Special reports

6. The Amsterdam Cohort Studies on HIV infection – Annual Report 2010

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The Amsterdam Cohort Studies (ACS) on HIV infection and AIDS were started shortly after the first cases of AIDS were diagnosed in the Netherlands in the early eighties. 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 2010, the cohorts reached 26 years of follow-up. The initial aim of the ACS was to investigate the prevalence of, 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 26 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, whilst more in-depth studies were performed later on 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) amongst 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.

De Amsterdamse Cohort Studies (ACS) naar HIV en AIDS zijn gestart kort nadat de eerste gevallen van AIDS in Nederland werden gediagnosticeerd. Sinds oktober 1984 worden mannen die seks hebben met mannen (MSM) gevolgd in een prospectieve cohortstudie. Een tweede cohort onder drugsgebruikers startte in 1985. In 2010 bestonden de cohorten 26 jaar. Het oorspronkelijke doel van ACS was het onderzoeken van de prevalentie en incidentie van, en risicofactoren voor HIV-1-infectie en AIDS, het natuurlijk beloop van de HIV-1-infectie en het evalueren van de effecten van interventies. De afgelopen 26 jaar zijn deze doelen min of meer gelijk gebleven maar is de nadruk van de studies wel verschoven. In het begin lag de focus vooral op het verkrijgen van inzicht in de epidemiologie van HIV-1. Later zijn meer verdiepende studies uitgevoerd naar met name de pathogenese van HIV-1. In de afgelopen jaren werden eveneens de epidemiologie en het natuurlijk beloop van andere bloedoverdraagbare en seksueel overdraagbare aandoeningen (SOA's) onder deelnemers aan de ACS bestudeerd.

Vanaf de beginfase heeft het onderzoek in de ACS zich onderscheiden door een multidisciplinaire aanpak (epidemiologie, sociale wetenschappen, virologie, immunologie en klinische geneeskunde). Deze unieke aanpak is erg productief gebleken en heeft in belangrijke mate inzicht gegeven in de verschillende aspecten van HIV-1. Deze expertise heeft direct bijgedragen aan de vooruitgang en verbetering van de preventie, diagnose en behandeling van de HIV-infectie.

As of 31 December 2010, 2447 MSM and 1657 (injecting) DU were included in the ACS. Every three to six months, participants completed a standardized questionnaire designed to obtain information regarding medical history, sexual and/or drug use behaviour, underlying cognitions, health care use, depression, psychological disorders, and demographics. In addition, they underwent a medical examination (HIV-positive participants and, in the past, HIV-negative drug users as well), and blood was drawn for diagnostic tests and storage. The ACS has 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 2447 MSM, 596 were HIV-positive at study entry, and 216 seroconverted during follow-up. For the 1657 DU, 322 were HIV-positive at study entry, and 98 seroconverted during follow-up. By 31 December 2010, 342 MSM and 452 DU had died, and several other participants were asked to leave the study or left at their own request. Almost 95% of the participants who visited the ACS during a given calendar year returned for a follow-up visit the next year. In total, the Public Health Service of Amsterdam was visited 49,647 times by MSM and 26,164 times by DU.

Collaborating institutes and funding

Within the ACS, different institutes collaborate to bring together the data and biological sample collections. 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 (Departments of Medical Microbiology, Experimental Immunology, and Internal Medicine, and the International Antiviral Therapy Evaluation Center) and the Jan van Goyen Medical Center (Department of Internal Medicine). Until 2007, the collection of blood cells was performed at the Sanquin Blood Supply Foundation, but this activity has since moved to the Department of Experimental Immunology at the AMC. However, the Sanquin Blood Supply Foundation is still affiliated with the ACS. Also, the ACS collaborates with many other research groups both within and outside of the Netherlands.

The ACS is a collaboration between the Public Health Service of Amsterdam, the Academic Medical Center of the University of Amsterdam, the Sanquin Blood Supply Foundation, the University Medical Center Utrecht, and the Jan van Goyen Medical Center. The ACS is

part of Stichting HIV Monitoring (SHM) and is financially supported by the Centre for Infectious Disease Control of the Netherlands National Institute for Public Health and the Environment.

The ACS in 2010

The cohort of men having sex with men

In 2010, 542 MSM were followed at the PHSA of Amsterdam. Thirty-six of them had been newly recruited since January 2010, and one participant died. From 2005, recruitment has been open to MSM of all ages with at least one sexual partner in the preceding six months. Of the MSM followed in 2010 at the PHSA, 473 men were HIV-negative, and 69 men were HIV-positive. The HIV-positive men, of whom 46 were HIV seroconverters, were followed according to the 'HIV Onderzoek onder Positieven' (HOP) protocol. This protocol was initiated in October 2003 for MSM who seroconverted or were HIV-positive at entry into the study cohort of young MSM after 1999. Since November 2008, all MSM followed at the PHSA have been routinely screened for sexually transmitted infections (STI), and as of July 2010, additional screening for human papillomavirus (HPV) was started among all MSM to investigate the prevalence, incidence, and clearance of anal, penile and oral HPV infections among HIV-negative and HIV-positive MSM (H2M study).

In 2010, 17 HIV-positive men were included in the HOP, of whom 7 were exclusively followed in an HIV treatment centre outside the PHSA. By the end of 2010, a total of 100 HIV-positive men were still in active follow-up according to the HOP protocol at the PHSA or in an HIV treatment centre outside the PHSA. From June 2006 onwards, HIV-positive steady partners of HIV-negative participants and all steady partners of HIV-positive participants have also been invited to participate in the ACS. Thirteen HIV discordant and 3 HIV-positive concordant couples were included in this partner study, of which 7 couples were still in active follow-up in 2010.

Plasma and cells from 58 of the 135 HIV-positive MSM in active follow-up at the Jan van Goyen clinic since 1999 were stored in 2010. Of these, 36 were HIV seroconverters, and the remaining 22 were defined as (1) slow or non-progressor or matched fast progressor in 1996 or (2) were HIV-positive for more than ten years and had a CD4 count greater than 400 cells/µl after ten years of follow-up after an HIV-positive result without effective therapy.

The cohort of drug users

In 2010, 351 drug users were followed at the PHSA. Of the 351 DU followed in 2010, 29 were HIV-positive at entry, 16 seroconverted for HIV during follow-up in the ACS, and 5 had their first study visit in 2010. Since December 2010, all DU followed at the PHSA have also been screened for STI as part of a pilot study to assess whether regular STI screening is indicated for this group.,

In 2005, a feasibility study (the Dutch-C project) was started within the DU cohort to evaluate the possibility of hepatitis C virus (HCV) testing and treatment combined with methadone programs. In 2010, as part of this project, 8 DU who were mono-infected with HCV and 1 with an HCV/HIV co-infection initiated HCV therapy, resulting in a total group of 73 DU treated for HCV. This project is one of the first studies specifically designed as an intervention to increase HCV assessment and treatment in a well defined cohort of DU.

Sub-studies

Primo-SHM study results

This randomized study compared no treatment during primary HIV infection (PHI) with 24 weeks or 60 weeks of antiretroviral treatment.

The optimal clinical management of PHI is controversial. Treatment during PHI may result in a more effective immune response to the virus, resulting in lowering of the viral set point and delaying the loss of CD4 T cells. Several ongoing randomized controlled trials in the combination antiretroviral therapy (cART) era have addressed the question whether such temporary treatment also has clinical benefits for the patient, but none have been published so far. The aim of the Primo-SHM study was to assess the clinical benefit of temporary cART during PHI.

The study was a multicenter, open-label, randomized controlled trial in which patients with laboratory evidence of PHI were randomly assigned to receive no treatment or 24 weeks or 60 weeks of cART. If therapy was clinically indicated, subjects were randomized over the 2 treatment arms. Patients were recruited in 13 Dutch HIV treatment centres. Recruitment started in May 2003 and continued until March 2010. Primary endpoints were the viral set point (defined as the plasma viral load [pVL] 36 weeks after randomization in the no-treatment arm and 36 weeks after treatment interruption in the treatment arms) and the total time that patients were off therapy (defined as the time between randomization and start of cART in the no-treatment arm and the time between treatment interruption and restart of cART in the treatment arms). cART was (re)started in the event of a confirmed CD4 count <350 cells/mm^3 or symptomatic HIV disease. Time off therapy was compared across study arms using Kaplan–Meier plots and multivariate Cox survival analyses adjusted for confounding factors.

The modified intention-to-treat-analysis comprised 168 patients: 115 were randomized over the three study arms and 53 were randomized over the two treatment arms only. The vast majority of patients randomized over the three study arms was MSM, had a negative or indeterminate Western blot and was symptomatic during PHI. Treatment in the treatment arms was well tolerated. The mean viral set point was significantly lower in the 24-week and 60-week treatment arms as compared to the no-treatment arm. The median total time off therapy was significantly longer in the 24-week and 60-week treatment arms as compared to the no-treatment arm; restart of cART during chronic HIV infection was

deferred by approximately 2 years. When all treated patients, including the patients randomized over the two treatment arms, were combined, the median total time off therapy did not differ between the 24-week and 60-week treatment arms. In the adjusted Cox analyses, temporary cART was independently associated with time to (re)start of cART.

The present randomized trial provides the first evidence of a clinical benefit of temporary cART during PHI. Temporary cART lowered the viral set point and deferred the need for initiation of cART during chronic HIV infection. These results were presented as an oral presentation at the 18th Conference on Retroviruses and Opportunistic Infections, February 2011 in Boston.

AgeHIV Cohort Study

In October 2010 the AgeHIV Cohort Study was started, a collaboration between the AMC Department of Infectious Diseases and the Department of Global Health and Amsterdam Institute of Global Health and Development, the PHSA and the SHM. This ongoing prospective cohort study aims to recruit 800 HIV-1-infected patients amongst AMC HIV outpatient clinic attendees and a control group of 400-600 HIV-uninfected individuals belonging to the same HIV exposure groups at the STI clinic of the PHSA and among participants of the Amsterdam Cohort Studies. Both groups will be aged ≥45 years and comparable as closely as possible in age, gender, ethnicity and risk behaviour. 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 aged 45 years or more and to determine the extent to which co-morbidities, as well as risk factors for co-morbidities and their relation to quality of life, differ between HIV-infected and uninfected individuals.

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 collaborators in the Departments of Obstetrics and Gynecology and Experimental Immunology at the AMC study factors involved in neonatal HIV-1 transmission. The children infected with HIV-1 are included in the Pediatric Amsterdam Cohort on HIV-1 (PEACH). The HIV-exposed children are studied in the context of the European Collaborative Study on Mother-to-Child Transmission (MTCT) of HIV (ECS), an ongoing birth cohort study that recently merged with the Pediatric European Network for Treatment of AIDS (PENTA).

The HIV epidemic

HIV incidence

Nine MSM and no DU participating in the ACS seroconverted for HIV in 2010. HIV incidence in 2010 was almost 2 per 100 person-years among MSM. The incidence has slowly increased since 1996, the year that cART became generally available in developed countries including the Netherlands.

The current trend in HIV incidence seen in the MSM cohort differs from that observed in the DU cohort. HIV incidence in drug users has continued to decline and is now less than 1.0/100 person-years. *Figures 6.1* and *6.2* show the yearly observed HIV incidence rates for MSM and drug users from the start of the ACS through 2010.

Figure 6.1: *HIV incidence per calendar year in the ACS among men having sex with men, 1984–2010*

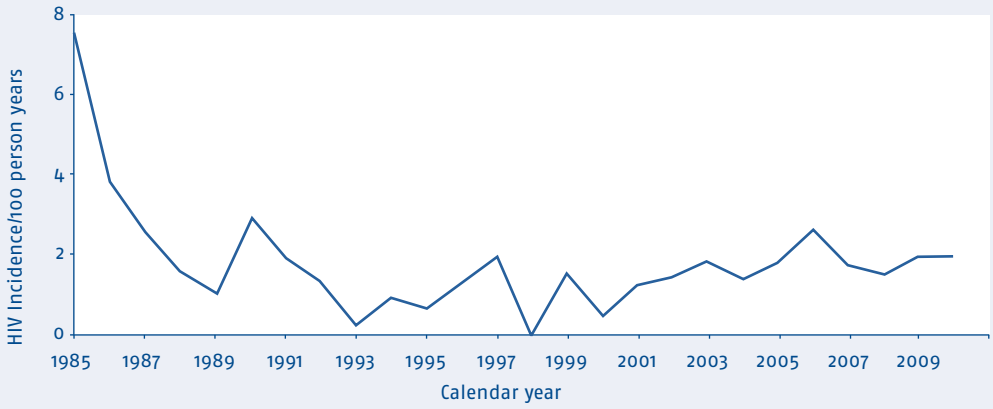
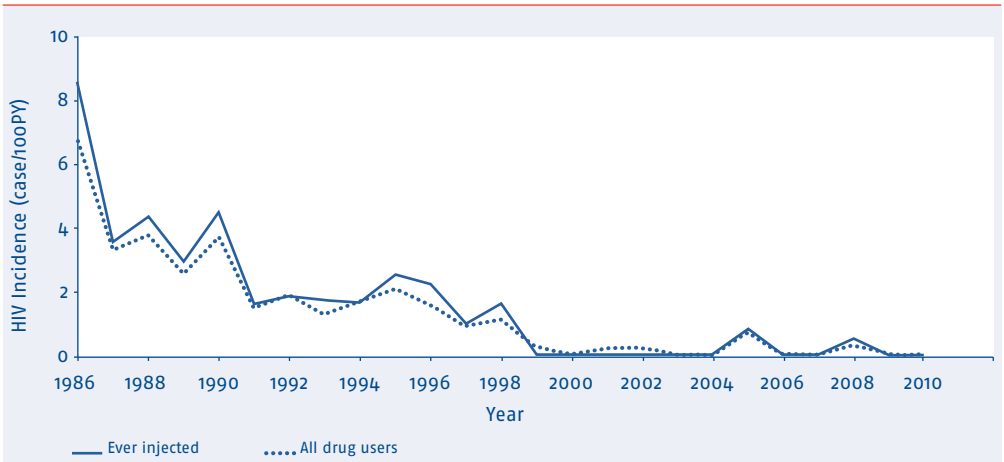


Figure 6.2: *HIV incidence per calendar year in the ACS among drug users, 1986–2010*



Transmission of therapy-resistant HIV strains

Surveillance of transmission of drug-resistant HIV-1 strains was performed for six MSM seroconverters and for four MSM seropositive at study entry in 2010. Two individuals were infected with virus harbouring resistance-associated mutations; a 41L, 210W and a so-called 215-revertant (215D) mutation were found in one of the seroconverters, and a 41L and 215D mutation were found in one of the seroprevalent participants. In the other eight individuals only a naturally occurring sequence variation was found. Phylogenetic analysis showed that nine individuals harboured subtype B HIV-1 strains, and one individual was infected with subtype CRF06-cpx.

In the cohort of drug users, one DU with a previous HIV-negative test result in the ACS was newly diagnosed with HIV. However, the seroconversion interval of this participant was 42 months; the last seronegative test was performed in 2006. In the first seropositive sample no HIV-1 RNA could be detected; therefore, baseline resistance testing could not be performed.

Combination antiretroviral therapy (cART) uptake

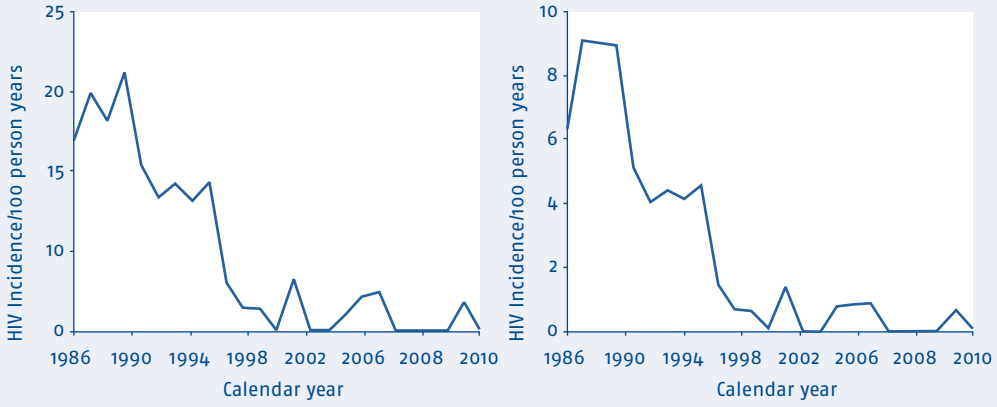
Of all 203 HIV-positive MSM visiting the Jan van Goyen Clinic or one of the other HIV treatment centers in the Netherlands according to the ACS protocols in 2010 and for whom treatment data were available in 2010, 190 (94%) received some form of antiretroviral therapy. Of 200 MSM for whom viral load results were available in 2010, 187 (94%) had a viral load of less than 50 copies/ml (assays: M²000rt).

Of the 45 HIV-positive DU who visited the PHSA in 2010, 31 (69%) received some combination of antiretroviral therapy. Of these 31, 29 (94%) had an undetectable viral load (≤150 copies/ml [assay: m²000rt]) at their latest visit. Of 14 HIV-positive DU not receiving HAART, 13 (93%) had an undetectable viral load.

Hepatitis C virus (HCV) incidence in drug users

In 2010 the HCV incidence was updated for the DU cohort. The HCV incidence strongly declined over a period of years amongst injectors only and in the total group; it was 0/100 person-years in 2010 (see *Figure 6.3*).

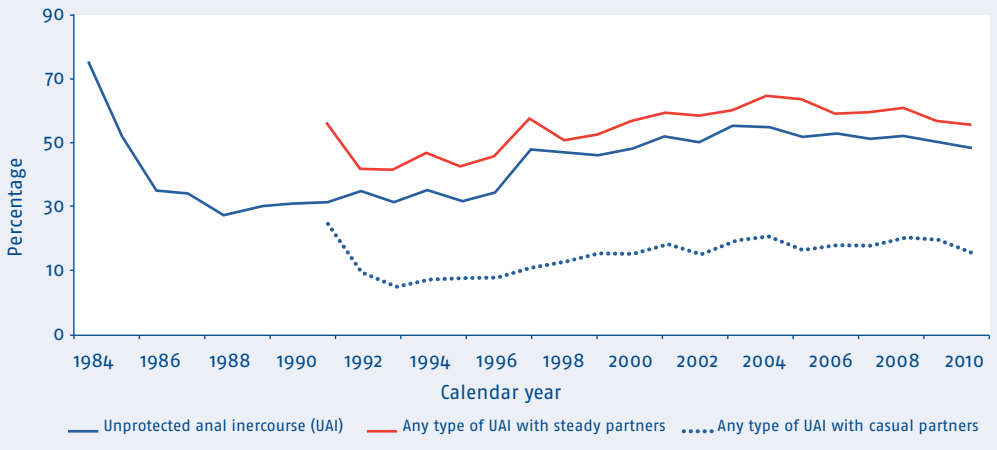
Figure 6.3: Hepatitis C virus incidence per calendar year in the ACS among all (left) and ever injecting (right) drug users, 1986–2010.



Risk behaviour of MSM

Information from the 867 questionnaires filled in by 473 HIV-negative MSM during cohort visits in 2010 resulted in 458 (53%) reports of unprotected anal intercourse (UAI) in the preceding six months. Highest rates of UAI were reported with steady partners (59%). Trends in UAI among HIV-negative MSM participating in the ACS have slowly increased since 1996, but have remained relatively stable in recent years.

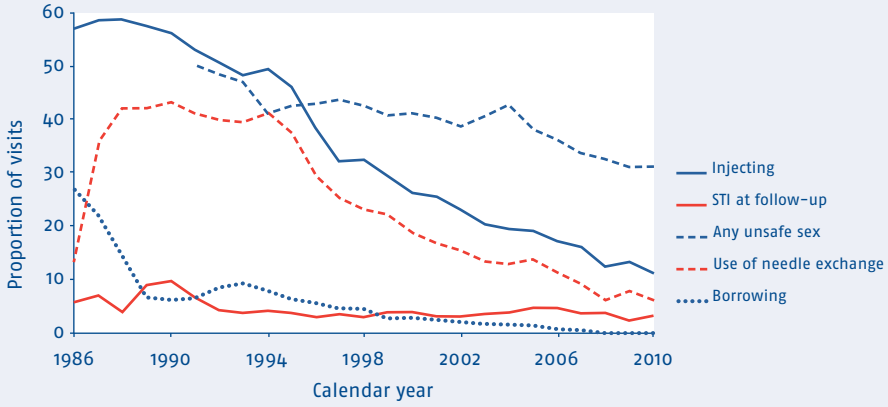
Figure 6.4: Trends in unprotected anal intercourse in the past six months amongst HIV-negative men having sex with men from the Amsterdam Cohort Study 1984–2010.



Risk behaviour of DU

In HIV-negative DU, reports of both injection and borrowing of needles significantly declined over the period 1985-2010. Reports of high-risk sexual behaviour at follow-up visits decreased before 1996, remained relatively stable until 2005 and further decreased to approximately 35% in 2010. Reports of STI have remained relatively stable around 5% in recent years (see *Figure 6.5*).

Figure 6.5: *Proportion of visits per calendar year at which injecting and high risk sexual behaviour was reported amongst 1315 drug users who were HIV-negative on ACS entry, 1986-2010.*



Legend: *STI=sexually transmitted infection*

STI screening among MSM in ACS

Since October 2008 all MSM in the ACS have been routinely screened for chlamydia and gonorrhoea using PCR techniques on samples from pharyngeal and rectal swabs and urine. Cases of syphilis are detected by *Treponema pallidum* haemagglutination assay (TPHA). In 2010 a total of 505 MSM from the ACS were screened for STI; 88 MSM once, 387 MSM twice and 30 MSM more than twice. The majority was HIV-negative (449 MSM, 835 visit), 40 MSM were HIV-positive (92 visits) and 16 had an unknown HIV status (31 visits). The prevalence of any STI at the first visit in 2010 was 9.5% (48/505), and the prevalence of any STI at the subsequent visit in 2010 was 8.6% (39/453). The prevalence of STI was significantly higher among HIV-infected MSM (24%) compared to HIV-uninfected MSM (7.5%).

ACS research highlights 2010

A key 2007 publication on the impact of participation in comprehensive harm reduction programmes of needle exchange, opiate substitution therapy, and social care on HIV and HCV transmission among DU was republished by invitation in 2010 [218]. Although we did not find evidence of any independent intervention effects, this was the first worldwide study demonstrating that the combination of interventions was effective not only in reducing HIV but also HCV. A recent meta-analysis and a systematic review have confirmed the ACS finding.

Recently, evidence has been provided that HIV-1 adapts over time to host cellular immune responses by losing epitopes restricted by the most abundant human leukocyte antigen types in a population. The hypothesis that, over the course of the epidemic, HIV-1 has also become more resistant to antibody neutralization was tested in participants from the ACS. HIV-1 variants obtained from participants who became infected at the beginning of the epidemic and from participants who recently contracted the virus were analyzed for their sensitivity to cross-reactive neutralizing antibodies. Over calendar time, HIV-1 developed an enhanced resistance to antibody neutralization, which was accompanied by an increase in the length of the variable loops and in the number of potential N-linked glycosylation sites on the HIV-1 envelope gp120 subunit [219].

A study was carried out examining the incidence of HIV-1 superinfection during the first year after infection amongst homosexual participants in the Amsterdam Cohort Studies on HIV infection and AIDS who seroconverted between 1985 and 1997. Sequence analysis of the viral env gene did not reveal evidence for superinfection, indicating that the incidence of HIV-1 superinfection soon after seroconversion in this cohort is low [220]. Risk reduction shortly after HIV-1 diagnosis early during the HIV-1 epidemic in the Netherlands may have contributed to the absence of HIV-1 superinfection observed in this study.

The ACS has participated in a genome-wide association study in a multiethnic cohort of HIV-1 controllers and progressors [221]. This study revealed >300 genome-wide significant single-nucleotide polymorphisms (SNPs) within the MHC and none elsewhere. Specific amino acids in the HLA-B peptide binding groove, as well as an independent HLA-C effect, explain the SNP associations and reconcile both protective and risk HLA alleles.

A longitudinal study was performed to assess the potential contribution of HIV-specific T-cell immunity in viral load containment after discontinuation of HAART [222]. Individuals who could maintain a low plasma viral load (<15,000 copies/mL) after treatment interruption (TI) were compared to those who could not do so (>50,000 copies/mL). Individuals maintaining a low viral load showed a more pronounced increase in HIV-specific CD8(+) T-cell numbers, leading to a significantly higher magnitude of the total HIV-1-specific CD8(+) T-cell response (IFN-γ(+) and/or IL-2(+) and/or CD107a(+)) 4 weeks after TI. Whether increased T-cell functionality is a cause or consequence of low viral load remains to be elucidated.

Xenotropic murine leukaemia virus-related virus (XMRV) is a recently discovered human gammaretrovirus with yet unknown prevalence and transmission route(s). Its presence in prostate stromal fibroblasts and prostatic secretions suggests that XMRV might be sexually transmitted. We searched for XMRV in seminal plasma, a compartment closely connected to the prostate, which is the only location where XMRV was unambiguously detected in independent studies. Seminal plasma from 54 HIV-1-infected men was analyzed. Although HIV-1 was amplified from 25% of the seminal plasma samples, no XMRV was detected, suggesting that either the prevalence of XMRV is very low in the Netherlands or XMRV is not naturally present in the seminal plasma [223].

Steering committee: The politburo

In 2010, the "politburo" met four times. Twenty-one proposals for use of data and/or samples (serum/PBMC) were submitted to the politburo: eight from AMC-Experimental Immunology, six from the AMC-Medical Microbiology, four from the UMCU, one from the GGD, one from AMC-internal medicine and one from researchers not affiliated with the ACS. Twenty requests were approved, some after revision, one request was resubmitted in 2011 after extensive revisions and one request was denied.

Publications in 2010 that include ACS data

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van Gils MJ, Bunnik EM, Burger JA, Jacob Y, Schweighardt B, Wrin T, Schuitemaker H. **Rapid escape from preserved cross-reactive neutralizing humoral immunity without loss of viral fitness in HIV-1-infected progressors and long-term nonprogressors.** *J Virol. 2010 Apr;84(7):3576-85. Epub 2010 Jan 13. PubMed PMID: 20071586; PubMed Central PMCID: PMC2838121.*

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Evelien Bunnik, March 5th 2010, "HIV-1 neutralizing humoral immunity, viral evolution and disease progression". Promotor is Prof. H. Schuitemaker.

Andrea Rachinger, April 16th 2010, "HIV-1 superinfection in homosexual men". Promotor is Prof. H. Schuitemaker; co-promotor is Dr A. B. van 't Wout.

Martijn Stax, October 1st 2010, "Characterization of DC-SIGN binding glycoproteins and the role in HIV-1 infection". Promotor is Prof. dr. B. Berkhout; co-promotor is Dr W.A. Paxton.

Edwin Heeregrave, October 15th 2010, "Influence of CD4+ cell types on HIV-1 infection". Promotor is Prof. dr. B. Berkhout; co-promotor is Dr W.A. Paxton.