The Amsterdam Cohort Studies on HIV infection: annual report 2015

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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 people who use drugs (PWUD) was initiated in 1985. In 2015, the cohorts reached 31 years of follow up. The initial aim of the ACS was to investigate the prevalence and incidence of HIV-1 infection and AIDS, the associated risk factors, the natural history and pathogenesis of HIV-1 infection, and the effects of interventions. During the past 31 years, these aims have remained primarily the same, although the emphasis of the studies has changed. Early on, the primary focus was to elucidate the epidemiology of HIV-1 infection, whereas later more in-depth studies were performed to investigate the pathogenesis of HIV-1 infection. In the past decade, research on the epidemiology of other blood-borne and sexually transmitted infections (STI) and their interaction with HIV has become an important component of the ACS research programme.

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.

Collaborating institutes and funding

Within the ACS, different institutes collaborate to bring together the data and biological sample collections and to conduct research. These include the Public Health Service of Amsterdam (Geneeskundige en Gezondheidsdienst Amsterdam; GGD Amsterdam) (Department of Infectious Diseases Research and Prevention), the Academic Medical Center (AMC) of the University of Amsterdam (Departments of Medical Microbiology, Experimental Immunology, and Internal Medicine (Division of Infectious Diseases), HIV treatment centre, Emma Kinderziekenhuis), Stichting HIV Monitoring (SHM), the Jan van Goyen Medical Centre (Department of Internal Medicine) and the HIV Focus Centre (DC Klinieken) Amsterdam. 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 at Sanquin Research. Sanquin financially supports the maintenance of the biobank of viable peripheral blood mononuclear cells (PBMC) at the Department of Experimental Immunology at the AMC. In addition, there are numerous collaborations between the ACS and other research groups both within and outside the Netherlands. The ACS is financially supported

by the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment (Centrum voor Infectieziektenbestrijding-Rijksinstituut voor Volksgezondheid en Milieu, CIb-RIVM).

Ethics statement
The ACS has been conducted in accordance with the ethical principles set out in the declaration of Helsinki. Participation in the ACS is voluntary and written informed consent is obtained from each participant. The most recent version was approved by the AMC Medical Ethics Committee in 2007 for the MSM cohort and in 2009 for the PWUD cohort.

The ACS in 2015

The cohort of men having sex with men
As of 31 December 2015, 2,713 MSM were included in the ACS. Every three to six months, participants complete a standardised questionnaire designed to obtain information regarding medical history, sexual and drug use behaviour, underlying psychosocial determinants, healthcare use, depression, psychological disorders, and demographics. Blood is collected for diagnostic tests and storage. Of the 2,713 MSM, 607 were HIV-positive at entry into the study, and 248 seroconverted during follow up. In total, the GGD Amsterdam was visited 56,184 times by MSM.

Until 1995, HIV-negative men of all age groups were eligible to participate if they lived in or around Amsterdam and had had at least two male sexual partners in the previous six months. During the period 1995–2004, only HIV-negative men aged ≤30 years with at least one male sexual partner in the previous six months could enter the study. Since 2005, recruitment has been open to HIV-negative MSM of all ages with at least one sexual partner in the preceding six months. In line with the advice issued by the international scientific advisory committee in 2013, the cohort made additional efforts to recruit young HIV-negative MSM. HIV-seroconverters within the ACS remained in the cohort until 1999, when follow up of a selection of HIV-positive MSM was transferred to the Jan van Goyen Medical Center. In 2003, the 'HIV Onderzoek onder Positieven' (HOP) protocol (HIV Research in Positive Individuals) was initiated. Individuals with a recent HIV infection at study entry at the GGD Amsterdam and HIV seroconverters within the cohort return for follow up at the GGD Amsterdam or at an HIV treatment centre. All behavioural data are collected on a six-monthly basis by questionnaires, coordinated by the GGD Amsterdam, and clinical data are provided by SHM.

In 2015, 654 HIV-negative and 69 HIV-positive MSM were in active follow up within the ACS (6-monthly visits to the GGD Amsterdam for STI testing, including HIV); 66 of these 69 MSM filled in behavioural questionnaires. Apart from the HIV-positive MSM visiting the GGD Amsterdam, 269 HIV-positive MSM were followed outside the GGD Amsterdam via the Jan van Goyen Medical Centre or at a HIV Focus Centre in Amsterdam. Behavioural questionnaires were filled in by 39 of them. The median age of the total group of MSM was 43.6 years (interquartile range [IQR] 36.6-51.1), 8.4% were non-Dutch, and 73.4% had attained a high level of education. The majority of the participants (84.7%) were residents of Amsterdam. Additional efforts to expand the HIV-negative cohort resulted in 64 newly

recruited HIV-negative participants in 2015. The median age in this group was 27.8 years (IQR 24.3-37.5).

The cohort of drug users

As of 31 December 2015, 1,680 people who use drugs (PWUD) were included in the ACS. Before 2014, participants visited the GGD Amsterdam every four to six months. They completed a standardised questionnaire designed to obtain information regarding medical history, sexual and drug use behaviour, underlying psychosocial determinants, healthcare use, depression, psychological disorders, and demographics. In addition, HIV-positive participants and, in the past, HIV-negative participants, underwent a medical examination. Blood was collected for diagnostic tests and storage.

In 2014, PWUD included in the ACS were divided into two groups, in line with the advice of the international scientific advisory committee in 2013. Group 1 consists of PWUD who visit the GGD Amsterdam once a year to complete questionnaires with no testing and blood sampling. In 2015, there were 130 PWUD in active follow up in this group. Group 2, the focus group, consists of PWUD who are 1) HIV positive; 2) hepatitis C virus (HCV) seroconverters; 3) multiple-exposed, non-infected with HIV and HCV, or 4) a random control group. This group visited the GGD Amsterdam twice a year for testing and blood sampling and to fill out questionnaires, as in the years before. In 2015, 61 PWUD were in active follow up in this focus group. The cohort was closed for new participants in January 2014. Therefore, no new participants were recruited in 2015.

Of the 1,680 PWUD, 323 were HIV-positive at entry, and 99 seroconverted during follow up. The last HIV seroconversion was seen in 2012. By 31 December 2015, 573 PWUD had died. In total, PWUD visited the GGD Amsterdam 28,002 times. The median age of the PWUD was 53.4 years (IQR 46.9-58.2), 7.1% had attained a high level of education, 11.5% were non-Dutch, and 182 (95.3%) were residents of Amsterdam.

<u>Subgroup studies and affiliated studies</u>

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 GGD Amsterdam, and SHM, was started in October 2010. The aim of the study is to assess the prevalence and incidence of a broad range of comorbidities and known risk factors for these comorbidities in HIV-infected patients aged ≥45 years, and to determine the extent to which comorbidities, their risk factors and their relation to quality of life differ between HIV-infected and uninfected groups. Participants undergo a comprehensive assessment for comorbidities and fill in a questionnaire at intake and 2 years afterwards. In total, 598 HIV-1-infected participants and 550 HIV-uninfected individuals completed a baseline visit between October 2010 and September 2012. HIV-1-infected participants were included through the AMC HIV outpatient clinic and HIV-uninfected participants from the same HIV exposure groups were included through the STI clinic of the GGD Amsterdam (n=486) or the ACS (n=64). All participants were aged ≥45 years and were as comparable as possible with respect to age, gender, ethnicity, and risk behaviour. By the end of September 2015, 498 HIV-1-infected participants and 482 HIV-uninfected individuals

had completed the second follow up visit. As of 31 December 2015, 368 HIV-1-infected participants and 226 HIV-uninfected participants had returned for their third visit. The third visiting round will continue until September 2016 and the fourth round of visits will commence in October 2016.

H2M Cohort Study

From 2010 to 2013, the H2M (HIV and human papillomavirus (HPV) in MSM) cohort study was conducted in a subset of the HIV-negative and HIV-positive ACS participants who were in active follow up and attending the STI clinic at GGD Amsterdam or the Jan van Goyen Medical Centre. The aim of the study was to compare the prevalence, incidence, and clearance of high-risk (hr) HPV infections between HIV-negative and HIV-infected MSM.

In 2015, a study based on the H2M cohort was initiated to identify potential predictors for high-grade anal intra-epithelial neoplasia in the HIV-infected MSM population. This study, the H2M2, is an Aids Fonds-supported project, and a collaboration between the GGD Amsterdam, HIV Focus Centre, the CIb-RIVM, VUmc, and the AMC. The study includes a subset of the HIV-positive ACS participants. Preliminary findings should be presented in 2016.

Since September 2014, collection of anal and genital swabs has been resumed in the ACS participants. The key aim of this second new study (the H2M3 study), which builds on the H2M study, is to study long-term incidence and clearance of anal and penile hrHPV infections. Samples at two time points (6 months apart) have been tested in the laboratory for HPV DNA and statistical analyses are underway. The study will investigate what proportion of MSM have long-term persistent hrHPV infections. This study is a collaboration between GGD Amsterdam, ACS, and Crucell.

ACS biobank

The ACS visits, together with data collection from several subgroup studies and affiliated studies, have resulted in a large collection of stored samples. In addition, the ACS biobank includes plasma/serum and PBMC samples collected within the context of the Primo-SHM study (a national randomised study on the effects of early temporary antiviral therapy as compared to no therapy among patients who presented with primary HIV-1 infection at the AMC outpatient clinic and among ACS seroconverters). These samples are stored at the AMC. At present, the biological samples are still being collected prospectively for Primo-SHM participants visiting the AMC clinic until one year after they have recommenced therapy. The ACS biobank also includes plasma and PBMC samples that were collected from 94 HIV-infected and HIV-exposed children at the Emma Kinderziekenhuis in the AMC until 2008. These are also stored at the AMC. In 2015, no new samples from children were collected within the ACS setting. All stored samples are available for ACS research

The HIV epidemic

HIV incidence

In 2015, 2 MSM participating in the ACS seroconverted for HIV. The observed HIV incidence among MSM has remained relatively stable in recent years and was 0.34 per 100 person

years in 2015. The HIV incidence in PWUD has been stable since 2008, with zero to less than one case per 100 person years. As follow up was restricted to a selection of PWUD in 2014 and inclusion of new PWUD stopped, the early observed incidence of PWUD can only be presented until 2013. Figures 8.1 and 8.2 show the yearly observed HIV incidence rates for MSM and PWUD from the start of the ACS through 2015 and 2013, respectively.

Transmission of therapy-resistant HIV strains
In 2015, surveillance of transmission of drug-resistant HIV-1 strains was only performed between January and June. During this period there was one MSM seroconverter and one MSM who was positive at study entry. None of the individuals were infected with a virus harbouring resistance-associated mutations in the protease and reverse transcriptase genes. In both individuals, naturally occurring sequence variation was found in the protease gene. HIV-1 subtypes were determined by phylogenetic analysis: the seroconverter harboured a subtype B HIV-1 strain; the seropositive MSM had a mosaic virus containing subtype A and unknown sequences.

Combination antiretroviral therapy (cART) uptake Of all 338 HIV-positive MSM from the ACS visiting the HIV Focus Centre, the Jan van Goyen Medical Centre or one of the other HIV treatment centres in the Netherlands in 2015, treatment data were available for 333 men. Of these, 328 (98%) received some form of antiretroviral therapy. Of the 330 MSM for whom viral load results were available in 2015, 306 (93%) had a viral load of <50 copies/ml (M2000rt assays). Of the 21 HIV-positive PWUD who visited the GGD Amsterdam in 2015 and for whom treatment data were available, 21 (100%) were receiving some combination of antiretroviral therapy. The 24 PWUD for whom viral load results were available all had an undetectable viral load (≤150 copies/ml [assay: M2000rt]) at their latest visit.

Risk behaviour of MSM in ACS
Information from the questionnaires completed by 654 HIV-negative MSM during cohort visits in 2015 showed higher proportions of condomless anal intercourse (CAI) with steady partners (39.6%) compared to casual partners (30.4%). Trends in CAI among HIV-negative MSM who are participants in the ACS, especially those with casual partners, continue to show a gradual increase from 1996 onwards. (Figure 8.3).

Risk behaviour of PWUD in ACS
As follow up was restricted to a selection of PWUD in 2014 and inclusion of new PWUD has been halted, trends in risk behaviour of PWUD can only be presented until 2013. In HIV-negative PWUD, reports of both injection and borrowing needles significantly declined over the period 1985-2013. Reports of high-risk sexual behaviour at follow-up visits decreased before 1996, then remained relatively stable until 2005, and further decreased to approximately 22% in 2013. Reports of STI have remained relatively stable at approximately 1% in recent years (see Figure 8.4).

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 using urine samples and pharyngeal and rectal swabs. Cases of syphilis are detected by Treponema pallidum haemagglutination assay (TPHA). In 2015, a total of 682 MSM from the ACS were screened

for STI. The overall prevalence of any STI (i.e., chlamydia, gonorrhoea, and syphilis) was 10.2% (63/619) among HIV-negative MSM and 23.8% (15/63) among HIV-positive MSM.

ACS 2015 research highlights

Preexposure prophylaxis (PrEP) is a new biomedical approach that offers HIV-negative individuals a regime of lower-intensity antiretroviral therapy to reduce their risk of HIV infection. Although PrEP is not yet registered in the Netherlands, its approval and implementation are expected in the near future. The aim of the study was to gain insight into PrEP awareness, the intention to use PrEP, and to identify sociodemographic and psychological determinants of a higher intention to use PrEP among MSM in the ACS. The intentions to use PrEP were relatively low: 27% of the ACS participants had a low intention, 60% a medium intention, and 13% a high intention. High-risk MSM (i.e., men who reported receptive condomless anal sex with casual partners, had ≥5 casual partners, or had been diagnosed with gonorrhoea) were more likely to have a higher intention to use PrEP than low-risk MSM. approximately one-third of the participants anticipated a decrease in their condom use during anal sex while using PrEP. When PrEP implementation starts in the Netherlands, PrEP costs and psychological determinants will influence acceptability and uptake of PrEP and should therefore be addressed in targeted information campaigns. PrEP implementation should be combined with other HIV and STI prevention strategies (185).

The envelope glycoprotein (Env) trimer mediates HIV-1 entry into cells. The trimer is flexible, fluctuating between closed and more open conformations and sometimes encountered in the fully open, CD4-bound form. Such conformational flexibility and transient exposure of non-neutralizing, immunodominant epitopes could hinder the induction of broadly neutralising antibodies. Researchers from the Laboratory of Experimental Virology (AMC) have therefore modified soluble Env trimers to stabilize the closed, ground states. These closed trimers may be useful components of vaccines aimed at inducing broadly neutralizing antibodies (186).

HIV-1 exploits the cellular machinery for replication and therefore several Interactions with cellular factors take place, some of which are yet unknown. We identified GTPase-activating protein-(SH3 domain)-binding protein 1 (G3BP1) as a cellular factor that restricts HIV-1, by analysing transcriptome profiles of in vitro cytokine-activated macrophages that are non-permissive to HIV-1 replication. Silencing of G3BP1 by RNA interference resulted in increased HIV-1 replication in primary T-cells and macrophages, but did not affect replication of other retroviruses. G3BP1 specifically interacted with HIV-1 RNA in the cytoplasm, suggesting that it sequesters viral transcripts, thus preventing translation or packaging. G3BP1 was highly expressed in resting naive or memory T-cells from healthy donors and HIV-1 infected patients, but significantly lower in interleukin 2 (IL-2)-activated T-cells. These results strongly suggest that G3BP1 captures HIV-1 RNA transcripts and thereby restricts mRNA translation, viral protein production and virus particle formation (187).

Steering committee

In 2015, the steering committee met four times. Seven proposals for use of data and/or samples (serum/PBMC) were submitted to the committee: four from the AMC Medical

Microbiology department, three from the GGD Amsterdam. All requests were approved, some after revision. Two of the approved proposals were collaborations with groups outside the ACS. Finally, a new ACS advisory board was installed in 2015 and a first meeting with this board took place in December 2015 and focused on future ACS data collection.

Publications in 2015 that included ACS data

1. van Aalderen MC, Remmerswaal EB, Verstegen NJ, Hombrink P, ten Brinke A, et al. Infection history determines the differentiation state of human CD8+ T cells. J Virol. 2015;89(9):5110-5123.

2. Bil JP, Davidovich U, van der Veldt WM, Prins M, de Vries HJ, et al. What do Dutch MSM think of pre-exposure prophylaxis to prevent HIV-infection? A cross-sectional study. AIDS. 2015;29(8):955-964.

3. Cobos Jiménez V, Martinez FO, Booiman T, van Dort KA, van de Klundert MA, et al. G3BP1 restricts HIV-1 replication in macrophages and T-cells by sequestering viral RNA. Virology. 2015;486:94-104.

4. Costa AI, Koning D, Ladell K, McLaren JE, Grady BP, et al. Complex T-cell receptor repertoire dynamics underlie the CD8+ T-cell response to HIV-1. J Virol. 2015;89(1):110-119.

5. De Taeye S, Ozorowski G, Torrents de la Peña A, Guttman M, Julien JP, et al. Immunogenicity of stabilized HIV-1 envelope trimers with reduced exposure of non-neutralizing epitopes. Cell. 2015;163:1702-1715.

6. Grady BP, Prins M, Rebers S, Molenkamp R, Geskus RB, Schinkel J. BMI, male sex and IL28B genotype associated with persistently high hepatitis C virus RNA levels among chronically infected drug users up to 23 years following seroconversion. J Viral Hepat. 2015;22(3):263-271.

7. Hajarizadeh B, Grady B, Page K, Kim AY, McGovern BH, et al. Patterns of Hepatitis C virus RNA levels during acute infection: The InC3 Study. InC3 Study Group. PLoS One. 2015;10(4):e0122232.

8. Hajarizadeh B, Grady B, Page K, Kim AY, McGovern BH, et al. Factors associated with hepatitis C virus RNA levels in early chronic infection: the InC(3) study. The InC3 Study. J Viral Hepat. 2015;22(9):708-717.

9. Jarrin I, Pantazis N, Dalmau J, Phillips AN, Olson A, et al. Does rapid HIV disease progression prior to combination antiretroviral therapy hinder optimal CD4+ T-cell recovery once HIV-1 suppression is achieved? CASCADE Collaboration in EuroCoord. AIDS. 2015;29(17):2323-2333.

10. Letko M, Booiman T, Kootstra N, Simon V, Ooms M. Identification of the HIV-1 Vif and Human APOBEC3G Protein Interface. Cell Rep. 2015;13(9):1789-99.

11. Marzolini C, Sabin C, Raffi F, Siccardi M, Mussini C, et al. Impact of body weight on virological and immunological responses to efavirenz- containing regimens in HIV-infected, treatment-naive adults. Obesity Project Team on behalf of Collaboration of Observational HIV Epidemiological Research Europe (COHERE) in EuroCoord. AIDS. 2015;29(2):193-200.

12. McLaren PJ, Coulonges C, Bartha I, Lenz TL, Deutsch AJ, et al. Polymorphisms of large effect explain the majority of the host genetic contribution to variation of HIV-1 virus load. Proc Natl Acad Sci U S A. 2015;112(47):14658-14663.

13. Mocroft A, Lundgren J, Antinori A, Monforte Ad, Brännström J, et al. Late presentation for HIV care across Europe: update from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study, 2010 to 2013. Euro Surveill. 2015;20(47)

14. Monge S, Jarrín I, Mocroft A, Sabin CA, Touloumi G, et al. Mortality in migrants living with HIV in western Europe (1997–2013): a collaborative cohort study. The Migrants Working Group on behalf of COHERE in EuroCoord. Lancet HIV. 2015;2(12): e540-549.

15. Oude Munnink BB, Cotten M, Deijs M, Jebbink MF, Bakker M, et al. A novel genus in the order Picornavirales detected in human stool. J Gen Virol. 2015;96(11):3440-3443.

16. Sacks-Davis R, Grebely J, Dore GJ, Osburn W, Cox AL, et al. Hepatitis C virus reinfection and spontaneous clearance of reinfection – the InC3 Study. InC3 study group. J Infect Dis. 2015; 212(9):1407-1419.

17. Setiawan LC, Gijsbers EF, van Nuenen AC, Kootstra NA. Viral evolution in HLA-B27-restricted CTL epitopes in human immunodeficiency virus type 1-infected individuals. J Gen Virol. 2015;96(8):2372-2380.

18. Sloot R, Schim van der Loeff MF, van Zwet EW, Haks MC, Keizer ST, et al. Biomarkers can identify pulmonary tuberculosis in HIV-infected drug users months prior to clinical diagnosis. EBioMedicine. 2014;2(2):172-179.

19. Spits HB, Grijsen ML, Steingrover R, Nanlohy NM, Kootstra N, et al. A lower viral set point but little immunological impact after early treatment during primary HIV infection. Viral Immunol. 2015;28(3):134-144.

20. Welling CA, Mooij SH, van der Sande MA, van Rooijen MS, Vermeulen-Oost WF, et al. Association of HIV infection with anal and penile low-risk human papillomavirus infections among men who have sex with men in Amsterdam: The HIV & HPV in MSM Study. Sex Transm Dis. 2015;42(6):297-304.

Theses in 2015 that included ACS data

Thijs Booiman – 5 February 2015: Host factors in HIV-1 replication: the good, the bad and the ugly. Supervisor: Prof. T.B. Geijtenbeek (AMC); co-supervisor: Dr N.A. Kootstra (AMC).

Xiomara Thomas – 13 March 2015: Epidemiological, immunological and virological aspects of acute and chronic hepatitis C virus infections. Supervisor: Prof. M.D. de Jong (AMC); co-supervisors: Dr C.J. Schinkel (AMC) and Dr R. Molenkamp (AMC).

Sofie Mooij – 27 March 2015: Epidemiology of anal and penile HPV infections. Supervisor: Prof. R.A. Coutinho (AMC/University Utrecht); co-supervisor: Dr M.F. Schim van der Loeff (GGD Amsterdam/AMC).

Bart Grady – 4 June 2015: Hepatitis C virus: risk factors and disease progression. Supervisor: Prof. M. Prins (GGD Amsterdam/AMC); co-supervisor: Dr D. van Baarle (University Utrecht/RIVM).

Rosa Sloot – 10 June 2015: Epidemiological studies on tuberculosis control and respiratory viruses. Supervisors: Prof. M.W. Borgdorff (AMC) and Prof. M.D. de Jong (AMC); co-supervisor: Dr M.F. Schim van der Loeff (GGD Amsterdam/AMC).

Amy Matser – 6 November 2015: Sexually transmitted infections: Unravelling transmission & impact. Supervisors: Prof. M. Prins (GGD Amsterdam/AMC) and Prof. M.E.E. Kretzschmar (University Utrecht/RIVM); co-supervisors: Dr M.F. Schim van der Loeff (GGD Amsterdam/AMC) and Dr R.B. Geskus (AMC/GGD Amsterdam).

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