



Ph.D. Thesis

HIV in Denmark and Greenland, 1995-2004:
The effect of highly active antiretroviral therapy and characteristics of
the HIV-infected population: An observational study

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This thesis is based on the following papers:

- I. Lohse N, Hansen AB, Jensen-Fangel S, Kronborg G, Kvinesdal B, Pedersen C, Larsen CS, Moller A, Willumsen L, Obel N. Demographics of HIV-1 infection in Denmark: results from the Danish HIV Cohort Study. *Scand J Infect Dis.* 2005;37(5):338-43.
- II. Lohse N, Ladefoged K, Pedersen L, Jensen-Fangel S, Sorensen HT, Obel N. Low effectiveness of highly active antiretroviral therapy and high mortality in the Greenland HIV-infected population. *Scand J Infect Dis.* 2004;36(10):738-42.
- III. Lohse N, Kronborg G, Gerstoft J, Larsen CS, Pedersen G, Pedersen C, Sorensen HT, Obel N. Virological control during the first 6-18 months after initiating highly active antiretroviral therapy as a predictor for outcome in HIV-infected patients: a Danish, population-based, 6-year follow-up study. *Clin Infect Dis.* 2006 Jan 1;42(1):136-44.
- IV. Lohse N, Obel N, Kronborg G, Laursen A, Pedersen C, Larsen CS, Kvinesdal B, Sorensen HT, Gerstoft J. Declining risk of triple-class antiretroviral drug failure in Danish HIV-infected individuals. *AIDS.* 2005 May 20;19(8):815-22.
- V. Lohse N, Obel N, Kronborg G, Jørgensen LB, Pedersen C, Larsen CS, Kvinesdal B, Sørensen HT, Gerstoft J. Declining prevalence of HIV-infected individuals at risk of transmitting drug-resistant HIV in Denmark during 1997-2004. *Antiviral Therapy*, *accepted for publication.*

Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral treatment
CD4	Cluster of differentiation 4
CRS	Danish Civil Registration System
DHCS	Danish HIV Cohort Study
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
IQR	Interquartile range
IR	Incidence rate
IRR	Incidence rate ratio
MR	Mortality rate
MRR	Mortality rate ratio
MSM	Men who have sex with men
NNRTI	Non-nucleoside analogue reverse transcriptase inhibitor
NRTI	Nucleoside analogue reverse transcriptase inhibitor
PI	Protease inhibitor
PYR	Person-years at risk
RCT	Randomized controlled trial
RNA	Ribonucleic acid
SMR	Standardized mortality ratio
SSI	Statens Serum Institut
STI	Structured treatment interruption
TCF	Triple-drug class failure
TI	Treatment interruption
VL	Viral load

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1. Preface

This thesis is based on five original research papers. As the thesis aims to supplement rather than replicate the original work, I have tried not to repeat too many details from the papers. The *Comments* sections contain additional analyses and methodological considerations specific to each paper.

2. Introduction

2.1. HIV surveillance in Denmark and Greenland

The first cases of acquired immunodeficiency syndrome (AIDS) were described in the USA in 1981 (1;2) and shortly thereafter in Denmark and Greenland (3-8). A few years later, the disease was linked to the human immunodeficiency virus (HIV) (9). Since then, HIV and AIDS have been under epidemiological surveillance by the Statens Serum Institut (SSI), Copenhagen (10;11) and the Chief Medical Officer in Greenland (12). The surveillance system initially was based on notification of new AIDS cases (starting in 1983), and anonymous notification of new HIV cases (starting in August 1990). The dramatic decline in the rate of AIDS diagnosis in recent years has diminished the value of this system as a surveillance tool (13). To better monitor the incidence and prevalence of the disease, date of birth and a Soundex code have been used to register new HIV cases since 1 January 2005 (14). This approach permits identification of double registrations while preserving individuals' anonymity.

2.2. Creation of an observational cohort

The HIV Cohort Study in Western Denmark was established in 1998, as an observational database for HIV research. The study collected prospective data on HIV-infected individuals from all clinics treating HIV in Western Denmark (Jutland and Funen counties). Its database included information retrieved from patient files from 1995 on. Besides its primary purpose of answering scientific questions about treatment with antiretroviral therapy (ART) (15-17), the study provided an additional valuable tool for disease surveillance (18). The study database had several advantages over the national surveillance system, *i.e.*, avoiding double registrations, recording emigration, and documenting deaths among HIV patients who have not developed AIDS. Since 2003 (when the research leading to this thesis began), the database has been expanded, and now encompasses HIV clinics throughout Denmark.

2.3 Therapeutic regimens

Highly effective antiretroviral treatment combinations (highly active ART, HAART) that stemmed the AIDS epidemic in Denmark and Greenland and throughout the Western world (19-22) were introduced in 1995-1996 (16). The first HAART regimens included one protease inhibitor (PI) and two nucleoside analogue reverse transcriptase inhibitors (NRTI). In later HAART regimens, the "backbone" of two NRTIs has been combined with other "third drugs", either a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI), a third NRTI, or a so-called "boosted PI", which is a PI supplemented by concomitantly administered ritonavir. Ritonavir – a PI itself – boosts the PI by inhibiting the cytochrome P-450 enzyme CYP3A4, thereby reducing the metabolism of the PI and leading to higher bioavailability of the drug (23;24). While other drug class combinations have been used as HAART, those mentioned above are the most common.

2.4. Treatment guidelines

As new treatment modalities emerged and therapeutic experience accrued, treatment guidelines have changed (25-38). Boosted PI regimens were shown to be superior to unboosted PI regimens (39-41), which are rarely used any more. The once promising triple-NRTI combinations turned out to be less effective than NNRTI- and PI-based regimens and have been phased out as a first-line treatment option except in rare individual cases (42-46). After dominating as the first-line therapy in Denmark in 1996-1997, unboosted PI regimens were gradually replaced by boosted PI regimens and NNRTI regimens in 1998-2000, and from 2001 on, 70-80% of patients initiating therapy received NNRTI-based HAART (47). Current Danish national guidelines recommend a first-line HAART regimen based on the NNRTI efavirenz, or, alternatively, a regimen based on a boosted PI or the NNRTI nevirapine (25). While Greenland follows the Danish guidelines, its geography, climate, culture, and health care system organization may hamper treatment of a chronic disease like HIV.

2.5. Direct effect of antiretroviral therapy

The direct effect of antiretroviral drugs is impairment of HIV replication, thereby lowering the plasma HIV RNA (viral load, VL) (48) to undetectable levels within weeks. This is followed by gradual restoration of immune function, measured by an increase in plasma CD4+ T-cell counts (CD4 count) that may continue for years (49) if VL remains fully suppressed. The dramatic decline in the risk of disease progression to AIDS and death observed in HIV-infected populations with access to therapy in the late 1990s (11;20;50;51) accords with subsequent findings that the most current CD4 count measurement is a strong predictor of short-term prognosis among patients on HAART (52-54).

2.6. Managing and monitoring antiretroviral therapy

2.6.1 Daily clinical treatment goals

The dramatic reduction in mortality has refocused treatment goals on maintenance of therapeutic *effectiveness* (a measure of the extent to which a health care intervention fulfills its objectives (55)). This raises the following questions: When is the best time to initiate therapy in relation to CD4 count, viral load, disease progression stage, and symptoms (56-58)? What initial combination regimen is optimal in terms of potency, tolerability, safety, risk of resistance development, available second line combinations when needed, and pill burden (57-59)? How do we manage and monitor life-long treatment (57;58;60)? VL is a predictor of disease progression in people on antiretroviral therapy (52-54;61-64), and a decrease in VL leads to a subsequent rise in CD4 count. As VL is easily and quickly measured, the primary goal in daily practice is to obtain an undetectable VL. Failure to fully suppress VL, despite complete drug-taking compliance, can be caused by low drug bioavailability (65;66) or a low-potency regimen (67;68). However, the most important factor seems to be adherence to therapy, with intake of more than 95% of prescribed doses necessary to obtain maximum clinical effectiveness (69-76).

2.6.2. Limits of long-term effectiveness of antiretroviral therapy

Incomplete virological suppression despite antiretroviral treatment may result in emergence of drug-resistant virus strains (76-82), whereas complete suppression is a sign of inhibited viral replication and is associated with a very low rate of new resistance mutations (close to zero) (83-87). The presence of a drug-resistant virus may result in a further increase in VL (virological failure). When virological failure occurs on the first-line regimen, a second-line regimen is chosen according to resistance tests and treatment history (35;36;88;89), but for those who have failed two or more regimens the number of available new drug combinations may be limited (90). The estimated prevalence of individuals harbouring drug-resistant HIV may increase with time on HAART (91), and it is above 75% in some populations (92;93). If individuals carrying one-drug- or multi-drug-resistant virus engage in unsafe practices, they may transmit the resistant virus. The recipients then will be infected with resistant HIV, limiting even their initial treatment options. Whether these effects indicate a threatening vicious cycle remains to be answered. In addition to development of drug resistance, use of antiretroviral drugs for several years may be associated with long-term drug toxicities, particularly metabolic abnormalities leading to lipodystrophy, hyperlipidemia, and increased risk of ischemic heart disease (38;94-96).

2.6.3. Interruption of antiretroviral therapy

One way to reduce accumulated drug intake has been to introduce structured treatment interruptions (STI): patients interrupt antiretroviral therapy when the status of the immune system does not confer an immediate risk of disease progression or death. They resume treatment at a pre-specified time (timed-cycle STI), or when the CD4 count reaches a pre-set lower limit (CD4-guided STI). It has been common practice in many treatment centers outside Denmark to recommend treatment interruptions (TI) to selected patient groups, even though there has been no strong evidence about the safety of the practice. A number of randomized and observational studies have addressed the problem, but a 2005 Cochrane review reported that evidence in support of STI was inconclusive (97). Recently, more definitive evidence was provided by the SMART trial, which included almost 5,500 patients randomized to either continuous treatment or to CD4-guided STI (98). In January 2006 the study was terminated early because patients in the STI arm had twice the risk of disease progression or death – the major study outcome (99). Interestingly, the increased risk was not confined to patients with low CD4 counts (oral presentation by El Sadr at the 13th Conference on Retroviruses and Opportunistic Infections in 2006, unpublished). This observation may challenge the established belief that disease progression is exclusively driven by immune deficiency, as measured by the CD4 count.

3. Aims

This thesis has the following aims:

- I. To estimate the annual number of new HIV-infections in Denmark and the annual number of deaths in the HIV-infected population. Further, to describe temporal trends among newly HIV-infected individuals in Denmark with special emphasis on race, route of transmission, age, and immunological status at time of diagnosis.
- II. To describe the demographic characteristics of the HIV-infected population in Greenland, and to examine the impact of HAART on immune status and mortality in this population.
- III. To examine the level of virological control during the period following HAART initiation as a predictor for long-term viral suppression, increases in CD4 counts, and mortality.
- IV. To examine the incidence, prevalence, and predictors of triple-class antiretroviral drug failure in the Danish HIV-infected population.
- V. To estimate acquisition of antiretroviral drug-resistant virus in the Danish HIV-infected population over time, and to examine temporal trends in the prevalence of patients at risk of transmitting drug-resistant HIV.

4. Methodological considerations

4.1. Cohort studies

A *cohort* is a group of individuals who are followed over a period of time (100). A cohort study may be *experimental*, for example a clinical trial, or *non-experimental* (synonymous with *observational study*). The Danish HIV Cohort Study (DHCS) is a non-experimental cohort study (55), and it is *prospective*, because it is assembled in the present and followed into the future (101). Individuals in the cohort compose the *study base*; in DHCS the study base is all HIV-infected persons in Denmark and Greenland. DHCS is *open*, because new individuals join the cohort over time, and it is *population-based*, because it aims to include all HIV patients in the geographic area under study (102). Outcomes in one cohort (*e.g.*, HIV patients) may be compared with outcomes in another cohort (*e.g.*, the general population) in a *double-cohort study* (Paper II) (103).

4.2. Outcome in epidemiological studies

The events of primary interest in clinical epidemiology are health *outcomes*, for example death, disease (*e.g.*, AIDS-defining illnesses), abnormal laboratory tests (low CD4 count, high VL, drug resistance), or discomfort (*e.g.*, lipodystrophy and other side effects) (101). Some outcomes are *surrogate measures* of the outcome of interest; low CD4 count and high VL are surrogate measures of clinical disease progression (50), and high VL is also a surrogate measure of increased infectivity (104-107)). Use of surrogate outcome measures saves time and money, but to have validity they must be strongly associated with the main health outcome of interest. CD4 count and VL are long-established measures of disease progression to AIDS or death. (50).

4.3 Descriptive and analytic studies

Observational studies can be *descriptive* or *analytic*, or both. A descriptive study examines patterns of health conditions in persons, places, and over time. In Papers I, II, and V, the prevalence of health outcomes was measured at pre-defined points in time to provide descriptive information on temporal trends. Analytic studies test one or more specific hypotheses, typically whether exposure to a given factor is a *risk factor* for a health outcome. Such exposure is a risk factor if exposed persons subsequently experience a particular outcome more often than similar people who are not exposed. As presence of the risk factor predicts the outcome, the risk factor is a *predictor*. However, while the term “risk factor” implies some degree of causality with an outcome, a “predictor” is a broader term that may not have a causal connection to the outcome (*e.g.*, yellow fingers are a predictor for development of lung cancer). Papers II-IV were mainly analytic studies. In Paper II, we used a double-cohort study design to compare the mortality of people who were “exposed” (infected with HIV) with mortality in people who were “unexposed” (the general population). In Papers III and IV we examined predictors of death, immunosuppression, virological suppression, and virological failure within a cohort in which everyone was infected with HIV. However, the distinction between descriptive and analytic studies is one of intent, objective, and approach, rather than one of design. Data obtained in an

analytic study may be explored in a descriptive mode, and data obtained in a descriptive study can be analyzed to test hypotheses. For example in Paper V, a primarily descriptive study, we applied an analytic approach to examine causes of the observed temporal trends in the prevalence of drug resistance carriers.

4.4. Measures of frequency and effect

In an epidemiological study, the key clinically relevant measures of event frequency are *incidence* and *prevalence* (101). Incidence is defined as the fraction of a group that develops a condition (an outcome) over a given period of time. Incidence is often reported per unit of time, as an *incidence rate* (IR). Prevalence is the fraction of a group possessing a condition at a given point in time. The prevalence depends both on the incidence and the duration of the condition. In a steady state, prevalence equals “IR x duration”. *Risk* is the probability that an event will occur in an individual during the observation period (100). *Incidence proportion* is used as the equivalent measure for a population and approximates “IR x time”. Measures of frequency – most commonly the risk and the incidence rate – can be compared to assess the effect of an exposure. The absolute effect is measured as the *risk difference*, *i.e.*, the difference in risk between the exposed and unexposed groups. The *incidence rate difference* can be calculated in a similar fashion. The relative effect is measured as a *relative risk*, *incidence rate ratio* (IRR), or – when the frequency measure is prevalence – as a *prevalence ratio* or *odds ratio*.

4.5. Random and systematic error

An observed outcome may be affected by random error or systematic error (*bias*) (100). Random error is due to chance, and its estimated magnitude is presented as confidence intervals and p-values in the statistical analysis. Bias can arise from the way people are selected into a study (*selection bias*), the way the variables are measured (*information bias*), or from an uncontrolled *confounder* (*confounding bias*). A confounder is an independent risk factor for the outcome of interest, is associated with the exposure, and must not be an intermediate variable. As shown in Table 1, a number of tools are available to deal with bias at the design and analytic stages (108).

Table 1

Tools to minimize different types of bias

Type of Bias	Tools
Selection bias (at the design stage only)	<ul style="list-style-type: none">Selecting only incident casesRestricting identification of incident cases to a given geographical area, to reduce referral biasMinimizing the number lost to follow upImplementing a procedure to track those who drop out
Information bias (at the design stage only)	<ul style="list-style-type: none">Standardizing the measurement processUsing objective, previously defined criteria for defining exposure and disease
Confounding (at the design or the analytic stage)	<ul style="list-style-type: none">RandomizationMatchingExclusionRestrictionStandardizationStratificationMultivariate analysis and modelling

5. Data sources and methods

5.1. Literature search

A versatile approach was used to stay current with literature in the quickly evolving field of HIV research. The most up-to-date and important literature was obtained from the following sources: 1) Regular searches on PubMed (www.pubmed.gov) using combinations of relevant MeSH (Medical Subject Heading) terms, *e.g.*, “HIV”, “AIDS”, “Denmark”, “Greenland”, “Mortality”, “Viral Load”, “CD4 Lymphocyte Count”, “Antiretroviral Agents”, “Treatment Outcome”, “Treatment Failure”, “Drug Resistance”, or “Transmission”; 2) Online links to “Related Articles” for selected records found in PubMed; 3) Reference lists in articles; 4) Regular literature updates from professional websites such as www.amedeo.com, www.clinicaloptions.com, and www.medscape.com; 5) Frequent communication with supervisors and other colleagues working in the fields of clinical epidemiology and HIV clinical care; 6) Participation in international scientific HIV conferences.

5.2. Data sources

5.2.1. Danish HIV Cohort Study

DHCS is a prospective cohort study, initiated in 1998 as the HIV Cohort Study in Western Denmark (18). This population-based study initially included all HIV-infected individuals in the western portion of the country (102), but was expanded by February 2004 to cover all clinics treating HIV in Denmark (Paper I). Data going back to 1 January 1995 were retrieved from patient files and entered into the database. Hence, the database includes all prevalent HIV cases as of 1 January 1995 and all incident cases since then. Types of data collected are comparable to other HIV cohort studies around the world (109-117), namely individual characteristics, biochemical test results, treatment history, and clinical events. DHCS is based at Odense University Hospital, headed by Professor Niels Obel. Physicians and research nurses collect clinical data at the participating clinics. While individual identity is kept anonymous in the database, an identification link exists locally at each participating clinic, to detect double counting when a patient moves between clinics. Cross-checking and validation algorithms are incorporated into the database in order to catch data retrieval and typing errors. In addition, 5-10 percent of records are monitored during annual visits to participating clinics.

5.2.2. DHCS Greenland

A database with the same design as DHCS was established in Greenland in 2003 (Paper II). A concerted effort was made to include every HIV-infected individual seen at Greenland’s clinics since 1995; personal contact was initiated with all 18 district health clinics. In order to retrieve old patient files, we searched the archives of the venereal disease clinic at Dronning Ingrid’s Hospital in Nuuk. This step was taken because HIV patients used to be seen by venereal disease specialists during regular consultations with districts outside of Nuuk. The files thus obtained were compared with the records collected by the Chief Medical Officer of Greenland. This provided presumably complete coverage in the study database of known HIV patients since

1995. Data is now updated through the Department of Internal Medicine at Dronning Ingrid's Hospital, which has assumed responsibility for all HIV treatment in Greenland.

5.2.3. Danish Civil Registration System (CRS)

CRS is a national registry of all residents of Denmark and Greenland, containing information on date of birth, gender, immigration, residency, emigration, and death (118). Each individual is assigned a 10-digit personal identification number (CPR number). CRS is updated within less than a week after a person is born, changes address, dies, or emigrates.

5.3 Methods

5.3.1. Sampling of general population controls

A population control cohort of 100 individuals for each HIV patient in Greenland was sampled from CRS, matched to patients on gender, age, and country of residence at the time of HIV diagnosis. The control cohort was used in a double-cohort study to compare mortality in HIV patients with that in the general population (Paper II).

5.3.2. Definitions

AIDS was defined using the 1993 expanded European AIDS case definition, based on the clinical definition of AIDS developed by the US Centers for Disease Control and Prevention (119;120) (Papers I, III, and IV). AIDS "at the time of diagnosis" was defined as any AIDS-defining illness within 30 days of diagnosis (Paper I). CD4 count or VL "at the time of diagnosis" was the first measurement up to 90 days following diagnosis (Papers I and II). HAART was defined as an antiretroviral regimen containing at least three drugs, including at least one PI, or one NNRTI, or abacavir (Papers II-V). CD4 count "at the time of HAART initiation" was the last measurement within the preceding 90 days or the first measurement up to 90 days after HAART initiation (Papers III and IV). VL "at the time of HAART initiation" was the last measurement within the preceding 90 days or the first measurement up to 14 days after HAART initiation (Paper IV). Treatment interruptions (Papers III-V) were documented if they lasted longer than 14 days, including temporary interruptions during infections and after pregnancy.

5.3.3. CD4 count and viral load as time-updated variables

Measurements of CD4 count and VL were unevenly spaced over time. To determine CD4 counts between measurements, we used the principle of "last observation carried forward" (Papers II and III). VL between measurements was determined using a conservative approach, depending on the study aim. When low VL (less than 400 copies per ml) was used as a marker of therapeutic success (Paper II), a missing value was considered low only if the preceding and subsequent values were both low. When high VL (above 1,000 copies per ml) was used as a marker of therapeutic failure (Papers IV and V), a missing value was considered high only if the preceding and subsequent values were both high. For studies in which we wished to determine

the exact VL at a given time point (Papers III and V), we developed a model in which each measured value was carried forward for 30 days or until the next measurement, whichever came first, and extrapolated back to 30 days following the previous measurement. This model presumes that a viral load tends to reflect the past more than the future.

5.3.4. Modelling of viral load as a proxy for acquired resistance and transmissibility

A high VL, defined as >1,000 copies per ml for 120 days during administration of antiretroviral drugs, was used to characterize prolonged virological failure and served as a proxy for acquired drug resistance (Papers IV and V) (63). A current high VL (>1,000 copies per ml) was used as a proxy for HIV transmissibility (Paper V).

5.3.5. Comparing individual characteristics

The distribution of individual characteristics between study groups was compared using the chi-square test for categorical variables (Papers III and V), and the Student's t-test (Paper III) or one-way analysis of variance (Paper III) for continuous variables.

5.3.6. Comparing study outcomes

Several statistical methods were employed to compare outcomes over time: Spearman's rank correlation and linear regression for examining changes in VL, CD4 count, and age (Papers I and III); Poisson regression for estimating incidence rate ratios and prevalence ratios (Papers I, IV, and V); time-to-event analyses (time to death and time to triple-class antiretroviral drug failure (TCF)) for estimating mortality rates (MR) (Papers II and III); incidence rates of TCF (Paper IV); and cumulative incidence proportions. The log-rank test and Cox proportional hazards regression were used for comparisons across groups. Proportions with undetectable VL were compared by logistic regression (Paper III). A Cox regression model with left truncation was used to compare incidence rates on two different timelines (time on HAART and calendar time) (Paper IV).

5.3.7. Confounder control

A number of the strategies listed in Table 1 were used to maximize control of confounding: matching in design (Paper II), restriction in design (Papers III-V), restriction in analysis (Papers III and V), stratification (Papers II-IV), and multivariate modelling (Papers II-IV). To control for confounding in the regression models, we initially performed unadjusted analyses (Papers II-IV). All variables were then examined individually in stratified analyses to assess their effect on the risk estimates (Papers II-IV) (121). If effect modification was detected, analytic results were presented for separate strata of those variables (Papers II and IV). Some variables were then forced into the multivariate models (Papers III and IV), based on their importance in the literature. Generally, the remaining variables were included in the final model only if they caused more than 10% change in the exposure effect (122).

6. The use of observational cohorts in HIV epidemiology

6.1. Primary applications of HIV observational cohorts

During the last 25 years, an unprecedented effort has been put forward to combat HIV. For example, in 1997 the U.S. Food and Drug Administration approved the use of surrogate markers (50) to measure clinical progression in randomized controlled trials (RCT, an experimental cohort study) for the first time, in part to speed up licensing of new antiviral compounds. RCTs are considered the gold standard for comparing the *efficacy* (a measure of the extent to which a specific health care intervention produces a beneficial result under ideal conditions (55)) of drugs and other treatments. Their results often pertain to a closely monitored population recruited using sometimes strict inclusion criteria, and their subjects do not always resemble the average population with the disease. Furthermore, the questions an RCT was designed to address often cannot be answered until years after trial initiation, and by that time the conclusions of even an elegantly designed RCT may be outdated (123). Parallel to RCTs, numerous clinical databases have been set up to study the course of HIV. These non-experimental cohort studies may be the best alternative when an RCT is not possible (124;125). They also confer a number of distinct advantages, *e.g.*, providing information on the clinical history and spectrum of HIV disease and an opportunity to examine questions as they crop up (126). Finally, in contrast to the efficacy examined by RCTs, observational studies shed light on the effectiveness of treatment. However, the databases established to study HIV have limitations (127), including some of the biases mentioned above.

6.2. Bias in selection

Selection bias may occur if groups of subjects characterized by an unusual and unequal relationship between exposure and outcome are selectively recruited into the study, or drop out before completion. Many HIV observational cohorts are “center-based cohorts”, in which patients are recruited from only one or a few HIV clinic(s). Even large clinics may have self-selected clientele, *i.e.* around larger cities where there are several clinics to choose from, or in cases where a clinic tends to serve a particular area or neighbourhood (as in Copenhagen, Paper I). In some settings, HIV patients are followed by their general practitioner or by an internist in a private clinic, and only those patients with complicated or advanced disease are referred to hospital clinics. In many cohorts, requirements for informed consent lead to selective recruitment. In addition, center-based cohorts may have a high dropout rate: patients enter a study when they first present for treatment, but later withdraw their consent or become lost to follow up. This problem often becomes more pronounced with increasing observation time. While the large Swiss HIV Cohort Study includes more than 13,500 individuals, 26% were lost to follow-up between 1988 and 2005 and it is estimated that 52% of all HIV patients under care in the country are not registered in the study (111). A patient who does not keep a regular visit at the HIV clinic, and is therefore considered lost to follow up, may not have moved from the area; rather, the patient may be hospitalised elsewhere for a non-HIV- related condition or may have died. This type of selection bias, in which censoring is associated with the outcome (*e.g.*, death),

is called *informative censoring* in survival analysis. Selection bias in DHCS was minimized by all of the four tools described in Table 1.

6.3 Information bias

Information bias may occur if the methods of measurements are consistently dissimilar in different groups of patients (101). Information in DHCS, and in most HIV observational cohorts, is retrieved from patient files. That most exposures (*e.g.*, antiretroviral drug intake) and outcomes (*e.g.*, AIDS-defining events, deaths, and laboratory test results) are defined from objective criteria will tend to minimize information bias (Table 1). However, some data depend on the patients' own information and are more prone to cause information bias (*e.g.*, an injection drug user or a homosexual may state heterosexual transmission, in order to avoid stigmatization).

6.4. Prevalent cohorts

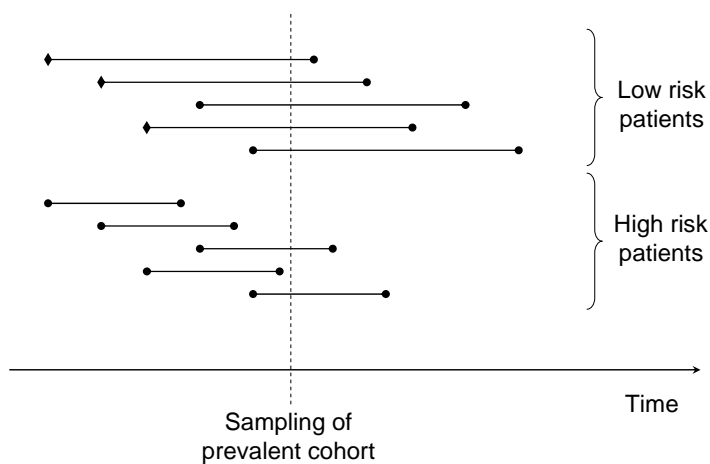
The starting point for inclusion in a cohort is also important (100;101;128;129). In an *inception cohort*, all individuals are followed from an initiating event, (*e.g.*, the date of infection with HIV). In a *prevalent cohort*, patients are included at some time point after the initiating event, but before the outcome of interest (*e.g.*, death). If the initiating event is HIV transmission, an inception cohort must include patients at the time of seroconversion. Such cohorts provide the best tool for studying the natural history of HIV disease, and for clarifying such issues as the optimal time to initiate HAART. A number of seroconversion cohorts exist (130-134), but none are geographically complete, as it is rare to know the seroconversion date. DHCS may be considered both as an inception and a prevalence cohort. If the initiating event is defined as diagnosis of HIV, DHCS comes close to being an inception cohort for patients diagnosed since 1995. If the initiating event is defined as initiation of HAART, DHCS is an inception cohort for patients initiating HAART in Denmark. If the initiating event is defined as HIV transmission, DHCS is a prevalent cohort.

6.5. Bias in prevalent cohorts

The chance of being included in a prevalent cohort reflects the duration as well as the incidence of the condition of interest. Prevalent cohorts are therefore subject to *length-biased sampling* (100;101). Patients at increased risk of death will have shorter disease duration between HIV infection and death and will therefore be underrepresented in the prevalent sample (Figure 1). Another type of bias, *onset confounding*, arises when a covariate is associated with the initiating event (*e.g.*, the date of infection with HIV). If a covariate is associated with earlier infection dates (*e.g.*, being a male homosexual), individuals with this covariate will have longer infection times, causing the covariate to appear associated with any outcome dependent on time from infection (*e.g.*, the risk of dying) (Figure 1). Results may be biased in both directions depending on the direction of the effect of the covariate. *Differential length-biased sampling* (128;129) may occur if the risk of death increases (or decreases) with the duration of the infection. Patients with a covariate that increases the risk of death tend to have a shorter prior duration of infection than

patients without the covariate. Low-risk patients thus will be infected for a longer time, causing them to have more advanced disease and therefore an increased risk of death. These countervailing factors reduce the disparity in risk between the two groups, biasing the relative risk estimate towards 1.0. In contrast, if the risk of death decreases with the duration of the infection, the relative risk estimate will be biased away from 1.0 (135). Risk of bias need not imply presence of bias, however. In one cohort of HIV patients, onset bias was identified but there was no evidence of differential length bias (129).

Figure 1



Inclusion of patients in a prevalent cohort. Each horizontal line represents one patient from the time of infection with HIV until death. Length-biased sampling is illustrated as follows: The high-risk patients and low-risk patients have the same incidence of acquiring HIV, but the low-risk patients live longer, and are therefore more likely to be included in the cohort at the time of sampling (5 vs. 2 patients). Onset confounding is illustrated as follows: If a covariate is associated with the date of infection, patients with earlier infection dates (marked with diamonds in low-risk patients) will have longer infection times, and the covariate will seem to be associated with the risk of dying.

6.6. Confounding by indication

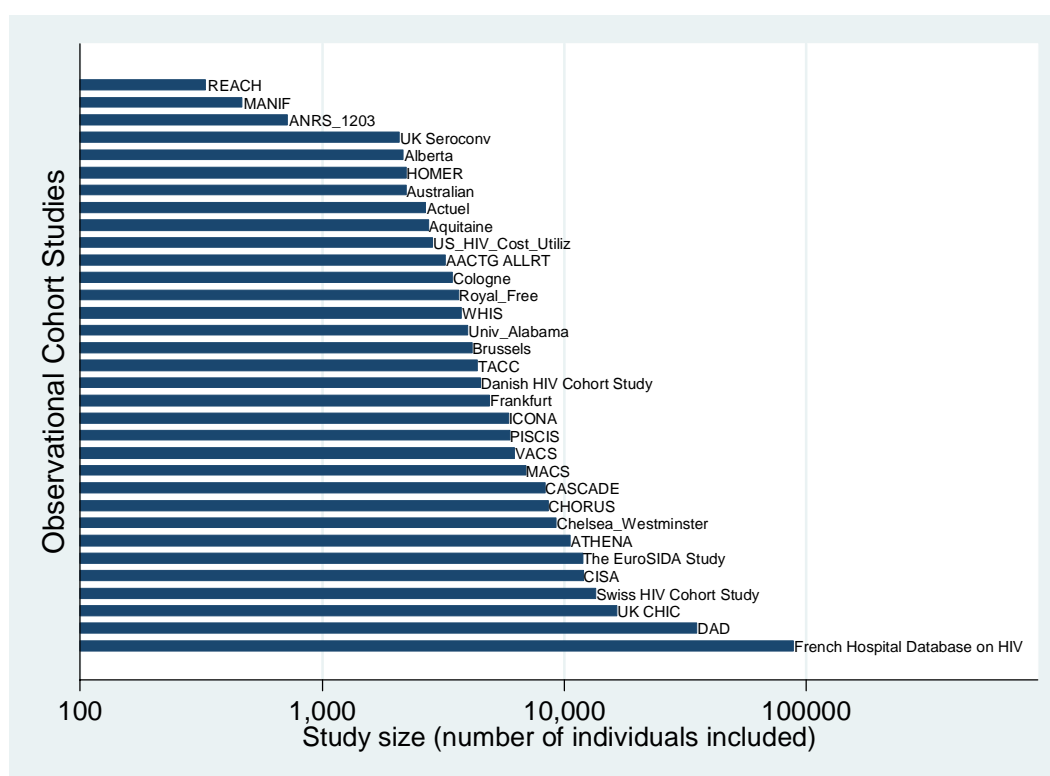
Observational studies are useful for exploring patterns of antiretroviral drug use (16) and monitoring the course of side effects (117;136). However, when their purpose is to examine and compare the effectiveness of different drugs (136), they are vulnerable to *confounding by indication*. This type of confounding may occur if an indication for a given treatment is associated with the outcome (137). As only a few available drug combinations have been compared in RCTs, it is tempting to compare regimens in observational cohorts. However, in such cohorts the chance of receiving a particular drug is not random, because physicians have a reason for prescribing a particular drug to a given patient. The indications for particular regimens change over time, and there are inter-cohort differences in use of different antiretroviral combination regimens (138). Thus, differences observed in observational studies (139-141) may not be confirmed in a subsequent RCT (142). Although hypotheses can be generated by

comparing effectiveness of different drugs in observational studies, care is needed in drawing conclusions.

6.7. DHCS and other HIV observational cohorts

The existence of numerous observational HIV cohorts has created a competitive research environment, resulting in a high number of excellent research papers. DHCS is one of many medium-sized cohorts (Figure 2), and its scientific aims must comply with its particular strengths – mainly complete geographical inclusion and a very low rate of loss to follow up (Papers III-V).

Figure 2



Study size of selected HIV observational cohorts.

A number of other cohorts cover well-defined geographic areas (51;109;111-114;143-145), but few are complete within their area. Apart from DHCS, only the Dutch ATHENA observational cohort may partially fulfil this criterion (145). Advantages of DHCS include the ability to study population-based prevalences (Papers IV and V) and incidences (Paper IV), as well as population trends after long-term use of HAART (Paper III). Naturally, the DHCS is ideal for examining questions of local interest in Denmark and Greenland (Papers I-II). Furthermore, the availability of numerous Danish registries (146) allows comparisons with the general population (Paper II), and linkage (by means of personal identification numbers) between registries containing hospital discharge diagnoses, cancer diagnoses, psychiatric diagnoses, causes of death, socio-economic factors, etc. The size of DHCS limits studies of rare events (*e.g.*,

leishmaniasis) or subgroups with rare characteristics (*e.g.*, patients infected with HIV type-2). International collaborations are more suited to answer scientific questions requiring larger numbers of patients and/or outcomes than a single cohort can provide (117;147). In this context, the current COHERE initiative, with nearly 250,000 HIV patients under observation, may become an invaluable scientific instrument. It is also important to note that results obtained in Denmark may not be generalizable to other countries because of regional differences in the composition of study populations (138;148). Finally, other cohorts may include data that are not collected in DHCS. DHCS does not contain detailed information on antiretroviral drug use prior to HAART (only the date when NRTI mono- or dual therapy was initiated), information on adherence is not collected systematically, and smoking status and blood pressure – important cardiovascular risk factors – have only recently been included as variables for routine data collection.

7. Demographics of HIV-1 infection in Denmark: results from the Danish HIV Cohort Study (Paper I)

7.1. Background

When this research was undertaken, the only source of information on new cases of HIV and AIDS in Denmark was the national surveillance system established by the SSI (11). Because this system was based on anonymous notification of HIV, deaths could be tracked only among patients with an AIDS-defining illness. As improved treatment options led to an increasing proportion of patients dying without having developed AIDS (149;150), SSI's death notifications became inaccurate estimates of total deaths among HIV-infected individuals. Furthermore, health policymakers in Denmark were concerned about a number of circumstances that could potentially worsen the AIDS epidemic: immigration from areas with a high prevalence of HIV-infection and different health-care seeking behaviour; increased risk behaviour among high-risk groups, particularly male homosexuals; and the lack of prevention campaigns aimed towards young people. The SSI system was unable to provide information on disease progression stage at the time of diagnosis and the number treated with antiretroviral drugs. The number of people living with HIV could be estimated only roughly from national surveillance data. We therefore decided to use DHCS to estimate the annual number of new HIV infections in Denmark and deaths in the HIV-infected population. We further aimed to describe temporal trends among newly HIV-infected individuals, with special emphasis on race, route of transmission, age, and immunological status at time of diagnosis.

7.2. Methods

Using data from DHCS, we summarized information on new HIV infections and deaths for each calendar year and examined temporal trends in individual characteristics using Poisson regression and linear regression.

7.3. Main results

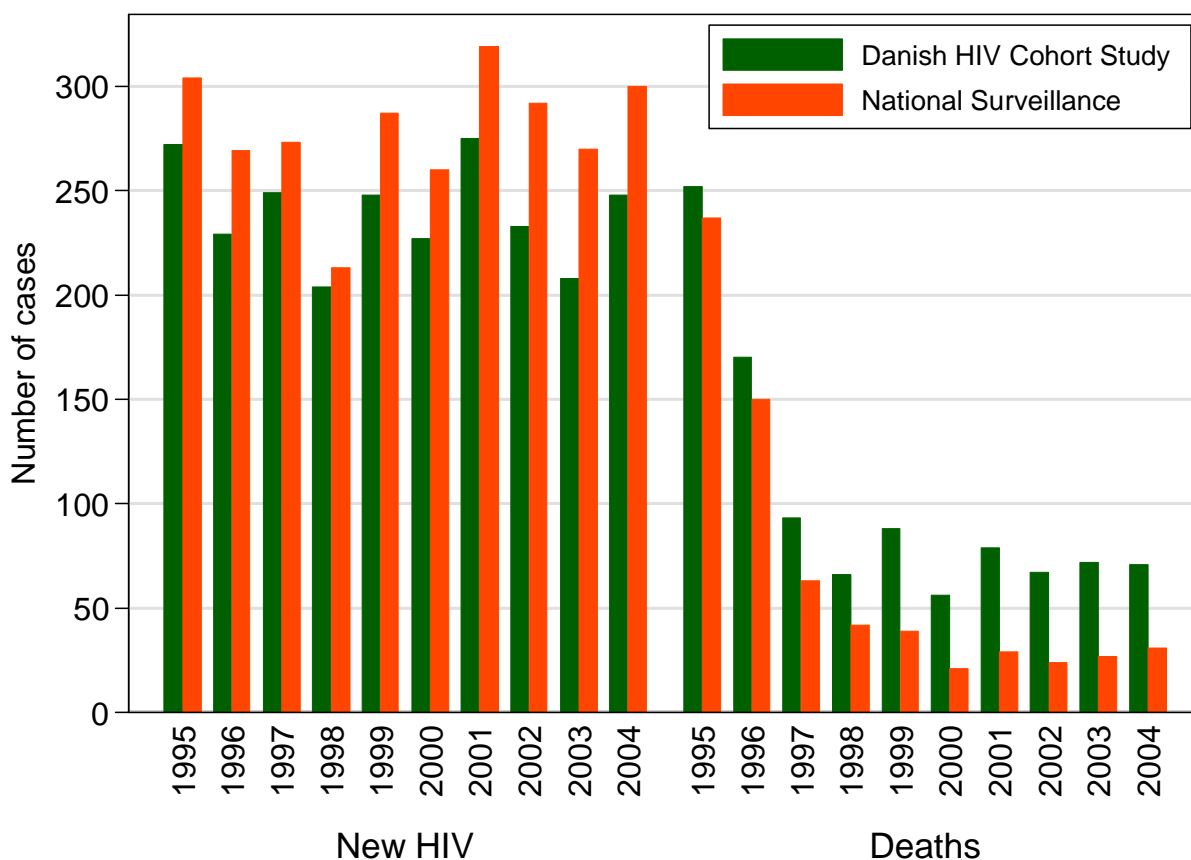
We estimated that 3,037 adult patients (>15 years old) with a known HIV infection were living in Denmark at the end of 2003, yielding a prevalence of 70 patients per 100,000 population. We observed no increase in the annual number of new HIV cases between 1995 and 2003 (median: 231). During the same period, the number of deaths initially decreased and then became stable from 1998 to 2003. The proportion of black Africans among newly infected patients did not increase, and the absolute number decreased. Among individuals born in Denmark, just less than half had acquired HIV through male homosexual activity. This was the most common risk factor, with no annual trend observed. The average age at HIV diagnosis increased from 33.1 years in 1995 to 38.7 years in 2003, and we saw a concomitant decrease in both the absolute number and the proportion (from 11.8% to 5.2%) of individuals aged under 25 at time of diagnosis. The prevalence of markers of advanced HIV disease was stable during the observation period: 38%-44% of cases had a low CD4 count (less than 200 cells pr μ l), 69%-84% had a high VL (more

than 10,000 copies per ml), and 11.4%-17.5% were diagnosed with AIDS within 30 days of being diagnosed with HIV.

7.4. Corrections

Continuous minor adjustments are made in the DHCS database in the course of ongoing data validation and monitoring. In most instances this is a positive reflection of our quality control efforts. Through this process, however, two significant errors have been detected. In the first instance, medical records of 95 individuals who died in 1995 were found after the completion of Paper I. The number of deaths in 1995 thereby increased from 166 to 251, as shown in Figure 3. The second instance involved an initiative to reanalyze laboratory specimens obtained from

Figure 3



Annual incidence of HIV diagnoses and deaths among HIV patients in Denmark. Comparison of registrations in the Danish HIV Cohort Study (DHCS) and in the National Surveillance System at the Statens Serum Institut. Deaths registered in the National Surveillance System include only deaths of patients with a previous AIDS diagnosis, while deaths registered in DHCS comprise deaths of patients with HIV, with or without a previous AIDS diagnosis.

individuals presumed to be infected with HIV type-2, using a better assay. Of the specimens from the 10 HIV type-1 and HIV type-2 coinfecting patients under study, four were not reanalysed, three were found to be monoinfected with HIV type-1, two were monoinfected with

HIV type-2, and possible coinfection was observed in just one patient (although cross-reactivity between HIV type-1 and type-2 could not be excluded). Of the specimens from the six HIV type-2 monoinfected patients under study, one was not reanalyzed, and five were confirmed to be monoinfected with HIV type-2.

7.5. Comments

In Figure 3, registrations of new HIV infections and deaths in DHCS are compared with those documented by SSI (11;151). As expected, SSI reports a larger number of new HIV infections than DHCS, most likely due to inclusion of patients diagnosed outside specialized HIV treatment centres and an inability to remove duplicate registrations because of the anonymity rule. The strikingly larger number of deaths registered in DHCS is also not surprising. This reflects the difference between deaths of patients with a previous AIDS-defining illness (as reported by SSI), and deaths of patients with HIV regardless of AIDS status (as in DHCS). Thus, until SSI has fully implemented the Soundex code system (14) in the National Surveillance System, the Danish HIV Cohort Study will remain a useful supplementary surveillance tool. However, it is important to note that data in DHCS is updated just once a year, so the most recent information may be as much as 15 months old.

8. Low effectiveness of highly active antiretroviral therapy and high mortality in the Greenland HIV-infected population (Paper II)

8.1. Background

Epidemiological studies of HIV in Greenland in the 1980s and 1990s focused on the prevalence, incidence, and geographical distribution of the disease (7;152-154). During the initial years of the epidemic, HIV was diagnosed in 11 of 16 health districts, but from the mid-1990s on, it was predominantly found in the two largest towns, Nuuk and Sisimiut. While the prevalence of HIV in the population was found to be low (155), that of other sexually transmitted diseases was high (10). Sexual activity without barrier protection was common among teenagers (156-158), creating the potential for rapid spread of HIV among young people. However, the majority of HIV-infected persons were middle-aged, burdened by unemployment, alcoholism and social problems, and most likely infected through heterosexual activity (154). Antiretroviral therapy had been available in Greenland as long as it had in Denmark, but its impact on Greenland's HIV-infected population remained unknown. We therefore aimed to describe the demographic characteristics of HIV-infected individuals in Greenland and to examine the impact of HAART on immune status and mortality in this population.

8.2. Methods

Using data from DHCS Greenland, we summarized the demographic characteristics of HIV-infected individuals, and compared their mortality with that of population controls using Kaplan-Meier life tables and Cox proportional hazards regression.

8.3. Main results

Among 103 HIV-infected patients in the study cohort, 55 were living in Greenland on 1 March 2004, yielding an adult (>15 years old) prevalence of 133 patients per 100,000 population. The youngest HIV-infected individual was 34.2 years old. In general, most HIV cases were found in the middle-aged (median age: 55.8 years) Inuit population (91%), living around the two largest towns of Nuuk and Sisimiut (85%). The impact of HAART was limited. At any time after initiating HAART, only 40-50% of patients reached the goal of a VL<400 copies per ml, even with a rising CD4 count. The MR was as high as 11.1% per year for HIV-infected individuals on HAART, and the mortality rate ratio (MRR) – using general population controls as the reference – was 10.6. Mortality among patients diagnosed before the HAART era was 2.5 times higher than among those diagnosed after HAART became available.

8.4. Comments

Standardized mortality ratios (SMR) are generally used to compare mortality in observational cohorts with that of the general population. The expected number of deaths is calculated by applying age- and gender-specific mortality rates from the general population to a cohort with the same distribution of age and gender as the “exposed” (diseased) cohort. The SMR is the observed number of deaths divided by the expected number of deaths. In Paper II, a different

approach was taken. Because the Danish Civil Registration System provided a unique opportunity to obtain individual-level survival data from both HIV patients and the general population, the MRR in this matched cohort study could be calculated using product-limit methods. This was convenient, as it allowed us to collect survival data on both patients and controls in the same manner. However, there is no evidence that mortality ratios obtained in this way are more accurate than those obtained using the usual standardization approach. As well, dropouts due to death and loss to follow up may bias outcome estimates (CD4 and VL) in the analyses of longitudinal data. This problem is addressed in the comments on Paper III.

9. Virological control during the first 6-18 months after initiating highly active antiretroviral therapy as a predictor for outcome in HIV-infected patients: a Danish, population-based, 6-year follow-up study (Paper III)

9.1. Background

A number of observational studies have examined the prognostic value of VL after HAART initiation, either at a pre-determined time point, *e.g.*, after six months on HAART (53;159), or by using time-updated values (52;63;64;160-162). A single VL measurement is an independent predictor of disease progression in patients on HAART (although the most recent CD4 count carries much more prognostic information) (54;64). In the HIV clinic setting, however, physicians have access to numerous VL measurements to assess therapeutic effectiveness. In such a series, some patients will show single or alternating episodes of incompletely suppressed VL. No previous studies had examined whether the level of VL suppression during a 12-month period was associated with long-term outcome. We therefore aimed to examine whether virological control during the first 6–18 months after HAART initiation was a predictor for clinical progression 18–90 months later.

9.2. Methods

Patients from DHCS who initiated HAART before 1 January 2002 and were alive 18 months later were divided into three predictor groups: detectable VL ($VL \geq 400$ copies per ml) during 0% of the 6-18 month period following HAART initiation (Group I), 1-99% of the period (Group II), or 100% of the period (Group III). We then examined three outcomes in the subsequent 18-90 month period: the proportion of patients with undetectable VL, CD4 count changes, and mortality, using logistic, linear, and Cox regression, respectively.

9.3. Main Results

We found that 93% of HIV patients with complete virological suppression during the 6-18-month period after initiating HAART could expect to be alive 6 years later, and that 96% of them would have a $VL < 400$ copies per ml at that time. Among HIV patients who did not achieve complete virological suppression during the 6-18 month period after HAART initiation, only 76% would be alive 72 months later, and only 57% of them would have a $VL < 400$ copies per ml 72 at that time. Of special interest was the predictor group with detectable VL during part, but not all, of the 6-18-month period following HAART initiation. This group of patients had a cumulative survival of 86% at 72 months, with 83% having a $VL < 400$ copies per ml. When we examined the possible influence of covariates on the survival estimates in this group, we found that a treatment interruption of at least two weeks was a strong predictor of death. Furthermore, even the lowest quartile of this predictor group (detectable VL during 1-25% of the 12-month period) had markedly higher mortality than the fully suppressed group (adjusted MRR = 2.17, 95% confidence interval 1.31-3.61). Finally, we found that the CD4 count continued to rise in all three predictor groups throughout the 72-month period, to the greatest extent in the group with full virological suppression during the 6-18-month period following HAART initiation.

9.4. Comments

9.4.1. Dropouts

Outcome in follow-up studies is conditional on the patient still being under observation at the time of measuring the outcome, but as surveillance time increases, the risk of dropouts also increases. If dropout patterns are in any way “informative”, *i.e.*, associated with the outcome of interest, the outcome estimates may be biased. There were three distinct reasons for dropouts in Paper III (and in Paper II): end of study period, loss to follow up, and death. Each is discussed below.

End of study period: Our analyses spanned the time period from “initiation of HAART” to “date of last clinic visit”, and since individuals initiated HAART at different dates, dropouts associated with end of study period were distributed throughout the observation period. We assume that this type of dropout is non-informative. Loss to follow up: As mentioned previously, dropouts due to loss to follow-up are likely to be informative. One strength of DHCS is the tiny fraction of individuals who drop out for this reason (3.2% in Paper III, and 1.1% in Paper II). Death: Dropouts due to death are certainly informative in many cases, and affect outcome measures. Examples include analyses of CD4 count progression over time and the proportion of patients with undetectable VL at 72 months. Those with the lowest CD4 count and highest VL are most likely to die before the next measurement.

Advanced statistical models dealing with problems of dropouts in longitudinal data exist (163-167), but they are rarely used in observational studies or RCTs in the HIV field. Many RCTs even suffer from investigator-induced bias, caused when patients who switch their assigned therapy are categorized as treatment failures (168;169). We addressed the problem in two ways in Paper III. When analyzing CD4 count changes between two time points, we calculated individual increases only for those observed at both time points. When analyzing the proportion of patients with undetectable VL during follow up, we multiplied the probability of survival with the proportion with undetectable VL, to obtain a combined and clinically relevant endpoint. The problem was not addressed in the data analysis in Paper II, but was mentioned as a study limitation.

9.4.2. Patients with intermittent viremia and treatment interruption

Although STIs are generally not recommended in Denmark, it is of particular interest to examine reasons for the increased mortality in the TI subgroup. Table 2 shows the characteristics of patients who were only partly virologically suppressed during the inclusion period (Group 2, Paper III), classified according to whether they experienced a TI during their first 18 months on HAART. The TI subgroup had a disproportionate number of intravenous drug users, patients with hepatitis C coinfection, patients who were antiretroviral drug-naïve prior to HAART, patients initiating HAART after 1998, and patients with a lower CD4 count at the time of

HAART initiation. However, the effect of TI was present in dichotomous strata of all variables shown in Table 2.

Table 2

Characteristics of patients in Group 2 (viral load \geq 400 copies per ml during 1-99% of the 6-18 month period following HAART initiation), by presence of a treatment interruption.

Treatment interruption during first 18 months on HAART	Yes	No	P-values †
N	92	454	
Gender			
Male	68%	76%	0.143
Female	32%	24%	
Median age at HAART initiation (IQR)	36 (30-43)	38 (32-46)	0.019
Median CD4 at HAART initiation	197 (100-299)	160 (70-270)	0.162
Median CD4 at baseline*	270 (125-389)	310 (195-450)	0.007
Median log(10) VL at HAART initiation	4.4 (3.2-5.2)	4.6 (3.4-5.2)	0.798
Mode of infection			
Intravenous drug use	23%	9%	<0.001
Other	77%	91%	
Hepatitis C coinfection			
Yes	32%	17%	0.003
No	68%	82%	
Unknown	0%	1%	
Antiretroviral exposure prior to HAART initiation			
Yes	40%	54%	0.013
No	60%	46%	
Race			
Caucasian	79%	81%	0.704
Other	21%	19%	
AIDS at HAART initiation			
Yes	28%	23%	0.272
No	72%	77%	
Date of HAART initiation			
Before 1 Jan 1999	58%	76%	<0.001
After 1 Jan 1999	42%	24%	

†: chi-square for binary variables; t-test for continuous variables

IQR: Interquartile range

* Baseline: 18 months after HAART initiation

To explore whether the predictive values of covariates in this group (Group 2, Paper III) were comparable to those found in other studies, we performed a multivariate regression analysis

(Table 3). The multivariate model found the following independent predictors of death at the 5% significance level (apart from TI): older age at HAART initiation; an AIDS diagnosis before HAART initiation; a low CD4 count at HAART initiation; and coinfection with hepatitis C. Indeed, this analysis indicated that the group was comparable to other research cohorts. If the underlying reasons for TI are also comparable, then our findings will probably apply to them as well.

Table 3

Cox regression analysis of mortality for patients in Group 2 ((viral load \geq 400 copies per ml during 1-99% of the 6-18 month period following HAART initiation)

	<u>Unadjusted analyses</u>			<u>Multivariate analyses</u>		
	Mortality rate ratio	95% confidence interval	P-value	Mortality rate ratio	95% confidence interval	P-value
Treatment interruption	2.94	1.78-4.84	<0.001	3.42	1.95-5.99	<0.001
Age \geq 40 years at HAART initiation	1.53	1.22-1.93	<0.001	1.87	1.44-2.42	<0.001
Intravenous drug use	3.52	2.08-5.97	<0.001	1.20	0.51-2.84	0.681
ART-experienced at HAART initiation	1.33	0.82-2.17	0.248	1.72	0.99-2.99	0.053
Diagnosed with AIDS before HAART initiation	2.79	1.75-4.45	<0.001	2.30	1.37-3.85	0.002
CD4 count <100 cells per μ l at HAART initiation	2.31	1.45-3.68	<0.001	2.15	1.29-3.57	0.003
Male gender	1.50	0.82-2.73	0.190	1.27	0.66-2.45	0.471
Initiation of HAART after 1 January 1999	1.18	0.66-2.13	0.576	1.15	0.58-2.28	0.688
Caucasian race	2.37	1.03-5.48	0.043	1.38	0.57-3.36	0.473
Hepatitis C positive	2.80	1.72-4.55	<0.001	3.54	1.58-7.92	0.002
Log10 viral load $>$ 4 at HAART initiation	1.49	0.85-2.62	0.162	1.74	0.95-3.19	0.071

10. Declining risk of triple-class antiretroviral drug failure in Danish HIV-infected individuals (Paper IV)

10.1. Background

As more patients are exposed to antiretroviral drugs, we may expect an increase in HIV that is resistant to drugs from multiple drug classes. However, as combination regimens improve in terms of potency and tolerability, the trend also could move in the opposite direction. In observational studies, in which resistance tests are not available for all patients, virological failure may be used as a proxy for resistance development (63;170). One earlier study examined the incidence and prevalence of triple-class antiretroviral drug failure (170), but not in a population-based setting. We therefore aimed to examine the incidence, prevalence, and predictors for TCF in the Danish HIV-infected population.

10.2. Methods

We included patients from DHCS who initiated HAART before 1 January 2004. TCF was defined as a minimum of 120 days with viral load >1000 copies per ml during treatment with each of the three major drug classes. We used person-year analysis to estimate TCF incidence rates and Cox regression analysis to examine risk factors for TCF.

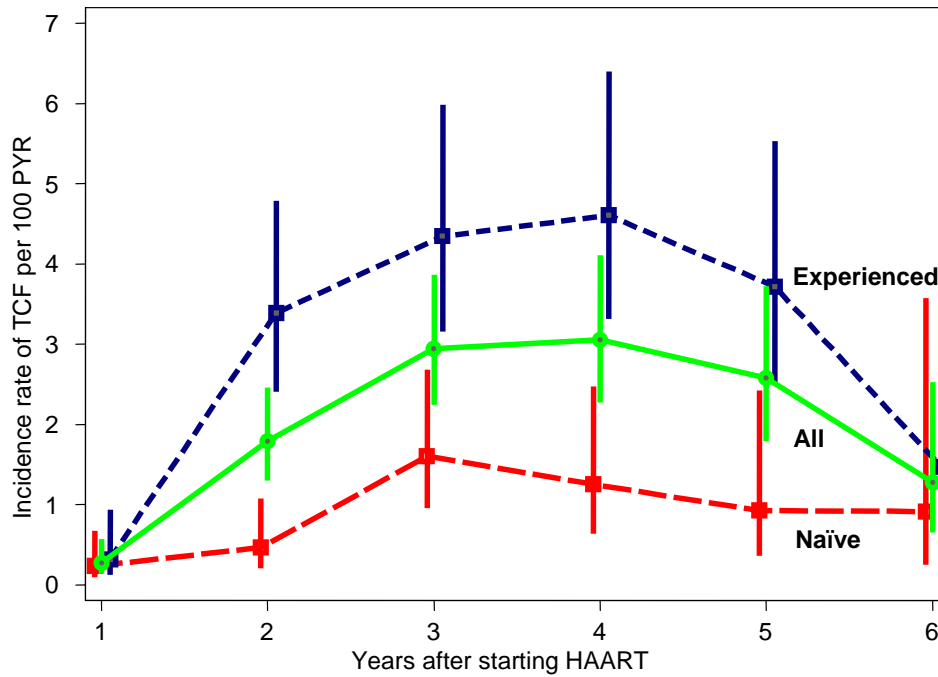
10.3. Main Results

The overall risk of experiencing TCF was 3.4 times higher among patients who were ART-experienced before HAART initiation, compared to ART-naïve patients. The annual IR of TCF among experienced patients increased with time on HAART up to 4.6% in the fourth year after starting HAART, followed by a decrease to 1.4% in the sixth year. Among ART-naïve patients, the annual IR increased up to 1.6%, and thereafter decreased to 0.8%. In calendar time, the highest annual IR was 3.7% in the year 2000, decreasing to 0.4% in 2003. Independent risk factors for TCF were an early year of HAART initiation, a low CD4 count at HAART initiation, and a high VL at HAART initiation (only among ART-experienced patients).

10.4. Comments

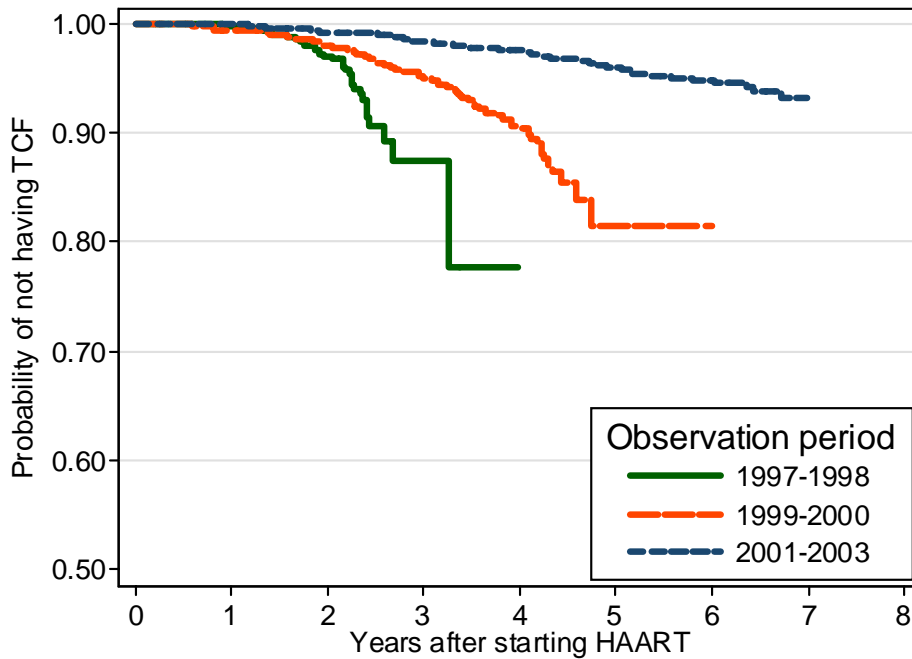
In observational HIV studies, one must take into account the complex interplay between different timelines. Any observation can be positioned according to *time with HIV infection*, *age (time since birth)*, *time on HAART*, and *date (calendar time)*. Placement on each of these four timelines may influence the risk of the outcome of interest, *e.g.*, death or TCF. In Paper IV, *time with HIV infection* was unknown, because DHCS is a prevalent cohort with regard to date of HIV infection. *Time since birth* was not the primary timeline of interest, but we entered *age* into the regression models as a possible confounder. The effect of *time on HAART* on the incidence of TCF is presented in Figure 4 (Figure 1 in Paper IV), showing an increase during the first four years, and then a decrease until the sixth year on HAART.

Figure 4



Incidence of triple-class drug failure for each year after initiation of highly active antiretroviral therapy. Vertical lines indicate 95% confidence intervals. HAART: highly active antiretroviral therapy. TCF: triple-class antiretroviral drug failure. PYR: person-years at risk.

Figure 5

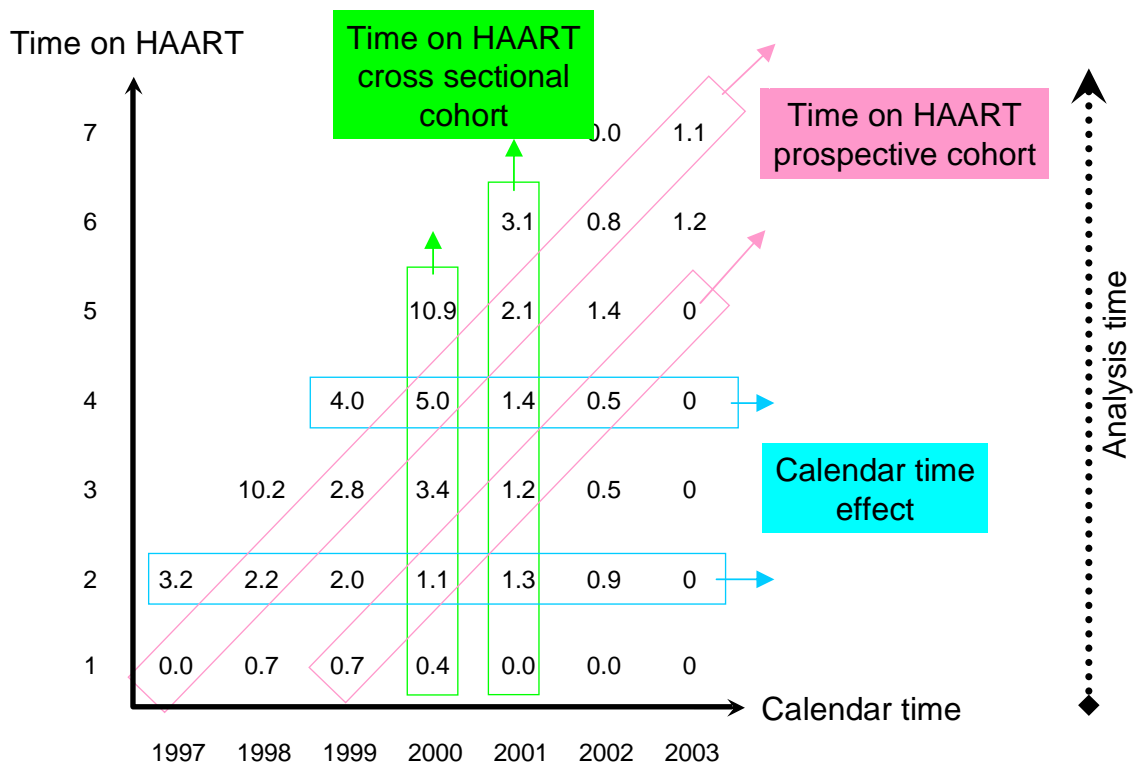


Kaplan-Meier survival curves for time to triple-class drug failure after initiation of highly active antiretroviral therapy, stratified by observation period. TCF: triple-class antiretroviral drug failure.

The effect of *calendar time* on the cumulative incidence of TCF is presented in Figure 5 (Figure 2 in Paper IV), showing that cumulative TCF-free survival, counting from HAART initiation, improved in later calendar years. Therefore, the crude incidence rates depicted in Figure 4 could be confounded by *calendar time*, if the observation dates were unevenly distributed on the timeline of *time on HAART*.

The stratified analysis described in Figure 6 explores this potential confounding problem. The incidence rates are stratified into cells, each summarizing observations from one *calendar year* and one year of *time on HAART*. As shown in blue (horizontal), the IR decreases with increasing *calendar time*; this also occurs when the analysis is restricted to observations from a single year on the timeline of *time on HAART*. As shown in pink (diagonal), single cohorts are followed from initiation of HAART through both *calendar time* and *time on HAART*. For every single cohort the IR first increases, then decreases. Thus, the IR pattern in Figure 4 was observed in all strata of HAART initiation year. Shown in green (vertical) is the interesting observation that if we examine the effect of *time on HAART* in a cross-sectional study with data from just one *calendar year*, we get the (false?) impression of a gradual increase in IR over time.

Figure 6



Incidence rates on two timelines. Each cell summarizes observations for patients who, during a given calendar year, initiated HAART a given number of years before. The number in each cell is the incidence rate of triple-class drug failure, per 100 person-years at risk.

Our model presumes that multi-drug resistance is likely to be present in patients fulfilling our criteria for virological failure. Indeed, other studies using the same criteria did show a poor prognosis for patients with TCF (63). However, the chance of developing resistance mutations varies by drug and drug combination (39;78), and in patients failing a two-drug regimen, resistance may not be present towards both drugs. Further, some patients may not have taken the prescribed drugs at all, in which case we would observe virological failure without emergence of drug resistance. To examine the prevalence of drug resistance mutations in our patients with TCF, we collected blood samples taken within one month before and 6 months after the date of TCF and performed genotyping of the reverse transcriptase gene and the protease gene. We were able to obtain samples from 79% of all patients with TCF. Eighty-eight percent had at least one mutation conferring resistance towards a major antiretroviral drug class, 77% had mutations affecting response to at least two drug classes, and 57% had mutations affecting all three drug classes (only major PI mutations were included). Because these results were obtained from a single test, they represent minimum estimations. For instance, patients receiving drugs from two drug classes at the time of testing might harbour undetected mutations that had emerged under pressure from a third drug class during a previous regimen.

11. Declining prevalence of HIV-infected individuals at risk of transmitting drug-resistant HIV in Denmark during 1997-2004 (Paper V)

11.1. Background

Transmission of drug-resistant HIV is as high as 20% in some countries, with large regional differences (171-179). Future increases in the prevalence of transmitted drug resistance are of major concern. While ineffective HAART may provoke drug resistance (76-82), successful HAART may reduce HIV transmission (180;181), most likely because low VL is associated with reduced infectivity (104;105). Most patients carrying drug-resistant virus will be on antiretroviral therapy, and if an increasing proportion of patients on HAART achieves low VL, transmission of drug-resistant HIV may ultimately decrease, even if use of HAART itself increases. A subtle balance may arise between the size of the infectious patient pool and the proportion of patients harbouring resistance mutations. Examining temporal trends in this balance would add to the understanding of the low prevalence (less than 5%) of transmitted resistance in Denmark (179). We therefore aimed to estimate acquisition of drug resistance over time and to examine temporal trends in the prevalence of patients at risk of transmitting drug-resistant HIV.

11.2. Methods

We included patients from DHCS seen during 1995-2004. Virological failure was defined as either having a VL>1,000 copies/ml for 120 days while on a HAART regimen, or taking NRTI for 180 days before HAART regardless of VL. Previous virological failure was considered a proxy for harbouring drug-resistant virus at subsequent observation points. Patients with a current VL>1,000 copies/ml were considered at risk of transmitting HIV. The prevalence of previous drug failures and transmission risks were computed on 1 January each year.

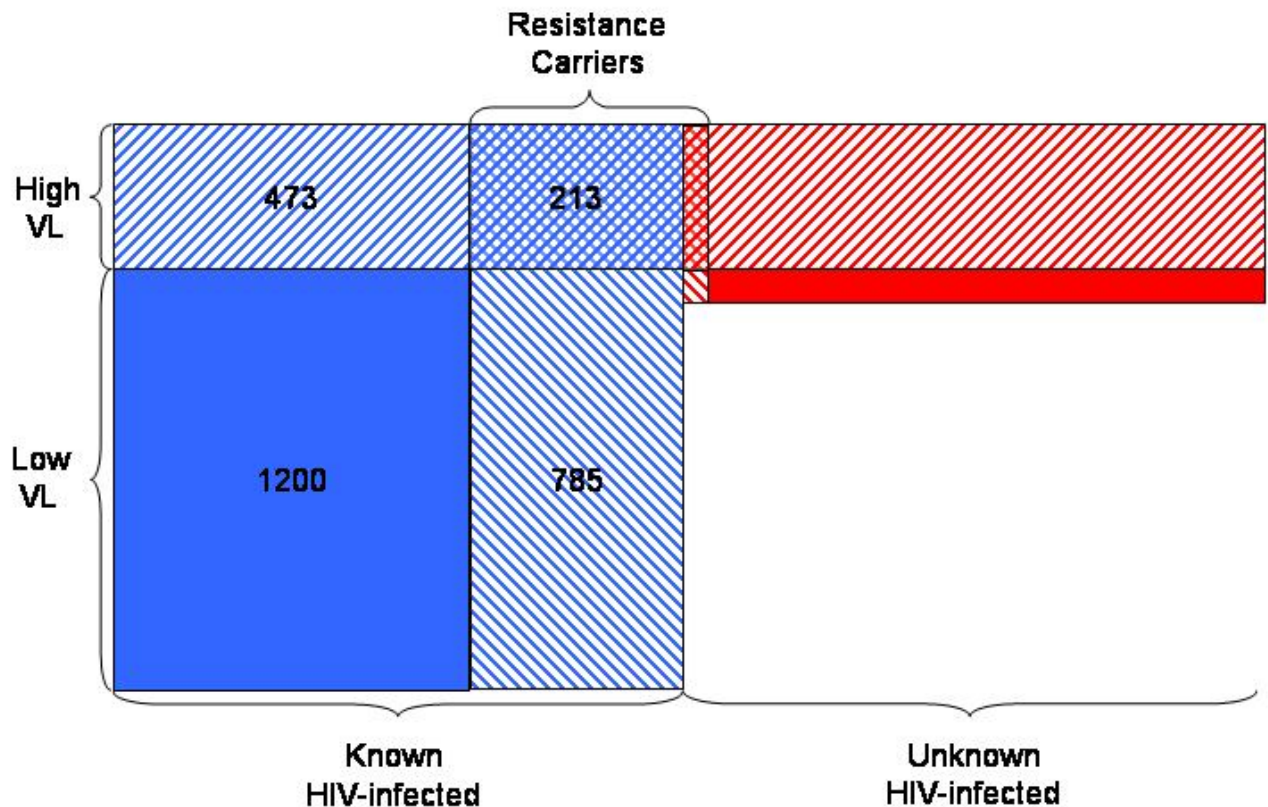
11.3. Main results

The pool of infectious patients (VL>1,000 copies per ml) decreased from 69% of all patients in 1997 to 26% of all patients in 2004. The proportion of patients with previous virological failure (who were therefore likely to harbour drug-resistant virus) decreased from 60% in 1997 to 31% in 2004. Thus, among 2,671 patients under observation on 1 January 2004, 686 were infectious (VL>1000 copies per ml), and 213 of the 686 had previous virological failure. Among patients who were ART-naïve before HAART initiation, the prevalence of previous NRTI failure was stable during the period 2000-2004. The prevalence of patients with previous PI failure decreased, whereas the prevalence of patients with previous NNRTI failure increased. Among 1,827 “ART-naïve before HAART” patients under observation on 1 January 2004, 160 (8.8%) had previous NRTI failure, 102 (5.6%) had previous PI failure, and 65 (3.6%) had previous NNRTI failure. From one year to the next, fewer new patients entered the risk group (PVF and VL>1,000 copies per ml), and fewer patients remained at risk.

11.4. Comments

Figure 7 depicts all HIV-infected individuals in Denmark, grouped by infectivity (VL above/below 1000 copies per ml), resistance carriers, and knowledge of HIV infection (known/unknown). We assume that 80% of undiagnosed individuals are infectious, and that a maximum of 5% carry drug-resistant virus acquired through transmission (179).

Figure 7



HIV-infected individuals in Denmark on 1 January 2004. The group of 2,671 patients who know that they are infected with HIV is depicted in blue. The estimated group of 1000 individuals who do not know that they are HIV-positive is depicted in red. The infectious pool (VL>1000 copies per ml) is marked with lines extending from bottom left to upper right. The group of potential carriers of drug-resistant HIV (those with previous prolonged virological failure) is marked with lines extending from upper left to bottom right. The size of the known (blue) groups is indicated with numbers.

If we further assume there are 1,000 undiagnosed patients, among them 40 (5% of 80%) will be infectious carriers of drug-resistant virus, and 760 (95% of 80%) will be infectious carriers of non-resistant virus. The total number of infectious resistance carriers will be 253 [213+40], and the proportion of the infectious pool carrying drug-resistant virus will be 17.0% [(213+40)/(686+800)]. However, at least two factors may influence the actual transmission risk.

First, drug-resistant viral strains may have reduced transmission capacity (182;183). Second, the amount of risky behaviour among individuals who are unaware that they are HIV-infected may be greater than among those who know they have HIV. Individuals in the latter group may have less unprotected sex and needle sharing because they know their HIV status or because they are too sick. A simple calculation, taking into account these two factors, can estimate the contribution from the 17% of individuals who are resistance carriers to the total risk of transmission. If we assume that risky behaviour is five times higher among those who do not know that they are infected with HIV, the resultant risk of transmission with drug-resistant HIV will be 8.8% $[(213+40*5)/(686+800*5)]$. If we further assume that the transmission capacity of drug-resistant virus is halved, the resultant risk of transmission with drug-resistant HIV will be 4.4% $[(213/2+40*5/2)/(686+800*5)]$. While these rough calculations are largely based on assumptions, they demonstrate that the 5% or lower rate of drug resistance transmission in Denmark (179) may be consistent with our observed prevalence of 31% of individuals in the infectious pool with previous virological failure.

12. Discussion of results in relation to the aims of the thesis

12.1. Aim I

(To estimate the annual number of new HIV-infections in Denmark and the annual number of deaths in the HIV-infected population. Further, to describe temporal trends among newly HIV-infected individuals in Denmark with special emphasis on race, route of transmission, age, and immunological status at time of diagnosis.)

Between 1995 and 1998 we observed a dramatic decrease in the number of deaths among HIV-infected patients in Denmark, reflecting the introduction of potent antiretroviral drug regimens (19;184). In the following five years, this number stabilized at around 60 deaths per year –more than twice the number of deaths among patients with AIDS as reported by SSI (11). A number of studies from other countries document a decrease in the proportion of deaths due to AIDS and other HIV-related causes in recent years (94;149;185-187). The observed differences between the reports from SSI and our findings could stem from an increased proportion of deaths unrelated to AIDS, but a study examining individual causes of death is needed to confirm this.

We observed a stable annual incidence of patients newly diagnosed with HIV and a low adult prevalence compared to other Western countries. The HIV incidence rate is twice as high in Western Europe and the prevalence is four times as high. In North America the incidence rate is five times higher than in Denmark, and the prevalence is eight times higher (188).

Prevention efforts lag behind the evolving HIV epidemic in several European countries. The spread of HIV in Europe is one of the main concerns of the World Health Organisation in 2006 (189). The majority of new HIV patients in Europe are infected through heterosexual contact, and a substantial proportion of these patients originate from high-prevalence countries, principally in sub-Saharan Africa (190-193). In contrast to the general European trend, we observed a decrease in non-Caucasians among patients newly diagnosed with HIV in Denmark. This may be explained by a decrease in the number of immigrants into the country due to government policy.

Another trend observed in industrialized countries is an increase in high-risk behaviour among men who have sex with men (MSM) (194-196;196). Surveillance data from SSI indicated a rise in Danish patients infected with HIV through male homosexual contact in 2004 (151). Together with an increase in syphilis diagnoses among MSM in Copenhagen from mid-2003 to mid-2004 (197), this could signal similar behavioural patterns in Denmark. However, this trend was not yet observed in our study in 2003.

In contrast to what was feared, we observed no annual increases in the proportion of young patients or patients with signs of advanced disease, such as a low CD4 count, a high VL, or

AIDS at the time of HIV diagnosis. The median age at diagnosis even increased, as well as the proportion of patients above age 25.

12.2. Aim II

(To describe the demographic characteristics of the HIV-infected population in Greenland, and to examine the impact of HAART on immune status and mortality in this population.)

The high proportion of middle-aged, heterosexually infected, socially marginalized patients found in Greenland's HIV-infected population accords with previous findings (154) and announcements as recently as late 2005 (198).

We found that mortality in patients receiving HAART in Greenland was more than ten times higher than mortality in the general population. Two studies published after the completion of our research compared the mortality in HIV-infected individuals with that in the general population in other settings. The Swiss HIV Cohort Study (199) reported a cohort-wide MR of 30 per 1000 person-years at risk (PYR) during 1997-2001, and the Dutch ATHENA cohort (200) reported a MR of 10.6 per 1000 PYR among antiretroviral-naïve patients who survived the first 24 weeks of a HAART-regimen. As in studies published prior to ours (17;201;202), these rates were considerably lower than the 111 per 1000 PYR found in patients receiving HAART in Greenland. The SMRs in the Swiss and Dutch studies cannot be compared with the MRR found in Greenland. These ratios are highly dependent on both the mortality in the background population and the age distribution in the exposed cohort. The short life expectancy in Greenland (64 years for men and 70 years for women (8)), combined with the median age of 55.8 years in the HIV cohort tends to reduce the *rate ratios* even though the *absolute* excess mortality was high (101 per 1000 PYR).

Part of the increased mortality among HIV patients in Greenland could be related to lifestyle, social status, and alcoholism. However, the magnitude of increased mortality due to these conditions was markedly lower in other studies (203-207), and 69% of the deaths were HIV-related. Furthermore, response to HAART was far from optimal, as measured by the disease markers CD4 count and VL. We therefore believe that the excess mortality observed in our study was mainly caused by HIV. HAART was freely available, but adherence to treatment was probably low, due both to limited personal resources among the HIV population and to the structure of the health care system.

Since the completion of our study, the prognosis for HIV-infected individuals in Greenland may have improved. After a median of 4.5 annual deaths during the years 1995-2002, only one HIV patient died in 2003, five died in 2004, and three died in 2005 (K Ladefoged, Nuuk, personal communication). In addition, the majority of viral loads measured in patients in Greenland have recently been below the detection limit of 200 copies per ml (LB Jørgensen, SSI, 2006, personal

communication). Reporting of cases to the HIV database may also have increased attention to HIV among health personnel and positively influenced the clinical course (198). Further analyses to be conducted after the 2006 update of cohort data may confirm these reports.

12.3. Aim III

(To examine the level of virological control during the initial period following HAART initiation as a predictor for long-term viral suppression, increases in CD4 counts, and mortality.)

To our knowledge no other studies have assessed the prognostic value of intermittent viremia taking into account all available VL measurements during a pre-determined period. We found that achieving an undetectable VL (<400 copies per ml) following six months of HAART and maintaining that level until 18 months was a strong predictor of long-term treatment success. The difference in outcome between these patients (Group 1) and those who never achieved an undetectable VL (Group 3) was striking, but not surprising.

Interestingly, the outcome among patients whose VL was only partly suppressed (Group 2) was distinctly different from the other two groups. There was a 4.5-fold increased mortality in Group 2 compared to Group 1, and even the lowest quartile of Group 2 (Group 2A) had a two-fold higher mortality than Group 1. The differences observed in mortality, immunologic outcome, and virological outcome between Group 2 and Group 1 could not be explained by an array of well-known prognostic covariates examined in multivariate regression models. The poor long-term outcome seemed to be caused by either the short viremic period itself (*e.g.*, through emergence of drug resistance), or by residual confounding due to unmeasured characteristics. Recent evidence indicates that single episodes of VL>50 copies per ml, so-called blips, are not associated with development of new resistance mutations, but rather represent random variation (208-210). Easterbrook *et al.* showed that following virological suppression six months after HAART, viremia of VL>400 copies per ml predicted impaired CD4 count rise and future sustained virological rebound (211). Napravnik *et al.* estimated that, in patients showing continuous detectable VL despite antiretroviral treatment, the incidence rate of acquiring a new resistance mutation was between 1.0 and 3.1 mutations per year, depending on VL level and number of mutations at baseline (77). Therefore, the amount of resistance developed in patients in Group 2, particularly Group 2A, during the short viremic period cannot solely explain the poor long-term outcome. We find it likely that viremia was a marker of poor adherence and therefore indicated a risk of future viremic episodes, which would in turn lead to emergence and accumulation of drug-resistance mutations. If poor adherence were also associated with non-HIV-related risk factors for death, the risk would increase further.

It is striking that a treatment interruption during the first 18 months of HAART was associated with a 3.5-fold increased risk of death among patients in Group 2. The increased mortality in these patients was constant throughout the 6-year post-baseline period, ruling out end-stage

AIDS or drug toxicities at the time of TI as explanations. Deaths due to non-HIV related conditions were less frequent in patients with TI than in those without TI, making it unlikely that non-HIV associated comorbidity could explain the findings. Again, this suggests that poor adherence underlies the high mortality rates. Alternatively, there may be a common cause of the increased risk of disease progression in our TI patients and the increased risk of disease progression in the STI arm of the discontinued SMART study (98;99), *e.g.*, the influence of these interruptions on the response to virus by the host's immune system.

12.4. Aim IV

(To examine the incidence, prevalence, and predictors of triple-class antiretroviral drug failure in the Danish HIV-infected population.)

We found a declining IR of TCF in the Danish population. The adjusted relative risk in patients who initiated HAART in 1999-2003 compared with those who initiated HAART in 1995-1996 was 0.4 in ART-experienced patients and 0.1 in ART-naïve patients. Changes in drug combination regimens are likely to have caused this improvement. Other possible reasons could be improved patient education on important issues such as adherence to therapy and treatment interruptions, and the increased number of drug combinations available for in-class substitutions of NRTI and PI, making it easier to create individually tolerable drug combinations.

One other study, by Mocroft *et al.* for the EuroSIDA group, has examined the development of TCF (170). The IR and six-year cumulative risk of TCF was approximately 1.5 times higher than the IR and seven-year cumulative risk observed in our study, both among ART-naïve and ART-experienced patients. The EuroSIDA investigators also observed a declining risk of TCF with later years of HAART initiation (0.89 per year in ART-experienced patients and 0.80 per year in ART-naïve patients), but this was not statistically significant at the five percent level. Patients from 72 centres in more than 25 countries were recruited for the EuroSIDA study, and its findings – which differed slightly from ours – may well be due to inter-country disparities, *e.g.*, in the use of antiretroviral drugs (138) both before and after introduction of HAART.

For ART-experienced patients, the risk of TCF increased with time on HAART until the fourth year. The following decline could be a sign of treatment improvements. Another explanation is that extensively pre-treated patients who harboured drug-resistant virus experienced their failures during the first years of HAART. For ART-naïve patients, the incidence rate was low, and no peak was observed.

Following a decreasing incidence rate, the prevalence of TCF among all patients on HAART in Denmark stabilized at around seven percent in 2001-2003. In the EuroSIDA study by Mocroft *et al.* the prevalence of TCF increased with calendar time (170). However, it is unclear how

prevalences were determined in this study, which is a prevalent cohort with regard to HAART initiation (144;202).

Based on the finding by Ledergerber *et al.* (63) that patients with TCF had a high three-year cumulative mortality of 15.3%, we assumed that patients meeting the definition of TCF would have a poor prognosis, probably because they harboured resistance mutations. We did indeed find multiple-drug class resistance in the majority of TCF patients, consistent with the rates of acquiring new resistance mutations estimated by Napravnik *et al.* in patients with continuous detectable VL (77).

12.5. Aim V

(To estimate acquisition of antiretroviral drug-resistant virus in the Danish HIV-infected population over time, and to examine temporal trends in the prevalence of patients at risk of transmitting drug-resistant HIV.)

To our knowledge, this study is the first to combine data on previous drug failure with data on current viral load to detect patients at risk of transmitting drug-resistant virus. Most other studies have estimated the prevalence of drug resistance using data from resistance tests (91;92;171;174-178), a method associated with several possible causes of bias: the indication for resistance testing has changed over time; the interpretation of mutations has changed; patients can harbour mutations undetected in a particular sample; assays are usually available only for a proportion of the HIV-infected population; and the assays are performed primarily at the decision of the treating physician. While our model was based on a number of assumptions, we attempted to make it consistent with current knowledge (77;104;105;180) and models used in other studies (63;170). Our model may have led to misclassification of both infectious patients and drug resistance carriers, but if this misclassification was constant over time, the temporal trends that the model estimated then mirrored actual decreasing trends in potential transmitters of antiretroviral drug resistance.

We found a decrease in the prevalence of infectious patients between 1997 and 2004, in particular a decrease in the prevalence of infectious patients with previous virological failure. The decrease stemmed from the declining incidence of prior virological failure, as well as successful re-suppression of viral load to below 1,000 copies per ml following failure. Declines in transmitted resistance in Canada (176) and Holland (177) may be signs of a similar reduction in potential transmitters in those countries. A poster presented at the 13th Conference on Retrovirus and Opportunistic Infections in 2006 drew comparable conclusions (212). The author applied a computer simulation model to a UK population, assuming 13% multi-drug resistance in the infectious pool in 2002. The model predicted a future decrease in the proportion of infectious patients with both one-drug and multi-drug resistant HIV.

We found a high prevalence of prior virological failure in 1997 (62%), which is comparable to the 78% prevalence of drug resistance reported in an ART-experienced US population in 1998 (92). The decline since then accords with the therapeutic success achieved in later years (202).

Among ART-naïve patients in our study, the decline in potential carriers of PI resistance and the increase in potential carriers of NNRTI resistance during 2000-2004 corresponded temporally with the shift from unboosted to boosted PI (39;41;47;213) and with the increased use of NNRTI instead of PI in initial regimens (25;47). A recent study from the UK (93) found similar trends in the prevalence of population resistance towards these two drug classes. The actual levels were higher than our estimates, but this is in line with the very high prevalence (>20%) of transmitted resistance in the UK (172;174). A recent trans-European study of transmitted resistance in 2,208 drug-naïve patients found that 10.4% carried resistant HIV, with underlying temporal increases in NNRTI-mutations and decreases in PI-mutations, and with large regional differences (0-23%) (178).

13. Conclusion, interpretation, and perspectives

13.1 Conclusion in relation to the aims of the thesis

- I. In Denmark, the HIV epidemic was stable during the period 1995-2003, and concerns about an unmonitored worsening of the epidemic were not confirmed. DHCS complements the national HIV surveillance system in monitoring trends in new HIV infections, deaths, and individual characteristics.
- II. In Greenland, the HIV-infected population was middle-aged, mainly living in Nuuk and Sisimiut, and predominantly infected by heterosexual contact. The marked reduction in mortality among patients on HAART observed in other Western countries had not yet occurred in 2003.
- III. Fully suppressed VL 6-18 months after HAART initiation was associated with long-term survival and clinical improvement. Viremic episodes increased the risk of death and poor immunologic outcome, and might be markers of poor adherence. Treatment interruptions further enhanced the risk.
- IV. The incidence rate of triple-class drug failure declined over calendar time, and its prevalence among patients on HAART was stable. This provides evidence that high drug pressure on HIV at the population level does not inevitably result in increasing rates of drug failure.
- V. The number of HIV-infected individuals at risk of transmitting drug-resistant virus was declining. Improved treatment appeared largely responsible for this positive development. However, while the prevalence of potential resistance decreased overall, potential NNRTI resistance increased in ART-naïve patients.

13.2 Interpretation

13.2.1. Preserved therapeutic options

As mentioned earlier in this thesis, the primary goal in daily clinical practice is to obtain an undetectable viral load, and the positive effects we observed were related to achieving this goal. The declining prevalence of mono-drug class failure (Paper V), triple-drug class failure (Paper IV), and risk of transmission (Paper V) relate directly back to the continuous increase in the proportion of patients with successful viral suppression. The importance of achieving an undetectable viral load was highlighted by the positive long-term outcome among patients with complete viral suppression in the first period of HAART, and by the negative outcome among those who did not reach this goal (Paper III). Studies from the UK Collaborative HIV Cohort (113) reported a 27% six-year cumulative risk of resistance mutations after HAART initiation (91) and an increase in the prevalence of treatment-experienced patients with virological failure (90). As these findings presage an exhaustion of available antiretroviral drug combinations, the investigators call for new drugs to combat the disease. Our findings contradict these prophecies. Treatment improvements over the years seem to have suspended and possibly ended the threatening accumulation of patients with multi-class drug failure (Paper IV). They also seem to have ended the accumulation of infectious patients with a history of virological failure (Paper V),

despite high drug pressure on HIV at the population level. A continuation of these trends would preserve initial therapeutic options for the majority of newly infected individuals in the future. Drug resistance from less successful therapeutic strategies in the past would not be carried forward, and the HIV-infected population would be able to fully benefit from new drugs on the market. However, drug failure – and NNRTI failure in particular – must be closely monitored in the future.

13.2.2. Generalization

Temporal trends observed in Denmark may not reflect trends in HIV-infected populations in other countries or settings, due to differing characteristics of infected persons (*e.g.*, mode of infection, age, gender, race, transmitted drug resistance, CD4 count, viral load), differing health care systems (*e.g.*, unlimited/limited access to HAART, education and experience of caregivers), and differing therapeutic strategies (*e.g.*, types of drugs and drug combinations used previously and/or currently, recommendations for STIs). At the same time, the encouraging trends which emerged from our study may stem from its population-based setting, which included all rather than a selected group of HIV-infected individuals. Reports of similar trends in other studies, particularly from Europe (177), suggest that the trends we identified are occurring in many other countries in the Western world. In any case, Denmark's experience may serve as an example of what is achievable.

13.3. Perspectives

13.3.1.. Future challenges

Knowledge of HIV has increased tremendously over the last 25 years, and our deeper understanding of HIV gives rise to more detailed scientific questions. First, how can we provide more effective therapy to HIV-infected individuals? We need to broaden our focus from whether a given drug is associated with an increased risk of a given event, to examining individual risk factors for adverse drug reactions, so we can tailor regimens to individuals. Identification of genetic markers for drug susceptibility may be another way to improve treatment. As well, as patients live longer and get older, they contract an increasing number of other diseases for which they need treatment. This demands more knowledge of the way antiretroviral drugs interact with other types of drugs. We also need deeper insight into the mechanisms of non-adherence to treatment, and we need more knowledge about the impact of HIV and HAART on the long-term risk of a number of other diseases. We also must ask about the future impact of HIV on the lives of the HIV-infected individuals and on the societies in which they live. How does increasing life expectancy affect the ability to work, the desire to have children, and what are the economic consequences? Finally, how do we transfer the success of HAART in the Western world to resource-poor settings? The spread of HIV in Sub-Saharan Africa is disastrous, and an uncontrolled epidemic is developing in Eastern Europe and Asia. While the use of antiretroviral drugs is increasing in those areas, poverty may force patients into drug-sharing and treatment interruptions, leading to a marked increase in the prevalence of drug resistance.

13.3.2.. Achieving the goals

Observational studies are necessary, but not sufficient to answer all of these questions. We need multi-cohort collaborations for studies of rare events and rare exposures (risk factors). Data extracted from patient files needs to be combined with banks of biological material to examine host genetics, viral genetics, and biological markers of disease progression and adverse drug effects. The development and use of advanced statistical methods that take into account time-varying exposures (214-218) and confounding by indication (219-221) may help to answer some of our questions. DHCS is needed in the future, both as an individual cohort, and as part of international cohort collaborations. Furthermore, with a stable incidence of around 250 new HIV-infections per year, and an annual number of deaths of just around 70, the prevalence of HIV in Denmark is expected to increase. DHCS must continue to monitor the HIV-infected population in Denmark and Greenland in order to detect changes in the HIV epidemic. Most studies of HIV are done – and will be done in the future - in wealthy parts of the world, examining scientific questions of specific interest to patients in these areas. However, despite the different geographical areas where the main research effort is conducted and where the majority of people who suffer from HIV live, it is my belief that any scientific gain in HIV research will ultimately – often with a lag in time – improve the prognosis for HIV-infected individuals in poor areas. Continued use of DHCS data to examine current scientific questions will have an effect not only in Denmark or in Europe – but will also play a role in the global fight against HIV.

14. Summary

This PhD thesis is composed of five articles and a review. The work leading to the thesis began in 2003. At that time, the dramatic decrease in mortality in HIV-infected individuals after the introduction of highly active antiretroviral therapy (HAART) in 1995-1996 was evident. However, concerns were raised that the effectiveness of HAART might wane due to long-term drug toxicity and emergence of drug-resistant virus, leading to multi-drug class treatment failure. Furthermore, the number of people receiving HAART in Denmark and Greenland was unknown, as was the disease progression stage at time of diagnosis. Aims of this thesis were therefore 1) to examine temporal trends in deaths, new infections, and individual patients characteristics among the Danish HIV-infected population, 2) to describe demographic characteristics and examine the impact of HAART on immune status and mortality among the HIV-infected population in Greenland, 3) to examine the level of complete plasma-HIV RNA (viral load) suppression during the initial period after HAART initiation as a predictor for long-term viral suppression, CD4 count increase, and mortality, 4) to examine the incidence, prevalence, and predictors for triple-class antiretroviral drug failure, and 5) to estimate acquisition of antiretroviral drug-resistant virus over time and examine temporal trends in the prevalence of patients at risk of transmitting drug-resistant HIV. Our studies were based on data from the Danish HIV Cohort Study – an observational study encompassing all HIV patients in Denmark and Greenland – and from the Danish Civil Registration System.

We found a decrease in the annual number of deaths in Denmark after introduction of HAART, and a stable number of new HIV infections with no sign of an increase in the proportion of patients with advanced HIV disease or young age. In Greenland, the HIV-infected population was middle-aged, mainly living in Nuuk and Sisimiut, and predominantly infected by heterosexual contact. Even in patients receiving HAART, mortality was more than ten times higher than in the general population, and only 40% had fully suppressed viral load. In another study, we found that the proportion of time with viral load suppression 6-18 months after HAART initiation was associated with long-term survival and clinical improvement. Episodes of non-suppression increased the risk of death and poor immunologic outcome and might be markers of poor adherence; treatment interruptions further enhanced the risk. We also found that the incidence rate of triple-class drug failure declined over calendar time, and that the prevalence among patients on HAART was stable. Finally, we found that the number of HIV-infected individuals at risk of transmitting drug-resistant virus was declining, and that improved treatment appeared to be responsible for this positive development. Although the prevalence of potential drug resistance decreased overall, potential resistance to non-nucleoside analogue reverse transcriptase inhibitors (NNRTI) increased in patients who were mono- or dual-drug antiretroviral therapy-naïve before HAART initiation.

The primary goal in the day-to-day treatment of HIV patients is to obtain an undetectable viral load, and the positive effects observed in these studies reflected clinical success. The declining

prevalence of drug failure and risk of transmission could be referred directly back to the continuous increase in the proportion of patients with successful viral suppression. The importance of achieving an undetectable viral load was underscored by the positive long-term outcome in patients with complete viral suppression in the first period of HAART treatment, and by the negative outcome among those who did not reach this goal. Other studies have predicted an exhaustion of available antiretroviral drug combinations, due to an increasing number of patients who have experienced viral failure on three drug classes. Our findings contradict these prophecies. Treatment improvements over the years seem to have suspended and possibly stopped the threatening accumulation of patients with multi-class drug failure. They also seem to have warded off the accumulation of infectious patients with a history of virological failure, despite high drug pressure on HIV at the population level. A continuation of these trends would preserve initial therapeutic options for the majority of newly infected individuals in the future. Drug resistance from less successful therapeutic strategies used in earlier years would not be carried on, and the HIV-infected population would be able to fully benefit from new drugs on the market. However, drug failure – and NNRTI failure in particular – must be closely monitored in the future.

15. Danish Summary

Ph.D.-afhandlingen indeholder fem publicerede originalarbejder og en oversigt. Arbejdet med afhandlingen påbegyndtes i 2003. På det tidspunkt var det tydeligt at der var sket et dramatisk fald i dødeligheden blandt HIV-smittede som følge af indførelsen af moderne kombinationsbehandling (highly active antiretroviral therapy, HAART) i 1995-1996. Der var dog bekymring for om effekten af HAART ville aftage på længere sigt på grund af langtidsbivirkninger og udvikling af virus med resistens overfor multiple antiretrovirale stofklasser, førende til behandlingssvigt. Antallet af HIV-patienter i behandling med HAART i Danmark og Grønland var ukendt, og det samme var sygdomsstadiet på diagnosetidspunktet. Formålet med denne afhandling var derfor 1) at undersøge ændringer over tid i antallet af dødsfald og nysmittede samt individuelle karakteristika blandt HIV-inficerede i Danmark, 2) at beskrive demografiske karakteristika samt undersøge indvirkningen af HAART på immunstatus og dødelighed blandt HIV-inficerede i Grønland, 3) at undersøge graden af komplet plasma HIV-RNA (viral load) suppression i den første periode efter opstart af HAART som langtidsprediktor for viral suppression, stigning i CD4 tal og dødelighed, 4) at undersøge incidensen, prævalensen og prediktorer for udvikling af tre-klasse behandlingssvigt samt 5) at estimere erhvervelsen af resistensmutationer over tid og undersøge tidsmæssige ændringer i prævalensen af patienter i risiko for at kunne smitte med resistent HIV. Studierne var baseret på data fra Den Danske HIV Kohorte, et observationelt studie af samtlige HIV-patienter i Danmark og Grønland, samt Det Centrale Personregister.

Vi fandt et fald i det årlige antal dødsfald i Danmark efter introduktion af HAART samt et stabilt årligt antal nye HIV-infektioner, uden at der var tegn til en øgning i andelen af patienter som var unge eller som havde tegn på fremskreden sygdom. I Grønland var den HIV-inficerede befolkning midaldrende, hovedsageligt bosiddende i Nuuk og Sisimiut, og overvejende smittet heteroseksuelt. Selv i patienter som var i behandling med HAART var dødeligheden mere end ti gange så høj som i baggrundsbefolkningen, og kun 40% havde fuldt supprimeret viral load. I et andet studie fandt vi at andelen af tid med viral load suppression i perioden 6-18 måneder efter start på HAART var associeret med overlevelse og klinisk bedring på længere sigt. Episoder med manglende suppression øgede risikoen for død og ringe immunologisk bedring og kunne være markører for dårlig adherence, og behandlingspauser øgede risikoen yderligere. Endvidere fandt vi at incidensraten af tre-klasse behandlingssvigt faldt over kalendertid, og at prævalensen blandt patienter i behandling med HAART var stabil. Endelig fandt vi at antallet af HIV-inficerede personer i risiko for at transmittere resistent virus var faldende, og at forbedret behandling syntes at være årsagen til denne positive udvikling. Selv om prævalensen af potentiel antiretroviral resistens faldt totalt set, var der en stigning i potentiel non-nukleosid analog revers transkriptase hæmmer (NNRTI)-resistens blandt patienter som var ét- eller to-stofs behandlingsnaive før start på HAART.

Det primære behandlingsmål i den daglige klinik er umåleligt viral load, og de positive resultater i disse studier var relateret til opnåelse af dette mål. Den faldende prævalens af behandlingssvigt og transmissionsrisiko kunne relateres direkte tilbage til den vedvarende stigning i andelen af patienter med succesfuld viral suppression. Vigtigheden af at opnå umåleligt viral load blev understreget af de positive langtidseffekter hos patienter med komplet viral suppression i den første periode efter start på HAART, og af de negative effekter hos patienter som ikke opnåede dette mål. Andre studier har forudsagt at de tilgængelige behandlingskombinationer ville blive udtømt på grund af et øget antal patienter med behandlingssvigt overfor tre forskellige stofklasser. Vores resultater er i modstrid med disse profetier. Forbedrede behandlinger gennem årene synes at have opbremset og muligvis endda stoppet den truende ophobning af patienter med multi-klasse behandlingssvigt. På trods af et højt antiretroviralt pres på HIV i den smittede befolkning synes også stigningen af infektiøse patienter med tidligere behandlingssvigt at være overvundet. En fortsat udvikling i denne retning vil betyde bevarede behandlingsmuligheder hos størstedelen af nye HIV-smittede i fremtiden. Resistens fra tidligere tiders mindre succesfulde behandlingsstrategier vil ikke blive videreført, og den HIV-inficerede befolkning vil kunne få fuldt udbytte af nye stoffer på markedet. Det er dog af største vigtighed at opretholde tæt overvågning af behandlingssvigt, især ved behandling med NNRTI.

16. References

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