (15-20)	1.01(1.01-1.02)^	<0.0001	17 (15-20)	0.99(0.99-1.01)^	0.32
HSV co-infection	568	568	1.29(1.14-1.47)	<0.0001	568	1.167(1.08-1.24)	<0.0001
HIV infection (%)	513	367 (72)	1.11(1.03-1.18)	0.007	312 (61)	1.12(1.00-1.24)	0.05
History of gonorrhoea in the past 5 years	1053	666 (63)	0.88(0.83-0.93)	0.0005	424 (40)	0.88(0.79-0.97)	0.02
History of syphilis in the past 5 years	278	219 (79)	1.15(1.07-1.24)	0.001	197 (71)	1.51(1.36-1.69)	<0.0001
Orogenital contact in the past 6 months**	1363	892 (65)	0.99(0.82-1.22)	0.46	565 (41)	0.69(0.55-0.87)	0.01
Anogenital contact in the past 6 months**	1222	819 (67)	1.11(1.14-1.60)	0.002	551 (45)	1.36(1.16-1.60)	0.0002
Oroanal in the past 6 months**	1081	700 (65)	1.00(0.91-1.09)	0.99	438 (41)	0.98(0.85-1.12)	0.80

*) SinceACS inclusion criteria have changed over times all analyses were adjusted for age (per 10-year increase) and HIV status at cohort entry.
**)analyses are also adjusted for the sexual techniques and number of partners to exclude residual confounding. ^) per 10 year of increase.

Table 2: multivariate model of risk factors associated with HSV-1 and HSV-2 infection.

	HSV1	Overall P-value	HSV2	Overall P-value
Year of study entry				
1984-1986	1		1	
1987-1991	0.91 (0.70-1.18)		0.86 (0.70-1.06)	
1992-1996	0.83 (0.66-1.02)		0.58 (0.48-0.71)	
>1997	0.75 (0.63-0.90)	<0.0001	0.47 (0.39-0.56)	<0.0001
Age	1.13(1.07-1.18)	<0.0001		
HIV serostatus:				
negative	1	0.01	1	<0.0001
positive	1.10 (1.02-1.18)		1.50 (1.37-1.68)	
HSV coinfection			1.15 (1.02-1.30)	0.02
Nationality:				
Dutch	1	0.0006		
Northern or Central European	1.05 (0.92-1.20)			
Non European	1.62 (1.12-1.36)			
Education:		0.02		
Low	1			
Middle	0.90 (0.79-1.00)			
High	0.84 (0.76-1.06)			
Sexual partners in lifetime				
1-20	1	0.003		
21-200	1.13 (1.05-1.25)			
>200	1.13 (1.04-1.23)			
History of Gonorrhoea in the past 5 yr	0.97 (0.90-1.03)	0.11		
History of Syphilis in the past 5 yr	1.21 (1.08-1.36)	0.001		
Orogenital contact in the past 6 months	1.12 (0.88-1.43)	0.42	0.69 (0.56-0.84)	<0.0001
Anogenital contact in the past 6 months	1.08 (0.97-1.20)	0.20	1.20(1.08-1.42)	0.02
Oroanal contact in the past 6 months	1.02 (1.07-1.13)	0.81	1.00(0.87-1.15)	0.72

Figure1: Comparison of HSV-2 serology by ELISA and Western Blot. 100 samples were tested with HSV-2 ELISA and re-tested with the Western blot to identify discrepancies between de two assays.

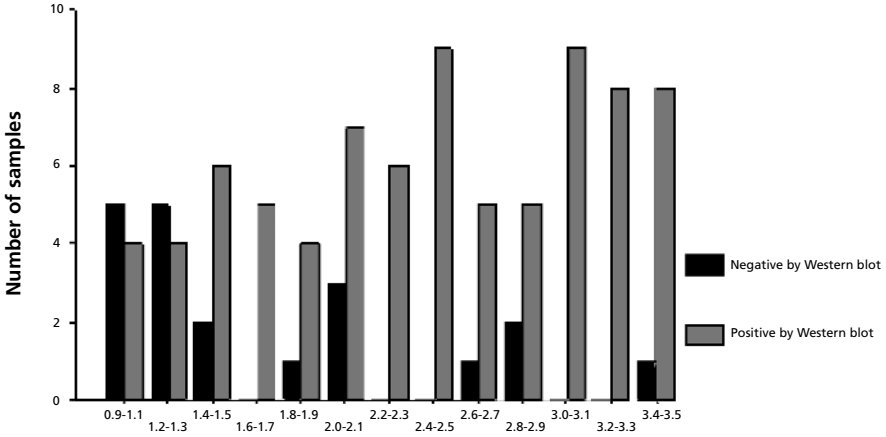


Figure 2a: HSV-1 prevalence in the Amsterdam Cohort Study among MSM, according to the HIV status (1 = HIV positive, 0 = HIV negative) and the 95% confidence interval.

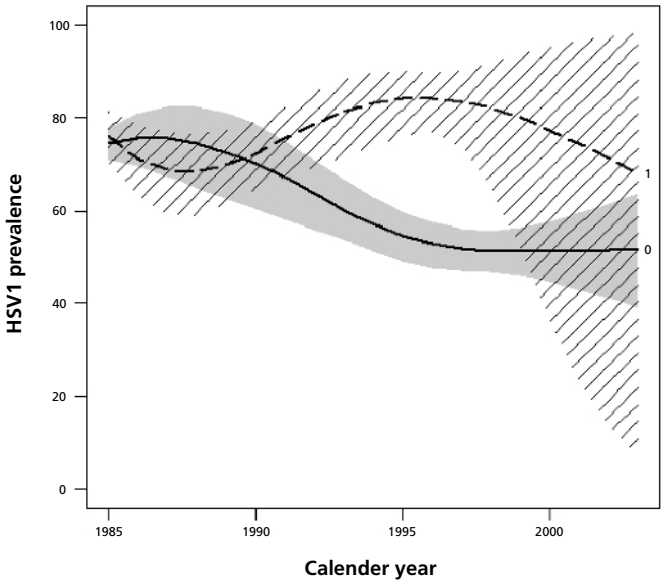


Figure 2b: HSV-2 prevalence in the Amsterdam Cohort Study among MSM, according to the HIV status (1 = HIV positive, 0 = HIV negative) and the 95% confidence interval.

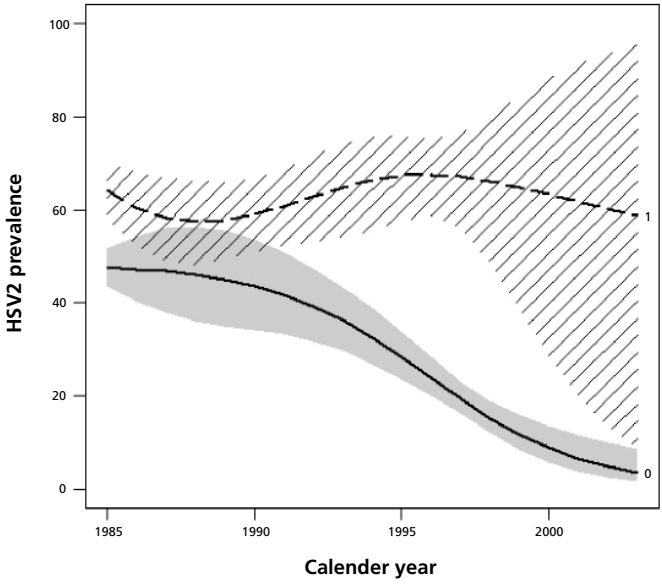


Figure 3a: Prevalence of HSV-1 and the 95% confidence inteval overtime among HIV negative MSM, according to number of lifetime sexual partners; 1 = 1-20, 2 = 21-200, 3 = >200 partners

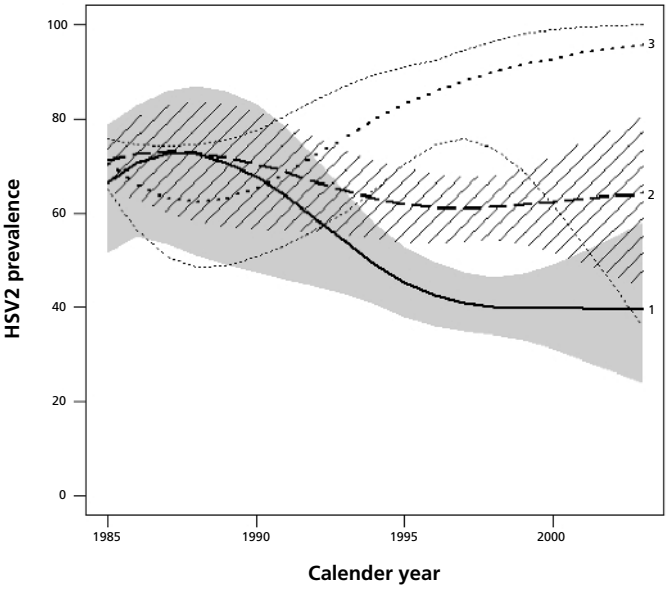
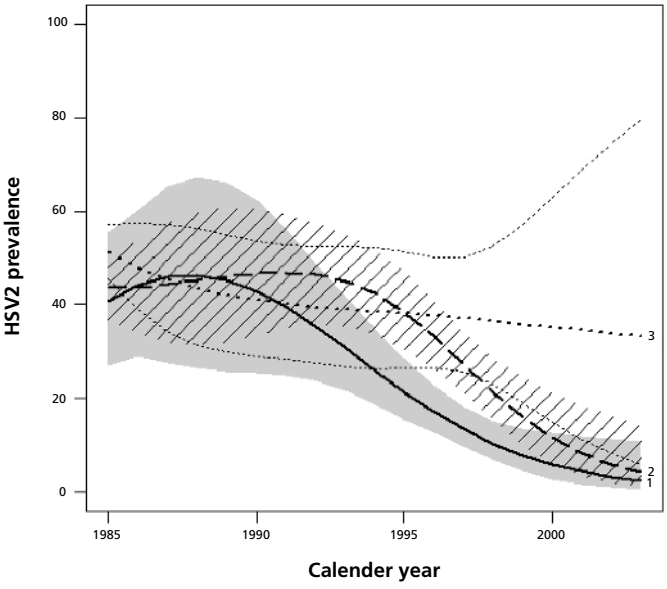


Figure 3b: Prevalence of HSV-2 and the 95% confidence inteval overtime among HIV negative MSM, according to number of lifetime sexual partners; 1 = 1-20, 2 = 21-200, 3 = >200 partners



4.1

Risk behaviour and HAART

Highly active antiretroviral therapy (HAART) among HIV-infected drug users: a prospective cohort study of sexual risk and injecting behaviour

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ABSTRACT

Aims To study sexual risk and injecting behaviour among HIV-infected drug users (DU) receiving highly active antiretroviral therapy (HAART). **Design and setting** As part of an ongoing prospective cohort study, HIV-infected DU who commenced HAART ($n = 67$) were matched with those not starting HAART ($n = 130$) on CD4 cell counts, duration of cohort participation, age and calendar year of visit. Immunological and virological responses of the HAART-treated DU were compared with the HAART-treated homosexual men from the same cohort ($n = 212$). **Measurements** Trends in behaviour and therapeutic response were tested with a logistic regression model adjusted for repeated measurements and a piecewise random effects model, respectively. **Findings** Non-HAART users reported more episodes of injecting than HAART users. In both groups injecting declined over time with no effect of HAART initiation. Before HAART initiation an increase in sexual risk behaviour was observed among those who had been assigned to receive HAART; their sexual risk behaviour declined thereafter. No change in sexual risk behaviour was found among non-HAART users. Relative to homosexual men, DU had a similar initial therapeutic response, but DU started HAART at lower CD4 cell counts and higher viral load levels. **Conclusion** DU who are treated with HAART are not increasing their risk behaviour, and their early response to HAART is similar to homosexual men. However, before the treated DU received HAART they were seen to inject less often than those not treated with HAART. This suggests that selection of potential HAART starters is based on limited drug use. Although the DU who commence HAART are a selected group, our results show that HIV-infected DU can be treated effectively.

Keywords Behaviour, therapeutic response, HAART, HIV infection, drug users.

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INTRODUCTION

In most western countries, highly active antiretroviral therapy (HAART) for HIV infection became widely available in 1996. HAART has been shown to be effective in reducing AIDS mortality and improving the quality of life [1]. However, after HAART was generally introduced, reports of an increase in sexual risk behaviour among homosexual men and women occurred [2–6], which may have reflected optimism regarding the effectiveness of this treatment [7–9]. Little is known about trends in risk behaviour among drug users (DU) after HAART became widespread [10–12] and results from published studies are conflicting. Relative to other HIV risk groups, DU appear to be less likely to commence HAART [13] and if they do, HAART initiation occurs later in the HIV infection [14]. A previous study in Amsterdam showed that 1 year after the introduction of HAART only one-third of eligible DU had started treatment [15]. The prescription of HAART by physicians tended to be influenced not only by decreasing CD4 cell counts and increasing HIV-RNA levels, but also by the expected adherence [16]. Some studies report that DU taking HAART do not differ in virological and immunological response from homosexual men [13], whereas other studies found that DU have an increased mortality rate [17–20]. By combining behav-

ioural and clinical outcomes, the present study was conducted to investigate outcomes of DU who initiate HAART. Our specific aims were to assess changes in injecting drugs and sexual risk behaviour as influenced by HAART availability and to examine virological and immunological response among HIV-positive DU using HAART, compared with those not and with homosexual men using HAART.

METHODS

Study population

The prospective Amsterdam Cohort Study (ACS) among DU began in December 1985 and is ongoing [21]. Recruitment is via local methadone outposts, sexually transmitted diseases clinics and word of mouth. Participants are asked to return for follow-up visits every 4–6 months at the Municipal Health Service. At each visit a standardized questionnaire is administered by specially trained nurses, and a blood sample is drawn. Blood samples are tested for HIV antibodies by enzyme-linked immunosorbent assay (ELISA), and when positive are confirmed using immunoblotting. HIV-positive participants undergo a clinical examination by a physician; they are not treated in the cohort setting but in one of the Amsterdam hospitals. Data about therapy use are self-reported during the clinical examination in the ACS and are supplemented by information from the Dutch HIV Monitoring Foundation [22] and from discharge letters, requested after hospital admission.

In blood samples of HIV-positive DU, CD4 cell counts are determined using flow cytometry and HIV RNA is determined using NASBA assays (Organon Teknika, Boxtel, the Netherlands). From 1997 onwards, a more sensitive test was adopted (Ultra Nuclisens, Organon Teknika) and participation in some clinical trials has led to the use of additional HIV RNA tests: AMPLICOR HIV, Roche (Almere, the Netherlands) and Quantiplex bDNA, Chiron (Mijdrecht, the Netherlands). In 1984, the ongoing ACS among HIV-positive and HIV-negative homosexual men was initiated [23]. Follow-up procedures are similar for both risk groups.

The ACS was conducted in accordance with the ethical principles set out in the Declaration of Helsinki and written informed consent is obtained prior to data collection.

Definition of variables

To study the effect of HAART initiation on behavioural changes in DU and to evaluate their therapeutic response, HAART was defined as an antiretroviral regimen that includes a combination of two or more antiretroviral agents, with at least one protease inhibitor (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI). HAART use was analysed as intention-to-treat.

At study entry, questions about current behaviour referred to the 6 months preceding cohort entry and at follow-up visits; these questions referred to the time period between the current and previous visit. Injecting was defined as injecting drugs at least once in the preceding period. The proportion of DU who reported borrowing needles and syringes (< 3%) was too small to analyse.

Sexual risk behaviour was defined as not always using a condom with a steady or casual partner in the preceding period. Unprotected sex with a steady HIV-infected partner was not considered to be sexual risk behaviour and was therefore excluded. Adherence to HAART is reported by the participant and referring to the period between visits. Patients were considered fully adherent if they reported having taken all prescribed medication and adherent for > 80% when taking at least 80% of all medication prescribed.

Statistical analysis

Matched study among drug users

Within the ACS, we matched HIV-positive DU using HAART with HIV-positive DU not using HAART [24], and compared the groups with respect to sexual risk and injecting behaviour. HAART users were matched on the last visit before HAART initiation with non-HAART users. One control could be matched more than once with various HAART users on different cohort visits. The matching criteria were age (± 7 years), number of ACS visits (± 2 visits) and CD4 cell count (± 150 cells/mm^3). Because, in preliminary analysis, calendar time appeared to be associated with behavioural changes, we also matched on the calendar year of visit.

If possible, a HAART user was matched with two non-HAART users. The visit on which matching occurred was designated as the reference visit. HAART users and non-HAART users were compared pairwise at the three visits before and after the reference visit. Visits were ranged from −3 to 3, with 0 being the matched (reference) visit; visits −3, −2, −1 and 0 are the visits before a HAART user initiated HAART, and visits 1, 2 and 3 are the visits after HAART initiation. Differences in sexual risk and injecting behaviour at each visit between HAART users and non-HAART users were tested using the MacNemar test.

Changes in risk behaviour among HAART users and non-HAART users were modelled within a logistic regression model, using the generalized estimating equation (GEE) method with an autoregressive covariance matrix to adjust for correlations between visits of the same individual. Trends in behaviour were modelled piecewise, allowing for a change in trend in risk behaviour at the reference visit.

Comparison of drug users with homosexual men with respect to HAART response

Immunological and virological therapeutic responses among DU were compared with these responses in homosexual men. The simultaneous development of CD4 cell counts and HIV RNA levels was modelled via a random effects model [25]. Its design allows for a random intercept and random slopes for CD4 cell counts and HIV RNA levels per individual, and the relation between CD4 and HIV RNA is modelled via the covariance matrix of the random effects. Because therapeutic response in patients is stronger shortly after therapy initiation and slows down thereafter, changes in CD4 cell count and HIV RNA load in the above-described models were modelled piecewise: the slope was allowed to change at the start of HAART (or matched time of controls), for HIV RNA levels 1.5 months after HAART initiation and for CD4 cell counts 3 months after HAART. Because the time between cohort visits varied between 3 and 6 months, we used time before and after the reference visit instead of visit numbers in this model. HIV RNA levels under the detection border were treated as left-censored assuming a normal distribution [26].

The analyses were performed using the Correlated Data Library in S-Plus 6.2 [27] and using WINBUGS [28].

RESULTS

Between 1 January 1996 and 31 March 2003, 202 HIV-positive drug users, including 65 (32.2%) women, were followed-up in the ACS. Of these 202 participants, 68 started HAART. More than a year later 74% of the DU who started HAART still used a HAART regimen.

Matched study

Of the 68 HAART users, 67 were matched with 130 non-HAART users into 67 pairs (Table 1). Two of the 68 HAART users were matched with one control person and one HAART user could not be matched at all.

Among those who initiated HAART, 51% had not used any antiretroviral agent before, the others were using or had used mono- or dual therapy at the point of starting HAART. At the reference visit 58% of the non-HAART users were treatment-naive (Table 1), whereas 17% had used mono- or dual therapy in the past. At the reference visit itself 25% used a NRTI-containing regimen.

At the first visit after HAART initiation 55% of the DU reported they were fully adherent, and 59% took more than 80% of all medication prescribed. Lower adherence was found at visit 2, followed by a higher adherence at visit 3 (Table 1).

At their reference visit, most DU used both heroin and cocaine. Among HAART users 15% used heroin only and

Table 1 Characteristics at reference visit for DU who initiated HAART and those who did not start.

	HAART users (n = 67)	*Non-HAART users (n = 130)*
Male (*n*%)	49 (73)	92 (71)
Age (years)	40 (36–43)	38 (35–41)
% Methadone	87	95
% Type of drug injected		
Heroin + cocaine	61	51
Heroin	15	28
Cocaine	20	12
% Injectors who report injecting at least three times per week	50	62
Follow-up time (years)	8.08 (4.6–10.2)	7.98 (4.2–10.0)
CD4 cell count ($10^{6/l}$)*	185 (143–268)	200 (130–280)
Viral load ($\log_{10}$ copies/ml)*	4.30 (3.30–4.83)	4.20 (3.58–4.64)
ART† naive (*n*%)	34 (51)	75 (58)
HAART regimen (n%)		
1 PI + 2 NRTIs	46 (69)	
1 NNRTI + 2 NRTIs	11 (16)	
2 PI 2 2 NNRTIs	9 (13)	
1 NNRTI + 1 NRTI	1 (2)	
Adherence per visit	Fully	> 80%
Visit 1	55%	59%
Visit 2	36%	50%
Visit 3	52%	62%

*Median, interquartile range; †ART: antiretroviral therapy.

20% used cocaine only (Table 1), while among the non-HAART users 28% used heroin only and 12% used cocaine only; 87% of the HAART users and 95% non-HAART users received methadone treatment. The median dosage of methadone was 70 mg per day (IQR: 50–100) among HAART users and 78 mg (IQR: 50–100) among the non-HAART users on methadone. Among those injecting, 50% of the HAART users injected at least three times per week, while 62% of the non-HAART users injected at least three times per week.

Behaviour among DU with HAART and without HAART

At their reference visit, 42% of the DU using HAART reported injecting since the last visit versus 61% among the non-HAART users. The proportion of DU that reported injecting drugs was significantly lower among HAART users than non-HAART users at all visits, except at the last two visits (Fig. 1a). The proportion of participants who reported injecting drugs declined from 51% at visit −3 to 30% at visit 3 in the HAART group and from 66% to 44% in the non-HAART group. When modelled piecewise, HAART users as well as non-HAART users showed a non-significant decline in injecting drugs over time. This decline did not change after HAART initiation.

Figure 1b shows the proportion of HAART users and non-HAART users who reported unprotected sex with a casual or steady partner having an unknown or HIV-negative serostatus. At the reference visit, 15% of the HAART users and 13% of the non-HAART users reported unprotected sex. Significant differences between HAART users and non-HAART users were seen at every visit. On visits −1, 0 and 1, HAART users reported more sexual risk behaviour than the non-HAART group. When HAART users were modelled piecewise, their sexual risk behaviour increased [OR = 1.67 per year: 95% CI = 0.98–2.83 (P = 0.06)] before HAART initiation. In the same group, a change in sexual risk was seen following HAART initiation [OR = 0.33 per year: 95% CI = 0.10–1.08 (P = 0.07)]. For the non-HAART group, sexual risk behaviour did not change over time.

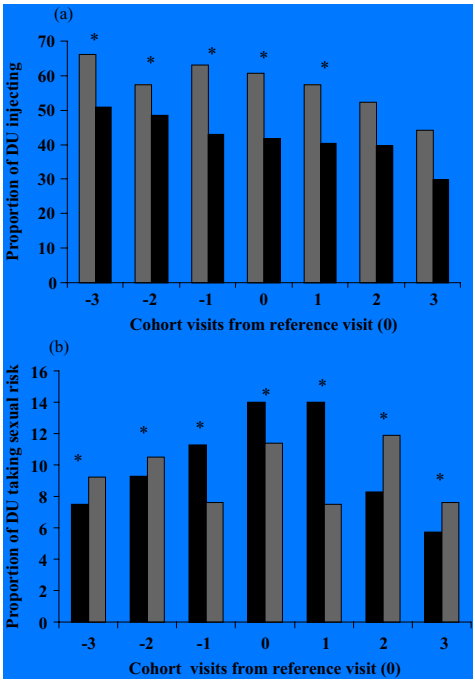

Figure 1 Proportion of HAART users (black) and non-HAART using DU (grey) who reported injecting drugs (a) since previous visit and unprotected sex (b). Where visit 0 is the reference visit (i.e. visits −3, −2, −1 before before matching and 1, 2, 3 after matching). Statistically significant difference ($P < 0.05$)

HIV RNA levels and CD4 cell counts

The immunological and virological trajectories of DU who used HAART were compared with homosexual men using HAART (n = 212, of whom 67 were treatment-naive) and with DU who did not use HAART (Fig. 2). In these figures time 0 represents the start of HAART among the HAART users and the reference visit among the DU not using HAART.

CD4 cell counts

Median CD4 cell count among DU is shown in Table 1. At time of HAART initiation, homosexual men had higher CD4 cell counts, median 360×10^6/l (IQR 180–510), relative to DU ($P < 0.01$).

The piecewise modelled immunological response is shown in Fig. 2a. Before time 0, CD4 cell counts declined in all groups, but more strongly among DU than among homosexual men.

In the first 3 months after therapy initiation (time = 0), CD4 cell counts increased significantly in both HAART-treated groups, and no significant difference in this increase was found between the DU and homosexual men. More than 3 months after time 0, CD4 cell counts continued to increase significantly among the HAART-treated homosexual men, while tending to remain stable among the DU with HAART; however, the slopes between DU on HAART and homosexual men on HAART did not differ significantly. CD4 cell counts continued to decrease significantly, among DU not on HAART, but their difference in slope with DU on HAART became insignificant.

HIV RNA levels

Table 1 shows the HIV RNA levels at the reference visit among DU. At that visit, homosexual men had a lower viral load, median HIV RNA 3.37 $\log_{10}$ copies/ml (IQR

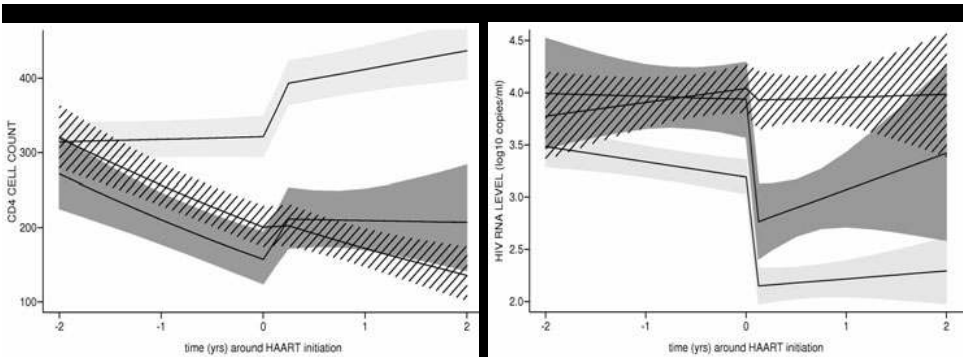

Figure 2 Therapy responses among DU (dark grey area) and homosexuals (grey area) on HAART and DU not on HAART (shaded area). Before and after time 0 (HAART initiation or matched time of non-HAART using DU). (a) Immunological response, piecewise modelled course of CD4 cell counts (10^6/l); slope was allowed to change at time 0 and at 3 months after time 0. (b) Virological response, piecewise modelled course of HIV RNA levels ($\log_{10}$ copies/ml); slope was allowed to change at time 0 and 1.5 months after time 0

2.60–4.68), relative to DU ($P < 0.01$). HIV RNA levels did not increase before time 0 among homosexual men, DU on HAART and non-HAART using DU.

During the first 1.5 months after time 0, a strong significant decline in HIV RNA levels was seen among DU on HAART and the homosexual men on HAART. This response in the first 1.5 months was comparable for homosexual men and DU, whereas the response of DU not using HAART differed significantly from DU on HAART after time 0. More than 1.5 months after time 0, the decrease in HIV RNA did not remain significant in either group on HAART. No significant differences in slope were seen between the homosexual men and DU on HAART. Although the rise was not significant, viral load levels tended to increase slightly among the DU on HAART after 1.5 months.

Adjustment for current injecting behaviour and frequency of injecting did not change these results.

DISCUSSION

In the present study, the impact of HAART was examined on behavioural changes and the response to HAART among HIV-infected DU. Because we observed a decline in injecting behaviour among both DU who had started HAART and those who had not, we considered this decline as a general trend without any relation to HAART initiation. Before HAART initiation, however, episodes of injecting were less often reported by the DU who would start HAART. This finding suggests that selection of DU to start HAART may be based on their current drug use. We observed a small decrease in sexual risk behaviour after HAART initiation, after an initial rise. Strong virological and immunological responses reflected by CD4 cell counts and HIV RNA levels were seen shortly after HAART initiation, and this short-term response did not differ between DU and homosexual men.

Longitudinal studies of homosexual men have shown an association between high-risk behaviour and HAART [2,3,7], suggesting that HAART efficacy may lessen concern about engaging in unsafe sex. To our knowledge, only three longitudinal studies have examined risk behaviour and HAART use among DU. Among American DU in Baltimore, no increase in injecting or needle sharing was seen after HAART initiation, but an increase in unprotected sex was observed [10]. In the same cohort, unprotected sex was associated with an improved immune status after HAART initiation [12]. In contrast, a decline in sexual risk behaviour was found in a prospective study among French HIV-infected DU [11]. We demonstrated in our study that sexual risk behaviour declined after HAART initiation.

Differences in results between these studies may be caused by their different methodological approaches and differences in selection into treatment or counselling. To rule out the possibility that calendar time might bias our results, we used HIV-infected DU not receiving HAART as a control group by matching them with treated DU on calendar year of visit. The other studies did not match by calendar year, a factor that may have confounded their data. To confirm our results, we conducted an additional ecological study, to investigate the association between HAART and calendar time with injecting and sexual risk behaviour (data not shown). In line with the matched study, calendar time and not the start of HAART was associated with behavioural changes.

In the present study HAART initiation among DU seems delayed, as shown by the low CD4 cell counts and high HIV RNA levels at the time of HAART initiation, and many HAART-eligible DU never started HAART during

our follow-up period. As the DU who would start HAART later reported fewer episodes of injecting than those who never started, it seems likely that DU on HAART are a selected group and that selection may be based on current injection of drugs. Relatively frequent injecting is reported by providers who prescribe HAART to be a barrier [29,30]; other barriers are perceived unreliability in managing appointments, alcohol use and housing. This association between current injecting and HAART initiation has been found in several studies [12,13,19,29]. It was not found in two previous studies among DU in Amsterdam but both were conducted shortly after HAART became generally available in Amsterdam [14,15], when its prescription could have been more lenient.

Several studies have shown a shorter survival of HIV-infected DU in the HAART era, relative to homosexual men [17–20]. In our study, among DU on HAART, the initial response of both HIV RNA and CD4 levels were similar to homosexual men. Although low CD4 cell counts at HAART initiation are associated with a faster progression to AIDS or death [19], the baseline CD4 and HIV RNA levels are less indicative than the early immunological and virological response to HAART and the levels a patient ultimately achieves over time [31]. Our DU subjects started HAART at higher HIV RNA levels and lower CD4 cell counts than homosexual men, and despite a similar initial response DU never reached the levels of the homosexual men. Therefore, it is likely that therapy will be less effective in the long term.

The relatively low adherence among DU might partly explain this less effective response [32]. Among DU initiating HAART in our study only 36–55% were fully adherent, whereas among HAART users in general adherence varies between 85 and 90% [33]. In addition, adherence of our DU might be overestimated, as we defined adherence as taking all medication prescribed every day. We did not also consider whether timing and dietary restrictions were followed, which is a more sensitive way to assess adherence. Poor adherence might be related to developing drug-resistant HIV strains, but results from a recent study among DU in this cohort showed a decrease in drug-resistant strains over time [34].

Most of the DU in the ACS are also participating in a low-threshold methadone programme by the Municipal Health Service [35]. In this programme illicit drug use is tolerated, which explains the high injecting rate among DU on methadone. The ultimate goal is to stop DU from using drugs. However, when this goal cannot be reached, the policy is to minimize the damage they cause to themselves and their environment. Our study indicates that the low-threshold methadone programme enables effective HIV treatment for those DU who continue using illicit drugs. With regard to HIV-related risk behaviour, an earlier study has shown that a high methadone dosage of more than 80 mg per day reduces injecting-related risk behaviour in the ACS [36]. However, in our study an association between methadone dosage and HAART response could not be found (data not shown). The immunological and virological responses to HAART may be stronger in those who had no prior exposure to antiretroviral therapy [37]. However, the percentages of DU HAART users and non-HAART users who were treatment-naive at the reference visit were almost the same (51.2% versus 57.7%). Among the homosexual men on HAART, only 31.6% were treatment-naive.

The findings from this prospective cohort study show that a large number of the HIV-infected DU eligible to initiate HAART are still not receiving HAART. Nevertheless, those who are treated with HAART are not increasing their risk behaviour, and their early response to HAART is similar to homosexual men. Although the DU who commence HAART are a selected group, our results show that HIV-infected DU can be treated effectively. Where there is an extremely low frequency of needle sharing and a low level of sexual risk behaviour among HIV-infected DU, as in Amsterdam, the potential for HIV spread is small. It therefore seems unlikely that poor adherence together with increased risk behaviour among DU will lead to transmission of drug-resistant HIV virus strains.

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5

General discussion

The introduction of HAART in the mid-1990s has changed the HIV epidemic, resulting in a decline in HIV-related morbidity and mortality. Since HIV-infected individuals live longer, morbidity and mortality linked to co-infection with HCV have become more important. Moreover, since HAART became generally available, increases in HIV-risk behaviour have been reported among homosexual men.
The studies presented in this thesis describe trends in mortality, risk behaviour and HIV-co-infections in various HIV risk groups, in particular homosexual men and injecting drug users.

Declining mortality in the era of HAART

Overall mortality
In this thesis much attention has been paid to mortality in HIV-infected individuals. After HAART became widespread, AIDS-related mortality started to decline in most Western countries. In Amsterdam as in other cities, the AIDS epidemic had a major impact on mortality, with AIDS becoming the most important cause of death in men aged between 25 and 54 years in the 1990s [1] [2]. After 1996, AIDS-related death became less important in Amsterdam, but mortality rates began to drop even before the widespread use of HAART. In chapter 2.1, we showed that although HAART greatly contributed to the declining mortality rates, the decrease in new HIV infections also contributed to this decline. In future evaluations of AIDS-related mortality in the general population, HIV incidence patterns must be taken into account. For example, among older homosexual men, the HIV incidence has started to rise again [3], and not taking this increase into account might result in an underestimation of future HAART-related declines in mortality rates.

Cause-specific mortality

The probability of dying has decreased for most specific causes of death in the era of HAART. Nevertheless, results from the CASCADE study discussed in chapter 2.2 showed that AIDS opportunistic infections remained the most important cause of death in the HIV-infected population. Thus that HIV-infected individuals continue to die from AIDS-related events, due to factors like late HIV diagnosis, delayed initiation of HAART and prior suboptimal mono or dual therapy [4] [5]. As also shown in chapter 2.2, the benefits of HAART were less strong among drug users across the specific causes of death, compared to homosexual men, which may be explained by the sub-optimal access to HAART in drug users. The

risk of dying from non-natural causes of death increased among drug users in the HAART-era, as noted in chapter 2.2 and 2.3. In chapter 2.3, we showed that fewer drug users are now dying from AIDS-related death, resulting in a larger population of drug users at risk of dying from the non-natural causes of death that are mainly a result of their risky life style.

In line with other studies, we found an increased risk of dying from hepatitis and liver-related disease [6] [7] [8] [9]. Since more HIV-infected individuals now survive, a larger number remain at risk to die from this competing cause. Infection with hepatitis C virus (HCV) is a common co-infection in HIV-risk groups, especially among drug users and haemophiliacs. Furthermore, we showed a higher risk of dying from hepatitis and liver related disease among HCV/HIV-co-infected drug users than in drug users solely infected with HCV, after correction for duration of injecting. This suggests that HIV continues to have a negative effect on the progression of HCV-disease (chapter 2.3). As the median time from HCV infection to liver cirrhoses is estimated to be 20 years [10] and most drug users became infected with HCV in the 1970's and 1980's, mortality from HCV in HIV-infected individuals is expected to further increase [11].

Life expectancy

Although the life expectancy of HIV-infected individuals has improved, mortality rates remain higher compared to the general population [12] [13]. An explanation for these increased mortality rates might be the large proportion of HIV-infected individuals who became infected before the introduction of HAART and could not fully benefit from HAART, due to prior suboptimal therapy or a delay in HAART initiation. We found lower mortality rates among those infected in the era of HAART than in the total population of HIV-infected individuals in this era, suggesting that the life expectancy in those newly infected will further increase in the future. However, follow-up time of those individuals who became infected in the HAART era (after 1996) is no more than 10 years. In addition, treatment strategies have been optimised and dosage simplified since 1996. As a consequence, mortality among HIV-infected individuals is probably overestimated, and studies that compare their life expectancy with that of the general population are under-estimating the life expectancy of those HIV-infected individuals who could fully benefit from HAART. The actual contribution of HAART to the decline in mortality and to the improvement of life expectancy cannot be assessed with the current available data. Therefore it is of interest to study in more detail the differences between those infected before the introduction of HAART and those infected after HAART became widespread. Such seroconverter studies are essential to determine whether the life expectancy of those infected with HIV in this era remains shorter than in the general population.

HIV co-infections

Due to infectious agents with overlapping routes of transmission, co-infections are prevalent among HIV-infected individuals [14] [15] [16] [17] [18]. In chapter 3.1, we have shown that 31% of HCV-infected drug users were co-infected with HIV, while chapter 3.2 demonstrates that 30% of the HSV-1-positive homosexual men and 41% of the HSV-2-positive homosexual were infected with HIV.

In chapter 3.1, we have shown a decline in the HCV incidence among drug users in Amsterdam that is comparable to the decline in HIV incidence in the same population, although the HCV incidence is more than four times the HIV incidence. This decline in HCV incidence is in contrast with other studies and might be explained by the long follow up time of the ACS [19] [20]. Most coinfected drug users first became infected with HCV and then with HIV. Chapter 2.3 describes an increased risk of dying from hepatitis and liver-related disease among HCV/HIV co-infected drug users compared to those infected with HCV only. HIV infection appears to accelerate the HCV disease progression even in the HAART era [21]. This finding emphasizes the importance of HCV treatment in HCV/HIV-co-infected individuals, even though it is complicated in HIV-infected individuals due to the interaction between anti-retroviral drugs. HIV-infected drug users, who are almost universally coinfected with HCV benefit less from HAART than other HIV risk groups [22] [23]. On the other hand, factors that are complicating the HIV treatment will act as barriers to HCV treatment as well. Further studies are needed to determine the barriers for HCV treatment in HCV/HIV-co-infected drug users.

Response to HCV treatment is associated with various factors, such as HCV genotype, CD4 cell count, and active drug use; therefore HCV treatment will be complex for most HCV/HIV-co-infected drug users. Concurrent treatment with methadone is no contraindication for HCV treatment [24]. Depending on their individual situation, drug users on methadone may be acceptable candidates for HCV treatment, although adjustments in the methadone dosage may be needed [25]. Much attention should be paid to programmes in which HCV treatment will be provided to drug users and a multidisciplinary team should consider each case [26]. Daily-observed treatment and the integration of HCV therapy into methadone programmes would be helpful to improve adherence. These approaches are currently being initiated in Amsterdam.

Two other important and common HIV co-infections are HSV-1 and 2 [14] [15]. The prevalence of HSV-1 and 2 decreased over time in HIV-uninfected homosexual men in the period 1984-2003 but remained high among those infected with HIV. Also, the association between HSV-2 and HIV became stronger over time. Several studies have recognised HSV-2 infection as a risk factor for HIV infection [27] [28] [29]. As 41% of the homosexual men in the ACS had antibodies to HSV-2, it is likely that HSV-2 infection is one of the driving factors in the HIV epidemic among homosexual men. Therefore, prevention of HSV-2 may play an important

role in the prevention of new HIV infections among them. There is a need to make homosexual men alert to HSV-2 infections, although a small proportion of HSV-2-infected individuals are able to recognise the symptoms. It has been shown that being aware of having an HSV-2 infection improved the recognition of its symptoms [30]. However, most shedding of HSV-2 occurs without symptoms [31]. Serological screening is thus crucial to identify those men infected with HSV-2. Commercially available serological assays have recently made possible the screening of high-risk populations. Their low specificity may be an argument against serological screening, but chapter 3.2 shows that using a higher cut-off value will decrease the number of false positive results, making the assays a reliable screening tool. Those HIV-uninfected homosexual men who are infected with HSV-2 should be counselled to avoid unsafe sex especially during HSV-2 symptoms to reduce their risk of acquiring HIV. More important, suppressive therapy is effective in reducing recurrent genital lesions [32] [33] and its use may play an effective role in the prevention of HIV infection.

Also in chapter 3.2, we demonstrate an increase in the HSV-1 prevalence among highly sexually active homosexual men between 1984 and 2003. This suggests that the sexual transmission of HSV-1 is increasing over time. HSV-1 infection has been associated with HIV infection, but not shown to be is a risk factor for HIV acquisition in earlier studies [27] [34] [29].However, we still consider that HSV-1 may be a risk factor since the population of homosexual men with genital HSV-1 infections is growing. Future longitudinally incidence studies are needed to study the association between HSV-1, HSV-2 and HIV infection. Unfortunately, no good quality serological assays for HSV-1 are available for conducting such studies at this time.

Risk behaviour and HAART

Since the introduction of HAART, increases in sexual risk behaviour have been reported among homosexual men [35] [36] [37] [38] [39], but little is known about changes in risk behaviour among drug users in the HAART era [40] [41] [42]. In chapter 4.1, we examined the behavioural changes among HIV-infected drug users, comparing those who had started HAART with those who had not, by matching them on pre-treatment CD4 cell counts and other relevant factors. Among those on HAART, we observed a decline in injecting behaviour that began before they started HAART and also continued thereafter. Because injecting behaviour declined comparable in the non-HAART group, we considered this decline to be a general trend that was not related to HAART.
Compared to other HIV risk groups, drug users are less likely to commence HAART and, if HAART is initiated, the start occurs later in the HIV infection. This delayed initiation of HAART might cause poorer immunologic and virologic therapeutic responses, as shown by some earlier studies. In chapter 4.1, we compared the immunologic and virologic responses among drug users who had started HAART with responses in homosexual men who had started HAART.

HAART initiation was indeed delayed among DU, as shown by a lower CD4 cell counts and higher HIV RNA level, but their short-term immunologic and virologic responses were comparable to those in homosexual men. It is nevertheless likely that the delayed initiation of HAART among drug users will negatively affect their long-term response. Therefore, earlier HAART initiation remains an important issue in treating HIV-infected drug users.
DU who had started HAART less often reported episodes of injecting than those who had not started HAART. This finding suggests that drug users who begin HAART are more stable in general or that the decision to begin is based on their current drug use. A high level of current drug use is, indeed, one of the reported barriers to prescribing HAART to drug users. Another important barrier is lack of adherence, which might will be associated with active drug use. We reported that a lower proportion of drug users were fully adherent compared to the general population of HAART users. However, studying the factors associated with adherence in this group might help to find ways to improve adherence. Also, insight into the reasons for delayed HAART initiation might help to improve the uptake and therapy outcome among drug users.
In the total group of DU in the ACS, a reduction in HIV and HCV incidences as well as risky injecting behaviour has been observed [43]. Therefore the argument that DU are likely to be reinfected with HCV is no reason to postpone HCV or HIV treatment.

HIV cohort studies in the future

The introduction of HAART has dramatically changed the face of the HIV epidemic. HIV infection has become a chronic disease and as a consequence, individuals with HIV live longer these days.
Important research issues now focus on the best time to initiate HAART, and concerns are shifting to its side effects and its effectivenes in the long term.
HIV-seroconverter cohorts will remain important for the study of research questions that require adjustment for the duration of infection and where long follow up time is required, as when life expectancy is compared between those infected before HAART became widespread and those infected after 1996, who have the potential to fully benefit from HAART. A known date of HIV-seroconversion makes it possible to follow HIV-disease progression through all its stages. HIV-seroconverter cohort studies are likewise needed to evaluate the role of HIV-co-infections on all stages of the HIV infection. For example, what will happen to the immunology and virology of HCV-infected individuals when they are subsequently infected with HIV? Results from such studies are needed to predict the future course of the HIV epidemic. However, as more HIV-infected individuals live longer and new HIV infections still occur, the population of HIV-infected persons is becoming larger. Also, since duration of exposure to antiretroviral therapy is increasing, a growing number of patients will face treatment-related side effects. Therefore, it becomes more important to conduct large observatio-

nal cohort studies in which the occurrence of therapy side effects and the effectiveness of long-term treatment can be occurred.
Individual cohorts tend to have relatively small numbers of patients that represent specific exposure groups or specific outcomes of interest, such as certain HIV- or non-HIV-related malignancies or rare therapy-related side effects. The resulting lack of power may reduce the ability of cohorts to examine research questions concerning study outcomes that take a long time to develop and thus need a long follow-up time. Therefore individual cohorts should work closely together on a collaborative basis. Combining data will increase follow-up time and the number of events, thereby increasing power.
Furthermore, with HCV and hepatitis B (HBV) being highly prevalent and leading to morbidity and mortality in HIV-risk groups, cohorts focused on HIV should also collect data on such topics as screening for HCV and HBV co-infection, HCV and HBV treatment, and HCV- and HBV-related morbidity and mortality.

Another important issue for future HIV research is the development of drug-resistant strains and the transmission of these resistant strains to newly HIV-infected persons, which may increase the number of patients with limited treatment options. Also of concern are the patients whose HIV can no longer be treated because there are no treatment options left for them. As the duration of exposure to HAART increase, this group of patients could well become larger.

In conclusion, although AIDS-related mortality has declined since the introduction of HAART, mortality remains an important issue for HIV-infected individuals. They still have higher mortality rates compared to the general population, and their risk of liver related-mortality is increased as a consequence of HCV and HBV co-infection. Future research must address the long-term effectiveness of HAART, taking the effect of HCV treatment into account. Insight into the related factors will contribute to an improvement in the prognosis of HIV-infected individuals.

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Summary

This thesis presents research on overall mortality, specific causes of death, HIV-related behaviour, and co-infections with HCV and HSV in the HIV epidemic, which started 25 years ago in Amsterdam. For all of the studies data was drawn from the Amsterdam Cohort studies on HIV and AIDS or the CASCADE collaboration. For one study additional data from the Amsterdam AIDS surveillance system, STI clinic surveys, and a trial of Hepatitis B vaccine were used (chapter 1).

Mortality

Decline in AIDS mortality
As a result of the success and widespread use of HAART, mortality resulting from AIDS has been declining in most industrialised countries. However, mortality trends are influenced by the HIV incidence pattern in the past. In chapter 2.1, the impact of HAART on mortality was evaluated, taking into account earlier HIV incidence patterns. AIDS surveillance data (1982-2000) were used to examine the observed course of the AIDS epidemic among homosexual men in Amsterdam; HIV incidence patterns (1980-2000) among homosexual men were used to estimate the course of the epidemic if HAART had not been introduced. The estimated course of the AIDS epidemic without the HAART benefits showed a decline in the AIDS mortality, this decline was not as strong as the observed decline. Although the introduction of HAART in 1996 greatly contributed to the decline, AIDS mortality rates had begun to drop before 1995 as a result of an earlier decline in the HIV incidence. Accounting for changes in the HIV incidence patterns, we estimated that 331 deaths were prevented by HAART in Amsterdam between 1996 and 2000.

Cause-specific mortality following HIV seroconversion
In Western countries, the life expectancy of HIV-infected individuals has improved since HAART became generally available. As HIV-infected individuals live longer these days, they are more likely to die from non-HIV related causes of death. Chapter 2.2 explored the impact of the introduction of HAART on cause specific mortality. Pooled data from the CASCADE collaboration was used to calculate the cumulative incidence for each cause of death in the presence of competing causes of death. Although the cumulative incidences of all causes of death decreased in the HAART era, AIDS opportunistic infections remain the most common cause of death; Thus AIDS-related events will continue to be an important cause of death in the future. In addition, this study shows a trend towards a higher probability of dying from hepatitis and liver-related disease in the HAART era across most HIV risk groups. As follow-up time since the introduction of HAART is limited, it might be too early to observe increases in non-AIDS-related death as

a consequence of therapy-related toxicities. Monitoring mortality trends will be essential for the future.

The progression of liver disease associated with HCV is known to be accelerated in HIV-infected individuals. Since HIV-infected drug users are almost universally co-infected with HCV, HCV/HIV-co-infection has a major impact on mortality in this group. In chapter 2.3, the cause-specific mortality was compared for drug users who are HCV-mono-infected, HCV/HIV-co-infected, HIV-mono-infected and HCV/HIV-uninfected, in the pre-HAART and HAART eras, over a twenty-year period. Those HIV-mono-infected or HCV/HIV-co-infected had the highest risk of dying. The co-infected group showed a sevenfold higher risk of dying from liver and hepatitis-related disease in the HAART era compared to the HCV-mono-infected group. These results highlight the need for HCV treatment in HCV/HIV-co-infected drug users.

HIV co-infections

Decline in HCV and HIV incidence rates
Injecting drug users are at high risk for infections with HCV and HIV. Under the threat of AIDS, those in Amsterdam reduced their injecting risk behaviour and consequently their HIV incidence. In chapter 3.1, the HCV incidence and related risk factors among drug users in the ACS were studied, and HCV incidence was compared with HIV incidence. Among ever-injecting drug users, the HCV prevalence was 85%, and 31% were co-infected with HIV. The HCV incidence declined considerably over a twenty-year period. Although the HCV incidence was 4 times the HIV incidence, its decrease was similar to the decrease in the HIV incidence. Drug users who started injecting drugs in a recent calendar period were at lower risk of HCV infection than those starting earlier, probably as a result of reduced risk behaviour among drug users at the population level.

HSV-1 and 2 among homosexual men
HSV-1 and 2 are both highly prevalent among homosexual men. HSV-2 is considered to be an important risk factor for HIV acquisition. Chapter 3.2 describes changes in HSV-1 and 2 prevalence over a 20-year time period. The overall prevalence of HSV-1 and 2 decreased over time among HIV uninfected homosexual men but not among HIV infected homosexual men. Moreover, the HSV-1 prevalence increased among HIV-uninfected men who are highly sexually active (i.e. those with at least 200 lifetime sexual partners). The association between HSV-2 and HIV in homosexual men became stronger over time. Since HSV-2 and HIV are now more strongly related than in the early days of the HIV epidemic, a determination is needed regarding the extent to which the prevention of HSV-2 can contribute to the controlling of the HIV epidemic. And because an increasing proportion of HSV-1 infections are transmitted sexually, the potential role of HSV-1 as a risk factor for HIV acquisition needs to be studied.

Risk behaviour and HAART

Since the introduction of HAART, sexual risk behaviour among homosexual men has increased. Study results on trends in risk behaviour among drug users are conflicting. Our own results on sexual risk and injecting behaviour and responses to HAART among HIV-infected drug users receiving HAART are discussed in chapter 4.1. Those who commenced HAART were matched with those not treated with HAART on CD4 cell counts and other important factors. Immunologic and virologic responses of the HAART treated drug users were compared to the responses of HAART-treated homosexual men. A general decline in injecting behaviour was observed among both HIV-infected drug users on HAART and a comparable group not receiving HAART. Those who are treated with HAART are not increasing their injecting risk behaviour, and their early response to HAART is similar to homosexual men. Although drug users who are receiving HAART are a selected group, because episodes of injecting were less often reported than by drug users not treated with HAART, results of this study show that HIV-infected drug users can benefit from HAART.

Although AIDS-related mortality has declined since the introduction of HAART, mortality remains an important issue for HIV-infected individuals.Their mortality rates are higher compared to the general population, and include an increased risk of liver-related mortality as a consequence of HCV and hepatitis B co-infection. Future research is needed to assess the long-term effectiveness of HAART and treatments for these and other coinfections.

Samenvatting

In Nederland werd AIDS voor het eerst gediagnosticeerd in 1982. De HIV epidemie trof met name homoseksuele mannen en in 1990 werd AIDS in Amsterdam de belangrijkste doodsoorzaak onder mannen in de leeftijd van 24 tot 55 jaar. Halverwege de jaren negentig kwam in de Westerse landen een effectieve HIV therapie (HAART) beschikbaar, dit leidde tot een drastische daling in de sterfte aan AIDS. Door de toename in de levensverwachting van HIV geïnfecteerde personen, neemt de ziekte en sterfte als gevolg van hepatitis co-infectie toe. Daarnaast is sinds het beschikbaar komen van HAART een toename in het risicogedrag onder homoseksuele mannen gerapporteerd. Dit proefschrift beschrijft de veranderingen in sterfte, risicogedrag en HIV co-infecties, zoals hepatitis C, in verschillende HIV risicogroepen, maar met name in homoseksuele mannen en drugsgebruikers.

Sterfte

Daling in AIDS gerelateerde sterfte
Als gevolg van de succesvolle HIV therapie daalde de sterfte door AIDS in de meeste geïndustrialiseerde landen. Trends in de AIDS gerelateerde sterfte worden niet alleen beïnvloed door het beschikbaar komen van deze therapie maar ook door het aantal nieuwe HIV infecties. De invloed van het aantal nieuwe HIV infecties op de daling in de AIDS gerelateerde sterfte is geëvalueerd in hoofdstuk 2.1. Om het werkelijk verloop van de AIDS epidemie onder homoseksuele mannen in Amsterdam in kaart te brengen is gebruik gemaakt van de gegevens van de AIDS surveillance (1982-2000). Het patroon van het aantal nieuwe HIV infecties (1980-2000) onder homoseksuele mannen is gebruikt om het verloop van de AIDS epidemie te schatten wanneer HAART niet beschikbaar was gekomen. Het geschatte verloop van de AIDS epidemie zonder de gunstige effecten van HAART liet een daling in de AIDS sterfte zien, maar deze daling was lang niet zo sterk als de werkelijke daling. Hoewel de introductie van HAART in 1996 een zeer grote bijdrage heeft geleverd aan de daling in de AIDS sterfte, daalde de AIDS sterfte cijfers al voor 1995 als een gevolg van een eerdere daling in het aantal nieuwe HIV infecties. Door rekening te houden met de veranderingen in het aantal nieuwe HIV infecties, hebben wij geschat dat in Amsterdam, tussen 1996 en 2000, 331 sterfgevallen door AIDS zijn voorkomen door HAART.

Specifieke doodsoorzaken na HIV-seroconversie.
De levensverwachting van HIV-geïnfecteerden is aanzienlijk toegenomen door het beschikbaar komen van HAART. Hierdoor is de kans dat HIV-geïnfecteerden sterven als gevolg van niet aan HIV gerelateerde doodsoorzaak toegenomen. Hoofdstuk 2.2 beschrijft de impact van de introductie van HAART op veranderin-

gen in de specifieke doodsoorzaken. Hoewel er een afname was in alle doodsoorzaken, bleef de sterfte aan AIDS opportunistische infecties de meest belangrijke doodsoorzaak. Deze studie liet ook een toename in de kans om te sterven aan hepatitis en levergerelateerde aandoeningen in het HAART tijdperk zien. Aangezien de follow-up tijd sinds het beschikbaar komen van HAART nog beperkt is, kan het te vroeg zijn om een toename in niet aan AIDS gerelateerde sterfte waar te nemen als gevolg van bijwerkingen door HAART. Het monitoren van sterfte trends blijft daarom van belang in de toekomst.

De progressie van lever gerelateerde ziekte, geassocieerd met hepatitis C, is versneld in HIV-geïnfecteerde personen. Bijna alle HIV-geïnfecteerde drugsgebruikers blijken ook geïnfecteerd te zijn met hepatitis C en de hepatitis C/HIV co-infectie heeft een grote impact op de sterfte in deze groep. In hoofdstuk 2.3 is de sterfte aan verschillende doodsoorzaken over 20 jaar vergeleken tussen drugsgebruikers met alleen een hepatitis C infectie, drugsgebruikers met zowel hepatitis C als een HIV infectie en drugsgebruikers zonder hepatitis C en HIV. Drugsgebruikers die alleen met hepatitis C of met zowel hepatitis C en HIV geïnfecteerd zijn, hadden de grootste kans op sterfte. In het HAART tijdperk is, in de groep met beide infecties, de kans op sterfte aan hepatitis en lever gerelateerde aandoeningen 7 keer groter vergeleken met diegenen die alleen met hepatitis C zijn geïnfecteerd. Deze resultaten benadrukken het belang van hepatitis C behandeling bij drugsgebruikers met een hepatitis C/HIV co-infectie.

HIV co-infecties

In HIV geïnfecteerde personen komen verschillende co-infecties, zoals seksueel overdraagbare aandoeningen, hepatitis B en C, tuberculose of herpes simplex virus infecties, vaker voor. Sommige van deze infecties vergemakkelijken de HIV transmissie, terwijl andere infecties de HIV ziekte progressie versnellen. In dit proefschrift is het voorkomen van hepatitis C en herpes simplex virussen beschreven.

Daling in nieuwe Hepatitis C en HIV infecties
Door het delen van gebruikte naalden hebben injecterende drugsgebruikers een hoog risico op infecties met hepatitis C en HIV. Onder de dreiging van AIDS, hebben drugsgebruikers in Amsterdam hun injecterend risicogedrag gereduceerd en daarmee ook het aantal nieuwe HIV infecties. Hoofdstuk 3.1 beschrijft het aantal nieuwe hepatitis C infecties en de daaraan gerelateerde risicofactoren bij drugsgebruikers in de Amsterdamse Cohort Studie. 85% van de drugsgebruikers die ooit hebben gespoten was geïnfecteerd met hepatitis C en 31% van hen bleek ook geïnfecteerd met HIV. Over 20 jaar is het aantal nieuwe hepatitis C infecties aanzienlijk gedaald. Hoewel het aantal nieuwe hepatitis C infecties 4 keer hoger is dan het aantal nieuwe HIV infecties, is de daling in hepatitis C gelijk aan de daling in HIV. Drugsgebruikers die in de recentere jaren voor de eerste keer hebben gespoten, hadden een kleiner risico op het krijgen van een hepatitis C infectie,

vergeleken met drugsgebruikers die langer geleden begonnen met injecteren. Heel waarschijnlijk is dit het gevolg van een reductie in het risicogedrag binnen de drugsgebruikers populatie.

HSV-1 en 2 bij homoseksuele mannen
Zowel herpes simplex virus type 1 (HSV-1) als HSV-2 zijn veel voorkomend onder homoseksuele mannen. HSV-2 wordt beschouwd als een belangrijke risicofactor voor het krijgen van HIV. Hoofdstuk 3.2 beschrijft de veranderingen in het voorkomen van HSV-1 en HSV-2 over een periode van 20 jaar. Het voorkomen van HSV-1 en HSV-2 daalde in de groep HIV negatieve homoseksuele mannen, maar niet in de groep homoseksuele mannen met een HIV infectie. Bovendien nam het voorkomen van HSV-1 toe onder homoseksuele mannen die meest seksueel actief waren. De associatie tussen HSV-2 en HIV is sterker geworden over de tijd en mogelijk kan de preventie van HSV-2 bijdragen aan de preventie van HIV. Vanwege het toenemende aantal HSV-1 infecties die seksueel worden overgedragen, zijn longitudinale studies nodig naar de mogelijk rol van HSV-1 in het krijgen van HIV.

Risico gedrag en HAART

Sinds de introductie van HAART is het risicogedrag onder homoseksuele mannen toegenomen. Verschillende studies naar veranderingen in het risicogedrag onder drugsgebruikers na HAART laten tegenstrijdige resultaten zien. In hoofdstuk 4.1 beschrijven we de resultaten van het seksueel risico en injecterende gedrag bij HIV geïnfecteerde drugsgebruikers in Amsterdam en de uitkomsten van behandeling van de drugsgebruikers die met HAART worden behandeld. Drugsgebruikers die met HAART starten zijn gematched met HIV positieve drugsgebruikers die niet met HAART beginnen. De immunologische en virologische veranderingen van de met HAART behandelde drugsgebruikers zijn vergeleken met die van HIV positieve homoseksuele mannen die ook met HAART worden behandeld.
Het injecterend gedrag daalde bij HIV positieve drugsgebruikers die HAART ontvingen, maar ook in de vergelijkbare groep drugsgebruikers die niet met HAART begonnen. De met HAART behandelde drugsgebruikers verhoogde dus niet hun injecterende risicogedrag en hun vroege respons op HAART was vergelijkbaar met die bij homoseksuele mannen. Ondanks dat drugsgebruikers, die met HAART worden behandeld, mogelijk een selecte groep is, laten de resultaten van deze studie zien dat ook drugsgebruikers kunnen profiteren van HAART.

Hoewel de AIDS relateerde sterfte is gedaald na de introductie van HAART, blijft de sterfte onder HIV geïnfecteerde personen een belangrijke onderwerp. Hun sterftecijfers zijn hoger vergeleken met de algemene bevolking, mede door een toegenomen risico in lever gerelateerde sterfte als gevolg van co-infectie met hepatitis C en B. Toekomstig onderzoek is nodig naar lange termijn effecten van HAART en naar de effecten van behandeling van deze en andere co-infecties.

Dankwoord

Dankwoord

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Curriculum vitae

Colette Smit werd geboren op 27 februari 1977 in Middelburg. In 1994 behaalde zij haar HAVO diploma aan de Scholengemeenschap De Breul, te Zeist. Vervolgens begon zij aan de opleiding Voeding en Diëtetiek aan de Hogeschool van Amsterdam. Na het afronden van deze opleiding begon zij de studie Voeding aan de Wageningen Universiteit, zij volgde hierbij de afstudeerrichting Epidemiologie, waarvoor zij een afstudeerscriptie schreef met als titel: *The Polyp Study: A case control study on diet and colorectal adenomas. Case definitions and response bias.* In 2001 studeerde zij af, waarna zij in dienst trad bij het Centrum voor Infectieziekten Epidemiologie (CIE) van het Rijksinstituut voor Volksgezondheid en Milieu. In 2002 startte zij met haar promotieonderzoek bij de GGD Amsterdam waarvan dit proefschrift het resultaat is. Tijdens deze periode heeft zij haar onderzoeksresultaten op verscheidene nationale en internationale congressen gepresenteerd en nam zij deel aan verschillende epidemiologische cursussen. Verder behoorde het begeleiden van collega onderzoekers, onderwijs geven en het coördineren van de cursus epidemiologie voor eerstejaars studenten aan de Universiteit van Amsterdam tot haar taken. Sinds februari 2006 werkt zij als epidemioloog bij de Stichting HIV Monitoring in Amsterdam.