Monitoring Report 2012

Human Immunodeficiency Virus (HIV) Infection in the Netherlands

HIV Monitoring

Contributing to the quality of HIV care

Stichting HIV Monitoring (SHM), the Dutch HIV monitoring foundation, was founded in 2001. Based in Amsterdam, SHM was appointed by the Dutch Minister of Health, Welfare and Sports (Ministerie van Volksgezondheid, Welzijn en Sport) as the national executive organization for the registration and monitoring of HIV-infected patients in follow-up in one of the Dutch HIV Treatment Centres.

Our Mission:
To further the knowledge and understanding of the epidemiology and the course of the treated and untreated HIV infection.

www.hiv-monitoring.nl

HIV Monitoring

Colophon

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on behalf of
the Netherlands
collaborative
HIV treatment
centres

HIV Treatment Centres

The monitoring of HIV-infected adults is a collaborative effort involving Stichting HIV Monitoring (SHM) and a total of 26 health institutes that are acknowledged by the Dutch Minister of Health, Welfare and Sport as HIV treatment centres or subcentres. In addition, HIV-infected children and adolescents are monitored in four institutes that are recognized as paediatric HIV treatment centres.

In 2012 the following health institutes were involved as (sub)centres for adult HIV care (in alphabetical order of town):

1. Medisch Centrum Alkmaar — Alkmaar
2. Flevoziekenhuis — Almere
3. Academic Medical Centre of the University of Amsterdam — Amsterdam
4. Onze Lieve Vrouwe Gasthuis — Amsterdam
5. Sint Lucas Andreas Ziekenhuis — Amsterdam
6. Slotervaartziekenhuis — Amsterdam
7. Stichting Medisch Centrum Jan van Goyen — Amsterdam
8. VU Medisch Centrum — Amsterdam
9. Rijnstate — Arnhem
10. HagaZiekenhuis (location Leyenburg) — Den Haag
11. Medisch Centrum Haaglanden (location Westeinde) — Den Haag
12. Catharina Ziekenhuis — Eindhoven
13. Medisch Spectrum Twente — Enschede
14. Universitair Medisch Centrum Groningen — Groningen
15. Kennemer Gasthuis — Haarlem
16. Medisch Centrum Leeuwarden — Leeuwarden
17. Leids Universitair Medisch Centrum — Leiden
18. MC Zuiderzee — Lelystad
19. Academisch Ziekenhuis Maastricht — Maastricht
20. Universitair Medisch Centrum Sint Radboud — Nijmegen
21. Erasmus Medisch Centrum — Rotterdam
22. Maasstad Ziekenhuis — Rotterdam
23. St Elisabeth Ziekenhuis — Tilburg
24. Universitair Medisch Centrum Utrecht — Utrecht
25. Admiraal De Ruyter Ziekenhuis — Vlissingen
26. Isala Klinieken (location Sophia) — Zwolle

Centres for the treatment and monitoring of paediatric HIV and AIDS were:

- A Emma Kinderziekenhuis, AMC-UvA — Amsterdam
- B Beatrix Kinderziekenhuis, UMCG — Groningen
- C Erasmus MC-Sophia — Rotterdam
- D Wilhelmina Kinderziekenhuis, UMCU — Utrecht


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Introduction

This report is the 12th in the series Stichting HIV Monitoring (SHM) has published since its founding in 2001. We again provide a description of the HIV-infected population in the Netherlands, but findings are updated per mid-June 2012. We show trends in AIDS-related and non-AIDS-related death, the effects of antiretroviral treatment, numbers of new infections, development of HIV-related and non-HIV-related disease, as well as trends in HBV and HCV co-infections, resistance when suppressive therapy fails and resistance transmission.

The year 2012 was a year of optimism, which was summarised most memorably during the XIX International AIDS Conference in Washington, D.C. With the motto "Turning the Tide Together", the conference focused on the possibility of 'delivering an AIDS-free generation' (U.S. Secretary of State Hillary Clinton, XIX International AIDS Conference, Washington D.C., U.S.A.) and on 'the beginning of the end of the AIDS epidemic'[1].

The reasons for this optimism are well founded, since antiretroviral drugs are more promising than we had previously envisioned and treatment of HIV in an earlier stage of infection seems feasible for a growing number of people. The barriers due to the logistics of providing continuous lifelong combination treatment to large numbers of infected people and convincing them to adhere to the regimen seem surmountable, provided funds are available and costs are within limits. However, when considering a small country like the Netherlands, with a concentrated epidemic amongst mostly well-educated individuals, one has to remain cautious.

Using our large set of data from an accumulated total of 19,985 infected individuals, of whom 16,169 (81%) are alive and under clinical observation, we have shown a few challenging results:

- Approximately half of the estimated number of 25,000 individuals with HIV in the Netherlands are currently on suppressive antiretroviral therapy;
- Although testing has improved over the last two decades, even today 43% of infected individuals are diagnosed late in the course of their infection. (Monitoring Report 2012, *Chapter 1*);
- Testing behaviour has improved, but 41% of those starting antiretroviral treatment with less than 350 CD4 cells/mm^3 had more than that level at the time of diagnosis. (Monitoring Report 2012, *Chapter 1*);
- Death rates have declined dramatically since the introduction of combination antiretroviral therapy, but even in recent years they are still higher than in the age- and sex-matched general population. (Monitoring Report 2012, *Chapter 2*);
- Antiretroviral treatment has a limiting effect on transmission of HIV, but it is out-competed by increased transmission risk behaviour; the number of new diagnoses per year is not declining [2, 3]; and
- Although the amount of HIV circulating in the treated population has decreased substantially, HIV-RNA levels measured at viral set point in recently infected populations seem to have increased [4].

Life with HIV has improved greatly over the last decade with better medications that have higher suppressive potential, are easier to take, have less short-term side effects and perhaps less longer-term toxicity (Monitoring Report 2012, *Chapter 3*). Nevertheless, it is still unclear if the damage to the immune system early in the infection plays a role in treated individuals in the appearance of certain co-morbidities earlier than would be expected on the basis of the patients' age [5]. In addition, it is largely unknown if low-level HIV production may induce other co-morbidities either directly or through ongoing inflammatory responses [6].

In addition to these uncertainties, a large proportion of the HIV-positive male homosexual population are living long enough to encounter the clinical effects of chronic infection with hepatitis B (HBV) or C virus (HCV) (Monitoring Report 2012, *Chapter 7*) [6]. And again, although treatment opportunities have improved for HBV, drugs that are directly active against HCV are becoming only sparsely available for clinical use.

So, yes, we are on our way to potentially achieving an AIDS-free generation and perhaps to the beginning of the end of AIDS. Huge improvements in HIV care have been made, but since we cannot yet eradicate HIV, the best we can do is test for and treat HIV as early as possible. In addition to limiting risk behaviour, these steps will contribute to preventing HIV disease and AIDS and slowing down transmission. However, these effects may be temporary; the rising viral load at set-point that indicates an increase in viral replicative fitness over time may be the result of earlier treatment. The window of opportunity for the virus to be transmitted is narrowed, and hence, it can adapt only by increasing its replicative capacity to enhance its chance of transmission. In other words, what we may have achieved is containing HIV rather than eradicating it.

Will pre-exposure prophylaxis (PrEP) help in these circumstances? Perhaps it will. According to our own preliminary model outcomes, PrEP aimed at high-risk groups would certainly decrease the number of new infections, perhaps to such an extent that transmission rates will decline to low levels, whereby the epidemic will retract. But, like sero-sorting or condom use, it means a high level of responsible behaviour towards sex partners, which is difficult to achieve and thus carries a risk of failure.

Close monitoring of testing, treating and PrEP, as well as monitoring of the long-term consequences of these pharmaco-therapeutic interventions, remains necessary to ensure that the best care for HIV-infected individuals is delivered and its spin-off of containing the epidemic is reached. We may be close to achieving containment of HIV and AIDS, but for eradication we still need a sterilising vaccine with a sufficient level of protection.

On 12 September 2001, the first HIV Monitoring Report was presented to the then-minister of Health, Welfare and Sport, Mrs Els Borst, and included the results of the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort study. This national clinical study on the

effect of highly effective combination antiretroviral therapy ran between 1998 and 2001 and became the foundation of our series of Monitoring Reports on Human Immunodeficiency Virus (HIV) Infection in the Netherlands, published yearly on 1 December, World AIDS Day.

The Monitoring Report 2012 is again a comprehensive overview of what we currently know about HIV and AIDS in the Netherlands and about the effect of antiretroviral treatment at both the individual and the population levels. I would like to thank the HIV-treating physicians, HIV nurse-consultants and the staff of various diagnostic laboratories and facilities in the HIV treatment centres, together with data collecting and monitoring staff both within and outside SHM, for their ongoing efforts and contribution. I would also like to thank the patients with HIV who provide data to SHM for their help and contribution. Through the contribution of professionals and patients, we continue to gain insight into HIV and HIV treatment that ultimately leads to improved care for people with HIV living in the Netherlands.

Prof. Frank de Wolf MD

Director, Stichting HIV Monitoring

Summary & recommendations

Frank de Wolf

The HIV epidemic in the Netherlands

As of June 2012, 16,169 HIV-infected patients, including 15,976 adults and 193 children and adolescents, were in clinical care in one of the 26 HIV treatment centres in the Netherlands. More than one third of the population in care was 50 years of age or older. In recent years, approximately 1100 new HIV infections have been diagnosed annually, and 700 to 750 of those have been in men who have sex with men (MSM). The increasing trend in the number of diagnoses amongst MSM, which has been observed since the turn of the millennium, appears to have come to an end. Indeed, amongst MSM between the ages of 35 and 44 years, the number of diagnoses is in a steady decline. On the other hand, the number of diagnoses is still increasing amongst young adults and amongst MSM 55 years of age or older. There is a decreasing trend in diagnoses in the group of patients infected via heterosexual contact, which is mainly due to a reduction in immigration from HIV-endemic regions.

Over a period of years, testing for HIV has become more common, as exemplified by an increase in CD4 cell counts in patients at the time of diagnosis and by a greater proportion of patients diagnosed with a recent infection. However, in recent years, 38% of MSM and approximately 60% of heterosexual men and women at the time of entry into care have had CD4 counts below the current threshold for starting treatment, 350 cells/mm^3. In addition, as a result of the increased age of women currently in follow-up, pregnancy rates have been lower compared to earlier calendar years. This is apparent in HIV-infected women from different geographic areas.

Despite all these positive developments (including more testing, earlier diagnosis and earlier start of treatment), the number of HIV diagnoses amongst MSM and heterosexuals is still not in a convincingly significant decline. To fully curb the epidemic, testing and treatment needs to be scaled up, whilst reductions in sexual risk behaviour are expected to have a much greater impact on the number of new infections.

Mortality and loss to follow-up

The death rates for both men and women with HIV-1 who have not started combination antiretroviral therapy (cART) are similar and have fallen in the last 10 years. These rates are currently low and comparable to those amongst age- and gender-matched samples from the general population. This suggests that HIV-1 infection is being diagnosed early in its course and patients are starting cART on time. The death rates for both men and women who have started cART and the proportion of those dying of AIDS have fallen in the last 15 years. In the group of those who have started cART, the death rate is higher and the time to

death shorter for men than women. In addition, the death rates for men and women are still higher than in gender- and age-matched samples from the general population. The rate of men and women becoming lost to follow-up before or after the start of cART is much higher for patients born outside the Netherlands than in this country. Even after correction for differences in age and the proportion born in the Netherlands, the women who started cART between 2007 and 2010 were more likely to become lost to follow-up than men.

AIDS and non-AIDS defining events in men and women

The incidence of AIDS defining conditions has decreased dramatically in the last 15 years and is currently similar for men and women. After correction for changes in the ages of men and women with HIV-1 infection, the incidences of renal insufficiency, non-AIDS defining malignancies and liver disease for both men and women, as well as cardiovascular disease and diabetes mellitus for women, have remained stable during the last 10 years. During this period, the incidence of diabetes mellitus and cardiovascular disease has decreased for men, whilst the incidence of osteoporosis has risen for men and women. The incidences of non-AIDS defining malignancies and osteoporosis for women were not significantly different from those in an age-matched sample from the general population, but were higher for men. The incidence of diabetes mellitus was lower for men and higher for women than in age- and gender-matched samples from the general population. It was not possible to compare the incidences of cardiovascular disease, renal insufficiency or liver disease with those in the general population.

Similar proportions of men and women who have lived with HIV-1 infection for at least 20 years have been diagnosed with diabetes mellitus, cardiovascular disease, renal insufficiency or a non-AIDS defining malignancy. However, more HIV-1-infected women have been diagnosed with liver disease or osteoporosis. Further research is needed to determine whether the prevalence of these conditions is higher amongst persons with HIV-1 infection than amongst the general population and whether it is higher between groups of men and women who have lived with HIV infection for longer or shorter periods.

Response to cART

CD4 counts at the time of cART initiation have increased since 2007, with a median of 310 cells/mm^3 in 2011. CD4 cell counts at the start of cART were lower amongst men from sub-Saharan Africa and amongst women. cART is currently recommended for effectively all HIV-infected patients, since restoration of CD4 counts to levels seen in uninfected patients has become feasible. Normal CD4 cell counts may be reached after 8 years of continuous HIV suppression, providing patients start cART before CD4 cell counts fall below 350 CD4 cells/mm^3. For patients to start cART on time, HIV testing rates still need improvement, especially in women and sub-Saharan African men. Suppression of plasma viral loads to below 50 HIV-RNA copies/ml is important, since high-level viraemia, as well as longer periods of low-level viraemia, are associated with smaller increases in CD4 cell count, higher probability of treatment failure and development of resistance.

Currently, almost half of the patients remain on their initial cART regimen for 3 years. Toxicity associated with the drugs used in the combination is still the main reason for changing the regimen, although the incidence of toxicity-driven changes has halved since the introduction of cART in 1996. The treatment-limiting adverse events most frequently recorded have shifted from lipodystrophy, rash or renal insufficiency in 2006 to nausea and diarrhoea in 2011, also indicating that the toxicity profile of antiretroviral drugs has improved.

The risk of virological failure has declined over time, but it increases in patients who start cART with higher CD4 cell counts, those who are younger, and those who are heterosexually infected from sub-Saharan Africa, the Caribbean and South America. Patients from sub-Saharan Africa, the Caribbean and South America continue to be at high risk of failure on subsequent second-line regimens. Measures to improve adherence in these patients are warranted. For instance, more individualized therapy strategies might help to reduce the risk of treatment-limiting toxicity.

Resistance

Virological failure is less common in the treated HIV-infected population in 2012 than it was in 2000, thanks to an improved availability of treatment options. This appears to be true even for patients pre-treated with mono- or dual therapy who now have the same rates of virological failure as patients who were previously therapy-naive. Nevertheless, because of a growing volume of treated HIV-infected patients, approximately 250 patients per year still experience virological failure.

Resistance patterns in sequences obtained at approximately the time of failure seem to indicate that in one quarter of previously therapy-naive patients, virological failure is the result of the patients not taking their prescribed medication, which could be due to, for instance, drug-related toxicity. In patients with a sequence obtained whilst failing on a protease inhibitor (PI)-based or a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based first-line regimen, overall levels of drug resistance are similar. However, PIs appear to be more resilient to the development of drug resistance than NNRTIs, most likely as a consequence of the larger number of mutations necessary to render the virus fully resistant. In patients on a PI-based regimen, resistance to lamivudine and emtricitabine (XTC) is most commonly observed, whereas in patients on NNRTI-based regimens, resistance to NNRTIs and, to a lesser extent, to XTC is most frequent.

Pol sequences are available for unfortunately only approximately 30% of the patients with virological failure. This makes it difficult to draw firm conclusions on the prevalence of resistance, since a thorough understanding of the conditions under which sequences are available is needed. Also, for some patients, virological failure may be caused by resistance to integrase or entry inhibitors, but sequences of the genes involved in this type of resistance are not yet routinely collected. To determine the true prevalence of resistance in treated

patients with virological failure, Stichting HIV Monitoring (SHM) is investigating the possibility of developing a study to obtain sequences and plasma drug concentrations at the time of failure in a randomly selected sample of patients.

Meanwhile, less than 2% of patients are infected with a virus that is already resistant to antiretroviral drugs. This may indicate that HIV still does not need resistance to survive in a population with easy access to antiretroviral treatment. If most new infections are caused by HIV-infected individuals who are not yet treated and who may not even be aware of their infection, as shown to be the case amongst MSM in the Netherlands, resistance as a survival mechanism would be unnecessary.

Conversely, the low prevalence of transmitted drug resistance indicates that transmission from the pool of resistant patients is limited. If, however, a resistant virus would be transmitted from the pool of patients on PI-based regimens, this would most likely be a virus with resistance to lamivudine and emtricitabine caused by an M184V mutation in reverse transcriptase. As this mutation comes at great cost to viral fitness, such a virus would quickly revert back to wild-type and defy detection at the time of diagnosis. On the other hand, new infections with a resistant virus arising from patients on NNRTI-based regimens would mostly involve resistance to NNRTIs and, to a lesser extent, to XTC. Mutations that play a role in resistance to NNRTIs also negatively impact the fitness of the virus, but at more moderate levels than M184V. In particular, K103N causes only a modest disadvantage in fitness compared to wild-type virus and may remain the dominant viral quasi-species in newly infected patients. As a result, viruses with K103N may have the potential to establish themselves as a subepidemic. Further studies and monitoring of resistance is necessary to confirm if this is already happening in the Netherlands.

HIV-infected children

Results of the monitoring of HIV-infected children in paediatric care have shown a substantial decline in mother-to-child transmission in the Netherlands since the introduction of national pregnancy screening. However, despite the high uptake of this pregnancy screening, a risk of mother-to-child transmission will always remain amongst women who become infected during the last two trimesters of their pregnancy. A limited number of vertical transmissions has occurred since the start of national screening. A second pregnancy screening in mothers at high risk of HIV infection may be beneficial in effectively increasing even further the prevention of mother-to-child transmission.

The majority of HIV-infected children in care are receiving cART. Exposure to cART will be lifelong, and therefore, virological failure and the development of drug-resistance during childhood may limit future treatment options. Although we observed a poorer early virologic response in vertically infected children aged 0 to 1 year at the time of cART initiation, the long-term virologic response was comparable to that in older children. In addition, the early response to cART improved over calendar time, probably as a result of the

introduction of improved regimens. For children with data on resistance testing, we found that 36% had high-level resistance mutations, and these were mainly children who experienced virological failure.

The survival of children benefits from this successful and improved response to cART. We observed low mortality in HIV-infected children in care in the Netherlands. A large proportion of the children have survived into adulthood and are now in care at one of the adult HIV treatment centres. All patients who have survived into adulthood are currently alive.

All HIV-infected children will face lifelong treatment with cART. For these children, it will be a challenge to maintain lifelong adherence to cART and achieve lifelong virologic suppression. Monitoring these HIV-infected children during their adolescence and into adulthood will be important in helping to take up that challenge.

Hepatitis B and hepatitis C co-infections

Since 2000, the number of hepatitis C virus (HCV) diagnoses in the Dutch HIV-infected population has increased. Most of these infections have developed in homosexual men. The increase in HCV diagnoses coincides with an increase in acute HCV infections in the same population. The acute HCV infections in homosexual men are probably caused by sexual transmission. The number of hepatitis B virus (HBV) diagnoses has remained stable over time.

Patients co-infected with HIV and HBV or HCV are at increased risk of the development of chronic liver disease. In the HIV-infected population, we observed a slow but steady increase in hepatocellular carcinoma (HCC) in patients with a chronic HBV or chronic HCV co-infection. Besides the impact of HBV and HCV on progression to liver disease, cART may have a protective effect on progression to liver fibrosis, but, on the other hand, it may enhance liver disease by drug-related hepatotoxicity. Screening for the presence of chronic HBV and chronic HCV infections and optimal management of HBV and HCV co-infection in individuals with HIV are needed to limit the impact of co-infection in the progression to severe chronic liver disease.

From July 2012 onwards, SHM data collection on hepatitis and liver-related disease has improved, and extended data on hepatitis and liver morbidity is now being collected on a regular basis.

A substantial decrease in HBV DNA levels has been observed. As a result of the long-term control of HBV replication, 16% of the patients with HIV treated for HBV co-infection showed HBsAg clearance.

The current treatment of HCV with a combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) has been found to clear HCV infection in 40% of the treated patients. The uptake of anti-HCV treatment in the HCV co-infected population was low, and a considerable number of patients dropped out early in the course of treatment.

As a result of the limited success rates of the current treatment with PEG-IFN and RBV, a large number of patients co-infected with HIV and HCV remain untreated. Two new, direct-acting protease inhibitors, boceprevir and telaprevir, have been recently licensed for the treatment of HCV in the Netherlands. When added to PEG-IFN and RBV, sustained rates of virologic response have improved substantially in patients with a chronic hepatitis C genotype 1 infection. However, both telaprevir and boceprevir have pharmacologic interactions with antiretroviral therapy that need to be taken into account when treating HCV co-infection in HIV-infected patients.

Other direct-acting anti-HCV drugs are being developed and will result in new therapeutic strategies that may provide new options for patients co-infected with HIV and HCV and, in the long run, may reduce development of severe chronic liver disease. Monitoring of HIV-infected patients who are co-infected with HCV or HBV will become increasingly important. In addition to our extended data collection on liver-related morbidity and mortality, SHM will monitor responses to new therapeutic anti-HCV and anti-HBV strategies.

The Amsterdam Cohort Studies

The Amsterdam Cohort Studies (ACS) are unique longitudinal and prospective studies focused on individuals at risk for HIV, providing insight into crucial viral and host factors that play a role in the transmission and pathogenesis of HIV. The ACS on HIV infection and AIDS were started shortly after the first cases of AIDS were diagnosed in the Netherlands. Since October 1984, MSM have been enrolled. A second cohort involving drug users (DU) was initiated in 1985.

To gain insight into the ongoing HIV transmission among MSM, sexual risk behaviour pre- and post-HIV-seroconversion was studied in 206 MSM, both before and after the introduction of cART. The risk of having unprotected anal intercourse (UAI) 1 year after HIV diagnosis decreased significantly when compared with 1 year before diagnosis in both the pre-cART era and the cART era. In contrast, the probability of UAI in the cART era increased again to pre-seroconversion levels 4 years after diagnosis. Recently seroconverted MSM reduced their sexual risk behaviour following HIV diagnosis both in the pre-cART era and in the cART period. However, in the cART period less reduction was found, and risk behaviour returned to pre-cART levels within 4 years. These findings not only confirm the need for early HIV testing, but stress the importance of recognising, counselling and possibly treating recently infected MSM.

Comparison of the number of cytotoxic T-cell (CTL) epitopes in HIV-1 strains isolated from individuals who seroconverted at the beginning of the HIV-1 epidemic in 1985 and from those who seroconverted in 2005 showed that HIV-1 has accumulated a number of adaptations to CTL responses within 20 years of the epidemic. Such adaptations are driven by the HLA-B molecules that provide the best protection against progression of HIV-1 disease.

In addition, HIV-1 variants obtained from recently infected individuals were more resistant to antibody neutralization than those from individuals infected in the beginning of the epidemic. A similar trend was observed when broadly neutralizing antibodies were studied, indicating that a changing environment in the host with progression of disease may favour the persistence of HIV-1 variants with the most fit and neutralization-resistant phenotype.

Curaçao

For more than 5 years, SHM has collected data on HIV-infected individuals in Curaçao. In recent years, the quality of care and treatment offered to HIV-infected patients in Curaçao has improved considerably and can now withstand a comparison with resource-rich settings. However, adherence to treatment and retention in care need to be improved in order to reduce the number of patients failing on treatment. Also, HIV infections need to be detected at an earlier stage, such that patients can start antiretroviral treatment in accordance with current recommendations.

Conclusion and recommendations

Trends in life expectancy and health of people living with HIV in the Netherlands have not changed substantially since our 2011 report. More than 70% of patients receiving cART achieve viral suppression within 9 months after starting, and near normal CD4 cell counts are reached within 8 years, provided cART is started when CD4 counts are still above 350 cells/mm^3. However, a substantial proportion of patients are diagnosed late in the infection and thus start cART late. Moreover, 41% of those who are diagnosed in time start cART late. Regular testing for HIV and, when the results are positive, the timely start of antiretroviral therapy still need attention.

Discontinuation of treatment occurs in three quarters of the population undergoing treatment; drug toxicity is the main reason for switching medications, followed by regimen simplification. Over calendar time, a larger proportion of patients continue longer on their first-line regimen. Frequency of virological failure of cART is relatively low and largely the result of decreasing adherence. However, when virological failure occurs, the risk of repeated failure on second-line cART is high. In almost 40% of those with virological failure whilst on cART, high-level resistance is found to at least one of the antiretroviral drugs used in the regimen. Monitoring of the effect of cART in individual patients and on the population level remains crucial for our understanding of trends in the development of resistance.

“Test and treat”, as a strategy, is growing. Currently, cART is started at CD4 cell counts higher than ever before, indicating a diagnosis of HIV made earlier in the infection and improved testing strategies amongst those at risk for HIV. An increasing proportion of the recently diagnosed population starts cART with CD4 cell counts above 350 cells/mm^3,, and more than 20% of those with 500 CD4 cells/mm^3 or higher start cART within 6 months of diagnosis.

With “test and treat”, the number of people with HIV receiving cART will increase again. This rise in the number of those being treated early in infection and without symptoms of HIV may increase the risk of reduced adherence and of treatment interruption and subsequently the risk of high-level resistance. This underlines the need for resistance measurement when virological failure occurs.

From 2009 onwards, the number of new HIV diagnosis has remained stable at around 1100 new diagnoses per year, indicating that the increase in frequency of testing and in the proportion of patients diagnosed early in the course of their infection has not yet been sufficient to have resulted in a significant reduction in the number of new infections and, thus, the number of new diagnoses. More worrying is the increase over calendar time of both the number and the proportion of diagnoses amongst young MSM. Changing risk behaviour in this group should remain a major goal in prevention policies.

To a certain degree, large-scale cART may help contain HIV spread, especially when adherence is high and “test and treat” becomes the preferred approach. On the assumption that HIV adapts itself continuously to be optimally transmitted, early treatment may change the virus’s fitness towards a shorter window of transmission with higher transmission potential. We have reported that over calendar time HIV-RNA loads at set-point (i.e., 9 to 24 months after the estimated date of infection) have increased. Such an increase could change the course of the HIV epidemic, and we are currently studying viral factors that could explain this change in viral load over time. Together with the uncertainties regarding adherence to lifelong cART and its toxicity and potential to increase resistance, these factors stress the need for continuing high quality standards of HIV care and monitoring of HIV.

Monitoring programme report

1. The HIV epidemic in the Netherlands

Ard van Sighem, Colette Smit

As of June 2012, 16,169 HIV-infected patients, including 15,976 adults and 193 children and adolescents, were in clinical care in one of the 26 HIV treatment centres in the Netherlands. More than one third of the population in care was 50 years of age or older. In recent years, approximately 1100 new HIV infections have been diagnosed annually, and 700 to 750 of those have been in men who have sex with men (MSM). Over the years, testing for HIV has become more common, as exemplified by an increase in CD4 T cell counts in patients at the time of diagnosis and by a greater proportion of patients diagnosed with a recent infection. However, in recent years, 38% of MSM and approximately 60% of heterosexual men and women had CD4 counts below 350 cells/mm^3 at the time of entry in care, which is currently the threshold for starting treatment. As a result of the increased age of women currently in follow-up, pregnancy rates were lower compared to earlier calendar years. This is apparent in HIV-infected women from different geographic areas.

Medio 2012 waren er 16.169 HIV-geïnfecteerde patiënten in follow-up in een van de 26 HIV-behandelcentra in Nederland: 15.976 volwassenen en 193 kinderen en adolescenten. Meer dan een derde van deze groep was 50 jaar of ouder. Het jaarlijks aantal nieuwe HIV-diagnoses dat in de afgelopen jaren werd gesteld, was ongeveer 1100, waarvan 700 tot 750 onder mannen die seks hebben met mannen (MSM).
In de loop der jaren wordt er steeds vaker op HIV getest. Dit blijkt uit hogere CD4-celaantallen op het moment van diagnose en een naar verhouding steeds grotere groep patiënten bij wie een recente HIV-infectie wordt vastgesteld. Desondanks had 38% van de MSM en ongeveer 60% van de heteroseksuele mannen en vrouwen de afgelopen jaren een CD4-celaantal van minder dan 350 cellen/mm^3 op het moment dat ze in zorg kwamen. Dit aantal is momenteel de grenswaarde voor het starten van behandeling. Door de stijgende leeftijd van vrouwen die nu in follow-up zijn, is het aantal zwangerschappen lager in vergelijking met vorige kalenderjaren. Dit geldt voor HIV-geïnfecteerde vrouwen afkomstig uit verschillende geografische gebieden.

For more than 10 years, Stichting HIV Monitoring (SHM) has collected demographic and clinical information from almost all HIV-infected patients who have been in care in one of the 26 HIV treatment centres in the Netherlands. One of the main achievements of this assiduous monitoring is a detailed knowledge of the characteristics of the HIV-infected population, which will be presented in this chapter. Here, the focus will be mainly on adult patients. Children and adolescents are described in more detail in *Chapter 5*.

Introduction

As of June 2012, 20,781 HIV-infected patients were registered by SHM; of that number, 19,985 were being followed in one of the HIV treatment centres in the Netherlands (*Figure 1.1*). The remaining 796 patients were registered in the St. Elisabeth Hospital in Willemstad, Curaçao, and are discussed in more detail in *Chapter 8*. Of the 19,985 patients, the majority were infected with HIV-1 (19,639; 98%), whilst 89 patients were infected with HIV-2, and 62 had antibodies against both HIV-1 and HIV-2. Serologic results were not yet available for 195 patients. The total follow-up time since diagnosis was 167,677 person-years. Although the majority of the 1269 patients registered since June last year were diagnosed in 2011 or 2012, 16% of those newly registered were diagnosed in or prior to 2010.

Population – in care

Patients in clinical care

In total, 16,169 (81%) of the 19,985 registered patients, including 15,976 adults and 193 minors below 18 years of age, were under clinical observation (*Figure 1.1*; *Table 1.1*; *Web Appendix Table 1.1*). Of the remaining 3816 patients who were no longer in clinical care, 1947 (51%) had died and 717 (19%) had moved abroad. Patients were considered to be in clinical care if data were available in 2011 or 2012 and if the patients were still alive. This definition differs from the one used in previous monitoring reports, in that it better reflects present-day clinical routine in which patients in several treatment centres who are doing well on treatment are seen only once a year by their treating physician [7].

Figure 1.1: Overview of the HIV-infected population registered by Stichting HIV Monitoring (SHM) as of June 2012.

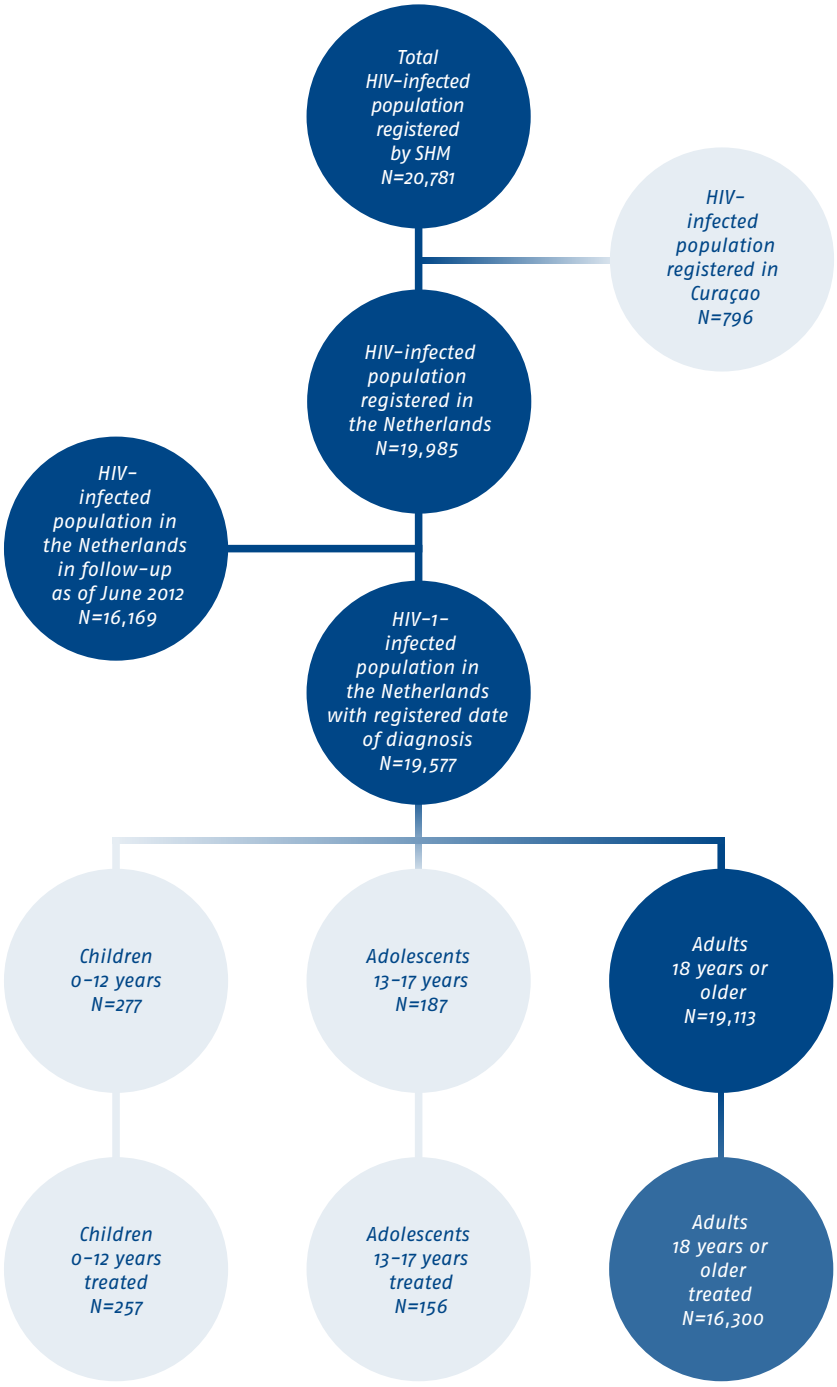


Table 1.1: Characteristics of the 16,169 patients in clinical care as of June 2012. An extended version of this table is available on the SHM website (Web Appendix Table 1.1).

	Men (N= 12,896, 80%)		Women (N= 3273, 20%)		Total (N=16,169)	
	N	%	N	%	N	%
Transmission						
MSM	9542	74	–	–	9542	59
Heterosexual	2134	17	2839	87	4973	31
IDU	264	2	98	3	362	2
Blood (products)	121	1	83	3	204	1
Other/unknown	835	6	253	8	1088	7
Age category (years)						
0–12	67	1	63	2	130	1
13–17	29	0	34	1	63	0
18–24	263	2	89	3	352	2
25–34	1554	12	720	22	2274	14
35–44	3531	27	1153	35	4684	29
45–54	4614	36	838	26	5452	34
55–64	2145	17	276	8	2421	15
≥65	558	5	81	3	639	4
Region of origin						
The Netherlands	8597	67	936	29	9533	59
Sub-Saharan Africa	1023	8	1414	43	2437	15
Western Europe	779	6	132	4	911	6
Latin America	863	7	284	9	1147	7
Caribbean	468	4	171	5	639	4
Other	1120	9	332	10	1452	9
Unknown	46	0	4	0	50	0
Years aware of HIV infection						
<1	680	5	112	3	792	5
1–2	1783	14	354	11	2137	13
3–4	1819	14	360	11	2179	13
5–10	3548	28	1065	33	4613	29
>10	4905	38	1347	41	6252	39
Unknown	161	1	35	1	196	1

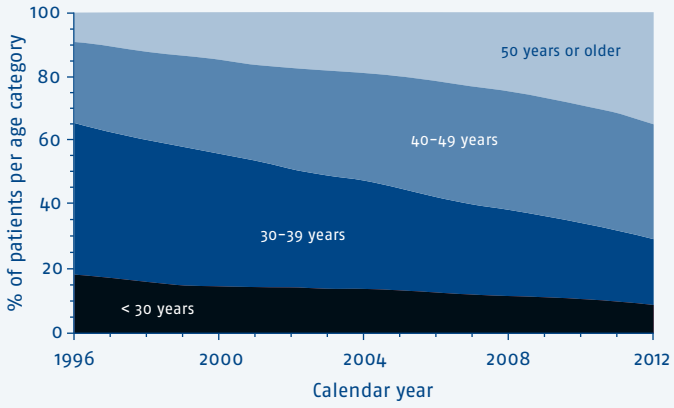
Legend: MSM=men who have sex with men; IDU=injecting drug user.

Ageing population

The median age of the population in clinical care was 46 years (interquartile range [IQR], 38-53) and has been increasing since 1996 (*Figure 1.2*). This increase in age is mainly a result of the improved life expectancy of HIV-infected patients after the introduction of

combination antiretroviral therapy (cART). In addition, patients are being diagnosed at increasingly older ages, as will be discussed later in this chapter. As a result, more than one third of the patients currently in clinical care, 35%, are 50 years or older, including 38% of the men and 22% of the women (*Web Appendix Table 1.1*). It is to be expected that in coming years the number of patients with age-related comorbidities will increase, thereby complicating the management of their HIV infection.

Figure 1.2: *The age of the HIV-infected population in clinical care has increased over calendar time. In 1996, 18% of the patients in follow-up were younger than 30 years of age, whereas 9% were 50 years or older. In 2012, these proportions were 9% and 35%, respectively. The proportion of patients in follow-up as of 1 June of each calendar year is shown according to those who were <30 years of age, 30 to 39 years, 40 to 49 years, and 50 years or older.*



Duration of infection

On average, patients in clinical care received their HIV diagnosis 9.2 years earlier. However, a large group (39%) of those in care had lived with HIV for more than 10 years, whilst 7% had done so for more than 20 years. The average time since diagnosis was 9.1 years for MSM, 8.4 years for heterosexual men, and 9.1 years for heterosexual women. For each of these three groups, roughly equal proportions were diagnosed less than 5 years ago, 5 to 10 years ago, and more than 10 years ago (*Web Appendix Table 1.1*). The majority of injecting drug users (78%) received their HIV diagnosis more than 10 years ago, which reflects the decreasing number of infections occurring via that route.

Treatment combinations

Most of the patients in care (86%) were treated with cART. The most frequently prescribed regimens, which accounted for 57% of all treatment combinations, were a combination of tenofovir/emtricitabine and either efavirenz (28%), nevirapine (14%), ritonavir-boosted

atazanavir (8%) or ritonavir-boosted darunavir (6%). Tenofovir as part of any treatment combination was used by 75% of the patients, whilst emtricitabine was used by 67%, efavirenz by 34%, nevirapine by 25%, atazanavir by 14% and darunavir by 11%.

Clinical condition

Partly as a result of treatment, the median CD4 counts were relatively high at 550 (IQR, 410-720) cells/mm^3. CD4 counts were similar between men and women, although men infected via heterosexual contact had lower CD4 counts than their female counterparts (*Web Appendix Table 1.1*). Of all patients in care, 80% had an HIV viral load below 500 copies/ml. About one fifth (22%) of the patients had been diagnosed with an AIDS-defining disease; 56% of these patients were diagnosed with AIDS concurrently with their HIV diagnosis.

Population – diagnosis

HIV-1-infected individuals

Having briefly discussed the HIV-infected population currently in clinical care, we will now focus on the 19,113 patients who were diagnosed with HIV-1 as adults and whose date of diagnosis was recorded (*Figure 1.1*). The majority of these patients were MSM (11,079, 58%); the rest were men (2700, 14%) or women (3308, 17%) infected via heterosexual contact (*Web Appendix Table 1.2*). For 707 (4%) of the patients the reported mode of transmission was injecting drug use, whilst 216 (1%) patients were infected by exposure to contaminated blood. Other and unknown modes of transmission accounted for the remaining 1103 (6%) infections.

No further increase

Since the 1990s, the annual number of diagnoses amongst MSM steadily increased to well above 800 in 2008. From 2009 onwards, however, the registered number of diagnoses was considerably lower and was confined to a range between 700 and 750 per year (*Figure 1.3*). Hence, it appears that the trend in an increasing number of diagnoses has halted[7]. Alternatively, the number of new diagnoses in 2007 and 2008 may exceed the long-term increasing trend because of the introduction of opt-out testing for HIV at major sexually transmitted infection (STI) clinics at about that time[8]. In either case, it appears that the increase in frequency of testing and in the proportion of patients diagnosed early in the course of their infection, which is discussed later on, has not yet been sufficient to have induced a significant reduction in the number of new infections and, as a result, the number of new diagnoses.

In the heterosexual population, the annual number of diagnoses has declined to approximately 300 cases per year in the last few years. This decline, as shown later, is largely a result of a reduced number of diagnoses in migrant populations. Injecting drug use is rarely reported any longer as the most probable mode of transmission, which reflects the decreasing popularity of injecting drugs.

Figure 1.3: Annual number of HIV-1 diagnoses per transmission risk group. In 2011, men who have sex with men (MSM) accounted for 67% of the diagnoses, infections via heterosexual contact for 26%, infections via injecting drug use (IDU) for 0.2%, and infections via other or unknown routes for 7% of the annual tally. The light coloured lines indicate the projected number of diagnoses when the backlog in registration of HIV cases (3% in 2010, 11% in 2011) is taken into account.

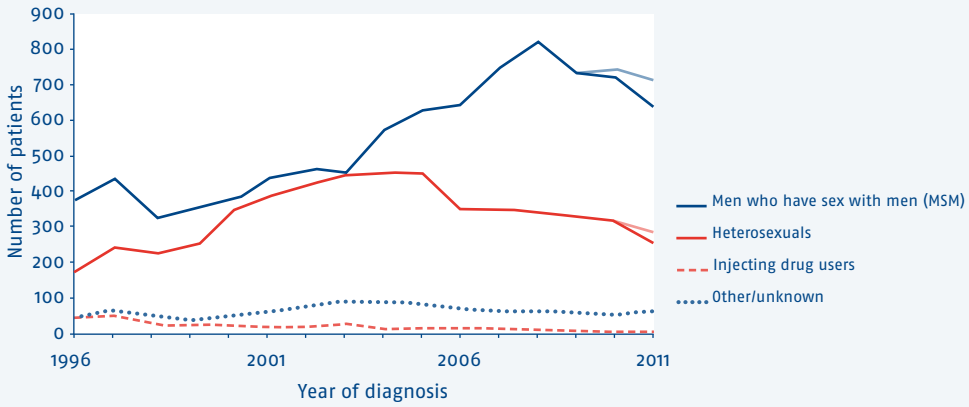
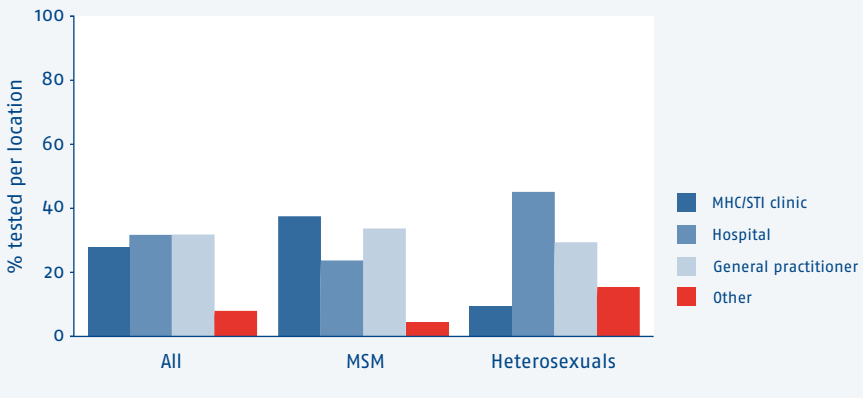


Figure 1.4: Proportion of patients diagnosed from 2008 onwards stratified by location of testing and route of transmission.



Legend: *MSM=men who have sex with men; MHC=municipal health service; STI=sexually transmitted infection.*

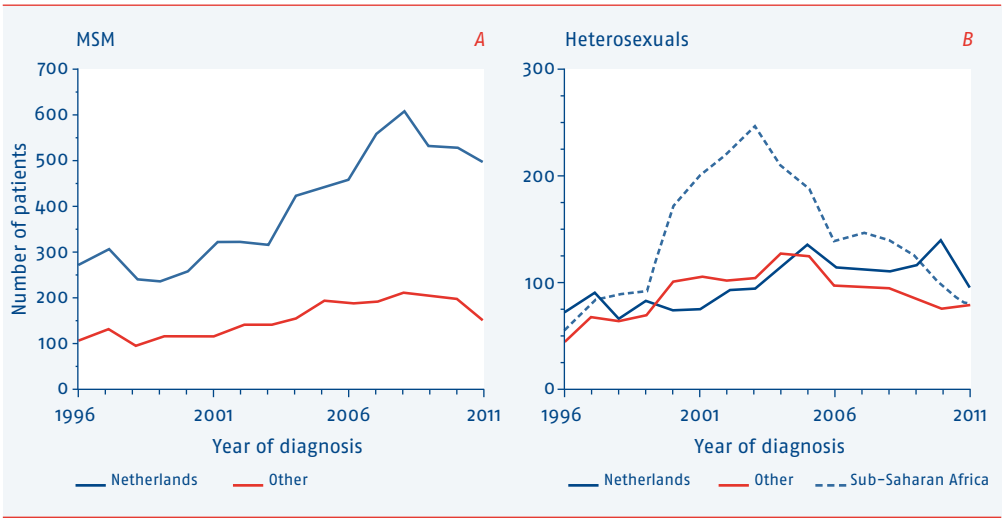
Testing location

For 95% of patients diagnosed in 2008 or later, information on the location of testing was available. Altogether, 28% received their HIV-positive test result at a Municipal Health Service or STI centre, 32% at a hospital, and 32% at a general practice (*Figure 1.4*). Amongst those tested at Municipal Health Services or STI centres, 89% were MSM, 5% were heterosexual men, and 5% were heterosexual women. These numbers are comparable with those directly reported by STI clinics in 2010 and 2011: 85% MSM, 8% heterosexual men, and 7% women [9, 10].

More patients of Dutch origin

Overall, 72% of the patients infected via homosexual contact originated from the Netherlands, 10% from other European countries, 6% from Latin America, and 3% from the Caribbean (*Figure 1.5A*). In recent years, the proportion of MSM of Dutch origin has increased to 75%. Minor changes over time have been observed in the proportion of patients from Central Europe, being 1% of the annual tally before 2010 and 3% afterwards, and in those of Western European origin, 8% before 2010 and 5% thereafter.

Figure 1.5: *Annual number of diagnoses amongst (A) men who have sex with men (MSM) and (B) patients infected via heterosexual contact stratified by country of birth. Of the 11,079 MSM, 7982 (72%) originated from the Netherlands, 1108 (10%) from other European countries, 715 (6%) from Latin America, and 357 (3%) from the Caribbean. Amongst the 6008 heterosexual patients, 2449 (41%) originated from sub-Saharan Africa, 1906 (32%) from the Netherlands, 587 (10%) from Latin America, 340 (6%) from the Caribbean, and 251 (4%) from South and Southeast Asia. Note: data collection for 2010 and 2011 is not yet finalised.*

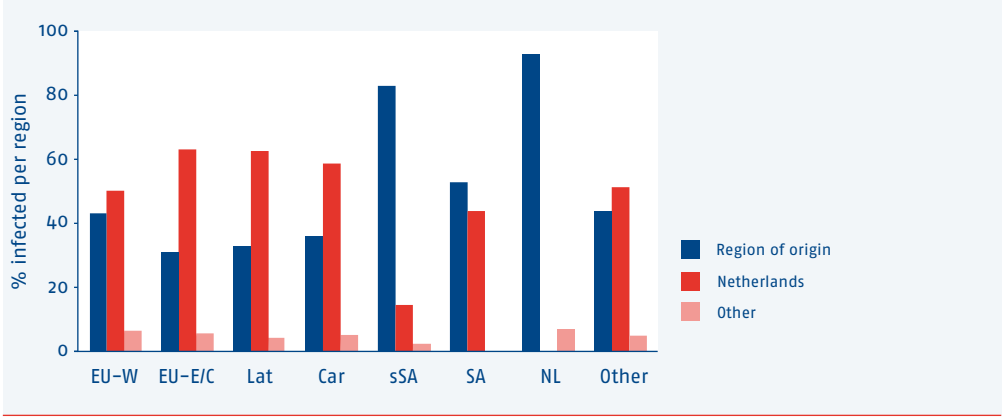


In the heterosexual population, only 32% originated from the Netherlands, whilst 41% originated from sub-Saharan Africa, 10% from Latin America, 6% from the Caribbean, and 4% from South and Southeast Asia (*Figure 1.5B*). However, the number of diagnoses amongst sub-Saharan Africans and, to a lesser extent, amongst patients from other regions dropped sharply after 2003, probably partially as a result of stricter immigration laws that came into effect in the Netherlands at approximately that time. In recent years, 41% of the diagnosed heterosexual population was of Dutch origin, and 33% originated from sub-Saharan Africa. The proportion of sub-Saharan Africans was higher amongst heterosexual women than amongst heterosexual men, whereas the proportion of patients of Dutch origin was correspondingly lower (*Web Appendix Table 1.3*). For other regions of origin, there were no prominent differences, except for a higher proportion of women than men, 6% compared to 2%, originating from South and Southeast Asia.

Country of infection

For 13,899 (73%) of the diagnosed patients, the most likely country of infection was reported. The majority of the patients born in the Netherlands (93%) reported having been infected in the Netherlands (*Figure 1.6*). Most of the patients born in sub-Saharan Africa were infected in that region (83%), but 15% of them were most likely infected in the Netherlands. Amongst patients from other regions, except those from South and Southeast Asia, the majority were infected in the Netherlands.

Figure 1.6: *Proportion of HIV-1-infected adults per region of origin who were infected in their own region of origin, in the Netherlands, or elsewhere.*



Legend: *EU-W=Western Europe; EU-E/C=Eastern and Central Europe; Lat=Latin America; Car=Caribbean; sSA=sub-Saharan Africa; SA=South and Southeast Asia; NL=the Netherlands; Other=other regions of origin.*

As may be expected from the heterogeneity in region of origin, there were also major differences in the region of infection between the major risk groups. The majority of homosexual men (88%) were infected in the Netherlands. Also, the majority of patients infected via injecting drug use (82%) were infected in the Netherlands, whilst 10% of them reported having been infected in other Western European countries. The reported distribution across regions of infection was compatible with HIV-1, which was the subtype in most of the patients. Overall, 94% of MSM and 91% of drug users were infected with subtype B virus, which is the dominant subtype found in Western countries.

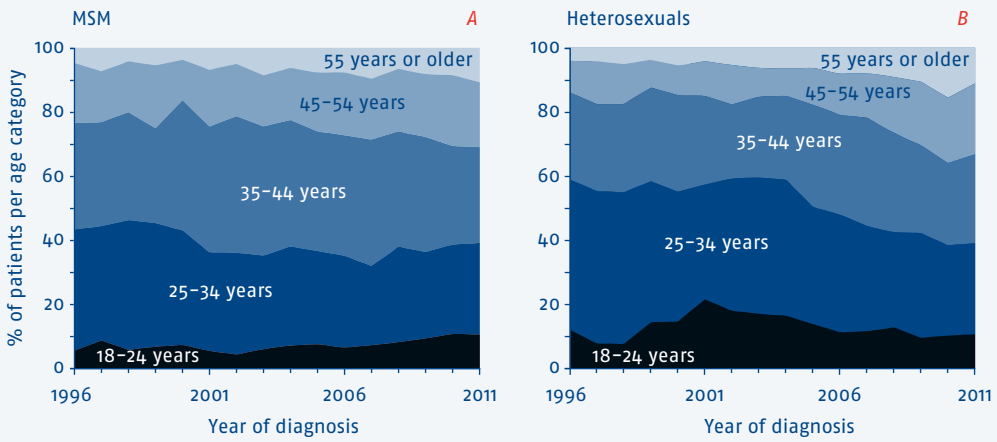
Amongst heterosexual patients, 46% were infected in the Netherlands, whilst 36% reported having been infected in sub-Saharan Africa. Altogether, 73% of the 826 Dutch heterosexual men with a reported country of infection were infected in the Netherlands, 13% in South and Southeast Asia, and 9% in sub-Saharan Africa. Amongst 692 Dutch women infected via heterosexual contact, 89% reported having been infected in the Netherlands and 6% in sub-Saharan Africa, whereas only 3 women were infected in South and Southeast Asia.

Increasing age

The age at which patients are diagnosed with HIV has been slowly increasing over time. In 1996, the average age at the time of diagnosis was 37 years; in 2011, it was 40 years. Of the adults who received their HIV diagnosis in 2011, 20% was 50 years or older, whilst this proportion was 14% over the entire period since 1996. There were, however, considerable differences between MSM and heterosexual men and women. MSM born in the Netherlands were diagnosed at a mean age of 40 years, whilst those of foreign origin were diagnosed at 35 years. Amongst heterosexual patients of Dutch origin, the average age at the time of diagnosis was 38 years for women and 43 years for men. Heterosexual patients born in sub-Saharan Africa (women: 31 years; men: 35 years) or elsewhere (women: 35 years, men: 41 years) were substantially younger than their Dutch counterparts.

For MSM, the age distribution at the time of diagnosis gradually changed over time, whilst amongst heterosexuals there were no notable changes up to 2003 (*Figure 1.7*). Thereafter, the age of heterosexuals at diagnosis started to increase concomitantly with the decreasing number of diagnoses amongst patients from sub-Saharan Africa, who are generally younger than heterosexuals of Dutch or other origin.

Figure 1.7: Age distribution at the time of diagnosis amongst HIV-1-infected men who have sex with men (MSM) (A) and heterosexual men and women (B). Between 1996 and 2010, the proportion of MSM aged 45 years or older at the time of diagnosis increased from 23% to 31%, whilst these proportions were 14% and 33% for heterosexuals. During the same period, the proportion of patients between 25 and 34 years of age decreased from 38% to 29% for MSM and from 47% to 28% for heterosexuals.



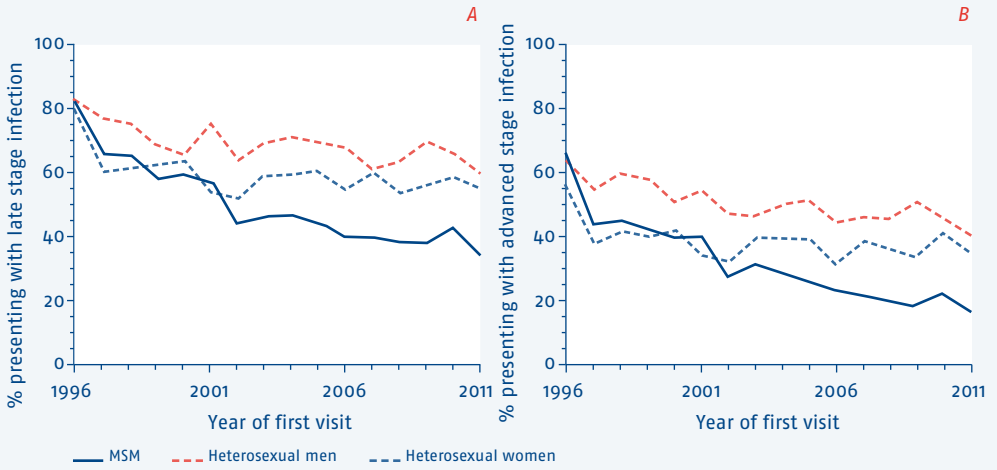
Young adults

The number of diagnoses amongst young adults less than 25 years of age infected via heterosexual contact was approximately 70 in the early 2000s and decreased to 27 in 2011, or 11% of the annual tally (*Figure 1.7*; *Web Appendix Figure 1.1*). Amongst MSM, both the number and the proportion of diagnoses amongst young adults increased over time and in 2011, young adults accounted for 11% of the annual tally as well.

Late presentation

Overall, 55% of the patients were late presenters, i.e., individuals either presenting for care with a CD4 count below 350 cells/mm^3 or presenting with an AIDS-defining event regardless of CD4 count [11]. Although the proportion of late presenters has decreased over time, in 2011 43% of patients entering clinical care did so only late in their infection (*Figure 1.8*; *Web Appendix Figure 1.2*). In recent years, between 10% and 15% of the patients already had AIDS at the time of entry into care. In the 2011 Monitoring Report, we reported an apparent increase in the proportion of late presenters in 2010, but this increase did not persist in 2011 [7]. Similar patterns were observed in the proportion of patients presenting for care with advanced HIV disease.

Figure 1.8: Proportion of patients classified as presenting with (A) late or (B) advanced HIV infection at the time of entry into care. Between 1996 and 2011, 55% presented with late HIV disease: men who have sex with men (MSM) 48%, heterosexual men 68%, heterosexual women 58%, injecting drug users (IDU) 68%. Overall, 36% were advanced presenters: MSM 30%, heterosexual men 49%, heterosexual women 37%, and IDU 48%. Note that stage of infection is assessed at the time of the first clinical visit instead of at the time of HIV diagnosis as in the report of 2011[7]. Late stage infection: CD4 counts below 350 cells/mm³ or having AIDS, regardless of CD4 count. Advanced stage infection: CD4 counts below 200 cells/mm³ or having AIDS.



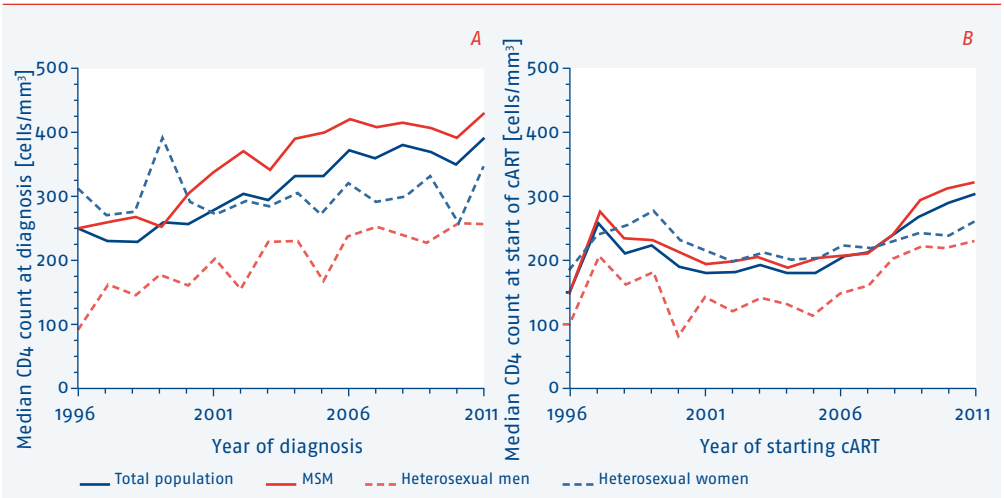
Amongst patients entering clinical care in 2008 or later, 38% of MSM, 65% of heterosexual men, and 55% of heterosexual women presented with a late stage infection. Patients of sub-Saharan African origin infected via heterosexual contact more often presented with a late-stage infection (67%) compared to their peers of Dutch origin (52%). Late stage infection at the time of entry into care was most often found in heterosexual patients originating from South or Southeast Asia, of whom 74% were late presenters. In this same group, 61% presented for care with advanced HIV infection compared to 44% of sub-Saharan Africans and 37% of Dutch patients.

Late presentation was also more common in patients entering care at older ages. Amongst those entering care at 45 years of age or older, 56% of MSM and 68% of heterosexuals were late presenters. In contrast, the proportion of late presenters was 30% amongst MSM and 46% amongst heterosexuals entering care at ages younger than 25 years. Generally, persons who are diagnosed with HIV at a young age enter care in an early stage of the infection, because their sex lives have been relatively short. Late stage infection was also more often observed in patients who received their HIV diagnosis at a hospital (65%) compared to those who were tested at a general practitioner (43%), at Municipal Health Services or STI clinics (28%), or at other testing locations (46%).

Increasing CD4 cell counts

Between 1996 and 2011, median CD4 counts in the total population at the time of diagnosis increased from 250 to 390 cells/mm³ (*Figure 1.9A*). This overall increase was mainly the result of a rise in CD4 counts in both homosexual and heterosexual men, whereas CD4 counts in women remained virtually unchanged. In the last few years, CD4 counts in MSM seem to have reached a plateau.

Figure 1.9: Changes over time in median CD4 counts (A) at HIV diagnosis and (B) at the start of combination antiretroviral therapy (cART). (A) Between 1996 and 2010, CD4 counts at diagnosis increased from 250 (interquartile range [IQR], 80–440) to 390 (IQR, 160–540) cells/mm³ in the total population. The increase was most apparent for men who have sex with men (MSM): 250 (IQR, 80–450) in 1996 and 427 (IQR, 275–584) cells/mm³ in 2011. During the same period, CD4 counts in heterosexual men increased from 100 (IQR, 26–390) to 255 (IQR, 80–450) cells/mm³, whereas CD4 counts in heterosexual women were 290 (IQR, 120–490) cells/mm³ and did not change over time. (B) In the total population, CD4 counts at the start of cART rose to 260 (IQR, 130–391) cells/mm³ shortly after cART became available, decreased to a plateau around 180 cells/mm³ between 2000 and 2005, and increased thereafter. In 2011, CD4 counts were 300 (IQR, 191–381) cells/mm³ in the total population, 320 (IQR, 230–400) for MSM, 230 (IQR, 60–340) in heterosexual men, and 259 (IQR, 140–345) cells/mm³ in heterosexual women.



Earlier diagnosis

The increase in CD4 counts at diagnosis in conjunction with a decreasing proportion of late presenters suggests that, on average, patients are being diagnosed increasingly earlier in the course of their HIV infection. For individual patients, however, the CD4 count at diagnosis may not always be a reliable marker of time since infection. In a large European cohort of seroconverters, for instance, one quarter of newly infected patients had CD4 counts below 350 cells/mm³ within only one year after seroconversion [12].

A further indication of earlier diagnosis was the increase in the proportion of MSM who were diagnosed with a recent infection (*Web Appendix Figure 1.3*). Diagnosis with a recent infection was less common in older MSM. Amongst homosexual men diagnosed in 2008 or later, 48% of the infections amongst those aged 18 to 24 years were classified as recent, whereas this held true for only 24% of those aged 55 years or older. Also, the proportion of recent infections appeared to increase amongst heterosexuals, but at a more moderate level.

Increasing frequency of testing

Apparently, since both the proportion of recent infections and CD4 counts at diagnosis have increased amongst those diagnosed with HIV, testing for HIV has become more common. An additional indication for this is the increasing proportion of patients with a previous HIV-negative test (*Web Appendix Figure 1.3*). In 2011, 69% of MSM and 32% of heterosexuals diagnosed in that year had a previous HIV-negative test. The proportion with a previous negative test was highest, 79%, amongst those diagnosed at Municipal Health Services or STI centres, whilst this proportion was 38% amongst those diagnosed in a hospital, 55% amongst those tested at a general practice, and 52% amongst those diagnosed elsewhere.

Population – start of cART

Treated population

Amongst the 19,113 adult patients with an HIV-1 infection, 16,300 patients had started cART by June 2012. The majority of these patients, 85%, started cART whilst being antiretroviral therapy-naive. For all patients, the total follow-up time since start of cART was 117,695 person-years.

Treatment combinations

According to the current guidelines, the recommended first-line antiretroviral regimens in therapy-naive patients include tenofovir/emtricitabine in combination with efavirenz, ritonavir-boosted darunavir, or ritonavir-boosted atazanavir [13]. In 2011 and 2012, these regimens accounted for 73% of all first-line regimens: 48% included efavirenz, 15% boosted darunavir, and 10% boosted atazanavir. A further 11% of the regimens used a combination of tenofovir/emtricitabine and nevirapine. Altogether, 28 patients, or 2%, started tenofovir/emtricitabine in combination with raltegravir. Although this combination is included in preferred first-line regimens in the American guidelines, it is not recommended by the Dutch guidelines, because little is known yet about the use of raltegravir, particularly its potential long-term side-effects in therapy-naive patients. More importantly, raltegravir is a twice-daily drug, whereas national guidelines favour once-daily regimens.

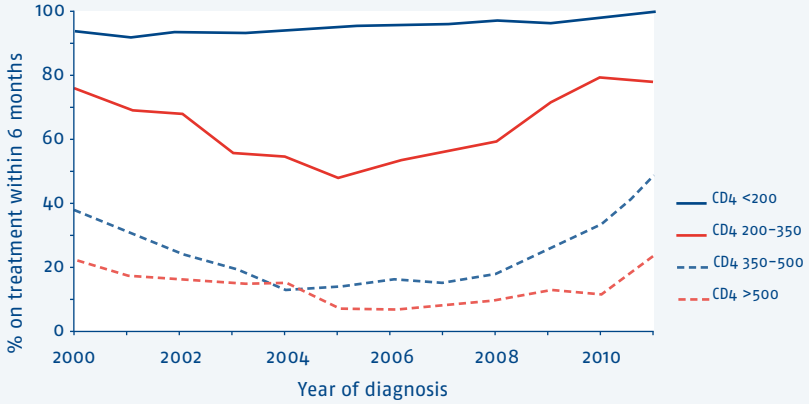
Earlier start

In the past few years, cART has been started increasingly earlier in the course of HIV infection as evidenced by higher CD4 counts at the start of treatment since the mid-2000s (*Figure 1.9B*). In 2011, median CD4 counts at the start of treatment had increased to 300

cells/mm³. About one third (35%) of the patients started treatment according to the current guidelines, which strongly recommend starting before CD4 counts cross the threshold of 350 cells/mm³ [13]. On the other hand, a large group of patients (25%) still began treatment with CD4 counts already below 200 cells/mm³, which is considered a late start.

The main reason for starting treatment too late appears to be a late diagnosis, because most patients who are able to start treatment on time do so. Patients with less than 200 CD4 cells/mm³ at diagnosis almost immediately started treatment: within 6 months after diagnosis more than 95% had started cART (*Figure 1.10*). The proportion who started treatment within 6 months was smaller for patients with higher CD4 counts, but has increased in recent years, reflecting changes in treatment guidelines.

Figure 1.10: *Proportion of patients who started combination antiretroviral treatment (cART) within 6 months after HIV diagnosis stratified by CD4 count at the time of diagnosis according to Kaplan-Meier estimates. Of all patients diagnosed in 2010, 97% (100% in 2011) of those with CD4 counts less than 200 cells/mm³ had started cART within 6 months after receiving their diagnosis, whilst 79% (77% in 2011) of those with CD4 counts between 200 and 350 cells/mm³, 33% (48% in 2011) of those with CD4 counts between 350 and 500, and 12% (23% in 2011) of those with CD4 counts exceeding 500 cells/mm³ had begun cART within 6 months of diagnosis.*



Fluctuating CD4 cell count

Of the patients who started cART in 2009 or later, 44% had CD4 counts between 200 and 350 cells/mm³ at the start of treatment, whilst 28% had CD4 counts below 200 cells/mm³. Overall, 59% of those starting with less than 350 CD4 cells/mm³ also had CD4 counts below that level at the time of diagnosis. On the other hand, 21% had more than 500 cells/mm³ at the time of diagnosis and thus appeared to have missed the opportunity to start on time. However, changes over time in CD4 counts are not always predictable. Amongst patients diagnosed with CD4 counts between 350 and

500 cells/mm^3 and thus not in immediate need of treatment, 21% had less than 350 cells/mm^3 6 months later, whereas for 25% CD4 counts had increased to more than 500 cells/mm^3 even though no antiretroviral treatment was given.

Immediate start of treatment

The most recent American guidelines recommend starting treatment irrespective of CD4 counts, although currently this recommendation is mainly based on expert opinion and indirect evidence [13]. The increase in the proportion of patients with more than 500 cells/mm^3 who were on treatment within 6 months of receipt of the diagnosis in 2011 may be an indication that this strategy of immediate treatment is also being adopted in the Netherlands (*Figure 1.10*). Some caution is required, however, as most patients diagnosed in 2011 do not yet have more than 6 months of recorded follow-up.

Short-term treatment outcomes

In the entire group who started cART, median CD4 counts increased from 220 cells/mm^3 at the start of treatment to 360 cells/mm^3 after 24 weeks. An increase of similar magnitude, albeit at higher CD4 counts, was observed in patients starting treatment in 2009 or later, that is, 283 cells/mm^3 at the start of cART and 430 cells/mm^3 after 24 weeks. Altogether, 87% of the patients achieved suppression of viral load to below 500 copies/ml. A more comprehensive overview of treatment outcome is presented in *Chapter 3*.

Pregnant women

Transmission of HIV from an infected mother to her child is the most common route of transmission amongst children aged 0-15 years worldwide [14]. Mother-to-child transmission (MTCT) can take place *in utero*, during labour and delivery and post-natally during breast-feeding. Without intervention, the risk of MTCT varies between 15 and 20% [15]. Since cART's introduction in the treatment of pregnant women, it has been shown to be beneficial in preventing MTCT, the occurrence of which as been dramatically reduced to 2% [16, 17].

Knowledge of HIV status during pregnancy is necessary for timely initiation of cART and thus for reduction of the risk of MTCT. In January 2004, voluntary HIV-antibody testing of pregnant women with the opting-out method was introduced in the Netherlands [18]. Since then, 233 women who were unaware of their HIV infection were diagnosed during their pregnancy and reported to the SHM. By June 2012, a total of 1734 pregnancies in 1114 women have been registered amongst the total 4016 HIV-infected women monitored by the SHM. Overall, 54% of the pregnant women were diagnosed with HIV before the onset of the pregnancy.

Maternal characteristics

Characteristics of HIV-infected women with a registered pregnancy are presented in *Table 1.2*. Of the 1114 women with a pregnancy, 174 (16%) originated from the Netherlands and 940 (84%) were of non-Dutch origin. The majority of women of non-Dutch origin were born in sub-Saharan Africa (70%) or in the Caribbean/Latin America (17%). Significant differences were found between women of different origins. Dutch women were more often aware of their HIV infection before they became pregnant compared to women of non-Dutch origin, 72% and 51% respectively ($p<0.0001$). Women of Dutch origin were significantly older at the time of their first registered pregnancy; the median age was 30 years (IQR: 26-35), whilst the median age of women of non-Dutch origin was 28 years (IQR: 24-33). Heterosexual contact was the most important route of HIV transmission (94%) in both Dutch and non-Dutch women. However, Dutch women were more often infected with HIV by another route compared to women of non-Dutch origin ($p=0.0002$); in 5% of the Dutch women, injecting drug use was reported as the route of transmission (5%).

Twenty mothers died during follow-up in the SHM database, with a median time between onset of the first registered pregnancy and death at 4.4 years (IQR: 1.8-9.0). Two mothers died within one month after parturition; for one mother the cause of death was an obstetric complication, and for the other the cause of death was unknown.

In total, 170 women became lost to follow-up; this happened more often in women of non-Dutch origin (17%) compared to women of Dutch origin (8%).

Pregnancy-related characteristics

The total 1114 women accounted for 1734 registered pregnancies. Sixty-two percent of the women had one registered pregnancy, and in 25% of the women two pregnancies were registered. Thirteen percent of the women had three or more registered pregnancies (*Table 1.2*). Of 1734 pregnancies, 1302 (75%) newborns resulted; 415 (24%) pregnancies ended in abortion, of which 189 were induced. Sixty percent of the newborns were delivered vaginally. Seventy percent of the Dutch women delivered vaginally compared to 58% of the women of non-Dutch origin ($p=0.002$). Five hundred and ten newborns were delivered via caesarean section. Elective caesarean delivery is known to reduce the risk of MTCT if the maternal viral load is detectable, but such a delivery is less beneficial if viral load suppression is achieved following successful treatment with cART [19, 20], when a vaginal delivery is possible. The proportion of elective caesarean deliveries has decreased over time from 52% in 2000 to 25% in 2010 ($p=0.0025$). In accordance with the decrease in elective caesarean sections, the proportion of women with an undetectable viral load has increased over time, from 56% in 1998 to 73% in 2010 ($p=0.006$). Although we observed a difference in the proportion of caesarean deliveries between Dutch and non-Dutch women, the proportion of women with a detectable HIV RNA load at time of delivery did not differ between Dutch women and women of non-Dutch origin (*Table 1.2*).

Table 1.2: Characteristics of HIV-infected pregnant women registered and monitored by the Stichting HIV Monitoring from January 1998 to 1 June 2012.

	Total N=1114 N (%)	Dutch N=174 (16%) N (%)	Non-Dutch N=940 (84%) N (%)
Maternal characteristics			
HIV diagnosis before pregnancy	606 (54)	126 (72)	*480 (51)*
Age at start of first pregnancy occurring in HIV infection (years, median, IQR)	29 (24-33)	30 (26-35)	*28 (24-33)*
HIV transmission route			
Heterosexual	1043 (94)	152 (87)	*891 (95)*
Other	71 (6)	22 (13)	*49 (5)*
Ever CDC class C event	188 (17)	28 (16)	*160 (17)*
Deaths	20 (2)	6 (3)	*14 (1)*
Lost to follow-up	170 (15)	14 (8)	*156 (17)*
Pregnancy-related characteristics			
Total number	1734	270	*1464*
Maximum number of pregnancies after HIV diagnosis			
1	695 (62)	107 (61)	*588 (63)*
2	275 (25)	47 (27)	*228 (24)*
3	105 (9)	13 (7)	*92 (10)*
≥4	39 (4)	7 (4)	*32 (3)*
Mode of delivery			
Vaginal	753 (44)	143 (53)	*610 (42)*
Caesarean	510 (29)	61 (23)	*449 (31)*
Unknown	468 (27)	66 (24)	*402 (28)*
Pregnancy outcome			
Partus	1302 (75)	209 (77)	*1093 (75)*
Abortion	415 (24)	61 (23)	*354 (24)*
Unknown	17 (1)	0	*17 (1)*
Pregnancy duration			
≥37 weeks	1056 (81)	177 (85)	*879 (80)*
32-37 weeks	147 (11)	21 (10)	*126 (13)*
<32 weeks	64 (5)	7 (3)	*57 (5)*
Missing	35 (3)	4 (2)	*31 (3)*
Birth weight (gram, IQR)	3090 (2690-3425)	3160 (2770-3458)	*3080 (2660-3420)*
Perinatal deaths	46 (3)	5 (2)	*41 (3)*
First $CD4$ cell counts (cells/mm^3) in first pregnancy (median, IQR)	400 (250-550)	520 (358-720)	*380 (236-525)*

	Total N=1114 N (%)	Dutch N=174 (16%) N (%)	Non-Dutch N=940 (84%) N (%)
Start cART			
Before pregnancy	959 (55)	152 (56)	*807 (55)*
During pregnancy	667 (38)	95 (35)	*572 (39)*
No cART during pregnancy*	108 (6)	23 (9)	*85 (6)*
HIV RNA plasma levels before delivery in first pregnancy (n=1114)			
HIV RNA available	952 (85)	152 (87)	804 (86)
Undetectable	688 (72)	117 (78)	574 (71)
Detectable	264 (28)	35 (23)	229 (28)
Unknown	162 (15)		

Legend: IQR=interquartile range; cART=combination antiretroviral therapy.

** 103 pregnancies were before cART became available for pregnant women.*

In 81% of the pregnancies, the duration was at least 37 weeks. A total of 211 newborns were born pre-term, with a pregnancy duration of 32-37 weeks for 147 newborns and <32 weeks for 64 newborns. The median weight of the newborns was 3090 grams (IQR: 2690-3425). Perinatal death occurred in 3% of the pregnancies. No significant differences in pregnancy duration, birth weight and perinatal death were found between Dutch and non-Dutch women.

The median first CD4 count measured in the pregnancy was significantly higher in Dutch women, compared to women of non-Dutch origin (p<0.0001). Differences in the proportion of Dutch and non-Dutch women diagnosed during pregnancy is probably responsible for the difference in median CD4 counts, as the median first CD4 count was significantly lower in women who were diagnosed with HIV in their pregnancy (340 cells/mm^3 (IQR: 205-520)) compared to women with a known HIV infection (430 cells/mm^3 (IQR: 300-570)) (p<0.0001). Dutch women were more often diagnosed with HIV before they became pregnant and were thus eligible for treatment before the onset of the pregnancy.

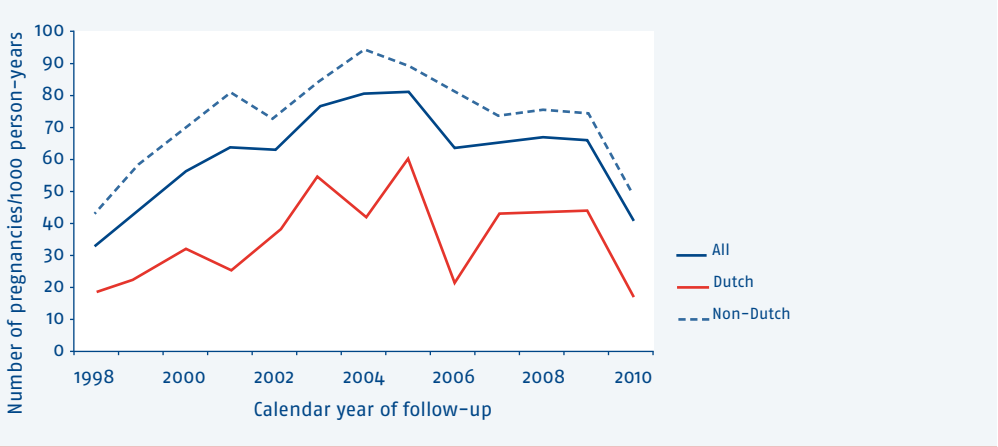
The majority of the women used cART during their pregnancy. Fifty-five percent initiated cART before the onset of the pregnancy, and 38% started while pregnant. As a result of cART treatment, the maternal viral load was undetectable at the time of delivery in 72% of the pregnancies. A detailed description of treatment and treatment outcome is presented in *Chapter 3*.

Trends in pregnancy rate amongst HIV-infected women

Overall, the occurrence of one or more pregnancies during or after HIV diagnosis amongst HIV-infected women aged 16-45 years was 56 pregnancies per 1000 person-years (95% CI: 53-60) (*Figure 1.11*). The highest pregnancy rates were found amongst women of non-Dutch

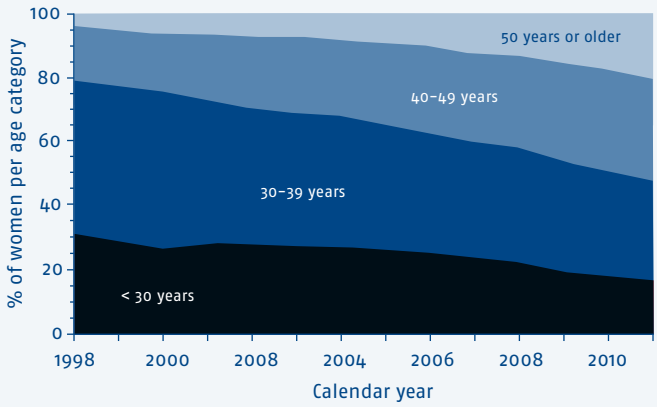
origin. Earlier studies have also shown differences in pregnancy rates amongst women of different geographic origins [21]. An HIV-infected woman's decision to become pregnant has been found to be socially and culturally related [22]. The annual incidence of pregnancies amongst HIV-infected women has steadily increased from 33 per 1000 person-years in 1998 to more than 80 per 1000 person-years in 2005. However, the incidence was considerably lower from 2006 onwards; it decreased to 64 per 1000 person-years in 2006 and remained stable until 2009. The lower number of pregnancies in 2010 might be a result of a backlog in the registration of pregnancies due to the visit-based data collection of pregnancy-related items.

Figure 1.11: *Incidence of pregnancies per 1000 person-years amongst HIV-infected women, overall and according to region of origin.*
The incidence of pregnancy in HIV-infected women in the Netherlands per calendar year of follow-up was calculated per 1000 person-years (PY). All women aged between 16-45 years were considered to be "at risk" for pregnancy. Person-years were calculated from the time of HIV diagnosis until last visit, death, the point at which the patient was lost to follow-up or 1 January 2011.



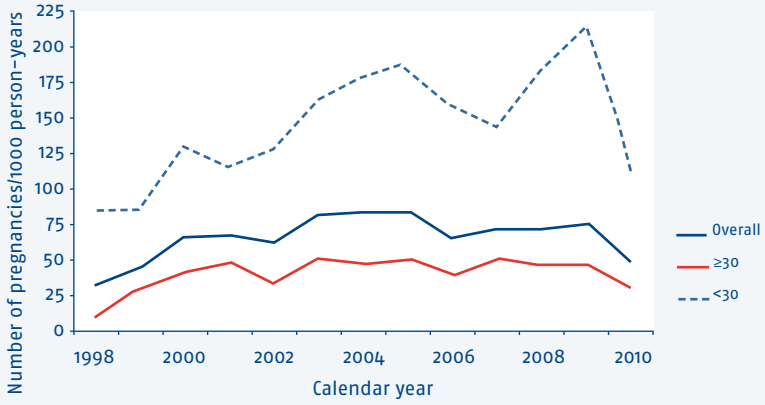
In both Dutch and non-Dutch women the pregnancy rates became lower after 2005, probably as a result of increasing age of the women in follow-up. The median age of HIV-infected women in follow-up increased from 34 years (IQR: 29-39) in 1998 to 41 (33-48) in 2011 (*Figure 1.12*). This increase in age is mainly a consequence of the improved life expectancy of HIV-infected patients after the introduction of cART. As a result of this increasing age, a smaller proportion of women of childbearing age is currently in clinical care; 52% of the women are currently 40 years or older.

Figure 1.12: The proportion of age categories of HIV-infected women in follow-up as of 1 June of each calendar year is shown. The age of the women in follow-up has increased over calendar-time. In 1998, 31% of the women in follow-up were younger than 30 years of age, whereas 4% were 50 years or older. In 2011, these proportions were 16% and 20%, respectively.



Pregnancy rates were significantly higher among women aged <30 years (112 pregnancies/1000 person-years of follow-up, 95% CI: 102-123) compared to women aged ≥35 years (25 pregnancies/1000 person-years, 95% CI: 22-28). The pregnancy rate amongst women aged <30 years increased over time (*Figure 1.13*). The majority of women who became pregnant when aged <30 years were newly diagnosed with HIV during their pregnancy (71%) compared to 45% of the older women. HIV screening during pregnancy is likely to detect infection in these women. From 2004 onwards, all pregnant women have been screened according to an opting-out strategy. However, before 2004, a selective screening policy was used, which aimed to screen women with an increased risk of HIV infection, such as originating from an high endemic region or (formerly) being an injecting drug user [23]. The number of HIV-infected women aged <30 years at the time of HIV diagnosis who were registered by the SHM dropped from 205 in 1999 to 93 in 2011. The decrease among women aged ≥30 years at time of HIV diagnosis was less marked; in this group the number of new registrations decreased from 202 in 1999 to 137 in 2011. The increase in pregnancy rates among young women is likely to be a result of the smaller number of women of child-bearing age due to the increasing age of HIV-infected women currently in care and to the influx of young pregnant women because of pregnancy screening.

Figure 1.13: Incidence of pregnancies per 1000 person-years of follow-up, overall and according to time-updated age of follow-up (<30 years, ≥30 years).



Conclusion

In recent years, the annual number of HIV diagnoses in the Netherlands has hovered around 1100. Meanwhile, the increasing trend in the number of diagnoses amongst MSM, which had been observed since the turn of the millennium, appears to have come to an end. Amongst MSM between the ages of 35 and 44 years, the number of diagnoses is even in a steady decline. On the other hand, the number of diagnoses is still increasing amongst young adults and in MSM 55 years of age or older. Diagnoses in the group of patients infected via heterosexual contact show a decreasing trend, which is mainly due to reductions in immigration from HIV-endemic regions.

HIV-infected patients are being diagnosed increasingly earlier in the course of their infection. A shrinking proportion of patients are diagnosed with CD4 counts below 350 cells/mm^3 and, conversely, the proportion diagnosed with a recent infection is on the rise. These changes are more pronounced, however, amongst MSM than amongst heterosexual men and women. In addition, in recent years, testing for HIV has appeared to be more frequent, because patients with a positive test more often have had a previous negative test. Testing rates appear to be highest amongst patients who have received positive results at Municipal Health Services or STI centres and lowest in those tested in a hospital. The population that has tested positive for HIV in a hospital also has the highest proportion of late testers. These observations illustrate that patients tested at Municipal Health Services or STI centres are more likely actively seeking testing for HIV on a regular base than patients diagnosed in a hospital.

Patients tested early in their infection generally start treatment in time, before CD4 counts have dropped below the threshold of 350 cells/mm^3. In most recent years, treatment uptake increased in patients with CD4 cells above this threshold. Interestingly, a considerable number of patients are diagnosed with more than 500 CD4 cells/mm^3, but still start treatment too late. Although the reason for such patients starting below the recommended threshold is unknown and may depend on factors not directly related to clinical condition, we also noted that changes over time in CD4 counts are often of a capricious nature. For instance, in a quarter of patients, CD4 counts increased in the first 6 months after receipt of an HIV diagnosis even though the patients were untreated. This effect probably reflects the impact of stress, in these cases caused by the dismay of first learning about one's positive status; the CD4 counts decrease initially with stress and then increase as the impact subsides.

Despite all these positive developments – more testing, earlier diagnosis, earlier start of treatment – the number of HIV diagnoses is still not in a convincingly significant decline, neither amongst MSM nor amongst heterosexuals. To fully curb the epidemic, testing and treatment needs to be scaled up. However, reductions in sexual risk behaviour are expected to have a much greater impact on the number of new infections.

2. Mortality, loss to follow-up, AIDS and non-AIDS defining events in men and women with HIV-1 infection

Rebecca Holman

In recent years, mortality rates for men and women with HIV-1 have fallen both before and after the start of combination antiretroviral therapy (cART). In fact, mortality rates for those who have not yet started cART are comparable with those in age- and gender-matched samples from the general population for men and women. This suggests that HIV infection is diagnosed when patients are still relatively healthy and that patients start antiretroviral therapy on time. However, mortality rates for patients who have started cART are significantly higher than those in the age- and gender-matched samples from the general population. This indicates that the duration of HIV infection, regardless of treatment, has an impact on life expectancy.

The rate of men and women becoming lost to follow-up is much higher in those born outside the Netherlands. This may be a result of individuals voluntarily leaving the Netherlands but it may also be a result of changes in immigration policy in the Netherlands.

The incidence of AIDS has decreased dramatically in the last 15 years and is currently similar for men and women. The age-corrected incidences of renal insufficiency, non-AIDS defining malignancies and liver disease for men and women has remained stable during the last ten years. The incidences of cardiovascular disease and diabetes mellitus has also remained stable over the last ten years for women but has decreased significantly for men. The incidence of osteoporosis has risen for both men and women. In comparison with an age-matched sample from the general population, the incidences of non-AIDS defining malignancies and osteoporosis in patients with HIV infection were not significantly different for women, but both were significantly higher for men. Compared to such samples, the incidence of diabetes mellitus in HIV-infected patients was significantly lower for men and higher for women. As in the general population, ageing plays a role in most of these conditions.

Men and women who have lived with HIV-1 infection for at least 20 years have been diagnosed in similar proportions with diabetes mellitus, cardiovascular disease, renal insufficiency or a non-AIDS defining malignancy. However, significantly more women have been diagnosed with liver disease or osteoporosis. The extent to which the incidence and prevalence of non-AIDS defining events in men and women living with HIV-1 are currently above levels in the general population needs further study.

In de afgelopen jaren is de mortaliteit onder HIV-geïnfecteerde mannen en vrouwen gedaald, zowel voor als na het starten met combinatie antiretrovirale therapie (cART). Het is zelfs zo dat het sterftepercentage bij patiënten die nog niet met cART zijn gestart vergelijkbaar is met het sterftepercentage bij mannen en vrouwen in de algemene bevolking van hetzelfde geslacht en dezelfde leeftijd. Dit veronderstelt dat de HIV-diagnose wordt gesteld op het moment dat patiënten nog relatief gezond zijn en dat zij op tijd starten met de antiretrovirale therapie. Echter, bij patiënten die al wel met cART zijn gestart, ligt het sterftepercentage significant hoger dan bij personen in de algemene bevolking van hetzelfde geslacht en dezelfde leeftijd. Dit geeft aan dat de duur van de HIV-infectie van invloed is op de levensverwachting, ongeacht behandeling.

Het percentage uitvallers (lost to follow-up), zowel mannen als vrouwen, ligt veel hoger bij patiënten die niet in Nederland zijn geboren. Mogelijk komt dit doordat mensen vrijwillig het land verlaten, maar het kan ook het gevolg zijn van veranderingen in het Nederlandse immigratiebeleid.

De AIDS-incidentie is in de afgelopen 15 jaar drastisch gedaald en is momenteel vergelijkbaar voor mannen en vrouwen. De voor leeftijd gecorrigeerde incidenties van nierinsufficiëntie, niet-AIDS-definiërende maligniteiten en leverziekten bij mannen en vrouwen zijn in de afgelopen 10 jaar stabiel gebleven. De incidenties van hart- en vaatziekten en diabetes mellitus zijn in de afgelopen 10 jaar ook stabiel gebleven bij vrouwen, maar zijn bij mannen significant gedaald. De incidentie van osteoporose is bij zowel mannen als vrouwen gestegen. De incidenties van niet-AIDS-definiërende maligniteiten en osteoporose bij HIV-geïnfecteerde vrouwen verschilden niet significant van die bij vrouwen van dezelfde leeftijd in de algemene bevolking, maar waren bij mannen significant hoger. Eenzelfde vergelijking wijst uit dat de incidentie van diabetes mellitus bij HIV-geïnfecteerde patiënten significant lager was bij mannen en hoger was bij vrouwen. Net als in de algemene bevolking speelt stijging van de leeftijd een rol bij het merendeel van deze aandoeningen.

Onder mannen en vrouwen die al ten minste 20 jaar met HIV geïnfecteerd zijn, zijn de percentages diabetes mellitus, hart- en vaatziekten, nierinsufficiëntie en niet-AIDS-definiërende maligniteiten gelijk. Er zijn echter significant meer vrouwen met een leverziekte of osteoporose gediagnosticeerd. De mate waarin de incidentie en prevalentie van niet-AIDS-definiërende voorvallen bij HIV-1-geïnfecteerde mannen en vrouwen momenteel uitsteekt boven deze zelfde incidentie in de algemene bevolking behoeft nader onderzoek.

Background

Increasing evidence has shown that the course of HIV-1 infection [24, 25], mortality rates [26] and incidence of and risk factors for AIDS [27] may differ for men and women. The same may be true for some non-AIDS defining events [28] and coping strategies [29]. In addition, the considerations when choosing antiretroviral treatment and the side effects of these

medications are different for men and women [30]. However, in the Netherlands, mortality rates, loss to follow-up and the incidence of AIDS and non-AIDS defining events are often not reported separately for men and women [5-7, 31]. As only approximately 20% of the people living with HIV-1 in the Netherlands are women (*Chapter 1*), important gender differences may be missed by presenting data in this way.

In this chapter, we consider the rates of mortality and loss to follow-up for both men and women living with HIV-1 before and after the start of combination antiretroviral therapy (cART). We also describe AIDS, diabetes mellitus, cardiovascular disease, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis after the start of cART. We describe the number of men and women experiencing each event for the first time. We present incidence rates and, where possible, risk factors separately for men and women. When considering rates of mortality, AIDS, diabetes mellitus, cardiovascular disease, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis, we concentrate on events between 2007 and 2011. When considering rates of loss to follow-up, we concentrate on events between 2007 and 2010. We believe that these choices give the best possible information on the risks of men and women currently in follow-up experiencing these events for the first time.

There is emerging evidence that patients who have lived with HIV-1 infection for many years have a greater burden of co-morbidities [32, 33], and that these develop in the patients earlier than in demographically similar subjects [34]. These patients may also exhibit different patterns of change in HIV and immunological parameters [35]. Hence, we consider men and women who have lived with HIV-1 for at least 20 years and describe the proportions of each who have experienced any and individual non-AIDS defining events.

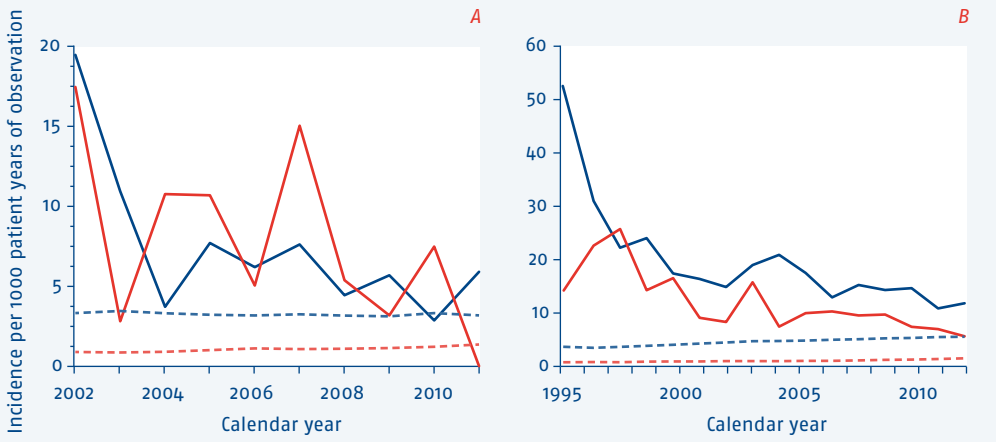
Mortality

When describing mortality rates before the start of cART, we include 9,434 men and 2,387 women who had a known date of HIV-1 diagnosis, no recorded HIV-2 infection, at least one instance of HIV-related care on or after 1 January 2002 and at least one recorded CD4 count. We define pre-cART care as starting on the date of an individual's first CD4 count, their 16th birthday or 1 January 2002, whichever is later, and ending on the date he or she started cART for the first time, date of death or 31 December 2011, whichever is earlier.

When describing mortality rates after the start of cART, we include 12,799 men and 3,350 women who had a recorded date of HIV-1 diagnosis, no recorded HIV-2 infection, started cART when they were at least 16 years old and whose last recorded contact with HIV-related care was in one of the HIV treatment centres in the Netherlands. We describe the characteristics of these patients in *Web Appendix Table 2.1*. We define on-cART care as starting on the date an individual started cART, their 16th birthday or 1 January 2002, whichever is later, and ending on the date of death or 31 December 2011, whichever is earlier. Patients, who have ever started cART remain in this group, even if they stop using cART either temporarily or permanently.

We modelled death rates using multilevel logistic regression models correcting for repeated observations on individuals and with observation periods corresponding to calendar years. We compared death rates before 2007 and between 2007 and 2011 using separate models for men and women and between men and women in the period 2007 to 2011. We corrected for potential differences in the age distribution between time periods or between men and women. We compared the number of deaths among men and women in this period with the expected number in an age- and gender-matched sample from the general population of the Netherlands using chi-squared statistics.

Figure 2.1: *The mortality rate before (A) and after (B) the start of combination antiretroviral therapy for men (blue) and women (red) per 1,000 years of follow-up (solid lines) and age-matched samples from the general population (broken lines).*



The numbers of men and women with HIV-1 who died never having started cART and who died after starting cART and the mortality rates are presented in *Web Appendix Table 2.2*. The mortality rates per calendar year and in gender- and age-matched samples from the general population [36] are presented in *Figure 2.1*.

Before the start of cART, the death rate between 2007 and 2011 for men was significantly lower than before 2007 (p=0.03), but not women (p=0.5). Between 2007 and 2011, there was no significant difference between the death rate for men and for women (p=0.4), and death rates were not significantly higher than in an age-matched sample from the general population for men (p=0.3) or women (p=0.1). Of the patients who died before the start of cART, 40 (29%) died of an AIDS-defining condition, 22 (16%) of a non-AIDS defining malignancy, 19 (14%) of non-natural causes, such as accidents, suicide, euthanasia and substance abuse, 8 (6%) of a non-AIDS defining infection and 49 (36%) of other, unclassifiable or unknown causes.

After the start of cART, the death rate between 2007 and 2011 for men was significantly lower than before 2007 for women (both p<0.0001). Between 2007 and 2011, the death rate was significantly higher for men than women (p=0.02), and the death rates were significantly higher than in age-matched samples from the general population for men (p=0.0005) and women (p=0.0095). The causes of death are presented in *Web Appendix Table 2.4*. Although both men and women with HIV-1 do still die of AIDS, that proportion has decreased significantly in men (p<0.0001) and in women (p=0.02).

We examined survival in patients from the start of cART using Cox regression analysis. After correction for all of the variables in *Web Appendix Table 2.1*, except the use of antiretroviral medication before 2007, the time to death was shorter for men than women (hazard ratio 1.4, 95% confidence interval [CI] 1.2 to 1.7, p<0.0001). In separate multivariate analyses for both men and women, all variables were significant for men (all p<0.0002) and women (all p<0.05) and factors associated with a shorter time to death were similar for men and women. The odds ratios for the individual categories of the covariates are presented in *Web Appendix Table 2.3*. In general, men and women survived for a shorter duration after starting cART if they were born in the Netherlands; they were older; had been HIV-1 positive for longer before they started cART; had an unknown HIV RNA load at the start of cART; had an unknown CD4 count or one under 200 cells/mm^3 at the start of cART; were underweight or had a normal body mass index; or had tested positive for hepatitis B or C. The shorter survival experienced by men and women born in the Netherlands may reflect variations in genetic make-up and lifestyle [78] between people born in the Netherlands or elsewhere, the raised level of loss to follow-up among men and women born outside the Netherlands, sick immigrants returning to their region of birth or the fact that people seeking opportunities in other countries are often in good general health [110].

Our results show that the death rates for both men and women with HIV-1 who have not started cART are currently low and comparable with those in the general population. This, in combined with the trends in median CD4 counts at the start of cART and the trends in diagnoses of AIDS-defining conditions before the start of cART (*Chapter 4*), suggests that patients in the Netherlands are currently being diagnosed with HIV-1 and starting treatment on time. Death rates among both men and women who have started cART have dropped dramatically, but are still higher than in the general population. Studies in Spain [37], Denmark [38], the U.S.A. [39] and a range of European countries [40] have described similar decreases in mortality through time. In addition, studies in the U.S.A. [41] and France [42] demonstrate similar risk factors for mortality.

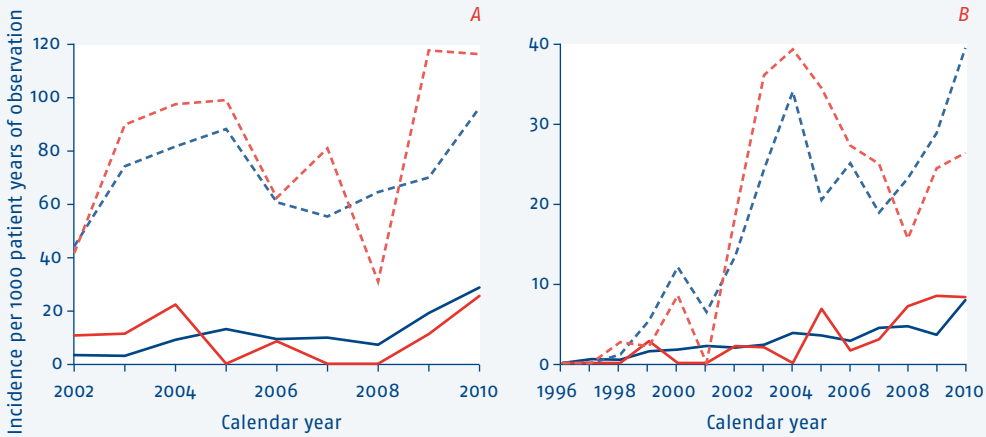
Loss to follow-up

Loss to follow-up is a problem in the monitoring and treatment of HIV because interruption of care increases the risk of HIV disease progression and death for individuals [43, 44], raises the probability of viral resistance to antiretroviral therapy [45] and increases the spread of HIV [46]. In this report, we define an individual as lost to follow-up if his or her last contact with a

HIV treatment centre was before 1 January 2011 and he or she is not known to have died. This means that he or she has not visited a HIV treatment centre for at least a year[7]. As an increased rate of loss to follow-up has been found in immigrants in other cohorts[47, 48] and the proportion of men and women who were born in the Netherlands is significantly different from that of patients born elsewhere (*Web Appendix Table 2.1*), we have chosen to perform analyses on the rates of loss to follow-up separately for men and women born in the Netherlands and elsewhere.

We describe rates of loss to follow-up for the selections of men and women described in and using similar methods to the section on mortality. The totals of men and women born in the Netherlands and elsewhere who became lost to follow-up before and after the start of cART and the incidence per 1,000 years of follow-up are presented in *Web Appendix Table 2.5*. The rates of loss to follow-up per calendar year are presented in *Figure 2.2*.

Figure 2.2: *The rates of loss to follow-up before (A) and after (B) the start of combination antiretroviral therapy for men (blue) and women (red) born in the Netherlands (solid lines) or elsewhere (broken lines) per 1,000 years of follow-up.*



Between 2002 and 2010, the incidence of loss to follow-up before the start of cART for men and women was significantly different between individuals born in the Netherlands and those born elsewhere (both $p<0.0001$). No significant difference was found for men or women in the rate of loss to follow-up before 2007 and between 2007 and 2010 (both $p=0.9$). After correction for differences in the ages of men and women and the proportion born in the Netherlands, there was no significant difference between the rate of loss to follow-up for men and women between 2007 and 2010 ($p=0.7$).

Between 2007 and 2010, the incidence of loss to follow-up after the start of cART for both men and women (both p<0.0001) was significantly different between those born in the Netherlands and those born elsewhere. After correction for differences in the ages of men and women and the proportion born in the Netherlands, there was no significant difference in the rate of loss to follow-up before 2007 and between 2007 and 2010 for men (p=0.2), but the rate was significantly higher before 2007 for women (p=0.0004). After correction for differences in the age distribution and the proportion born in the Netherlands, women were significantly more likely to become lost to follow-up than men (p=0.04).

Our results show a huge difference in the proportion of men and women born in the Netherlands and those born elsewhere who became lost to follow-up without receiving cART and after starting cART. This may be a result of individuals voluntarily leaving the Netherlands, but it may also be a result of changes in immigration policy in the Netherlands [49]. An elevated rate of loss to follow-up has also been found in immigrants in Switzerland [47] and France [48] and in patients who became infected with HIV abroad [50] or were members of particular ethnic groups [51] in the United Kingdom.

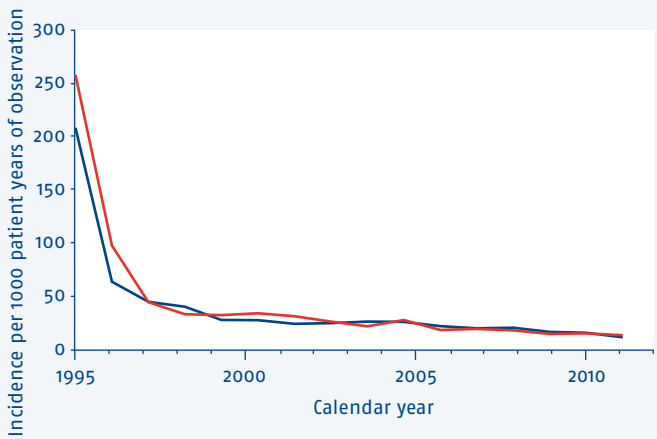
AIDS and non-AIDS defining events

We report on the incidence of any AIDS-defining event, separate AIDS-defining events, diabetes mellitus, cardiovascular diseases, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis, recorded by the physicians treating HIV. We define AIDS as any Centers for Disease Control (CDC) category C condition [52]. We define cardiovascular diseases as myocardial infarction, stroke, coronary artery by-pass grafting, coronary angioplasty/stenting and carotid endarterectomy, and liver disease as cirrhosis, fibrosis (METAVIR scores F1, F2, F3 and F4) and hepatocellular carcinoma. Apart from cardiovascular diseases, the definitions are the same as used previously [7]. We report on these events as they represent systemic conditions, which may result from the impact and progression of HIV-1 infection, chronic inflammatory processes [6] and specific cART regimes, as demonstrated in the D:A:D Study [53-59]. In addition, the occurrence of these events may be influenced by infections, such as cytomegalovirus [60, 61] and hepatitis B and C [62, 63] virus, which often occur in men and women with HIV. Furthermore, the incidence of these events increases with age [64-66], meaning that they may provide insights into the role of HIV infection in the ageing process.

In these analyses, we report on data from the 12,799 men and 3,350 women who started cART and are also described in the sections on mortality and loss to follow-up. For the analyses on incidence per calendar year and period, we consider all events after an individual started cART and after routine data collection on the condition started, whichever was later. In these analyses, except those considering AIDS, we exclude men and women who experienced the event before they started cART or routine data collection was started. This is similar to a previous approach [6].

When constructing the models, we used the variables, except for gender, described and categorized in *Web Appendix Table 2.1* as covariates. We also included the time-updated number of years on cART as a covariate using the following categories: less then one year; one to five years; five to ten years; and more than ten years. We performed univariate analyses on each of the covariates and then included the covariates with a univariate p-value smaller than 0.1 in a multivariate analysis and performed a stepwise backwards procedure, eliminating one variable per step until all variables had a p-value of 0.05 or less. If there were at least 40 events for men and women separately between 2007 and 2011, we performed separate analyses for men and for women. Otherwise, we included gender as a covariate and retained it in the model at all stages of modelling, regardless of significance. When the results of the analyses are considered, it is important to remember that the number of women and numbers of events that they experience are much smaller than for men. This can mean that the results are not directly comparable because of differences in statistical power. We regarded p-values of less than 0.05 as statistically significant.

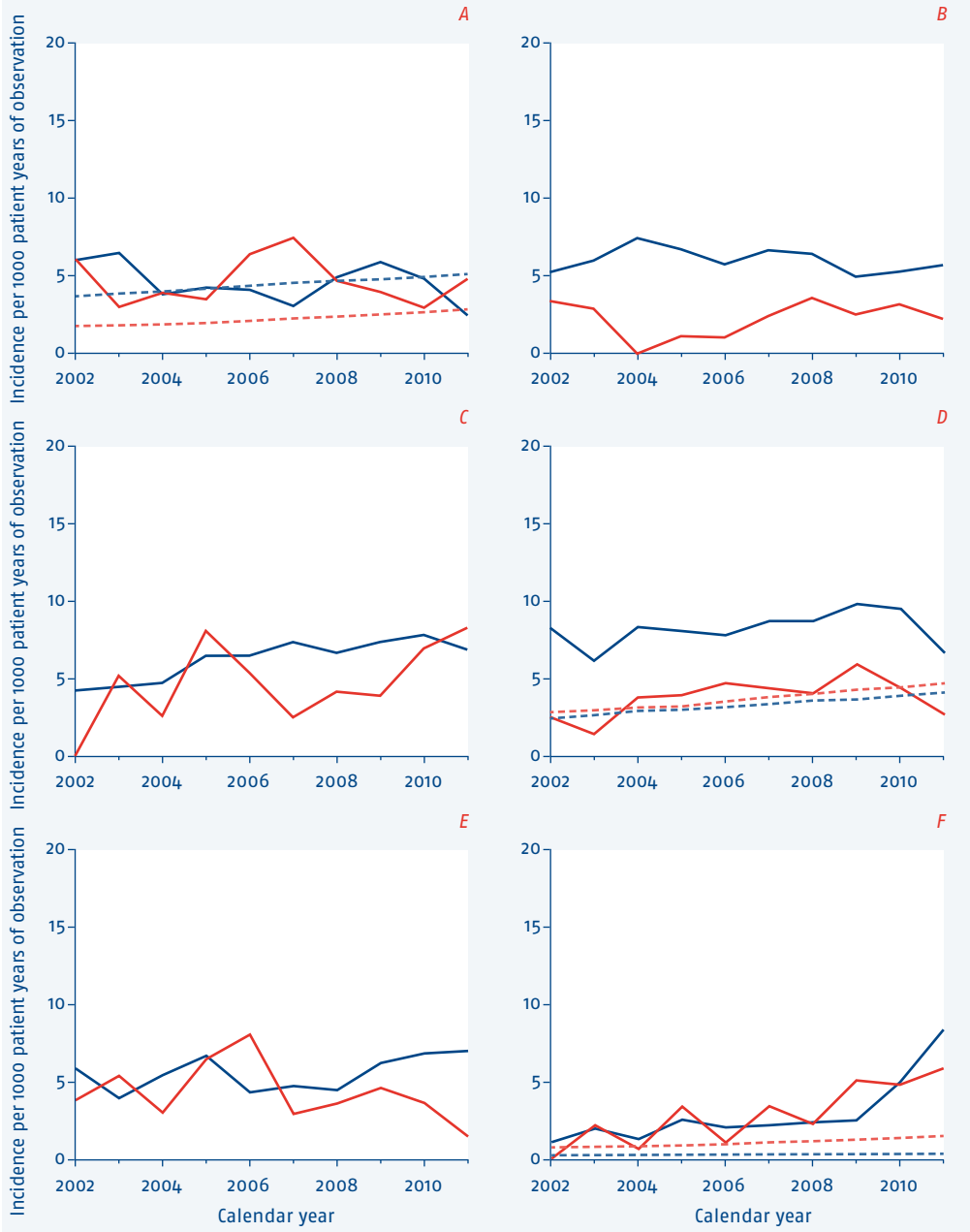
Figure 2.3: *The incidence of first AIDS defining event after the start of cART for men (blue) and women (red) per 1,000 years of follow-up.*



The incidence of the first occurrence of any and individual AIDS defining events after the start of cART are presented in *Web Appendix Table 2.6*. In these analyses, we concentrate on the first occurrence of any AIDS defining event after the start of cART. The incidence per calendar year is presented in *Figure 2.3*. The incidence between 2007 and 2011 was significantly lower than before 2007 for men and women (both $p<0.0001$). Between 2007 and 2011, there was no significant difference between the incidence for men and women ($p=0.7$). We present the p-values of the values of the variables remaining in the multivariate logistic regression analysis in *Web Appendix Table 2.7*. The results of this analysis show that men were more likely to experience their first AIDS defining event if they had become HIV-1 positive due to blood contact, had intravenous drug use or unknown risk factors, had been diagnosed with HIV-1 less than one year before the start of cART, had been on cART for less than one year, had an unknown HIV RNA viral load, had an unknown number of or fewer than 200 CD4 cells/mm^3, were hepatitis B virus negative, or had a known positive or negative hepatitis C virus status. Women were more likely to experience an AIDS defining event if they had been on cART for less than one year, had fewer than 200 CD4 cells/mm^3, and had a known hepatitis C virus status.

We present the number of men and women experiencing diabetes mellitus, cardiovascular diseases, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis for the first time and the incidence per 1,000 years of observation in *Web Appendix Table 2.8* and the incidence per calendar year in *Figure 2.4*. After correction for potential changes in age distribution, the incidences in men of diabetes mellitus and cardiovascular disease were significantly lower between 2007 and 2011 than between 2002 and 2006, whilst the incidence of osteoporosis was significantly higher. The incidences in men of renal insufficiency, liver disease and non-AIDS defining malignancies were similar during both periods. The incidences in women of liver disease and osteoporosis were significantly higher between 2007 and 2011 than between 2002 and 2006. The incidences in women of diabetes mellitus, cardiovascular disease, renal insufficiency and non-AIDS defining malignancies were similar during both periods. Between 2007 and 2011 and after correction for potential differences in the ages of men and women, the incidences of cardiovascular diseases, non-AIDS defining malignancies and liver disease were significantly higher for men than for women. The incidence of osteoporosis was significantly higher for women than men. There were no differences in the incidences of renal insufficiency or diabetes mellitus between men and women. We present the p-values of the values of the variables remaining in the multivariate logistic regression analyses in *Web Appendix Table 2.7*.

Figure 2.4: The incidence of diabetes mellitus (A), cardiovascular disease (B), renal insufficiency (C), non-AIDS defining malignancies (D), liver disease (E) and osteoporosis (F) for men (blue) and women (red) per 1,000 years of follow-up (solid lines) and age-matched samples from the general population (broken lines).



The incidence of diabetes mellitus was significantly lower for men and higher for women than in age- and gender-matched samples from the general population [64]. Increased rates of diabetes mellitus among people with HIV-1 have been previously reported [67, 68], although these may have been associated with the use of stavudine, indinavir and didanosine [69]. The use of these antiretroviral medications is currently low in the Netherlands [70], which may partly explain why the incidence of diabetes mellitus in men with HIV-1 does not appear to be currently elevated. A decreasing incidence of diabetes mellitus through time has also been reported in France and the D:A:D study [68, 69]. Men were more likely to develop diabetes mellitus if they had become HIV-1 positive due to blood contact, intravenous drug use, heterosexual contact or an unknown route; were aged 45 years or older; and were underweight or overweight. These three risk factors were also found in the D:A:D study [68], a cohort in France [69] and the Swiss Cohort Study [71], as well as age and overweight in a cohort in the United States [72]. Increasing age and body mass index are traditional risk factors for diabetes mellitus for HIV-negative individuals, as well [69]. For women, no variables remained in the multivariate analysis. This may reflect the smaller number of women with HIV, and hence, lower statistical power to detect these effects. However, older age and obesity have been reported as risk factors for incident diabetes mellitus among women with HIV infection [73].

The incidence of non-AIDS defining malignancies was significantly higher than in the general population [74] for men, but not for women. An increased incidence of grouped non-AIDS defining cancers has also been reported in other cohorts consisting mainly of men [75-77]. Increased incidences of non-AIDS defining malignancies may result from immune deficiency [5, 78], the use of cART [76, 79], and behavioural [75] or family-related risk factors [80, 81]. Men were more likely to experience non-AIDS defining malignancies if they were aged 45 years or older, were born in the Netherlands, had an unknown HIV RNA load, had a CD4 count less than 200 cells/mm^3 or were underweight. Women were more likely to experience non-AIDS defining malignancies if they were aged 45 years or older, were born in the Netherlands or had been using cART for over ten years. An increased risk of non-AIDS defining malignancies for older patients, those born in the Netherlands and those with a low CD4 count has been previously reported for our cohort [5]. An increase in incidence with age has also been reported earlier for our cohort [5] and the Swiss Cohort Study [82] and in deaths from non-AIDS defining malignancies in the D:A:D Study [58]. However, the effects of immunological parameters may be stronger for infection-related malignancies [5].

The incidence of osteoporosis was significantly higher than in the general population [65] for men, but not women. Previous studies have shown that osteoporosis is more common in women with HIV-1 infection than men [82] and in men [83] and women [84, 85] with HIV-1 infection than without. The increased incidence among men may result from factors related to HIV infection [86], a premature decline in serum testosterone levels [28, 87] and the use of antiretroviral medication, such as tenofovir [84, 88, 89]. The results of the multivariate analysis show that men were more likely to develop osteoporosis if they were aged 45 years or older,

were born in the Netherlands, had been using cART for more than ten years, had an unknown HIV RNA or one under 1000 copies/ml or were underweight. Women were more likely to develop osteoporosis if they were aged 45 years or older or had been using cART for longer than one year. Increasing age was also associated with an increased risk of osteoporosis in the Swiss Cohort Study [82]. Caucasian race, increasing age and the use of cART increased the risk of low energy fractures in a cohort of patients with HIV infection in Denmark [90].

The incidences of myocardial infarction [91] and stroke [66] were not significantly different from that in age-matched samples from the general population for men or women. Previous studies have reported that patients with HIV-1 infection have an increased risk of myocardial infarction [7, 92-94] and stroke [95]. The contrast with our data may be due to improvements in the treatment and monitoring of HIV-1 infection and antiretroviral medications or better management of classical risk factors. Patients were more likely to experience cardiovascular disease if they were aged 45 years or more, had used antiretroviral therapy before 2007, were born in the Netherlands or had an unknown HIV RNA. These results are similar to those presented previously [6] in the Swiss Cohort Study [82], in a cohort in Italy [96] and the D:A:D Study [55].

There are no published figures on the incidence of renal insufficiency as defined in this report in the Netherlands. However, people with HIV-1 infection may have an increased risk of renal insufficiency [97]. The increased risk may be associated with the use of certain antiretroviral medications, such as tenofovir and indinavir [89, 98, 102, 103] although people with HIV-1 may also have other conditions that increase risk for the development of renal disease [99], such as diabetes mellitus [100] or hepatitis B and C virus co-infection [101]. Men were more likely to experience renal insufficiency if they had become HIV-1 positive via blood contact, intravenous drug use or an unknown route; were aged 45 years or older; had been using cART for less than one or more than 10 years; had an unknown HIV RNA load; had fewer than 200 CD4 cells/mm^3 or had an unknown hepatitis C status. Women were more likely to experience renal insufficiency if they had become HIV-1 positive via blood contact or intravenous drug use or an unknown route, were aged 45 years or older or had an unknown hepatitis C status. Increasing age was also a risk factor in cohorts in Italy [96], Denmark [98], France [102] and the United States [100].

There are no published figures on the incidence of liver disease as defined in this chapter in the general population of the Netherlands. However, substantial increases in liver disease have been reported in patients with HIV-1 infection [104, 105] and may occur earlier in these patients [106]. There may also be substantial differences with regard to gender [106], hepatitis B [105] and C [107] virus status, injecting drug use, the period in which patients started cART [107] and ethnic groups [108]. In our cohort, patients were more likely to develop liver disease if they were male, had a HIV RNA load of under 1,000 particles/ml, had fewer than 200 CD4 cells/mm^3, or were hepatitis B or C positive. These results are similar to those our cohort has presented previously [6] and in the Swiss Cohort Study [82].

Our analyses show that men who were born in the Netherlands were more likely to experience cardiovascular diseases, non-AIDS defining malignancies and osteoporosis than men born elsewhere. As the transmission route did not remain in the multivariate analyses for any of these conditions, it is unlikely that this is a result of the high proportion of homosexual men (*Chapter 1*) among patients with HIV infection in the Netherlands. It may reflect variations in genetic make-up and lifestyle between men born in the Netherlands and elsewhere or the epidemiologic paradox [109]. It may also be partly a result of the 'salmon effect', where sick immigrants return to their region of birth, or the 'healthy immigrant effect', where the most healthy people seek opportunities in other countries [110]. Even after correction for age, men who used antiretroviral therapy before 2007 also had a higher risk for the development of renal insufficiency, non-AIDS defining malignancies or osteoporosis. This may reflect long-term exposure to high levels of HIV RNA or suboptimal levels of CD4 cells, risks associated with long-term use of antiretroviral medication or long-term side-effects of early antiretroviral medications [58, 59, 82]. In addition, CD4 cell counts in men were associated with the incidence of renal insufficiency, non-AIDS defining malignancies and liver disease, and HIV RNA levels were associated with the incidence of cardiovascular diseases, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis. These results show that there may be links between the amount of exposure to certain levels of HIV RNA or CD4 cell counts may influence the development of these conditions [58, 82]. Unknown HIV RNA load and hepatitis B or C status may reflect suboptimal engagement in care by patients who return to HIV-related care upon noticing that they have other health problems [111].

Our analyses show that for men the incidence of diabetes mellitus and cardiovascular disease and renal insufficiency, non-AIDS defining malignancies and osteoporosis for both men and women increases as patients become older. This is similar to the situation in the general population of the Netherlands [64-66, 74, 91, 112, 113]. Increasing age increases the risk that a range of non-AIDS defining co-morbidities, including diabetes mellitus [71, 72], cardiovascular disease [93, 94], renal insufficiency [100], non-AIDS defining malignancies [77, 114], liver disease [104, 105] and osteoporosis [28, 115], develop in patients with HIV infection [82, 116, 117] above that of individuals without HIV-1 infection. However, the aging process in individuals with HIV-1 infection may also be reflected in cognition [118], neuropsychiatric illness [119], neuropsychological [120] and neurodegenerative processes [121, 122] and sexual functioning [123]. In general, older patients with HIV infection have a substantial burden of co-morbidities [33], and this burden may be greater [32] and develop at a younger age than in subjects without HIV [34]. Furthermore, HIV infection may enhance the negative effects of ageing and lifestyle factors on general health status [124] and on cardiovascular disease [125]. However, more research is needed to find out whether these conditions appear at a younger age or the prevalence of these conditions among men and women with HIV-1 in the Netherlands [34] and elsewhere [96, 126] is actually higher than in the general population. Future research should also examine the role of HIV-1 infection and cART in neurodegenerative processes and psychiatric illness and take into account the potential role of other epidemiological factors, including drug use [32], smoking [125], co-infections [60, 61, 101], other co-morbidities [100] and changing social circumstances [127] in men and women.

Men and women who have lived with HIV-1 for at least 20 years

Of the 12,799 men and 3,350 women who started cART and are described earlier in this chapter, 845 (7%) men and 162 (5%) women were diagnosed with HIV-1 before or on 1 January 1992, were still alive on 1 January 2012 and had had at least one contact with HIV-related care on or after 1 January 2011. Hence, these men and women have lived with HIV-1 infection for at least 20 years. We present their characteristics in *Table 2.1*. There are significant differences between men and women with respect to the proportion born in the Netherlands, HIV-1 transmission route, age at HIV-1 diagnosis, age on 1 January 2012 and the number of years since the start of cART. There are no significant differences with respect to the proportion with a last known HIV RNA load under 100 copies/ml, years between HIV-diagnosis and the start of cART and last known CD4 cell count.

Table 2.1: *The characteristics of patients who have lived with HIV-1 infection for at least 20 years.*

	Men N = 845	Women N = 162	P-value*
Born in the Netherlands	603 (71%)	92 (57%)	0.0002
Route of HIV-1 transmission			
Homosexual contact	674 (80%)	0	< 0.0001
Heterosexual contact	51 (6%)	92 (57%)	
Blood contact or intravenous drug use	94 (11%)	53 (33%)	
Other or unknown	26 (3%)	17 (10%)	
Age at HIV-1 diagnosis (years, median, IQR)	31 (26-36)	27 (24-30)	< 0.0001
Age at start of cART (years, median, IQR)	41 (36-47)	37 (32-42)	< 0.0001
Time between HIV-diagnosis and start of cART (years, median, IQR)	9 (7-12)	9 (7-12)	0.7658
Age on 1 January 2012 (years, median, IQR)	54 (50-60)	50 (47-53)	< 0.0001
Years since start of cART, on 1 January 2012 (median, IQR)	15 (12-15)	14 (12-15)	0.0264
Last known HIV RNA load under 100 copies/ml	787 (93%)	146 (90%)	0.3599
Last known CD4 cell count (cells/mm^3, median, IQR)	570 (418-762)	610 (440-840)	0.1110

** Chi-squared tests for categorical variables and Kruskal-Wallis test for continuous variables.*

The numbers and proportions of men and women who have ever experienced diabetes mellitus, cardiovascular disease, renal insufficiency, non-AIDS defining malignancies, liver disease and osteoporosis are presented in *Table 2.2*. There were no significant differences in the proportion of men and women who were diagnosed with diabetes mellitus, cardiovascular disease, renal insufficiency or a non-AIDS defining malignancy or experienced a stroke. In addition, there was no significant difference between the proportion of men and women who experienced at least one of the adverse events. However, a significantly larger proportion of women were diagnosed with liver disease or osteoporosis.

The differences and similarities between men and women largely reflect the incidences of these events. However, more women were living with liver disease and fewer men with cardiovascular diseases and non-AIDS defining malignancies than is to be expected. This may reflect differences in survival between men and women after these events. More research is needed to examine whether, after correction for demographic and HIV-related factors, the prevalence of these events is higher among men and women who have lived with HIV infection for a longer, rather than shorter, period.

Table 2.2: The numbers and percentages of men and women who have lived with HIV-1 infection for at least 20 years and have experienced at least one adverse event.

	Men N = 845	Women N = 162	P-value (Chi-squared test)
Diabetes mellitus	62 (7%)	8 (5%)	0.2714
Cardiovascular disease	77 (9%)	8 (5%)	0.0800
Renal insufficiency	63 (7%)	18 (11%)	0.1171
Non-AIDS defining malignancy	78 (9%)	11 (7%)	0.3161
Liver disease	68 (8%)	24 (14%)	0.0062
Osteoporosis	35 (4%)	14 (9%)	0.0147
At least one adverse event	299 (35%)	60 (37%)	0.6875

Conclusion

The death rates for both men and women with HIV-1 who have not started cART are similar and have fallen in the last ten years. These rates are currently low and comparable with those among age- and gender-matched samples from the general population. This suggests that HIV-1 infection is diagnosed early in its course and patients start cART on time. The death rates for both men and women who have started cART and the proportions dying of AIDS have fallen in the last 15 years. In this group, the death rate is higher and the time to death shorter for men than women. In addition, the death rates for men and women are still higher than in gender- and age-matched samples from the general population. The rate of men and women becoming lost to follow-up before or after the start of cART is much higher for patients born outside rather than in the Netherlands. Even after correction for differences in age and the proportion born in the Netherlands, women who had started cART were more likely to become lost to follow-up than men between 2007 and 2010.

The incidence of AIDS defining conditions has decreased dramatically in the last 15 years and is currently similar for men and women. After correction for changes in the ages of men and women with HIV-1 infection, the incidences of renal insufficiency, non-AIDS defining malignancies and liver disease for both men and women and cardiovascular disease and diabetes mellitus for women have remained stable over the last ten years. In this period, the incidence of diabetes mellitus and cardiovascular disease has decreased for men, while the incidence of osteoporosis has risen for men and women. The incidences of

non-AIDS defining malignancies and osteoporosis for women were not significantly different from those in an age-matched sample from the general population, but were higher for men. The incidence of diabetes mellitus is lower for men and higher for women than in age- and gender-matched samples from the general population. It was not possible to compare the incidences of cardiovascular disease, renal insufficiency or liver disease with those in the general population.

Similar proportions of men and women who have lived with HIV-1 infection for at least 20 years have been diagnosed with diabetes mellitus, cardiovascular disease, renal insufficiency or a non-AIDS defining malignancy. However, more HIV-1-infected women have been diagnosed with liver disease or osteoporosis. More research is needed to examine whether the prevalence of these conditions is higher among individuals with HIV-1 infection than in the general population and between groups of men and women who have lived with HIV infection for longer or shorter periods.

3. Response to cART

Luuk Gras, Colette Smit

Response to cART in adults

In 2011, 1014 HIV-1-infected patients started combination antiretroviral therapy (cART). From 2007 onwards the CD4 count at the start of cART has increased in heterosexually infected individuals to a median of 255 cells/mm^3 in 2011, but has shown a greater increase among men who have sex with men (MSM) to a median of 310 cells/mm^3. Currently, the United States Department of Health and Human Services (US DHHS) treatment guidelines, which are followed in the Netherlands, recommend cART for all HIV-infected individuals, as it has been shown to prevent further transmission. To improve testing rates in this patient group, major barriers for testing, such as fear of the test result, need to be addressed.

Currently, 72% of patients achieve HIV RNA plasma concentrations below 50 copies/ml within 9 months after starting cART. Women and patients 50 years of age or older have a better short- and long-term virological response than men and patients younger than 50. This may be due to improved adherence, but it may also be because of gender and age differences in pharmacokinetics. Women and persons 50 years or older have a higher rate of toxicity related to stopping antiretroviral therapy, which further suggests pharmacokinetics may be different in women and persons of older age.

A resistance test after virological failure of first-line cART is needed to determine whether resistant mutations have appeared, and if so, it must be known which ones, so that an active second-line cART can be selected. In 64% of patients with first-line virological failure and a subsequent switch to a second-line regimen, such a resistance test was available. Most patients who started second-line cART after first-line virological failure switched from a protease inhibitor (PI)-based regimen to an non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen. A high percentage of patients starting second-line cART also fail this new regimen (18% within one year), especially patients from the Caribbean, South America or sub-Saharan Africa. Virological failure under cART is mainly due to non-adherence. To avoid repeated virological failure, it is important to address the factors contributing to this non-adherence.

As it is now recommended to offer cART to all HIV-infected individuals, it is important to monitor patients with high CD4 counts (≥500 cells/mm^3) who may have a low perceived necessity of anti-HIV treatment. When cART is started with high CD4 cell counts the probability of virological suppression is lower than when started with CD4 counts of

200-500 cells. However, this difference has diminished in more recent years, possibly due to the changing perception of a benefit from cART when CD4 counts are still high. cART started at CD4 counts between 350 and 500 cells/mm^3 results in an increase in CD4 count to a median of 800 cells/mm^3 after 8 years of virologically successful therapy. This level is comparable to normal values seen in uninfected individuals. Increases were smaller when cART was started at lower CD4 counts (550 cells/mm^3 after 8 years virologically successful cART was achieved when cART was started between 50 and 200 CD4 cells/mm^3) and during long-term periods of viraemia higher than 50 copies/ml.

Currently, almost half of the patients can stay on the initial regimen for 3 years. Toxicity has remained the main reason for changes in antiretroviral therapy (49% of all changes), although the incidence of toxicity-driven therapy changes has been reduced by more than half since the introduction of cART in 1996. Due to the introduction of less toxic drugs, the most frequently recorded treatment-limiting adverse events has shifted from lipodystrophy, rash or renal insufficiency in 2006 to nausea and diarrhoea in 2011.

From our monitoring results of the effect of cART in the infected adult population, we conclude that improvement in HIV-testing rates among heterosexuals at high risk for HIV infection is needed to make a timely start of cART possible. Both efficacy and tolerability of cART has improved over time, such that the majority of patients can remain on first line cART for years. Starting cART with high CD4 counts (≥350 cells/mm^3) is important, since counts similar to those seen in uninfected individuals may be reached after 8 years of continuous viral suppression. Monitoring of viral load is crucial in all HIV-infected individuals, as adherence may diminish over time with increasing CD4 cell counts. Individually based treatment strategies, especially for older patients and women, may help to reduce toxicity. Besides the monitoring of changes in CD4 cell count, monitoring of changes in viral load is needed to identify patients at risk for treatment failure and also to study the effect of the introduction of new drugs and of adapted viral load assays.

Response to cART in pregnant women

In this section, we report on the virological response to cART in HIV-infected pregnant women. In 395 (39%) women, cART was initiated before they became pregnant, and in 616 women it was initiated during their pregnancy. At the time of cART initiation, CD4 count and HIV RNA levels were significantly lower in women who started cART before pregnancy compared to those who started during their pregnancy. At the time of delivery, 74 women (9%) had a detectable HIV RNA level. By 6 months after starting cART, 86% of the women had experienced a virological response (two consecutive HIV RNA levels <50 or 500 copies/ml). The strongest response was observed among women who started cART during their pregnancy from 2001 onwards. Overall, 18% of the women experienced virological failure after initial suppression. The highest failure rates were observed among pregnant women

who started cART during the pregnancy. Lower adherence rates post partum might cause the increased risk of virological failure after delivery. Intervention to improve adherence during the postpartum period of HIV-infected women is needed to prevent virological failure after pregnancy.

In this report, response to cART in children is covered in *Chapter 5*.

CART-respons bij volwassenen

In 2011 zijn 1014 HIV-1-geïnfecteerde patiënten gestart met combinatie antiretrovirale therapie (cART). Vanaf 2007 is het CD4-celaantal bij start van de behandeling onder heteroseksuele HIV-geïnfecteerden, maar vooral onder MSM, gestegen (naar een mediaan aantal van respectievelijk 255 en 310 cellen/mm^3). De US DHHS (U.S. Department of Health and Human Services) behandelrichtlijn, die in Nederland wordt gevolgd, beveelt momenteel behandeling met cART aan voor alle HIV-geïnfecteerden, omdat is aangetoond dat het de overdracht van verdere transmissie voorkómt. Het percentage mensen dat op HIV wordt getest is in de loop van de tijd verbeterd maar loopt onder heteroseksuelen nog achter. Om ook bij deze groep de testpercentages te verbeteren, is het nodig om barrières, zoals angst voor de testuitslag, aan te pakken.

Momenteel bereikt 72% van de patiënten binnen 9 maanden na start van de cART-behandeling een HIV-RNA-concentratie in plasma tot onder de 50 kopieën/ml. De virologische respons op zowel de korte als de lange termijn is beter bij vrouwen en bij patiënten die ouder zijn dan 50 jaar, dan bij mannen en bij patiënten onder de 50. Dit gunstiger resultaat is mogelijk het gevolg van een betere therapietrouw maar kan ook worden verklaard door verschillen in geslacht en leeftijd in de farmacokinetiek. Vrouwen en personen van 50 jaar en ouder stoppen vaker met de antiretrovirale behandeling wegens toxiciteit en dit zou ook kunnen wijzen op farmacokinetische verschillen bij deze groepen.

In geval van virologisch falen op het eerstelijns cART-regime is een resistentietest nodig om te achterhalen of, en zo ja welke resistente mutaties er zijn opgetreden zodat een actieve tweedelijns cART-therapie gekozen kan worden. Bij 64% van de patiënten met eerstelijns virologisch falen gevolgd door een switch naar een tweedelijns regime werd een dergelijke test uitgevoerd. De meeste patiënten die na virologisch falen op een eerstelijns cART-regime starten met een tweedelijns cART-regime switchen van een regime met als basis (een) proteaseremmer(s) naar een regime met als basis (een) NNRTI(s). Een hoog percentage patiënten dat start met een tweedelijns cART-regime faalt opnieuw (18% binnen een jaar), vooral patiënten afkomstig uit het Caraïbisch gebied, Zuid-Amerika of sub-Sahara Afrika. Virologisch falen op cART-behandeling komt vooral door onvoldoende therapietrouw. Om herhaling van virologisch falen te voorkomen, is het van belang de factoren die bijdragen aan verminderde therapietrouw te onderzoeken.

Omdat de huidige richtlijn aanbeveelt cART aan alle HIV-geïnfecteerden aan te bieden, is het belangrijk om patiënten met een hoog CD4-celaantal (≥500 cellen/mm^3) te monitoren aangezien zij de noodzaak voor anti-HIV-behandeling mogelijk te laag inschatten. Bij starten van cART met een hoog CD4-celaantal is de kans op virologische suppressie lager dan bij starten met een CD4-celaantal tussen de 200 en 500 cellen/mm^3. In de afgelopen paar jaar is dit verschil echter kleiner geworden, mogelijk door veranderende inzichten omtrent het voordeel van de cART-behandeling bij nog hoge CD4-celaantallen. Het starten van cART bij een CD4-celaantal tussen 350 en 500 cellen/mm^3 leidt tot een mediane stijging naar 800 cellen/mm^3 na acht jaar virologisch succesvolle behandeling - een waarde die vergelijkbaar is met normale waarden bij niet-geïnfecteerden. De stijging is minder wanneer cART wordt gestart bij een lager CD4-celaantal (550 cellen/mm^3 na acht jaar virologisch succesvolle cART-behandeling als cART was gestart bij een CD4-celaantal tussen 50 en 200 cellen/mm^3) en de viral load tijdens de cART-behandeling langdurig boven de 50 kopieën/ml ligt.

Op dit moment kan ongeveer de helft van de patiënten drie jaar lang het initiële regime blijven gebruiken. Hoewel de incidentie van therapieveranderingen door toxiciteit meer dan gehalveerd is sinds de introductie van cART in 1996, is toxiciteit nog steeds de belangrijkste reden om te stoppen (in 49% van therapie stops in 2011). Door de introductie van minder toxische anti-HIV-geneesmiddelen is het percentage patiënten dat stopt met cART vanwege lipodystrofie, huiduitslag of nierinsufficiëntie sinds 2006 afgenomen. Nu zijn misselijkheid en diarree de meest gemelde korte termijn bijwerkingen waardoor middelen worden gestopt.

Uit onze resultaten van het monitoringprogramma over het effect van cART in de populatie van geïnfecteerde volwassenen kan worden geconcludeerd dat het aantal HIV-testen bij heteroseksuelen die een hoog risico lopen op een HIV-infectie verhoogd moet worden om een tijdige start met cART-behandeling mogelijk te maken in deze groep. Zowel de effectiviteit als de verdraagbaarheid van cART zijn in de loop van de tijd zodanig verbeterd dat de meerderheid van de patiënten jarenlang hetzelfde eerstelijnsregime kan blijven gebruiken. Het is belangrijk om al bij een hoog CD4-celaantal (≥350 cellen/mm^3) met cART te starten omdat pas na 8 jaar continue virusonderdrukking CD4-celaantallen behaald kunnen worden die vergelijkbaar zijn met die in de niet-geïnfecteerde bevolking. Monitoring van de viral load is cruciaal voor alle HIV-geïnfecteerden onder behandeling omdat de therapietrouw met het stijgen van de CD4-celaantallen kan afnemen. Behandelregimes moeten worden afgestemd op het individu om toxiciteit te verminderen, met name bij ouderen en vrouwen. Behalve het monitoren van veranderingen in CD4-celaantallen is het van belang om veranderingen in de viral load te monitoren om patiënten die risico lopen op therapiefalen te identificeren en om het effect van nieuwe middelen en assays te kunnen beoordelen.

CART-respons bij zwangere vrouwen

In deze paragraaf beschrijven we de virologische respons op behandeling met cART bij zwangere vrouwen. Bij 395 vrouwen (39%) werd de cART-behandeling gestart vóór de zwangerschap en bij 616 vrouwen tijdens de zwangerschap. Bij vrouwen die met cART startten vóór hun zwangerschap waren het CD4-celaantal en de viral load significant lager dan bij vrouwen die tijdens de zwangerschap startten. Op het moment van de bevalling hadden 74 vrouwen (9%) een detecteerbare HIV-RNA-spiegel. Zes maanden na het starten van de cART-behandeling had 86% van de vrouwen een virologische succes bereikt (twee opeenvolgende HIV-RNA-metingen < 50 of 500 kopieën/ml). De sterkste respons werd gezien bij vrouwen die tijdens hun zwangerschap met cART waren gestart, zowel in de periode 2001-2006 als na 2006. Achttien procent van alle vrouwen faalde op de behandeling na initiële virologische suppressie. Therapiefalen kwam het meest voor bij vrouwen die tijdens de zwangerschap startten met cART. Een verminderde therapietrouw na de bevalling zou de oorzaak kunnen zijn van het verhoogde risico op virologisch falen na de bevalling. Interventies die de therapietrouw van HIV-geïnfecteerde vrouwen na de zwangerschap bevorderen zijn nodig om virologisch falen na de zwangerschap te voorkomen.

De respons op cART bij kinderen wordt in dit rapport apart behandeld in Hoofdstuk 5.

Response to cART in adults

The primary goal of combination antiretroviral therapy (cART) is to prevent HIV disease progression [128]. Studies in HIV-serodisconcordant heterosexual couples have shown that when HIV RNA levels are lower, transmission of HIV is less common [129-131]. A recent randomized controlled trial in HIV-serodisconcordant couples in which the HIV-infected partner had a CD4 count between 350 and 500 cells/mm^3 showed that immediate antiretroviral therapy (ART) as compared to deferred ART is effective in preventing transmission of HIV [132]. Thus, besides prevention of disease progression, a secondary goal of cART is to prevent HIV transmission. US guidelines for when to start cART have been changed accordingly, and starting cART is now recommended for all HIV-infected individuals, which implies that all HIV-infected individuals should start treatment immediately after diagnosis. The strength of the recommendation varies according to the latest CD4 cell count. Evidence for starting cART with 350 CD4 cells/mm^3 or less comes from several observational studies and a randomized trial. Evidence of the benefit of cART when started between 350 and 500 CD4 cells/mm^3 comes only from observational studies. Observational studies have reported conflicting results regarding the benefit of starting cART when the CD4 count is still above 500 cells/mm^3 [133-135]. However, as untreated HIV increases the risk of several non-AIDS defining diseases, newer drugs are generally better tolerated, and early cART exerts a beneficial effect on prevention of HIV transmission, it is recommended that cART be started when CD4 counts are 500 cells/mm^3 or higher. A disadvantage of an earlier start of cART is a longer exposure to antiretroviral drugs. Some drugs are associated with development

of cardiovascular disease [56, 136, 137], loss of bone density [138-140], renal disease [59, 98, 141] and liver disease [142, 143]. Short-term toxicity is less frequent with newer drugs [144-146], but certain complications may take a long time to emerge. Another disadvantage of an early start of cART is the extended inconvenience of taking daily lifelong medication. When individuals do not yet experience life-limiting effects of HIV infection and have high CD4 cell counts, this inconvenience and occurrence of therapy-induced adverse events may lead to less than optimal adherence to therapy [147], possible emergence of resistant mutations [148-150], virological failure and disease progression [151]. In this chapter we describe trends over time in the virological and immunological response, tolerability of cART and trends in the emergence of adverse events and signs and symptoms of toxicity, according to demographic and clinical characteristics, including CD4 cell count, at the start of cART.

Demographic and clinical characteristics at the start of cART

Of the 19,577 patients with an HIV-1 infection and a known date of diagnosis registered by Stichting HIV Monitoring (SHM), 16,149 were 16 years of age or older when they started cART between January 1995 and December 2011. Of these, 2535 were mono- or dual ART-experienced at the start of cART, and 13,614 were ART-naïve. We divided patients according to starting year of cART: 5202 started between 1995 and the end of 2000, 5063 between 2001 and the end of 2006, 4870 between 2007 and the end of 2010 and 1014 started in 2011 (*Table 3.1*). Patients starting in 2012 were not included, as follow-up for these patients was too short to report a virological and immunological response to cART.

Table 3.1: *Baseline characteristics of 16,149 patients starting combination antiretroviral therapy (cART) between 1 January 1995 and 31 December 2011.*

	Year of starting cART							
	1995–2000		2001–2006		2007–2010		2011	
	N	%	N	%	N	%	N	%
Total	5202		5063		4870		1014	
Male gender	4271	82.1	3648	72.1	4006	82.3	874	86.2
Transmission risk group								
MSM	3123	60.0	2299	45.4	3062	62.9	695	68.5
Heterosexual contact	1332	25.6	2163	42.7	1465	30.1	260	25.6
IDU	364	7.0	177	3.5	75	1.5	5	0.5
Blood or blood products	109	2.1	74	1.5	30	0.6	7	0.7
Vertical transmission			1	0.0	5	0.1		
Unknown	274	5.3	349	6.9	233	4.8	47	4.6
Region of origin								
Netherlands	3239	62.3	2378	47.0	2945	60.5	656	64.7
W-Europe/N-America/Australia	597	11.5	370	7.3	325	6.7	59	5.8
Caribbean/S-America	484	9.3	634	12.5	525	10.8	108	10.7
Sub-Saharan Africa	567	10.9	1268	25.0	663	13.6	106	10.5
Other	315	6.1	413	8.2	412	8.5	85	8.4

	Year of starting cART							
	1995–2000		2001–2006		2007–2010		2011	
	N	%	N	%	N	%	N	%
AIDS diagnosis at the start of cART	1729	33.2	1399	27.6	867	17.8	134	13.2
HCV*								
Negative	3909	75.1	4131	81.6	4183	85.9	896	88.4
Positive	483	9.3	394	7.8	398	8.2	58	5.7
Unknown	810	15.6	538	10.6	289	5.9	60	5.9
HBV**								
Negative	4404	84.7	4443	87.8	4384	90.0	913	90.0
Positive	378	7.3	344	6.8	295	6.1	46	4.5
Unknown	420	8.1	276	5.5	191	3.9	55	5.4
Other drug class next to NRTI in initial regimen								
NNRTI	780	15.0	2648	52.3	3398	69.8	615	60.7
PI	4302	82.7	1940	38.3	1171	24.0	328	32.3
NNRTI+INSTI			1	0.0	29	0.6	12	1.2
PI+INSTI					30	0.6	6	0.6
INSTI					49	1.0	23	2.3
Other***	120	2.3	474	9.4	193	4.0	30	3.0
Daily frequency of initial regimen								
1	37	0.7	1472	29.1	2609	73.6	1951	83.4
2	2362	45.4	3426	67.7	899	25.4	365	15.6
3	2632	50.6	100	2.0	13	0.4	2	0.1
≥4	111	2.1	26	0.5	3	0.1	2	0.1
Unknown	60	1.2	39	0.8	22	0.6	18	0.8
ART-experienced at start cART	2132	41.0	308	6.1	85	1.7	10	1.0
cART started during pregnancy	92	1.8	394	7.8	192	3.9	29	2.9
cART started during primary infection	129	2.5	289	5.7	289	8.2	283	12.1
Previously tested for HIV (within 1.5 yrs before HIV diagnosis)	371	7.1	453	8.9	1011	20.8	293	28.9
	Med	IQR	Med	IQR	Med	IQR	Med	IQR
Age at starting cART (years)	37.8	32.2–44.5	37.9	31.5–45.2	40.8	33.4–47.7	40.9	33.0–48.8
CD4-cell count at start cART (cells/mm^3)	200	80–350	190	80–280	254	180–332	310	200–380
HIV RNA at start cART (log_{10} cps/ml)	4.80	4.10–5.30	5.00	4.45–5.34	4.94	4.38–5.35	4.93	4.44–5.40

Legend: cART= combination antiretroviral therapy; MSM=men having sex with men; IDU=injecting drug use; W-Europe=Western Europe; N-America=North America; HCV=hepatitis C virus; HBV=hepatitis B virus; NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor; INSTI=integrase strand transfer inhibitor; Med=median; IQR=interquartile range.

* *Hepatitis C RNA or if absent, antibody.*

** *Hepatitis B surface antigen.*

*** *Other includes regimens including only NRTIs, regimens including both PIs and NNRTIs and other combinations.*

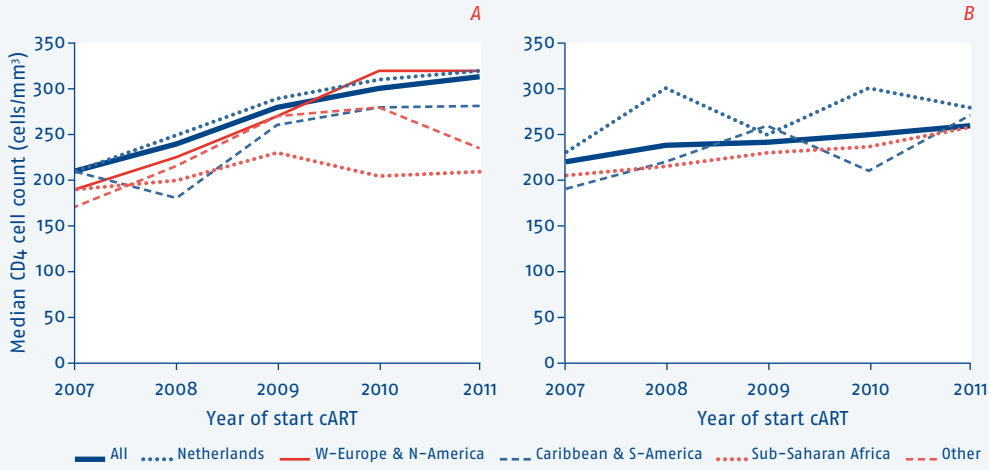
Of the 16,149 patients 16 years of age or older who started cART between January 1995 and December 2011, 3350 were women (21%), and of those, 26% were from the Netherlands. Of the 1014 patients who started cART in 2011, 695 (69%) were infected through homosexual contact, a higher percentage than between 2007 and 2010 (63%, difference p=0.0006). Among the 108 patients from the Caribbean or of South American origin who started cART in 2011, 41 individuals were from the former Netherlands Antilles (38%) and 59 from Surinam (55%). The 85 individuals from other regions of origin who started in 2011 were from Central and Eastern Europe (n=36), Southeast Asia (n=33), North Africa and the Middle East (n=11), and Oceania and Pacific (n=3); for 2 patients, the region of origin was unknown. Out of 140 women who started cART in 2011, 29% were born in the Netherlands.

CD4 cell count at the start of cART

The CD4 count threshold below which cART should be started increased to 350 cells/mm^3 in 2007 and 500 cells/mm^3 in 2009, per the US DHHS treatment guidelines, which are followed in the Netherlands. Currently cART is recommended for all HIV-infected patients [152, 153]. Likewise, the median CD4 count at the start of cART increased from 210 cells/mm^3 in 2007 to 300 cells/mm^3 in 2010 and to 310 cells/mm^3 in 2011 (Cuzick test for trend p<0.0001). Also, the percentage of patients with an AIDS diagnosis at the start of cART declined over time (test for trend p<0.0001). Of patients starting cART in 2011 with a known CD4 count, 37% had a count of 350 cells/mm^3 or more, which was an increase from 2010 (30%, p=0.002). Among women, the median CD4 count at the start of cART increased from 220 cells/mm^3 in 2007 to 260 cells/mm^3 in 2011. The increase was more pronounced among men; it rose from 210 cells/mm^3 in 2007 to 310 cells/mm^3 in 2011. The increase was strongest among men from the Netherlands, Western Europe and North America, most of whom were infected through homosexual contact. The increase was lowest among women and sub-Saharan African men.

In an adjusted logistic regression analysis among patients starting cART in 2011, the probability of starting with 350 cells/mm^3 or more was lower among heterosexually infected individuals than among men who have sex with men (MSM), odds ratio [OR] 0.53 (95% confidence interval [CI] 0.34-0.84, p=0.006), among individuals from the Caribbean and South America, and among individuals from other regions, OR 0.60 (95% CI 0.37-0.98, p=0.04) and OR (95% CI 0.37-0.98, p=0.04), respectively, compared to Dutch individuals. Once the model was further adjusted for individuals repeatedly tested (defined as having a negative test at 1.5 years prior to HIV diagnosis), transmission risk group and region of origin were no longer significant. Repeated testing for HIV is less frequent in the heterosexual population compared to the homosexual population (*Chapter 1*). It has been shown that repeated testing for HIV may lead to a diagnosis at a less advanced stage, making a timely start of cART more likely [31]. HIV testing is part of routine sexually transmitted infection (STI) screening unless patients decline. The most frequently reported barriers for HIV testing are fear of the test result and no perceived risk for HIV infection [8, 154, 155].

Figure 3.1: Median CD4 cell count at the start of cART (2007–2011) according to region of origin for men (A) and women (B).



Starting regimens

The percentage of patients starting a boosted protease inhibitor (PI)-containing regimen increased from 25% in 2010 to 33% in 2011. The percentage of patients starting on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen declined from 69% to 62%. The four most frequently used starting regimens in 2011 were tenofovir, emtricitabine combined with efavirenz (50%), darunavir/ritonavir (15%), atazanavir/ritonavir (10%) or nevirapine (10%). The fixed-dose combination of tenofovir and emtricitabine was used in 92% of all starting regimens in 2011. The fixed-dose combination of abacavir and lamivudine, currently recommended as the starting regimen in patients with 100,000 HIV RNA copies or less, was used in 2011 in only 2% of starting regimens.

Over time, daily dosing of the initial regimen has shifted from three times daily (54% amongst patients starting cART between 1995 and 1999) to once daily (83% amongst those starting in 2011). All recommended first-line regimens (efavirenz/tenofovir/emtricitabine [NNRTI-based], ritonavir-boosted atazanavir/ tenofovir/emtricitabine or ritonavir-boosted darunavir/ tenofovir/emtricitabine [both PI-based]) are once-daily regimens [153]. Once-daily regimens have been associated with a modestly better adherence compared to twice-daily regimens [156]. Since 2009, the integrase inhibitor raltegravir, not recommended in starting regimens because it needs to be taken as part of a twice-daily regimen, was used in 4% of starting regimens.

In summary, patients currently start cART with higher CD4 cell counts than ever before. The increase in CD4 counts at the start of cART from 2007 onwards was strongest among MSM and less strong among men from sub-Saharan Africa and among women. As cART is currently recommended for all HIV-infected individuals, the diagnosis of HIV in an early stage is of high importance. Repeated testing for HIV will inevitably result in an early diagnosis and will make a timely start of cART more likely. Testing rates are especially low in the heterosexual transmission risk group. Fear of a positive test result is the most important barrier for testing for HIV in high-risk populations [8, 154, 155].

Short-term virological response

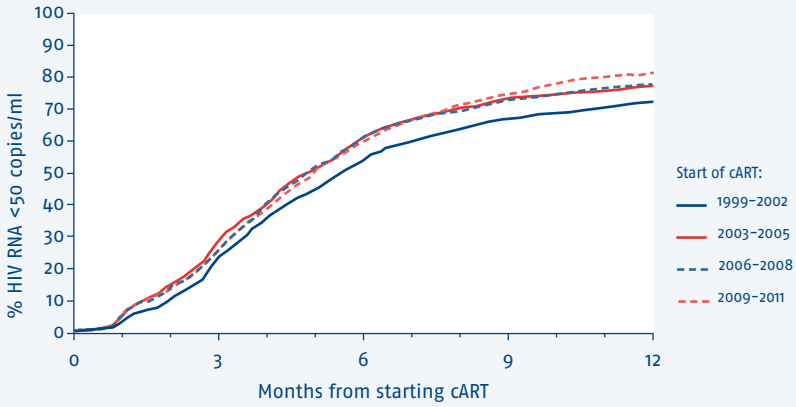
The short-term virological response to cART is an important marker for longer-term clinical outcome. We therefore monitor the time to virological suppression to below 50 copies/ml during the first year after the start of cART. We included data from 8608 patients with at least two plasma viral loads measured with an assay with a lower detection limit of 50 copies/ml or less after the start of cART. Overall, the percentage of patients with initial virological suppression to below 50 copies/ml (first of 2 consecutive measurements <50 copies/ml) increased from 59% (95% CI, 58-60%) at 6 months to 72% (71-73%) at 9 months and 77% (76-78%) at 12 months. The percentage of patients with a plasma viral load less than 50 copies/ml 9 months after starting cART was 67% (95% CI, 64-69%) between 1999 and 2002, 73% (71-75%) between 2003 and 2005, 72% (95% CI 71-74%) between 2006 and 2008 and 75% (95% CI 73-75%) between 2009 and 2011 (*Figure 3.2*). Differences in time to initial suppression after the start of cART across the four periods were small, but significant (overall log rank test p<0.0001).

These results were influenced by the increasing use of the Roche COBAS AmpliPrep COBAS TaqMan HIV-1 assay, version 2.0 (CAP/CTM v2.0) from 2009. The CAP/CTM 2.0 assay was used as the standard test to determine the viral load in 29% of patients starting cART in 2009, with an increase to 57% in 2011. The assay has been known to give higher results of plasma viral load when the viral load is at levels close to the lower detection limit [157]. This also has an effect on the percentage of patients with <50 copies/ml 9 months after starting cART. For those starting cART between 2009 and 2011, the overall percentage of patients with <50 copies/ml was 75%, but it was lower in laboratories where the CAP/CTM v2.0 was the standard assay (71%, 95% CI 68-74%) and higher in laboratories where other assays were used (77%, 95% CI 75%-79%).

To study factors associated with a shorter time to initial suppression to HIV RNA <50 copies, we performed Cox regression using demographic and clinical data from the 8608 patients, as well as data on the daily frequency (once daily, twice daily, and three times daily or more) and type of initial regimen (NNRTI-based, PI-based, protease inhibitors pharmacologically boosted with ritonavir [PI/r]-based, triple nucleoside reverse transcriptase inhibitor [NRTI], and other), use of an integrase inhibitor (yes/no) and type of the viral load assay (CAP/CTM v2.0 vs. other assays). In adjusted analyses, the probability of suppression to ≤50 copies in

patients starting between 2003 and 2005 and between 2009 and 2011 was slightly higher compared to those starting between 2006 and 2008 (both, hazard ratio [HR] 1.09, 95% CI 1.02-1.17, p=0.02). When treatment variables were included in the model, differences between calendar year of starting remained, suggesting that other changes over time also play a role. Starting a regimen that included an integrase inhibitor was significantly associated with a shorter time to suppression (HR 1.39, 95% CI 1.09-1.77). Compared to once-daily NNRTI, starting a twice-daily PI-based regimen or twice-daily NNRTI-based regimen was associated with a longer time to suppression (HR 0.90, 95% CI 0.84-0.97, p=0.005 and HR 0.90, 95% CI 0.82-0.99, p=0.03, respectively). As previously reported, the hazard was lower when the CAP/CTTM v2.0 assay was used to measure viral load (HR compared with other assays 0.85, 95% CI, 0.78-0.93, p=0.0005). In agreement with other studies [158,159], female patients had a shorter time to suppression than male patients (HR 1.12, 95% CI 1.03-1.21, p=0.001), most likely because drug plasma concentration reaches higher levels in women than in men [160, 161]. Time to suppression was inversely associated with age at the start of cART; patients younger than 30 years of age had a longer time to suppression compared to those aged 30-39 years (HR 0.89, 95% CI 0.82-0.96, p=0.002), and patients from sub-Saharan Africa, the Caribbean and South America or North America and Western Europe had a longer time to suppression than patients from the Netherlands (HR 0.90, 95% CI 0.83-0.99, p=0.02, HR 0.92, 95% CI0.85-1.00, p=0.05 and HR 0.89, 95% CI 0.81-0.99, p=0.04, respectively). HIV RNA plasma levels at the start of cART showed, as expected, the strongest association with time to suppression.

Figure 3.2: *Kaplan–Meier estimates of the percentage of patients with initial suppression to <50 copies/ml during the first year after starting combination antiretroviral therapy (cART).*



Independent of HIV RNA, we found an association between CD4 cell count at the start of cART and duration to suppression. Starting cART with CD4 counts of 500 cells/mm³ or more was associated with a longer time to suppression compared to counts between 200 and 500 cells/mm³ (HR 0.83, 95% CI 0.75-0.93, p<0.0001), possibly because of decreased adherence to cART with high CD4 cell counts and a reduced perceived necessity for therapy [147]. Interestingly, there was evidence that the association between CD4 cell counts at the start of cART and time to viral suppression diminished over time (see *Table 3.2*, test for interaction between CD4 cell count and period of starting cART p=0.0003). The HR of starting cART with 500 CD4 cells/mm³ or more compared to starting with a CD4 count of between 200 and 500 cells/mm³ was 0.60 when treatment was started between 2003 and 2005, but the HR increased to 0.98 between 2009 and 2011. As in the therapy guidelines, the threshold of CD4 cell count below which antiretroviral therapy is recommended has increased since 2007. Accordingly, the perception of the necessity for therapy may have changed, and adherence to therapy may have improved in those starting cART with CD4 counts of 500 cells/mm³ or higher.

Table 3.2: Adjusted hazard ratios of time from start of cART to viral suppression to 50 HIV RNA copies/ml or less for different levels of CD4 cell count at the start of cART and for different periods of starting cART.

	Year of starting cART			
CD4 cell count at start of cART (cells/mm³)	**1999–2002** HR (95% CI)	**2003–2005** HR (95% CI)	**2006–2008** HR (95% CI)	**2009–2011** HR (95% CI)
<200	1.04 (0.91–1.20)	1.02 (0.91–1.15)	0.95 (0.87–1.05)	0.81 (0.72–0.90)
200–500 (ref)	1.00	1.00	1.00	1.00
≥500	0.80 (0.62–1.03)	0.60 (0.46–0.78)	0.83 (0.67–1.04)	0.98 (0.83–1.15)

Long-term virological response

After initial virological success, more than 30% of patients on cART experienced episodes of viraemia [162]. Monitoring of longer-term virological response is of importance as high-level viraemia has been associated with a poorer clinical outcome and smaller increases in CD4-cell count [162-164], but low-level viraemia seems to be of limited clinical significance. Short-term low-level viraemia was not associated with AIDS, non-AIDS defining events, death or response to CD4 cell count [6, 162, 165-167]. It is thought that release of virus from latently infected cells rather than ongoing viral replication is what mostly occurs at this level of HIV RNA [168]. Furthermore, short-term low-level viraemia is assay-dependent [169] and has been found more frequently when new assays with a lower limit of detection have been introduced [170, 171].

However, frequent or persistent periods of low-level viraemia have been reported to be associated with treatment failure and emergence of drug resistance [172, 173]. Also, detectable plasma viral load (<50 copies/ml) measured with more sensitive viral load assays was associated with a lower probability of sustained virological suppression [174]. Here we report on the long-term virological response in the cART-treated population.

Figure 3.3: Percentage of patients with a plasma HIV RNA concentration <50 and between 50 and 500 copies/ml at month 9, 12, 18 and at every 6 months of follow-up thereafter. Only plasma samples measured with assays with a lower detection limit of ≤50 copies are included. Plot A shows results from all patients after starting combination antiretroviral therapy (cART) and plot B shows a subgroup of patients continuously on cART, allowing for a therapy interruption of <2 weeks. In total, 8720 patients starting cART were included, but this number diminished over time due to differences in length of follow-up.

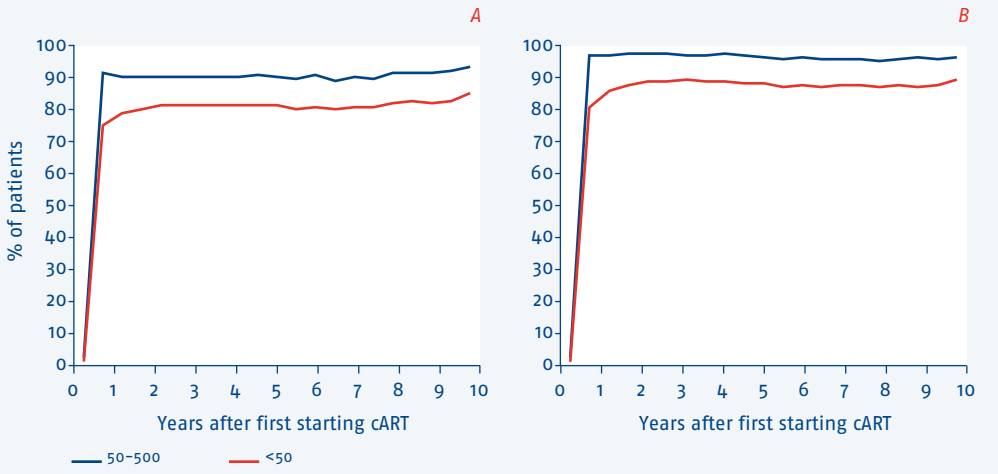


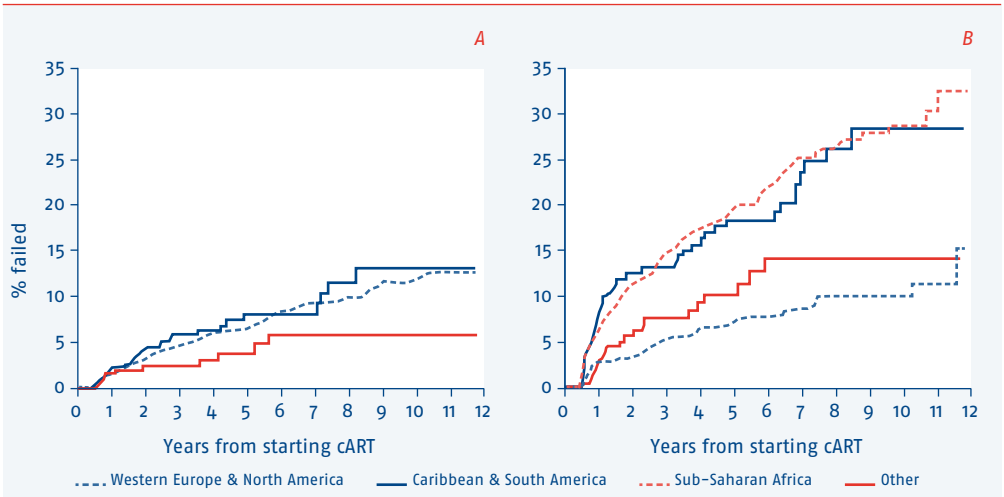
Figure 3.3 shows that 82% to 84% of the patients had a plasma viral load <50 copies/ml after week 36 from the start of cART. This percentage was 88% to 90% for those continuously on cART. After 9 years these percentages seemed to increase slightly. This is probably because of selection of patients who do well and remain in follow-up.

As these plots do not show the total percentage of patients with virological failure over time, we also analysed time to virological failure using Cox proportional hazard models. Virological failure was defined as time to the first of two consecutive plasma viral HIV RNA levels >200 copies/ml after 24 weeks on antiretroviral therapy, as defined in the US guidelines [13]. cART interruptions shorter than 2 weeks did not count as an interruption. In total, 664 patients (8.7%) out of 7625 who started cART from 1999 onwards met the definition of virological failure.

Twelve years after first starting cART, 17% (95% CI 15-19%) of patients had experienced virological failure. Among MSM, there were no significant differences in time to virological failure between patients from Western Europe (including the Netherlands) and North America, the Caribbean and South America, and other regions of origin (plot A in *Figure 3.4*, overall logrank p=0.10). However, among the heterosexual risk group, the risk of failure was

higher among patients from sub-Saharan Africa and the Caribbean and South America compared to Western Europe and North America and other regions (plot B in *Figure 3.4*, overall logrank p<0.0001). At 12 years, the risk of failure in heterosexually infected patients (13.7%) and MSM (12.2%) from Western Europe and North America was comparable (logrank p=0.75).

Figure 3.4: *Kaplan–Meier estimates of the percentage and 95% confidence intervals (CI) of patients with virological failure according to transmission risk group (A: men who have sex with men, B: heterosexual) and region of origin.*



In adjusted analyses, the risk of failure decreased with later calendar years of the start of cART (HR 1999-2004 and 2005-2011 0.48, 95% CI 0.40-0.58, p<0.0001), and with older age at the start of cART (HR per 10-year increase 0.81, 95% CI 0.74-0.89, p<0.0001). Those with a higher viral load at the start had an increased risk of failure (HR >100,000 copies/ml compared to 10,000-100,000 1.39, 95% CI 1.15-1.68, p=0.0007). An increased risk was also found in patients with high CD4 counts at the start (HR >500 cells/mm^3 compared with 200-500 cells/mm^3 1.49, 95% CI 1.08-2.05, p=0.02). Furthermore, there was no evidence that the higher risk of virological failure, with CD4 cell counts of 500 cells/mm^3 or more at the start of cART, had changed with later calendar years as one perhaps might expect because of the higher CD4 cell count threshold for cART in more recent years. Low CD4 counts were also associated with an increased risk of virological failure (HR <200 cells/mm^3 compared with 200-500 cells/mm^3 1.51, 95% CI 1.25-1.82, p<0.0001), most likely due to lesser adherence to antiretroviral drugs in those starting with low CD4 cell counts. Risk of failure was not significantly different between men and women after adjustment for transmission risk group and region of origin.

The lower risk of virological failure in later calendar years after the start of cART may be partly attributable to availability of newer, better tolerated drugs that permit easier adherence. Adherence is also better among older patients [175]. Short- and long-term virological response to cART has improved over time. As it is now recommended to treat all HIV-infected individuals regardless of CD4 cell count, it is reassuring that the short-term virological response when cART is started at high CD4 cell counts has improved over time and is not significantly different from the response when therapy is started at intermediate CD4 cell counts. However, a higher rate of virological failure was found after initial success when cART was started at CD4 cell counts >500 cells/mm^3. It is of importance to keep adherence at high levels, and special attention must be paid to patients with high CD4 cell counts, younger patients, and heterosexually infected patients from sub-Saharan Africa and the Caribbean and South America.

Second-line cART

The goal of a second-line highly active antiretroviral therapy (HAART) regimen remains to suppress plasma HIV-RNA below 50 copies/ml [166], because it is thought that drug resistant strains do not emerge below this level [176], although it appears to be possible [177]. Persistent low-level viraemia, defined as between 50 and 1000 copies/ml [178], and also between 50 and 500 copies/ml [179], is associated with drug resistance, although it is not clear whether this is due to emergence of archived mutations or new resistant mutations generated during viral evolution. After confirmation of virological failure, therapy should be changed as soon as possible to avoid accumulation of resistance mutations. The new regimen should contain at least 2, but preferably 3, active drugs. Apart from results of resistance testing, previous drug history should also be considered when selecting the new regimen because of viruses that may have reverted back to wild-type [13].

In *Chapter 4* a more general discussion on virological failure and transmission of drug-resistant virus can be found. Our objectives in this section are to describe characteristics of patients who start a second-line regimen after experiencing first-line virological failure, to describe the proportion of patients who also experience virological failure on the second-line regimen and to investigate characteristics associated with a poor virological response.

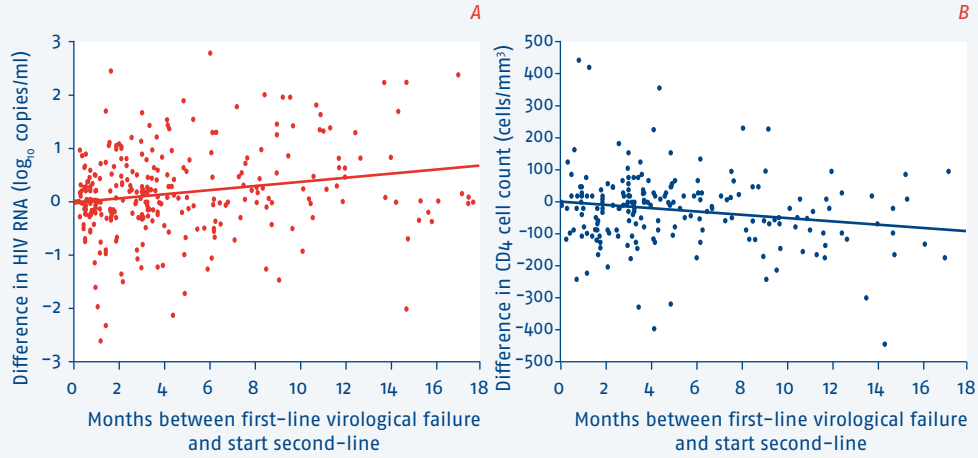
Of the 664 patients who met the definition for virological failure (see previous section on virological failure), 116 did not change the regimen (70 patients [60%] of these failed in or after 2010), 7 patients died, 22 were lost to follow-up, and a further 67 patients had managed to suppress viral load to levels below 200 copies/ml before switching to a second-line regimen (the most frequently recorded reasons in this last group were toxicity [28%] and simplification [27%]). The characteristics of the remaining 452 patients who changed to a new second-line regimen are shown in *Table 3.3*.

Table 3.3: Baseline characteristics at the start of second-line combination antiretroviral therapy (cART) after virological failure in 452 patients.

	N	%
Total patients starting second-line cART	452	100.0
Male gender	309	68.4
Transmission risk group		
Men who have sex with men (MSM)	178	39.4
Heterosexual	226	50.0
Other	48	10.6
Region of origin		
Netherlands	180	39.8
Caribbean and South America	66	14.6
Western Europe and North America	27	6.0
Sub-Saharan Africa	155	34.3
Other	24	5.3
AIDS diagnosis prior to start 2nd line	193	42.7
Hepatitis C virus (HCV)		
Negative	369	81.6
Positive	36	8.0
Unknown	47	10.4
Hepatitis B virus (HBV)		
Negative	383	84.7
Positive	42	9.3
Unknown	27	6.0
Year second-line regimen started		
1999-2002	53	11.7
2003-2005	127	28.1
2006-2008	137	30.3
2009-2012	135	29.9
Resistance test available	288	63.7
	Median	**IQR**
Age at starting second-line cART	39.0	31.5-46.8
Years since start cART	2.8	1.4-4.9
Years since failure	0.4	0.1-1.1
CD4 cell count at starting cART (cells/mm^3)	130	52-260
CD4 cell count at failure (cells/mm^3)	340	210-480
CD4 cell count at start second regimen (cells/mm^3)	300	180-440
HIV-RNA at starting cART (copies/ml)	5.00	4.66-5.51
HIV-RNA at failure (copies/ml)	3.58	2.84-4.56
HIV-RNA at start second regimen (copies/ml)	4.03	2.97-4.75

The majority were men (68%), with more heterosexually infected patients than MSM. The median age at start of second-line cART was 39 years. The second-line regimen was started with a median of 300 CD4 cells/mm^3 and 4.03 $\log_{10}$ HIV RNA copies/ml. Calendar year of starting was uniformly distributed over the period 2003-2012. The median time between first-line cART initiation and start of the second-line regimen was 2.8 years, and it was 5 months (IQR 1-13 months) between virological failure and start of the second-line regimen. *Figure 3.5* shows that the estimated mean increase in HIV RNA between first-line virological failure and start of second-line cART was 0.039 $\log_{10}$ copies/ml/month. Mean CD4 cell count decreased by 5.2 cells/mm^3/month during the same period.

Figure 3.5: *Months between first-line virological failure and the difference between HIV RNA (A) and difference in CD4 cell count (B) at failure and at the start of second-line cART, in the 367 patients who switched within 18 months. The regression lines show a mean increase of 0.039 $\log_{10}$ copies/ml/month in HIV RNA and decrease of 5.2 CD4 cells/mm^3/month between failure and starting second-line cART.*



Compared to the characteristics of all patients who started cART (described in *Table 3.1*), the median CD4 count at the start of cART in patients who started second-line cART after first-line virological failure was low (310 vs. 130 cells/mm^3). This is because virological failure was more frequent before 2005, when cART was initiated at lower CD4 counts. Also, because failure was more frequent among those from sub-Saharan Africa and the Caribbean and South America, median CD4 cell counts at the start of cART in these patient groups were relatively low. In total, 60 patients were using mono- or dual therapy at the time of failure. The most frequently recorded reasons for switching to mono- or dual therapy were patient decision or poor compliance in 21 patients (35%), pregnancy-related choices in 15 patients (25%), and toxicity in 8 patients (13%).

In total, 160 patients started a new NRTI, 153 started two new NRTI's and 11 patients started three new NRTI's. *Table 3.4* gives an overview of switching patterns for the 366 patients who started a new PI or NNRTI in the second-line regimen. Raltegravir was added in the second-line regimen in 25 patients, and this was the only change made in five of them. Maraviroc was started in two patients. Changing from single pills to a fixed-dose combination was not considered as the start of a new drug.

Table 3.4: *Overview of changes in the PI or NNRTI part of the regimen after failure of the first-line regimen.*

				Second-line regimen	
Failing regimen	**NNRTI**	**PI**	**PI/r**	**NNRTI+PI**	**Total**
	N (%)	**N(%)**	**N(%)**	**N(%)**	
NNRTI	9 (7)	4 (3)	107 (80)	13 (10)	133 (36)
PI	16 (36)	3 (7)	16 (36)	9 (20)	44 (12)
PI/r	19 (30)	5 (8)	32 (50)	10 (6)	64 (19)
3 NRTI	41 (55)		17 (23)	17 (23)	75 (20)
Mono-/dual	20 (42)	3 (6)	19 (40)	6 (13)	48 (13)
Other			1 (50)	1 (50)	2 (1)
Total	105 (29)	13 (4)	192 (52)	56 (15)	366

Legend: *NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor, PI/r=ritonavir boosted protease inhibitor; NRTI=nucleoside reverse transcriptase inhibitor.*

When a sequence for resistance testing was available (in 288 [64%] of patients), we used a genotypic resistance interpretation algorithm to infer a drug susceptibility score for each sequence according to a five-level scheme: susceptible, potential low-level resistance, low-level resistance, intermediate resistance and high-level resistance [180].

Of the 288 patients tested, 122 (42%) did not have or had only low-level resistance; 145 (50%) had intermediate or high-level resistance to NRTI (mainly to emtricitabine or lamivudine, see *Chapter 4*), 4 (1%) had both PI- and NNRTI-associated mutations and 17 (7%) had mutations associated with all three major drug classes. Often, viruses with major resistance mutations have reduced replication rates. Patients without or with low-level resistance had a higher mean plasma viral load (3.87 $\log_{10}$ copies/ml (95% CI 3.70-4.04)) compared to those with intermediate/high-level resistance to NRTI (3.50 $\log_{10}$ copies/ml (3.09-3.91; difference p=0.02)) or two or three drug classes (3.60 $\log_{10}$ copies/ml (3.44-3.76); p=0.10)).

We scored the new second-line regimen as the maximum of all of the individual drugs in the second-line combination. According to this score, 124 (44%) patients harboured intermediate or high-level resistant mutations to at least one of the NRTI's (mainly emtricitabine or lamivudine) in the new regimen. Only four patients had intermediate or high-level resistance to one of the PI's or NNRTI's in the new regimen.

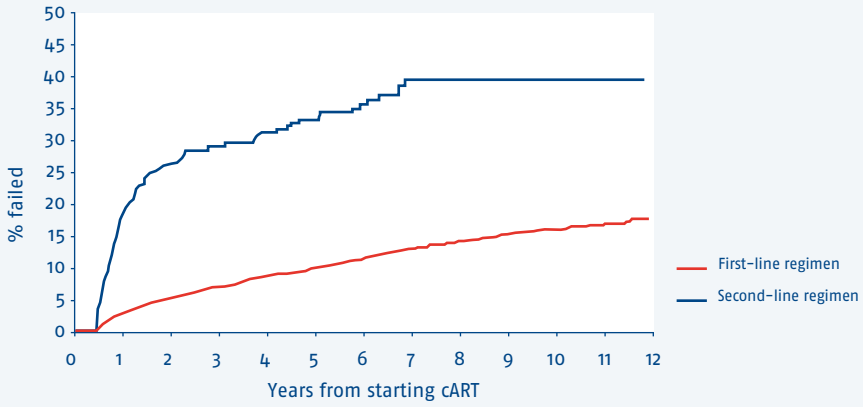
Failure of second-line cART

Out of the 452 patients starting a new second-line regimen, 129 met the definition for virological failure on the second-line regimen. *Figure 3.6* shows that within 1 year after starting the second-line regimen, 18% of the patients had experienced failure again. *Figure 3.6* also shows that the rate of failure on the second-line cART regimen was higher than that on the first-line regimen. This lower virological success rate of second-line regimens can be explained by cross-resistance between antiretroviral drugs in consecutive regimens, but it is more likely due to a selection of patients with less than optimal adherence, which was the cause of the failure of first-line HAART. In an adjusted Cox regression analysis, region of origin was the only variable associated with the risk of virological failure on the second-line regimen. Compared to patients from Western Europe (including the Netherlands) and North America, patients from the Caribbean and South America had a higher risk of failure (HR 1.94, 95% CI 1.19, 3.16; p=0.008), as did those from sub-Saharan Africa (HR 1.67, 95% CI 1.11, 2.52; p=0.01).

Other characteristics that have been found to be associated with first line virological failure, such as low CD4 cell counts or a high plasma HIV-1 concentration and the time between first-line virological failure and start of the second-line regimen, were not significantly associated with second-line failure in our analysis.

The risk was not significantly different between patients with or without an available resistance test (p=0.82). When the analysis was restricted to those with a resistance test, we found an increased risk of virological failure in the four patients with resistance to a PI or NNRTI in the new regimen (HR 2.76, 95% CI 0.65-11.80, p=0.17), but not in those who had resistance to one of the NRTI's in the regimen. This is most likely because there were other, still active NRTI's in the second-line regimen.

Figure 3.6: Kaplan-Meier estimates of the percentage of patients failing a first-line regimen (red line) or second-line regimen (blue line).



Each of the factors that contribute to failure of an initial regimen needs to be addressed before there can be a likelihood of a successful response to any subsequent regimen. Patients who could not adhere to a simple initial regimen are unlikely to manage more complicated second-line regimens. Thus, those with failure on an initial regimen are at high risk of subsequent failure, especially patients from the Caribbean, South America or sub-Saharan Africa. Failure due to pre-existing resistance to any of the drugs in the second-line regimen appears to be of limited significance. A resistance measurement during failure is needed to know if drug resistance has occurred, and if so, which drugs are involved.

Immunologic response

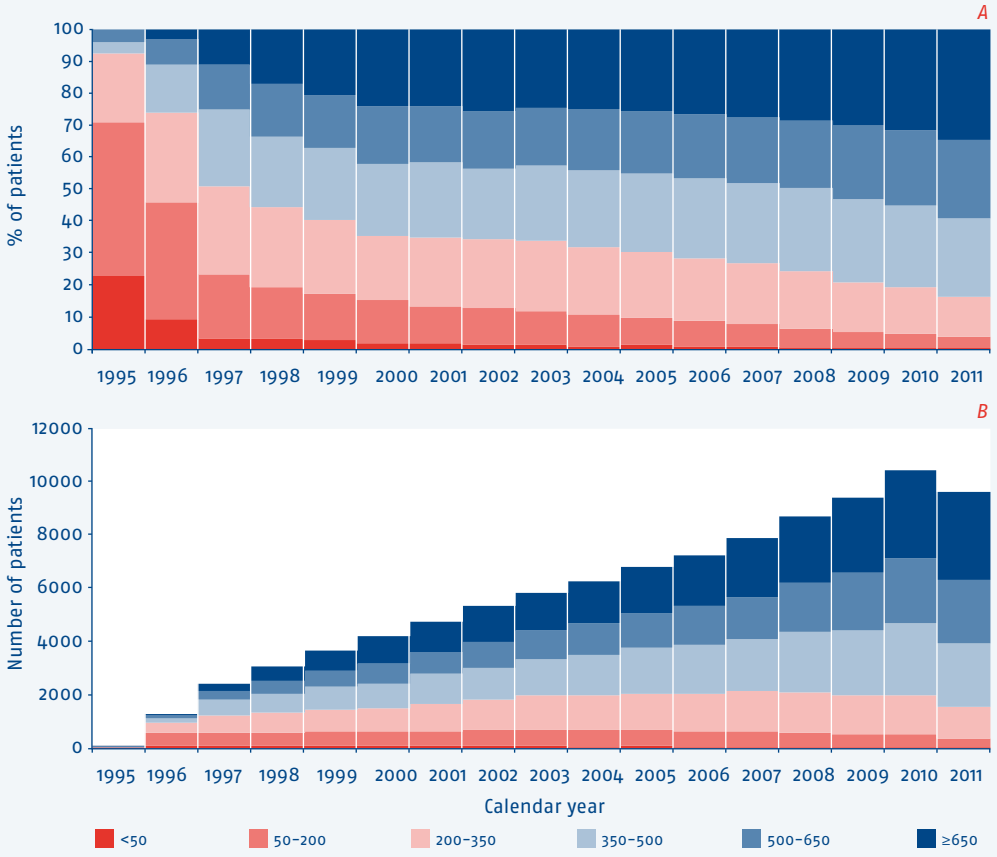
After initiation of cART, most individuals suppress HIV viral load to levels below the detection limit of HIV assays, and this is accompanied by an increase in CD4 cell count. Failure to suppress viraemia is associated with a poorer recovery of CD4 cell count [181, 182]. However, incomplete immunological recovery may also occur when the plasma viral load is below the limit of detection [183]. As the clinical benefit of cART is strongly related to the level of recovery of CD4 cell count (*Chapter 2*) [184-189], we report in this section on the immune status in the treated population in the cART era and long-term CD4 cell count responses after the start of cART.

Immune status in the treated population by calendar year

Figure 3.7 shows the immune status of patients in each calendar year after the start of cART. After starting cART, the percentage of patients with counts <200 cells/mm^3,a level that puts them at higher risk for AIDS, dropped from 46% in 1996 to 5% in 2011. Likewise, the number of patients with low CD4 counts (<200 cells/mm^3) at the end of each calendar year decreased from 710 in 2003 to 526 in 2010. Figures for 2011 should be interpreted with caution as they

are not yet complete. The trend of starting cART with higher CD4 cell counts that was begun in 2007 partly explains the drop in the absolute number of patients with low CD4 cell counts at the end of each calendar year.

Figure 3.7: Last available CD4 cell count (cells/mm³) in each calendar year after the start of cART. The percentage (A) and the number (B) of patients is shown. For each patient the last available CD4 cell count between July and December of each year and after starting cART was selected.



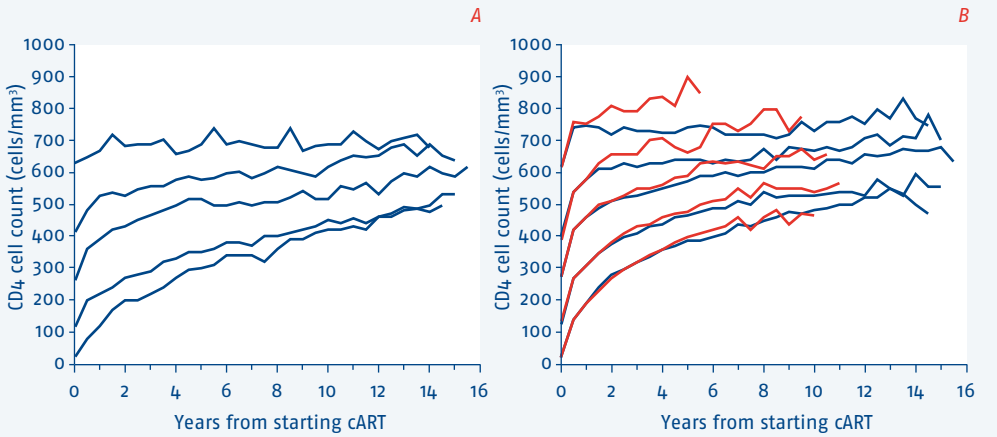
Longitudinal CD4 cell count changes after starting cART

Out of the 16,149 patients who started cART, a CD4 cell count at the start of therapy or thereafter was available for 16,078 (99.6%), and they were included in further analyses. We studied CD4 cell count changes after starting cART in this group, which included both patients on cART and those during a therapy interruption. In patients

who received antiretroviral mono- or dual therapy before starting cART, median CD4 counts increased from 200 cells/mm³ (IQR, 80-340) at the start to 320 CD4 cells/mm³ (IQR, 180-490) at 1 year, 450 (IQR, 280-630) at 5 years, 510 (IQR, 330-730) at 10 years and 550 (IQR, 390-750) at 15 years.

The median CD4 count at the start of cART in ART-naïve patients was slightly higher compared to that in ART-experienced patients, with a median of 230 cells/mm³ (IQR, 100-320) at the start of cART, and there were greater increases: to 400 (IQR, 270-560) at 1 year, 520 (IQR, 370-690) at 5 years, 580 (IQR, 420-780) at 10 years, and 640 (IQR, 470-860) at 15 years. *Figure 3.8* shows the median CD4 cell count after the start of cART stratified by the CD4 count at the start of cART. In the ART-experienced group, median CD4 counts for patients starting cART with <50 and 50-200 cells/mm³ and for those with 350-500 and 500 cells/mm³ or more seemed to converge after 12 years. In the group of patients who started with low CD4 cell counts, this may because patients with a poor immunological response after the start died and only patients who did well remained in follow-up. In the two groups with high CD4 cell counts at the start, this may be because patients reached normal CD4 cell counts and remained at normal levels without a further increase.

Figure 3.8: *Median CD4 count according to the count at the start of combination antiretroviral therapy (cART) in ART-experienced patients (A) and ART-naïve patients (B) determined by CD4 count at the start of cART (<50, 50-200, 200-350, 350-500 and ≥500 cells/mm³). Blue coloured lines show the median CD4 cell counts in all patients after starting cART, including patients on cART and those experiencing a therapy interruption. Red coloured lines in plot B show the median CD4 counts for patients with an initial suppression to <50 copies/ml within 9 months after starting cART and with plasma HIV RNA concentration levels <50 copies/ml thereafter. In this subgroup, CD4 counts were censored at the first of two consecutive measurements of HIV RNA >50 copies/ml after the initial suppression of <50 copies/ml. The trend line was stopped when the number of patients in a subgroup dropped below 50 patients.*



Increases in the initial phase, during the first few months after starting cART, are higher in women and in patients from Western Europe and North America compared to other regions, as well as when pre-cART HIV viral load is higher [181]. Initial CD4 increases are lower with a hepatitis C virus (HCV) co-infection [181]. Increases in CD4 cell count thereafter are known to depend on the level of virological suppression during cART [181, 182, 190, 191], and although the increases are higher in younger patients, they do not seem to depend on other demographic or pre-cART variables [181]. To study the maximum capacity of cART to restore CD4 cell counts, we restricted analyses to therapy-naive patients with continuous viral suppression (<50 copies/ml). Only those patients who had reached HIV RNA levels of <50 copiers/ml within 9 months after the start of cART were included; CD4 cell counts after virological failure (defined as two consecutive viral-load measurements >50 copies/ml) were excluded. Median CD4 counts at 9.5 years were 470 cells/mm^3 for patients starting with <50 cells/mm^3, 553 cells/mm^3 for those starting with 50 to 200 cells/mm^3, 620 cells/mm^3 for those starting with 200 to 350 cells/mm^3 and 775 cells/mm^3 for patients starting with 350 to 500 cells/mm^3 (*Figure 3.8 B*, red coloured lines). Because fewer patients remained in follow-up when cART was started with a CD4 count of 500 cells/mm^3 or higher, numbers for this group are reported only up to 5.5 years. Although median CD4 cell counts fluctuated over time and did occasionally decrease, the trend over time was an increase in these counts in patients succeeding on cART. Median CD4 cell counts for each of the subgroups with 5 CD4 counts at the start of cART did not converge with each other, because a highly select group of patients included only those who continued to suppress viral load, resulting in changes in CD4 cell count that showed the best possible response to cART. For patients starting cART with <50 CD4 cells/mm^3, the median CD4 counts did not differ greatly from those for patients with suppressed viral load. The difference in median CD4 cell counts over time between all patients and those with a suppressed viral load increased with higher CD4 counts at the start of cART. This is because a substantial proportion of patients starting cART with high CD4 cell counts either interrupt therapy or do not maintain a viral load <50 copies/ml whilst on therapy, as shown earlier.

Our results presented in this section show that the mean CD4 count of patients starting cART with 350 to 500 cells/mm^3 after 8 years of virologically successful cART is near normal. Patients starting at CD4 cell counts between 50 and 200 cells/mm^3 reached levels higher than 500 cells/mm^3 after 6 years of continuous virologically suppressive cART, which places them at low risk of AIDS or non-AIDS-defining events, but well below normal levels. Normal CD4 levels in uninfected subjects have been reported to be 1050, 840, and 800 cells/mm^3 for women, heterosexual men, and MSM, respectively [192], with a likely geographic variation in normal ranges [193]. Normal ranges in healthy individuals are also reported to be lower with older age [194-197].

Therapy switches and incidence of toxicity-driven regimen change during the first 3 years after start of cART

Antiretroviral therapy is associated with adverse clinical events and laboratory toxicities. This may lead to poor adherence and treatment discontinuation, which are major reasons for treatment failure and development of resistant strains [198-200]. The avoidance of toxicity is important, as switching to sequential regimens leads to less effective virological suppression than the first-line regimens and a higher number of treatment switches has been associated with smaller gains in CD4 cell count [201, 202]. In this section we report on trends over time in treatment switches and especially treatment-limiting toxicities during the first 3 years after starting cART.

Among patients in follow-up, the percentage of patients who remained on cART after the initial start increased from 63% in 1996 to 94% in 2011 (*Figure 3.9*). The increasing proportion over time may be due to more tolerable drugs and easier dosing schedules. In 2011, 90% of women and 96% of men in follow-up after starting cART were still using cART. This is partly because women with sufficiently high CD4 cell counts stopped cART at the end of pregnancy (see paragraph 'Response to cART in pregnant women' later in this Chapter). Long-term treatment interruption outside the setting of a clinical trial, is not recommended in any patient with HIV infection, regardless of clinical status [13]. Staying on cART is important, as therapy interruptions have been linked to adverse outcomes such as death, opportunistic infection, cardiovascular and renal disease [203, 204]. cART induced suppression of HIV may have a favourable effect on other co-morbidities via suppression of inflammation or pro-inflammatory cytokines or both [205].

Figure 3.9: *Percentage of patients on combination antiretroviral therapy (cART), antiretroviral therapy (but not cART) or not on therapy as of 31 December of each year after starting cART.*

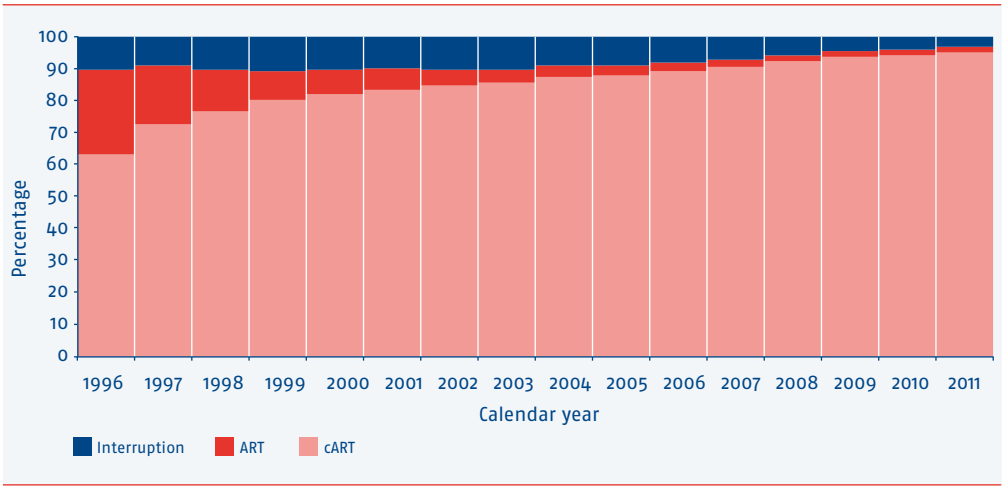
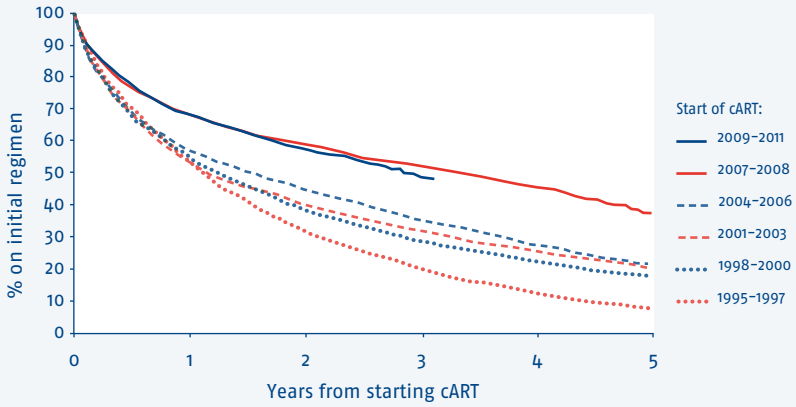
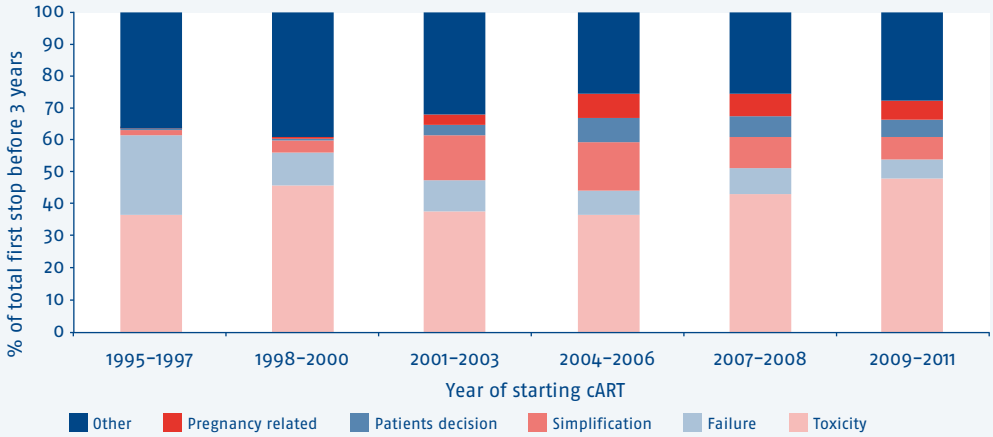


Figure 3.10: Kaplan–Meier estimates of the percentage of patients still on the initial combination antiretroviral therapy (cART) regimen by period of initiation. Planned switches according to protocol and changes to fixed co-formulation dosages were not counted as a regimen change.



There is a clear trend over time towards a longer interval before discontinuation of the initial cART regimen, as *Figure 3.10* shows. Of the 16,148 patients who had started cART, 11,951 discontinued the initial regimen. The percentage of patients still on the initial cART regimen 3 years after starting was 20% (95% CI, 19-22%) for those starting during 1995-1997, 29% (95% CI, 27-31%) during 1998-2000, 32% (95% CI, 30-34%) during 2001-2003, 35% (95% CI, 34-37%) during 2004-2006, 52% (95% CI, 50-54%) during 2007-2008, and 48% (95% CI, 45-51%) for those starting in or after 2009.

Figure 3.11: Relative distribution of reasons for stopping cART within 3 years of initiation among those who stopped the first-line regimen. Failure includes virological, immunological and clinical failure; "other" includes new medication available, pharmacokinetic reasons, precautionary reasons, problems with adherence and unknown reasons.



Overall, the most common reasons for discontinuation were toxicity (39%), treatment failure (12%), patient decision (8%), and simplification (8%), which are similar to results reported by others [206]. *Figure 3.11* shows trends over time in reasons for stopping. Failure was the reason for stopping in 22% among those who had initiated cART 1995-1997, but was lower thereafter (6% of all stops within 3 years of initiating cART in or after 2009 were due to failure). Over time, toxicity has remained the major reason for stopping treatment.

Specific adverse events associated with toxicity-driven therapy changes

As toxicity is the most common reason for discontinuing not only the first regimen but also subsequent regimens, we now focus on trends over time in toxicity-driven therapy changes during the first 3 years of cART. These changes partly reflect the toxicity of cART, but they also reflect the number of treatment options a patient has when experiencing side effects that limit the quality of life.

During the first 3 years after the start of cART, patients were followed for a total of 38,266 person-years (PY); of that number, 37,349 person-years (97.6%) included cART (PYcART). The overall incidence of toxicity-driven regimen changes was 202 (95% CI, 197-206) per 1000 PYcART. Patients could change the regimen more than once. During follow-up, 10,975 of the 16,108 patients (68.1%) did not change the regimen because of toxicity. The maximum number of changes because of toxicity in a single patient was 14.

Table 3.5: Toxicity-driven changes in therapy during the first 3 years after the start of cART. Ninety-five percent confidence intervals (95% CI) for the incidence per 1000 PYcART were obtained by the Poisson distribution. Adjusted estimates of relative risk were obtained with logistic regression models including age, gender, region of origin, transmission risk group, and time after starting cART (0-6, 6-12, 12-24 and 24-36 months).

Year of starting cART	PYcART	N	Incidence per 1000 PYcART (95% CI)	Adjusted relative risk (95% CI)
1995	217	80	368 (292-458)	2.24 (1.64-3.07)
1996	3911	1229	314 (297-332)	1.71 (1.47-2.00)
1997	3124	799	256 (238-274)	1.39 (1.19-1.61)
1998	2107	495	235 (215-257)	1.25 (1.08-1.45)
1999	2059	450	219 (199-240)	1.17 (1.00-1.36)
2000	1890	506	268 (245-292)	1.36 (1.17-1.57)
2001	2094	396	189 (171-209)	1.02 (0.88-1.19)
2002	2000	372	186 (168-206)	0.96 (0.82-1.11)
2003	2051	386	188 (170-208)	1.04 (0.90-1.22)
2004	2162	395	183 (165-202)	1.06 (0.92-1.23)
2005	2250	361	160 (144-178)	1.00
2006	2137	337	158 (141-175)	1.06 (0.91-1.24)
2007	2610	364	139 (125-155))	0.96 (0.83-1.13)
2008	3339	432	129 (117-142)	0.96 (0.82-1.12)
2009	2906	421	145 (131-159)	0.99 (0.85-1.14)
2010	1896	347	183 (164-203)	1.06 (0.90-1.24)
2011	595	167	281 (240-327)	1.15 (0.94-1.39)

***Legend:** cART=combination antiretroviral therapy; PYcART=person-years on cART during the first 3 years following the start of cART; N=number of toxicity-driven therapy changes; CI=confidence interval*

The incidence was highest (519 per 1000 PYcART) during the first 3 months after the start of cART; it declined to 226 per 1000 PYcART between 3 and 6 months, 179 per 1000 PYcART between 6 and 12 months, and 130 per 1000 PYcART between 24 and 36 months (p<0.0001). The incidence of toxicity-driven therapy changes during the first 3 years following cART initiation declined from 2000 to 2008. The increase thereafter can be largely attributed to patients not yet having 3 years of follow-up after starting cART, although in analyses adjusted for timing after starting cART and other confounders, the risk for a toxicity-driven therapy change remained higher in 2010 and 2011 compared to 2008, albeit non-significantly. The risk was higher when weight at the start of cART was lower (p=0.007). The risk was 0.72 times lower in men than in women, independent of weight (95% CI 0.66-0.78, p<0.0001). There was no evidence that the difference between men and women had changed over time (p value for interaction=0.48). Several studies have found evidence that plasma drug levels are higher in women than in men, although not for all drugs [161]. The risk of toxicity was 10% higher when CD4 cell counts were 500 cells/mm^3 or higher (relative risk [RR] compared to 200-350 cells/mm^3 1.10, 95% CI 1.00-1.210, p=0.04).

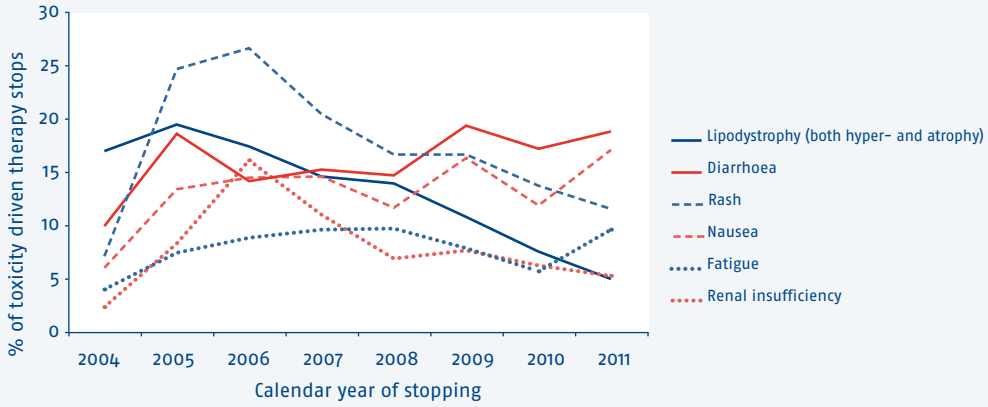
A previous toxicity-driven therapy change was associated with a 46% increased risk of a new toxicity-driven therapy change (95% CI, 39-54%; p<0.0001) compared to no previous change. The most likely explanation for this finding is that some patients had underlying conditions not accounted for in the analysis [207]. The risk increased when patients were older than 40 years at the start of cART. Compared to patients aged 30-40 years, the risk was increased by 12%, 17% and 30% for patients aged between 40-50, 50-60 and 60 years or more, respectively. Older age has been associated with an increased risk for a toxicity-driven therapy change in other reports [208]. Whether this is because of a different pharmacokinetic profile of drugs in plasma in older patients compared to younger patients is not known yet [209]. There was no interaction between age and year of starting. Finally, patients with an HCV co-infection when cART was started had a 25% increased risk (95% CI, 13-37%, p<0.0001) and MSM compared to heterosexually infected patients with a 15% increased risk (95% CI, 7-34%; p=0.0002). MSM may have a higher awareness of other treatment options than heterosexually infected individuals when they experience adverse effects.

We have shown that although the incidence of toxicity-driven therapy changes has declined over time, toxicity remains the major reason for stopping therapy. In the next section, we give an overview of changes in the most frequently recorded adverse events associated with these therapy stops due to toxicity.

Web Appendix Table 3.1 shows that the number of patients with at least one therapy stop due to toxicity-driven change has remained relatively stable between 2004 and 2011. Numbers were highest in 2010 when 997 patients stopped a drug due to toxicity and lowest in 2004 when 842 patients did so. *Figure 3.12* shows the change in distribution of the six most frequently registered adverse events over time. Most remarkable was the absolute and relative decline in treatment-limiting toxicity due to lipodstrophy (both peripheral fat loss and central fat accumulation) from 168 patients in 2005 (19% of all toxicity-driven therapy stops in 2005) to 42 (5%) in 2011. In most of these discontinuations due to lipodystrophy, lamivudine/zidovudine was stopped (in 31%), followed by stavudine (27%) and lamivudine/zidovudine/abacavir (14%). These were replaced by tenofovir/emtricitabine (in 39% of the therapy stops, which were followed by a new cART regimen), emtricitabine/tenofovir (24%) or abacavir/lamivudine (18%). The percentage of stops because of rash (in 27%) or renal insufficiency (16%) was highest in 2006, and both percentages declined thereafter. The percentage of therapy stops because of nausea, diarrhoea and fatigue was relatively stable between 2005 and 2011.

An overview of the number of other adverse events associated with a toxicity-driven therapy stop is given in *Web Appendix Table 3.1*. The percentage of patients who stopped because of a neurological or psychosocial type of adverse event increased from 168 out of 997 patients with a toxicity-driven therapy stop in 2010 (17%) to 286 out of 837 patients in 2011 (34%). Among those 286 patients, efavirenz was stopped due to neurological adverse events in 242 patients (85%). Of the 230 patients (95%) who subsequently switched to another cART regimen, 48% switched to nevirapine, 23% to ritonavir boosted atazanavir, and 13% to ritonavir boosted darunavir.

Figure 3.12: Relative distribution over time of the six most frequently recorded adverse events associated with a toxicity-driven therapy stop of at least one of the drugs in the combination antiretroviral therapy (cART) regimen. For every toxicity-driven therapy stop, one to three adverse events can be recorded; therefore, percentages do not total 100%.



Dyslipidemia in the cART-treated population

An elevated triglyceride level has been associated with an increased risk of myocardial infarction [54] and, together with an increased waist circumference, an increased risk for metabolic syndrome, insulin resistance and cardiovascular risk [210]. As HIV-infected patients continue to age, management of these issues will become increasingly important. Here we report on changes in triglyceride levels and both total and high density lipoprotein (HDL) cholesterol following the start of cART.

Early in the course of HIV infection, both HDL cholesterol and low density lipoprotein (LDL) cholesterol levels decrease, and triglyceride levels increase [211]. After the start of ART, triglyceride levels increase further whilst HDL cholesterol typically remains low and LDL cholesterol increases, usually to levels higher than before HIV infection [54]. The prevalence of hypercholesterolemia (total cholesterol level above 6.2 mmol/l) was 27% in patients on cART that included PIs and 23% of those on cART that included NNRTIs, as compared to 8% in untreated patients. Prevalence for hypertriglyceridemia (triglyceride level above 2.3 mmol/l) was 40% in patients on cART that included PIs, 32% when NNRTIs were included and 15% in untreated patients [136, 211]. Exposure to certain drugs or drug classes has been associated with an increased risk of dyslipidemia [212, 213], but hypertriglyceridema was also commonly observed before the cART era [214]. Severe changes in lipid levels, as well as other metabolic alterations, have been associated with the use of ART, especially with inclusion of PIs. Dyslipidemia has been defined as a total cholesterol level of ≥6.2 mmol/l, HDL cholesterol level of ≤0.9 mmol/l or triglyceride level of ≥2.3 mmol/l or receipt of

lipid-lowering drugs [56], and it may increase the risk of cardiovascular disease [215]. The independent effect of elevated triglyceride levels on the risk of myocardial infarction after adjustment for total and HDL cholesterol seems small. Here we report on changes over time in total cholesterol, HDL cholesterol and triglyceride levels in the cART-treated population.

Figure 3.13: Last available total cholesterol value in each calendar year after the start of combination antiretroviral therapy (cART). Plot A shows the percentage and plot B the number of patients. For each patient the last available total cholesterol measurement between July and December of each year and after the start of cART was selected.

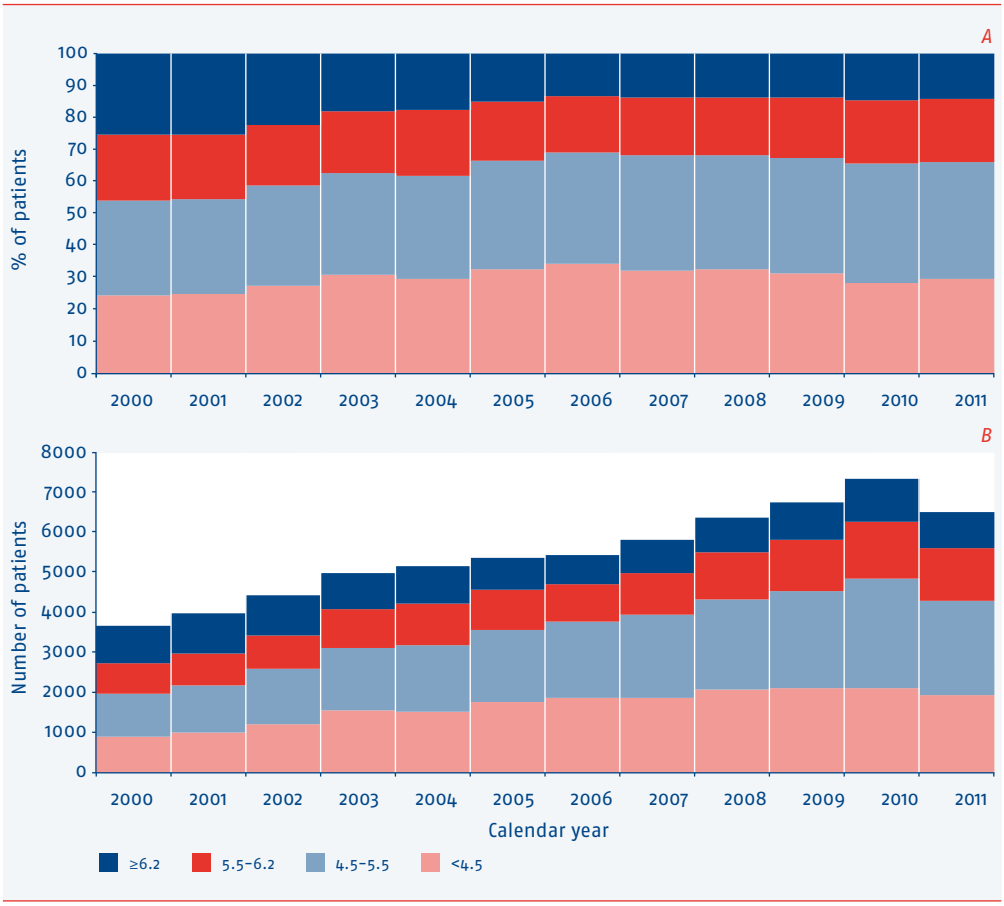


Figure 3.14: Last available total high density lipoprotein (HDL) cholesterol in each calendar year after the start of combination antiretroviral therapy (cART). Plot A shows the percentage and plot B the number of patients. The last available HDL cholesterol measurement between July and December of each year and after the start of cART was selected for each patient.

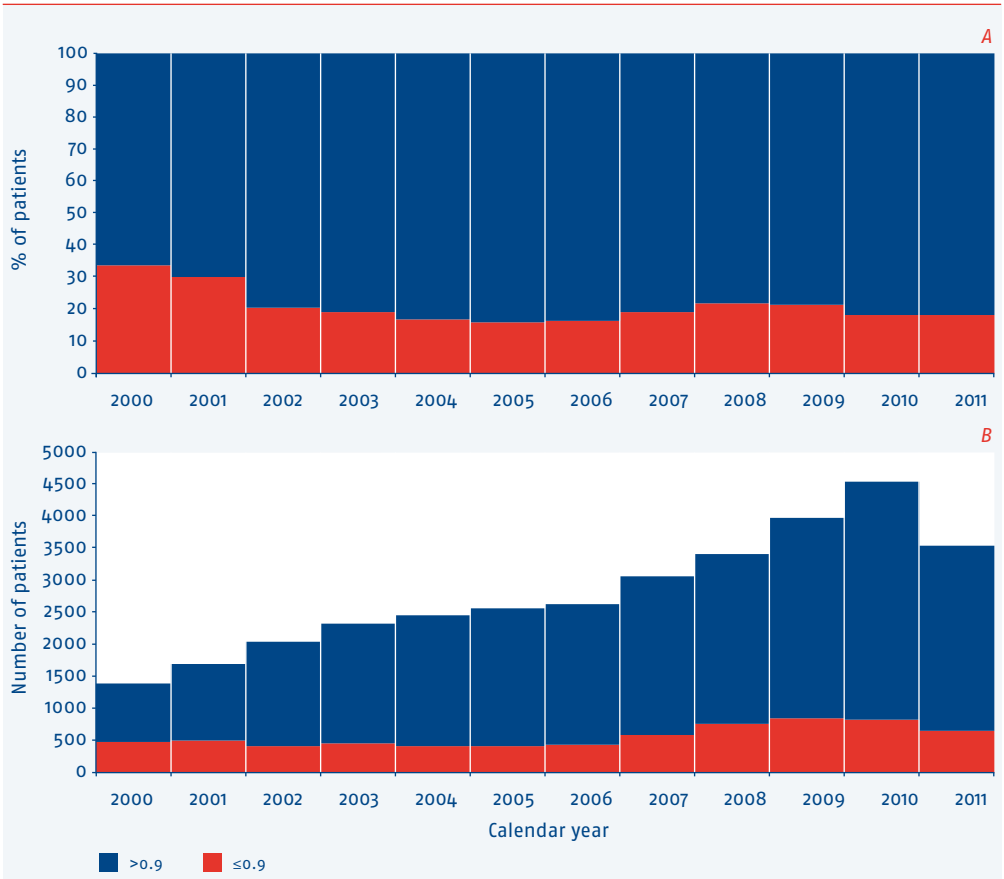
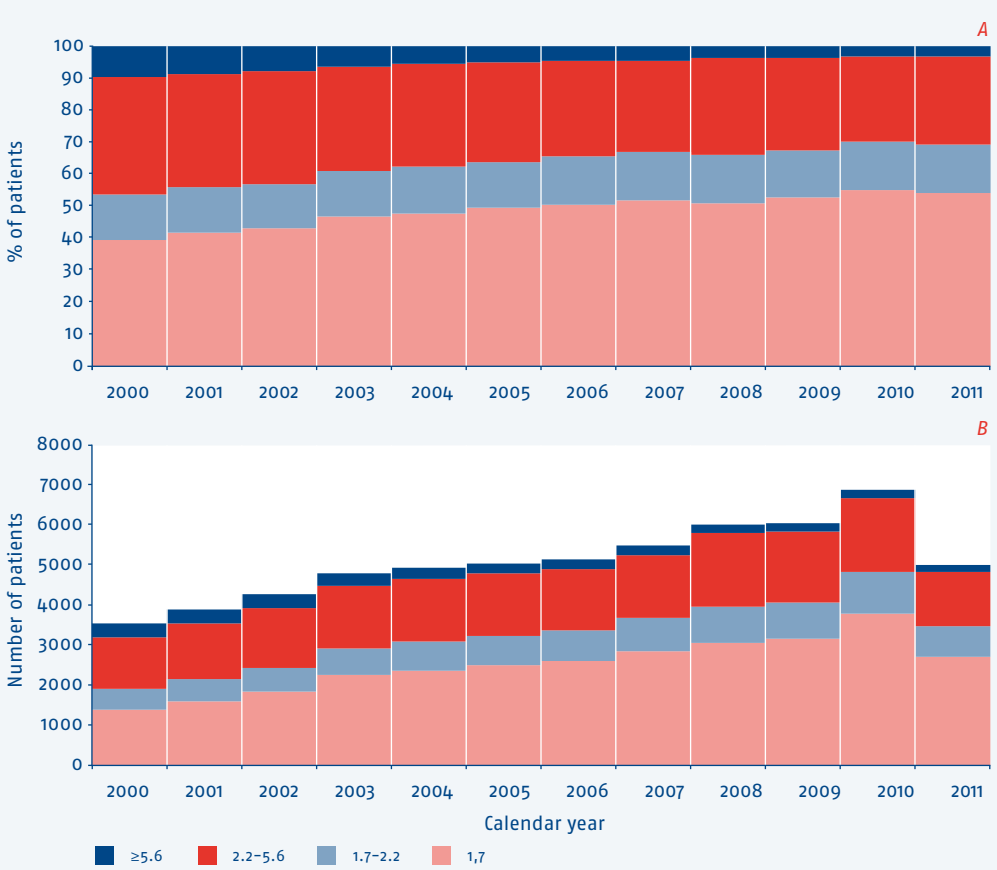


Figure 3.15: Last available triglyceride level in each calendar year after the start of combination antiretroviral therapy (cART). Plot A shows the percentage and plot B the number of patients. The last available total triglyceride measurement between July and December of each year and after the start of cART was selected for each patient. No information on fasting was recorded.

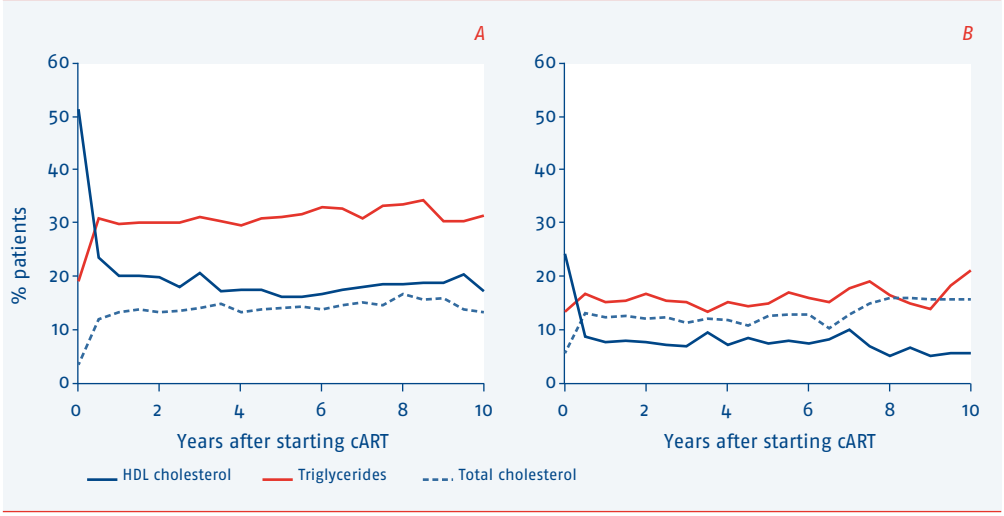


Figures 3.13-3.15 show that the proportion of patients with high total cholesterol and triglyceride levels declined with later calendar years. The percentage of patients with hypercholesterolemia between 2005 and 2011 showed a stable level of 13% to 15% (14% in 2011). Over the same period, the percentage with hypertriglyceridemia declined from 36% to 31%. The number of patients with an available HDL cholesterol measurement is approximately half that of patients with cholesterol or triglyceride levels. The number of patients with low levels of HDL cholesterol increased from 410 in 2004 to 837 in 2009. The proportion of patients with low-level HDL cholesterol was lowest in 2005 and

2006 with 16% of patients; it increased to 21% in 2009 and was 18% in 2010 and 2011. Although the percentage of patients with elevated total cholesterol and triglyceride and low HDL cholesterol levels did not increase over time, the absolute number of patients with these levels did increase. Although elevated lipid levels are associated with longer exposure to ART, *Figure 3.16* shows that the percentage of patients with high total cholesterol and triglyceride and low HDL cholesterol levels did not increase with longer time after the start of cART (apart from the period immediately after the start). This might be because of the substitution of drugs associated with increases in lipid levels for drugs that have a lesser effect on lipids; more frequent monitoring of lipid levels; lifestyle alterations including smoking cessation, diet and exercise; and a clearer understanding of pathways over time. Furthermore, the figure also shows that an increased triglyceride level was the most pronounced lipid abnormality. The percentage of men with abnormal triglyceride and HDL cholesterol levels was twice as high as it was among women.

Other risk factors for cardiovascular disease include hypertension and diabetes mellitus, both of which are associated with older age. An increase in the number and percentage of patients with these conditions has been observed in the Data Collection on Adverse events of Anti-HIV Drugs (D:A:D) study [216]. As the patient population ages, management of patients at risk for dyslipidemia and other risk factors of cardiovascular disease will become increasingly important.

Figure 3.16: *Changes in the percentage of men (A) and women (B) with total cholesterol ≥6.2 mmol/l (blue dashed line), HDL cholesterol ≤0.9 mmol/l (blue solid line) and triglyceride ≥2.3 mmol/l (red solid line) after starting combination antiretroviral therapy (cART) in or after 2000. No information on fasting was recorded.*



Lifelong use of ART requires tolerable and durable regimens, so it is important to study such durability. Approximately 50% of patients currently starting cART can remain on the first-line regimen for more than 3 years. Toxicity remains the main reason for therapy discontinuation, although the incidence of therapy changes driven by toxicity has dramatically declined since the introduction of cART. Patients with high CD4 cell counts, women and older patients had a higher risk for a toxicity-driven therapy change. To overcome these problems, more individualized patient management strategies are needed.

Discussion

In summary, CD4 counts at the time of cART initiation have increased since 2007, with a median of 310 cells/mm^3 in 2011. CD4 cell counts at the start of cART were lower among men from sub-Saharan Africa and women. cART is currently recommended for all HIV-infected patients, and if the goal of antiretroviral therapy is to restore CD4 counts to levels seen in uninfected patients, it is important to start cART with ≥350 CD4 cells/mm^3 or more, as normal cell counts with virological successful cART are approached only after 8 years of continuous therapy. Therefore, HIV testing rates will need to continue to improve, especially in women and sub-Saharan African men. Ensuring a quick suppression of plasma viral load with a maintenance level of <50 copies/ml is important, as high-level viraemia or longer periods of low-level viraemia are associated with smaller CD4 cell count increases, higher probability of treatment failure and development of resistance. The risk of virological failure has declined over time, but it increases when cART is started with higher CD4 cell counts and in younger patients and heterosexually infected patients from sub-Saharan Africa, the Caribbean and South America. Patients from sub-Saharan Africa, the Caribbean and South America continue to be at high risk of failure on subsequent second-line regimens. Measures to improve adherence in these patients are warranted. More individualized therapy strategies might help to reduce the risk of treatment-limiting toxicity in women and older patients. Monitoring of lipid levels in an aging HIV-1-infected population will remain important in identifying patients at higher risk of cardiovascular disease and other serious non-AIDS-defining diseases.

Response to cART in pregnant women

Without intervention, the risk of mother-to-child transmission (MTCT) in HIV-infected pregnant women is 15% to 20% [217]. A detectable HIV RNA level is an important risk factor for MTCT of HIV infection. cART has reduced MTCT rates by successful suppression of maternal HIV RNA levels [16]. In the Netherlands a large proportion of women have been identified with HIV and treated for their infection before pregnancy (*Chapter 1*). However, as also described in *Chapter 1*, a substantial proportion (46%) of the women received an HIV diagnosis during pregnancy and started cART for the first time during this pregnancy to prevent MTCT.

Between 1 January 1998 and 1 June 2012, 1011 women with a registered pregnancy started cART. Women were categorised into two groups according to the time of cART initiation in relation to when they became pregnant: before they became pregnant and during pregnancy. In 395 (39%) of the women, cART was initiated before they became pregnant, and in 616 (61%) women it was initiated during their pregnancy (*Table 3.6*).

At the time of cART initiation, CD4 counts were significantly lower in women who started cART before pregnancy compared to those who started during their pregnancy (p<0.0001). Differences in CD4 counts might be explained by differences in the indications for starting treatment, such as low CD4 counts for women who started cART before they became pregnant, whereas cART has been initiated in all women during pregnancy since the availability of this therapy, regardless of CD4 counts. Also, median HIV RNA levels were significantly lower in women who started cART during their pregnancy compared to women who started cART before they became pregnant (p<0.0001).

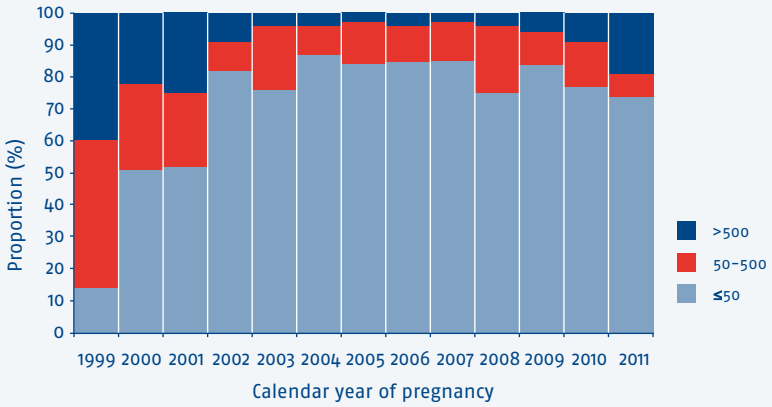
***Table 3.6:** Characteristics of HIV-infected pregnant women aged between 16 and 45 years who initiated combination antiretroviral therapy (cART) between 1 January 1998 and 1 June 2012.*

	cART initiation	
	Before pregnancy	**During pregnancy**
Total	395	616
Median age at start of cART (years, IQR)	29 (24-32)	28 (24-32)
Region or origin		
Netherlands	69 (17)	85 (14)
Other	326 (83)	531 (86)
Calendar year of cART initiation		
≤2000	118 (30)	53 (9)
2001-2007	232 (59)	402 (65)
≥2007	45 (11)	161 (26)
At start of cART		
CD4 cell counts (cells/mm^3, median, IQR)	200 (109-310)	353 (220-513)
HIV RNA levels (log_{10} copies/ml, median, IQR)	4.7 (4.0-5.3)	4.0 (3.3-4.5)
Undetectable HIV RNA plasma levels at cART initiation	16 (4)	29 (5)
At parturition		
CD4 counts (cells/mm^3, median, IQR)	420 (290-570)	460 (296-640)
HIV RNA levels (log_{10} copies/ml)	1.7 (1.6-1.7)	1.7 (1.7-1.9)
Detectable HIV RNA levels	36 (9)	44 (7)

At the time of delivery, 74 women (9%) had a detectable HIV RNA level. An elective caesarean section in women with detectable HIV RNA levels will lower the risk of MTCT [16, 218]. Of the 74 women who had a detectable HIV RNA level at time of parturition, 59 babies were delivered, of which 36 were by caesarean section. *Figure 3.17* shows the percentage of women

over time with an undetectable load at the time of delivery; HIV RNA levels were categorised as <50 copies/ml, 50-500 copies/ml and >500 copies/ml. Overall, 75% of the women had an HIV RNA level <50 copies/ml at the time of delivery, and 16% of the women had an HIV RNA level between 50 and 500 copies/ml. The proportion of women with an HIV RNA level <50 copies/ml increased from 14% in 1999 to 87% in 2004 (p<0.0001). Between 2005 and 2011, the proportion of women with an HIV RNA level <50 copies/ml varied between 74% and 85%. Women who started cART before they became pregnant had an HIV RNA level below 50 copies/ml (79%) relatively more often than women who started cART during their pregnancy (72%) (p=0,003).

Figure 3.17: *Distribution of women with HIV RNA levels <50 copies/ml, 50-500 copies/ml and >500 copies/ml at delivery over time.*



Time to initial virological success

Time from cART initiation to the first of two consecutive plasma HIV RNA concentrations of <50 copies/ml (or 500 copies/ml, depending on the detection border of the HIV RNA assay used) in pregnant women was compared between women who started cART before they became pregnant and those who started cART during pregnancy; the women were further categorised according to the calendar time of cART initiation (≤2000, 2001-2006, ≥2007).

By 6 months after the start of cART, 86% of the women had experienced a virological response (two consecutive HIV RNA levels <50 or 500 copies/ml). The strongest response was observed among women who started cART during their pregnancy after 2007 (92%; 95% CI: 86-96%) and women who started cART during their pregnancy between 2000 and 2006 (91%; 95% CI: 86-94%), with poorer responses among pregnant women who started cART before pregnancy (*Figure 3.18*, p<0.0001). Hazard ratios for the time to initial virological success were significantly higher for the women who started cART from 2001 onwards compared to women who initiated cART ≤2000 (*Table 3.7*).

Figure 3.18: Time to initial viral suppression of HIV RNA to 50 (or 500) copies/ml after the start of cART amongst HIV-infected pregnant women. Pregnant women were divided into those who initiated combination antiretroviral therapy (cART) before pregnancy and those who initiated cART during pregnancy and by calendar time of initiation. The Kaplan–Meier method was used to estimate the time between the start of cART and virological suppression.

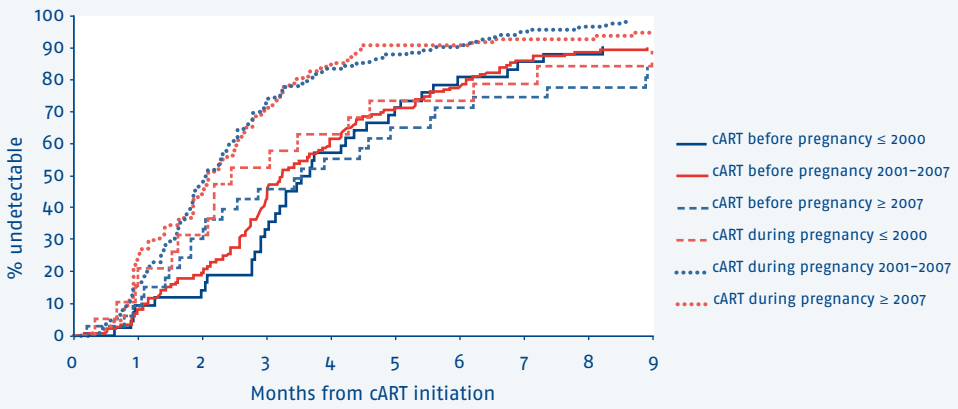


Table 3.7: Risk estimates for the hazard of treatment success. Time from start of combination antiretroviral therapy (cART) until initial viral suppression of HIV RNA to 50 (or 500) copies/ml was analysed with a Cox regression model.

cART initiation:	Hazard ratio*	95% Confidence interval	p-value
Before pregnancy ≤2000	1		<0.0001
Before pregnancy 2001–2007	1.46	(1.11–1.92)	
Before pregnancy ≥2007	1.87	(1.26–2.78)	
During pregnancy ≤2000	1.01	(0.69–1.47)	
During pregnancy 2001–2007	1.58	(1.22–2.05)	
During pregnancy ≥2007	2.52	(1.86–3.41)	

** Adjusted for region of origin, CD4 counts and HIV RNA levels at time of cART initiation.*

Time to virological failure after initial virological suppression

Figure 3.19 shows the time to virological failure after initial suppression amongst pregnant women. Overall, the Kaplan–Meier estimate for the time to virological failure after initial suppression was 18% (95% CI: 15-20%).

The highest failure rates were observed amongst pregnant women who started cART in pregnancy, 23% (95% CI: 13-38) for those who started cART ≤2000, 26% (21-31%) for 2001-2007 and 24% (17-34%) for women who started from 2007 onwards. In women who started cART

before they became pregnant and before 2000, 7% failed after virological suppression (95% CI: 5-12%), and the lowest failure rate was observed among pregnant women who started cART after 2007 before they became pregnant (3%; 95% CI:0.4-18%, p<0.0001). These findings are confirmed by the hazard ratios for the time to virological failure (*Table 3.8*).

When we repeated the analyses by taking into account time since parturition, we found that 6% (95% CI: 3-11%) of the women who started cART before pregnancy reached a detectable HIV RNA level after parturition. Amongst women who started cART during the pregnancy, 39% (95% CI: 33-46) reached a detectable HIV RNA level within 6 months after parturition.

In a total of 644 of the women who started cART in pregnancy, the cART regimen was switched after parturition. Among the women who experienced virological failure after parturition, 81% switched their cART regimen.

The median time between parturition date and date of switch was 2.5 months (IQR: 0.5-15). Most common reasons for switching were end of pregnancy (23%) and simplification of the regimen (17%). Amongst the remaining 97 women who ended cART and did not switch, 24 became lost to follow up and 5 women died, 68 women did not restart cART.

Overall, 1135 genotypic sequences were available for 888 HIV-infected pregnant women. At least one high-level resistance mutation to an NRTI was found in 161 (14%) of the sequences, according to the Stanford interpretation algorithm. A high-level resistance to an NNRTI was found in 135 (12%) of the sequences and a high-level resistance to a PI in 44 (4%) of the sequences. The majority of high-level resistance sequences showed a resistance to lamivudine (n=152), emtricitabine (n=152), nevirapine (n=134) and delavirdine (n=106). Poor virological response and virological failure are associated with the presence of resistance-associated mutations. In women with an available genotypic sequence, the proportion of high-level resistance mutations was significantly higher (30%) amongst women who experienced virological failure compared to women who did not (5%) (p<0.0001).

Figure 3.19: Time to virological failure (HIV RNA >500 copies/ml) after initial suppression amongst HIV-infected pregnant women. Pregnant women were divided into groups by initiation of combination antiretroviral therapy (cART) either before pregnancy or during pregnancy and by calendar time of cART initiation. Kaplan–Meier method was used to estimate the time between start of cART and virological suppression.

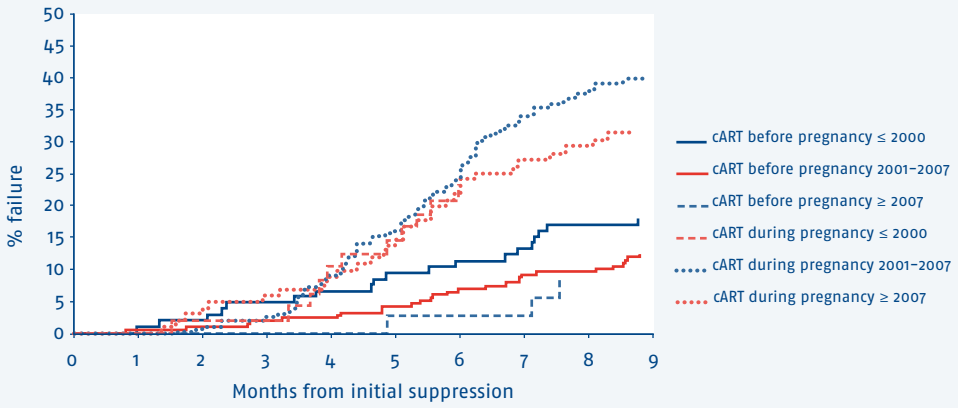


Table 3.8: Risk estimates for the hazard of virological failure after initial suppression. Time from date of virological suppression until viral failure (HIV RNA> 500 copies/ml) was analysed in a Cox regression model.

cART initiation	Hazard ratio*	95% Confidence interval	p-value
Before pregnancy <2000	1		<0.0001
Before pregnancy 2001–2007	0.78	(0.54–1.12)	
Before pregnancy ≥2007	0.51	(0.22–1.19)	
During pregnancy <2000	1.50	(0.96–2.35)	
During pregnancy 2000–2007	2.00	(1.45–2.76)	
During pregnancy ≥2007	1.78	(1.13–2.76)	

** Adjusted for region of origin, CD4 counts & HIV RNA levels at time of cART initation.*

Discussion

Viral load, the most important factor in preventing MTCT, was generally low around the time of delivery. However, 9% of the women had a detectable HIV RNA level at time of delivery, in particular, those who started cART during their pregnancy.

The proportion of women with non-suppressed HIV RNA levels at time of delivery in our data was lower compared to other reports [219]. In our data, time to virological suppression improved over calendar time. In the recent calendar years, time between start of cART and viral suppression became shorter compared to the early years of cART. Factors associated with a detectable viral load at delivery are lower CD4 counts and higher HIV RNA levels at the start of pregnancy [220, 221]. Improvement in virological response might be a result of more effective and safer cART regimens that have become available over time.

We found a stronger response to treatment among women who started cART during their pregnancy compared to those who were already on cART before conception, but risk of virological failure after initial suppression was higher amongst those who started during pregnancy. The majority of women who experienced virological failure did so after parturition. The strong response to treatment during pregnancy may be due to improved adherence during pregnancy as a result of increased clinical and social support or increased motivation to adhere to therapy [222]. Lower adherence rates post partum might have caused the increased risk of virological failure after delivery. Although we do not have data on treatment adherence, lower rates post partum compared to rates during pregnancy have been reported elsewhere [223].

Women who become pregnant require a high level of clinical support not only during their pregnancy, but certainly also after delivery. Continued monitoring of HIV-infected women after pregnancy is necessary for prevention of decreased motivation in adherence and early detection of virological failure.

4. Resistance

Ard van Sighem

Generally, HIV-infected patients on combination antiretroviral treatment (cART) nowadays achieve sustained suppression of viral load, such that replication of the virus is virtually blocked. Each year, however, approximately 3% of the treated patients experience virological failure, defined as a viral load level above 500 copies/ml despite treatment, which may be a marker of inadequate adherence to therapy and may herald the presence of drug resistance. Amongst patients experiencing virological failure, the proportion of sequences with high-level resistance decreased from 91% in 2000 to 39% in 2011. Altogether, 9% of patients currently in follow-up are resistant to at least one antiretroviral drug, although this proportion is probably an underestimate since a genotypic sequence is obtained in less than one third of patients with virological failure. Evidence of transmission of resistant virus is found in less than 2% of patients, indicating that infections from the reservoir of treated patients with resistance are relatively rare and that new infections occur mainly via untreated HIV-infected individuals who may not yet be aware of their infection.

Het gros van de HIV-geïnfecteerden die met combinatietherapie (cART) worden behandeld, bereikt tegenwoordig langdurige onderdrukking van hun viral load, zodat de HIV-replicatie praktisch gestopt wordt. Toch krijgt ieder jaar ongeveer 3% van de behandelde patiënten te maken met virologisch falen, hier gedefinieerd als een viral load van meer dan 500 kopieën/ml, ondanks de behandeling, wat kan duiden op onvoldoende therapietrouw en kan wijzen op de aanwezigheid van geneesmiddelresistentie.
Bij de patiënten met virologisch falen nam het percentage sequenties met een hoge mate van resistentie af, van 91% in 2000 naar 39% in 2011. In totaal is 9% van de patiënten die momenteel in follow-up zijn resistent tegen ten minste één antiretroviraal geneesmiddel. Waarschijnlijk is dit echter een onderschatting van het werkelijke aantal, omdat bij minder dan een derde van de patiënten met virologisch falen een resistentieprofiel bepaald is. Minder dan 2% van de patiënten is geïnfecteerd met een resistente virusvariant. Dit duidt erop dat het aantal infecties door behandelde, resistente patiënten relatief zeldzaam is en dat nieuwe infecties derhalve hoofdzakelijk plaatsvinden via onbehandelde HIV-geïnfecteerden die zelf misschien nog niet weten dat ze geïnfecteerd zijn.

Introduction

Treatment with combination antiretroviral therapy (cART) generally results in sustained suppression of HIV viral loads to levels below the threshold of quantification. It is believed that in a majority of patients, cART inhibits viral replication virtually completely [224]. However, patients may have difficulty maintaining optimal adherence to the treatment regimen because of drug-related toxicities, for example. As a result, drug concentrations may be too low to completely halt the replication of HIV, and mutations in the viral genome may be selected that confer resistance to one or more drugs in the regimen. Here we report on the development of resistance in the treated HIV-infected population followed by Stichting HIV Monitoring (SHM) and the extent to which resistant virus strains are transmitted to uninfected individuals.

Resistance during treatment

Residual replication

In clinical practice, residual replication of HIV despite cART, known as virological failure, usually betrays itself by quantifiable viral load levels, typically above 50 copies/ml. Many patients, however, who have viral load levels consistently below 50 copies/ml may occasionally have a single measurement above 50 copies/ml [225, 226]. The clinical relevance of these so-called blips appears to be limited, and their occurrence may be related partially to random assay variations or to release of virus from the latent reservoir, neither of which herald the presence of resistance-associated mutations [162, 167]. Moreover, rates of virological failure may have increased since 2008, when a new quantification assay was introduced in several treatment centres across the country. With this new assay, HIV RNA levels below 50 copies/ml measured with earlier assays are frequently found to be greater than 50 copies/ml (see also *Chapter 3*) [7].

Less virological failure

To minimise the effect of blips and the new quantification assay, we used a viral load of 500 copies/ml as a marker of virological failure. Since 2000, the annual proportion of patients with virological failure has decreased to approximately 3%. During the same time, the difference between patients pre-treated with mono- or dual therapy and patients starting cART whilst being antiretroviral therapy-naive has disappeared (*Web Appendix Figure 4.1*). The sudden decline in virological failure amongst pre-treated patients from 10% in 2007 to 5% in 2008 coincides with the introduction of new antiretroviral drugs. These drugs are able to suppress viral load, even in patients who have had multiple episodes of virological failure and who are resistant to many of the older drugs.

Scanning for drug resistance

In patients who experienced virological failure, resistance to antiretroviral drugs was ascertained by scanning genotypic sequences of the reverse transcriptase (RT) and protease genes obtained at the time of the failure for specific mutations known to be associated

with resistance to the four original classes of drugs: lamivudine and emtricitabine (XTC), other nucleoside RT inhibitors (NRTI), non-nucleoside RT inhibitors (NNRTI), and protease inhibitors (PI) [227]. Although in recent years new drug classes have been introduced, including integrase inhibitors and entry inhibitors, genotypic sequences of the relevant genes are not yet routinely obtained. A genotypic resistance interpretation algorithm developed by Stanford University was used to infer a drug susceptibility score for each sequence according to a five-level scheme: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance [180].

Sequences

In total, 3973 sequences were obtained from 2483 patients after they started cART. Pre-treated patients were disproportionally represented with 1566 sequences, or 39%. In recent years, however, only 16% of the sequences were from pre-treated patients, whereas 12% of all treated patients in clinical care were pre-treated. Altogether, 3283 sequences, or 83%, were obtained whilst patients were on treatment. In 74% of these 3283 sequences, high-level resistance to at least one antiretroviral drug was found, including in 89% of the sequences obtained from pre-treated patients and in 63% of those from patients who started cART whilst being antiretroviral therapy-naive. Of note, 7% of the sequences from pre-treated patients and 24% of those from previously therapy-naive patients were susceptible to all antiretroviral drugs, indicating that the patients probably did not take their prescribed medication.

Less resistance

Altogether, the proportion of sequences with high-level resistance at the time of virological failure decreased from 91% in 2000 to 39% in 2011 (*Web Appendix Figure 4.2*). Generally, patients who were pre-treated with mono- or dual therapy had higher levels of resistance at the time of failure compared to previously therapy-naive patients. Differences in proportions with resistance were most apparent for PIs and NRTIs, whereas the proportions with resistance to lamivudine and emtricitabine and to NNRTIs were comparable between pre-treated and previously therapy-naive patients (*Figure 4.1*; *Web Appendix Figure 4.3 and 4.4*). From 2008 onwards, the proportion of sequences with resistance showed a sharp decrease for pre-treated patients, which was concomitant with the decrease in the proportion with virological failure.

Figure 4.1: Annual proportion of sequences with high-level resistance to (A) lamivudine and emtricitabine (XTC), (B) other nucleotide reverse transcriptase inhibitors (NRTI), (C) non-nucleoside reverse transcriptase inhibitors (NNRTI), and (D) protease inhibitors (PI). In total, 3283 sequences were obtained from patients whilst they were on treatment, distinguishing patients who started combination antiretroviral therapy (cART) whilst being antiretroviral therapy-naive and patients who were pre-treated with non-cART regimens. High-level resistance was found in 2430 (74%) sequences, including 1219 (89%) sequences from pre-treated patients and 1211 (63%) sequences from previously therapy-naive patients.

Type of regimen

In total, 262 sequences were obtained from patients who started cART from 2000 onwards whilst being antiretroviral therapy-naive and who were still on their first-line regimen. The proportion of sequences with high-level resistance to at least one antiretroviral drug

was similar for patients on NNRTI-based regimens, 60%, and for patients on PI-based regimens, 57%. In 59% of the patients on an NNRTI-based regimen, high-level resistance to an NNRTI was found. In contrast, only 18% of the patients on a PI-based regimen were resistant to a protease inhibitor. Resistance to lamivudine and emtricitabine was found in 52% of patients on a PI-based regimen and in 43% of those on NNRTI-based regimens, whilst resistance to other NRTIs was observed in 4% and 16%, respectively.

Overall prevalence of resistance

Altogether, as of June 2012, resistance-associated mutations had been found in 2304 patients, or 14%, of the 16,169 HIV-infected patients who were still in clinical care [227]. For 1530 patients, or 9%, including 623 patients who were pre-treated with non-cART regimens, these mutations resulted in high-level resistance to at least one antiretroviral drug. Since resistance tests were performed in only 30% of patients with virological failure in or after 2000, probably the true prevalence of resistance is higher. A crude estimate would put the true prevalence at approximately 30%, which would be more in line with findings in other European countries. For instance, in Switzerland, approximately 40% of the antiretroviral therapy-exposed population have been found to harbour resistance-associated mutations [228].

Of the 1530 patients with evidence of high-level resistance, 72% had resistance to lamivudine and emtricitabine, whilst 49% had resistance to at least one other NRTI. Resistance to at least one PI was found in 31% and to at least one NNRTI in 60%. High-level resistance to drugs from one class was observed in 33% of patients, resistance to two classes in 32%, to three classes in 24%, and resistance to all four original drug classes in 11%. Inferred levels of resistance for each antiretroviral drug are shown in *Web Appendix Tables 4.1 and 4.2*.

Transmission of drug resistance

Limited treatment options

Treatment options may be limited when patients are infected with an HIV virus strain that is already resistant to one or more of the currently available antiretroviral drugs. In such patients, standard treatment combinations may not be the most efficacious ones, and as a result, patients may experience delayed viral suppression or have an increased risk of virological failure. It is, therefore, important to screen patients for the possible presence of drug resistance so that the initial treatment regimen can be optimised [229, 230].

Back-mutation

Although a resistant virus strain may change to a drug-susceptible virus by back-mutation, tiny concentrations of resistant variants will remain dormant in resting CD4 cells and other reservoirs awaiting better conditions for replicating once treatment is started. The presence of resistance, therefore, needs to be established as close to the moment of infection as

possible [231-233]. In particular, the M184V mutation in RT, which is associated with high-level resistance to lamivudine and emtricitabine, can revert back relatively quickly after transmission. Other mutations will reverse at a much slower pace or not reverse at all, depending on the extent to which the virus becomes capable of replicating.

Screening for resistance

In 2003, screening for resistance at the time of entry into care was incorporated in the treatment guidelines. Since that time, 4271 patients have been screened for resistance, which comprises 41% of all 10,391 patients diagnosed with HIV during that period. In order to reduce a possible effect of back-mutation on observed levels of resistance, only patients who had a genotypic sequence within 1 year of diagnosis and who had not started antiretroviral treatment were considered for screening. In addition, the patients were divided into two groups, one with patients with recent infection (32%) and one with patients with long-standing infection (68%). These two groups were quite different regarding patient characteristics. Dutch homosexual men represented 69% of the recently infected group, but only 42% of the group with long-standing infection. In contrast, sub-Saharan Africans accounted for 18% of the long-standing infections, but only 4% of the recent infections.

Transmitted drug resistance

In total, 84 patients had high-level resistance to drugs from one class, 11 patients to drugs from two classes, and 6 patients to drugs from 3 classes. No patients with resistance to antiretroviral drugs from all four classes have been observed so far. It should be emphasised that this does not mean that entire drug classes are rendered unsuitable for use in antiretroviral combinations. All classes except lamivudine and emtricitabine include drugs with little cross-resistance between them. Moreover, other classes of drugs have become available in recent years. As a result, even for patients with resistance to all four classes, there may still be fully efficacious cART combinations.

High-level resistance to at least one antiretroviral drug was found in 2.4% of the diagnosed patients, whilst 2.1% had intermediate levels of resistance (*Table 4.1*). The proportions of patients with resistance were similar between those with a recent or a long-standing infection, although resistance to NNRTIs appeared to be somewhat more common amongst those with long-standing infection. Overall, intermediate or high-level resistance to zidovudine and stavudine were most frequently observed, but both drugs are no longer commonly used (*Figure 4.2*). In addition, 1.1% of the patients had high-level resistance to efavirenz, whilst 1.7% were resistant to nevirapine.

Table 4.1: Number of patients with intermediate or high-level resistance to any drug, protease inhibitors (PI), lamivudine and emtricitabine (XTC), other nucleoside reverse transcriptase inhibitors (NRTI), or non-nucleoside reverse transcriptase inhibitors (NNRTI), according to the Stanford genotypic interpretation algorithm [180]. Only patients diagnosed in 2003 or later are included.

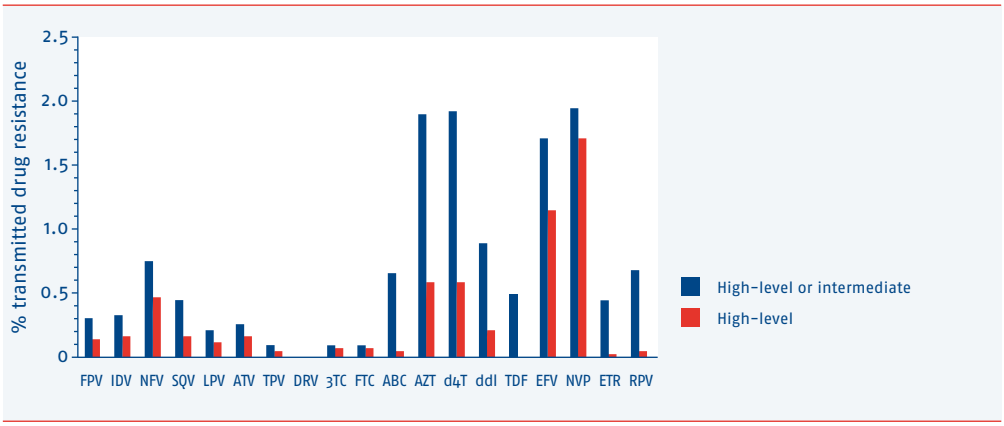
	Recent infection, N=1376		Long-standing infection, N=2895		All infections, N=4271	
	N	%	N	%	N	%
Any drug						
Intermediate	30	2.2	59	2.0	89	2.1
High-level	29	2.1	72	2.5	101	2.4
PI						
Intermediate	5	0.4	9	0.3	14	0.3
High-level	7	0.5	13	0.4	20	0.5
XTC						
Intermediate	1	0.1	0	0.0	1	0.0
High-level	1	0.1	2	0.1	3	0.1
NRTI						
Intermediate	21	1.5	42	1.5	63	1.5
High-level	8	0.6	20	0.7	28	0.7
NNRTI						
Intermediate	7	0.5	13	0.4	20	0.5
High-level	17	1.2	56	1.9	73	1.7

In recent years, no changes were observed in the proportion of patients with predicted transmitted drug resistance. However, there were indications that the percentage of patients with a K103N mutation in RT, which confers resistance to efavirenz and nevirapine, has been increasing since 2008, being 0.8% (95% confidence interval, 0.5-1.2) between 2003 and 2008 and 1.3% (0.8-2.0) in 2009 or later [234].

Sex and the subtype

The proportion of HIV infections with evidence of intermediate or high-level resistance was similar between men and women. However, transmission of virus strains fully susceptible to all NRTIs was less frequent amongst men, 92%, than amongst women, 97%. In contrast, virus strains susceptible to all PIs were observed in 87% of men, but in only 65% of women. These differences between the sexes can be explained by the HIV-1 subtype with which patients were infected. When we considered patients infected with either a subtype B virus or with any other subtype, differences in levels of resistance between men and women disappeared.

Figure 4.2: The predicted proportion of patients with high or intermediate levels of transmitted drug resistance, according to the Stanford interpretation algorithm, was 1.9% for AZT and d4T (two drugs that are no longer commonly used) and 1.9% for NVP and 1.7% for EFV [180]. High-level or intermediate resistance to other drugs was observed in less than 1% of new infections. Only patients with an HIV diagnosis in 2003 or later were included.



Legend: *FPV=fosamprenavir; IDV=indinavir; NFV=nelfinavir; SQV=saquinavir; LPV=lopinavir; ATV=atazanavir; TPV=tipranavir; DRV=darunavir; 3TC=lamivudine; FTC=emtricitabine; ABC=abacavir; AZT=zidovudine; d4T=stavudine; ddI=didanosine; TDF=tenofovir; EFV=efavirenz; NVP=nevirapine; ETR=etravirine; RPV=rilpivirine.*

Altogether, 2.7% of subtype B viruses had intermediate or high-level resistance to NRTIs, whereas this was true for only 0.3% of non-B viruses. These higher levels of resistance to NRTIs were largely due to subtype B strains with revertant mutations in RT such as 215S or 215D, which have established themselves as subepidemics [235]. Revertant mutations at position 215 in RT were found in 159 (5%) subtype B infections, but in only 3 out of 997 non-B infections. Full susceptibility to all protease inhibitors was found in 93% of subtype B sequences, but in only 52% of non-B viruses, most likely because of naturally occurring polymorphisms at minor resistance-associated positions in the protease gene [227].

Conclusion

In terms of percentages, virological failure is less common nowadays than it was in 2000, thanks to an improved availability of treatment options. This appears to be true even for patients pre-treated with mono- or dual therapy who now have the same rates of virological failure as previously therapy-naive patients. Nevertheless, due to a growing volume of treated HIV-infected patients, approximately 250 patients per year still experience virological failure.

Resistance patterns in sequences obtained at approximately the time of failure seem to indicate that in one quarter of previously therapy-naive patients, virological failure is

the result of the patients not taking their prescribed medication, which could be due to, for instance, drug-related toxicity. In patients with a sequence obtained whilst failing on a PI-based or an NNRTI-based first-line regimen, overall levels of drug resistance are similar. However, PIs appear to be more resilient to development of drug resistance than NNRTIs, most likely as a consequence of the larger number of mutations necessary to render the virus fully resistant [236]. In patients on a PI-based regimen, resistance to lamivudine and emtricitabine is most commonly observed, whereas in patients on NNRTI-based regimens, resistance to NNRTIs and, to a lesser extent, to lamivudine and emtricitabine is most frequent.

Unfortunately, sequences are available for only approximately 30% of the patients with virological failure. Without a thorough understanding of the conditions under which sequences are available is needed, it is difficult to draw firm conclusions on the prevalence of resistance. Also, for some patients, virological failure may be caused by resistance to integrase or entry inhibitors, but sequences of the genes involved in this type of resistance are not yet routinely collected. In order to determine the true prevalence of resistance in treated patients with virological failure, SHM is investigating the possibility of developing a study to obtain sequences and plasma drug concentrations at the time of failure in a randomly selected sample of patients.

Meanwhile, less than 2% of patients are infected with a virus that is already resistant to antiretroviral drugs. This may indicate that HIV still does not need resistance to survive in a population with easy access to antiretroviral treatment. Resistance as a survival mechanism would be unnecessary if most new infections are caused by HIV-infected individuals who are not yet treated and who may not even be aware of their infection, as seems to be the case amongst men who have sex with men in the Netherlands [2, 237].

Conversely, the low prevalence of transmitted drug resistance indicates that transmission from the pool of resistant patients is limited [235]. If, however, a resistant virus would be transmitted from the pool of patients on PI-based regimens, this would most likely be a virus with resistance to lamivudine and emtricitabine caused by an M184V mutation in RT. As this mutation comes at great cost to viral fitness, such a virus would quickly revert back to wild-type and defy detection at the time of diagnosis. On the other hand, new infections with a resistant virus arising from patients on NNRTI-based regimens would mostly involve resistance to NNRTIs and, to a lesser extent, to lamivudine and emtricitabine. Mutations that play a role in resistance to NNRTI also negatively impact on the fitness of the virus, but at more moderate levels than M184V. In particular, K103N causes only a modest disadvantage in fitness compared to wild-type virus and may remain the dominant viral quasispecies in newly infected patients [232, 238]. As a result, viruses with K103N may have the potential to establish themselves as a subepidemic. Further studies and monitoring of resistance would be necessary to confirm if this is already happening in the Netherlands.

5. HIV-1 infected children in the Netherlands

Colette Smit

Health care for HIV-infected children living in the Netherlands is provided in four specially designated paediatric HIV treatment centres. The number of children born in the Netherlands and infected through mother-to-child transmission has declined over time, most likely because of the introduction of HIV screening amongst pregnant women introduced in 2004. Still, a limited number of vertical transmissions have occurred since the start of the national screening. Most HIV-infected children in care are receiving combination antiretroviral therapy (cART). We observed a poorer early virologic response in vertically infected children aged 0-2 years at the time of cART initiation compared to older children; long-term immunologic and virologic responses were comparable with the older children. In 36% of the 124 children tested, resistance-associated mutations were found, mainly in the children who experienced virological failure. Children's survival benefits from this successful response to cART, as we observed low mortality in HIV-infected children in care in the Netherlands. A large proportion of the children have survived into adulthood and are now receiving care in one of the general HIV treatment centres. All children who have survived into adulthood are currently alive.

De zorg voor HIV-geïnfecteerde kinderen in Nederland wordt gegeven in vier speciaal aangewezen pediatrische HIV-behandelcentra. Het aantal kinderen dat in Nederland wordt geboren en door moeder-kindtransmissie besmet raakt met HIV is in de loop van de tijd afgenomen, hoogstwaarschijnlijk door de introductie van HIV-screeningsmethoden voor zwangere vrouwen in 2004. Toch raakt een klein aantal kinderen, ondanks deze nationale screening, nog besmet via verticale transmissie.
De meeste met HIV-geïnfecteerde kinderen worden behandeld met cART. Tijdens behandeling werd bij verticaal geïnfecteerde kinderen in de leeftijd van 0 to 2 jaar bij aanvang van de behandeling een slechtere vroege virologische respons gezien vergeleken met oudere kinderen. De immunologische en virologische respons op de lange termijn was vergelijkbaar met die bij de oudere kinderen. Bij 36% van 124 geteste kinderen werden met resistentie geassocieerde mutaties gevonden, met name bij kinderen die virologisch faalden op de therapie.
De mortaliteit onder HIV-geïnfecteerde kinderen in Nederland is laag, wat aangeeft dat ze baat hebben bij behandeling met cART. Een groot aantal kinderen is inmiddels volwassen geworden en wordt nu behandeld in een van de algemene HIV-behandelcentra. Al deze volwassen geworden kinderen zijn momenteel in leven.

Total population

Health care for HIV-infected children living in the Netherlands is provided in four paediatric HIV treatment centres. As with adult patients, diagnosis, treatment and follow-up of these children are monitored by the Stichting HIV Monitoring (SHM).

As described in *Chapter 1*, 464 patients aged 0 through 17 years at time of HIV diagnosis have been registered with the SHM. As of June 2012, 309 of these HIV-infected children were in care in one of the four paediatric HIV treatment centres. The remaining 155 patients were in care in a general HIV treatment centre and were infected by sexual contact, with a median age at diagnosis of 17 years (interquartile range [IQR]: 16-18), 57% of the patients were female.

Demographical and clinical data were collected for 289 out of the 309 children monitored in one of the four paediatric treatment centres (*Table 5.1*). For this group of children, the total follow-up time since diagnosis was 9 years (IQR: 5-12).

Table 5.1: *Demographic characteristics of HIV-infected children in care in the Netherlands.*

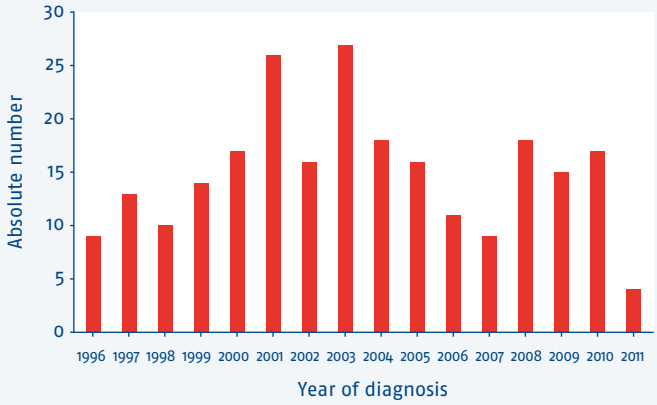
Variables	Vertically infected children	Non-vertically infected HIV-1 infected children
Total n=289		
Number (n,%)	233 (81%)	56 (19%)
Gender (n,%)		
Boy	114 (49)	28 (50)
Girl	119 (51)	28 (50)
Country of origin, child (n,%)		
The Netherlands	105 (45)	8 (14)
Sub-Saharan Africa	101 (43)	43 (77)
Other	27 (12)	5 (9)
Country of origin, mother (n,%)		
The Netherlands	22 (9)	5 (9)
Sub-Saharan Africa	146 (63)	33 (59)
Other	65 (28)	14 (25)
Unknown		4 (7)
Year of HIV diagnosis (n,%)		
<1998	60 (26)	20 (36)
1998-2004	83 (36)	18 (32)
≥2004	90 (39)	18 (32)
CDC event at HIV diagnosis (n,%)		
CDC-b	20 (9)	5 (9)
CDC-c	35 (15)	5 (9)
Current age in years (median, IQR)	12 (7-17)	17 (9-19)

Legend: *CDC=Centers for Disease Control and Prevention*

Diagnoses

Figure 5.1 shows the number of registered diagnoses amongst children according to the year of diagnosis.

Figure 5.1: *Number of HIV-infected children according to their year of HIV diagnosis.*



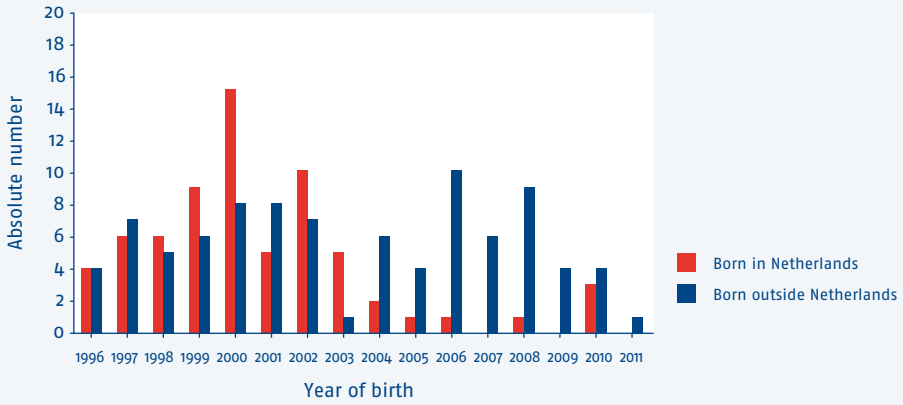
Most HIV-infected children (81%) in care in the Netherlands were vertically infected (*Table 5.1*), and absolute numbers varied over time from 7 in 1996 to 24 in 2003. The number of HIV-infected children that were not infected by mother-to-child transmission (MTCT) varied between 1 and 7 per calendar year. The median age at diagnosis was 2 years (IQR: 0.4-5). Although 45% of the vertically infected children were born in the Netherlands, for only 4% did both parents originate from the Netherlands, and for 65% (151 out of 233) of the children, at least one parent originated from sub-Saharan Africa. For the 56 children who were non-vertically infected with HIV, the routes of transmission were sexual contact (n=15) and blood contact (n=11). An unknown mode of transmission accounted for 30 infections; these children were included in the group of children who were non-vertically infected. The median age of diagnosis was 11 years (IQR: 7-15). The majority of the non-vertically infected were born in sub-Saharan Africa.

Vertical transmission of HIV in the Netherlands

The number of children born in the Netherlands and infected through MTCT has declined since 2004 (*Figure 5.2*). This decline is most likely due to compulsory HIV screening amongst pregnant women introduced in 2004 [23, 239]. Eight children born with HIV in the Netherlands have been reported to the SHM since the introduction of this screening. Two of them were born in 2004 from women who became pregnant before 1 January 2004. Four children were born to mothers who tested positive after giving birth; the mothers of two children tested negative during screening and became infected during the pregnancy. One child was born

to a mother who was known to be infected with HIV but who was not receiving treatment during her pregnancy for an unknown reason. The remaining child was born to a mother without a known screening or known HIV status during pregnancy.

Figure 5.2: Number of vertically HIV-infected children according to their year of birth, stratified by region of origin.



Although the number of registered vertically infected children born in the Netherlands has declined since the introduction of national pregnancy screening in 2004, MTCT cannot be completely excluded. Screening for HIV only in the first trimester does not totally rule out maternal infection, as it can occur during the second or third trimester. In addition, when testing is performed shortly after primary infection of the mother, test results may still be negative.

However, because the prevalence of HIV amongst pregnant women in the Netherlands is between only 0.04 and 0.08% [239], a nationwide second pregnancy screening is not likely to be very effective. Since a large majority of vertically infected children born in the Netherlands had at least one parent originating from sub-Saharan Africa, the Caribbean or central Europe, it may be beneficial to perform a second screening later in pregnancy amongst women who originate from countries where HIV is endemic.

Treatment

The majority of HIV-infected children in the Netherlands are now receiving cART (*Table 5.2*), Most children (68%) were treated with a first-line regimen including a protease inhibitor (PI) and 2 or more nucleoside reverse transcriptase inhibitors (NRTI's); 30% of the children received a non-nucleoside reverse transcriptase (NNRT)-based first-line regimen with 2 or more NRTI's, and 2% of the children started cART with a combination of an NNRT+PI and 2 NRTI's. The median time on these first-line regimens was 14 months (IQR: 4-30).

Table 5.2: Clinical characteristics of HIV-infected children in care in the Netherlands.

Variables	Vertically infected children	Non-vertically infected HIV-1 infected children
Number	233	56
cART treated (n,%)	215 (92)	41 (73)
CDC event at start cART (n,%)		
CDC-b	25 (11)	4 (7)
CDC-c	34 (15)	7 (13)
Regimen (n,%)		
NNRTI + ≥2 NRTI's	64 (30)	13 (31)
PI + ≥2 NRTI's	148 (69)	26 (63)
NNRTI +PI + 2 NRTI's	3 (1)	1 (2)
Time from HIV-1 diagnosis to cART initiation (months, median, IQR)		
0-2 years	0.8 (0.4-2.3)	
2-5 years	10 (3-24)	
>5 years	15 (2-64)	12 (3-56)
CD4 count at start cART (cells/mm^3)		
0-2 years	1410 (850-1790)	
2-5 years	670 (370-1130)	
>5 years	367 (217-560)	343 (270-400)
HIV RNA load at diagnosis at start cART (log_{10} copies/ml)		
0-2 years	5.4 (4.4-5.8)	
2-5 years	4.4 (3.5-5.1)	
>5 year	4.3 (3.2-4.9)	4.0 (3.5-4.9)
High level drug resistance (n,%)		
No	65 (28)	14 (25)
Yes	37(16)	8 (14)
No data	131 (56)	34 (61)

***Legend:** cART=combination antiviral therapy, CDC=Centers for Disease Control and Prevention; NNRTI=non-nucleoside reverse transcriptase inhibitor, NRTI=nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, IQR=interquartile range*

cART initiation

The World Health Organization (WHO) recommends starting cART in all children aged 0-1 years, irrespective of CD4 counts or clinical condition [240]. Amongst the 233 vertically infected children registered with the SHM, 119 were aged 0-1 year at the time of HIV diagnosis, and 73% of these children received cART before the age of 2 years.

In children aged 2-4 years cART initiation is recommended in those with CD4 counts ≤750 cells/mm^3 or a CD4 percentage below 20 [240]. Amongst the children in whom cART was initiated between 2 and 5 years of age, the median CD4 count was 670 cells/mm^3 (IQR: 370-1130) (*Table 6.2*). For children aged 5 years or older, cART is recommended when CD4 counts reach a threshold of 350 cells/mm^3 or a CD4 percentage below 15, which is the same as in adult patients [240]. In children aged 5 years or older, the median CD4 count at the start of cART was 367 cells/mm^3.

When we took the current recommendations for starting cART in children into account, those aged younger than 2 years and those 5 years or older at the start of cART initiated therapy in time to achieve a good response. A substantial proportion of children aged between 2 and 4 years at the start of cART initiated treatment with less than 750 CD4 cells/mm^3. However, the majority of these children started cART <2007 (72%) before the current guidelines became available, and according to the guidelines at that time, cART initiation was in time. Other reports have also shown that early treatment in asymptomatic children is not widespread clinical practice in many centres [241], which has been in line with the earlier guidelines [242].

The most important reasons for changes in cART regimens or reasons for stopping therapy were toxicity, virological failure and poor adherence. Thirty-two percent of the children on cART switched their regimen due to toxicity and 23% due to virological failure. No significant difference in the occurrence of toxicity and virological failure was found amongst the three age groups. Children aged 5-17 years more often changed or discontinued their regimen because of poor adherence compared to children aged 0-1 year and those 2-4 years (32%, 19%, and 16% respectively) (p-value=0.04).

Immunologic response

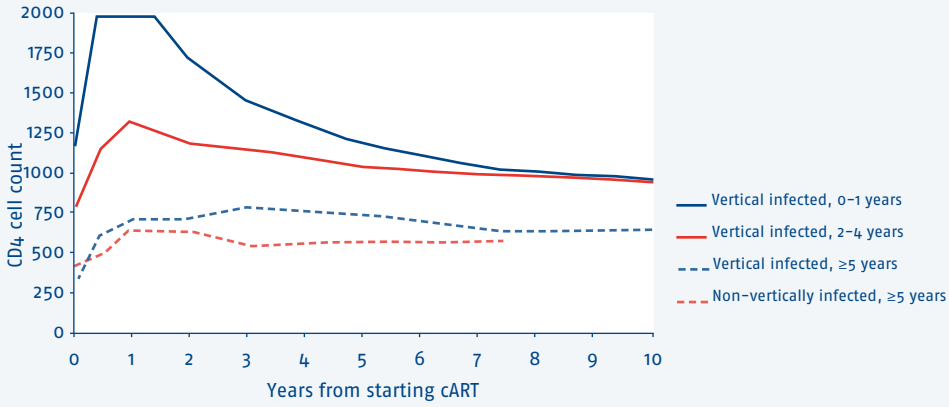
The clinical benefit of cART is strongly related to the level of recovery of CD4 cell count [187]. Taking into account that CD4 cell counts in children aged less than 5 years are higher compared to older children and adults and that CD4 counts decrease with increasing age [243], we stratified the children on cART according to their age at the time of cART initiation: (1) vertically infected, 0-1 year; 2) vertically infected, 2-4 years; 3) vertically infected, ≥5 years; 4) non-vertically infected or unknown mode of HIV transmission and ≥5 years).

Younger, vertically infected children had significantly higher CD4 cell counts at the time of cART initiation compared to older and non-vertically infected children (*Table 5.2*), which are normal age-related values and irrespective of HIV.

Figure 5.3 shows the longitudinally modelled changes in CD4 counts during 10 years after cART initiation amongst HIV-infected children, stratified by age at cART initiation. Amongst all three age groups of vertically infected children, CD4 counts significantly increased in the

first 6 months after cART initiation. Although not statistically significant, CD4 counts also increased during these first 6 months among the non-vertically infected children. Amongst vertically infected children aged 0-1 year, CD4 counts remained stable during the second year on cART, and then the counts steadily decreased over the next 5 years on cART before they became stable. This decrease is age-related [243].

Figure 5.3: Changes in absolute CD4 counts (cells/mm³) amongst HIV-infected children, stratified by age at cART initiation. Immunologic trajectories were assessed in a random effect model, and time is in years since start of cART.



A slow decrease in CD4 counts was also observed amongst vertically infected children aged 2-5 years, which also reflects the age-related decrease [243]. In this group, CD4 counts became stable and remained constant during the second half of the first decade on cART. After an increase in CD4 counts during the first year on cART, CD4 counts became relatively stable over time for the next 5 to 10 years amongst vertically and non-vertically infected children aged 5 years or older.

Although CD4 counts steadily decreased during the first 5 years on cART among children aged 0-1 year and 2-4 years at the time of cART initiation, these children still had higher CD4 counts compared to children who started cART when they were ≥5 years of age.

Virologic response to cART

At the time of cART initiation, young children (aged 0-1 year) had significantly higher HIV RNA levels compared to older children (p=0.0023) (*Table 5.2*). Twelve months after starting cART, 75% of the children had experienced a virologic response (two consecutive HIV RNA levels <500 copies/ml). Taking into account that a large number of viral load measurements were determined using the HIV RNA assays with a detection limit of

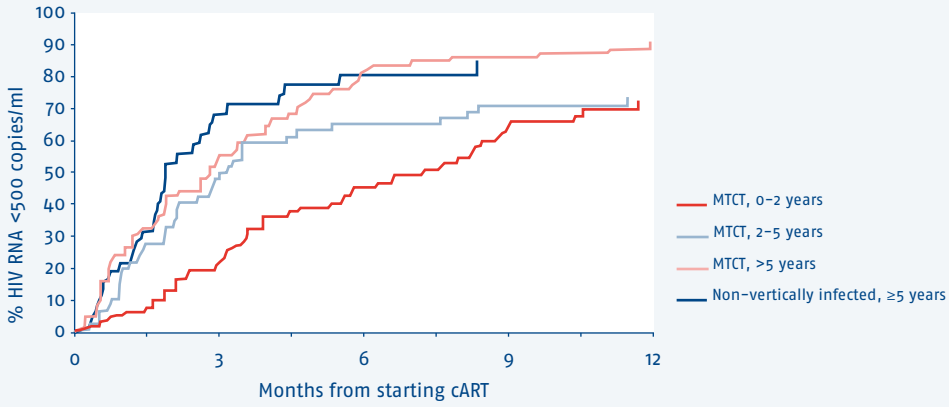
500 copies/ml, we used 500 copies/ml. The poorest responses were observed amongst those aged 0-1 year (71%; 95% confidence interval (CI); 60-80%) and those aged 2-4 years (72%; 95% CI: 60-83%); the best responses were amongst vertically infected children aged ≥5 years (88%; 95% CI: 79-94%) and those non-vertically infected (85%; 95% CI: 71-95) (*Figure 5.4*). These differences were confirmed by the Cox proportional hazard model (*Table 5.3*). *Figure 5.5* shows the longitudinally modelled long-term virologic response to cART over a period of 10 years. In all groups HIV RNA levels significantly decreased during the first 6 months on cART (p<0.0001), with a slower decrease amongst children aged 0-2 years (p<0.0001). However, 2 years after the start of cART, virologic response to cART no longer differed between the groups. This slower decrease in HIV RNA levels in young children compared to older children and adults has also been shown by other observers (*244*). The lower initial virologic response to cART might be explained by poor adherence or by difficulties in the regular dosing adjustments needed for young children (*245*). However, cART regimens for young HIV-infected children have improved substantially over time (*246*). After the changes in viral load for all age groups by calendar year of cART initiation (<2000, ≥2000) were stratified, this improvement was shown by the decline in viral load, which was significantly more rapid during the first year on cART amongst those who started cART from 2000 onwards (p=0.0004).

Table 5.3: *Results from an adjusted Cox proportional hazard model of the time from the start of cART to the first of 2 consecutive plasma HIV RNA concentrations of <500 copies/ml in vertically infected children aged 0-2 years at the time of starting cART; those aged 2-5 years; those aged >5 years; and those with other routes of transmission and aged ≥5 years at the start of cART.*

	Hazard ratio*	95% Confidence interval	p-value
According to age at time of cART initiation			
Vertically infected, 0-1 years	1		0.02
Vertically infected, 2-4 years	1.24	(0.83-1.83)	
Vertically infected, ≥5 years	1.90	(1.24-2.92)	
Other mode of transmission ≥5 years	1.55	(0.95-2.53)	

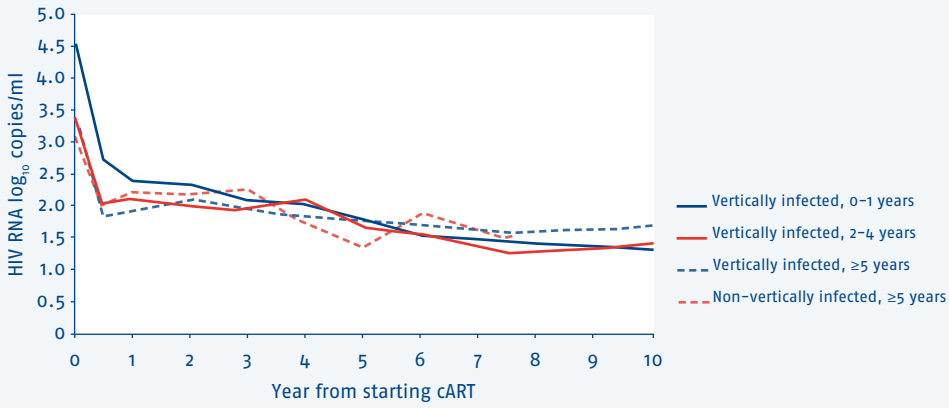
** Adjusted for gender, calendar year of HIV diagnosis, region of origin, time between HIV diagnosis and cART initiation, baseline log RNA levels and baseline CD4 cell counts.*

Figure 5.4: Kaplan-Meier estimates of the percentages of HIV-infected children with initial suppression (<500 copies/ml) during the first year after starting combination antiretroviral therapy (cART), by age at cART initiation and HIV transmission mode.



Legend: MTCT=mother-to-child transmission

Figure 5.5: Changes in HIV RNA levels amongst HIV-infected children, stratified by age at combination antiretroviral therapy (cART) initiation. Virologic responses were assessed in a random effect model, time is in years since start of cART.



Drug resistance

Overall, 236 genotypic sequences were available for 124 HIV-infected children; in 86 sequences (36%) at least one high-level resistant mutation to an NRTI, according to the Stanford interpretation algorithm, was found. These sequences were found in 74 vertically infected children and 12 children either non-vertically infected or with an unknown route of HIV transmission.

In 47 (20%) sequences high-level resistance to a PI was found; 44 of the 47 children were vertically infected, and in 95 (40%) sequences there was high-level resistance to an NNRTI. The majority of high-level resistance sequences showed a resistance to lamivudine (n=82), emtricitabine (n=82), nevirapine (n=36), efavirenz (n=31) and delavirdine (n=33).

In 3 (4%) of the 79 sequences obtained before the start of cART, at least one high-level resistance-associated mutation was found. After the start of cART, 83 (53%) high-level resistance mutations were found in 157 sequences. The proportion of drug resistance found in HIV-infected children varied over calendar time. In 2000, a high-level resistance mutation was found in 66% of the sequences, whereas no sequences with a mutation were found in 2011.

Poor virologic response and virological failure is associated with the presence of resistance-associated mutations. The proportion of high-level resistance mutations was significantly higher among children who experienced virological failure after initial virologic success (32/109, 29%) compared to those who did not experience failure (13/161, 8%) ($p<0.0001$).

Mortality

During follow-up in the paediatric HIV treatment centres, 3 (1%) deaths occurred in the 289 children. The median time between date of HIV diagnosis and date of death was 2.3 years (IQR: 1.5-3.1). One boy who was born in sub-Saharan Africa died of an HIV-related cause when he was 5 months old. Two other boys died at 11 and 12 years of age; both children were born outside the Netherlands. One of those boys was non-vertically infected and was diagnosed with HIV when he was 10 years old; the cause of death was multi-organ failure, and the child never received cART. The other boy was vertically infected with HIV and diagnosed when he was 9 years of age; he died of an HIV-related event whilst being treated with cART.

Transfer to adult HIV treatment centre

As of 1 June 2012, 210 out of the 289 HIV-infected children were still in care in one of the Dutch paediatric HIV treatment centres. As of 1 June 2012, 79 children who have been followed in paediatric care were no longer in such care; 3 patients died, and 21 patients became lost to follow-up. The median age at the time these patients became lost to follow-up was 9 years (IQR: 6-13). The remaining 55 of the 79 children transferred to an adult HIV treatment centre. The median age at transfer was 19 years (IQR: 18-20 years). The median

time in care after transfer was 3 years (IQR: 0.7-6 years). Four patients became lost to follow-up, and the other 51 are currently alive and in care. Currently, 43 patients are on a cART regimen, of which 15 (34%) were not able to suppress their viral load; the current median CD4 count is 525 cells/mm^3 (IQR: 365-665).

Discussion

Results of the monitoring of HIV-infected children in paediatric HIV care show a substantial decline in mother-to-child transmission in the Netherlands since the introduction of national pregnancy screening. However, despite the high uptake of this pregnancy screening, a risk of mother-to-child transmission will always remain amongst women who become infected during the last two trimesters of their pregnancy. A limited number of vertical transmissions has occurred since the start of the national screening. A second pregnancy screening in mothers at high risk of HIV infection may be beneficial in making prevention of mother-to-child transmission even more effective than it already is.

The majority of HIV-infected children in care were receiving cART. Exposure to cART will be lifelong, and therefore, virological failure and the development of drug-resistance during childhood may limit future treatment options. Although we observed a poorer early virologic response in vertically infected children aged 0-1 year at time of cART initiation, the long-term virologic response was comparable to that in older children. In addition, the early response to cART improved over calendar time, most probably as a result of the introduction of improved regimens. For children with resistance test data, we found that 36% of the children had high-level resistance mutations, mainly those who experienced virological failure [247].

Children's survival benefits from this successful and improved response to cART. We observed low mortality in HIV-infected children in care in the Netherlands. A large proportion of the children have survived into adulthood and are now in care at one of the adult HIV treatment centres. All patients who have survived into adulthood are currently alive.

All HIV-infected children will face lifelong treatment with cART. For these children, it will be a challenge to maintain lifelong adherence to cART and achieve lifelong virologic suppression. Monitoring these HIV-infected children during their adolescence and into adulthood will be important in helping to take up that challenge.

Acknowledgements

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6. Hepatitis B and hepatitis C co-infections

Colette Smit, Jan T.M. van der Meer, Marc van der Valk

Hepatitis B (HBV) and hepatitis C (HCV) infections occur frequently in HIV-infected patients. HBV and HCV are associated with progression to chronic liver disease. Amongst HIV-infected patients in the Stichting HIV Monitoring (SHM) database who were screened for co-infection, 8% were chronically infected with HBV, 12% tested positive for HCV antibodies and 8% had a HCV RNA-confirmed HCV infection and were considered to be chronically infected with HCV. Most co-infected patients were male and infected with HIV through homosexual contact. The proportion of deaths was 14% in the patients chronically co-infected with HBV and 15% amongst those with a chronic HCV co-infection; 2% of the patients co-infected with HBV and 3% of those co-infected with HCV died from progression to chronic viral hepatitis. Optimal management of co-infection of HBV and HCV in persons with HIV is needed to limit the impact of co-infection in the progression to severe chronic liver disease and death. Fifty-eight percent of the patients co-infected with HBV received anti-HBV treatment, and 44% of the patients with a chronic HCV co-infection received anti-HCV therapy. A substantial decrease in HBV DNA and HCV RNA levels was observed, and as a result of the long-term control of HBV replication, 16% of the treated patients co-infected with HBV experienced clearance of hepatitis B surface antigen (HBsAg). The current treatment of HCV with a combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) has been found to clear HCV infection in only a small proportion of the treated patients, which stresses the need for improved antiviral treatment of HCV co-infection.

Hepatitis B (HBV) en hepatitis C (HCV) zijn vaak voorkomende infecties bij HIV-patiënten. HBV en HCV worden geassocieerd met progressie naar chronische leveraandoeningen. Van de HIV-patiënten die in de database van de Stichting HIV Monitoring database zijn gescreend op de aanwezigheid van een co-infectie, is medio 2012 8% chronisch geïnfecteerd met HBV, bij 12% werden HCV-antistoffen aangetroffen, en bij 8% werd HCV-RNA aangetoond. Deze laatste groep werd beschouwd als chronisch met HCV geïnfecteerd. De meeste co-geïnfecteerde patiënten waren mannen die via homoseksueel contact met HIV geïnfecteerd waren geraakt. Het sterftepercentage bij de chronisch HBV- en HCV-co-geïnfecteerde patiënten was respectievelijk 14% en 15%. Twee procent van de HBV-co-geïnfecteerden en 3% van de HCV-co-geïnfecteerden overleed ten gevolge van progressie naar chronische virale hepatitis. HIV-geïnfecteerden hebben optimale behandeling van hun HBV- of HCV-co-infectie nodig om de impact van de co-infectie op progressie naar ernstige chronische leveraandoeningen en overlijden te beperken.

Van de HBV-co-geïnfecteerden werd 58% behandeld voor HBV en van de patiënten met een chronische HCV-co-infectie kreeg 44% anti-HCV-behandeling. Er werd een substantiële daling gezien in HBV-DNA-spiegels en als gevolg van de langdurige onderdrukking van HBV-replicatie werd bij 16% van de HBV-co-geïnfecteerden klaring van HBsAg geconstateerd. Tijdens anti-HCV behandeling steeg het aantal patiënten met een negatieve HCV RNA test. Het feit dat de huidige anti-HCV-behandeling, bestaande uit een combinatie van gepegyleerd interferon (PEG-IFN) en ribavirine (RBV), de HCV-infectie bij slechts een klein aantal patiënten blijkt te klaren, benadrukt het belang van betere antivirale behandeling voor HCV-co-geïnfecteerden.

Background

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are common infections in the Netherlands. It is estimated that 30,000 to 60,000 (0.2%-0.4%) persons of the total population living in the Netherlands are infected with HBV and 15,000 to 60,000 (0.1%-0.4%) persons are infected with HCV [248].

These HBV- and HCV-infected persons are at high risk for the development of chronic liver disease. Liver fibrosis, cirrhosis or hepatocellular carcinoma (HCC) will develop in 15% to 40% of the persons chronically infected with HBV or HCV [249, 250]. Progression to serious liver disease takes 20 to 25 years of chronic HBV or HCV infection [251, 252]. These long-term complications of chronic HBV and HCV infection are increasing mortality rates in HBV and HCV mono-infected individuals [253].

As a result of shared routes of transmission, chronic HBV and HCV are highly prevalent amongst HIV-infected persons. Progression of HBV- and HCV-associated liver disease is accelerated in the presence of HIV [254, 255].

In the early years of the HIV epidemic, the faster progression of untreated HIV infection masked the progression to serious liver disease in patients co-infected with hepatitis. Since progression of HIV infection and death dramatically declined after combination antiretroviral therapy (cART) became available, liver disease has become apparent as a frequent cause of death in HIV-infected populations [256]. However, some studies have shown a protective effect of cART on progression to liver fibrosis, whilst cART, on the other hand, may enhance liver disease by drug-related hepatotoxicity [257, 258].

The SHM monitors HBV and HCV co-infection of HIV-infected patients. In this chapter, we report on the demographic and clinical characteristics of patients co-infected with HBV and HCV and the response to HBV and HCV treatment.

Demographics and clinical characteristics

Of the 19,113 HIV-1-infected patients in care in the Netherlands who were aged 18 years or older at the time of HIV diagnosis, 94% were screened for the presence of hepatitis B surface antigen (HBsAg) at least once and 92% of the HIV-infected persons were tested at least once for HCV antibodies or HCV RNA.

We defined chronic HBV co-infection by a positive test for HBsAg, whereas a chronic HCV co-infection was defined as a positive test result on a qualitative or a quantitative HCV RNA test.

HBV

Co-infection with HBV was found in 8% (n=1374) of the screened population, which is considerably higher than in the general Dutch population [259]. Patients co-infected with HBV were predominantly male; 59% were infected with HIV through homosexual contact and born in the Netherlands. Twenty-two percent of these patients were born in sub-Saharan Africa. The majority of sub-Saharan Africans are probably perinatally infected with HBV. In regions such as sub-Saharan Africa where HIV is highly endemic, transmissions primarily occur perinatally or in childhood [260]. Amongst the HIV-infected women, 199 (6%) were co-infected with HBV. The number of new diagnoses varied between 5 and 22 per calendar year. The majority of these women became infected with HIV by heterosexual contact (85%) and originated from sub-Saharan Africa (n=120, 60%). Of the patients co-infected with HBV, 91% were receiving cART (*Table 6.1*).

Table 6.1: Characteristics of patients co-infected with hepatitis B virus (HBV) and hepatitis C virus (HCV).

	Total	HBV	HCV RNA positive
Number	19,113	1374*	1388*
Number screened		17,986	17,500
Prevalence of those screened		8%	8%
Male gender (n,%)	15348 (80)	1,175 (86)	1169 (84)
Age at HIV diagnosis (years, median, IQR)	36 (29-44)	34 (29-42)	
Region (n,%)			
Netherlands	10,901 (57)	705 (51)	888 (64)
Europe	1785 (9)	128 (9)	254 (18)
Sub-Saharan Africa	2940 (15)	297 (22)	61 (4)
Caribbean/Latin America	2146 (11)	141 (10)	83 (6)
Southeast Asia	625 (3)	52 (4)	40 (3)
Other	716 (4)	51 (4)	62 (4)
HIV transmission group (n,%)			
Homosexual	11,085 (58)	810 (59)	684 (49)
Heterosexual	6008 (31)	388 (28)	138 (10)
Injecting drug user	707 (4)	76 (6)	400 (29)
Other	1313 (7)	100 (7)	166 (12)

	Total	HBV	HCV RNA positive
Combination antiretroviral therapy (cART) (n,%)	16301 (85)	1244 (91)	1283 (92)
Calendar year of diagnosis co-infection (n,%)			
<1998	4,967 (26)	504 (37)	603 (43)
1998-2002	3,941 (21)	293 (21)	274 (20)
2003-2007	5,569 (29)	372 (27)	355 (26)
2008-2012	4,636 (24)	205(15)	156 (11)
Time between HBV or HCV and HIV diagnosis (years, median, IQR)		0.13 (0.003-3.1)	3 (0.3-8.0)
HCV genotype (n,%)			
Total determined in 1388 HCV RNA confirmed cases			1168 (82)
1			761 (65)
2			72 (6)
3			163 (12)
4			172 (15)
Other**			263 (45)
Total liver fibrosis (n,%)	654 (3)	179 (13)	242(17)
Severe chronic liver disease*** (n,%)	251 (1)	69 (5)	96 (7)
Hepatocellular carcinoma (n,%)	35 (0.18)	16 (1)	17(1)
Deaths (n,%)	1897 (10)	188 (14)	203 (15)
Cause of death related to viral hepatitis (n,%)	88 (0.5)	28 (2)	46 (3)

* *194 patients had a hepatitis B surface antigen (HBsAG) and HCV antibodies or HCV RNA positive test result.*

** *One genotype 5 and one genotype 6.*

*** *Severe liver disease defined as clinical symptoms of end-stage liver failure based on the diagnosis documented in a clinical note including bleeding from gastric or oesophageal varices, hepatic encephalopathy or hepatorenal syndrome, confirmed with a pathology report or FibroScan report documenting severe liver fibrosis or cirrhosis (Metavir f3-f4 or FibroScan stiffness ≥8kPa) or clinical evidence of chronic liver disease based on radiographic or endoscopic documentation of the presence of portal hypertension by oesophageal varices, ascites, splenomegaly and reversal of portal blood flow, confirmed by a pathology report or FibroScan report documenting severe liver fibrosis or cirrhosis or a probable diagnosis of severe chronic liver disease on the basis of clinical symptoms, clinical evidence or a pathology report. Data updated to 1 June 2011.*

HCV

In total, 2112 (12%) of the patients screened for the presence of HCV had a positive HCV antibody or HCV RNA test result. In 1388 (8%) of the patients, the HCV co-infection was confirmed by a positive HCV RNA test result, and 15,388 patients had a negative HCV test result. The patients with a positive HCV RNA test result were considered as chronically infected with HCV and will be described in this chapter.

As a result of shared transmission routes, the HCV prevalence in HIV-infected patients is considerably higher than in the general Dutch population [259]. Most of the patients co-infected with HCV were male and originated from the Netherlands (*Table 6.1*). Forty-nine percent of the

patients chronically co-infected with HCV were infected with HIV by homosexual contact and were the largest group of co-infected patients. The second largest group of patients with a chronic HVC co-infection consisted of patients infected with HIV by injecting drug use (29%). Of the HIV-infected women followed in care in the Netherlands, 6% (n=219) were co-infected with chronic HCV. The number of new HCV diagnoses in women increased from 10 in 1998 to 19 in 2003. Among these co-infected women, 35% were infected with HIV by heterosexual contact and 43% by injecting drug use. The majority of the women originated from the Netherlands or another European country (n=278, 72%), whereas 66 (17%) women were born in sub-Saharan Africa.

Most patients were infected with the HCV genotype 1 (65% of the available HCV genotype test results). Almost all the patients (91%) were treated with cART.

Chronic HBV and HCV diagnoses over time

The number of HBsAg tests in HIV-infected patients registered in the SHM database increased from 962 in 1998 to more than 1900 per year since 2004. The number of HBsAg-positive test results varied between 52 and 97. The percentage of HBsAG-positive test results decreased from 8% in 1999 to 3% from 2009 onwards. This decrease might be a result of the possible impact of cART on the clearance of HBV [261]. Between 1998 and 2004, the number of HBV-positive test results was approximately the same in homosexual men and heterosexual patients. From 2004 onwards, there was a small decrease in the number of new HBsAg diagnoses amongst heterosexuals, from 96 in 2004 to 50 in 2011. In 2007, we observed a small peak in the number of new occurrences of HBsAg diagnosed amongst homosexual men; the number of HBsAg-positive test results increased from 34 in 1998 to 61 in 2007 among this group and then subsequently decreased to 31 in 2011. The screening for HBV co-infection in the HIV-infected population has improved over time. In 2006, 67% of the HIV-infected patients were screened for HBV co-infection, and this proportion increased to 94% of the patients in 2011.

Besides HBV, HCV antibody testing has increased over time. In 1998, 966 HIV-infected patients were screened for the presence of HCV antibodies. The number of HCV antibody tests increased to 4261 in 2011. The number of HCV-positive test results varied between 70 in 2001 to 131 positive results in 2011, and the percentage of positive results declined from 9% in 1999 to 3% in 2011.

Also, HCV RNA testing has increased over time. In 1998, 86 HIV-infected patients were tested for the presence of HCV RNA. The number of HCV RNA tests increased to 1077 in 2009. The number of patients with positive HCV RNA test results for the first time varied between 46 in 2000 and 132 in 2008.

The screening for HBV and HCV co-infection in HIV-infected patients in care in the Dutch treatment centres has improved over time. In 2006 only 64% of the HIV-infected patients were screened for the presence of HCV antibodies in the first year after HIV diagnosis [262]. Recently the proportion of patients screened for HCV co-infection has increased to 92%.

In the past few years, several studies have shown an increase in the number of newly acquired HCV infections amongst HIV-infected homosexual men [263, 264]. The number of patients with a newly acquired HCV infection has significantly increased since 2003, with 1 diagnosis in 2000 to 52 in 2011 (p<0.0001).

Morbidity and mortality

Chronic liver disease caused by chronic HBV and HCV infection is currently an important cause of death in HIV-infected patients [256, 265]. Some studies have shown an accelerated progression to liver disease caused by HBV and HCV in the presence of HIV infection [266-268].

Amongst the patients chronically co-infected with HBV registered with the SHM, 13% progressed to liver fibrosis, including all Metavir scores (*Table 6.1*) [269]. In the HCV-co-infected population, 405 patients (19%) progressed to liver fibrosis (*Table 6.1*). Of the HBV co-infected patients, severe chronic liver disease developed in 5%, and it developed in 7% of the patients with a chronic HCV co-infection. Severe chronic liver disease is defined as clinical symptoms of end-stage liver failure confirmed with: 1) a pathology report or FibroScan report, or 2) clinical evidence of chronic liver disease based on radiographic or endoscopic documentation. A detailed description of the definition is presented in *Table 6.1*.

HCC was diagnosed in 35 patients; 16 of these patients were chronically co-infected with HBV, and 17 diagnoses of HCC were made in patients chronically infected with HCV (*Table 6.1*). Although not statistically significant, HCV co-infected patients were less likely to have HCC compared to HBV co-infected patients (adjusted hazard ration [HR]: 0.81 (0.31-2.15)) (*Figure 6.1*).

The proportion of death was 14% in the patients chronically co-infected with HBV and 15% amongst those with a chronic HCV co-infection; 2% of the patients co-infected with HBV and 3% of those co-infected with HCV died from progression to chronic viral hepatitis or from a complication of viral hepatitis (*Table 6.1*). Ten years after cART initiation, 15% (95% CI: 13-18%) of the patients co-infected with HBV and 14% (12-17%) of those co-infected with HCV had died (*Figure 6.2*). The proportion of death was significantly lower in HIV mono-infected patients, where 12% (11-12%) had died 10 years after cART initiation. Without adjustment for demographical and clinical differences, the risk of death was significantly higher in patients chronically co-infected with HCV (HR: 1.25, 95% CI: 1.05-1.50), the risk of death was non-significantly higher in patients chronically co-infected with HBV (HR: 1.10, 95% CI: 0.90-1.35) compared to HIV mono-infected patients. *Table 6.2* shows the adjusted hazard ratios for the risk of death. After adjustment for differences in demographical and clinical differences, co-infected patients did not have a higher risk of death than HIV mono-infected patients. Several studies have shown an increased risk of death in the HCV co-infected population, which was mainly found amongst injecting drug users [270, 271]. The increased risk of death in patients with HIV co-infected with HCV remains controversial, as other reports did not find an impact [272]. In the SHM database, the risk of death was no longer increased in patients co-infected with HCV after adjustment for demographical and clinical characteristics,

including HIV transmission risk group. We observed an increase in the number of newly acquired HCV infections amongst homosexual men. These recently acquired HCV infections had probably not yet progressed to advanced liver disease that results in death.

Figure 6.1: Probability of the development of hepatocellular carcinoma (HCC) amongst patients co-infected with HIV and hepatitis B virus (HBV) or hepatitis C virus (HCV). Kaplan–Meier method was used to estimate the time to death. Follow-up time was from the date of combination antiretroviral therapy (cART) initiation to the date of last contact, date of HCC diagnosis or 1 June 2012.

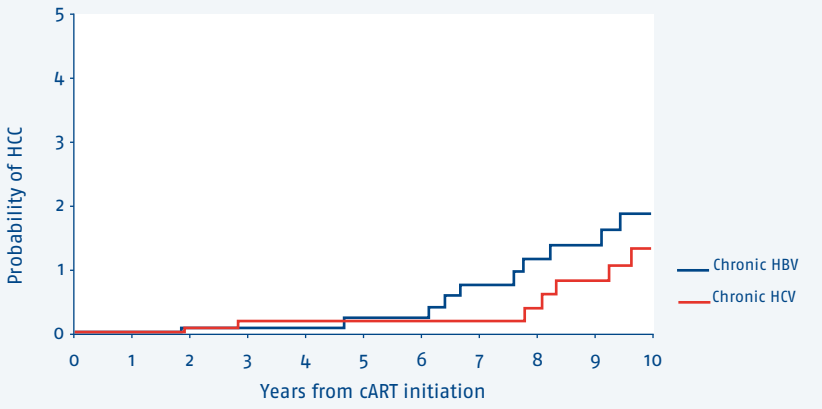


Figure 6.2: Probability of death amongst patients with HIV only, patients co-infected with HIV and hepatitis B virus (HBV) or hepatitis C virus (HCV) and patients with triple infections (HIV/HBVHCV). Kaplan–Meier method was used to estimate the time to death. Follow-up time was from the date of cART initiation to the date of last contact, date of death or 1 June 2012.

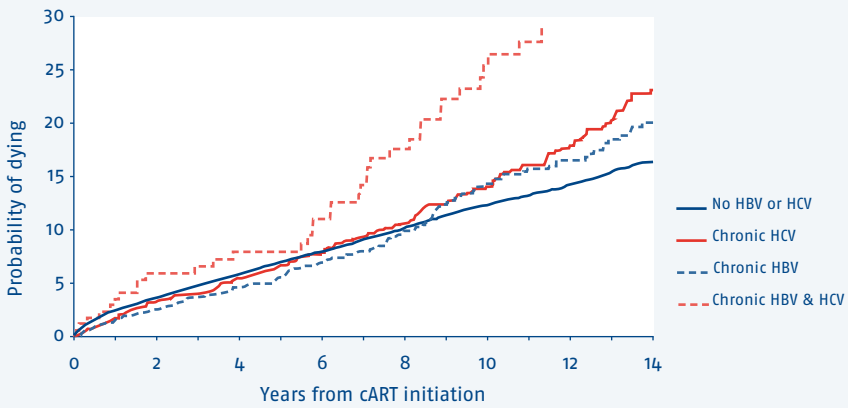


Table 6.2: Risk of progression to liver fibrosis and death amongst combination antiretroviral therapy (cART)-treated HIV-infected patients with hepatitis co-infection compared to patients infected with HIV only. To evaluate the impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection on risk of liver disease and death, time to liver disease or death was estimated by a Cox proportional hazard model. Follow-up time was from the date of cART initiation until date of last contact, most recent follow-up visit, death or 1 June 2012.

				Risk of dying
	Hazard ratio* (95% CI)	p-value	Hazard ratio* (95% CI)	p-value
HIV	1	0.08	-	0.08
HIV/HBV	1.14 (0.93-1.39)		1	
HIV/HCV	0.82 (0.66-1.00)		0.71 (0.54-0.94)	
HIV/HBV/HCV	1.10 (0.77-1.55)		0.96 (0.65 -1.43)	

Legend: CI=confidence interval

** Adjusted for age, gender, region of origin, transmission risk group, calendar year of cART initiation, baseline CD4 and HIV RNA levels, alcohol use and smoking.*

Treatment

HBV

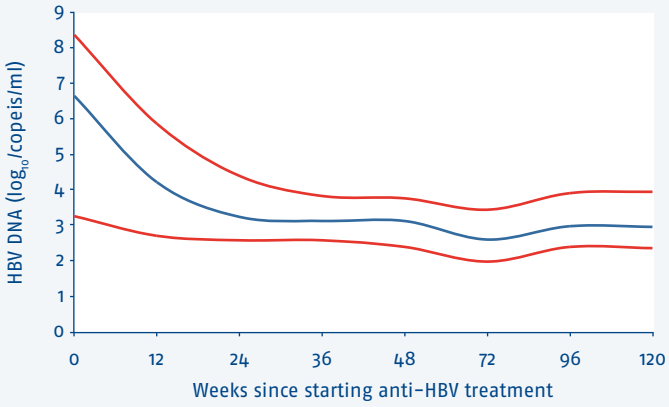
At present, anti-HBV treatment is aimed at suppressing HBV production, thereby delaying progression to liver fibrosis and cirrhosis [273]. Several antiretroviral agents used for HIV treatment, such as lamivudine, emtricitabine and tenofovir, are also active against HBV [274].

In the SHM database, a total of 796 (58%) HIV and HBV co-infected patients received a cART regimen that included an agent that was also active against HBV (*Table 6.3*). The median duration of a first-line cART regimen containing an anti-HBV active agent was 4.7 years (IQR: 1.6-7.7). In most of the HBV-treated patients, lamivudine was the initial anti-HBV agent (n=688, 86%). Twenty percent of the patients switched from lamivudine to tenofovir (n=136) after a median of 13 months (IQR: 6-33 months) of lamivudine treatment.

Table 6.3: Characteristics of HIV and hepatitis B virus (HBV) co-infected patients receiving anti-HBV treatment.

	Untreated	Total treated
Number (n,%)	578 (42)	796 (58)
Age at start anti-HBV therapy (years, median, IQR)		37.7 (32.6-43.8)
Gender (n,%)		
Male	490 (85)	685 (86)
Female	88 (15)	111 (14)
HIV risk group (n,%)		
Men who have sex with men (MSM)	338 (58)	472 (59)
Heterosexual contact	182 (31)	206 (26)
Injecting drug use	19 (3)	57 (7)
Other	39 (7)	61 (8)
Region (n,%)		
Netherlands	285 (49)	420 (53)
Europe	43 (7)	85 (11)
Sub-Saharan Africa	147 (25)	150 (19)
Other	103 (18)	141 (18)
Duration of first anti-HBV regimen (median months, IQR)		18.6 (6.9-37.5)
Anti-HBV agent (n,%)		
Lamivudine		688 (86)
Tenofovir		97 (12)
Other		11 (1)

Figure 6.3: Changes in HBV DNA plasma levels (log_{10} copies/ml) since the start of anti-hepatitis B (HBV) treatment in patients co-infected with HBV and HIV, median with interquartile range (IQR).



HBV treatment outcome

Figure 6.3 shows the time course of HBV DNA plasma levels after the initiation of HBV treatment. At the start of HBV treatment, the median HBV DNA level was 6.4 (IQR: 3.1-8.0) $\log_{10}$ copies/ml. Median HBV DNA levels decreased to 4.0 (IQR: 2.6-5.6) $\log_{10}$ copies/ml at week 12 and 3.1 (2.5-4.2) $\log_{10}$ copies/ml at week 24. HBV DNA levels further decreased to 2.5 (IQR: 1.9-3.3) $\log_{10}$ copies/ml when patients were treated for more than 72 weeks. From week 72 onwards, median HBV DNA level remained stable over time. HBsAg clearance with receipt of anti-HBV therapy was achieved by 128 (16%) patients. The median time between the start of anti-HBV therapy and the first negative test result for HBsAg was 38 weeks (IQR: 10-77).

In total, 111 out of the 199 (56%) women co-infected with HBV received a cART regimen with an anti-HBV active agent. The median HBV DNA level at the start of anti-HBV treatment was 5.3 $\log_{10}$ copies/ml (IQR: 2.3-6.8) and decreased to 2.9 (2.5-4.5) $\log_{10}$ copies/ml after 24 weeks on treatment. Twenty-four weeks after the start of anti-HBV treatment, 64% of the women cleared HBV DNA.

HCV

A combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) is the first choice of therapy for HCV. Since April 2012, two direct acting agents administered orally for the treatment of HCV are registered in the Netherlands, boceprevir and telaprevir [275]. Both agents could be added to the standard anti-HCV treatment, which is the PEG-IFN/RBV combination. Currently, only data regarding patients co-infected with HCV treated with a combination of PEG-IFN and RBV are available in the SHM database. The primary aim of anti-HCV treatment is to achieve undetectable serum HCV-RNA levels 24 weeks after completing a course of treatment, which is defined as a sustained virologic response (SVR) [276].

A total of 613 (44%) out of the 1388 patients chronically co-infected with HCV were prescribed anti-HCV treatment, according to the SHM database. Of these, 540 patients completed the full course of therapy or ended their anti-HCV therapy because of side effects or lack of response. The remaining 73 patients are currently being treated for their HCV infection. HCV RNA follow-up data were available for 527 out of the 540 patients.

As response to treatment and treatment duration differs between acute and chronic infected patients [277, 278], we reported HCV treatment responses for only HCV chronically infected patients (n=461). Patients were assumed to be treated in the chronic phase of their HCV infection when they had no evidence of a newly acquired HCV infection (defined as less than 2 years between a registered negative test result for HCV and a positive test result). In the SHM database, out of the 527 patients receiving anti-HCV therapy, 66 (13%) patients were treated during the acute phase of their HCV infection and 461 (87%) patients in the chronic phase of their infection.

Patients who received anti-HCV treatment were more often male, infected with HIV by homosexual contact and of Dutch origin (*Table 6.4*). For the patients receiving anti-HCV treatment, HCV genotype was determined in 94% of persons. Eighty-four percent of the HCV-treated patients were already receiving cART before the start of anti-HCV therapy. The median duration of anti-HCV treatment was 30 (IQR: 21-48) weeks. Seventy-eight (16%) of the patients ended anti-HCV treatment within 14 weeks, 32 (7%) of these stopped in the first 4 weeks. In the group of patients who dropped out within 14 weeks, 14 (18%) still responded to treatment (undetectable HCV RNA load at week 12).

***Table 6.4:** Characteristics of patients co-infected with HIV and hepatitis C virus (HCV) receiving anti-HCV treatment.*

	Total treated	SVR	No SVR	p-value
Number (n,%)	461	183 (40)	278 (60)	
Age at start anti-HCV therapy (years, median, IQR)	42 (37-48)	42 (36-48)	43 (38-48)	0.15
Gender (n,%)				
Male	412 (89)	165 (90)	247 (89)	0.65
Female	49 (10)	18 (10)	31 (11)	
HIV risk group (n,%)				
Men who have sex with men (MSM)	274 (59)	122 (67)	152 (55)	0.03
Heterosexual contact	44 (10)	10 (5)	34 (12)	
Injecting drug use	87 (19)	30 (16)	57 (21)	
Other	56 (12)	21 (11)	35 (13)	
Region (n,%)				
Netherlands	319 (69)	127 (69)	192 (69)	0.51
Europe	65 (14)	29 (16)	36 (13)	
Other	27 (17)	27 (15)	50 (18)	
Combination antiretroviral therapy (cART) (n,%)				
Before anti-HCV	388 (84)	153 (84)	235 (85)	0.79
After anti-HCV	73 (16)	30 (16)	43 (15)	
HCV genotype (n,%)				
1&4	349	132	217	0.34
2&3	86	39	47	
Other/unknown	26	12	14	
Duration (median weeks, IQR)	30 (21-48)	47 (25-48)	25 (14-48)	<0.0001

***Legend:** IQR=interquartile range; SVR=sustained virologic response*

HCV treatment outcome

The outcome of HCV treatment is frequently measured with a quantitative HCV RNA test. The percentage of patients with an undetectable HCV RNA level increased from 3% at the start of anti-HCV treatment to 36% at week 4, 62% at week 12 and 77% at week 24 (*Figure 6.4*). The median decline in HCV load during the first 12 weeks of anti-HCV treatment was 7.2 $\log_{10}$ copies/ml (IQR: 6.5-7.8).

Figure 6.4: Anti-HCV treatment response in patients co-infected with HIV and hepatitis C virus (HCV): percentage of patients with an undetectable HCV RNA level at the start of treatment and at week 4, week 12, week 24 and week 48 after the start of treatment and a sustained virologic response (SVR, undetectable HCV RNA levels 24 weeks after completion of anti-HCV treatment).

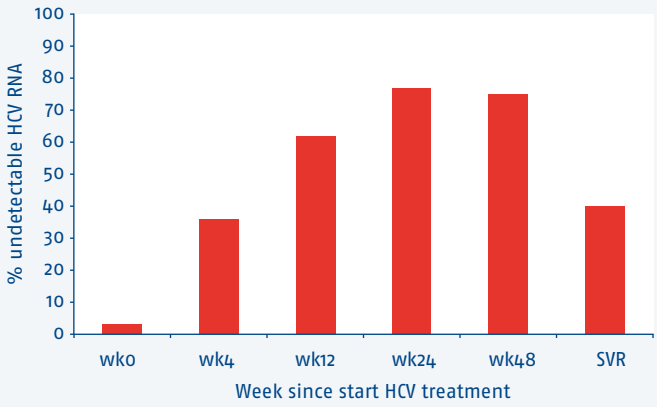
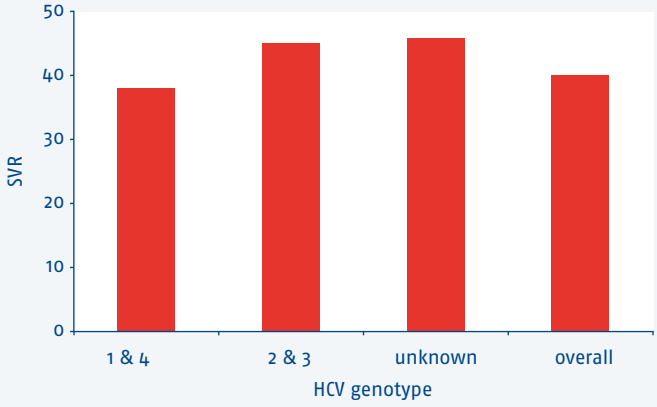


Figure 6.5: Anti-HCV treatment response in patients co-infected with HIV and hepatitis C virus (HCV): sustained virologic response (SVR, undetectable HCV RNA levels 24 weeks after completion of anti-HCV treatment) stratified by HCV genotype.



Forty percent of the patients who started anti-HCV treatment received an SVR (138/461), an undetectable HCV RNA level measured 6 months after the completion of ant-HCV treatment. Men who have sex with men (MSM) were more likely to achieve an SVR (*Table*

6.4). SVR varied amongst patients with different genotypes. Amongst patients infected with HCV genotype 1 or 4, the SVR was 38%, whereas 45% of the patients with genotype 2 or 3 achieved a SVR (*Figure 6.5*).

Of the women co-infected with HIV and HCV, 49 (6%) were treated for their HCV co-infection. The SVR rate was 41% for women receiving anti-HCV treatment; 20 out of the 49 women had undetectable HCV RNA 24 weeks after the stop date of anti-HCV treatment.

Conclusion

Since 2000, the number of HCV diagnoses in the Dutch HIV-infected population has increased. Most of these infections have been in homosexual men. The increase in HCV diagnoses coincides with an increase in acute HCV infections in the same population. The acute HCV infections in homosexual men are probably caused by sexual transmission [264]. The number of HBV diagnoses has remained stable over time. As a substantial proportion of the HBV co-infected patients are born in sub-Saharan Africa where HBV is highly endemic in this region, transmission primarily occurs perinatally or in childhood [260]. As a result of perinatal transmission of HBV, these patients are likely to have experienced lifelong exposure to HBV. Patients co-infected with HIV and HBV or HCV are at increased risk of the development of chronic liver disease [251, 252]. In the HIV-infected population, we observed a slow but steady increase in hepatocellular carcinoma in patients with a chronic HBV or chronic HCV co-infection. Besides the impact of HBV and HCV on progression to liver disease, cART may have a protective effect on progression to liver fibrosis, but, on the other hand, it may enhance liver disease by drug-related hepatotoxicity [257, 258]. Screening for the presence of chronic HBV and chronic HCV infections and optimal management of HBV and HCV co-infection in individuals with HIV is needed to limit the impact of co-infection in the progression to severe chronic liver disease. We had difficulty obtaining a complete and updated picture of severe chronic liver disease, as data on diagnostics were not part of the SHM data collection. As additional data to assess severe chronic liver disease has been available up to June 2011, but not up to June 2012, the reported numbers do not represent a complete and updated picture of liver morbidity in the population co-infected with HIV and hepatitis. From July 2012 onwards, data collection on hepatitis and liver-related disease has improved, and extended data on hepatitis and liver morbidity is now being collected by SHM, which will make possible further specification of the cases of liver fibrosis.

As a result of these recent improvements in data collection, the current collection regarding HBV and HCV infection has been extended to include detailed information on diagnostics, treatment, treatment responses and side effects, clinical and diagnostic data on liver diseases such as radiographic or endoscopic reports of the presence of portal hypertension, varices, ascites, splenomegaly and reversal of the portal flow and pathology reports and documentation of clinical symptoms.

In this chapter, we reported treatment responses to HBV and HCV treatment. A substantial decrease in HBV DNA levels was observed. As a result of the long-term control of HBV replication, 16% of the patients with HIV treated for HBV co-infection experienced HBsAg clearance.

The current treatment of HCV with a combination of PEG-IFN and RBV has been found to clear HCV infection in less than half of the HCV-treated patients, as shown by our data in which 40% of the treated patients cleared their HCV infection and by the results in the reports of others [279, 280]. The uptake of anti-HCV treatment in the HCV co-infected population was low and a considerable number of patients dropped out early in the course of treatment.

Future of HCV treatment

As a result of the limited success rates of the current treatment, a large number of patients co-infected with HIV and HCV remain untreated. New anti-HCV drugs are urgently needed and are now being developed.

Two protease inhibitors have been recently licensed for the treatment of HCV in the Netherlands [275]. The availability of the protease inhibitors boceprevir and telaprevir, when added to pegylated interferon and ribavirin, has dramatically improved SVR rates for the treatment of patients with a chronic hepatitis C genotype 1 mono-infection. The SVR was 68% in untreated patients and 88% in previously treated patients receiving a combination of peg-IFN/RBV with boceprevir [281, 282] Recent studies in HIV and HCV co-infected persons that have been presented only during conferences and are not yet published look promising with regard to similar rates of SVR compared to those in HCV-mono-infected patients [283-286].

Although the SVR rates are improved compared to those achieved with the current treatment option, both telaprevir and boceprevir have numerous pharmacological interactions with antiretroviral therapy that need to be taken into account [287, 288]. In the next few years, several new classes of direct acting anti-HCV agents will become available. It is expected that combination anti-HCV therapy without the use of pegylated interferon will be introduced in the near future for HCV mono-infected individuals. Data about these new compounds in persons who are co-infected with HIV and HCV have not yet been presented, but several early clinical trials are currently being performed.

These new therapeutic strategies may provide more treatment options for patients co-infected with HIV and HCV and may contribute to reducing severe chronic liver disease. Monitoring of patients co-infected with HIV and HCV, together with monitoring of the co-infected population for the development of liver disease and mortality, will remain important in the future. Besides carrying forward the extended data collection on liver-related morbidity, the SHM will follow the uptake and response to new therapeutic strategies and the interaction between these new drugs with cART.

Special reports

7. The Amsterdam Cohort Studies on HIV infection – Annual Report 2011

Ineke Stolte, Maria Prins for the ACS

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 2011, the cohorts reached 27 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 27 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 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.

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 2011 bestonden de ACS 27 jaar. Het oorspronkelijke doel van de ACS was het onderzoeken van de prevalentie en incidentie van, en de risicofactoren voor HIV-1-infectie en AIDS en van het ontstaan en natuurlijk beloop van de HIV-1-infectie en het evalueren van de effecten van interventies. In de afgelopen 27 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 laatste paar jaar zijn 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 en kennis verschaft over de verschillende aspecten van HIV-1. Deze expertise heeft direct bijgedragen aan de vooruitgang en verbetering van de preventie, diagnose en behandeling van HIV.

As of 31 December 2011, 2473 men who have sex with men (MSM) and 1658 (injecting) drug users (DU) were included in the Amsterdam Cohort Studies (ACS). Every 3 to 6 months, participants have completed a standardized 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 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 2473 MSM, 614 were HIV-positive at study entry, and 228 seroconverted during follow-up. For the 1658 DU, 322 were HIV-positive at study entry, and 98 seroconverted during follow-up. By 31 December 2011, 353 MSM and 466 DU had died, and several other participants were asked to leave the study or left at their own request. In total, the Public Health Service of Amsterdam was visited 50,493 times by MSM and 26,554 times by DU.

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 (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 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) (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 2011

The cohort of men having sex with men

In 2011, 564 MSM were followed at the PHSA. Twenty-seven of them were newly recruited, and two died in 2011. 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 in active follow-up by the end of 2011 at the PHSA, 480 men were HIV-negative, and 84 men were HIV-positive. The HIV-positive men were followed at the PHSA or in an HIV treatment centre outside the PHSA according to the 'HIV Onderzoek onder Positieven' (HOP) protocol, which is comparable to the HIV-negative protocol. This protocol was initiated in October 2003 for MSM who seroconverted or who tested HIV-positive at entry into the study cohort of young MSM after 1999. Of the 84 MSM in active follow-up in 2011, 6 were newly included, 56 were HIV seroconverters, and 40 were being followed at an HIV treatment centre outside the PHSA. 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 2011.

In 2011, 152 HIV-positive MSM were in active follow-up at the Jan van Goyen clinic since 1999. Of these, 41 were HIV seroconverters, and 30 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 following an HIV-positive result without effective therapy.

Since November 2008, all MSM followed at the PHSA have been routinely screened for STI. Furthermore, between July 2010 and July 2011 all these MSM were invited to participate in the H2M study. In this study MSM are additionally screened for Human Papillomavirus (HPV) during five consecutive 6-monthly visits to investigate the prevalence, incidence and clearance of anal, penile and oral HPV infections among HIV-negative and HIV-positive MSM (H2M study).

The cohort of drug users

In 2011, 327 drug users were followed at the PHSA. Of the 327 DU followed in 2011, 24 were HIV-positive at entry, 15 seroconverted for HIV during follow-up in the ACS. 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, nobody was recruited in 2011, which might be explained by the unpopularity of injecting drugs in Amsterdam.

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. 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. Treatment for HCV in a multidisciplinary setting is still offered to drug users at the PHSA.

Sub and affiliated studies

Primo-SHM study

In addition to the cohorts previously described, from May 2003 until March 2010 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". The Primo-SHM study is a national randomized study on the effect of early temporary quadruple antiviral therapy as compared to no therapy. Some of these patients were seronegative men in the MSM cohort at the PHSA who seroconverted during follow-up. Some of them are also still followed with the HOP protocol of the ACS at the PHSA. All 466 samples that are collected within the Primo-SHM study are part of the ACS and stored at the Department of Experimental Immunology.

AGE$_h$IV Cohort Study

The Age$_h$iv 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 aged 45 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 2011, about 525 HIV-1-infected participants were included through the AMC HIV outpatient clinic, and 400 HIV-uninfected individuals belonging to the same HIV exposure groups were included through the STI clinic of the PHSA or the Amsterdam Cohort Studies. All participants are aged ≥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 collaborators in the Departments of Obstetrics and Gynecology and Experimental Immunology at the AMC analyse factors involved in neonatal HIV-1 transmission. The children infected with HIV-1 are included in the Paediatric 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 Paediatric European Network for Treatment of AIDS (PENTA). All samples that are collected within the study until 2008 are part of the ACS and stored at the Department of Experimental Immunology.

The HIV epidemic

HIV incidence

Five MSM and no DU participating in the ACS seroconverted for HIV in 2011. The observed HIV incidence among MSM declined to 1.2 per 100 person-years in 2011.

Figure 7.1: HIV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among men who have sex with men (MSM), 1984–2011.

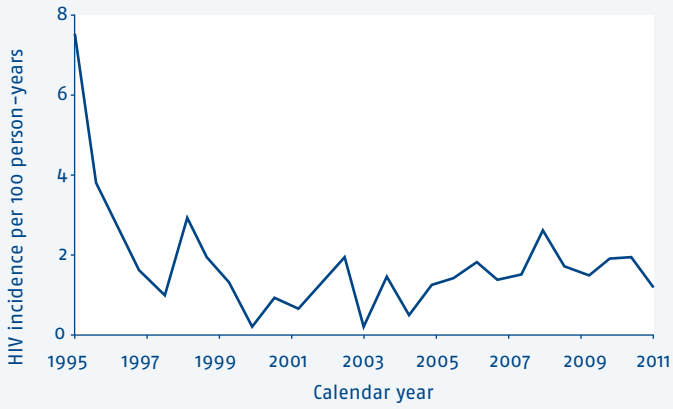
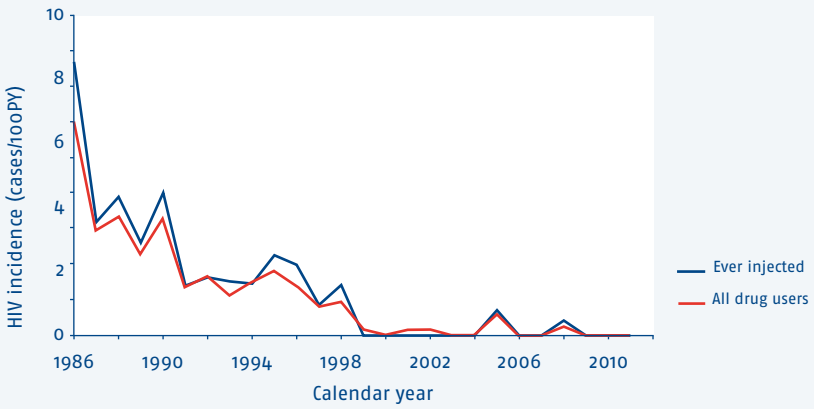


Figure 7.2: HIV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among drug users, 1986–2011.



The HIV incidence in drug users has continued to decline and is now less than 1.0/100 person-years. *Figures 7.1* and *7.2* show the yearly observed HIV incidence rates for MSM and drug users from the start of the ACS through 2011.

Transmission of therapy-resistant HIV strains

Surveillance of transmission of drug-resistant HIV-1 strains was performed for seven MSM seroconverters and for four MSM who were seropositive at study entry in 2011. Two individuals were infected with virus harbouring resistance-associated mutations: a so-called 215-revertant (215E) mutation was found in one of the seroprevalent participants, and a virus carrying multiple protease (30N, 88D) and reverse transcriptase (41L, 215C) mutations was found in one of the seroconverters. In eight individuals only naturally occurring sequence variation was found, and no sequence could be obtained from one individual because of a low viral load. Phylogenetic analysis showed that all individuals harboured subtype B HIV-1 strains.

In the cohort of drug users, no seroconversions or seropositive entries appeared.

Highly active antiretroviral therapy (HAART) uptake

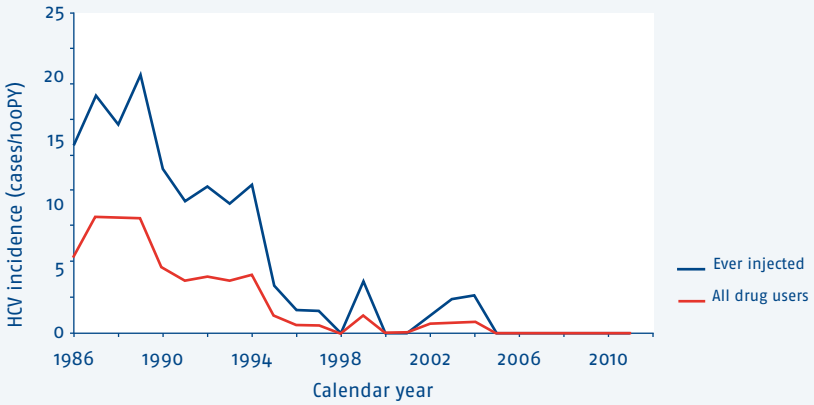
Of all 193 HIV-positive MSM visiting the Jan van Goyen Clinic or one of the other HIV treatment centers in the Netherlands according to the ACS protocols in 2011 and for whom treatment data were available, 185 (96%) received some form of antiretroviral therapy. Of 192 MSM for whom viral load results were available in 2011, 158 (82%) had a viral load of less than 50 copies/ml (assays: M2000rt).

Of the 30 HIV-positive DU who visited the PHSA in 2011 and for whom treatment data were available, 29 (97%) received some combination of antiretroviral therapy. Of the 30 DU, 28 (93%) had an undetectable viral load (less than or equal to 150 copies/ml [assay: M2000rt]) at their latest visit.

HCV incidence in drug users

In 2011 the HCV incidence was 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 7.3*).

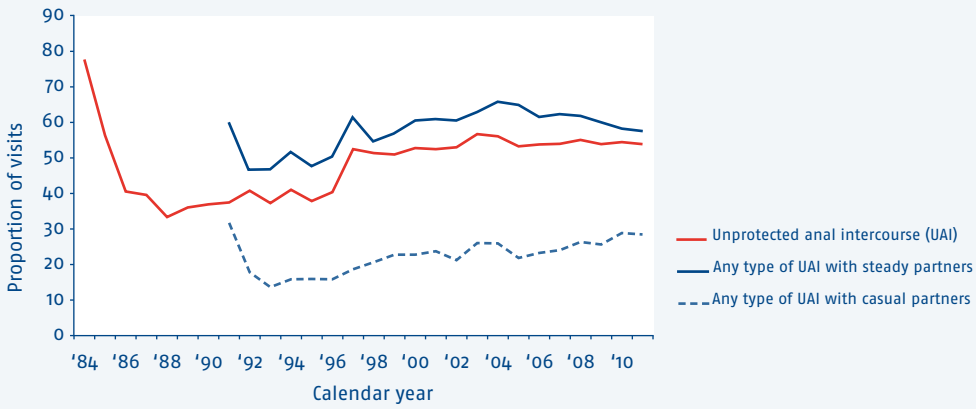
Figure 7.3: HCV incidence per calendar year in the Amsterdam Cohort Studies (ACS) among drug users, 1986–2011



Risk behaviour of MSM

Information from the 895 questionnaires filled in by 479 HIV-negative MSM during cohort visits in 2011 resulted in 457 reports (54%) of unprotected anal intercourse (UAI) in the preceding 6 months. Higher proportions of UAI were reported for steady partners (57%) compared to casual partners (28%). Trends in UAI among HIV-negative MSM participating in the ACS have slowly increased since 1996, but they have remained relatively stable in recent years (*Figure 7.4*).

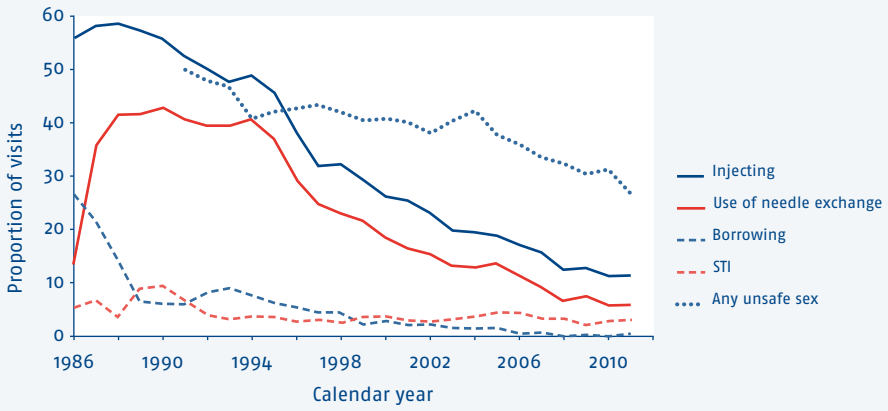
Figure 7.4: Trends in unprotected anal intercourse (UAI) in the past 6 months amongst HIV-negative men having sex with men (MSM) from the Amsterdam Cohort Studies (ACS), 1984–2011.



Risk behaviour of DU

In HIV-negative DU, reports of both injection 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 35% in 2011. Reports of STI have remained relatively stable at approximately 6% in recent years (see *Figure 7.5*).

Figure 7.5: 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), 1986-2011.



Legend: STI=sexually transmitted infection

STI screening among MSM and DU 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 2011, a total of 527 MSM from the ACS were screened for STI; 95 MSM were screened once, 394 twice and 5 more than twice. The prevalence of any STI at the first visit in 2011 was 8.7% (46/527), and the prevalence of any STI at the subsequent visit in 2011 was 7.8% (37/475). The prevalence of STI was significantly higher among HIV-infected MSM (18.3%) compared to HIV-uninfected MSM (7.0%).

Between November 2010 and June 2011, 197 (72%) of the 272 DU followed at the PHSA were also screened for chlamydia, gonorrhoea and syphilis as part of a pilot study to assess whether regular STI screening is indicated for this group. No infectious syphilis or gonorrhoea was found. The prevalence of chlamydia was 1.5% (3/197). On the basis of these results, it was decided that regular STI screening is not indicated for the DU in the ACS.

ACS research highlights 2011

To gain insight into the ongoing HIV transmission among MSM, we compared sexual risk behaviour pre- and post-HIV-seroconversion in 206 MSM participating in the Amsterdam Cohort Studies (1984-2008), both before and after the introduction of combination antiretroviral therapy (cART). MSM completed behavioural questionnaires and were tested for HIV antibodies every 6 months. Trends in anal intercourse and number of sex partners from 4 years before HIV seroconversion until 4 years after diagnosis were analysed with latent class random effects logistic regression models. We found that the risk of having UAI one year after HIV diagnosis decreased significantly when compared with 1 year before diagnosis in both the pre-cART era (difference=30% [95%CI:22%-36%]) and the cART era (difference=19% [95%CI:9%-30%]). In contrast to a continuing decrease of UAI in the pre-cART era, the probability of UAI in the cART era increased again to pre-seroconversion levels (61% (95%CI: 48%-74%)) 4 years after diagnosis. This study provides evidence that recently seroconverted MSM have reduced their sexual risk behaviour following HIV diagnosis both in the pre-cART era and in the cART period. However, in the cART period there has been less reduction in sexual risk behaviour, and it has returned to pre-cART levels within 4 years. These findings not only confirm the need for early HIV testing, but they also make it clear that much more effort should go into identifying, counselling and possibly treating recently seroconverted MSM who have been found to be one of the most important drivers of HIV transmission amongst MSM [289].

We investigated whether adaptations to cellular immunity have accumulated during the HIV-1 epidemic. To this end, we compared the number of CTL epitopes in HIV-1 strains isolated from individuals who seroconverted at the beginning of the HIV-1 epidemic (1985) and from individuals who seroconverted in recent calendar time (2005). The number of CTL epitopes in HIV-1 variants restricted by the most common HLA alleles in the population did not change significantly during the epidemic. In contrast, we found a significant loss of CTL epitopes restricted by HLA-B alleles associated with a low relative hazard of progression of HIV-1 disease during the epidemic. Such a loss was not observed for CTL epitopes restricted by HLA-A alleles. Thus, despite the large degree of HLA polymorphism, HIV-1 has accumulated adaptations to CTL responses within 20 years of the epidemic. The fact that such adaptations are driven by the HLA-B molecules that provide the best protection against progression of HIV-1 disease has important implications for our understanding of HIV evolution [290].

It was previously shown that HIV-1 has adapted to both the host cellular and humoral immune responses over the course of the epidemic. HIV-1 variants of recently infected individuals were more resistant to antibody neutralization than those from individuals infected in the beginning of the epidemic. With the recent discovery of more potent and cross-reactive broadly neutralizing antibodies, it was thought that these antibodies would overcome this phenomenon. However, the same trend was also observed with the broadly neutralizing antibodies VRC01, PG9 and PG16 [291]. This increased neutralizing resistance

could be attributed to longer V1-V2 regions and an increased number of potential N-linked glycosylation sites (PNGS), as viruses isolated from recently infected individuals could be made sensitive for neutralization if these regions were exchanged between viruses isolated from the beginning of the epidemic [292].

The pressure of the humoral immune system on the virus evolution within one individual was underscored by the analysis on longitudinally sampled HIV-1 env sequences from a patient with cross-reactive neutralizing activity in serum [293]. In this study, it was shown that virus variants with different sensitivities to neutralizing antibody pressure and with different replication fitness may coexist for a certain amount of time but that a changing environment in the host with progression of disease may favour the persistence of HIV-1 variants with the most fit and neutralization-resistant phenotype.

To test whether cellular subsets of the innate arm of the immune response are affected early after HIV-1 transmission in terms of numbers and infection with HIV-1, we used a cohort of HIV-1-infected individuals from the ACS. We analyzed various cellular populations during acute infection. We observed that plasmacytoid DCs (pDCS) represented a non-negligible HIV-1 DNA reservoir and that CD16(+) monocytes contained higher HIV-1 DNA loads than their CD16(-) counterpart during acute infection. Our results demonstrate that cell populations of the innate arm of the immune response are a major reservoir for HIV-1 from very early after transmission. The infection of such cell types will likely contribute to subsequent disease progression and immunodeficiency seen with HIV-1 [294].

Steering committee: The politburo

In 2011, the "politburo" met three times. Twenty-nine proposals for use of data and/or samples (serum/PBMC) were submitted to the politburo: 12 from AMC-Experimental Immunology, 13 from the AMC-Medical Microbiology, 2 from the UMCU, 1 from the GGD and 1 from the AMC-Internal Medicine. All twenty-nine requests were approved; however, one request was withdrawn after approval.

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Theses in 2011 that include ACS data

Diana Edo-Matas, 21 January 2011, "Within-host HIV-1 evolution in relation to viral coreceptor use and host environment". Promotor is Prof. H. Schuitemaker; co-promotor is Dr A. B. van 't Wout.

Marit van Gils, 1 April 2011, "Cross-reactive neutralizing humoral immunity in HIV-1 disease: dynamics of host-pathogen interactions". Promotor is Prof. H. Schuitemaker.

Danielle van Manen, 24 June 2011, "The influence of host genetic factors on HIV-1 infection". Promotor is Prof. H. Schuitemaker; co-promotor is Dr A. B. van 't Wout.

Sebastian Bol, 7 July 2011, "Host genetic effects on HIV-1 replication in macrophages". Promotor is Prof. H. Schuitemaker; co-promotor is Dr A. B. van 't Wout.

8. Curaçao

Ard van Sighem, Daniela Bezemer, Ashley Duits

For more than 5 years, Stichting HIV Monitoring (SHM) has collected data on HIV-infected individuals in Curaçao. As of June 2012, a total of 796 patients were registered; of those, 59% were still in clinical care. At the time of entry into care, 64% already had AIDS or had CD4 counts below 350 cells/mm^3. Hence, for many patients, antiretroviral treatment could be started only when CD4 counts were already below the recommended threshold for treatment initiation, although this situation has improved in recent years. Approximately 80% of patients who started treatment achieved sustained viral suppression. In contrast to patients starting treatment before 2003, those starting after that time had consistently high levels of viral suppression, probably as a result of improved treatment regimens in the past 5 years; the majority of patients are now being treated with a combination of tenofovir/emtricitabine and either lopinavir or efavirenz. Nevertheless, in 2011 virological failure occurred in 11% of treated patients. For a third of those patients, the absence of resistance to any antiretroviral drug suggested that they did not take their medication as prescribed. The frequency of follow-up was according to the recommended guidelines. Thus, on the whole, care and treatment of HIV-infected patients in Curaçao withstands comparison with settings having more resources.

Al meer dan vijf jaar verzamelt de Stichting HIV Monitoring data van HIV-geïnfecteerden op Curaçao. Begin juni 2012 waren er 796 patiënten geregistreerd; 59% van hen is momenteel in klinische zorg. Op het moment van in zorg komen, had 64% van deze patiënten al AIDS of een CD4-celaantal van minder dan 350 cellen/mm^3. Antiretrovirale behandeling kon derhalve bij veel patiënten pas ingezet worden op een moment waarop hun CD4-celaantal onder de aanbevolen grens lag. Dit is de laatste jaren wel verbeterd. Ongeveer 80% van de patiënten die met therapie zijn gestart, slaagt erin langdurige virale onderdrukking te bereiken. In tegenstelling tot de patiënten die voor 2003 met behandeling waren gestart, hadden degenen die daarna startten aanhoudend een hoge mate van virusonderdrukking. Dit is waarschijnlijk een gevolg van de verbeterde behandelregimes in de laatste vijf jaar waarin de meerderheid van de patiënten behandeld wordt met een combinatie van tenofovir/emtricitabine plus lopinavir of efavirenz. Toch kwam virologisch falen in 2011 nog bij 11% van de behandelde patiënten voor. Een derde van de patiënten met therapiefalen heeft tegen geen enkel antiretroviraal geneesmiddel resistentie ontwikkeld. Dit duidt erop dat zij hun voorgeschreven medicatie hoogstwaarschijnlijk niet hadden ingenomen. De frequentie van follow-up is overeenkomstig de aanbevolen behandelrichtlijnen, wat betekent dat de zorg en behandeling van HIV-geïnfecteerden in Curaçao in het algemeen vergelijkbaar is met die in rijkere landen.

Introduction

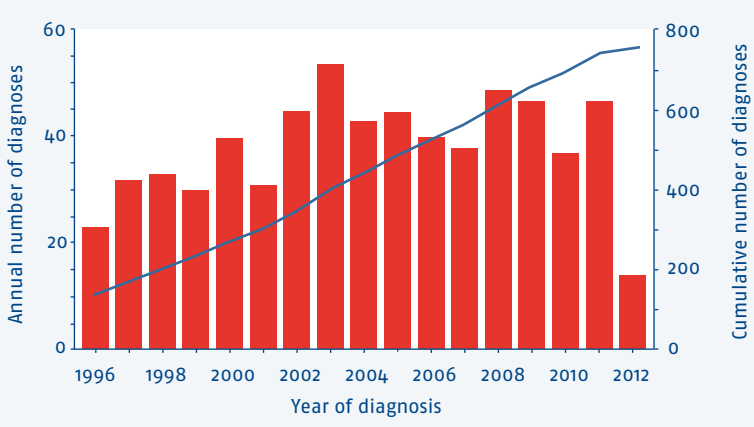
For more than 5 years, Stichting HIV Monitoring (SHM) has collected demographic and clinical data about HIV-infected individuals in clinical care at the St. Elisabeth Hospital in Willemstad in Curaçao. As a result of this registration and monitoring, an extensive database has been established from which a clear picture emerges of the HIV-infected population, the effectiveness of HIV care, and the challenges that are still present in this small Caribbean setting. This special report endeavours to present a concise overview of the current state of HIV infection in Curaçao.

HIV-infected population

Out of the total of 796 HIV-infected patients registered in Curaçao as of June 2012, since the initial registration 157 (20%) have died. The total follow-up for the entire group of 769 patients was 5100 person-years since HIV diagnosis. Of the 639 patients who were still alive, 466 (73%) were also still in clinical care and had at least one contact with the treating physician in Curaçao since January 2011.

In total, 231 (29%) of the registered patients were diagnosed with HIV in or before 1999; 72 (31%) of those patients died before June 2012 (*Figure 8.1*; *Web Appendix Table 8.1*). Between 2000 and June 2012, 530 patients were diagnosed, whereas for the remaining 35 patients, no information regarding the date of their first positive HIV test was available. By far, the majority of the patients were infected with HIV-1, whilst two patients were infected with HIV-2, and four other patients had antibodies against both HIV-1 and HIV-2. Almost three-quarters of the registered population originated from the former Netherlands Antilles, and two-thirds reported being infected via heterosexual contact (*Table 8.1*).

Figure 8.1: *Annual and cumulative number of HIV diagnoses amongst 796 HIV-infected patients in Curaçao registered by Stichting HIV Monitoring as of June 2012. In total, 113 patients were diagnosed prior to 1996, whilst for 35 patients the year of diagnosis was unknown or not yet recorded.*



Legend: *Bars=annual number of diagnoses; line=cumulative number of diagnoses since the start of the HIV epidemic*

Table 8.1: Characteristics of the HIV-infected population in Curaçao registered by Stichting HIV Monitoring as of June 2012.

	Alive, N=639		Dead, N=157		Total, N=796	
	N / median	% / IQR	N / median	% / IQR	N / median	% / IQR
Sex						
Male	387	61	109	69	496	62
Female	252	39	48	31	300	38
Transmission						
MSM	120	19	15	10	135	17
Heterosexual	437	68	98	62	535	67
Other/unknown	82	13	44	28	126	16
Country of birth						
Antilles	446	70	139	89	585	73
Haiti	77	12	7	4	84	11
Dominican Republic	55	9	6	4	61	8
Other	61	10	5	3	66	8
Treated with cART						
No	160	25	57	36	217	27
Yes	479	75	100	64	579	73
Diagnosis						
CD4 (cells/mm^3)	342	140–505	94	33–315	321	94–482
RNA (log_{10} copies/ml)	4.5	3.8–5.0	4.9	4.0–5.5	4.5	3.9–5.0
Age (years)	38	30–46	41	32–55	38	30–47
AIDS	35	5	31	20	66	8
Time to cART	1.1	0.3–4.3	0.8	0.2–4.1	1.0	0.2–4.2
Follow-up (years)	5.8	1.6–11.1	2.7	0.3–7.1	5.0	1.2–10.2
Start of cART						
CD4 (cells/mm^3)	173	56–287	79	12–189	155	46–274
RNA (log_{10} copies/ml)	4.9	4.4–5.4	4.8	4.3–5.4	4.9	4.4–5.4
Age (years)	42	34–49	46	38–57	43	35–51
AIDS	67	10	51	32	118	15
Follow-up (years)	4.5	1.7–9.1	1.6	0.1–4.5	3.9	1.3–8.0
Present (June 2012)[a]						
CD4 (cells/mm^3)	469	316–647	-	-	469	316–647
RNA <500 copies/ml	306	71[b]	-	-	306	71[b]
Age (years)	48	40–56	-	-	48	40–56

[a] *for 466 patients still in clinical care;*

[b] *percentage of 428 patients with a viral load measurement.*

Legend: *IQR=interquartile range; MSM=men having sex with men; cART=combination antiretroviral therapy.*

Children and adolescents

At the time of diagnosis, 15 patients were younger than 13 years of age ('children') and 16 were aged 13 to 18 years ('adolescents'). Most of the children, 12 in total, were infected by mother-to-child transmission. Adolescents were mainly infected via either heterosexual contact (11) or homosexual contact (4 patients). As a result of universal testing of pregnant women in Curaçao, only two children were diagnosed with HIV in or after 2000. During the same period, 12 adolescents were diagnosed. In total, 9 children and 1 adolescent have died. Of the other 6 children, 3 were still in clinical care as were 9 adolescents.

Country of infection

For 501 patients, or 63% of the registered population, the most likely country of infection was known. For 448 (89%) of those patients, the country of infection was the former Netherlands Antilles. This percentage was even higher (96%) amongst the 393 patients who were also born in the Antilles. Of the 501 patients, 15 reported that they were infected in the Netherlands, 15 in Haiti, and 10 in the Dominican Republic. All but four of the 238 patients with a known HIV-1 subtype were infected with a subtype B virus, which is the most prevalent subtype in both the Caribbean and the Netherlands amongst patients of non-African origin.

Hepatitis B and C

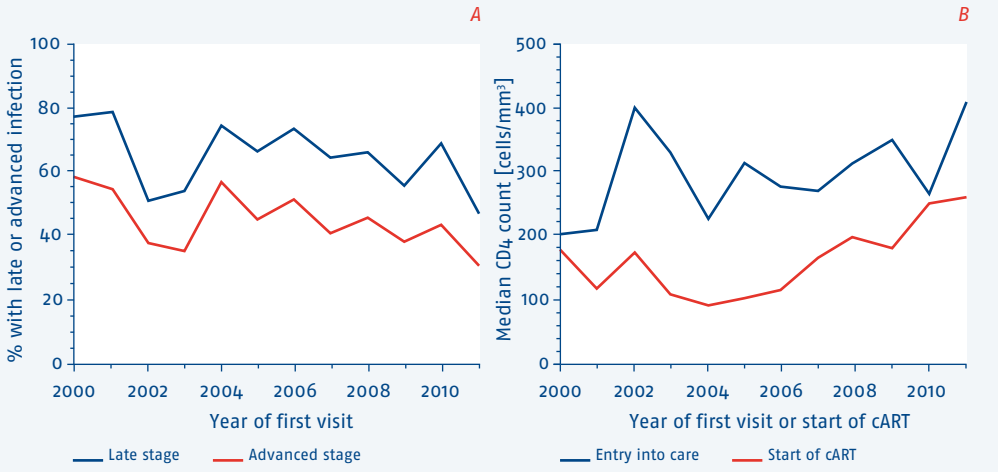
In total, 44 patients, or 8%, of the 578 who were tested were co-infected with hepatitis B. The prevalence of hepatitis B did not differ by transmission risk group, but it appeared to be somewhat higher amongst men (9%) than amongst women (4%). Co-infection with hepatitis C was found in only 7 patients, or 1% of the 510 tested.

Late presentation and start of treatment

At the time of the first visit to the hospital, 400 (64%) of the 627 patients who could be classified presented with late-stage HIV infection, that is, with a concurrent AIDS diagnosis or with CD4 counts below 350 cells/mm^3 (*Figure 8.2A*) [11]. Of these 400 patients, 278 (70%) were already in an advanced stage of their infection. Late and advanced entry into care probably reflects a combination of late testing and a delay between HIV diagnosis and entry into care.

As a result of late entry into care, median CD4 counts at the start of cART were low, 155 cells/mm^3, which is well below the threshold of 200 cells/mm^3 at which treatment definitely should be started. Nevertheless, only 15% of the patients had been diagnosed with an AIDS-defining event by the time treatment was started. In recent years, there has been an increase in CD4 cell counts at the start of cART (*Figure 8.2B*). Between 2009 and 2012, 43% of the patients for whom a CD4 count was available at the start of cART had less than 200 cells/mm^3, whilst 44% had counts between 200 and 350 cells/mm^3.

Figure 8.2: (A) From 2000 onwards, 64% of patients entered clinical care with late-stage HIV infection, whilst 43% had advanced HIV infection. Late-stage infection: CD4 counts below 350 cells/mm³ or having AIDS, regardless of CD4 count. Advanced-stage infection: CD4 counts below 200 cells/mm³ or having AIDS. (B) From 2000 onwards, median CD4 counts at the time of entry were 301 cells/mm³ (interquartile range [IQR], 106-460), whilst they were 165 cells/mm³ (IQR, 51-274) at the start of cART. In recent years, both CD4 counts at the time of entry into care and at start of cART clearly increased to 408 and 260 cells/mm³, respectively, in 2011, indicating more timely diagnosis and start of treatment.



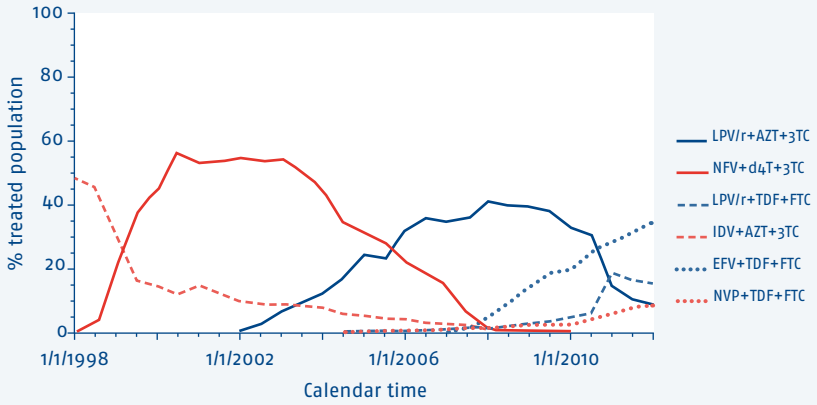
Patient monitoring

Current guidelines recommend monitoring HIV-infected patients two or three times a year, depending on CD4 count and treatment status [13]. In most recent years, these guidelines have been generally well followed. Between 2007 and 2011, on average, 2.2 immunology measurements were performed annually per patient. During the same period, the viral load was monitored 1.8 times per year, whilst follow-up visits for each patient averaged 2.8 per year.

Combination treatment

In total, 579 (73%) patients started cART. Of the 254 who did so between 2007 and 2012, 44% started with a combination of combivir and ritonavir-boosted lopinavir and 38% with a combination of tenofovir/emtricitabine and efavirenz. Over time, there have been clear shifts in the treatment regimens (*Figure 8.3*). Since 2008, a combination of tenofovir/emtricitabine with efavirenz, nevirapine, or lopinavir has become more popular. Of the 396 patients who started cART and were still in clinical care as of June 2012, 36% were receiving efavirenz, 31% lopinavir, and 14% nevirapine, whilst 69% were receiving tenofovir/emtricitabine.

Figure 8.3: Percentage of patients treated with combination antiretroviral therapy (cART) by specific regimens over calendar time. The proportion of patients taking IDV+AZT+3TC decreased from 48% in 1998 to almost 0% after 2007. This decrease was counterbalanced by an increase in the proportion of patients treated with NFV+d4T+3TC. Since 2002, a combination of LPV/r+AZT+3TC has been used increasingly until 2010. The use of EFV+TDF+FTC and LPV/r+TDF+FTC increased from 2008 onwards, and at the beginning of 2012, 34% of the patients were receiving EFV+TDF+FTC, 15% LPV/r+TDF+FTC, and 9% LPV/r+AZT+3TC.



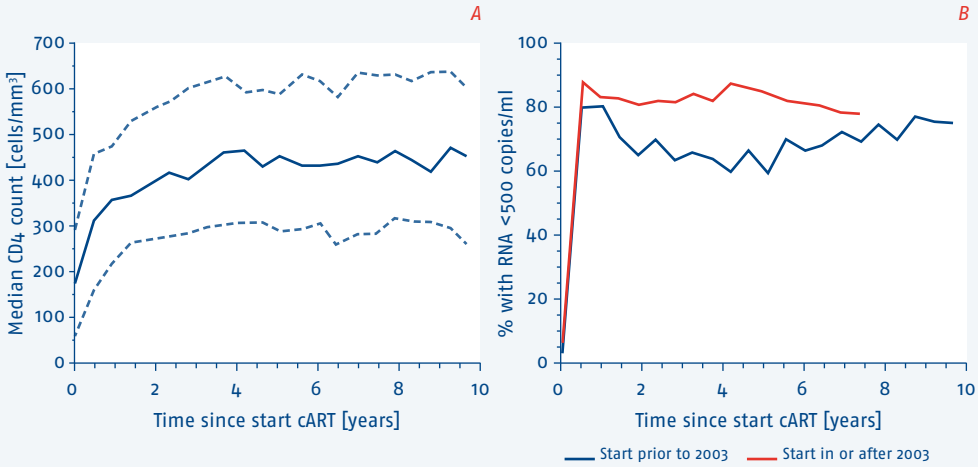
Legend: LPV/r=ritonavir-boosted lopinavir; AZT=zidovudine; 3TC=lamivudine; NFV=nelfinavir; d4T=stavudine; EFV=efavirenz; TDF=tenofovir; FTC=emtricitabine; NVP=nevirapine; IDV=indinavir.

Treatment outcome

For 47% of the 537 antiretroviral therapy-naïve patients who started cART, CD4 counts increased by at least 150 cells/mm^3 during the first 6 months of treatment; after 2 years, this proportion increased to 78%. At the same time, 80% of the patients reached a viral load level below 500 copies/ml within 6 months after starting treatment.

In patients who were still in clinical care as of June 2012, CD4 counts reached a plateau between 450 and 500 cells/mm^3 after 5 years of cART (*Figure 8.4A*). During the same period, the proportion of patients with a viral load below 500 copies/ml decreased from 82% after 48 weeks to 72% after 5 years of treatment. However, amongst those who started cART in 2003 or later, i.e., when more efficacious treatment combinations came into use in Curaçao, the proportion of patients who were able to retain viral suppression remained approximately 80% (*Figure 8.4B*). For 81% of the patients still in clinical care, the most recent viral load result was below 500 copies/ml, and this proportion was the same irrespective of the period in which cART was started.

Figure 8.4: CD4 cell counts and viral load in 389 treated patients who were still in clinical care as of June 2012. (A) Median CD4 counts (solid line; dotted line: interquartile range [IQR]) increased from 181 (IQR, 56–287) cells/mm³ at the start of combination antiretroviral therapy (cART) to 316 (167–465) cells/mm³ after 24 weeks and reached a plateau between 450 and 500 cells/mm³ after 5 years. (B) The proportion of patients with HIV RNA <500 copies/ml was 82% after 48 weeks, and it remained at a high level amongst those who started cART in 2003 or later, but gradually declined to levels between 60% and 75% after 5 years for those who started prior to 2003.



Virological failure

As viral suppression rates appear to have increased, one may presume that, conversely, rates of virological failure have decreased. Indeed, when considering HIV RNA above 500 copies/ml despite at least 4 months of continuous treatment as virological failure, the proportion of patients with virological failure steadily declined from approximately 32% between 2000 and 2004 to 11% in 2011. Nevertheless, failure rates are still higher than in the Netherlands.

Mortality and survival

Of the group of 663 patients who were still alive as of 1 January 2005 or were diagnosed after that date, 22 patients died within 6 months, whilst 77 had died by June 2012. Overall, the survival probability after 6 years of follow-up was 85%. Altogether, 325 patients started cART in or after 2005, and out of this group, 36 died, 17 of those died within 6 months of starting cART. After six years, the survival probability was 85%, with no recorded deaths after the fourth year.

Drug resistance

With so many patients experiencing virological failure, it may be expected that resistance to one or more antiretroviral drugs will have developed in some patients. For 158 patients, 237 genotypic sequences of the protease and reverse transcriptase (RT) gene were examined for drug resistance after the start of treatment. In total, 86 patients, or 54%, of those sequenced, had high-level resistance to at least one antiretroviral drug, according to the Stanford interpretation algorithm [180].

In total, 200 out of the 237 genotypic sequences were obtained when the 158 patients were supposedly being treated. Altogether, 62% of these 200 sequences had high-level resistance to at least one antiretroviral drug. Resistance to lamivudine and emtricitabine was observed in 42% of the sequences; resistance to at least one other nucleoside RT inhibitor was found in 20%, to protease inhibitors in 32% and to non-nucleoside RT inhibitors in 24%. On the other hand, 31% of the sequences indicated full susceptibility to all drugs. Most likely, this means that about a third of the patients who experienced treatment failure did so because they did not take their medication as prescribed, for instance, because of drug-related toxcity.

Infection with resistant virus

Infection with a resistant virus, and consequently the preclusion of certain drugs from the antiretroviral arsenal, does not seem to be a major problem at the moment. Infection with resistant virus was investigated in 62 patients who had a genotypic sequence within 1 year of diagnosis but before the start of treatment. Resistance-associated mutations were detected in five patients, which is two more than reported previously, most likely as a result of updates in the drug-resistance mutations list of the International Antiviral Society-USA (IAS-USA) [7, 227]. These mutations gave rise to high-level resistance in only one patient; this may have been, however, because a pre-treatment sample from this patient in which no resistance was found could have been confused with a sample with resistance mutations obtained during treatment.

Transmission networks

Geographic mobility between the Netherlands and Curaçao is relatively high, and intra-Caribbean migration and tourism are also common. A phylogenetic tree was constructed with the first polymerase subtype B sequence available as of June 2011 from 219 persons in Curaçao, together with sequences from patients in the Netherlands and the rest of the world as available online [295, 296]. Selecting clusters with ≥5 Curaçao sequences identified 96 (44%) sequences from patients on Curaçao in 7 transmission clusters in the phylogenetic tree, together with 73 sequences from patients in the Netherlands. Of the patients from the Netherlands, 55% (40) were born on Curaçao. All 7 clusters showed a mix of sequences from both the Netherlands and Curaçao. One of the clusters was embedded in a cluster of sequences from Honduras. The networks are estimated to have been spreading on Curacao since the 1980s or early 1990s.

Conclusion

In recent years, the quality of care and treatment offered to HIV-infected patients in Curaçao has improved considerably and can now withstand a comparison with resource-rich settings. However, adherence to treatment and retention in care need to be improved in order to reduce the number of patients failing on treatment. Also, HIV infections need to be detected at an earlier stage, such that patients can start antiretroviral treatment in accordance with current recommendations.

List of tables & figures

Web Appendix list of tables and figures

An appendix containing tables and figures supplementary to this report can be found on the Stichting HIV Monitoring website, *www.hiv-monitoring.nl*.

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Publications & presentations 2012

Publications

HCV viremia increases the incidence of chronic kidney disease in HIV-infected patients.
Peters L, Grint D, Lundgren JD, Rockstroh JK, Soriano V, Reiss P, Grzeszczuk A, Sambatakou H, Mocroft A, Kirk O; for EuroSIDA in EuroCoord. *AIDS. 2012 Sep 24;26(15):1917-1926.*

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Smit M, Smit C, Cremin I, Garnett GP, Hallett T, de Wolf F. *AIDS. 2012 Sep 24;26(15):1953-9.*

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van Sighem A, Jansen I, Bezemer D, De Wolf F, Prins M, Stolte I, Fraser C. *AIDS. 2012 Sep 10;26(14):1840-3.*

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The HIV-CAUSAL Collaboration. *AIDS. 2012 Aug 24;26(13):1691-705.*

No advantage of quadruple or triple-class antiretroviral therapy as initial treatment in patients with very high viraemia.
Grijsen ML, Holman R, Gras L, Wit FW, Hoepelman AI, van den Berk GE, de Wolf F, Prins JM; the ATHENA National Observational Cohort Study. *Antivir Ther. 2012 Aug 20. doi: 10.3851/IMP2321. [Epub ahead of print]*

Effect of hepatitis C treatment on CD4 cell counts and the risk of death in HIV-hepatitis C co-infected patients; COHERE collaboration.
The HCV working group of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord. *Antivir Ther. 2012 Jul 24. doi: 10.3851/IMP2263. [Epub ahead of print]*

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Zhang S, van Sighem A, Kesselring A, Gras L, Smit C, Prins JM, Kauffmann R, Richter C, de Wolf F, Reiss P. *J Acquir Immune Defic Syndr. 2012 Jul 1;60(3):265-272. Epub 2012 Apr 23.*

The clinical interpretation of viral blips in HIV patients receiving antiviral treatment: are we ready to infer poor adherence?
Chun-Hai Fung I, Gambhir M, van Sighem A, de Wolf F, Garnett GP. *J Acquir Immune Defic Syndr. 2012 May 1;60(1):5-11. Epub 2012 Jan 19.*

Impact of Antiretroviral Therapy on Tuberculosis Incidence Among HIV-Positive Patients in High-Income Countries.
The HIV-CAUSAL Collaboration. *Clin Infect Dis. 2012 May;54(9):1364-72. Epub 2012 Mar 28.*

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The Primo-SHM study group. *HIV Med. 2012 Apr 25. doi: 10.1111/j.1468-1293.2012.01020.x. [Epub ahead of print]*

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Lewden C, Bouteloup V, De Wit S, Sabin C, Mocroft A, Wasmuth JC, van Sighem A, Kirk O, Obel N, Panos G, Ghosn J, Dabis F, Mary-Krause M, Leport C, Perez-Hoyos S, Sobrino-Vegas P, Stephan C, Castagna A, Antinori A, d'Arminio Monforte A, Torti C, Mussini C, Isern V, Calmy A, Teira R, Egger M, Grarup J, Chêne G voor The Collaboration of Observational HIV Epidemiological Research Europe (COHERE) in EuroCoord. *Int J Epidemiol. 2012 Apr;41(2):433-445.*

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Snijdewind IJ, Smit C, Godfried MH, Nellen JF, de Wolf F, Boer K, van der Ende ME. *J Infect. 2012 Apr;64(4):409-16. Epub 2011 Dec 23.*

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Nakagawa F for the PLATO II group for COHERE in EuroCoord. *JAIDS 2012 Mar 1;59(3):294-9.*

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The Pursuing Later Treatment Option II (PLATO II) project team* for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Group*. *Lancet Infect Dis. 2012 Feb;12(2):119-27. Epub 2011 Oct 9.*

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Jarrin I, Pantazis N, Gill MJ, Geskus R, Perez-Hoyos S, Meyer L, Prins M, Touloumi G, Johnson A, Hamouda O, de Olalla PG, Porter K, del Amo J; CASCADE Collaboration in EuroCoord. *Clin Infect Dis. 2012 Jan 1;54(1):111-8. Epub 2011 Nov 21.*

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van Manen D, Gras L, Boeser-Nunnink BD, van Sighem AI, Maurer I, Mangas Ruiz MM, Harskamp AM, Steingrover R, Prins JM, de Wolf F, van 't Wout AB, Schuitemaker H; Dutch HIV monitoring foundation HIV-1 Host Genetics study. *AIDS. 2011 Nov 28;25(18):2217-26.*

Comparative Effectiveness of Initial Antiretroviral Therapy Regimens: ACTG 5095 and 5142 Clinical Trials Relative to ART-CC Cohort Study.
Mugavero MJ, May M, Ribaudo HJ, Gulick RM, Riddler SA, Haubrich R, Napravnik S, Abgrall S, Phillips A, Harris R, Gill MJ, de Wolf F, Hogg R, Günthard HF, Chêne G, D'arminio Monforte A, Guest JL, Smith C, Murillas J, Berenguer J, Wyen C, Domingo P, Kitahata MM, Sterne JA, Saag MS; on behalf of the AIDS Clinical Trial Group DACS 241 team AIDS Clinical Trial Group Study 5095 team AIDS Clinical Trial Group Study 5142 team and the Antiretroviral Cohort Collaboration. *J Acquir Immune Defic Syndr. 2011 Nov 1;58(3):253-60, Epub ahead of print Aug 18.*

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Damond F, Benard A, Balotta C, Böni J, Cotten M, Duque V, Ferns B, Garson J, Gomes P, Gonçalves F, Gottlieb G, Kupfer B, Ruelle J, Rodes B, Soriano V, Wainberg M, Taieb A, Matheron S, Chene G, Brun-Vezinet F; for the ACHIEV2E Study group. *J Clin Microbiol. 2011 Oct;49(10):3491-7. Epub 2011 Aug 3.*

The comparison of the performance of two screening strategies identifying newly-diagnosed HIV during pregnancy.
Boer K, Smit C, van der Flier M, de Wolf F; ATHENA cohort study group. *Eur J Public Health. 2011 Oct;21(5):632-7. Epub 2010 Nov 4.*

Oral presentations

ECDC work on estimating HIV prevalence in EU/EEA/EFTA countries
Van Sighem A, Fraser C, Garnett G, Phillips A, Lodwick R, Keiser O, Egger M, de Wolf F, Asikainen T, Salminen M, van de Laar M, Bergroth T. *Networks: stochastic models for populations and epidemics, Edinburgh, Scotland, 12-16 September 2011.*

Immigrants and HIV
De Wolf F, Nellen J, Prins J, Wit F, Lange J, Jurriaans S. *6th Ethnic Minority Conference, Amersfoort, the Netherlands, 14 October 2011.*

Developments in the HIV epidemic in the Netherlands
Van Sighem A, Gras L, Smit C, Homan R, Bezemer D, Zaheri S, de Wolf F. *NCHIV 2011, Amsterdam, the Netherlands, 29 November 2011.*

Comorbidity and Ageing in HIV-1-infection
Schouten J, Wit F, Stolte I, Van der Valk M, Geerlings S, de Wolf F, Prins M, Reiss P, on behalf of the AGEhIV Study Group. *NCHIV 2011, Amsterdam, the Netherlands, 29 November 2011; 14th International AIDS Conference, Washington DC, USA, 22-27 July 2012.*

Delay of entry into care in HIV-positive individuals
Veen M, Heijman R, Götz H, de Wolf F, Zaheri S, Fennema J, van der Sande M. *NCHIV 2011, Amsterdam, the Netherlands, 29 November 2011.*

Tenofovir/emtricitabine versus abacavir/lamivudine
Holman R, Gras L, Prins J, de Wolf F. *Winter meeting NVHB, Amsterdam, the Netherlands 13 January 2012.*

Estimating HIV prevalence in European countries
Van Sighem A on behalf of the HIV modelling project team. *HIV in Europe, Copenhagen, Denmark, 19 March 2012.*

Effect of hepatitis C treatment on the risk of death in HIV/HCV co-infected patients; European Cohort Collaboration (COHERE) in EuroCoord
Smit C, on behalf of the HCV working group of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Temporal changes and regional differences in treatment uptake of hepatitis C therapy in EuroSIDA
Grint D on behalf of EuroSIDA. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Higer rates of AIDS and death during the first year of therapy among those born outside Europe, the United States, and Canada: the importance of tuberculosis
Sheperd B on behalf of ART-CC. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Emulating randomized trials with dynamic strategies: an empirical comparison between effect estimates from randomized and observational studies
Young J on behalf of the HIV-CAUSAL Collaboration. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

When to switch cART: Novel methods for the comparison of switchting strategies using observational data
Cain L on behalf of the HIV-CAUSAL Collaboration. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Time to virologic response: performance of parametric survival models under non-random interval censoring
Pantazis N on behalf of CASCADE. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Comorbidity and Ageing in HIV-1 infection
Schouten J on behalf of The AGEhIV cohort study. *16th International Workshop on HIV Observational Databases, Athens, Greece, 29-31 March 2012.*

Advanced renal disease and end-stage renal disease in Europe
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