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Intro duction

Introduction

The short-term beneficial effect of highly active antiretroviral therapy (HAART) on survival and development of AIDS has now been well established[1-4]. Since the introduction of HAART in 1996 as part of the regular treatment in the Netherlands, HIV-related morbidity and mortality has declined dramatically[5]. Besides an increase in life expectancy, the quality of life of patients improved as well[6]. In the final report of the Foundation's preceding project, ATHENA, we presented the first tentative figures to the contrary of the success of HAART treatment: adverse events, toxicity of the drugs used[7,8] and, as a possible consequence, the appearance of HIV strains that became resistant, all resulting in therapy failure[5].

In the present report we further the contribution of the ATHENA project to our knowledge of the epidemic and treatment of HIV in the Netherlands. Within the framework of the registration programme of the HIV Monitoring Foundation, the main objective is to study the natural history and the effect of antiretroviral treatment on the HIV infection. With this objective in mind a number of operational questions were asked:

- What changes have been seen since the introduction of HAART in 1995/1996 on HIV-related morbidity and mortality?
- What determines therapy success and what determines therapy failure?
- What, after a first or second failure on antiretroviral therapy, determines therapy outcome?
- To what extent does toxicity of the drugs or drug combinations used influence the course of a treated HIV infection?
- To what extent does resistance play a role in the course of a treated HIV infection?

In this report we will present results of the monitoring of the HIV infection and its HAART treatment over a period of six to seven years. In addition, we will present data obtained from the monitoring of infected patients who are treated with a combination of antiretroviral drugs that cannot be defined as HAART and patients who do not receive any treatment at all. Based on these results we will review a number of the recommendations that were made in the final report of the ATHENA project.

The HIV Monitoring Foundation largely continued the organisational structure that had been established for the monitoring of HIV in the ATHENA project. However, a few important changes were made. In the ATHENA project only HAART-treated patients who signed an informed consent form and who were eighteen years old or older were included. At present, in accordance with one of the recommendations made in the final report, all patients are included, irrespective of treatment and irrespective of age. Moreover, as HIV monitoring is considered to be part of patient care, an informed consent form is no longer required. This does not imply that patients are kept ignorant. Before entry patients are informed about the monitoring and the various procedures by their treating physician and also through written information produced by the HIV Monitoring Foundation.

As of 1 October 2002, 6302 patients have been registered in the monitoring system, which is 2734 more than in the ATHENA project and approximately 37% of the estimated (WHO/UNAIDS 2002) total population of 17000 HIV-infected individuals in the Netherlands. Of these registered patients, approximately 1.5% refuses participation in the monitoring of HIV, mainly for reasons of privacy. The remaining group of patients form an observational cohort, and the data obtained, despite being biased to some degree owing to this observational character and the way in which the data are collected, have resulted in research into the impact of antiretroviral treatment on the course of infection and public health.

Summary

Summary

Further to the ATHENA project, the monitoring of the course and treatment of the HIV infection in the Netherlands is continued in the HIV Monitoring Foundation. Inclusion of patients is now no longer restricted to patients on highly active antiretroviral therapy (HAART) who signed an informed consent. Instead, the inclusion procedure has been simplified since January 2002, so that all HIV-infected individuals can be included, whether or not they have been treated with antiretroviral drugs. Clinical, epidemiological, socio-demographic, virological as well as immunological data are obtained from these patients at every visit to the treating physician in one of the 21 HIV Treatment Centres. Coordination of the monitoring of HIV and collection, quality control, management and analysis of the data is organised within the framework of the HIV Monitoring Foundation in Amsterdam. The former ATHENA system of consultative working groups and meetings is being continued. Performance of the HIV Monitoring Foundation is reviewed on a regular basis by the foundation's international advisory board. The national advisory board is key to reviewing the requests for access to the collected data. In addition, the Dutch Association of AIDS Physicians provides organisational support.

Per 1 October 2002, 6302 patients had been registered in the monitoring system, 2734 more than in the ATHENA project and approximately 37% of the estimated total population of HIV-infected individuals in the Netherlands. Of these registered patients, approximately 1.5% refused participation in the monitoring of HIV, mainly for reasons of privacy. The remaining group of patients formed an observational cohort of whom data were collected with a frequency of on average once every three months.

In the present report data were used from patients included in the HIV Monitoring Foundation up to 1 April 2002. At that moment, a total of 5102 patients had been registered, 4156 (81.5%) of whom had been included between 1998 and 2001 during the ATHENA project and 356 (7%) since January 2002. Of 590 (11.5%) patients the monitoring of HIV had already started prior to the ATHENA project. Compared to the ATHENA final report, data of an additional 1754 patients were used in the present analysis.

The effect of HAART in the HIV-infected population remains impressive. An ongoing decline in HIV-related morbidity and mortality in the HAART-treated population is found. Large proportions of HAART-treated patients reach plasma HIV RNA levels <1000 copies/ml and achieve an increase in CD4+ T cells of at least 100 cells per mm^3. This effect in the population remains over a prolonged period of time. However, as in the ATHENA cohort there are differences between antiretroviral-therapy-pre-treated and -naïve patients. In general, the time to a positive response is shorter, therapy failure occurs less frequently, and if it does, the time to therapy failure takes longer in naïve patients. Moreover, naïve patients change their therapy less frequently than pre-treated patients. Therapy failure and therapy change, including therapy interruption, are predictive of new CDC-C events and death.

No major differences in treatment effect were found between men and women, and gender did not significantly influence treatment outcome. As the inclusion of non-western patients, especially patients from endemic areas, has only just started, we were unable to draw conclusions about differences in effect of treatment between groups of patients of different origin. From the limited available data, so far a relatively large proportion of non-B HIV-1 infections appears to exist among non-western patients, and the proportion of patients with heterosexual contact as risk factor and the proportion of women in this group is larger as well.

At present only a small number of patients without antiretroviral treatment or with a non-HAART treatment regimen (ART-treated patients) have been included and results obtained point at positive selection, reason why no comparisons were made with the HAART-treated groups.

Toxicity due to the antiretroviral drugs used was frequent, also on a longer time scale, although there were no indications that toxicity was of significant influence on mortality in the HAART-treated group. Signs and symptoms of toxicity remained the same. Transmission of antiretroviral-drug-resistant HIV was low, although the data available were still limited and underestimation because of sampling failures remained an issue. Resistance was found especially in patients who had been sub-optimally treated before they started HAART. Whether or not the level of resistance will increase with the increasing number of HAART-regimen changes is still to be investigated. The finding that large proportions of HAART-treated patients experienced a second and third regimen change after the first change, together with the correlation of therapy change with therapy failure and subsequently outcome, is of major concern.

Based on the findings of the present report it is recommended to initiate the following projects through the framework of the HIV Monitoring Foundation:

- Improved registration of resistance combined with data on adherence and plasma drug levels:
  - Active measurement of resistance of HIV a) at entry for every newly diagnosed HIV infection and b) at virological therapy failure. Initiation and support of an organisational structure among the HIV Treatment Centres for adequate storage of patient material to perform resistance measurements and for the collection, storage and analysis of resistance data.
  - Active measurement of adherence in relation to patterns of antiretroviral drug use in a subgroup of patients, in addition to self-reported adherence.
  - Structured collection of data regarding plasma levels of antiretroviral drugs.
- Improved registration of toxicity of the various drugs used in HAART combinations and of the frequency of therapy changes or interruptions.
- Improved registration of non-treated and non-HAART-treated patients.
- Continuation of the inclusion of treated and untreated patients from non-western, endemic areas and improved registration of HIV-1 subtypes.

Samenvatting

Samenvatting

De monitoring van HIV in Nederland is een vervolg op het ATHENA project dat in 2000 werd afgesloten. In tegenstelling tot de situatie in het ATHENA project is er geen sprake meer van beperking van de monitoring tot alleen die HIV-geïnfecteerde patiënten die werden behandeld met HAART en een *informed consent* hadden getekend. In plaats daarvan wordt sinds januari 2002 een simpeler inclusieprocedure gehanteerd, waarbij patiënten in de monitoring kunnen worden opgenomen onafhankelijk van hun antiretrovirale behandeling.

Van deze patiënten werden bij elk bezoek aan hun behandelend arts in een van de 21 erkende HIV Behandel Centra klinische, epidemiologische, socio-demografische, virologische en immunologische gegevens verzameld. Coördinatie van de monitoring werd uitgevoerd door de Stichting HIV Monitoring (SHM) in Amsterdam. De SHM was bovendien verantwoordelijk voor de uitvoering en bewaking van de kwaliteitscontrole van de gegevensverzameling, het datamanagement en de bewerking en analyse van de gegevens.

De in het ATHENA project opgebouwde organisatie van adviserende werkgroepen werd gecontinueerd. Bovendien werd een adviesraad van de SHM ingesteld die een sleutelrol speelt in de goedkeuring van aanvragen voor het gebruik van gegevens die door de SHM in het kader van de monitoring van patiënten worden verzameld. De Nederlandse Vereniging van Aids Behandelaren (NVAB) was betrokken bij de organisatie van werkgroepen en adviesraad.

Op 1 oktober 2002 waren 6302 HIV-geïnfecteerde patiënten geregistreerd, 2734 patiënten meer dan in het ATHENA project. Het aantal geregistreerde patiënten is naar schatting 37% van de totale groep personen met een HIV-infectie in Nederland. Van de geregistreerde patiënten weigerde ongeveer 1.5% verdere deelname aan de monitoring van de HIV-infectie, voornamelijk om privacy-redenen. De overblijvende groep patiënten kan worden beschouwd als een observationeel klinisch cohort, waarin van patiënten gemiddeld elke drie maanden gegevens werden verzameld.

In dit rapport worden gegevens gebruikt van patiënten die werden geïncludeerd tot 1 april 2002. Op dat moment waren gegevens van in totaal 5102 patiënten in de Stichting HIV Monitoring opgenomen. Van deze groep werden er 4156 (81.5%) geregistreerd in de ATHENA projectperiode mei 1998 – december 2001 en 356 (7%) vanaf 1 januari 2002. Van 590 (11.5%) patiënten waren al gemonitorde gegevens beschikbaar in de periode voorafgaande aan het ATHENA project. In vergelijking tot het ATHENA eindrapport bevatte het analysebestand voor het nu voorliggende rapport gegevens van 1754 extra patiënten.

Het effect van 'highly active antiretroviral therapy' (HAART) in de HIV-geïnfecteerde groep bleef indrukwekkend. HIV-gerelateerde morbiditeit en mortaliteit daalde ook na de laatste rapportagedatum (november 2000). De HIV-1 RNA concentratie in bloed daalde naar waarden <1000 kopieën/ml in een groot deel van de met HAART behandelde patiënten. Tegelijkertijd steeg bij een groot deel het aantal CD4+ T cellen met ten minste 100 per mm^3. Dit effect bleef ook over een langere follow-upperiode zichtbaar. Maar net als in het ATHENA project waren er ook verschillen in effect tussen patiënten die voor de start van hun HAART-behandeling nooit met antiretrovirale middelen waren behandeld en patiënten die wel ooit antiretrovirale behandeling hadden gehad. Over het algemeen deden patiënten zonder enige behandeling voorafgaand aan HAART het beter dan de 'voorbehandelde' patiëntengroep. De responstijd van niet-voorbehandeld patiënten was korter, er was minder vaak sprake van therapiefalen, en de periode tot falen duurde langer. Bovendien waren er minder vaak wijzigingen in het HAART-regiem. Dat is van belang omdat falen van therapie, maar ook verandering van therapieregiem, inclusief onderbreking van behandeling, voorspellend bleek voor het verdere beloop van de infectie.

Er werd geen verschil in het antivirale of klinische effect van HAART gevonden tussen mannen en vrouwen. Geen conlusies konden worden getrokken over eventuele verschillen tussen allochtone en autochtone patiëntengroepen, aangezien de inclusie van allochtone HIV-geïnfecteerde patiënten pas zeer recent was gestart. Wel werd duidelijk dat in vergelijking met de autochtone groep een relatief groot deel van de allochtone patiënten besmet is met een non-B HIV-subtype, heterosekueel contact als een belangrijker risicofactor voor besmetting heeft, en het aandeel vrouwen in de allochtone groep groter is.

In het huidige gegevensbestand waren vooralsnog weinig patiënten opgenomen die helemaal geen of 'geen-HAART'-therapie kregen. De resultaten die uit deze twee groepen werden verkregen, wijzen vooral op positieve selectie: de groep patiënten zonder therapie krijgt geen therapie omdat ze het virologisch en immunologisch goed doen. Hetzelfde geldt voor de niet-HAART-behandelde patiënten.

Toxiciteit als gevolg van de antiretrovirale behandeling bleek, ook op langere termijn, een vaak voorkomend probleem. Er waren echter geen aanwijzingen dat toxiciteit van invloed was op de mortaliteit in de met HAART behandelde groep. Over het algemeen bleven de aan toxiciteit gerelateerde symptomen dezelfde als die welke in het ATHENA project werden gerapporteerd. Overdracht van resistente HIV bleek nog steeds beperkt, maar de beschikbare hoeveelheid gegevens was relatief klein en het probleem van onderschatting als gevolg van het onbekend zijn met het infectiemoment van de meeste patiënten werd nog niet opgelost. Resistentie bleek zeker een probleem bij voorbehandelde patiënten. Of resistentie ernstiger wordt naarmate het aantal therapiewijzigingen toeneemt, zal nog moeten blijken. Maar het grote aantal patiënten dat na een eerste therapiewijziging voor een tweede en zelfs een derde keer van therapie verandert, baart in dit verband zorgen.

Op basis van de bevindingen in het onderhavige rapport wordt aanbevolen om in SHM-verband een aantal projecten uit te voeren:

- Verbeter de registratie van resistentie en combineer resistentiedata met die over therapietrouw en geneesmiddelenspiegels in bloed:
  - Meet resistentie van HIV bij a) elke nieuwe HIV-diagnose en b) therapiefalen. Initieer en ondersteun de opslag van patiëntmateriaal om deze bepalingen uit te voeren en om resistentiedata te verzamelen, op te slaan en te analyseren, en zorg voor een goede afstemming op deze punten tussen de HIV Behandelcentra.
  - Meet, naast de zelfrapportage van patiënten, actief therapietrouw in een subgroep van patiënten en relateer dat aan het gebruik van antiretrovirale middelen.
  - Verzamel aktief de gegevens over geneesmiddelenspiegels in bloed.
- Verbeter de registratie van toxiciteit en van verandering en onderbreking van antiretrovirale behandeling.
- Verbeter de registratie van niet-behandelde en niet-HAART-behandelde patiënten.
- Continueer de inclusie van allochtone patiënten en verbeter de registratie van HIV-1 subtypes.

Data Collection

4.1 Data

Socio-demographic data are collected from patients at entry into the HIV Monitoring Foundation. Next to date of birth and gender, the risk factors for acquiring HIV, date of first HIV diagnosis, country of birth and nationality are collected. In addition, data regarding the identification of the hospital and the treating physician are recorded.

Clinical data, including the registration of HIV disease according to CDC classification[9], are collected every time the patient is seen by his or her treating physician. Next to data on the immune status of the patient, data on the level of viral replication (the concentration of HIV-1 RNA in blood) are collected. Antiretroviral treatment data include data on the antiretroviral drugs used, changes in drug regimens and drug combinations, and changes in the doses of the various drugs used. The use of co-medication is also registered, although not as detailed as of antiretroviral medication. Specific attention is given to the collection of clinical and laboratory data on adverse events and toxicity that are or might be connected to the antiretroviral drugs used. Finally, genotypic data are collected that might indicate the development of resistance to the drugs used.

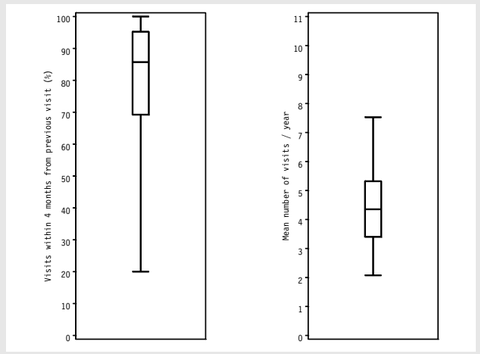

Figure 4.1.1 *Percentage of visits within four months from previous visit (left) and mean number of visits per year (right) in all patients*

The frequency of data collection is on average once every three months. A median of 85.7% of the patients has a follow-up visit within four months of the previous visit (IQR 69.2-95.2; Figure 4.1.1). The treating physicians on average see their patients 4.5 times (SD±1.8) per year.

4.2 Data collection

Patient data are collected in 23 hospitals that are part of 21 specifically appointed HIV Treatment Centres by the Dutch Minister of Health. Data are obtained directly from the patient's medical file, and a limited number of case report forms, filled in by or under the responsibility of the patient's treating physician, are used to support the data collection and entry into the local and the national database.

The treating physician in each of the HIV Treatment Centres is responsible for the collection and quality of the data obtained from his or her patients. So-called data collectors, who work under the supervision of the treating physician and who have access to the patient's file, enter the data into local databases and subsequently add these data to the national database on a regular basis. In addition, the physicians are supported by data monitors of the HIV Monitoring Foundation in the execution of quality control procedures, which is done by comparing the data added to the national database with the source documents, i.e. the patient's medical file. Differences between source documents and data in the database are discussed and clarified and, if needed, corrected, again under supervision of the treating physician.

At present, due to financial limitations, the quality control procedure is restricted to the follow-up data of a random selection of 10% of the patients and to the retrospectively collected data of a random selection of 10% of the newly admitted patients per year.

Data are collected and stored in the national database under a unique code that identifies the patient. On a national level no other identifying information is available than the date of birth. None other than the treating physician can combine the name and identification code of the patient. Patients are not asked for consent, but are informed about the possibility to object against the adding of data on their HIV infection to the national database. Abiding by the rules and regulations of the HIV treatment centre, the treating physician informs the patient on the ins and outs of the monitoring of their HIV infection. In addition, the HIV Monitoring Foundation provides written information in many different languages.

4.3 Inclusion

The Minister of Health appointed the 21 HIV Treatment Centres on 5 February 2002. Most centres simply continued the entry of follow-up data into the ATHENA database and added data of new patients. However, a few centres that were responsible for large numbers of patients stopped data entry, awaiting a formal agreement with the HIV Monitoring Foundation to continue data collection.

Per the first of October 2002, 6302 patients were included in the national database of the HIV Monitoring Foundation. The inclusion had started under the management of the ATHENA project in 1998 and was continued by the Foundation in 2001 (see Figure 4.3.1). The present report contains data from patients that were included in the HIV Monitoring Foundation before 1 April 2002. At that time a total of 5102 patients had been registered, 4156 (81.5%) of whom had already participated in ATHENA and 356 (7%) were newly registered. Of 590 (11.5%) patients, the monitoring of HIV had already started before the ATHENA project.

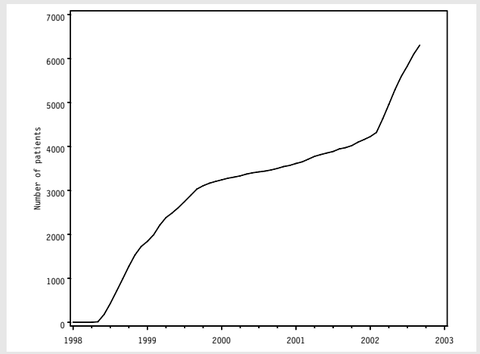

Figure 4.3.1 *Number of patients included over time in the ATHENA project (1998-2001) and the HIV Monitoring Foundation*

4.4 Conclusion

Although there may be differences between subgroups, on average the patients included in the HIV Monitoring Foundation form a rather coherent observational clinical cohort, wherein comparable clinical and laboratory data are collected in a well-structured fashion and at more or less regular intervals for purposes of treatment follow-up.

Data collection with the support of dedicated data collectors remains operational after the ATHENA project is closed in 2001.

An agreement about the delicate position of data collectors and data monitors with respect to the protection of the privacy of participants is reached between the HIV Treatment Centres and the HIV Monitoring Foundation.

Procedures for the quality control of data that are added to the national database remain valid. Finally, the inclusion of new patients in the HIV Monitoring Foundation since the beginning of 2002 is largely on schedule.

The observational clinical cohort

5.1 Introduction

Highly active antiretroviral therapy (HAART) became available for regular treatment of HIV-infected individuals in the Netherlands on 1 July 1996[5]. In clinical trials[10-14], however, and as so-called salvage therapy[15] it had already been administered to patients since 1994. HAART is defined here as a combination of antiretroviral drugs consisting of at least two of the three classes that to date encompass the officially registered antiretroviral drugs. Those classes are:

- Nucleoside[16] (and nucleotide[17]) analogue inhibitors of HIV-1 reverse transcriptase (RT), abbreviated as nRTI
- Non-nucleoside analogue inhibitors of HIV-1 RT, abbreviated as NNRTI[18]
- HIV-1 protease inhibitors, abbreviated as PI[19].

RT is necessary for the translation of HIV-1 RNA into DNA that will be incorporated in the cellular DNA, an early step in the replication cycle of the virus. Protease is needed for the production of infectious viral particles at the end of the replication cycle of HIV-1. Inhibition of those steps in the replication cycle of HIV-1 appears to be very effective for both the individual patient and on a population level as well. In individual patients the plasma levels of HIV-1 RNA decline to undetectable levels within a few days to weeks after the start of HAART and CD4+ T cell counts tend to increase[20;21]. The quality of life of patients treated with HAART improves, although not to the level of age-matched non-infected individuals[22]. On a population level, HIV-related morbidity and mortality has declined dramatically since the large-scale introduction of HAART in Europe and the USA in 1995/1996[3;5;23;24]. Nevertheless, HAART does not stop viral replication completely and is not effective enough for the eradication of HIV-1 from long-lived non-activated T cells[13;25-27]. The incomplete blocking, frequently enhanced by limited adherence of patients to the drugs prescribed[28], ultimately results in selection of virus strains that are less sensitive to the drugs used[29;30]. Moreover, cross-resistance between drugs of the same class is common[31-35].

Antiretroviral drug class	Available	In trials
Non-Nucleoside RTIs	Delavirdine	Calanolide A
	Efavirenz	Capravirine
	Nevirapine	DPC 083
		TMC125
Nucleoside/-tide RTIs	Abacavir (ABC)	DAPD
	Didanosine (ddI)	FTC
	Lamivudine (3TC)	L-Fd4C
	Stavudine (d4T)	
	Tenofovir	
	Zalcitabine (ddC)	
	Zidovudine (AZT)	
	Combivir (3TC/AZT)	
	Trizivir (ABC/3TC/AZT)	
Protease Inhibitors	Amprenavir	Atazanavir
	Indinavir	GW433908
	Kaletra	Tipranavir
	Nelfinavir	TMC114
	Ritonavir	
	Saquinavir	

Table 5.1.1 *Antiretroviral drugs available for regular treatment and drugs currently in trials*

To date, HAART still consists of a combination of drugs from two of the three classes that were already known in 1994-1996. Since this time, however, the number of drugs per class has increased substantially (see Table 5.1.1)[36]. Moreover, combining two drugs from the same class has become common practise, especially in the class of PIs, thereby using the enhancing pharmacological effect of one PI when given together with another[37]. In addition, the dosing of the drugs has changed since 1994-1996. Further insights into the pharmacokinetics and -dynamics of the various drugs improved the daily intake schedules, diet constraints and dosage of drugs[38].

New classes of drugs that are almost approved for regular treatment of HIV-1 are drugs involved in blocking virus entry into the cell and integrating viral DNA into the cellular DNA[36]. These new classes of drugs are not only necessary to limit the development of resistance of HIV-1 to antiretroviral treatment, but also to improve the various treatment options in case of severe adverse events and toxicities that are known to be related to the currently available antiretroviral drugs[39-41]. Peripheral neuropathy, lipodystrophy/atrophy, pancreatitis, diabetes mellitus, hepatic steatosis, nephrolithiasis and loss of libido are frequently reported as being associated with one or more of the antiretroviral drugs used in a HAART combination[42-46]. In the ATHENA final report we showed that clinical signs and symptoms of toxicity were seen in a continuous 10% of the population treated with HAART combinations[5].

In this chapter we will report on the effects of long-term HAART treatment in an enlarged population of HIV-infected individuals. Apart from patients treated with HAART, patients treated with non-HAART combinations of antiretroviral therapy (abbreviated as ART) and non-treated patients are now included in the study population as well, although the latter two are relatively small groups. The baseline characteristics of the three groups will first be described, in order to discern between the various sub-groups. Emphasis will be placed on the possible differences in the course of the (treated) HIV infection between men and women. Subsequently, we will present results of long-term HAART treatment and discuss various variables that might influence therapy as well as disease outcome. In two separate paragraphs we will discuss the issues of toxicity and resistance. Overall aim is to get insight into changes in the course of the HIV infection since the introduction of large-scale HAART treatment in 1996.

5.2 Study population

Data were obtained from HAART-treated patients entering the ATHENA project during 1998-2001. These patients were monitored after they signed an informed consent. From 1 January 2002, however, patients were included irrespective of their antiretroviral treatment. Moreover, patients did no longer have to sign an informed consent form, but could object to having data on the course and treatment of their infection added to the national database. Inclusion of patients is still ongoing.

When possible and useful, the study population was subdivided into specific groups, with an emphasis on possible differences in the course of infection between men and women. The latter was done because the proportion of women among the population of HIV-1-infected patients increased over the years and their socio-demographic background might be different from the male HIV-infected population in the Netherlands.

5.3 Material and methods

Data, definitions and endpoints

For the present report the database was frozen for analysis on 1 April 2002.

The following definitions were used to describe the course of the (treated) HIV-1 infection:

- Non-treated patients: patients who did not receive any antiretroviral treatment during the monitoring period in this report.
- ART: patients who were treated with a single antiretroviral drug or a combination of antiretroviral drugs that did not reach the HAART definition without ever having been treated with a HAART combination of antiretroviral drugs.
- HAART: patients who started treatment with three or more antiretroviral drugs of at least two classes. Patients were defined as treatment-naïve if they started HAART without any previous treatment with antiretroviral drugs. Patients were defined as pre-treated or antiretroviral-drugs-experienced if they had been treated with a single or non-HAART combination of antiretroviral drugs before the start of HAART.

Clinical parameters

An important surrogate endpoint in monitoring antiretroviral therapy is the level of suppression of the virus production as measured by the effect on the concentration of HIV-1 RNA in plasma. In addition, the immunological status of the patient and thereby the effect of therapy on immunological parameters such as the CD4+ T cell count is of great importance to the course of the (clinical) infection.

In the present analysis, therapy success was defined by the following parameters:

- Reaching a plasma HIV-1 RNA level below 1000 copies/ml
- A 1 log decrease from the baseline plasma HIV-1 RNA level (log copies/ml)
- An increase of 100x10^6 cells/l from the baseline CD4+ T cell count
- A 0.5 log increase from the baseline CD4+ T cell count (log x10^6 cells/l).

Therapy failure was defined as:

- A 0.5 log increase from baseline viral load (log copies/ml)
- A decrease of 50x10^6 cells/l from the baseline CD4+ T cell count.

In addition to immunological and virological parameters of therapy success and failure, we considered the changes in HIV-related morbidity[47] and mortality.

Change of HAART

Therapy switches were studied by analysing the time to the first, second and third change of regimen. These switches were scored regardless of the reason for switching, e.g. virological failure, toxicity. Dose changes and therapy interruptions were not taken into account. If a patient started with a new regimen after a therapy interruption, this was considered a switch only if the new regimen was different from the patient's regimen prior to the therapy interruption.

Virological failure

The 'cumulative failing time' was calculated by summing the time periods between two subsequent HIV-1 RNA measurements that were >1000 and by taking half of the time between two measurements of which only the last or the first one was >1000 copies/ml. In case a patient stopped or changed HAART before reaching the criteria for failure, the cumulative failing time was reset to zero. If the cumulative failing time was >112 days (i.e. the average time between two patient visits) while on the same HAART regimen, the patient was labelled as having failed on therapy. The date of the first visit or viral load measurement recorded in the database at or after these 112 days was considered the date of failure. The HAART regimen, the viral load, and the CD4+ T cell count at the time of failure were determined. Therapy interruption was defined as a period of at least one month without any antiretroviral drug treatment following and being followed by HAART treatment.

Salvage therapy

Patients who failed on three different regimens were considered as a separate group in order to investigate changes in drug regimen after the third failure, the maximum number of drugs before and after the third failure, and the occurrence of new CDC-C events and survival. In this analysis the date of third failure was considered as baseline.

Laboratory measures

Two laboratory measures play a key role in the monitoring of HIV: the concentration of HIV-1 RNA in plasma and the number of CD4+ T cells in peripheral blood.

HIV-1 RNA

Determination of the HIV-1 RNA concentration in plasma, representative of the amount of virus produced, was measured in each of the centres by using one of the commercially available quantitative tests. The quantification limit of these assays changed since their introduction in the mid nineties. For the present report a set point of 1000 HIV-RNA copies/ml was used.

CD4+ T cell count

Absolute numbers of CD4+ T cells were determined by using immune-fluorescence techniques and flow cytometry.

Unless otherwise stated, viral load measurements (plasma HIV-1 RNA) are reported in $\log_{10}$ copies/ml and CD4+ T cell counts in 10^6 cells/l.

5.4 Statistical analyses

Characteristics of the population at different time points in the course of the (treated) infection are presented. For continuous variables the median and inter-quartile range or mean and standard deviation were calculated. For categorical variables the percentage of the total was calculated.

Box plots of the viral load and CD4+ T cell counts over time were constructed. Log HIV-1 RNA values and CD4+ T cell counts were plotted at 0, 6, and 12 months and subsequently at every 12 months until 96 months of follow-up (long-term). In the analysis of the data obtained from patients on HAART, only patients with a repeated measurement within 168 days of the target date were included.

Baseline characteristics were compared between men and women and, in case of HAART treatment, between pre-treated and naïve patients. T-tests were used for continuous variables and the chi-square test was used for categorical variables. Kruskal Wallis tests were used for not-normally distributed variables.

Kaplan-Meier survival curves were plotted for time to switch of regimen, virological failure, therapy success and failure, new CDC-C event and death, stratified by baseline characteristics. Cox proportional hazards model was used to study predictors of time to switch of regimen, virological failure, therapy success and failure, new CDC-C event and death. Breslow's method for handling of tied values was used. Predictors considered were gender, age at baseline, pre-treatment with antiretroviral drugs prior to the start of HAART, therapy interruptions longer than one month during the course of treatment, baseline CD4+ T cell count and baseline viral load. In addition, age at failure and CD4+ T cell counts and viral loads at first and second failure were also taken into account in the analysis of time to failure. Treatment interruptions and therapy switches were included in the analysis of time to failure as time-dependent variables. In the analysis of time to death, virological failure and treatment interruptions were included as time-dependent variables.

Mortality and incidence of AIDS were calculated per 100 person-years of follow-up after start of HAART or after start of the first antiretroviral drug. Poisson's distribution was used to calculate 95% confidence intervals for rates. Expected death rates were calculated for an age- and gender-matched group from the general Dutch population (Statistics Netherlands. Available at: http://www.cbs.nl).

All analyses were performed using the SAS software for Windows, version 8.02 (SAS Institute Inc, USA).

5.5 Results at baseline

Characteristics of the total study population

Data of in total 5102 patients had been entered into the database when it was frozen for analysis on 1 April 2002. The clinical, immunological and virological data of 94 patients were missing due to very recent inclusion into the HIV Monitoring Foundation. These patients were therefore not included in the further analyses.

Of the remaining 5008 patients, 1809 (36%) started with an ART regimen but switched to HAART during subsequent follow-up; 299 patients (6%) were treated with ART during follow-up and never switched to HAART; 2555 patients (51%) started with HAART as their first antiretroviral treatment; and 345 patients (7%) never received antiretroviral therapy during the reported follow-up period.

The number of men and women newly diagnosed each year is shown in Figure 5.5.1. The first one of the two peaks coincides with the introduction of commercially available screening assays for the diagnosis of HIV infection, the second one with HAART. The total absolute number of newly diagnosed male patients started to decline in 1997. The proportion of HIV-1-infected women in the population increased over time (see Figure 5.5.2). In the male population the proportion of patients infected through heterosexual contact increased (see Figure 5.5.3), whereas

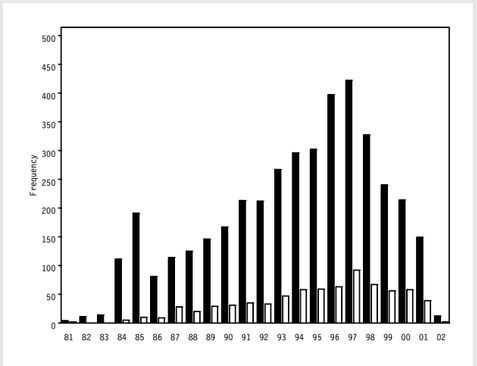

Figure 5.5.1 *Year of diagnosis for men (black bars) and women (white bars). Patients with missing data on gender are not included.*

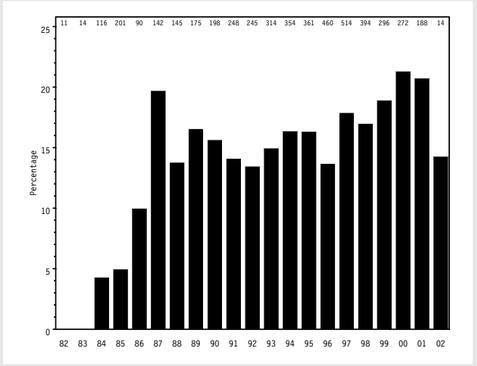

Figure 5.5.2 *Proportion of infected female patients in the HIV-1-positive population by year of diagnosis. Numbers on top are the absolute number of newly diagnosed patients per year. Patients with missing data on gender or year of diagnosis are not included.*

the proportion of men infected through homosexual contact and intravenous drug use slowly declined. The female HIV-1-infected population in the early epidemic consisted mainly of intravenous drug users. Over the years the proportion of women who acquired their infection through heterosexual contact increased (Figure 5.5.4).

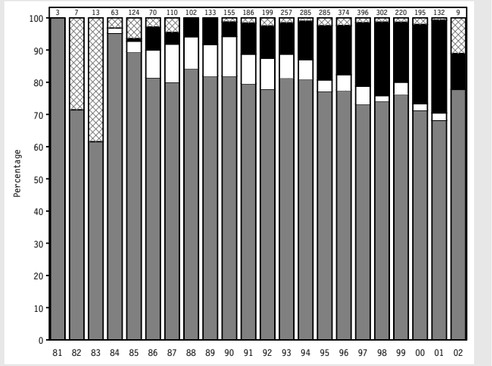

Figure 5.5.3 *Mode of transmission by year of diagnosis for men. Grey bars: men having sex with men; white bars: intravenous drug use; black bars: heterosexual contact; and crossed bars: other. The numbers above bars are the number of patients newly diagnosed each year. Patients with missing data on transmission mode or year of diagnosis are not included.*

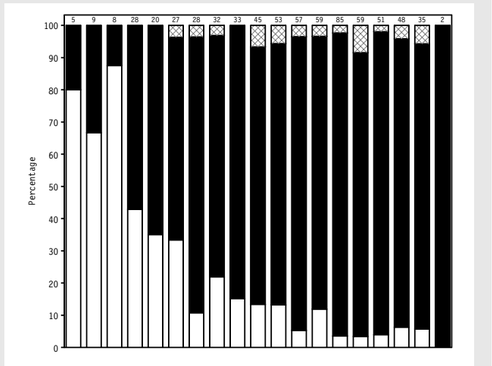

Figure 5.5.4 *Mode of transmission by year of diagnosis for women. White bars: intravenous drug use; black bars: heterosexual contact; and crossed bars: other. The numbers above bars are the number of patients newly diagnosed each year. Patients with missing data on transmission mode or year of diagnosis are not included.*

Figures 5.5.5 and 5.5.6 show the region of origin of heterosexually infected men and women, respectively. Overall, 48% of heterosexual patients were from Dutch origin while 27% was from Sub-Saharan African origin and 8% from Latin American origin.

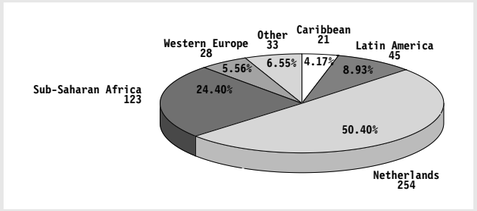

Figure 5.5.5 *Region of origin of men infected with HIV through heterosexual contact*

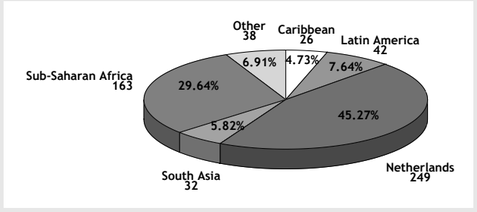

Figure 5.5.6 *Region of origin of women infected with HIV through heterosexual contact*

Untreated patients

Of the 345 untreated patients, 99 are not discussed any further in this section because of missing data on gender. Of the 246 remaining untreated patients, 76 (31%) were diagnosed with HIV early in the epidemic and died before HAART was introduced in 1994-1996. Forty-one (24.1%) of the now remaining 170 patients were diagnosed after 2000 and data on possible further treatment are not available. The last 129 patients (75.9%) did not receive antiretroviral treatment for other reasons. The amount of missing data, especially with respect to socio-demographics, is large (Table 5.5.1).

	Male		Female		Total	
	N	%	N	%	N	%
Total	**207**	**84.1**	**39**	**15.9**	**246**	**100.0**
Region of origin						
Netherlands	127	61.3	13	33.3	140	56.9
Caribbean	1	0.4	2	5.1	3	1.2
Central Europe	3	1.4			3	1.2
Western Europe	5	2.4	2	5.1	7	2.8
Latin America	7	3.3			7	2.8
North America	2	0.9			2	0.8
North Africa & Middle East			1	2.5	1	0.4
Sub-Saharan Africa	14	6.7	14	35.8	28	11.3
South Asia	7	3.3	1	2.5	8	3.2
Unknown	41	19.8	6	15.3	47	19.1
Age at diagnosis (years)						
0-25	14	6.7	8	20.5	22	8.9
26-35	71	34.2	12	30.7	83	33.7
36-45	51	24.6	10	25.6	61	24.7
46 or more	24	11.5	1	2.5	25	10.1
Unknown	47	22.7	8	20.5	55	22.3
Transmission group						
Homo-/bisexual	68	32.8			68	27.6
IDU	5	2.4	3	7.6	8	3.2
Heterosexual	19	9.1	23	58.9	42	17.0
Blood transfusion	1	0.4			1	0.4
Unknown	114	55.0	13	33.3	127	51.6

Table 5.5.1 *Characteristics of non-treated patients at entry of clinical follow-up. Patients with missing data on gender are not included.*

Of 107 patients HIV-1 RNA measurements were available before 1 January 2002. Their median HIV RNA level in plasma was 4.0 $\log_{10}$ copies/ml (IQR 3.0-4.8) and was significantly (p=0.002) higher in the 88 men (4.2; 3.0-5.0) than in the nineteen women (3.0; 2.6-4.4). When the first available CD4+ T cell count that was measured in the same year as the first viral load was taken, the median number of CD4+ T cells was 500x10^6 cells/l in 75 patients and was higher in the ten women (530; 230-680) than in the 65 men (470; 270-590).

ART-only-treated patients

Of the 299 patients treated with ART only, 16 are not discussed any further in this section because of missing data on gender. Of the 283 remaining patients, 91 (32%) started their ART treatment early in the epidemic and died before the introduction of

HAART. Two hundred and fifty four of these 283 patients were male (90%) and 65% of patients were of Dutch origin. Figure 5.5.7 shows the number of ART-treated patients by year of diagnosis that was still alive in 1994; Table 5.5.2 summarises the baseline characteristics of the ART-treated patients.

Men having sex with men was the main risk factor for acquiring HIV in male patients, whereas female patients acquired their infection mainly through heterosexual contact. Fifty-nine percent of the patients were diagnosed in the eighties, yet there still continues to be a number of recently diagnosed patients who started ART and have not (yet) received HAART (24 patients since 1999).

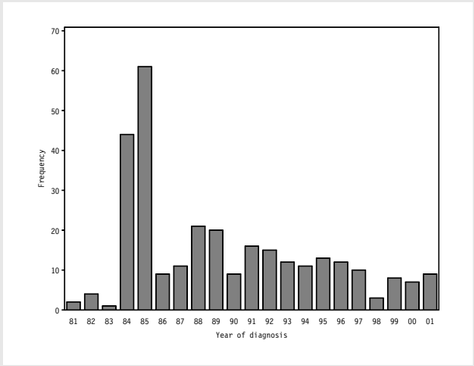

Figure 5.5.7 *Year of diagnosis of ART-treated patients*

The time between diagnosis and start of ART was less than a year in 29% of the patients. At start of ART CD4+ T cell counts were unknown for 40/283 patients and plasma HIV-1 RNA concentrations were unknown for 113/283 patients. The median HIV-1 RNA level at start of ART in the remaining patients was 4.7 $\log_{10}$ copies/ml plasma (IQR 4.2-5.2) and was higher in men than in women (4.8, 4.3-5.3, and 3.6, 3.0-5.0, respectively). CD4+ T cell counts at start of ART were median 210x10^6/l and did not differ

	Male		Female		Total	
	N	%	N	%	N	%
Total	**1504**	**100.0**	**258**	**100.0**	**1762**	**100.0**
Region of origin						
Netherlands	173	68.1	12	41.3	185	65.3
Australia & New Zealand	1	0.3			1	0.3
Caribbean	1	0.3			1	0.3
Central Europe	1	0.3	1	3.4	2	0.7
Western Europe	12	4.7	5	17.2	17	6.0
Latin America	2	0.7			2	0.7
North America	2	0.7	1	3.4	3	1.0
North Africa & Middle East	1	0.3			1	0.3
Sub-Saharan Africa	4	1.5	7	24.1	11	3.8
South Asia	2	0.7	1	3.4	3	1.0
Unknown	55	21.6	2	6.8	57	20.1
Age at diagnosis (years)						
0-25	24	9.4	9	31.0	33	11.6
26-35	120	47.2	11	37.9	131	46.2
36-45	83	32.6	7	24.1	90	31.8
46 or more	25	9.8	2	6.8	27	9.5
Unknown	2	0.7			2	0.7
Transmission group						
Homo-/bisexual	95	37.4			95	33.5
IDU	18	7.0	7	24.1	25	8.8
Heterosexual	9	3.5	17	58.6	26	9.1
Other	1	0.3	2	6.8	3	1.0
Unknown	131	51.5	3	10.3	134	47.3
First ART regimen						
Mono	167	65.7	12	41.3	179	63.2
Dual	57	22.4	9	31.0	66	23.3
Triple	30	11.8	8	27.5	38	13.4
Maximum number of drugs during ART						
1	79	31.1	1	3.4	80	28.2
2	133	52.3	19	65.5	152	53.7
3	39	15.3	9	31.0	48	16.9
4	3	1.1			3	1.0
CD4 count (x10^6/l) at start ART						
< 50	39	15.3			39	13.7
50 – 200	75	29.5	13	44.8	88	31.0
200 - 400	71	27.9	5	17.2	76	26.8
> 400	37	14.5	3	10.3	40	14.1
Not available	32	12.5	8	27.5	40	14.1
RNA (copies/ml) at start ART						
0-10,000	24	9.4	9	31.0	33	11.6
10,000-60,000	55	21.6	2	6.8	57	20.1
60,000-200,000	39	15.3	3	10.3	42	14.8
>200,000	36	14.1	2	6.8	38	13.4
Not available	100	39.3	13	44.8	113	39.9
CDC event at start ART						
No event	136	53.5	17	58.6	153	54.0
B	37	14.5	9	31.0	46	16.2
C	81	31.8	3	10.3	84	29.6
Years between diagnosis and start ART						
< 1	74	29.1	9	31.0	83	29.3
1-3	78	30.7	6	20.6	84	29.6
3-5	38	14.9	9	31.0	47	16.6
> 5	63	24.8	5	17.2	68	24.0
Unknown	1	0.3			1	0.3

Table 5.5.2 *Baseline characteristics of ART-only-treated patients at start of ART. Patients with missing data on gender are not included.*

significantly between men and women (210, 100-320, and 200, 150-370, respectively).

When compared to the baseline values of the HAART-treated patients, the HIV-1 RNA levels in the ART-treated men was lower (p<0.001) than in the HAART-treated men. In women no difference was found. Mean CD4+ T cell counts at start of ART did not differ from those of patients at start of HAART.

CDC-C events at start of ART were found in 32% of the men and 10% of the women (p=0.02).

Although most patients (179/283, 63%) started with a single drug (monotherapy), 99 of them were at least once treated with two drugs at the same time (dual therapy). Fifty-one patients were at some time treated with three or four drugs (within the same drug class). These triple or quadruple regimens are listed in Table 5.5.3.

ART Combination	Number of patients
AZT + ddI + ddC	1
AZT + ddI + 3TC	8
AZT + ddC + 3TC	3
AZT + ddI + ddC + 3TC	1
AZT + d4T + 3TC	1
ddI + d4T + 3TC	9
AZT + ddC + d4T + 3TC	1
AZT + 3TC + IDV	1
AZT + ddI + 3TC + IFNA	1
AZT + ddC + 3TC + loviride	1
AZT + ddI + ABC	1
AZT + 3TC + ABC	27
ddI + 3TC + ABC	2
d4T + 3TC + ABC	2
AZT + d4T + 3TC + ABC	1
AZT + ddI + 3TC + HU	1

Table 5.5.3 *Triple or quadruple ART regimens used*

HAART-treated patients

Of the 4364 patients who received HAART, 80 (2%) are not discussed any further in this section because of missing data on gender. Of the remaining 4284 patients, 1762 (41%) had been treated with antiretroviral therapy prior to the start of HAART, whereas 2522 patients (59%) were therapy-naïve (Tables 5.5.4 and 5.5.5).

The majority of recently diagnosed HIV-infected individuals started with HAART as their first antiretroviral treatment regimen. The proportion of patients who had been pre-treated before the start of HAART decreased from 1996 on (Figure 5.5.8).

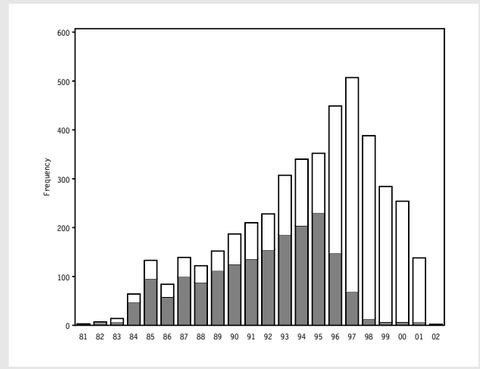

Figure 5.5.8 *Pre-treated (grey bars) and naïve patients (white bars) by year of diagnosis at start of HAART*

Seventy-one percent of the treatment-naïve and 73.5% of the pre-treated men were Dutch. For both therapy-naïve and pre-treated men the main risk factor for infection was having sex with men. The proportion of men who acquired their infection heterosexually was higher in the naïve group (16% versus 10%, respectively; (p<0.0001)). The majority of women (524/683, 77%) acquired their infection heterosexually, although in the pre-treated group a relatively large proportion reported intravenous drug use as the main risk factor (53/258, 21%).

A relatively large proportion of the antiretroviral-therapy-naïve male patients came from Sub-Saharan Africa in comparison with the pre-treated male patients (129/2097 versus 42/1504, respectively; (p<0.0001)). The proportion of Dutch patients in the female patient group was smaller than in the male patient group: 304 of

the 683 women (45%) and 2595 of the 3601 men (72%) were Dutch (p<0.0001). Of the 683 women, 180 (26%) came from Sub-Saharan Africa. At diagnosis, women were on average 5.1 years younger (95% CI: 4.4, 5.9) than men (31.2 and 36.3 years, respectively).

Three hundred and ten (45%) out of 683 female patients were treated within a year from date of diagnosis. This percentage was higher than in men (1482/3601 (41%); (p=0.04)). The majority of patients started with an nRTI + PI combination (overall 84%). However, naïve patients more often started with an nRTI + NNRTI combination than pre-treated patients (18% versus 7%; p<0.0001). Figure 5.5.9 shows that the number of pre-treated patients declines over the years; however, a small group of patients who do not start with HAART as their first therapy remains.

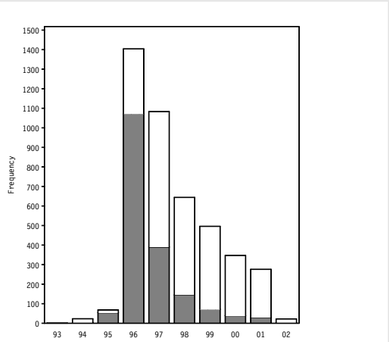

Figure 5.5.9 *Year of start of HAART in pre-treated (grey bars) and naïve patients (white bars)*

There was no significant difference between pre-treated men and women with respect to CD4+ T cell counts at start of HAART (Table 5.5.4). Treatment-naïve men had higher CD4+ T cell counts than pre-treated men, as was the case in women. The difference in HIV-1 RNA concentration at start of HAART was higher in the therapy-naïve than in the pre-treated group in both men and women (0.72 $\log_{10}$ copies/ml; 95% CI: 0.65 – 0.78 in men and 0.46 $\log_{10}$ copies/ml; 95% CI: 0.29 – 0.63 in women). The difference between men and women was 0.09 $\log_{10}$ copies/ml (95% CI: -0.07 – 0.25) in the pre-treated and 0.35 (0.26 – 0.43) in the naïve patients.

In the year prior to the start of HAART 23.4% of pre-treated and 25% of the naïve patients were diagnosed with having a CDC-C event. A CDC-C event prior to the start of HAART was more frequent in men (22.2%) than in women (16.4%; 95% CI: 2.9-8.7).

HIV-1 Subtypes

Using the nucleotide sequences of protease and RT that were obtained for the detection of resistance-associated mutations (see paragraph 5.8), the HIV-1 subtype could be determined for 660 patients. For each patient one sequence was used, preferably the one before therapy. Sequences were compared pair-wise using the Kimura 2 parameter model[48] for distances between nucleotides. A representative set of reference sequences for each known HIV-1 subtype was obtained from the Los Alamos sequence database (hiv-web.lanl.gov) and included in the distance calculations. Sequences were clustered using the neighbour-joining method[49] and were assigned a specific subtype when the bootstrap value of the cluster containing the measured sequences and a reference sequence exceeded 85%.

The majority of patients (585/660, 89%) were infected with HIV-1 B subtype. Twenty patients (3%) had subtype C, fourteen (2%) had subtype AG, thirteen (2%) had subtype REC, eight (1%) had AE, seven (1%) had A, five (0.8%) had D, four (0.6%) had G. Subtypes DF and F1 were each detected in two patients (0.3%).

	Male		Female		Total	
	N	%	N	%	N	%
Total	**1504**	**100.0**	**258**	**100.0**	**1762**	**100.0**
Region of origin						
Netherlands	1106	73.5	129	50.0	1235	70.0
Australia & New Zealand	1	0.0			1	0.0
Caribbean	29	1.9	11	4.2	40	2.2
Central Europe	11	0.7	2	0.7	13	0.7
Eastern Europe	1	0.0			1	0.0
Western Europe	125	8.3	23	8.9	148	8.3
Latin America	56	3.7	16	6.2	72	4.0
North America	29	1.9	2	0.7	31	1.7
North Africa & Middle East	6	0.3	3	1.1	9	0.5
East Asia & Pacific	1	0.0			1	0.0
Sub-Saharan Africa	42	2.7	54	20.9	96	5.4
South Asia	37	2.4	11	4.2	48	2.7
Unknown	60	3.9	7	2.7	67	3.8
Age at diagnosis						
0-25	175	11.6	84	32.5	259	14.6
26-35	647	43.0	118	45.7	765	43.4
36-45	476	31.6	41	15.8	517	29.3
46 or older	206	13.6	14	5.4	220	12.4
Unknown			1	0.3	1	0.0
Transmission group						
Homo-/bisexual	1164	77.3			1164	66.0
IDU	96	6.3	53	20.5	149	8.4
Heterosexual	145	9.6	183	70.9	328	18.6
Blood transfusion	32	2.1	8	3.1	40	2.2
Other	6	0.3	2	0.7	8	0.4
Unknown	61	4.0	12	4.6	73	4.1
First HAART combination						
nRTI & PI	1343	89.2	227	87.9	1570	89.1
nRTI & NNRTI	95	6.3	27	10.4	122	6.9
nRTI & PI & NNRTI	33	2.1	3	1.1	36	2.0
Unknown	33	2.1	1	0.3	34	1.9
Years between diagnosis and start HAART						
< 1	195	12.9	33	12.7	228	12.9
1-5	656	43.6	108	41.8	764	43.3
5-10	445	29.5	93	36.0	538	30.5
> 10	208	13.8	24	9.3	232	13.1
CD4 count (x10⁶/l) at start HAART						
< 50	311	20.6	53	20.5	364	20.6
50 – 200	496	32.9	68	26.3	564	32.0
200 - 400	401	26.6	82	31.7	483	27.4
> 400	184	12.2	36	13.9	220	12.4
Not available	112	7.4	19	7.3	131	7.4
RNA (copies/ml) at start HAART						
0-10,000	407	27.0	82	31.7	489	27.7
10,000-60,000	263	17.4	56	21.7	319	18.1
60,000-200,000	222	14.7	27	10.4	249	14.1
>200,000	152	10.1	31	12.0	183	10.3
Not available	460	30.5	62	24.0	522	29.6
CDC event at start HAART						
No event	931	61.9	159	61.6	1090	61.8
B	214	14.2	46	17.8	260	14.7
C	359	23.8	53	20.5	412	23.3

Table 5.5.4 *Baseline characteristics of pre-treated patients at start of HAART. Patients with missing data on gender are not included.*

	Male		Female		Total	
	N	%	N	%	N	%
Total	**2097**	**100.0**	**425**	**100.0**	**2522**	**100.0**
Region of origin						
Netherlands	1489	71.0	175	41.1	1664	65.9
Australia & New Zealand	4	0.1	1	0.2	5	0.1
Caribbean	51	2.4	16	3.7	67	2.6
Central Europe	25	1.1	2	0.4	27	1.0
Eastern Europe	5	0.2	1	0.2	6	0.2
Western Europe	131	6.2	27	6.3	158	6.2
Latin America	109	5.1	33	7.7	142	5.6
North America	39	1.8	1	0.2	40	1.5
North Africa & Middle East	21	1.0	6	1.4	27	1.0
East Asia & Pacific	4	0.1	1	0.2	5	0.1
Sub-Saharan Africa	129	6.1	126	29.6	255	10.1
South Asia	51	2.4	24	5.6	75	2.9
Unknown	39	1.8	12	2.8	51	2.0
Age at diagnosis						
0-25	190	9.0	101	23.7	291	11.5
26-35	855	40.7	202	47.5	1057	41.9
36-45	661	31.5	87	20.4	748	29.6
46 or older	391	18.6	35	8.2	426	16.8
Transmission group						
Homo-/bisexual	1499	71.4			1499	59.4
IDU	89	4.2	33	7.7	122	4.8
Heterosexual	338	16.1	341	80.2	679	26.9
Blood transfusion	18	0.8	6	1.4	24	0.9
Other	18	0.8	8	1.8	26	1.0
Unknown	135	6.4	37	8.7	172	6.8
First HAART combination						
nRTI & PI	1680	80.1	346	81.4	2026	80.3
nRTI & NNRTI	369	17.5	77	18.1	446	17.6
nRTI & PI & NNRTI	46	2.1	2	0.4	48	1.9
Other	1	0.0			1	0.0
Unknown	1	0.0			1	0.0
Years between diagnosis and start HAART						
< 1	1287	61.3	277	65.1	1564	62.0
1-5	463	22.0	84	19.7	547	21.6
5-10	243	11.5	53	12.4	296	11.7
> 10	104	4.9	11	2.5	115	4.5
CD4 count (x10⁶/l) at start HAART						
< 50	360	17.1	47	11.0	407	16.1
50 – 200	518	24.7	105	24.7	623	24.7
200 – 400	595	28.3	133	31.2	728	28.8
> 400	372	17.7	71	16.7	443	17.5
Not available	252	12.0	69	16.2	321	12.7
RNA (copies/ml) at start HAART						
0-10,000	289	13.7	56	13.1	345	13.6
10,000-60,000	152	7.2	88	20.7	240	9.5
60,000-200,000	499	23.7	101	23.7	600	23.7
>200,000	560	26.7	98	23.0	658	26.0
Not available	597	28.4	82	19.2	679	26.9
CDC event at start HAART						
No event	1210	57.7	294	69.1	1504	59.6
B	328	15.6	56	13.1	384	15.2
C	559	26.6	75	17.6	634	25.1

Table 5.5.5 *Baseline characteristics of naïve patients at start of HAART. Patients with missing data on gender are not included.*

Tables 5.5.6 and 5.5.7 show the subtypes by gender and mode of transmission. While 523/551 (95%) male patients had subtype B, only 43/78 (55%) female patients had subtype B (p<0.0001). Subtype B was found in 455 of the 460 homosexual patients (99%) and in all of the sixteen intravenous drug users. In contrast, only 65/113 patients (58%) who had acquired HIV-1 through heterosexual transmission had subtype B (p<0.0001).

Conclusions

The baseline characteristics show that the population of patients from whom data are collected and stored in the ATHENA and subsequently the HIV Monitoring Foundation database is still largely a HAART-treated population. A small number of HIV-1-infected patients, however, does not start with HAART as first therapy but with ART, and an even smaller number of patients is not treated with antiretroviral drugs at all. Male homosexuals form the largest proportion of patients, but in the last few years the proportion of patients who were infected through heterosexual contact has increased. Moreover, the proportion of women in the HIV-infected population is increasing as well. Together with the finding that only half of the women is of Dutch origin, these findings indicate that the population of HIV-infected individuals in the Netherlands is changing from a homosexual-male-dominated one to one in which heterosexual transmission is becoming a major risk factor and in which, as a consequence, men and women will have an equal share. This change is confirmed by the changes found in the composition of the HIV-1-subtype population. Although subtype B is still most prevalent, other subtypes emerge and half of the women are infected with a non-B subtype. In 40% of the non-B infection the mode of transmission is 'heterosexual.' The data on the non-treated and the ART-treated infection in the present data set represent patient groups that are only partly comparable with the HAART-treated group. Especially the ART-treated patients are apparently doing well on ART alone, both clinically and with respect to the level of antiretroviral treatment effect. Comparisons with the HAART-treated group should therefore be made with care.

	Male		Female		Total	
	N	%	N	%	N	%
Not done	3577	86.6	702	90.0	4279	87.2
Done	551	13.4	78	10.0	629	12.8
A	1	0.2	3	3.8	4	0.6
AE	7	1.3	1	1.3	8	1.3
AG	3	0.5	8	10.3	11	1.7
B	523	94.9	43	55.1	566	90.0
C	9	1.6	7	9.0	16	2.5
D			4	5.1	4	0.6
DF	1	0.2	1	1.3	2	0.3
F1			1	1.3	1	0.2
G			4	5.1	4	0.6
REC	7	1.3	6	7.7	13	2.1

Table 5.5.6 *HIV-1 subtypes by gender. Patients with missing data on gender are not included.*

	Mode of transmission													
	Homo-/ Bisexual		IDU		Hetero-sexual		Blood transfusion		Vertical		Other		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Not done	2432	84.1	302	95.0	982	89.7	54	80.6	13	50.0	35	92.1	3818	86.1
Done	460	15.9	16	5.0	113	10.3	13	19.4	13	50.0	3	7.9	618	13.9
A					4	3.5			2	7.7			6	1.0
AE	3	0.7			4	3.5							7	1.1
AG					10	8.8			2	7.7			12	1.9
B	455	98.9	16	100.0	65	57.5	12	92.3	5	19.2	1	33.3	554	89.6
C					12	10.6			3	11.5	1	33.3	16	2.6
D					3	2.7	1	7.7					4	0.6
DF					2	1.8							2	0.3
F1									1	3.8			1	0.2
G					4	3.5							4	0.6
REC	2	0.4			9	8.0					1	33.3	12	1.9

Table 5.5.7 *HIV-1 subtypes and mode of transmission. Patients with missing data on mode of transmission are not included.*

At the start of HAART therapy, pre-treated patients were at a more advanced stage of HIV-1 infection than treatment-naïve patients, and male therapy-naïve patients were at a more advanced stage of disease than female therapy-naïve patients.

5.6 Follow-up

Before analysing the observational data collected through ATHENA and the subsequent HIV Monitoring Foundation, it is of importance to report on the frequency of data collection in the population studied.

Data were collected on at least a four-monthly basis in a median 78% (IQR 71, 96) of the male and 82% (IQR 62, 92) of the female patients in our cohort. Mean number of visits per year was 4.6 for men (SD 1.7), which was higher (95% CI 0.2, 0.5; p<0.0001) than the 4.2 visits per year for women (SD 1.7). Homosexual men still formed the largest group of the study population and half of them had at least 87% of their follow-up visits within four months of the previous visit (IQR 74, 96). For heterosexual patients this was 83% (IQR 62, 94) and for intravenous drug users 78% (IQR 56, 90). Mean number of visits per year for homosexual men was 4.7 (SD 1.7), which was higher than for heterosexual patients (4.2, SD 1.7 visits/year; 95% CI 0.4, 0.9; p<0.0001) and for intravenous drug users (4.0, SD 1.7 visits/year; 95% CI 0.4, 0.6; p<0.0001).

Data collection on at least a four-monthly basis occurred more frequently when patients were from the Netherlands, North America or Western Europe than from other parts of the world. Median percentage of visits within four months was 86% (IQR 71, 95) and 83% (IQR 62, 93), respectively. Mean numbers of visits per year was 4.6 (SD 1.7) for patients from the Netherlands, North America and Western Europe and higher (95% CI 0.3, 0.6; p<0.0001) than the 4.1 (SD 1.7) visits of patients from other parts of the world.

Data were more frequently collected from patients receiving HAART at any time than patients who never received HAART (median percentage of visits within four months 86%, IQR 70, 95 for HAART-treated patients, and 80%, IQR 57, 100 for patients never treated with HAART). Mean number of visits per year was 4.6 for HAART-treated patients (SD 1.8) and 3.4 for patients who never received HAART (SD 1.4; 95% CI 0.9, 1.5; p<0.0001). Patients older than 35 years at diagnosis visited outpatient clinics more frequently than younger patients. Median percentages of visits within four months were 87% (IQR 71, 97) for patients older and 84% (IQR 67, 94) for patients younger than 35 years of age and visit frequencies were 4.6 (SD 1.8) and 4.4 (SD 1.7) per year, respectively (95% CI 0.1, 0.3; p=0.0002).

These data show that in this observational cohort data were collected frequently and largely in a three-monthly schedule, although differences in frequency of follow-up did exist between subgroups.

Untreated patients

A subgroup of 108 untreated patients who were still alive in 1994 and had at least three months of follow-up and an HIV-1 RNA measurement before 2001 was selected. This latter criterion for eligibility was imposed, as it was not improbable that for untreated patients with a first viral load measured in 2001 therapy data were still missing in the database. Of the 32 patients who were alive on 1 January 1994 or became eligible in 1994, seven died in the same year and three died in 1995. None of the remaining 76 patients who became eligible from 1995 onwards died before the end of follow-up.

Nine patients (8%) had at least one AIDS-defining event in or before the year in which they became eligible. Two patients progressed to AIDS after becoming eligible, one from the group that became eligible in 1994 – this patient died one month later – and one from the group that became eligible in 1996.

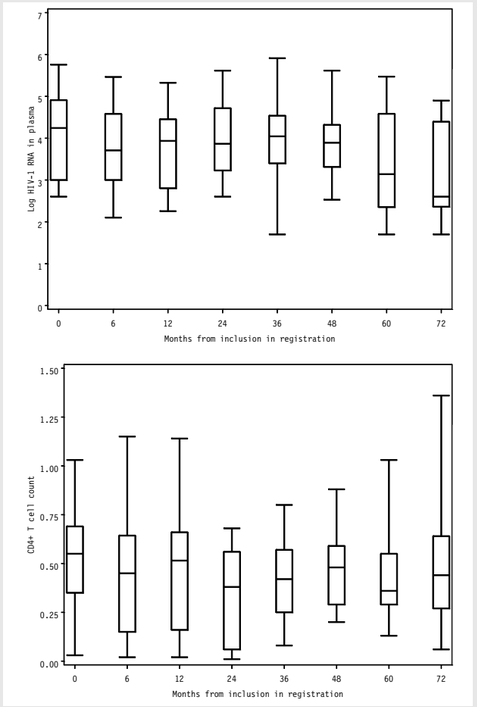

Figure 5.6.1 *HIV-1 RNA (log_{10} copies/ml) and CD4+ T cell count ($x10^6$ cells/mm^3) over time among untreated patients who were included in the registration after 1994 or before 1994 and still alive in 1994*

Viral load and CD4+ T cell count remained relatively stable over time in the untreated population from the moment the patients became eligible (Figure 5.6.1). The slightly elevated load at first measurement was caused by those patients in the 1994 group who were at the end-stage of their HIV infection and died quickly after having become eligible. Any further inferences from these data cannot be made unless it is carefully checked for all untreated patients whether they did not use therapy or whether therapy data are missing in the database.

ART-only-treated patients

A total of 299 patients initiated non-HAART therapy (ART) and never switched to HAART during follow-up. Of these patients, 207 were still alive on or after 1 January 1994.

To evaluate the effect of ART on viral load and CD4+ T cell counts among the latter group, the ART-treated population was divided into two groups, one group starting ART before 1994 and the second group starting ART in 1994 or thereafter. Figure 5.6.2 shows the effect of ART on the plasma viral load in these two groups. In the group starting ART before 1994 the viral load decreased by 0.8 log_{10} copies/ml in the first six months but rebounded at 12 months and stayed 0.3 log_{10} copies/ml below the baseline viral load until 36 months after start. This behaviour reflects the introduction of AZT monotherapy in this population, which only has a short lasting effect on the viral load. After 36 months the viral load decreased again, reflecting the increasing use of dual therapy.

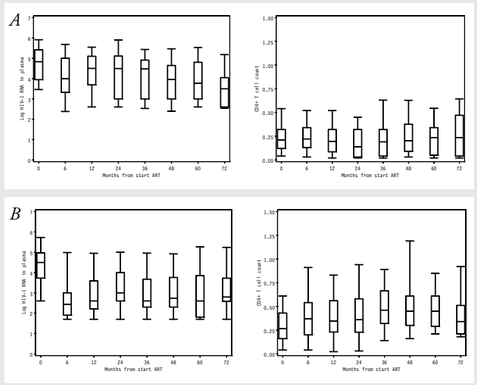

Figure 5.6.2 *HIV-1 RNA (log_{10} copies/ml) and CD4+ T cell count ($x10^6$ cells/mm^3) over time among patients who were alive in 1994 and started antiretroviral (non-HAART) treatment A) before 1994 and B) after 1994*

The decrease in viral load in the population starting ART in 1994 or thereafter was more pronounced. In the first six months after start the viral load was reduced by a factor 100 and only slightly rebounded thereafter. Apparently patients in this group started with a better therapy than patients in the group that had started before 1994. It should be noted that patients taking Trizivir, which in our definition is not HAART, are part of the second group.

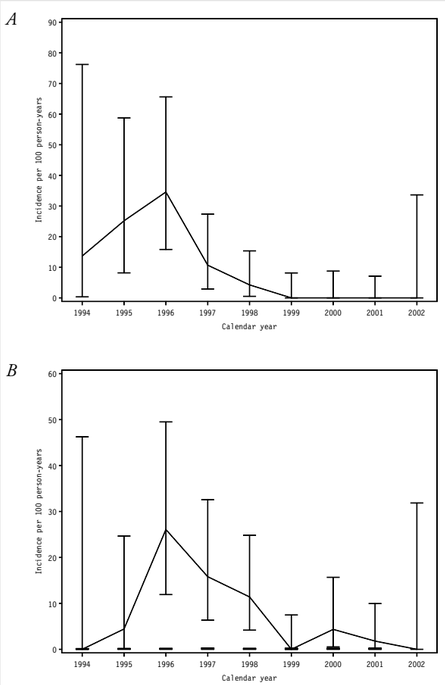

Figure 5.6.3 *Incidence and 95% confidence intervals of A) AIDS and B) death among patients who started ART therapy after 1 January 1994. Black bars represent the death rate in the age- and gender-matched general Dutch population.*

Administration of ART did not have an effect on the number of CD4+ T cells in the first group, which remained stable between 200 and 300x10^6 cells/l (Figure 5.6.2). CD4+ T cell counts in the second group increased from 267 at baseline to 370 at six months, and further, though slightly, in the years thereafter. This positive effect was clearer when patients who died shortly after starting ART were omitted from the analysis, as these generally had low CD4+ T cell counts and were at the end-stage of their HIV infection.

Of the 103 patients who initiated non-HAART therapy after 1 January 1994 and never switched to HAART during follow-up, 26 (25%) died and 21 (20%) progressed to AIDS. Figure 5.6.3 shows the mortality rate per 100 person-years since start of first drug. Mortality declined from 26 incidences in 1996 to 1.8 in 2001 but remained about eight times larger than in the gender- and age-matched general Dutch population. Incidence of AIDS peaked in 1996 (Figure 5.6.3) and declined afterwards. After 1998 no new AIDS diagnoses were observed in the ART-treated group.

HAART-treated patients

Between 1 January 1994 and 1 April 2002, 4363 HIV-positive patients started a HAART regimen. Patients were on average 38 years old (IQR: 32-45) and 58.6% had not been pre-treated with mono- or dual therapy. At the start of HAART the median CD4+ T cell count among those who were naïve was 230x10^6 cells/l (100-380) and among those who had been pre-treated 180x10^6 cells/l (IQR: 70-310). The median viral loads (in log copies per ml) were 5.0 (IQR: 4.5-5.4) and 4.4 (IQR: 3.4-5.0), respectively.

Figure 5.6.4 represents the CD4+ T cell count and viral load in this population after the start of HAART, stratified by treatment status at the start of HAART, and shows a steep decline in median viral load immediately after the start of therapy. The effect of

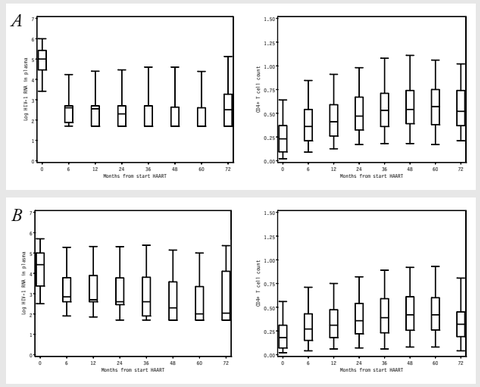

Figure 5.6.4 *HIV-1 RNA (log_{10} copies/ml) and CD4+ T cell count ($x10^3$ cells/mm^3) over time among patients who were A) naïve and B) pre-treated at start of HAART therapy*

HAART is sustained over the entire period of follow-up. The median HIV-1 RNA plasma levels decrease from 4.8 log copies/ml at baseline to 2.6 (IQR: 2.2-4.3) at six months in the entire population. Almost immediately after the start of HAART, an increase in the median CD4+ T cell count ($x10^6$ cells/l) in the population was observed: from 210 at baseline to 320 after six months and to 470 (IQR: 270-610) at 48 months of follow-up. Thereafter the increase in CD4+ T cell count tended to level off or even showed a slight decrease, although the number of measurements at 72 months was small. This pattern of increase and stabilisation at 48 to 60 months occurred regardless of pre-treatment with antiretroviral drugs prior to the start of HAART and of CD4+ T cell count at baseline.

Morbidity and mortality among HAART-treated patients

The total number of first CDC-C events registered in the HAART-treated population after the initiation of HAART was 517. The incidence of a first CDC-C event per 100 person-years since start of HAART steadily declined from sixteen in 1996 to 1.3 in 2001 (Figure 5.6.5). The total number of recorded deaths in the HAART-treated population was 404. Mortality rates decreased from 5.3 per 100 person-years in 1996 to 1.7 in 2001 (Figure 5.6.5) and this decrease seemed to continue in 2002. Despite this decline, mortality remained six to seven times higher than in the general population.

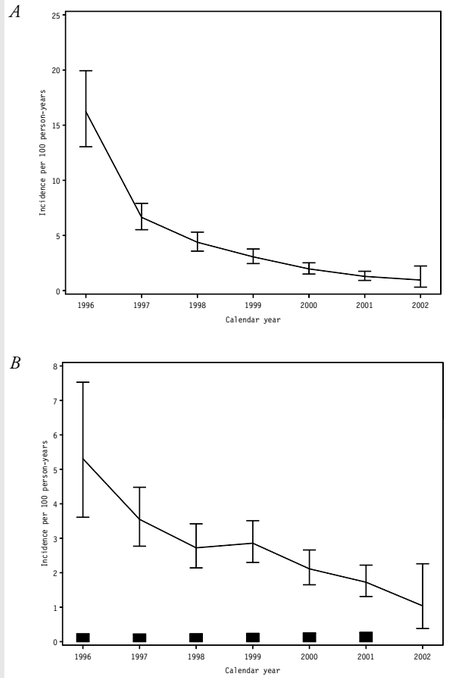

Figure 5.6.5 *Incidence and 95% confidence intervals of A) AIDS and B) death among patients on HAART. Black bars represent the death rate in the age- and gender-matched general Dutch population.*

Time to AIDS and death in the HAART-treated population

Time to AIDS (new CDC-C event)

New CDC-C events occurred among 517 patients on HAART during follow-up. After 48 months, 17% of the pre-treated patients versus 9% of the naïve patients had developed a new CDC-C event (Figure 5.6.6).

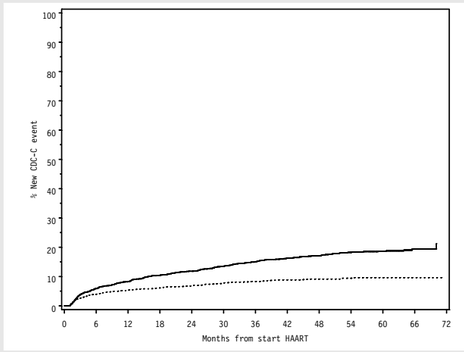

Figure 5.6.6 *Kaplan-Meier curve of time to new AIDS (CDC-C event) for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

In the multivariate analysis, pre-treatment prior to the start of HAART was predictive of the time to a new CDC-C event (HR=0.64, CI: 0.52-0.79 for naïve versus pre-treated patients; Table 5.6.1, column A). Moreover, patients who experienced virological failure (HR=2.3, CI: 1.8-2.9), as well as patients with a high viral load and a low CD4+ T cell count at baseline, had a higher risk of developing a new AIDS event. Gender and age at baseline showed no association with the occurrence of a new AIDS event. There was no additional risk associated with a second failure among patients who had experienced a first failure (HR=1.1, CI: 0.72-1.6).

When treatment interruption was included in the model, the effect of virological failure remained (HR=1.9, CI: 1.5-2.5; Table 5.6.1, column B). In addition, interrupting treatment was predictive of developing a new CDC-C event (HR=4.0, CI: 3.2-5.0). However, it could not be determined from this analysis whether the interruption was the consequence or the cause of a virological and immunological deterioration that led to a new CDC-C event.

	A		B	
	HR	**95% CI**	**HR**	**95% CI**
Naïve at start HAART *(compared with pre-treated)*	0.64	0.52-0.79	0.64	0.51-0.77
CD4+ T cell count at start HAART *(per 100 $x10^6$ cells/l increase)*	0.69	0.64-0.75	0.68	0.63-0.74
Viral load at start HAART *(per 1 log_{10} copies/ml increase)*	1.4	1.3-1.7	1.4	1.2-1.6
First virological failure *(compared with no virological failure)*	2.3	1.8-2.9	1.9	1.5-2.5
Treatment interruption *(compared with no treatment interruption)*	-	-	4.0	3.2-5.0

Table 5.6.1 *Predictors of time to a new CDC-C event among patients who started HAART. All hazard ratios are adjusted for the other variables in the table: A) excluding and B) including treatment interruptions.*

Time to death

Twelve months after the start of HAART, 98% of the patients who were naïve at the start of HAART therapy and 96% of those who had been pre-treated were still alive. After 72 months these percentages had decreased to 93% and 82%, respectively (Figure 5.6.7).

Table 5.6.2, column A shows that several factors were associated with the risk of dying. Older patients, patients who had been pre-treated prior to the start of HAART, and patients who had a high viral load and a low CD4+ T cell count at baseline had a higher risk of death. Moreover, this risk increased once patients experienced virological failure (HR=1.8, CI: 1.4-2.2). When the analysis was restricted to patients who had experienced a first failure, we found an even greater risk of death among patients who had experienced a second failure (HR=1.4, CI: 1.0-2.0).

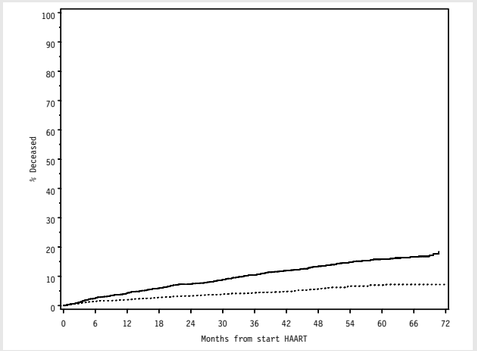

Figure 5.6.7 *Kaplan-Meier curve of time to death for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

	A		B	
	HR	**95% CI**	**HR**	**95% CI**
Naïve at start HAART				
(compared with pre-treated)	0.59	0.46-0.74	0.59	0.46-0.74
Age at start HAART				
(per 1 year increase)	1.03	1.02-1.04	1.03	1.02-1.05
CD4+ T cell count at start HAART				
(per 100 x10^6 cells/l increase)	0.70	0.64-0.77	0.70	0.64-0.76
Viral load at start HAART				
(per 1 $\log_{10}$ copies/ml increase)	1.2	1.0-1.4	1.2	1.0-1.4
First virological failure				
(compared with no virological failure)	1.8	1.4-2.2	1.5	1.2-2.0
Treatment interruption				
(compared with no treatment interruption)	-	-	2.4	1.9-3.0

Table 5.6.2 *Predictors of time to death among patients treated with HAART. All hazard ratios are adjusted for the other variables in the table: A) excluding and B) including treatment interruptions.*

Column B in Table 5.6.2 shows the results of the analysis including treatment interruption. Treatment interruptions were predictive of death, i.e. once the patient had interrupted therapy the risk of death increased (HR=2.4, CI: 1.9-3.0). Again, it should be noted that besides being a true risk factor for death, it could very well be that the interruption is merely a sign of the poor state the patient is in. Although we excluded those interruptions that were not followed by a new HAART regimen (i.e. that had a strong correlation with death or censoring), we cannot entirely rule out this effect.

Markers of therapy success and therapy failure

Therapy success

The four charts of Figure 5.6.8 depict the time to achieving therapy success, characterised by several markers of immunological and virological response to therapy. Almost the entire population reached a plasma HIV-1 RNA level below 1000 copies/ml after starting therapy. However, the rate at which this happened differed between naïve patients and pre-treated patients: the median time to reach a viral load <1000 copies/ml is 3.0 months (IQR: 1.1-11.5) among pre-treated patients and 1.8 months (IQR: 0.95-3.5) among naïve patients. After 12 months, 76% of the pre-treated and 92% of the naïve patients had a viral load <1000 copies/ml.

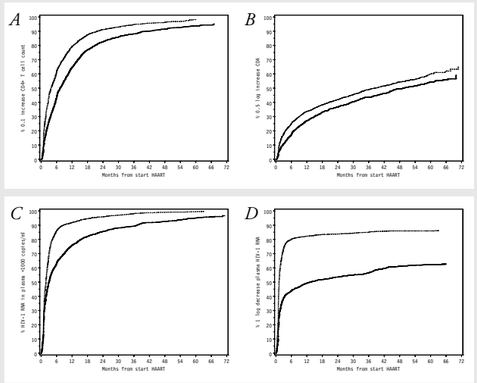

Figure 5.6.8 *Markers of therapy success: Kaplan-Meier curves of time to A) an increase of 0.100 x10^3 CD4+ T cells/mm^3 from baseline; B) an increase of 0.5 log CD4+ T cell count from baseline; C) an HIV RNA plasma load of less than 1000 copies/ml; and D) a decrease in HIV RNA of 1 log copies/ml from baseline for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

	HIV-1 RNA				CD4+ T cells			
	< 1000 copies/ml		**1 $\log_{10}$ decrease ***		**100 x10^6 cells/l increase ***		**0.5 $\log_{10}$ increase ***	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Naïve at start HAART								
(compared with pre-treated)	1.7	1.6-1.8	1.3	1.2-1.5	1.3	1.3-1.4	1.6	1.5-1.8
Age at start HAART								
(per 1 year increase)	1.007	1.003-1.01	1.004	1.00-1.008	0.995	0.992-0.999	-	-
CD4+ T cell count at start HAART								
(per 100 x10^6 cells/l increase)	1.04	1.02-1.05	1.07	1.05-1.09	-	-	0.28	0.26-0.30
Viral load at start HAART								
(per 1 $\log_{10}$ copies/ml increase)	0.77	0.74-0.80	2.2	2.2-2.3	1.1	1.1-1.2	1.3	1.2-1.4

Table 5.6.3 *Predictors of therapy success among patients treated with HAART.*
** Patients with missing values for these variables at start HAART were not included in the analysis, as these values are relative to the values at start HAART.*

This difference between naïve and pre-treated patients is also observed for other parameters of therapy success. After 12 months of therapy, 49% of the pre-treated patients and 82% of the naïve patients had achieved a 1 log decrease from the baseline plasma HIV-1 RNA level (copies/ml).

An increase of 0.1x10^3 cells/mm^3 from the baseline CD4+ T cell count was reached within 12 months by 65% of the pre-treated patients and 79% of those who were naïve. Moreover, after 12 months 27% of the pre-treated and 34% of the naïve patients had experienced a 0.5 log increase from the baseline CD4+ T cell count (x10^6 cells/l).

Pre-treatment status was a predictor of therapy success, regardless of the marker chosen to define success (Table 5.6.3). In general, patients with a low viral load and a high CD4+ T cell count at baseline achieved therapy success after a shorter period of time. Somewhat in contrast to this general finding, a low CD4+ T cell count at baseline was predictive of reaching a 0.5 log increase in CD4+ T cell count, and a high viral load at baseline was predictive of reaching a decrease of 1 log. This effect stems from the fact that all parameters are relative to the baseline status of patients, and that patients who are doing poorly at baseline do have more room for improvement. In addition, it has to be noted that patients with the worst immunological and virological status often do not have baseline measurements. This is very apparent from the hazard ratio for declining to <1000 HIV RNA copies/ml among persons with versus persons without a baseline viral load measurement (HR=8.9, 95%CI: 7.3-10.8).

Therapy failure

The time to therapy failure was longer among treatment-naïve patients than among pre-treated patients (Figure 5.6.9). The percentage with a 0.5 log increase from baseline viral load within 12 months from the start of HAART was 13% in naïve and 35% in pre-treated patients. Furthermore, within that same period a decrease of 0.050x10^3 cells/mm^3 from the baseline CD4+ T cell count was more often observed among pre-treated patients (20%) than among naïve patients (15%; Table 5.6.4).

	HIV-1 RNA		CD4+ T cells	
	0.5 log increase		**0.050 x10^6 cells/l decrease**	
	HR	95% CI	HR	95% CI
Naïve at start HAART				
(compared with pre-treated)	0.79	0.70-0.88	0.71	0.62-0.81
CD4+ T cell count at start HAART				
(per 100 x10^6 cells/l increase)	0.91	0.88-0.94	1.31	1.29-1.33
Viral load at start HAART				
(per 1 $\log_{10}$ copies/ml increase)	0.41	0.39-0.42	0.82	0.76-0.88

Table 5.6.4 *Predictors of markers of therapy failure among patients treated with HAART.*
** Patients with missing values for these variables at baseline were not included in the analysis, as these values are relative to the values at baseline.*

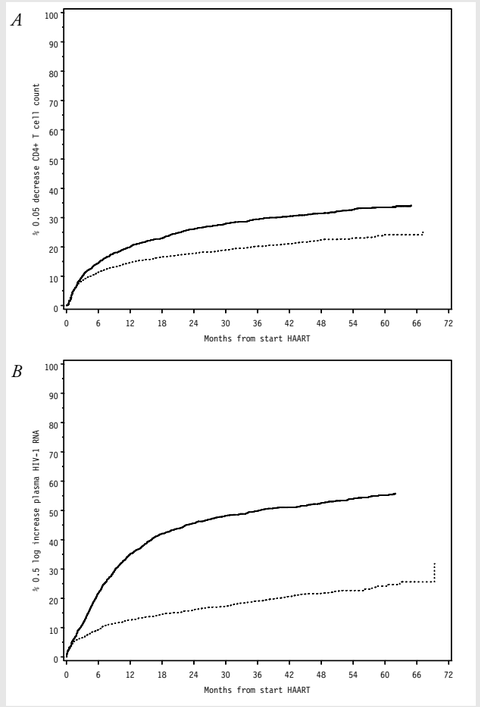

Figure 5.6.9 *Markers of therapy failure: Kaplan-Meier curves of time to A) a decrease of 0.050 x10³ CD4+ T cells/mm³ from baseline, and B) an increase of 0.5 log from baseline viral load for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

Virological failure while on HAART

Of the population of 4363 patients on HAART, 1595 (36.6%) experienced virological failure during follow-up. The risk of failing was higher among patients who had been pre-treated than among patients who were naïve (Figure 5.6.10). In the Cox proportional hazards model, predictors of failure were found to be pre-treatment, age at start of HAART, low CD4+ T cell count at start of HAART and high viral load at start of HAART. In addition, a therapy switch prior to virological failure was associated with an increased risk

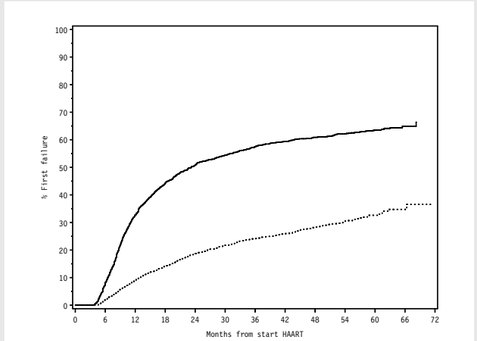

Figure 5.6.10 *Kaplan-Meier curve of time to first virological failure from start of HAART for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART (n=4363)*

	A		B	
	HR	**95% CI**	**HR**	**95% CI**
Naïve at start HAART *(compared with pre-treated)*	0.41	0.37-0.46	0.41	0.36-0.46
Age at start HAART *(per 1 year increase)*	0.99	0.99-1.00	0.99	0.99-1.00
CD4+ T cell count at start HAART *(per 100 x10⁶ cells/l increase)*	0.93	0.90-0.96	0.93	0.90-0.96
Viral load at start HAART *(per 1 log copies/ml increase)*	1.2	1.1-1.2	1.2	1.1-1.2
Therapy switch *(compared with no therapy switch)*	4.6	4.1-5.1	4.4	3.9-4.9
Therapy interruption *(compared with no treatment interruption)*	-	-	1.1	1.0-1.3

Table 5.6.5 *Predictors of first failure among patients who started HAART (n=4363). All hazard ratios are adjusted for the other variables in the table: A) excluding and B) including treatment interruptions.*

(HR=4.6, CI: 4.1-5.1; Table 5.6.5). This association remained virtually unchanged when therapy interruptions were taken into account.

Characteristics of the population at the time of first failure are described in Table 5.6.6. Among patients who had been pre-treated 66% had failed therapy at least once after 66 months of follow-up, compared to 37% among those who were naïve. At the time of failure the median CD4+ T cell count in the naïve group was 360x10⁶ cells/l (IQR: 230-540) and in the pre-treated group 260x10⁶ cells/l (IQR: 140-410; Table 5.6.6). Of the naïve patients who failed therapy 34.3% had interrupted therapy for one month or longer. This number was lower (13.1%) among pre-treated patients.

	Naïve		**Pre-treated**	
Median (IQR) CD4+ T cell count at first failure (x10⁶ cells/l)	360	(230-540)	260	(140-410)
Median $\log_{10}$ HIV RNA copies/ml (IQR) at first failure	3.8	(3.1-4.5)	3.9	(3.1-4.6)
Number (%) of periods of interrupted HAART use before first failure				
0	372	(65.7)	889	(86.9)
1	153	(27.0)	104	(10.2)
≥2	41	(7.3)	30	(2.94)
Number (%) of regimen switches before first failure				
0	257	(45.4)	475	(46.4)
1	150	(26.5)	249	(24.3)
2	75	(13.3)	140	(13.7)
≥3	84	(14.8)	159	(15.5)

Table 5.6.6 *Characteristics of patients who experienced a first virological failure while on HAART (n=1595)*

Among the 1595 patients who experienced a first virological failure, 507 (31.9%) failed again after having started on a new regimen: 8% percent of the naïve patients and 50% of the pre-treated patients. Predictors of a second failure in the Cox proportional hazards model were pre-treatment (see also Figure 5.6.11), a low CD4+ T cell count, and a high viral load at the time of the first failure (Table 5.6.7). In addition, regimen switches were strongly related to the occurrence of a second virological failure (HR 7.4; 95% CI 6.1-9.1). In contrast to the increase in risk of first failure, treatment interruptions seemed to delay the occurrence of a second virological failure (0.61; 0.46-0.80). In the present analysis, we were not able to distinguish whether this effect was caused by the treatment interruptions or merely indicative of a form of selecting relatively healthy patients.

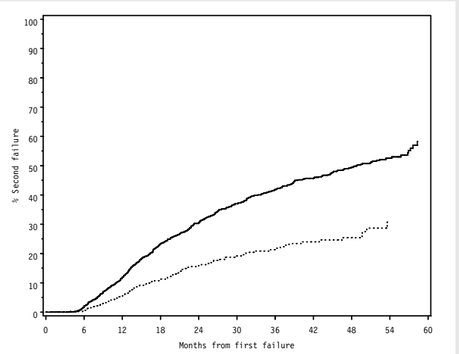

Figure 5.6.11 *Kaplan-Meier curve of time to second virological failure from first failure for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART (n=1595)*

	A		B	
	HR	**95% CI**	**HR**	**95% CI**
Naïve at start HAART *(compared with pre-treated)*	0.77	0.61-0.98	0.77	0.61-0.98
Viral load at first failure *(per 1 $\log_{10}$ copies/ml increase)*	1.4	1.2-1.5	1.4	1.3-1.6
Therapy switch between first and second failure *(compared with no switch)*	6.6	5.4-8.0	7.4	6.1-9.1
Therapy interruption between first and second failure *(compared with no interruption)*	-	-	0.61	0.46-0.80

Table 5.6.7 *Predictors of second failure among patients who experienced a first virological failure (n=1595). All hazard ratios are adjusted for the other variables in the table: A) excluding and B) including treatment interruptions.*

At the time of the second failure, 48.9% of the naïve patients had switched regimens at least once since the start of a new regimen after the first failure and 11.4% had interrupted therapy at least once. Among pre-treated patients, these numbers were 64.7 and 13.6%, respectively (Table 5.6.8). The median CD4+ T cell count among naïve patients remained higher and the median viral load lower than among pre-treated patients.

	Naïve		Pre-treated	
Median (IQR) CD4+ T cells (x10^6 cells/l) at second failure	360	(240-550)	260	(150-410)
Median $\log_{10}$ HIV RNA copies/ml (IQR) at second failure	3.8	(3.4-4.4)	4.1	(3.5-4.8)
Number (%) of periods of interrupted HAART use between first and second failure				
0	78	(88.6)	362	(86.4)
1	8	(9.1)	43	(10.3)
≥2	2	(2.3)	14	(3.4)
Number (%) of regimen switches between first and second failure				
0	45	(51.1)	148	(35.3)
1	23	(26.1)	142	(33.9)
2	10	(11.4)	52	(12.4)
≥3	10	(11.4)	77	(18.4)

Table 5.6.8 *Characteristics of patients who experienced a second virological failure while on HAART (n=507)*

Twenty-four months after the second failure, 35% of the patients who had started a new regimen after the second failure experienced a third virological failure (Figure 5.6.12). Patients who experienced a third failure did not switch regimens, nor did they interrupt their HAART therapy for a month or longer after having started a new regimen after the second failure. At the time of the third failure, the median CD4+ T cell counts among naïve and pre-treated patients were 360 and 230x10^6 cells/l, respectively.

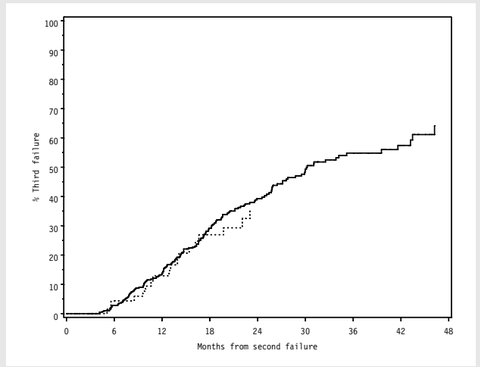

Figure 5.6.12 *Kaplan-Meier curve of time to third virological failure from second failure for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART (n=507)*

The only predictor of time to third failure was a high viral load at second failure (HR=1.7, CI: 1.4-2.0). No associations were found with other factors, such as CD4+ T cell counts, gender and treatment prior to start of HAART.

Salvage therapy after three virological failures

At the time of censoring (1 April 2002), 175 patients had experienced three virological failures while on therapy. Of these patients, 90% were male and 10% were naïve when they started HAART. Characteristics of the patients who failed on their HAART regimen three times are shown in Table 5.6.9.

The median duration of follow-up after a third failure was 17.6 months (IQR: 8.5-26.0). Of the patients who failed, 33.6% interrupted their therapy completely (i.e. did not start a new regimen at any time during the remainder of their follow-up). Furthermore, 65.6% (82/125) switched to a new regimen immediately after their third failure without interrupting therapy, and of these patients 55 (67.1%) eventually changed regimens again.

	Total	
Median (IQR) age at third failure	41	(36-48)
	N	**%**
Year of diagnosis		
before 1990	40	(22.9)
1990-2000	135	(77.1)
Treatment status at start HAART		
Naïve	18	(10.3)
Pre-treated	157	(89.7)
Gender		
Male	154	(90.1)
Female	17	(9.9)
Median (IQR) CD4+ T cells (x10^6 cells/l) at third failure	230	(120-410)
Median (IQR) $\log_{10}$ HIV-RNA copies/ml at third failure	4.3	(3.8-4.9)
Year of start HAART		
before 1998	146	(83.4)
1998-1999	28	(16.0)
after 1999	1	(0.6)
Maximum number of drugs used simultaneously		
3	21	(12.0)
4	96	(54.9)
≥5	58	(33.1)

Table 5.6.9 *Characteristics of the population that failed three times while on HAART (n=175)*

Among seventeen (13.6%) patients the maximum number of drugs taken simultaneously after their third failure was higher than the maximum number in any regimen before this failure. Among 66 patients (52%) the maximum number of drugs taken after the third failure was lower than in any previous regimen, excluding those who had stopped therapy altogether.

The viral load and CD4+ T cell counts at the end of follow-up were 4.1 $\log_{10}$ copies/ml (IQR: 3.0-4.9) and 240x10^6 cells/l (IQR: 110-410), respectively.

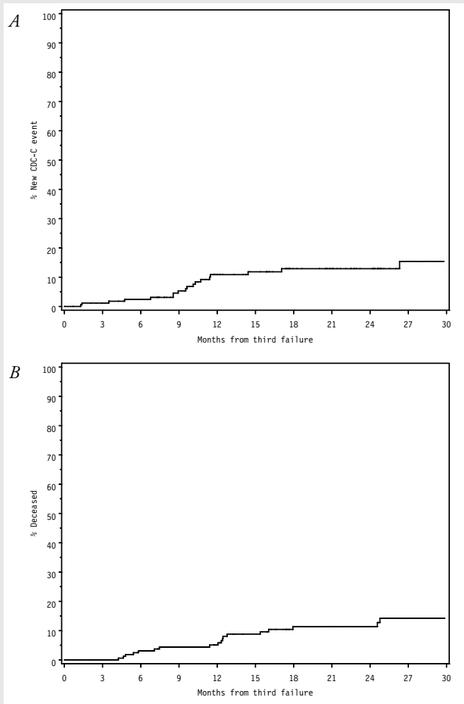

Figure 5.6.13 *Kaplan-Meier curves of time to new CDC-C event (left) and death (right) among patients who experienced three virological failures while on HAART*

Within 12 months of the third failure, 11% of the patients developed a new CDC-C event. In addition, 5% of the patients who failed three times died within one year of the third failure. After 24 months, 89% was still alive (Figure 5.6.13).

Therapy switches

A large proportion of the population changed regimens during the course of their therapy. Patients who had been pre-treated with either mono- or dual therapy switched therapies more often than patients who were naïve at the start of therapy (Log-rank, p<0.0001). After 12 months, 53% of the pre-treated patients and 42% of the naïve patients had changed regimens. This increased to 98% and 93% after 66 months of follow-up (Figure 5.6.14). The median time until the first change was 16.1 months among naïve patients (IQR: 5.3-33.7) and 11.1 months among pre-treated patients (IQR: 4.0-26.2).

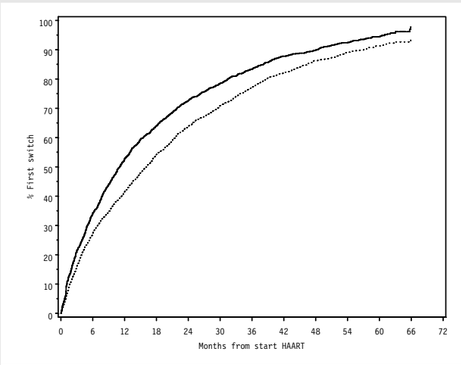

Figure 5.6.14 *Kaplan-Meier curve of time to first therapy switch after start of HAART for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART.*

The majority of the patients for whom a reason for regimen change was recorded switched regimens due to toxicity (40.0% pre-treated versus 47.7% naïve). Other important

reasons for switching were failure (26.7% pre-treated versus 9.8% naïve) and patient's decision (11.1% pre-treated versus 15.4% naïve).

Of the 3375 patients who had switched once, 2341 switched therapy a second time. The median duration from first switch to second switch was 9.4 months (IQR: 2.0-23.3; Figure 5.6.15) for pre-treated patients. Again, a significant difference was found between naïve and pre-treated patients (Log-rank, p<0.0001). The main reasons for switching the second regimen were similar to the reasons for switching the first regimen: toxicity (43.6% pre-treated versus 48.6% naïve) and failure (22.3% pre-treated versus 10.2% naïve).

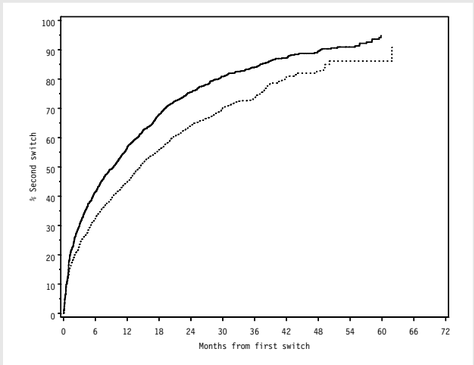

Figure 5.6.15 *Kaplan-Meier curve of time to second therapy switch after first switch for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

In total, 1620 patients changed therapy a third time. Twelve months after starting their second regimen 56% of the pre-treated patients and 49% of the naïve patients switched therapy again (Figure 5.6.16). This difference between pre-treated and naïve patients was statistically significant (Log-rank, p=0.0006). Again, the most important reasons for switching therapy were toxicity (45.0% pre-treated versus 52.5% naïve) and failure (23.6% pre-treated versus 9.9% naïve).

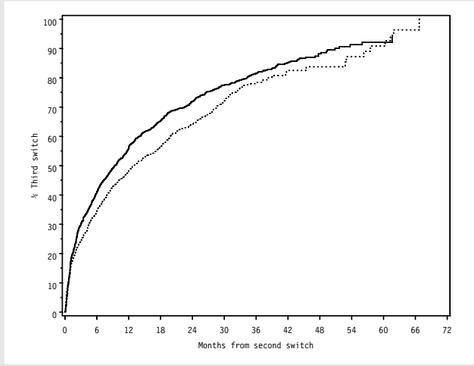

Figure 5.6.16 *Kaplan-Meier curve of time to third therapy switch after second switch for patients who are pre-treated (solid line) and therapy-naïve (dashed line) at start of HAART*

Conclusions

The untreated and ART-treated patients are insofar specific groups that they are small and that their results are relatively positive because they respond well to non-treatment or ART treatment, whereas those patients who do not respond well are already part of the large group of HAART-treated patients. In other words: the standard of HIV patient care is what can be achieved with HAART treatment, which in small subgroups may be reached with treatment regimens less than HAART or even no treatment at all.

In addition to the known predictors of therapy outcome, such as CD4+ T cell count, HIV RNA plasma levels at baseline and changes from baseline[4;50-53], and clinical status at baseline[24], switching HAART regimens as well as interrupting treatment[54] (Van Sighem, A.I., Van de Wiel M.A., Ghani A.C., et al. Mortality and progression to AIDS after starting highly active antiretroviral therapy. 2002, submitted for publication) are also important outcome predictors. Changing HAART regimens appears to be a common practice and increases the risk of virological failure and thereby the risk of a (new) CDC-C event and death[55]. No differences with respect to therapy outcome were found between men and women[56].

5.7 Toxicity

One of the main questions to be answered by monitoring the HIV-1 infection is: to what extent does toxicity of the drugs or drug combinations used influence the course of a treated HIV infection? As shown in the final report of ATHENA[5] and in the previous chapter of the present report, therapy regimens are changed rapidly and frequently. The ATHENA final report showed that, unfortunately, toxicity of the drugs used for antiretroviral treatment are an important reason for short-term change of therapy and more important than therapy failure. In this chapter, we again report on the signs and symptoms of toxicity of the antiretroviral drugs used in HAART-treated patients, though after a longer period of treatment. In addition we report on toxicity related to ART treatment.

Material and methods

As part of the monitoring of the HIV infection, the start and stop date, and the reason for interruption or therapy change (if available) were recorded for each antiretroviral drug. Clinical signs and symptoms possibly related to the drug or drug combinations used were recorded as well. For each drug we first determined whether it was given as part of an ART or HAART regimen. If so, then for each patient the number of drug changes and the reason for change were analysed. If toxicity was the reason for change, the related signs and symptoms were categorised into tract-related classes and the number of patients per class were computed.

Results

Of the 4663 treated patients, 4366 patients (93.6%) were treated at least once with a HAART regimen and 2648 patients (56.8%) were treated at least once with an ART regimen (including patients who started HAART and changed to ART later). In both groups toxicity was the most frequently reported reason for change or interruption of the regimen (2326/4663 patients, 49.9%; see Table 5.7.1). Of the 4366 patients on HAART 2075 (47.5%) changed or stopped at least once because of toxicity, in contrast to patients on ART where this number was only 704/2648 (26.6%) (p<0.0001). Other reasons for interruption or change were therapy failure and the patient's own decision. Among the HAART-treated patients, 19.6% interrupted or changed therapy because of failure compared to 18.7% of the ART-treated patients, and 22.0% versus 9.0% did so because of their own decision.

Reason for change	Patients on HAART or ART		Patients on HAART		Patients on ART	
	N	%	N	%	N	%
Total	**4663**	**100**	**4366**	**93.6**	**2648**	**56.8**
Toxicity	2326	49.9	2075	47.5	704	26.6
Failure	1140	24.4	857	19.6	496	18.7
Patient's decision	1101	23.6	960	22.0	238	9.0
Pharmacology	280	6.0	247	5.7	44	1.7
Dose-related	49	1.1	40	0.9	9	0.3
Other	1237	26.5	1034	23.7	313	11.8

Table 5.7.1 *Reason for therapy change by HAART / ART regimen*

A cumulative number of in total 10026 antiretroviral drugs administered to the 2326 patients were interrupted or changed due to toxicity. For 2768 of the 10026 drugs (27.6%) no link with a sign or symptom could be established, partly as a result of database complications. An overview of the most important signs and symptoms that were related to change of therapy because of toxicity is given in Table 5.7.2. Within the category of gastrointestinal signs or symptoms, nausea (369 patients), vomiting (326 patients) and diarrhoea (183 patients) were most frequently reported. In 242 patients lipodystrophy/atrophy was reported and in 82 other liver abnormalities. In total 480 patients were recorded as having neuropsychiatric signs and symptoms, 260 of whom were diagnosed with peripheral neuropathy. Anaemia was the most frequently recorded haematological disorder (257/343 patients). General fatigue, malaise and fever were recorded in 221 patients. Nephrolithiasis was recorded in 112 patients.

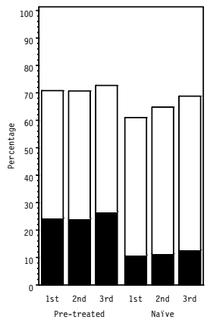

Figure 5.7.1 *Failure (black) and toxicity (white) as reasons for 1st, 2nd and 3rd change of regimen in pre-treated and therapy-naïve HAART-treated patients*

	N	%
Total	4663	100
Never stopped a regimen due to toxicity	2337	50.1
Once or more stopped a regimen due to toxicity	2326	49.9
Signs and symptoms of:		
Gastrointestinal disorders	844	36.3
Lipodystrophy	242	10.4
Liver abnormalities	82	3.5
Peripheral neuropathy	260	5.6
Transient paraesthesia	72	1.6
Depression	27	0.6
Other neuropsychiatric disorders	160	3.4
Hematological disorders	343	14.7
Rashes	277	11.9
Fatigue, malaise, fever	221	9.5
Urogenital disorders	185	8.0
Muscle/joint disorders	73	3.1
PNE	51	2.2
Pancreatitis	27	1.2
Cardiovascular disorders	16	0.7
Pulmonary disorders	16	0.7
Diabetes mellitus	7	0.3
Visual disorders	4	0.2
Other	59	2.5

Table 5.7.2 *Recorded signs and symptoms*

Toxicity appeared to be the most important reason for change of HAART regimen on the first, second, and third occasion (Figure 5.7.1) and more important than therapy failure. Remarkably, the proportion of toxicity and failure as reason for change remained stable over time. HAART regimens were more often changed because of failure in pre-treated than in naïve patients. In 2722 of the 7652 regimen changes (36%) the reason for change was unknown.

The most important toxicity-related signs or symptoms and the drugs they were linked to are summarised in Table 5.7.3. Gastrointestinal signs and symptoms were frequently reported as related to toxicity of protease inhibitors, nRTIs or NNRTIs, although the nature and severity differed per class and within class. Haematological abnormalities, mainly anaemia, were, as expected, seen more frequently in the nRTI class, whereas urogenital abnormalities, almost exclusively nephrolithiasis, were especially found in relation to indinavir use. Pancreatitis was infrequent. General fatigue and malaise were recorded on average at the same frequency for each of the three classes. Neuro-psychiatric signs and symptoms due to toxicity mainly consisted of (peripheral) neuropathy and paraesthesia and were more frequent in the nRTI and NNRTI classes. Lipodystrophy/atrophy was recorded as a result of the use of PIs, but also of the nRTIs stavudine and lamivudine.

Conclusion

Toxicity remains the most important reason for changing the HAART regimen in both pre-treated and treatment-naïve patients and more so than therapy failure[14;54]. Pre-treated patients change their regimen more often because of failure than naïve patients. However, in the present cohort the reason for change of HAART regimen frequently appeared to be unknown, which is why the results presented here should be interpreted cautiously.

Patients on HAART regimens are more likely to change their regimen for reasons of toxicity than patients on ART regimens, indicating that a combination of antiretroviral drugs used in HAART is more toxic than in ART. However, the ART-treated patients are a selected group of patients who are doing well on their regimen and can therefore not very well be compared to the

	Fatigue, malaise, fever	Skin abnormalities (rash)	Gastrointestinal abnormalities	Liver abnormalities	Pancreatitis	Lipodystrophy	Hematological abnormalities	Urogenital abnormalities	Paraesthesia (transient)	Peripheral neuropathy	Muscle/joint abnormalities	Depression	Other neuropsychiatric abnormalities	Therapy change not matched with toxicity	Total
Protease Inhibitors															
Ritonavir	60	44	364	22	4	90	9	65	22	23	8	5	29	431	1051
	5.7	4.2	34.6	2.1	0.4	8.6	0.9	6.2	2.1	2.2	0.8	0.5	2.8	41.0	
Indinavir	39	58	200	26	8	68	14	170	5	19	13	6	24	273	790
	4.9	7.3	25.3	3.3	1.0	8.6	1.8	21.5	0.6	2.4	1.6	0.8	3.0	34.6	
Saquinavir/Invirase	31	13	156	10	3	58	11	4	5	13	4	1	11	233	545
(HGC)	5.7	2.4	28.6	1.8	0.6	10.6	2.0	0.7	0.9	2.4	0.7	0.2	2.0	42.8	
Saquinavir/Fortofase	4	1	28		1	8	1			1			1	28	69
(SGC)	5.8	1.4	40.6		1.4	11.6	1.4			1.4			1.4	40.6	
Nelfinavir	13	24	149	5	5	25	9	1	6	4	5	1	8	95	329
	4.0	7.3	45.3	1.5	1.5	7.6	2.7	0.3	1.8	1.2	1.5	0.3	2.4	28.9	
Kaletra	4	9	20		4	4	1			1		3	10	8	53
	7.5	17.0	37.7		7.5	7.5	1.9			1.9		5.7	18.9	15.1	
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors															
Stavudine	41	40	153	27	19	109	14	21	24	172	20	8	36	291	869
	4.7	4.6	17.6	3.1	2.2	12.5	1.6	2.4	2.8	19.8	2.3	0.9	4.1	33.5	
Zidovudine	39	29	179	12	2	2	241	9	8	17	28	5	29	221	702
	5.6	4.1	25.5	1.7	0.3	0.3	34.3	1.3	1.1	2.4	4.0	0.7	4.1	31.5	
Lamivudine	53	47	153	18	9	32	34	24	5	29	14	10	33	226	595
	8.9	7.9	25.7	3.0	1.5	5.4	5.7	4.0	0.8	4.9	2.4	1.7	5.5	38.0	
Didanosine	20	33	157	14	19	7	12	13	11	69	7	3	14	194	516
	3.9	6.4	30.4	2.7	3.7	1.4	2.3	2.5	2.1	13.4	1.4	0.6	2.7	37.6	
Combivir	29	35	83	6	1	5	72	11	1	8	11	2	14	104	351
	8.3	10.0	23.6	1.7	0.3	1.4	20.5	3.1	0.2	2.3	3.1	0.6	4.0	29.6	
Zalcitabine	15	21	43	2	2	1	25		20	54	9		9	80	280
	5.4	7.5	15.4	0.7	0.7	0.4	8.9		7.1	19.3	3.2		3.2	28.6	
Abacavir	41	30	36	2	2	4	2	4		3	1	1	13	39	149
	27.5	20.1	24.2	1.3	1.3	2.7	1.3	2.7		2.0	0.7	0.7	8.7	26.2	
Non-nucleoside/Nucleotide Reverse Transcriptase Inhibitors															
Nevirapine	27	109	76	23	5	6	15	2	1	16	9	3	28	146	430
	6.3	25.3	17.7	5.3	1.2	1.4	3.5	0.5	0.2	3.7	2.1	0.7	6.5	34.0	
Efavirenz	18	18	15	2	3	2		2				4	28	20	102
	17.6	17.6	14.7	2.0	2.9	2.0		2.0					27.5	19.6	

Table 5.7.3 *Number and percentages for each group of signs and symptoms of toxicity recorded per antiretroviral drug used*

HAART-treated patients.

Gastrointestinal signs and symptoms (nausea, vomiting, diarrhoea), liver abnormalities and peripheral neuropathy were most frequently recorded as being related to toxicity of the antiretroviral drugs used.

5.8 Resistance

Although HAART can suppress plasma virus to undetectable levels, replication is still going on, albeit at a lower rate than in untreated patients[57-60]. In the end, this might result in a selection of HIV-1 viruses that escape suppression by antiretroviral drugs – in other words, that are resistant to the drugs. Prolonged treatment with these drugs and consequently selection of resistant virus could result in transmission of these resistant viruses to uninfected persons.

Resistance is measured in the study population by genotyping HIV-1 RT and protease for resistance-associated mutations. Thus far, resistance measurements were only part of the follow-

up of treated patients in trials or studies. Since January 2002 it is possible to determine resistance as part of regular clinical practice. In this paragraph we report on the available resistance data in the whole group of HAART-treated patients. These data are still limited as only the data obtained from the sub-group of 600 patients of the ATHENA project[5] and a small number of more recent clinical cases can be used.

Material and Methods

Resistance measurements were based on isolation of HIV-1 RNA from plasma of patients and amplification of the protease and (part of) the RT gene of the virus. Successful amplification was only achieved in patients with a viral load above 1000 copies/ml. HIV-1 RT and protease were genotyped by using the amplified genes in a sequencing procedure. Results were compared to subtype B wild-type virus and scanned for mutations. Mutations directly related to resistance (primary mutations) and mutations associated with resistance in combination with other mutations (secondary mutations) were scored[61].

A sequencing procedure was requested for 1446 plasma samples, of which 42 (3%) were not successful because the load was too low for the assay (34 requests) or the sample was not available (six requests). The majority of the sequencing (764 sequences, 53%) was done within the framework of the ATHENA study. The other sequences were requested by the treating physician or were done for other dedicated studies.

Baseline resistance

For 509 patients (229 pre-treated and 280 therapy-naïve) a sequence was measured before start of HAART. In 470 patients both RT and protease could be successfully sequenced, while in 24 patients only protease was obtained and in one patient only RT. For fourteen patients the HIV-RNA load in the plasma sample was too low to obtain any sequence. Primary resistance-associated mutations were found in RT in 93 (43%) out of 216 pre-treated patients with an RT sequence. Most prevalent primary mutations were T215Y/F (67%), M184V/I (19%) and K70R (39%). Two patients had a two amino acid insertion between positions 69 and 70. Among the secondary mutations found in the 216 pre-treated patients were M41L (22%), D67N (16%) and L210W (16%). In only one patient out of 255 pre-treated patients with a protease sequence a primary mutation in protease (V82A) was found. In the naïve group primary RT mutations were found in twelve patients and primary protease mutations in one patient (see next section).

These findings show that primary mutations were mainly found in patients pre-treated with an nRTI and that mutations in protease were limited, the latter indicating that protease inhibitors were infrequently used before the introduction of HAART.

Transmission of drug-resistant viruses

In recent years the prevalence of drug-resistant viruses in newly infected patients varied from 5% to 25% in Western Europe and the United States[59;62-64]. Resistance to drugs from one or more classes is increasing over calendar time[65].

In the Netherlands, resistance to AZT was found in approximately 9% of homosexual men and intravenous drug users participating in the Amsterdam cohort studies[66]. An indication of the present situation in the Netherlands can be deduced from samples of treatment-naïve patients, despite the fact that for the majority of these patients the approximate date of seroconversion is unknown.

In 23 out of 355 patients with an RT sequence before start of any therapy, primary mutations were found in RT. Seven patients had V118I and one had E44D as single primary mutation, which causes resistance to 3TC only in combination with each other. Another three patients had V108I as single mutation, which is associated with resistance to nevirapine; two patients showed mutation 69D, and one patient mutations 67N and 219Q[61]. The remaining nine

T215	M41	D67	K70	L210	K219
Y	L	N		W	
F			R		Q
D	L			W	
D	L				
D					
S	M/L				
S					
C					
V		N			Q

Table 5.8.1 *Resistance-associated mutations found in HIV-1 RT obtained from nine infected patients before start of antiretroviral therapy*

patients (4%) had a mutation at position 215 and are listed in Table 5.8.1.

The T215Y and T215F mutation directly cause resistance to AZT, while the Y215C mutation causes resistance to ddC. The T215D and T215S mutations reflect evolution from a transmitted AZT-resistant virus[67]. One of the patients with a 215D mutation, however, had two undetectable viral load measurements four years before start of therapy, which might indicate a use of therapy that was not recorded in the database.

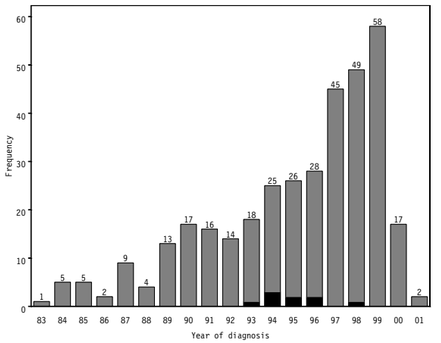

Figure 5.8.1 *Number of RT sequences measured by year of diagnosis. The number of RT sequences with primary mutations at position 215 is indicated in black.*

Figure 5.8.1 shows the number of patients who were diagnosed in each calendar year and who had an RT sequence somewhere during follow-up but before initiation of therapy. Patients with a mutation at position 215 were mainly diagnosed in the mid-1990s and were thus probably infected when mono- and dual therapy was already widely used.

Among the 333 patients with a protease sequence, one patient was found with an M46I mutation. This patient was diagnosed in 1991, making it improbable that this was a resistant-virus transmission.

Development of resistance during treatment with HAART

HIV-1 RT and protease sequences were obtained from thirty naïve and 78 pre-treated patients who were genotyped at least four weeks after start of their first treatment regimen and who were still using this regimen. For two pre-treated and three naïve patients no RT sequence was available. In six (8%) pre-treated and nine (30%) naïve patients no primary resistance-associated mutations were found. Apparently these fifteen patients were not fully adherent to their therapy.

The remaining patients had on average 1.6 and 3.3 primary mutations for the naïve and pre-treated patients, respectively. Primary protease mutations are listed in Table 5.8.2, categorised by the protease inhibitor in the first treatment regimen.

	Pre-treated						Naïve		
	IDV	NFV	RTV	SAQ	SAQ/ RTV	NVP	NFV	other PI	NVP
N Patients	28	8	12	26	3	1	13	10	7
D30N		2					6		
M46I	6	1	2	1					
G48V	3			6					
V82A/F/S/T	10		8			1			
I84V	2		2		1				
L90M	5	2	3	13	1	1	1		

Table 5.8.2 *Primary mutations found in patients failing their first HAART regimen*

In the naïve group only protease mutations causing resistance to nelfinavir were found (D30N and L90M). The most prevalent mutations in the pre-treated group were the V82A/F/S/T mutations, found in ten (35%) patients using indinavir and in eight (67%) patients using ritonavir. The L90M mutation was mostly found in patients using saquinavir. The one pre-treated patient with protease mutations, who was treated with nevirapine, had been pre-treated with saquinavir and ritonavir.

The most frequent RT mutation was M184V/I, which was found in nineteen naïve and 42 pre-treated patients. This mutation causes resistance to 3TC, which was used by 29 naïve and 53 pre-treated patients in their first HAART regimen. Three pre-treated patients had an M184V without using 3TC but they had been pre-treated with 3TC. Other frequently found mutations in pre-treated patients were T215Y/F (51), K70R (23) and the secondary mutations M41L (43), D67N (40) and L210W (36).

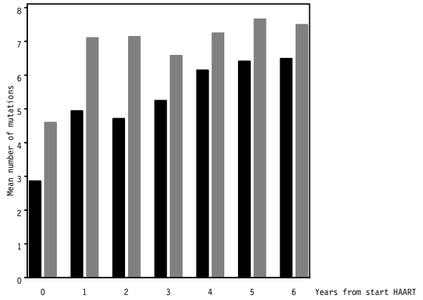

Figure 5.8.2 *Mean number of primary (black bars) and secondary (grey bars) resistance-associated mutations after start of HAART in the group of patients who eventually fail three times on therapy*

Therapy failure has been defined in paragraph 5.3 and described in paragraph 5.6. In total 175 (157 pre-treated and eighteen naïve) patients in the HAART group failed therapy at least three times. In 52 (fifty pre-treated patients) of these patients an RT and protease sequence were obtained at least once after the start of HAART.

The mean number of primary and secondary resistance-associated mutations in the fifty pre-treated patients since start of HAART is depicted in Figure 5.8.2. The number of mutations increases in the first year after start of HAART reflecting the introduction of protease inhibitors in the population. In the years thereafter the number of primary mutations slightly increases at a rate of 0.6 mutations per year, while the number of secondary mutations is more or less constant.
These findings show that after three therapy failures patients harbour resistant virus strains with six to seven primary mutations, which severely reduces the spectrum of possible therapy strategies. Already in the first year on therapy the number of mutations in these patients increases from three to four or five primary mutations. This clearly indicates that in these patients first-line therapy failed, most probably because patients were already resistant to one or more of the drugs in the first-line regimen before starting the regimen[68;69].

Conclusions

It is essential that for pre-treated patients the first-line therapy is carefully chosen. Should patients fail on this therapy then they are very likely to fail again on the second or subsequent follow-up therapies. Therefore, a genotypic determination of the resistance profile is recommended for sub-optimally treated patients who switch to HAART. By using this information the treating physician can carefully select the appropriate first-line regimen for these patients.
Transmission of drug-resistant HIV-1 virus strains seems to be limited in the Netherlands, but is probably underestimated, as people were not always genotyped close to the time of infection. It is to be expected that, as in other countries, the transmission of resistant strains will increase as the number of people on treatment increases. A dedicated study in patients with a primary infection might give more insight into the prevalence of transmission of drug-resistant strains.

General conclusions, discussion and recommendations

6.1 Conclusions

With the data collected in the ATHENA project from 1994/1995 until 2001 and the subsequent continuation of the data collection of HAART-treated patients and the inclusion of non-HAART and untreated HIV-infected patients, a huge observational clinical cohort of HIV-infected patients has been established in the Netherlands. At present, data collection and monitoring is performed within the framework of the HIV Monitoring Foundation, embedded in regular patient care. Twenty-one hospitals with 23 locations have been specifically appointed as HIV treatment centres and participate in the collection of these data, which is relevant for the monitoring of the HIV infection in an era of large-scale antiretroviral treatment. Recently, the Medical Centre Alkmaar has regained its status as HIV treatment centre, which brings the total number of these centres to 22.

The ATHENA organisation has changed into the HIV Monitoring Foundation and has expanded to cover the data collection of an additional number of HAART-treated patients, as well as the group of treated and untreated HIV-infected patients. Altogether, the number of patients monitored is twice the size of the number of patients followed in the ATHENA cohort.

As in the ATHENA project we were again able to answer questions regarding changes in the course of the HIV infection, yet over a longer period of follow-up.

What are the changes since the introduction of HAART in 1995/1996 in HIV-related morbidity and mortality?

Both HIV-1-related morbidity and mortality are still decreasing since the introduction of HAART in the HAART-treated group of patients – to 1.3 and 1.7 per 100 person-years in 2001, respectively. Although data obtained in 2002 are limited to the first quarter, they show a further decreasing tendency. However, mortality in the HAART-treated HIV-infected patients is still six to seven times higher compared to the age- and gender-matched general Dutch population. Interestingly, morbidity and mortality in the ART-treated group decreases to the same extent, indicating that in this selected group of patients a less intensive treatment regimen is already sufficient. In the group of untreated patients, none died after 1995, which is when they could have had antiretroviral treatment, pointing at selection bias in this group. Partly, this could be explained by their late inclusion into the HIV Monitoring Foundation and partly because this group represents a small number of patients selected over time because of their survival. Comparisons to the ART- and especially the HAART-treated group were not performed because of this (and other) selection biases.

In addition to the well-known variables, such as pre-HAART treatment status, CD4+ T cells and viral load at baseline, time to AIDS or death after start of HAART appeared to be dependent on virological failure under therapy and therapy interruption (Van Sighem A.I., et al. 2002 submitted for publication).

What determines therapy success and what therapy failure?

Determinants of therapy success and failure were studied mainly in the HAART-treated group of patients. Again, the pre-HAART treatment status of patients was an important determinant of success. Almost the entire population reached a plasma HIV-1 RNA level below 1000 copies/ml after starting therapy; however, naïve patients reached that point earlier (median 1.8 months) than pre-treated patients (median three months). When using other therapy success parameters, like an increase in CD4+ T cells, this difference between treatment-naïve and pre-treated patients was found as well. Treatment status in combination with CD4+ T cell count and viral load at baseline largely determined therapy success. In addition, no differences were found between men and women with regard to therapy success.

When analysing therapy failure, time to treatment failure was longer among naïve patients than among pre-treated patients. Time to an increase in viral load and a decrease in CD4+ T cells took much longer in naïve than in pre-treated patients.

Interestingly, the number of patients who were antiretroviral-therapy-naïve when starting HAART is increasing over time. In combination with the significantly higher CD4+ T cell counts at baseline in naïve patients, this indicates a shift towards a start of HAART earlier in the HIV infection.

What, after a first or second failure on antiretroviral therapy, determines therapy outcome?

In an analysis based on data obtained from the ATHENA study it was shown that there might be differences in therapy effect among first-drug regimens (Van de Wiel, et al. 2002 submitted for publication). In the present report we analysed determinants of first, second and third failures and subsequently looked at the preliminary results of so-called salvage therapy in the group of HAART-treated patients that failed on their third therapy regimen. More than one third of the population on HAART experienced virological failure on their first HAART treatment regimen and age, low CD4+ T cell count and high viral load at start of HAART were predictive of failure. Moreover, therapy interruptions were associated with failure, as was switching antiretroviral therapy regimen, which give a five times higher risk of virological failure, irrespective of treatment interruptions.

Again, one third of the population that fails a first time fails antiretroviral therapy a second time, and again, pre-treated HAART patients are at greater risk of failure than naïve patients. Change of HAART regimen is a risk factor for second failure as well; however, treatment interruption is not. Third failures seem easier to define: the main factor determining third failure is viral load at second failure, indicating a relative collapse of the effect of antiretroviral drugs on the replication of HIV. Thus, virological failure is frequent and there is a high risk of second and third failure following a first one. In addition, it is important to note that this risk is influenced by therapy interruptions. The risk of a new AIDS-related CDC-C event after a third failure is also high, as is the risk of death within one year after a third failure, irrespective of salvage therapy.

Impressive is the proportion of patients that change their therapy regimen during the course of treatment: 98% of the pre-treated and 93% of the naïve patients change their regimen within 66 months after the start of HAART. Multiple changes are common and the main reason for change is toxicity, rather than treatment failure.

To what extent does toxicity of the drugs or drug combinations used influence the course of a treated HIV infection?

Toxicity remains the most important reason for change of the HAART regimen in both pre-treated and treatment-naïve patients, and patients on HAART regimens are more likely to change their regimen for reasons of toxicity than those on ART regimens. This

indicates that a combination of antiretroviral drugs as used in HAART might be more toxic than in ART. Gastrointestinal signs and symptoms (nausea, vomiting, diarrhoea), liver abnormalities and peripheral neuropathy are most frequently recorded as related to toxicity of the antiretroviral drugs used. As change of HAART regimen is an important variable in predicting the outcome of treatment, toxicity of the drugs used in a HAART regimen influences the course of a treated infection in that it increases the risk of new CDC-C events and eventually death. Assuming that toxicity will negatively influence adherence to the drugs prescribed, development of resistance due to insufficient inhibition of viral replication might be an effect as well, although further studies on the relationship between toxicity, adherence and resistance within the HIV Monitoring Foundation are needed.

To what extent does resistance play a role in the course of a treated HIV infection?

From the data on therapy success and failure it is clear that the carefully chosen first-line HAART combination is essential for both naïve but especially for pre-treated patients. If patients fail on this therapy, they are very likely to fail on the second or subsequent therapies as well. Although the number of RT and protease sequences obtained from pre-treated patients at the start of HAART is still limited, the results indicate that a large proportion of these patients who fail on their first HAART regimen do have primary resistance-associated mutations in, especially, RT. Therefore, a genotypic determination of the resistance profile of sub-optimally treated patients who switch to HAART is recommended. By using this information the treating physician can carefully select the appropriate first-line regimen for these patients.

Based on the sequence data obtained so far from naïve patients at the start of HAART, the conclusion already drawn from the ATHENA project holds true: transmission of drug-resistant HIV-1 virus strains seems to be limited in the Netherlands. However, these results are an underestimation, as resistance measurements could not be performed on samples close to the time of infection. Measuring resistance in the first positive blood sample of patients newly diagnosed with HIV will provide more insight into the prevalence of transmission of drug-resistant strains.

6.2 Discussion

As with the ATHENA study, a number of cautionary statements have to be drawn up for a proper evaluation of the validity of the answers presented to the research questions.

The first one is of course that the patients followed within the HIV Monitoring Foundation form an observational cohort, which can result in serious biases when the outcomes from the use of antiretroviral therapy in such a cohort are compared. There are several indications that patients in the cohort have a different prognosis at the time they start new antiretroviral therapy. For the ATHENA cohort we developed a specific approach to deal with this issue of different baseline hazards for outcome (Van de Wiel, et al. 2002 submitted for publication) and in the near future this approach is to be extended to the new, enlarged HIV Monitoring Foundation.

Another serious bias stems from differences between the design of the ATHENA study and the design of the HIV Monitoring Foundation. Moreover, merging the population studied in ATHENA with the population followed outside ATHENA will bias results as well and this effect will only disappear after a certain amount of time.

In contrast to the ATHENA study, non-treated patients or ART-only-treated patients are now included in the cohort. Results obtained from these small groups so far point at a positive selection and we did not compare these groups with the HAART-treated groups. In the HAART group no major differences in treatment effect were found between men and women and gender did not significantly influence treatment outcome. At present, it is not possible to draw any conclusions with respect to differences in effect of treatment between patients from Dutch, Western European and North American origin versus patients from other parts of the world, in particular Sub-Saharan Africa. The inclusion of foreign (non-western) patients into the HIV Monitoring Foundation has just started. However, from the limited data available it is clear that there is a relatively large proportion of non-B HIV-1 infections among non-western patients. Moreover, the proportion of patients with heterosexual contact as risk factor and the proportion of women in this group is larger as well.

The effect of HAART in the population is impressive. Morbidity and mortality in the HAART-treated population still decline. Large proportions of HAART-treated patients reach plasma HIV RNA levels <1000 copies/ml and achieve an increase of CD4+ T cells of at least 100 cells per mm^3. This effect in the population remains over a prolonged period of time. Nevertheless, there are differences between pre-treated and naïve patients. In general, naïve patients do better in terms of the time to positive response as well as the time to therapy failure, but also with respect to frequency and time to therapy failure as well as therapy change. Therapy failure and therapy change, including therapy interruption, have shown to be predictive of new CDC-C events and death, in addition to the now well-known predictors such as viral load and CD4+ T cell counts at baseline.

Further to the ATHENA study, it is clear that toxicity of the drugs used is frequent, also on a longer time scale, although there are no indications that toxicity is a significant factor for mortality in the HAART-treated group (Van Sighem, et al. 2002 submitted for publication). Signs and symptoms of toxicity remain the same, which is not surprising given the relative stability of the various antiretroviral drugs that can be prescribed. Unfortunately, we cannot link toxicity to the results of adherence and quality of life studies, as these studies are not part of the present report; neither can we judge the possible influence on the development and spread of resistance.

Compared to the results of the ATHENA study, the number of resistance measurements did not increase substantially and the availability of results was limited. Transmission of resistance is still limited, although underestimated due to the well-known sampling issue. In order to adequately estimate the transmission of resistant HIV, sampling should be done as closely as possible to the moment of transmission or at least that moment should be relatively well defined. Resistance is a problem in patients who have been sub-optimally treated before they start HAART, and in that respect the so-called ART-treated group might add to this problem. The question to what extent resistance plays a role in second and third therapy failure remains.

The number of patients that at present is included in the HIV Monitoring Foundation approaches 7000. The majority of these patients is treated with HAART and for most of them this treatment is beneficial, also over a longer period of time. On a population level, the amount of HIV viral load found in infected patients after the introduction of HAART is low and the immunological status of patients, as measured by an increase in CD4+ T cells, improves, although it still does not reach normal levels. This might be an expression of the ongoing low-level replication of HIV despite HAART. Toxicity of the drugs used is frequently reported. Longer-term results show an increasing failure of treatment with an increasing number of HAART regimen changes. The implication might be that, apart from resistance, transmission of HIV will increase, which together with the HIV-infected individuals entering from endemic areas will add to the total number of infected patients in the Netherlands.

6.3 Recommendations

Although we were able to describe the HIV-infected population in the Netherlands up to and including the first quarter of 2002, it appears to be less feasible to answer a number of important questions. These questions regard the extent to which resistance develops, toxicity and adverse events occur, and salvage therapy is feasible and effective in the growing group of patients with multiple failures on HAART. Resistance data are still limited and data on toxicity and adverse events are only partly linked to the antiretroviral drugs used. Results on the effect of salvage therapy after multiple failures are to be awaited, as the follow-up period of the HAART-treated population is still too short.

Based on the findings of the present report it is recommended to initiate the following projects in 2003 through the framework of the HIV Monitoring Foundation:

- Improved registration of resistance combined with data on adherence and plasma drug levels:
  - Active measurement of resistance of HIV a) at entry for every newly diagnosed HIV infection and b) at virological therapy failure. Initiation and support of an organisational structure among the HIV Treatment Centres for adequate storage of patient material to perform resistance measurements and for the collection, storage and analysis of resistance data. The HIV Monitoring Foundation will coordinate this part of the project, in collaboration with Dr Rob Schuurman and Dr Charles Boucher of the Department of Virology of the University Medical Centre in Utrecht.
  - Active measurement of adherence in relation to patterns of antiretroviral drug use in a subgroup of patients, in addition to self-reported adherence. A proposal for this project will be prepared by the HIV Monitoring Foundation in collaboration with Professor Roy Anderson of the Department of Infectious Disease Epidemiology of the Imperial College in London.
  - Structured collection of data regarding plasma levels of antiretroviral drugs. A proposal for collecting plasma level data will be prepared in collaboration with Dr David Burger of the Department of Pharmacology in Nijmegen and professor Jos Beijnen of the Slotervaart Hospital in Amsterdam.
- Improved registration of toxicity of the various drugs used in HAART combinations and of the frequency of therapy changes or interruption and improved registration of patients on so-called salvage therapy. At present, due to the way in which the database is organised, the available toxicity data are too limited to recognise antiretroviral-drug-related toxicity, in particular over a prolonged period of time. These projects will be performed in collaboration with Dr Peter Reiss of the Department of Internal Medicine of the Academic Medical Centre in Amsterdam, Dr Jens Lundgren, coordinator of the international DAD study, Dr Bruno Ledergerber of the Swiss Clinical Cohort Study, and Professor Andrew Phillips of the University College in London, within the framework of the international PLATO collaboration.
- Improved registration of non-treated and non-HAART-treated patients.
- Continuation of the inclusion of treated and untreated patients from non-western, endemic areas and improved registration of HIV-1 subtypes. This project will be coordinated in collaboration with Dr Jan Prins, Dr Ineke van der Ende and Dr Margriet Schneider of the departments of Internal Medicine of the AMC in Amsterdam, the UMCU in Utrecht and the EMC in Rotterdam, respectively.

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