8. The Amsterdam Cohort Studies on HIV infection - Annual Report 2009

Ineke Stolte, Maria Prins for the ACS

The Amsterdam Cohort Studies (ACS) on HIV infection and AIDS 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 2009, the cohorts reached 25 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 25 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 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.

De Amsterdamse Cohort Studies (ACS) naar HIV en Aids zijn gestart kort nadat de eerste gevallen van Aids gediagnosticeerd werden in Nederland. Sinds oktober 1984 worden mannen die seks hebben met mannen (MSM) gevolgd in een prospectieve cohort studie. Een tweede cohort onderdruggebruikers startte in 1985. In 2009 bestonden de cohorten 25 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 HIV-1 infectie en het evalueren van de effecten van interventies. De afgelopen 25 jaar zijn deze doelen min of meer gelijk gebleven maar is de nadruk van de studies wel veranderd. In het begin lag de focus vooral op het ophelderen van epidemiologie van HIV-1. Later zijn meer verdiepende studies uitgevoerd, met name naar de pathogenese van HIV-1. Afgelopen jaren worden eveneens de epidemiologie en het natuurlijke beloop van andere bloed-overdraagbare en seksueel overdraagbare (SOA's) aandoeningen 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 inzicht gegeven in de verschillende aspecten van HIV-1. Deze expertise heeft direct bijgedragen aan de vooruitgang en verbetering in preventie, diagnose en behandeling van HIV infecties.

As of 31 December 2009, 2420 MSM and 1652 (injecting) DU were included in the ACS. Every 3 to 6 months, participants have 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 undergo a medical examination (HIV-positive participants and, in the past, HIV-negative drug users 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 2420 MSM, 585 were HIV-positive at study entry, and 216 seroconverted during follow-up. For the 1652 DU, 322 were HIV-positive at study entry, and 96 seroconverted during follow-up. By 31 December 2009, 342 MSM and 439 DU had died, and several other participants were asked to leave the study or left at their own request. About 90% 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 48,577 times by MSM and 25,791 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, 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, many collaborations exist between the ACS and other research groups both within and outside of the Netherlands.

The ACS is part of Stichting HIV Monitoring (the Netherlands HIV monitoring foundation) 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 2009

The cohort of men having sex with men

In 2009, 525 MSM were followed at the PHSA of Amsterdam. Fourteen of them were newly recruited in 2009. From 2005, recruitment has been open for MSM of all ages with at least one sexual partner in the preceding 6 months. Of the MSM followed in 2009, 473 men were HIV-negative, and 53 men were HIV-positive. The HIV-positive men, of whom 39 were HIV seroconverters, were followed according to the 'HIV Onderzoek onder Positieven' (HOP) protocol, which 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 also been screened for STI.

In 2009, 21 HIV-positive men were included in the HOP, of whom 12 were exclusively followed in an HIV treatment centre outside the PHSA. By the end of 2009, 45 HIV-positive men were still in active follow-up in an HIV treatment centre outside the PHSA and were being followed according to the HOP protocol. 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. By the end of 2009, 13 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 2009, 258 HIV-positive MSM were seen at the Jan van Goyen Clinic or at one of the 22 other HIV treatment centres in the Netherlands. Ninety-one of them were HIV seroconverters. Plasma and cells from 60 of the 141 HIV-positive MSM in active follow-up at the Jan van Goyen clinic in 2009 were stored. Of these, 38 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 10 years and had a CD4 count greater than 400 cells/μl after 10 years of follow-up following an HIV-positive result without effective therapy.

The cohort of drug users

In 2009, 364 drug users were followed at the PHSA of Amsterdam. Forty-nine were young drug users aged 30 years or less, they were recruited after 2000 and had used cocaine, heroin, or amphetamines at least 3 times a week in the 2 months preceding enrolment. Of the 364 DU followed in 2009, 34 were HIV-positive at entry, 17 seroconverted for HIV during follow-up in the ACS, and 5 had their first study visit in 2009.

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 2009, as part of this project, 10 DU who were mono-infected with HCV initiated HCV therapy, resulting in a total group of 60 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.

Substudies

The ACS Open project*

During the past 25 years vast amounts of data on social-scientific, demographic, clinical, and biomedical information have been obtained from the participants of the ACS by the various collaborating institutes. In 2005, the "ACS Open" project group, composed of data managers and scientists from all of the participating research groups, started to connect these data sets and build an easily accessible, multidisciplinary database that comprises all longitudinally obtained epidemiological, social-scientific and biomedical information, and contains data regarding the availability of stored samples in the repositories. In 2010 these data sets will be available to scientists in the collaborating institutes and their colleagues.

The ACS data are also very suitable for universities and research institutes to teach students in epidemiology, biomedicine and social science how to analyze longitudinal data sets. Therefore, a multidisciplinary data set with limited information has been made available for general use and launched on the Internet: www.amsterdamcohortstudies.org.

**This project, 'The opening up of the Amsterdam Cohort Studies (ACS Open)', has been funded by MaGW and ZonMw (grant number 91104002).*

Primo-SHM study

In addition to the cohorts previously described, the ACS also includes patients who present with primary HIV-1 infection at the outpatient clinic of the AMC in the so-called "primo-SHM study". Some of these patients are seronegative men who seroconverted during follow-up in the MSM cohort at the PHSA. Some of them are also still followed with the HOP protocol of the ACS at the PHSA. The primo-SHM study is a national randomized study on the effect of early temporary quadruple antiviral therapy as compared to no therapy. As of December 2009, 238 patients were already included as patients with primary infection, of whom 173 participated in the randomized clinical trial (RCT). Inclusion in the RCT stopped in early 2010, and its results are expected in early 2011.

Blood is collected from all patients for storage of plasma and peripheral-blood mononuclear cells (PBMC), and sampling is more frequent early after entry into the study. Individuals are followed until they have to (re) start highly active antiretroviral therapy (HAART) because of a CD4+ T cell decline <350 cells/μl.

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. Of the 59 HIV-infected children currently being followed, 58 were infected with HIV-1 and 1 with HIV-2. Two patients were co-infected with hepatitis B virus (HBV). 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 at the PHSA had a first HIV-positive test in 2009 after a previous HIV-negative test. HIV incidence in 2009 was 2 per 100 person-years among MSM, and it has slowly increased since 1996, the year that combination antiretroviral therapy (cART) became generally available in high-income countries including the Netherlands, followed by a strong decrease in HIV-related morbidity and mortality rates.

The trend in HIV incidence among DU in the ACS differed from that observed among MSM; HIV incidence has substantially declined to less than 1 per 100 person-years in most recent years. Figures 8.1 and 8.2 show the yearly observed HIV incidence rates for MSM and drug users from the start of the ACS through 2009.

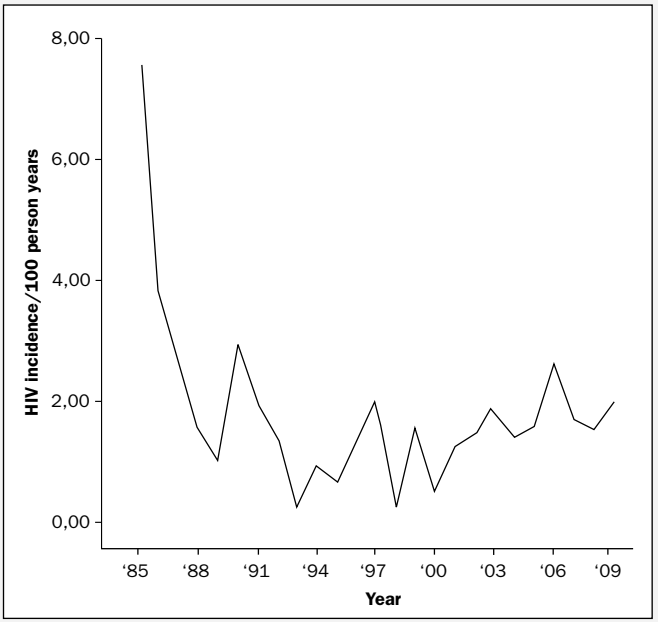

Figure 8.1: HIV incidence per calendar year among MSM in the ACS, 1984-2009

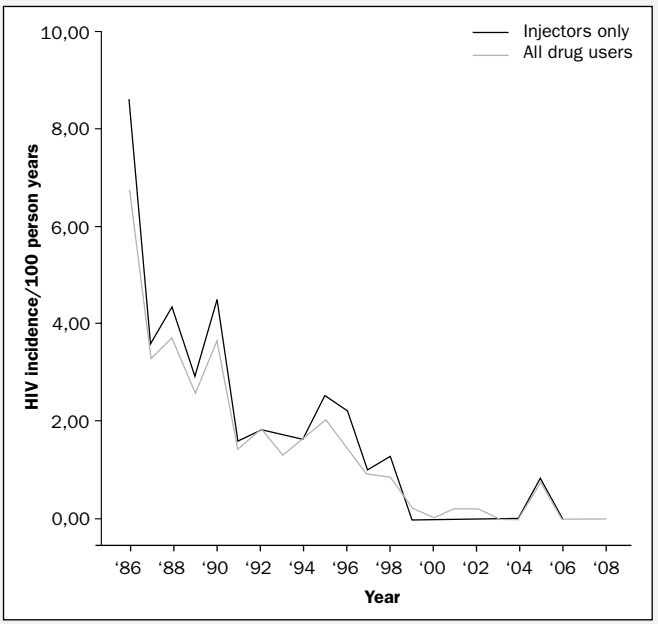

Figure 8.2: HIV incidence per calendar year among drug users in the ACS, 1986-2009

Transmission of therapy-resistant HIV strains

Surveillance of transmission of drug-resistant HIV-1 strains was performed for 9 MSM seroconverters and for 1 of 2 MSM seropositive at study entry in 2009. None of the individuals was infected with virus harbouring resistance-associated mutations; only a naturally occurring sequence variation was found in the predominant circulating virus populations. Phylogenetic analysis showed that 9 individuals harboured subtype B HIV-1 strains and 1 individual was infected with subtype CRF02-AG.

HAART uptake

Of all 258 HIV-positive MSM visiting the Jan van Goyen Clinic or one of the other HIV treatment centres in the Netherlands in 2009, 235 (91%) received some form of antiretroviral therapy. Of 258 MSM for whom viral load results were available in 2009, 228 (88%) had a viral load of less than 50 copies/ml (assay: M2000rt).

Of the 41 HIV-positive DU who visited the PHSA in 2009 and for whom treatment data were available, 35 (85%) received some combination of antiretroviral therapy. Of these 35, 33 (94%) had an undetectable viral load (less than or equal to 150 copies/ml [assay: M2000rt]) at their latest visit. Of 6 HIV-positive DU not receiving HAART, 3 (50%) had an undetectable viral load.

Risk behaviour of MSM

Among HIV-1-negative MSM practising anal sex the percentage of men practising unprotected anal intercourse (UAI) reached 55% in 2009. Similar to the HIV incidence, trends in UAI among HIV-negative MSM participating in the ACS have slowly increased since 1996.

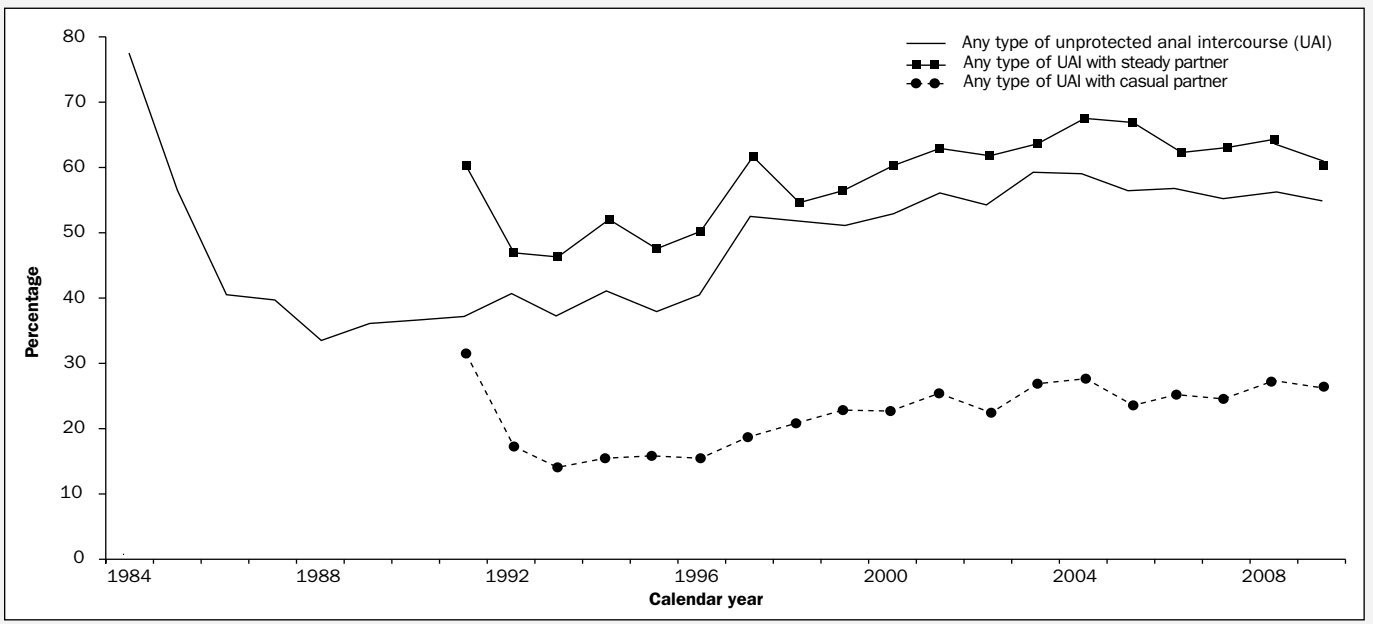

Figure 8.3: Trends in unprotected anal intercourse in the past 6 months amongst HIV-negative MSM who engaged in anal sex. ACS 1984-2009

Risk behaviour of DU

In HIV-negative DU, reports of both injection and borrowing needles significantly declined over the period 1985-2009. Reports of high risk sexual behaviour and sexually transmitted infections at follow-up visits decreased before 1996, but they remained relatively stable after 1996 at approximately 35% and 8% respectively (see Figure 8.4).

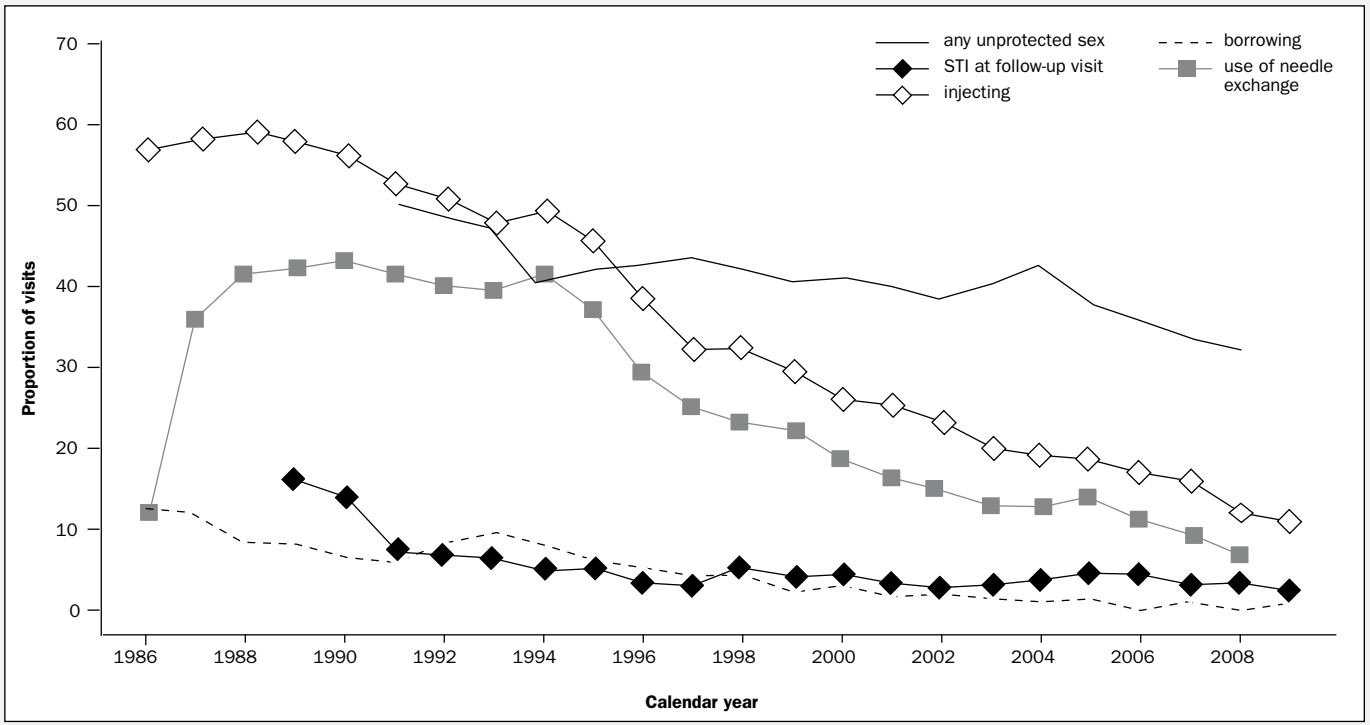

Figure 8.4: Proportion of visits per calendar year at which injecting and high risk sexual behaviour was reported amongst 1315 DU who were HIV-negative on ACS entry, 1986-2009. STI=sexually transmitted infection

Hepatitis B co-infections

The ACS has expanded in recent years to include studies of other blood-borne and sexually transmitted infections among the participants of the ACS, opening up new avenues for further research. In 2009, the first results on the retrospective longitudinal testing for HBV infections became available[211, 212]. Between 1984 and 2003, sera of MSM and DU in the ACS with a history of at least two visits were retrospectively screened for anti-hepatitis B core (HBc) antibodies. After 2003, most MSM and DU participating in the ACS were vaccinated against HBV, making further testing redundant.

After screening the sera of 1862 MSM, 1042 MSM proved to be negative for anti-HBc antibodies at study entry; 64 of the 1042 subsequently seroconverted during follow-up at a median age of 32 years. At the moment of seroconversion, 31 MSM were HIV-positive. HBV incidence declined dramatically in the first years and then remained stable throughout the study period. Although HBV is generally considered more infectious than HIV, this study shows that the trend and magnitude

in HBV and HIV incidence among MSM are similar. With the exception of 3 MSM, all were infected with an identical genotype A strain. This strain has been circulating not only amongst MSM of the ACS but also amongst the general MSM population in the Netherlands for at least 2 decades.

Sera of 1268 DU were screened for anti-HBc antibodies, and of the 598 participants who were anti-HBc–negative at entry, 83 subsequently seroconverted for anti-HBc antibodies. The incidence of HBV declined from 5.9 per 100 person-years between 1985 and 1993 to 0 per 100 person-years in 2002. Of the acutely infected injecting and non-injecting DU, 88% were infected with the same genotype D, serotype ayw3 strain. Current injecting was the most important risk factor for HBV infection. The decline in the incidence of HBV amongst DU in Amsterdam was probably caused by the decline in injecting behaviour. Injecting and non-injecting DU were infected with the same strain, indicating that DU infect one another, regardless of their risk behaviour. No reports of new cases among DU and the disappearance of the specific genotype D strain suggest that DU may no longer be a high-risk group for HBV infection in Amsterdam. However, trends in drug use need to be monitored in case injecting drug use regains popularity in the Netherlands, thereby increasing HBV transmission risk among DU.

ACS research highlights 2009

Since 2000, there has been a marked rise in acute hepatitis C virus (HCV) in HIV-positive MSM. We conducted an international phylogenetic study to investigate the existence of an HCV transmission network among MSM. This analysis revealed a large international network of HCV transmission. The rapid spread of HCV among neighbouring countries is supported by the large proportion (74%) of European MSM infected with an HCV strain co-circulating in multiple European countries, the low evolutionary distances among HCV isolates from different countries, and the trend toward increased country mixing with increasing cluster size. Temporally, this epidemic coincides with the introduction of HAART and associated increases in sexual risk behaviours. International collaborative public health efforts are needed to mitigate HCV transmission among this population[213].

Interestingly, we were able to study HCV-specific T cell responses during acute HCV infection in the presence of existing HIV-1 infection in four MSM infected with HIV-1. Three patients with near normal CD4+ T cell counts either resolved their HCV infection (n=1) or temporarily suppressed HCV RNA, and one patient with low CD4+ T cell had sustained high HCV RNA levels. All four patients had low HCV-specific CD8+ T cell responses and similar magnitudes of CD4+ T cell responses. Interestingly, individuals with resolved infection or temporary suppression of HCV-RNA had HCV-specific CD4+ T cell responses predominantly against nonstructural (NS) proteins[176].

HIV-infected participants of the ACS were screened for the presence of cross-reactive neutralizing activity in their serum. Cross-reactive neutralizing activity was observed in both rapid and slow progressors[214]. Longitudinal analysis revealed that the potency and breadth increased with duration of infection and correlated with CD4 counts at set point. In the first genome-wide association studies (GWA) on HIV-1 infection, single nucleotide polymorphisms (SNPs) in the HLA-C and the HCP-5 gene region have been described as major determinants in host control of HIV-1. We observed that the described SNPs were also associated with viral load and the clinical course in participants of the Amsterdam Cohort Studies[215].

The main clinical objective of cART is suppression of HIV-1 plasma viremia to below the lowest detection limit of commercial assays. In most patients on cART,

this objective is achieved and therefore, plasma viremia in these patients cannot predict the therapeutic outcome. Hence, additional markers have to be identified that are associated with the outcome of therapy in patients with fully suppressed plasma viremia. We demonstrated that the level of HIV-1 unspliced RNA in PBMC from such patients is predictive of subsequent virological rebound. So, a viral parameter measured in a patient receiving cART during a period of undetectable plasma viremia is predictive of the therapeutic outcome[216].

Steering committee: The politburo

In the 2009, the "politburo" met four times. Seventeen proposals for use of data and/or samples (serum/PBMC) were submitted to the politburo: 12 from AMC-Experimental Immunology, 1 from the AMC-Medical Microbiology, 3 from the UMCU, and 1 from researchers not affiliated with the ACS. Sixteen requests were approved, some after revision, and one request was denied.

To mark the 25th anniversary of the ACS, a successful and moving symposium for ACS participants and professionals affiliated with the ACS was held in Amsterdam, November 28, 2009.

Publications in 2009 that include ACS data

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Other publications:

The Amsterdam Cohort Studies on HIV infection and AIDS; A summary of the results 2001-2009. Heijnis & Schipper, Zaandijk, 2009; ISBN 978-90-9024893-6.

Theses in 2009 that include ACS data

Ingrid Schellens. *"Impact of HLA Class I restricted T cells on HIV-1 disease progression*". Promotor is prof dr F Miedema (UMCU), copromotores are dr J Borghans and dr D van Baarle.

M Navis. *"Cellular immunity driving HIV-1 evolution".* Promotor is prof dr H Schuitemaker (AMC). Copromotor is dr NA Kootstra (AMC).

Rogier van Gent. *"Lymphocyte dynamics in health and disease"*. Promoter is prof dr F Miedema (UMCU), copromotores are dr K Tesselaar and dr J Borghans (UMCU).

Jolanda Scherrenburg. *'T cell immunity to herpesviruses in immune disorders"*. Promoter is prof dr F Miedema (UMCU) and copromotor is dr D van Baarle (UMCU).

Maarten Rits. *"Cellular factors involved in HIV-1 replication"*. Promotor is prof dr H Schuitemaker (AMC). Copromotor is dr NA Kootstra (AMC).

Robin van Houdt. *"Molecular epidemiology of hepatitis B in the Netherlands"*. Promotor is prof dr RA Coutinho (RIVM/AMC). Copromotor is dr SM Bruisten (GGD Amsterdam).

Charlotte van den Berg. *"Hepatitis C virus epidemiology and immunology.* Promotor is prof dr RA Coutinho (RIVM/AMC). Copromotores are dr M Prins (AMC/GGD Amsterdam) and dr D van Baarle (UMCU).

Thijs van Montfort. *"Interaction of HIV-1 with dendritic cells: implications for pathogenesis"*. Promotor is prof dr B Berkhout (AMC). Copromotor is dr WA Paxton (AMC).

Daniela Bezemer. *"Impact of antiretroviral therapy on HIV-1 transmission dynamics"*. Promotores are prof dr RA Coutinho (AMC) and prof dr M Sabelis (AMC). Copromotores are dr M Prins (AMC/GGD Amsterdam) and dr F de Wolf (AMC).

K Kozaczynska. *"HIV-1 superinfection"*. Promotor is prof dr B Berkhout (AMC), copromotores are dr M Cornelissen (AMC) and dr T van der Kuyl (AMC).