Special reports

8. The Amsterdam Cohort Studies on HIV infection – Annual Report 2012

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Introduction

The Amsterdam Cohort Studies (ACS) on HIV infection and AIDS were started shortly after the first cases of AIDS were diagnosed in the Netherlands. Since October 1984, men who have sex with men (MSM) have been enrolled in a prospective cohort study. A second cohort involving drug users (DU) was initiated in 1985. In 2012, the cohorts reached 28 years of follow-up. The initial aim of the ACS was to investigate the prevalence and incidence of, and risk factors for, HIV-1 infection and AIDS, the natural history and pathogenesis of HIV-1 infection, and the effects of interventions. During the past 28 years, these aims have remained mostly the same, although the emphasis of the studies has changed. Early on, the primary focus was to elucidate the epidemiology of HIV-1 infection; more in-depth studies were performed later to investigate the pathogenesis of HIV-1 infection. In recent years, the focus has shifted to also include the epidemiology and natural history of other blood-borne and sexually transmitted infections (STI) among the participants in the ACS.

From the beginning, research in the ACS has taken a multidisciplinary approach (epidemiology, social science, virology, immunology and clinical medicine). This unique collaboration has been very productive, significantly contributing to the knowledge and understanding of many different aspects of HIV-1 infection. This expertise has contributed directly to advances in prevention, diagnosis and management of HIV infection.

As of 31 December 2012, 2511 men who have sex with men (MSM) and 1661 (injecting) drug users (DU) were included in the Amsterdam Cohort Studies (ACS). Every 3 to 6 months, participants complete a standardised questionnaire designed to obtain information regarding medical history, sexual and drug use behaviour, underlying cognitions, health care use, depression, psychological disorders, and demographics. In

addition, they have undergone a medical examination (HIV-positive participants and, in the past, HIV-negative DU, as well), and blood is drawn for diagnostic tests and storage. The ACS have been conducted in accordance with the ethical principles set out in the declaration of Helsinki, and participation in the ACS is voluntary; written informed consent (the most recent version approved by the AMC Medical Ethics Committee in 2007 for the MSM cohort and in 2009 for the DU cohort) is obtained for every participant.

Of the 2511 MSM, 614 were HIV-positive at study entry, and 232 seroconverted during follow-up. For the 1661 DU, 322 were HIV-positive at study entry, and 99 seroconverted during follow-up. By 31 December 2012, 354 MSM and 510 DU had died, and several other participants were asked to leave the study or left at their own request. In total, MSM visited the Public Health Service of Amsterdam 51,502 times, and DU visited 27,007 times.

Collaborating institutes and funding

Within the ACS, different institutes collaborate to bring together the data and biological sample collections and to conduct research. These are the Public Health Service of Amsterdam (PHSA) (Cluster Infectious Diseases, Department of Research), the Academic Medical Center (AMC) of the University of Amsterdam (UvA, Departments of Medical Microbiology, Experimental Immunology, Internal Medicine/Division of Infectious Diseases, Tropical Medicine and AIDS, and HIV treatment centre of Emma Children's Hospital), University Medical Center Utrecht (UMCU, Department of Immunology), Stichting HIV Monitoring (SHM), and the Jan van Goyen Medical Center (Department of Internal Medicine). From the start, Sanquin Blood Supply Foundation has been involved in the ACS, and until 2007 research in the ACS was conducted by the Department of Clinical Viro-Immunology of Sanquin Research. Sanquin financially supports the maintenance of the biobank of viable peripheral-blood mononuclear cells at the Department of Experimental Immunology at the AMC. Also, the ACS is involved in a significant amount of collaborative work with other research groups both within and outside the Netherlands. The ACS is financially supported by the Centre for Infectious Disease Control of the Netherlands National Institute for Public Health and the Environment.

The ACS in 2012

The cohort of men having sex with men

In 2012, 612 MSM were in active follow-up within the ACS. Of the MSM in active follow-up by the end of 2012, 498 were HIV-negative, and 114 were HIV-positive MSM who

filled in behavioural questionnaires. The median age of the MSM was 38.8 years (interquartile range [IQR]: 34.4-43.8), 9.5% were non-Dutch and 78.6% had attained a high level of education (college degree or higher). The majority of the participants (94.5%) were residents of Amsterdam. Thirty-eight of them were newly recruited, and one died in 2012.

Until 1995, men of all age groups were eligible to participate if they lived in or around Amsterdam and had had at least 2 male sexual partners in the previous 6 months (see Annex 4, Figure 1). In the period 1995–2004, only men 30 years or less with at least 1 male sexual partner in the previous 6 months could enter the study. From 2005, recruitment has been open for MSM of all ages with at least one sexual partner in the preceding 6 months.

In 1999, follow-up of HIV-positive participants was transferred from the PHSA to the Jan van Goyen Medical Center in Amsterdam and 6-monthly behavioural follow-up ceased. However, since 2000, HIV-infected MSM in follow-up at the Jan van Goyen Medical Center have again been asked to complete behavioural ACS questionnaires once a year. In 2012, 150 of the HIV-positive MSM had been in active follow-up at the Jan van Goyen clinic since 1999. Of these, 41 were HIV seroconverters, and 29 were defined as (1) slow or non-progressor or matched fast progressor in 1996 or (2) were HIV-positive for more than 10 years and had a CD4 count greater than 400 cells/mm^3 after 10 years of follow-up without antiretroviral therapy. In total, 57 MSM in active follow-up at the Jan van Goyen clinic completed a behavioural questionnaire.

Behavioural and clinical follow-up of individuals with a recent HIV infection at study entry at the PHSA and of HIV seroconverters in the period after 1999 was newly initiated in October 2003, in accordance with the 'HIV Onderzoek onder Positieven' (HOP) protocol. These participants return for follow-up at the PHSA or at an HIV treatment centre, and all ACS behavioural data are collected on a 6-monthly basis, with clinical data provided through the SHM. Of the 69 HIV-positive MSM in active follow-up in 2012 in accordance with the HOP protocol, 3 were newly included and 45 were HIV seroconverters. A behavioural questionnaire, as required by the HOP, was completed by 57 HIV-positive MSM.

In 2006, HIV-positive steady partners of HIV-negative participants and all steady partners of HIV-positive participants were also invited to participate in the ACS. Thirteen HIV-discordant and 3 HIV-positive concordant couples were included in this partner study, of which 5 couples were still in active follow-up in 2012.

Since November 2008, all MSM followed at the PHSA have been routinely screened for STI.

The cohort of drug users

In 2012, 285 DU were followed at the PHSA. The median age of the DU was 50.5 years (IQR: 44.4-55.2), 16.2% were non-Dutch, and 9.5% had attained a high level of education. 326 (99.7%) were residents of Amsterdam. Of the 285 DU followed in 2012, 18 were HIV-positive at entry, 13 seroconverted for HIV during follow-up in the ACS and 20 DU died. Inclusion criteria are individuals between 18 and 30 years who regularly use hard drugs in Amsterdam and individuals older than 30 years who started injecting hard drugs in the preceding 2 years in Amsterdam. Although the cohort is open and efforts were made to include new participants, only 3 were recruited in 2012, which might be explained by the unpopularity of injecting drugs in Amsterdam.

Affiliated studies and studies linked to the ACS

Primo-SHM study

In addition to the cohorts previously described, the ACS also included 238 patients who presented with primary HIV-1 infection at the outpatient clinic of the AMC in the so-called "Primo-SHM study" from May 2003 until March 2010. The Primo-SHM study is a national randomised study on the effects of early temporary (24 or 60 weeks) antiviral therapy as compared to no therapy. Some of these patients were seronegative men in the ACS amongst the MSM who seroconverted during follow-up. Some of them are also still in follow-up in accordance with the HOP protocol of the ACS at the PHSA. Plasma and peripheral-blood mononuclear-cell samples that are collected within the Primo-SHM study are part of the ACS and stored at the AMC. At present, biological samples are still prospectively collected for Primo-SHM participants visiting the AMC clinic until 1 year after recommencing therapy. ACS researchers make use of these samples for their studies.

The Dutch-C study

The Dutch-C (Drug Users Treatment for Chronic Hepatitis C) study was started within the DU cohort to evaluate the possibility of HCV testing and treatment combined with methadone programmes. This project aimed to offer HCV screening and treatment to all DU participating in the ACS and to develop guidelines for HCV treatment of active DU outside a clinical setting. Drug users were offered HCV testing and, if chronically infected, medical and psychiatric screening and HCV treatment. Various specialists collaborated to

provide optimal HCV care at the PHSA. Almost 60% of DU tested positive for HCV antibodies, and 64% of them were positive for HCV RNA. Of 57 chronically infected DU that started treatment and had sufficient follow-up after a treatment stop in 2010, 37 (65%) achieved a sustained virological response. On account of successful results seen in ACS DU, it was decided in 2007 to extend HCV treatment to DU who were not participants in the ACS, and they were referred from methadone clinics and other addiction clinics in Amsterdam. A total of 88 DU from the ACS and methadone clinics were treated for HCV by the end of 2011. The first active DU chronically infected with HCV genotype 1 started treatment at the PHSA in 2012 with telaprevir combined with peginterferon and ribavirin. We will continue to evaluate HCV treatment uptake and the short- and long-term outcomes amongst ACS participants, using the rich data collection and infrastructure of the ACS.

AGEhIV Cohort Study

The AGEhIV Cohort Study, a collaboration between the AMC Department of Infectious Diseases, Department of Global Health and Amsterdam Institute of Global Health and Development, the PHSA, and the SHM, was started in November 2010. The aim of the study is to assess the prevalence and incidence of a broad range of co-morbidities and known risk factors for these co-morbidities in HIV-infected patients 45 years and older and to determine the extent to which co-morbidities, their risk factors and their relation to quality of life differ between HIV-infected and uninfected groups. Participants undergo a comprehensive assessment for co-morbidities and fill in a questionnaire at intake and 2 years afterwards. By the end of 2012, the first data wave was completed and the second data wave started. In total, 597 HIV-1-infected participants were included through the AMC HIV outpatient clinic, and 550 HIV-uninfected individuals belonging to the same HIV exposure groups were included through the STI clinic of the PHSA (n=486) or the Amsterdam Cohort Studies (n=64). All participants are ≥45 years and are as comparable as possible with respect to age, gender, ethnicity and risk behaviour.

HIV-infected and HIV-exposed children

At the Emma Children's Hospital in the AMC, both HIV-infected and HIV-exposed children are in follow-up. Data from both groups are collected by the SHM and colleagues in the Departments of Obstetrics and Gynaecology and Experimental Immunology at the AMC analyse factors involved in neonatal HIV-1 transmission. The children infected with HIV are included in the Paediatric Amsterdam Cohort on HIV-1 (PEACH, n=60). The HIV-exposed children (30-40 annually) are studied in the context of the European Collaborative Study on Mother-to-Child Transmission of HIV (ECS), an ongoing birth cohort study that recently merged with the Paediatric European Network for Treatment of

AIDS (PENTA in EuroCoord). Plasma and peripheral mononuclear-cell samples that were collected within the study until 2008 are part of the ACS and stored at the AMC. Currently, no new samples are being collected. The stored samples are available for ACS research.

H2M study

The H2M (HIV and HPV in MSM) study is a successful collaboration between the Center for Infectious Disease Control (Cib), PHSA, the Jan van Goyen Medical Center, VUmc and the AMC. The study aims to compare the prevalence, incidence and clearance of high-risk (hr) HPV infections between HIV-negative and HIV-infected MSM. It also aims to investigate whether anal or penile hrHPV infections may be a risk factor for acquiring HIV. In July 2010, the H2M study started recruiting. The participants are recruited from three sites: the ACS (n=520; mostly HIV-negative), the STI clinic of the PHSA Amsterdam (n=120; all HIV-infected), and the Jan van Goyen Medical Center (n=160; all HIV-infected). Participants answer additional questions regarding sexual behaviour, smoking and circumcision and provide self-collected anal and penile-shaft swabs, as well as oral rinse-and-gargle specimens. These are tested for the presence of HPV DNA, and if positive, HPV types are determined. Serum is tested for L1 HPV antibodies. Two years of follow-up per participant was completed in July 2013.

The HIV epidemic

HIV incidence

Three MSM and no DU participating in the ACS seroconverted for HIV in 2012. The observed HIV incidence among MSM declined to 0.6 per 100 person-years in 2012. The HIV incidence in DU has continued to decline with less than 1 case per 100 person-years since 1999. *Figures 8.1* and *8.2* show the yearly observed HIV incidence rates for MSM and DU from the start of the ACS through 2012.

***Figure 8.1**: HIV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among men who have sex with men (MSM), 1984-2012.*

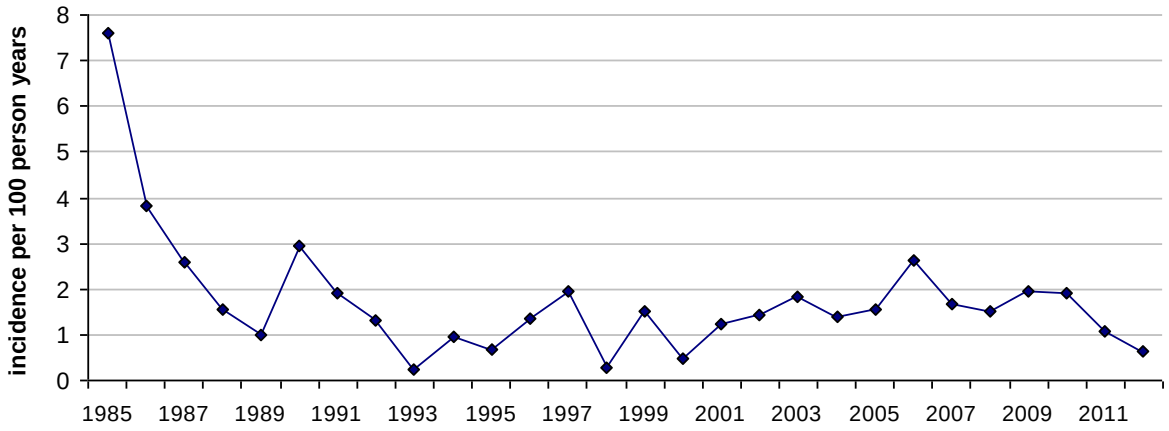
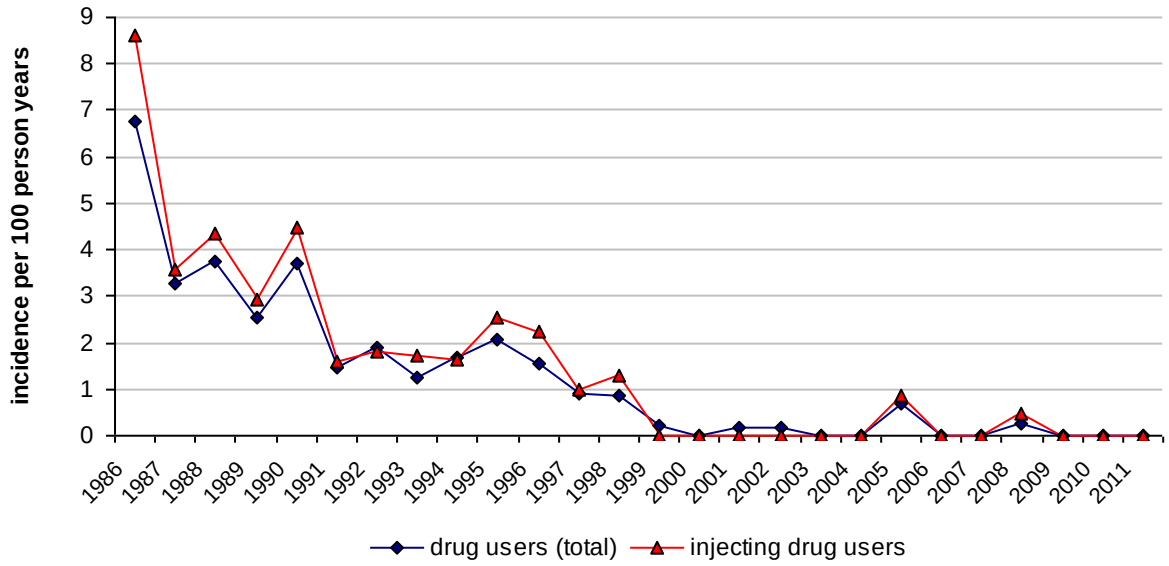


***Figure 8.2**: HIV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among drug users, 1986-2012.*



Transmission of therapy-resistant HIV strains

Surveillance of transmission of drug-resistant HIV-1 strains was performed for 3 MSM seroconverters. None of the individuals were infected with virus-harbouring resistance-associated mutations; only naturally occurring sequence variation was found. Phylogenetic analysis showed that two individuals harboured subtype B HIV-1 strains and one subtype F1.

In the cohort of DU no seroconversions or seropositive entries appeared.

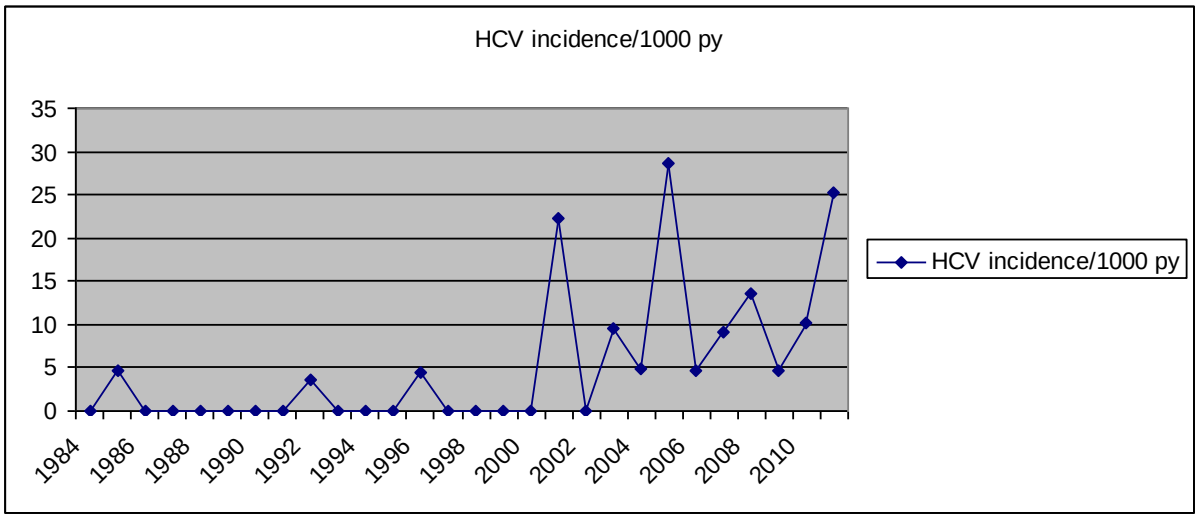
Highly active antiretroviral therapy (HAART) uptake

Of all 218 HIV-positive MSM visiting the Jan van Goyen Clinic or one of the other HIV treatment centers in the Netherlands according to the ACS protocols in 2012 and for whom treatment data were available, 211 (97%) received some form of antiretroviral therapy. Of 211 MSM for whom viral load results were available and were on therapy in 2012, 191 (91%) had a viral load of less than 50 copies/ml (assays: M2000rt).
Of the 31 HIV-positive DU who visited the PHSA in 2012, 25 (81%) received some combination of antiretroviral therapy. Of the 25 DU, 22 (88%) had an undetectable viral load (less than or equal to 150 copies/ml [assay: M2000rt]) at their latest visit.

HCV incidence in MSM and DU

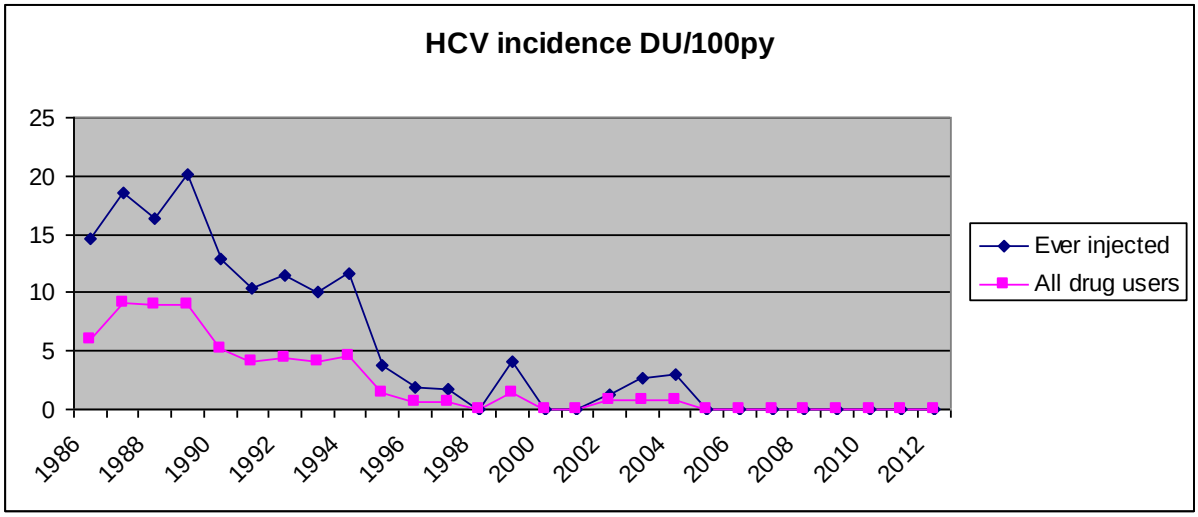
In 2012 the HCV incidence was updated for the MSM cohort through 2011. No incident HCV infections were recorded among HIV-uninfected MSM. Among HIV-infected MSM, HCV incidence rates increased significantly after 1999. However, the incidence seems to have levelled off in recent years around 10/1,000 person years. This stabilizing incidence is in line with recent findings from the STI clinic of the PHSA.

***Figure 8.3**: HCV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among MSM, 1986-2011.*



In 2012 the HCV incidence was also updated for the DU cohort. The HCV incidence in the total group and among injectors has strongly declined over the years to 0/100 person years since 2005 (see *Figure 8.4*).

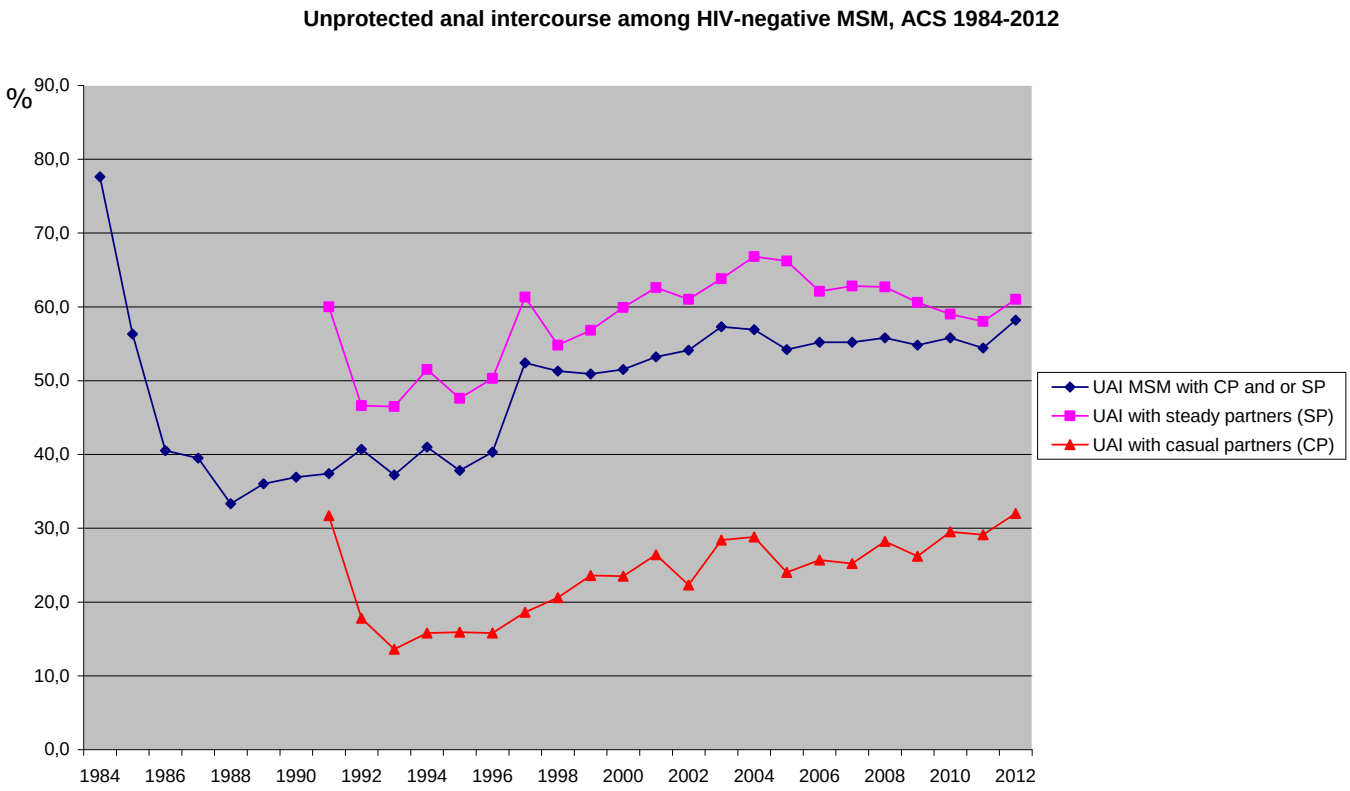
***Figure 8.4**: HCV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among drug users, 1986-2012*



Risk behaviour of MSM

Information from the 895 questionnaires completed by 498 HIV-negative MSM during cohort visits in 2012 resulted in 485 reports (54%) of unprotected anal intercourse (UAI) in the preceding 6 months. Higher proportions of UAI were reported for steady partners (37%) compared to casual partners (22%). Trends in UAI, especially with casual partners, among HIV-negative MSM participating in the ACS have slowly increased since 1996. (*Figure 8.5*).

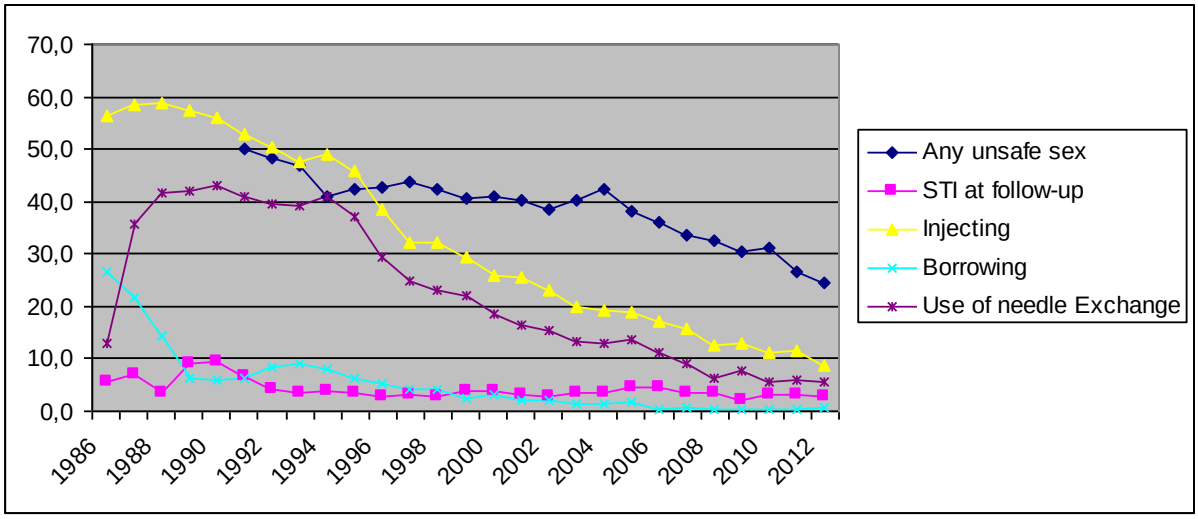
***Figure 8.5**: Trends from the Amsterdam Cohort Studies (ACS) in unprotected anal intercourse (UAI) in the past 6 months amongst HIV-negative men having sex with men (MSM) with a casual and/or steady partner, 1984-2012.*



Risk behaviour of DU

In HIV-negative DU, reports of both injecting and borrowing needles significantly declined over the period 1985-2011. Reports of high-risk sexual behaviour at follow-up visits decreased before 1996, remained relatively stable until 2005 and further decreased to approximately 24% in 2012. Reports of STI have remained relatively stable at approximately 3% in recent years (see *Figure 8.6*).

***Figure 8.6**: Proportion of visits per calendar year at which injecting and high-risk sexual behaviour was reported amongst 1315 drug users (DU) who were HIV-negative on entry to the Amsterdam Cohort Studies (ACS), 1986-2012.*



STI screening among MSM in ACS

Since October 2008, all MSM in the ACS have been routinely screened for chlamydia and gonorrhoea by polymerase chain reaction (PCR) techniques on samples of urine and pharyngeal and rectal swabs. Cases of syphilis are detected by TPHA (Treponema pallidum haemagglutination assay). In 2012, a total of 545 MSM from the ACS were screened for STI; 130 MSM were screened once, 396 twice and 19 more than twice. The overall prevalence of any STI was 8.8% (87/989). The prevalence of any STI was significantly higher among HIV-infected MSM (23.1%) compared to HIV-uninfected MSM (6.8%).

ACS research highlights 2012

Infection with HIV-1 may result in severe cognitive and motor impairment, referred to as HIV-1-associated dementia (HAD). Whilst its prevalence has dropped significantly in the era of combination antiretroviral therapy, milder neurocognitive disorders persist with a high prevalence. To identify additional therapeutic targets for treating HIV-associated neurocognitive disorders, several candidate gene polymorphisms have been evaluated, but few have been replicated across multiple studies. We tested seven candidate gene polymorphisms and five recently identified single nucleotide polymorphisms (SNPs) affecting HIV-1 replication in macrophages for their association with HAD in a case-control study. A significant difference in genotype distribution among all cases and controls irrespective of the year of AIDS diagnosis was found for only an SNP in candidate gene Prep1 ($p = 1.2 \times 10(-5)$). Prep1 has recently been identified as a transcription factor preferentially binding the -2,518 G allele in the promoter of the gene encoding MCP-1, a protein with a well established role in the etiology of HAD. (ref 2) Previously we established that at 3 years post-seroconversion, approximately 30% of HIV-infected individuals have cross-reactive neutralizing activity (CrNA) in their sera.

Here we studied the kinetics with which CrNA develops and how these relate to the development of autologous neutralizing activity as well as viral escape and diversification. We found that CrNA can rapidly develop after HIV-1 infection is established, even within the first year after seroconversion, in an elite neutralizer as opposed to five other patients in whom CrNA was first detected at 20 to 35 months post-seroconversion. The kinetics with which CrNA developed paralleled the development of autologous neutralizing activity, as well as gp160 sequence diversity. Viral escape occurred in all individuals, despite the CrNA in their sera, which was reflected by the increasing gp160 sequence diversity that was higher in individuals with CrNA, especially in the elite neutralizer. This implies that CrNA in sera adds extra pressure on the virus to escape this potent immune response. The rapid escape was in line with the absent effect of CrNA on the clinical course of infection. (ref 8)

The HIV-1 characteristics associated with transmission are still poorly defined, but a better understanding of which viruses are selected would aid in the development of drugs or vaccines aimed at preventing infection. At the Laboratory of Experimental Virology of the AMC, mothers infected with HIV-1 subtype A or C viruses, including several who infected their children, were studied. The genotypic characteristics of the V1-V5 region of the gp120 envelope proteins of viruses, found not only in transmitting mothers and their infected children, but also in non-transmitting mothers, were investigated. An association with transmission was identified; viruses with a potential N-glycosylation site on position AA339 were preferably transmitted, not only in transmitting mothers, but also in acute sexual transmissions. The function of the potential N-glycosylation site at AA339 in the HIV-1 envelope protein remains to be determined; however, AA339 is situated in the α2-helix region of C3 of the gp120 molecule. Residues within that region of subtype C viruses have a unique mutational pattern, which may be associated with neutralization by antibodies. (ref. 1)

Trends in HIV incidence among MSM who have recently had post-exposure prophylaxis (PEP) prescribed in Amsterdam were compared with MSM participating in the ACS. We used data from MSM who were prescribed PEP in Amsterdam between 2000 and 2009, who were HIV-negative at the time of PEP prescription, and who had follow-up HIV testing 3 and/or 6 months after PEP prescription (n=395). For comparison, cohort data from MSM participating in the ACS in the same period were used (n=782). Between 2000 and 2009, among MSM who were prescribed PEP, an overall HIV incidence of 6.4 [95% confidence interval (CI) 3.4–11.2] per 100 person-years was found, compared with an HIV incidence of 1.6 (95% CI 1.3–2.1) per 100 person-years among MSM participating in the ACS (P<0.01). In both cohorts, an increasing trend in HIV incidence over time was

observed (incidence rate ratio [IRR per calendar year] 1.3 [95% CI 0.9–1.7] and 1.1 [95% CI 1.0–1.2] among MSM prescribed PEP and MSM of the ACS, respectively). Particularly in more recent years, MSM who were recently prescribed PEP had a higher HIV incidence compared with MSM participating in the ACS, indicating ongoing sexual risk behaviour. (ref 21)

The hepatitis C virus (HCV) disease burden among injecting drug users (IDUs) is determined by HCV incidence, the long latency period of HCV, competing mortality causes, presence of co-infection and HCV treatment uptake. We examined the effect of these factors and estimated the burden of HCV disease in Amsterdam. A Markov model was developed, incorporating HCV and human immunodeficiency virus (HIV), and parameterized with data of the IDU population of Amsterdam from the ACS, surveillance studies and literature. HCV infection was simulated from its acute phase to HCV-related liver disease (i.e., decompensated cirrhosis and hepatocellular carcinoma).
We found that the HCV prevalence among IDUs in Amsterdam increased to approximately 80% in the 1980s. From 2011 to 2025, the HCV-related disease prevalence will accordingly rise by 36%. In total, HCV-related liver disease will develop in 945 (95% range 617–1309) individuals. This burden would have been 33% higher in the absence of HIV. In Amsterdam, 25% of HIV-negative IDUs receive successful HCV treatment, reducing the cumulative disease burden by 14%. Further reduction of 36% can be achieved by improving treatment, resulting in 603 cases (95% range 384–851). The hepatitis C virus burden among IDUs in Amsterdam has been reduced by a high competing mortality rate, particularly caused by HIV infection, and to a smaller extent by hepatitis C virus treatment. Improved hepatitis C virus treatment is expected to contribute to reduce the future hepatitis C virus disease burden. (ref 32)

Steering committee: The politburo

In 2012, the "politburo" met four times. Twenty-five proposals for use of data and/or samples (serum/PBMC) were submitted to the politburo: five from AMC-Experimental Immunology, nine from the AMC-Medical Microbiology, four from the UMCU, six from the PHSA**,** one from AMC-internal medicine and one from researchers not affiliated with the ACS. Twenty-four requests were approved, some after revision, and one request was denied. Three of the approved proposals were collaborations with groups outside the ACS of whom two were from groups abroad.

Publications in 2012 that include ACS data

1. Baan E, de Ronde A, Luchters S, Vyankandondera J, Lange JM, Pollakis G, Paxton WA. **HIV Type 1 Mother-to-Child Transmission Facilitated by Distinctive**

Glycosylation Sites in the gp120 Envelope Glycoprotein. *AIDS Res Hum Retroviruses. 2012 Jul;28(7):715-724.*

2. Bol SM, Booiman T, van Manen D, Bunnik EM, van Sighem AI, Sieberer M, Boeser-Nunnink B, de Wolf F, Schuitemaker H, Portegies P, Kootstra NA, van 't Wout AB. **Single nucleotide polymorphism in gene encoding transcription factor Prep1 is associated with HIV-1-associated dementia.** *PLoS One. 2012;7(2):e30990.*
3. van den Boom W, Stolte I, Sandfort T, Davidovich U. **Serosorting and sexual risk behaviour according to different casual partnership types among MSM: the study of one-night stands and sex buddies.** *AIDS Care. 2012 Feb;24(2):167-73.*
4. Bunders MJ, van der Loos CM, Klarenbeek PL, van Hamme JL, Boer K, Wilde JC, de Vries N, van Lier RA, Kootstra N, Pals ST, Kuijpers TW. **Memory CD4+CCR5+ T cells are abundantly present in the gut of newborn infants to facilitate mother-to-child transmission of HIV-1.** *Blood. 2012 Nov 22;120(22):4383-90.*
5. Cornelissen M, Pasternak AO, Grijsen ML, Zorgdrager F, Bakker M, Blom P, Prins JM., Jurriaans S, and van der Kuyl AC. **HIV-1 dual infection is associated with faster CD4+Tcell decline in a cohort of men with primary HIV infection.** *Clinical infectious diseases 2012:54, 539-547.*
6. Edo-Matas D, Rachinger A, Setiawan LC, Boeser-Nunnink BD, van 't Wout AB, Lemey P, Schuitemaker H. **The evolution of human immunodeficiency virus type-1 (HIV-1) envelope molecular properties and coreceptor use at all stages of infection in an HIV-1 donor-recipient pair.** *Virology. 2012 Jan 5;422(1):70-80.*
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9. Geskus RB. **Which individuals make dropout informative?** *Stati Methods Med Res. 2012 Apr 25. [Epub ahead of print]*
10. Gijsbers EF, van Nuenen AC, Schuitemaker H, Kootstra NA. **Gag sequence variation in an HIV-1 transmission cluster influences viral replication fitness.** *J Gen Virol. 2012 Nov 7. [Epub ahead of print].*
11. Grady BP, Vanhommerig JW, Schinkel J, Weegink CJ, Bruisten SM, Lindenburg CE, Prins M. **Low incidence of reinfection with the hepatitis C virus following treatment in active drug users in Amsterdam.** *Eur J Gastroenterol Hepatol. 2012 Nov;24(11):1302-7.*
12. Grebely J, Prins M, Hellard M, Cox AL, Osburn WO, Lauer G, Page K, Lloyd AR, Dore GJ; on behalf of the International Collaboration of Incident HIV and Hepatitis C in

Injecting Cohorts (InC(3)). **Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine.** *Lancet Infect Dis. 2012 May;12(5):408-414.*

13. Grebely J, Morris MD, Rice TM, Bruneau J, Cox AL, Kim AY, McGovern BH, Shoukry NH, Lauer G, Maher L, Lloyd AR, Hellard M, Prins M, Dore GJ, Page K; on behalf of the InC Study Group. **Cohort Profile: The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC3) Study.** *Int J Epidemiol. 2012 Nov 30. [Epub ahead of print].*
14. Grijsen ML, Vrouenraets SM, Wit FW, Stolte IG, Prins M, Lips P, Reiss P, Prins JM. **Low bone mineral density in men who have sex with men regardless of HIV status.** *J Infect Dis. 2012 Nov 12. [Epub ahead of print].*
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