The Road to Success

Long-term prognosis for persons living with HIV in Denmark - time trends and risk factors

by

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Copenhagen
2014
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This review is based on the following papers


Papers previously included in academic theses

Papers III, IV, and VI were three of the five papers included in my PhD 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, University of Southern Denmark, 2006. Paper V was part of Anne Audelin’s PhD thesis Molecular-epidemiological studies of HIV-1 and antiretroviral resistance in Denmark, Copenhagen University, 2011. Papers I, II, VII, VIII, and IX have not previously been submitted for obtaining of an academic degree.
1 INTRODUCTION

1.1 The HIV epidemic

The HIV epidemic is a marvellous example of a new disease hitting the medical community – and the whole world - by surprise, and then going through the stages from being overwhelming to gradually becoming a disease, which is largely manageable with regard to both prevention and treatment. The immense progress would not have been possible were it not for a concerted action by patients, civil society groups, doctors, scientists, donors, politicians, the pharmaceutical industry, and many others. Fortunately, the HIV epidemic possessed the right cocktail of scientific challenge, urgency, despair, human discrimination, and geographical distribution to stimulate these various groups.

In 2003, when I started the work providing the basis for this thesis, the global HIV epidemic was out of control, increasing in incidence in most parts of the World(10), with the prevalence being kept down only due to the high death rates. Antiretroviral therapy (ART) combinations showing high efficacy towards HIV had been known for 6-7 years (11), but only a minority of all persons with HIV were getting the full benefit (10). In many places, treatment was not available, people could not afford it, or they did not have access to trained health care professionals. For those who did have access, some were burdened by considerable side effects, or they were infected with virus that had developed drug resistance after previous years’ exposure to less effective single drugs or drug combinations. Many found it extremely difficult to adhere to the strict requirements for taking the medication at specific times of the day, and without interruptions – not made easier by the often large pill burden of the drug combinations. Finally, it was unknown how long the drugs could maintain their efficacy in the individual even if administered as intended.

Thus, despite many individual stories of success, there was reasonable doubt as to whether these successes would translate into a positive population effect, and result in decreased morbidity and mortality. On the contrary, there was a fear that the increased drug pressure would increase the prevalence of drug resistance in the population, subsequently leading to transmission of resistant virus from one individual to another, and thereby waning the treatment options available.

Absolute prevention of new infections will always be the key to eradicating this epidemic (12), and for those already infected, finding a cure is the optimal goal(13–18). However, until we have overcome these obstacles, we must optimize infection control and management both in the individual and at the population level. Ideally, an HIV-infected individual should know immediately that he/she is infected, should have access to specialized medical and social support, receive a drug combination which effectively suppresses the virus and has no side effects, and should be without comorbid conditions both before and after he/she gets infected.

As a country with free access to health care including treatment for HIV infection, and a limited number of highly specialized HIV clinics, Denmark is one of few countries providing the basic ingredients for optimal HIV control at the population level (19). Further, the systematized collection of clinical and paraclinical data on all persons with HIV in the Danish HIV Cohort Study (DHCS) (20,21), combined with access to excellent population-based administrative databases that can all...
be linked to DHCS through a unique person identification number, makes Denmark an ideal place to study the prerequisites for and effects of good population control.

2 AIMS OF THIS THESIS

2.1 Specific aims

The papers on which this thesis is based each aimed to provide new knowledge to different aspects of the above. Accordingly, the aims of the thesis were:

i. To explore the potential for an indicator disease-based HIV testing strategy (paper I).
ii. To compare temporal trends in quality and quantity of ART introduction in countries with optimal and sub-optimal health systems for HIV care (paper II).
iii. To assess temporal trends of virological failure and the importance of virological control at the population level (papers III and IV).
iv. To assess temporal trends of drug resistance development and drug resistance transmission at the population level (papers V and VI).
v. To assess the implications of drug resistance at the population level (paper VII).
vi. To project long-term survival in an HIV population with excellent access to treatment and care (paper VIII).
vii. To assess the impact of non-HIV related morbidity on the prognosis for HIV patients in a health system delivering high-quality HIV care (paper IX).

2.2 Scope of this work related to my PhD thesis

The work that was part of my PhD thesis (papers III, IV, and VI) was focused primarily on biomarkers, i.e. virological control and virological failure, and associated risk factors and prognosis, and was based on DHCS only. Papers V and VII added resistance data from DHSD, allowing these studies to detail and explore previous findings of time trends and risks by relating these to specific resistance mutations. Papers I, II, VIII, and IX took a much broader view and compared findings from DHCS with other populations. This expansion in scope reflected the evolving focus from the individual with HIV to populations with HIV. By taking advantage of the unique availability of other databases, the latter papers allowed us to study pertinent questions such as: How do we identify those at increased risk of HIV in a mixed clinical population? (paper I); What is the positive effect of being diagnosed with HIV within a good healthcare system? (paper II); How long can persons with HIV expect to live compared to the general population? (paper VIII); and What would have been the impact of HIV if the comorbidity pattern had been similar to that of the general population? (paper IX).
3 DATA SOURCES

3.1 The Danish HIV Cohort Study (DHCS)

DHCS is an open, prospective, population-based cohort (20, 22), initiated in 1998 as a collaborative effort by Denmark’s eight HIV treatment centres (23). Data going back to 1 January 1995 were retrieved from patient files and entered into the database. Hence, the cohort 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 (24–38), namely individual characteristics, biochemical test results, treatment history, and clinical events. DHCS was founded at Aarhus University Hospital. It was later moved to Odense University Hospital and is currently, as of 2014, based at Copenhagen University Hospital Rigshospitalet. Physicians and research nurses collect clinical data at the participating clinics. The individual identity is kept anonymous, but an identification link exists locally at each participating clinic, to detect double counting when a patient moves between clinics. Crosschecking 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. DHCS covers the whole country and is virtually complete.

3.2 DHCS Greenland

A database with the same design as DHCS was established in Greenland in 2003 (39). To include every HIV-infected individual seen at Greenland’s clinics since 1995, personal contact was initiated with all 18 district health clinics, and old patient files were retrieved by searching the archives of the Venereal Disease Clinic at Dronning Ingrid’s Hospital in Nuuk. Doctors from this clinic were responsible for HIV treatment and care during the first years of the epidemic (40). The files thus obtained were compared with the records collected by the Chief Medical Officer of Greenland (41–44). 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.

3.3 Danish National Patient Registry (DNPR)

DNPR was established in 1977 and covers all Danish hospitals and records all hospital admissions, and diagnoses. Since 1995, all outpatient and emergency visits are registered as well (45). The DNPR covers both private and public hospitals.

3.4 Danish Civil Registration System (DCRS)

DCRS is a national registry of all residents of Denmark and Greenland, containing information on date of birth, sex, immigration, residency, date of migration, and death (46, 47). Each individual is assigned a 10-digit personal identification number (CPR number). DCRS is updated within less than a week after a person is born, changes address, dies, or emigrates. We used CPR numbers to link data between the registries.
3.5 Danish Cancer Registry (DCR)

DCR has recorded all incident cancers in Denmark since 1943, classifying cancers registered after 1977 according to ICD-10(48).

3.6 Danish HIV Sequence Database (DHSD)

DHSD is a prospective, nationwide, population-based database of all genotypic HIV drug resistance tests performed in Denmark after 31 December 1999.

4 METHODOLOGICAL CONSIDERATIONS

4.1 Utility of the DHCS cohort

A cohort is a group of individuals who are followed over a period of time (49). A cohort study may be experimental, for example a randomized clinical trial (RCT), or non-experimental (synonymous with observational study). The Danish HIV Cohort Study (DHCS) is a non-experimental cohort study(50), and it is prospective, because it is assembled in the present and followed into the future(51). Individuals in the cohort compose the study base; in DHCS the study base is all HIV-infected persons in Denmark and Greenland(20,22,39). 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 (52). Even though RCTs are considered the gold standard for comparing the efficacy(53) of drugs and other treatments, observational studies of HIV confer a number of distinct advantages over RCTs(54,55). They provide information on the clinical history and spectrum of HIV disease, they are useful for exploring patterns of antiretroviral drug use (56) and monitoring the course of side effects (37,57), and they give an opportunity to examine questions as they crop up (58). Further, in contrast to the efficacy(53) examined by RCTs, observational studies shed light on the effectiveness of treatment. Thus, advantages of DHCS include the ability to study population-based prevalences and incidences, as well as population trends over time (papers II, III, V, and VI). Furthermore, the unique personal identifier enables linkage to numerous Danish registries (papers I, VIII, IX) (59). The size of DHCS limits studies of rare events or subgroups with rare characteristics; and results obtained in Denmark may not be generalizable to other countries because of regional differences in the composition of study populations(60,61). Data in one cohort may be compared with data in another cohort in a double-cohort study (62). Papers I, II, VIII, and IX used a double-cohort study design to compare the outcome of interest in people who were “exposed” (infected with HIV) with mortality in people who were “unexposed” (the general population). Papers III, IV, V, VI, and VII were single-cohort studies based on DHCS, with papers V and VII expanding the available information by including resistance mutation data from DHSD. An overview of data sources and study design is shown in Table 1.

Many HIV cohorts are prevalent cohorts, in which patients are included at some time point after the initiating event(63,64). In an inception cohort, all individuals are followed from the time of an initiating event, (e.g., the date of infection with HIV) (49,65). DHCS may be considered both an inception and a prevalence cohort. If the initiating event is defined as initiation of combination ART,
DHCS is an inception cohort for HIV patients initiating ART in Denmark. 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 HIV transmission, DHCS is a prevalent cohort.

Table 1

Data sources and study design for each of the nine papers.

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4.2 Outcomes

The events of primary interest in clinical epidemiology are health outcomes, for example death, disease, abnormal laboratory tests, or discomfort(51). Some outcomes are surrogate measures of the outcome of interest; low CD4+ cell count and high viral load are surrogate measures of clinical disease progression(66), and high viral load is also a surrogate measure of increased infectivity (67–70). 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+ cell count and viral load are long-established measures of disease progression to AIDS or death. (66).
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. Papers II, III, V, and VI provided descriptive information on temporal trends in health outcomes. Analytic studies test one or more specific hypotheses, typically whether exposure to a given factor is a risk factor for a health outcome. Papers I, IV, VII, VIII, and IX were mainly analytic studies. 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 VI, a primarily descriptive study, we applied an analytic approach to examine causes of the observed temporal trends in the prevalence of drug resistance carriers; and in paper III we described temporal trends in triple class virological failure and performed a multivariable analysis to identify risk factors for virological failure.

4.4 Measures of frequency and effect

In an epidemiological study, the key clinically relevant measures of event frequency are incidence and prevalence (51). Incidence is defined as the fraction of a group that develops a condition (an outcome) over a given time period. 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 on both 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 (used in paper IX) is 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 (used in paper VIII). The relative effect is measured as a relative risk, an incidence rate ratio (used in papers II, III, IV, VII, and VIII), or – when the frequency measure is prevalence – as a prevalence ratio or odds ratio (used in paper I).

4.5 Bias and confounding

An observed outcome may be affected by random error or systematic error (bias)(49). 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). Observational studies are particularly prone to bias and confounding, and rigorous assessment and control of these is imperative.

4.5.1 Selection bias

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. Selection bias has to be dealt with at the design stage, for example by selecting
only incident cases, restricting inclusion to a geographical area, minimizing the number lost to follow up or implementing a procedure to track those who drop out. Selection bias in papers I-IX was dealt with by all of the above. Many HIV observational cohorts recruit from only one or a few HIV clinic(s) where the clientele may be self-selected, may be predominantly patients with complicated or advanced disease referred from general practitioners, or may be lost to follow-up. 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. In some cohorts, requirements for informed consent may lead to selective recruitment, and persons may later withdraw their consent, leading to selective dropout.

4.5.2 Information bias

Information bias may occur if the methods of measurements are consistently dissimilar in different groups of patients(51). The study design is crucial for minimizing information bias, for example by ensuring a standardized measurement process, and by using objective, pre-defined criteria for exposure and outcome. Most HIV observational cohorts, including DHCS, retrieve information from patient files, and many exposures and outcomes (e.g., AIDS-defining events, deaths, and laboratory test results) are defined from objective criteria, all of which will tend to minimize information bias. Some data depend on the patients’ own information and are more prone to cause information bias, e.g., information on alcohol use or mode of HIV transmission.

4.5.3 Confounding

To be a potential confounder, a variable has to be an independent risk factor for the outcome of interest, it must be associated with the exposure, and it must not be an intermediate variable. Several variables are related to health outcomes and therefore commonly act as confounders in HIV cohort studies.

These include for example AIDS, mode of infection, co-infection with hepatitis C virus (HCV), age, time on ART, time since HIV diagnosis, and observation year. Figure 1 shows an analysis of the cohort used in paper VIII and depicts how the prevalence of confounders in DHCS vary according to age, for all persons observed during 1995-2006. It draws attention to the complexity of clinical epidemiological studies of HIV. The fatal natural history counteracts with the continuous emergence of improved treatment options, rendering it highly important for researchers to know the details of their cohort. Incidence rates may change considerably when analyses are stratified by the observations’ position on different time scales (papers III and VIII).

Confounding control can take place both at the design stage or analytical stage, using tools such as randomization, matching, exclusion, restriction in design, restriction in analysis, standardization, stratification, and multivariable analysis and modelling(71). With the exception of randomization, all of the above methods were used in papers I-IX.
Potential confounders in DHCS and their variation over time. X-axis: observation year, time scale = age. Y-axis: prevalence of covariates at each observation time point.

4.5.4 Bias in prevalent cohorts

Some types of bias relate specifically to prevalent cohorts. Length-biased sampling (49,51) may occur because patients at increased risk of death will have shorter disease duration between HIV infection and death and therefore be underrepresented in the prevalent sample. Differential length-biased sampling (63,64) 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 (e.g., HCV coinfection) 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 (72). Another type of bias, onset confounding arises when a covariate is associated with the initiating event. 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). Results may be biased in both directions depending on the direction of the effect of the covariate. To avoid bias related to prevalent cohorts, we restricted our study populations to either incident cases of HIV diagnosis (papers I and IX) or incident cases of ART initiation (papers III-VII).

4.6 Statistical analyses

4.6.1 Comparing individual characteristics

Individual characteristics between study groups were compared using the chi-square test for categorical variables, and the Student’s t-test or one-way analysis of variance for continuous variables.

4.6.2 Comparing outcomes

Time-to-event analyses were used to estimate incidence rates of TCF, mortality rates, and cumulative incidence proportions (papers III, IV, VII, VIII, and IX), using the Cox proportional hazards regression and log-rank test to compare outcomes between groups. A time-to-event model with left truncation was used to estimate incidence rates on two different timelines (papers III and VIII). Logistic regression was used to compare proportions with undetectable viral load (paper IV), and conditional logistic regression was used in a matched case control design to compare the odds of subsequent HIV diagnosis (paper I). Trends over time in incidence rates and prevalence were estimated with Poisson regression (papers II, III, V, and VI), and changes over time in CD4+ cell count were estimated in a linear regression model (paper IV). Population attributable risk was used to estimate the proportion of deaths attributable to comorbidity acquired before HIV diagnosis, and interaction risk was used to estimate the interaction between the effects of HIV and comorbidity on mortality (paper IX).

4.7 Data safety end ethics

Establishment of DHCS and the linkage to other registries were approved by the Danish Data Protection Agency (journal number 2012-41-0005). As none of the studies included direct patient contact, approval from the national or regional committees on health research ethics were not required. Data were handled and protected in compliance with Danish law (73).

5 EARLY DETECTION

5.1 Background

Early detection is one of the cornerstones of optimal HIV management both at the individual and on the population level(74). Detecting persons with HIV as early as possible will allow for timely initiation of ART and lower the risk of disease progression in the individual(74,75). Persons initiating ART at very low CD4+ cell counts are at higher risk of death(76,77) and take longer to experience good immune reconstitution(78–80) than those commencing therapy with higher CD4+ cell counts.
Most current guidelines, including those published by the World Health Organization (WHO), recommend ART initiation when the CD4+ cell count falls below 350 cells/mcl \((81,82)\). Further, timely and adequate prevention efforts require knowledge of where the next new infection is most likely to be \((83–85)\). As local HIV epidemics change over time, early detection is an important tool in mapping this. Finally, with recent evidence that ART can effectively reduce the risk of HIV transmission\((86)\), there are speculations that bringing down the population viral load by comprehensive treatment of all persons infected will lead to fewer new infections and thereby have a positive effect on the HIV incidence \((87–90)\). Whether such a “treatment as prevention” strategy is feasible\((91)\), cost-effective \((92,93)\), and ethically acceptable\((94,95)\), and in which populations and areas it might be recommended \((96,97)\), is still up for discussion\((98–102)\). Also not known is the coverage level required, and the potential added impact on HIV-associated comorbidities such as tuberculosis \((103,104)\). Numerous modelling exercises are being conducted, and at least three cluster-randomized trials are underway to give answers to some of the above-mentioned questions \((97,105–111)\).

### 5.2 Trends in late diagnosis and late presentation

#### 5.2.1 Epidemiology

A considerable barrier to optimal HIV care in both high-income countries (HIC) and low- and middle-income countries (LMIC) are the many late presenters \((112–117)\), defined as presenting to HIV care with a CD4+ cell count below 350 cells/mL or with an AIDS-defining event \((74,118,119)\). These are either diagnosed late \((120–124)\), or the time from diagnosis until they reach clinical care is long. Although improvements are observed in some countries \((112,114,121,125)\), recent reports estimate late diagnosis and/or late presentation to occur in 35-60% of newly diagnosed \((112,126,127)\), similar to 2005 figures for Denmark \((128)\).

#### 5.2.2 Risk factors

Those who are diagnosed late are more often males, older, with low education level and low socioeconomic status, and belong to marginalized groups such as immigrants \((115,120,121)\). They often do not perceive themselves at risk of infection or have not gone for testing due to fear of the disease itself and of stigmatization\((90,115)\), and they have not routinely been offered HIV testing\((121)\). In addition, many do not have easy access to HIV testing facilities. Timely diagnosis, on the other hand, has been associated with belonging to a known risk group such as men who have sex with men (MSM) or injecting drug users (IDUs) \((120,129)\), and perceived effectiveness of treatment \((115)\).

Those who are late presenters will naturally share the risk factors of those who are diagnosed late \((121)\). Specific additional conditions associated with longer time from diagnosis until care are IDU \((129)\), lack of disclosure of HIV status to spouse or partner, and being unmarried \((130)\). Associated with early presentation are current pregnancy, having young children, and consuming alcohol in the previous year \((130)\).
5.2.3 Clinical and economic consequences:

The consequences of late presentation are grave (131). Late presenters have higher rates of morbidity (132) and mortality (75,133–135), and they are more likely to be admitted to the Intensive Care Unit (123). They have higher likelihood of poor adherence, exacerbated by the same factors that contribute to their late diagnosis such as lack of knowledge on HIV and the benefits of highly active antiretroviral therapy (74,116). They are also more likely to transmit HIV, not only because of the high viral load when not on ART (68,136), but also because they have low general awareness of the risk of transmission (137). Finally, these medical conditions translate into higher medical costs (138–140).

5.3 Boosting early diagnosis and presentation for care (paper I)

5.3.1 Testing strategies

More than 30% of persons with HIV in Europe are estimated to be undiagnosed (141). To turn the epidemic, we need to diagnose more persons earlier and make sure they present to clinical care without undue delay. The optimal screening strategy will depend on the nature of the local epidemic: transmission patterns, risk groups, healthcare system, and cultural norms. Client-initiated screening (opt-in) voluntary counselling and testing has been the dominant form of testing for many years. However, due to the often disappointingly low uptake of testing by this strategy, provider-initiated “opt-out” counselling and testing (142,143) is now being widely introduced in various forms (144).

A number strategies that could permit earlier testing are currently being recommended or used in low-prevalence countries (90,143,145,146). These include screening of high-risk groups such as MSM, IDUs, and sex workers; universal screening in selected healthcare facilities such as patients in sexually transmitted disease (STD) clinics (147), pregnant women in antenatal care facilities, and persons newly-diagnosed with viral hepatitis or tuberculosis (121); and symptom-guided screening in all healthcare facilities based on selected indicator conditions associated with high risk of HIV infection (87,148,149). Further, newer self-testing technologies recently being approved in the United States (150–153) might be able to reach populations who would not reach a medical facility for testing, or be used more frequent than facility-based services and thereby lead to earlier detection of persons with HIV (154). To have the desired effect, however, emphasis must be on linking the self-test to timely HIV care.

5.3.2 Indicator condition-based HIV testing

Conditions and diseases that should lead to HIV testing fall into three basic categories: conditions for which, in case the presence of HIV infection is identified, their clinical management can have deleterious consequences for the individual; conditions that are AIDS defining in persons with HIV; and conditions that are otherwise associated with high prevalence of undiagnosed HIV. The latter two categories are called indicator conditions. As the prevalence threshold above which testing has been shown to be cost-effective in high-income settings is 0.1% (155–157), this has become the target prevalence in studies of new indicator conditions. With the greatly varying economic status and HIV prevalence in countries affected by HIV, the set of indicator conditions that should lead to
an offer of HIV testing will naturally be different from one setting to another. Apart from AIDS-defining illnesses, some of the conditions first shown to be associated with HIV prevalence higher than 0.1% were Guillain Barré syndrome / acute inflammatory demyelinating polyneuropathy (158,159), unexplained fever (160), visceral leishmaniasis (161,162), candidaemia (163), community-acquired pneumonia (164) mononucleosis-like illness(165–167), HCV infection (168,169), anal or cervical cancer or dysplasia(170–173), herpes zoster (174,175), malignant lymphoma (176), and psoriasis (177). As the population effect of modern ART kicked in during the first decade of this millennium, HIV and public health experts and advocates expressed several calls for action to identify and implement better strategies for early detection (178,179).

With access to complete diagnostic history for in-patients in Danish hospitals since 1977, we used a case-control design to study the association between potential indicator conditions and HIV diagnosis one, three, and five years later (paper I). We identified a broad range of conditions with an adjusted odds ratio (aOR) of being diagnosed with HIV at between 3.0 and 94.7. With the controls in our study population identified by incidence density sampling, the OR was a direct estimate of the relative risk of HIV. We confirmed already known associations between HIV and polyneuropathy (aOR=4.52), candida infection (aOR=25.5), lower respiratory tract infections (aOR=3.98), mononucleosis (aOR=8.64), hepatitis B and C (aOR=23.6), herpes zoster (aOR=33.7), and lymphoma (aOR=5.83). Other broader disease groups which we identified as having an increased risk of HIV were “STIs and viral hepatitis” (aOR=12.3), “CNS infections” (aOR=3.44), “skin infections” (aOR=3.05), “other infections” (aOR=4.64), and “haematological diseases” (OR=4.28). Detailing the above disease groups, we identified a number of specific potential indicator conditions who all had adjusted ORs above 10: opioid abuse (aOR=43.5), hepatitis A (aOR=41.6), thrombocytopenia (aOR=24.0), endocarditis (aOR=23.2), bacterial meningitis (aOR=14.7), seborrheic dermatitis (aOR=11.8), and drug poisoning (aOR=11.2). Our data allowed us to look at future HIV risk at various distances in time from the occurrence of the indicator condition (Figure 2). Thrombocytopenia, seborrheic dermatitis, and bacterial infections are manifestations of the HIV infection and were highly associated with being diagnosed with HIV during the coming year and less so during the 3 to 5-year period. Substance abuse, hepatitis A, and drug poisoning, on the other hand, were associated with an almost constant 5-year long increased risk of HIV diagnosis. These conditions share behavioural risk factors with HIV and are therefore indicators of not only current HIV but also of future HIV acquisition.

As a response to the urgent need for guidance, the HIV in Europe Initiative, with contributions from the European Center for Disease Control and the World Health Organization published in late 2012 a guidance for indicator-based HIV testing (180). While there is evidence of undiagnosed HIV prevalence of >0.1% for some of the recommended indicator conditions, many of the indicators are included based on the opinion of experts who consider them likely to be associated with an HIV prevalence of >0.1%. The document acknowledges the paucity of evidence to robustly identify indicator conditions, and the document is likely to be modified during the coming years as we gain more knowledge.

Published in 2013, a case-control study using the UK-based general practice database THIN (The Health Improvement Network) tested the 37 indicator conditions recommended in the UK National
Guidelines for HIV testing 2008 (178), and found 12 of these to be associated with HIV infection (181). Another recent study from England and Wales found an HIV prevalence of 2.4% among persons with invasive pneumococcal disease (182), while a small Spanish study tested a strategy of four indicator diseases and found an HIV prevalence of 4.7% (95% CI 1.3%-11.6), corresponding to a cost per new diagnosis of only €129 (183).

**Figure 2**

*Forest plot of selected indicator diseases showing how some risk estimates may vary depending on time to future HIV diagnosis. (Source: paper I)*

<table>
<thead>
<tr>
<th>Disease category</th>
<th>aOR (95% CI)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-5 y prior to i.d.</td>
<td>&lt;1 y prior to i.d.</td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>11.2 (7.74–16.2)</td>
<td>25.0 (16.5–37.7)</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>1.56 (0.89–2.71)</td>
<td>10.5 (7.53–14.7)</td>
</tr>
<tr>
<td>CNS infections</td>
<td>1.86 (0.42–8.25)</td>
<td>5.25 (1.96–14.1)</td>
</tr>
<tr>
<td>Skin infections</td>
<td>3.96 (2.91–5.37)</td>
<td>4.81 (3.44–6.73)</td>
</tr>
<tr>
<td>Other infections</td>
<td>2.49 (1.81–3.43)</td>
<td>11.6 (8.94–15.0)</td>
</tr>
<tr>
<td><strong>Hematological diseases and cancers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>2.05 (1.07–3.92)</td>
<td>8.80 (5.73–13.5)</td>
</tr>
<tr>
<td>Non-AIDS defining cancers</td>
<td>0.81 (0.38–1.71)</td>
<td>2.37 (1.50–3.74)</td>
</tr>
<tr>
<td><strong>Substance abuse and poisoning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance abuse</td>
<td>3.33 (2.44–4.56)</td>
<td>3.07 (2.15–4.39)</td>
</tr>
<tr>
<td>Poisoning</td>
<td>3.37 (2.41–4.69)</td>
<td>3.65 (2.42–5.51)</td>
</tr>
<tr>
<td><strong>Other disease categories</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear, nose, and throat diseases</td>
<td>1.87 (1.41–2.47)</td>
<td>2.46 (1.81–3.35)</td>
</tr>
<tr>
<td>Skin diseases</td>
<td>1.43 (0.93–2.20)</td>
<td>2.57 (1.70–3.89)</td>
</tr>
<tr>
<td>Gastrointestinal diseases</td>
<td>1.18 (0.94–1.48)</td>
<td>2.08 (1.67–2.58)</td>
</tr>
<tr>
<td>Eye diseases</td>
<td>1.48 (1.01–2.19)</td>
<td>1.31 (0.82–2.11)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>1.11 (0.66–1.85)</td>
<td>1.61 (0.99–2.59)</td>
</tr>
<tr>
<td>Kidney diseases</td>
<td>1.21 (0.67–2.18)</td>
<td>0.93 (0.49–1.77)</td>
</tr>
<tr>
<td>Non-IHD vascular diseases</td>
<td>0.97 (0.69–1.36)</td>
<td>1.50 (1.12–2.01)</td>
</tr>
<tr>
<td>IHD</td>
<td>0.82 (0.39–1.70)</td>
<td>1.14 (0.62–2.08)</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>0.61 (0.37–1.01)</td>
<td>1.34 (0.90–1.98)</td>
</tr>
<tr>
<td>Trauma</td>
<td>0.92 (0.81–1.04)</td>
<td>1.05 (0.91–1.21)</td>
</tr>
<tr>
<td>Rheumatological diseases</td>
<td>0.84 (0.67–1.05)</td>
<td>0.70 (0.54–0.91)</td>
</tr>
<tr>
<td>Non-diabetic endocrine diseases</td>
<td>0.69 (0.38–1.25)</td>
<td>0.78 (0.46–1.30)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.69 (0.35–1.35)</td>
<td>0.27 (0.11–0.66)</td>
</tr>
</tbody>
</table>

The adjusted odds ratio of subsequent HIV diagnosis for 22 major disease categories with 95% confidence intervals was determined by conditional logistic regression (with adjustment for all other disease categories in the same observation period). Only cases and their respective controls under observation from the beginning of the strata up to the index date were included in each of the analyses. For each disease category three risk estimates are shown as squares with the corresponding 95% confidence intervals shown as lines. In the order from top to bottom the three observation periods are: <1 year prior to the index date; 1–2 years prior to the index date; 3–5 years prior to the index date. Cases (n=2,036) and controls (n=35,718). The exact risk estimates for the first and last observation period are shown in the two columns. aOR, adjusted odds ratio; CI, confidence interval; i.d., index date; STIs, sexually transmitted infections; IHD, ischaemic heart disease.
The HIV in Europe Initiative (149) is currently running the HIV Indicator Diseases across Europe Study (HIDES), which aims to identify indicator diseases and develop a model for implementation of targeted HIV testing in clinical settings. Eight conditions tested in HIDES I were all found to be associated with an HIV prevalence of >0.1% (148). These included STIs, malignant lymphoma, cervical dysplasia or anal cancer, herpes zoster, hepatitis B or C, ongoing mononucleosis-like illness, unexplained leukocytopenia/thrombocytopenia, and seborrhoeic dermatitis/exanthema. HIDES II is now expanding to >50 sites across Europe and will include, in addition, the diseases hospitalized pneumonia, unexplained lymphadenopathy, peripheral neuropathy, lung cancer, and recalcitrant psoriasis (184).

5.4 Conclusion

Late diagnosis and late presentation to clinical care continue to be major barriers to improved HIV management, and we need to find ways to identify those who are not yet diagnosed. New testing strategies must be tailored to the settings in which they are to be used to ensure they are feasible and cost-effective. The body of knowledge on indicator conditions is increasing rapidly, and studies confirming previous findings and adding new pieces to the puzzle continue to improve our knowledge on where and when indicator condition-based testing can be applied and have a positive effect. Thus, indicator condition-based testing may turn out to be a valuable addition to the existing practice in many settings.

6 ACCESS TO GOOD HEALTH CARE

6.1 Background

Before a person with HIV can get the life-saving medication, the drugs need to be available, accessible, and affordable. While this is the case in most high-income countries, successful roll-out of antiretroviral therapy (ART) furthermore requires a functional health care system with trained medical staff (185,186). Some evidence suggests that personal support from friends, family and peers improves drug effectiveness even further (187). Sub-optimal health care systems are most prevalent in poorer parts of the world (188), but they also exist in wealthy countries. Washington DC has recently been portrayed as an example of a city with poor access to care for many of its HIV-infected population (189). The causes of poor ART uptake are often complex and not immediately visible, and there is an increasing need to identify these barriers.

6.2 Uptake of antiretroviral therapy (paper II)

6.2.1 Factors associated with ART uptake

Uptake of ART is influenced by individual as well as health system-related factors. Characteristics such as sex (being male), age (being younger), and employment status (being unemployed) and homelessness are associated with lower ART uptake (190–192), but also less visible, psycho-socio-cultural issues such as illness ideology, unfamiliarity with chronic disease management, depression, interpersonal challenges, stigma, and values of church or marriage have been shown
to provide barriers to ART initiation (192,193). System-related factors include distance to the nearest clinic (191), waiting times for medical care (194–196) poor linkage between HIV testing and HIV care and treatment services, and shortage of HIV/AIDS specialists (197). While psycho-socio-cultural barriers are found in both LMIC and HIC, the economic and health system barriers are predominantly described in LMIC (198).

**Figure 3**

*ART introduction in Denmark and Greenland 1996-2006. Numerator, proportion of patients who were receiving antiretroviral therapy as part of a highly effective ART regimen on Jan 1st each year. Denominator, all patients under observation. NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.* (Source: paper II)
6.2.2 ART uptake in Greenland

The health care system in Greenland is well funded, public, and with free access for all citizens (39). Health care provision, however, is challenged by the vast geography and staffing issues. Fifty-six thousand people live in 74 towns and settlements between which transportation is only possible by air or sea, making them frequently inaccessible due to bad weather; only 18 of these have facilities with permanent physician staffing, and frequent use of locums impedes the consistency of care for patients with chronic conditions. We compared the uptake of ART and changes in HIV mortality over time in Greenland with Denmark, its former colonial power (paper II). Both are high-income countries with public health care offered free of charge, and the Greenland health care system is staffed with physicians trained in Denmark. We found that ART introduction had been delayed in Greenland, with the total coverage level among persons with HIV only catching up in 2003, and with newer combination regimens such as those including ritonavir-boosted protease inhibitors reaching levels in Denmark only in 2006 (Figure 3).

These patterns were also found among the proportion with suppressed viral load, and reflected in the mortality rates that dropped dramatically in Denmark in years 1998-2000 to 29/1,000 per year, and although steadily declining in Greenland were still 59/1,000 per year in 2004-2006. A later study found a slight further decline in mortality until 2011 of 53.4/1,000 per year (199).

6.3 Conclusion

Despite similar levels of health worker education and economic resources, ART implementation and mortality decline in Greenland lacked several years behind Denmark. Geography, lack of consistency in clinic staffing, and difficult infrastructure with less access to advanced laboratories, hospital care, and HIV specialist clinics most likely bear part of the cause. Furthermore, the HIV epidemic in Greenland is characterized by a mainly middle-aged, heterosexually transmitted population with low socio-economic status, and these issues related to the individual might have further challenged timely and effective introduction of new treatments. While we were not able to single out the main reason for the observed differences, the study reminded us that although economy may be a prerequisite for implementing an effective HIV care system, it is certainly not all it takes.

7 VIROLOGICAL CONTROL

7.1 Background

The goal of modern antiretroviral therapy is continuous suppression of HIV replication in the body, so-called virological control. This will delay the HIV-induced deterioration of the immune system (81) and postpone or even avoid immune-deficiency-related morbidity and eventually death. Assessing the drug efficacy in persons on ART is done by regular measurements of the amount of virus (HIV RNA) in the blood (viral load), which serves as a proxy measure for HIV replication in the cells.
Obtaining and sustaining virological control can be challenging. Primarily, the prescribed drugs must be efficacious and able to suppress HIV replication, but HIV is a chronic disease, and the drugs must remain effective and acceptable to the patient also after long-term use. Factors that determine long-term effectiveness include properties of the ART combination used, the genotype and phenotype of the dominant HIV strain in the individual, and the person’s ability to adhere to the prescribed treatment. Some drugs have lower efficacy and are less forgiving if one or several doses are missed, some are more likely to cause side effects, and some should be taken twice or even three times daily. Some viral strains are resistant to the most commonly used drugs, and some patients are highly burdened by side effects, find it very difficult to take medication at designated hours, or find that psychosocial aspects of their life keep them from adhering to a rigorous treatment scheme.

7.2 ART combinations

The first ART combinations that were able to induce virological control for more than a few months consisted of a PI and two nucleoside reverse transcriptase inhibitors (NRTIs)(11), so-called PI-based regimens. Later came regimens based on NNRTIs or ritonavir-boosted PIs (200–202), and more recently we have seen the advent of the newer drug classes (203) such as fusion inhibitors (204), integrase inhibitors (205–209) and entry inhibitors(210,211). Each new drug or drug combination has offered improvements in terms of fewer side effects, lower long-term drug toxicity, lower pill burden, or more forgivingness to inconsistent adherence with lower risk of drug-induced resistance development (212–216). Other new drugs have found their place in “salvage therapy” to persons who harbour multi-drug resistant virus, often due to a long and complex treatment history (217,218).

7.3 Triple-class virological failure (paper III)

Along with the advent of efficacious drug combinations around the millennium came a decrease in mortality (77,219–221), but also a concern about how long the effect would last. When people failed their first regimen, they had to start so-called second-line or third-line therapy because their virus had become drug-resistant and thereby rendered previously administered ART combinations ineffective. Experts were uncertain as to whether this evolvement would be avoidable even in the individual with perfect treatment adherence, and whether the invention of new antiretroviral drugs could keep up with the rate of resistance development and drug failure in the population. Early studies showed multi-drug class failure to be associated with poor prognosis (222), but only little was known about the incidence and prevalence of virological failure (223).

In paper III, we estimated time trends in both incidence and prevalence of drug failure towards three drug classes, so-called triple-class failure (TCF). Many studies of time trends in failure up to 2005 had been rather pessimistic with regard to failure rates and prevalence at the population level (224,225), but most of these studies had been prone to bias because their design was cross-sectional and based on observations from a single clinic. These cohorts are often ill suited for measuring temporal trends because they have a higher accumulation of difficult cases. DHCS, with its nation-wide design, is much less prone to this type of bias. In paper III, we found a declining
The population incidence of TCF during the years 1997-2003. When looking at incidence rates according to *time since ART initiation*, the incidence of TCF was declining from the 4th year onwards. Later studies have come to similar conclusions. The Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Group found a rising TCF incidence until 2005, followed by a decline during the subsequent 4 years until 2009. In another large study containing almost 46,000 observation years, the same group found the cumulative incidence of TCF to be 3.4% at 5 years and 8.6% at 9 years after starting ART, and a study from the UK Collaborative HIV Cohort (UK CHIC) containing >27,000 observation years found a 9.2% risk of TCF after 10 years of ART (226). These results correspond well to the cumulative incidence of 7.0% after 7 years of ART that we found in paper III (227). Of note, DHCS is part of COHERE but contributes less than 10% of total patient years, so this cohort overlap has only contributed marginally to the similar findings in the two studies.

### 7.4 Consequences of suboptimal virological control (paper IV)

The risk of virological failure in HIV populations with access to well-functioning health care is now quite low (paper III), but a proportion does not obtain full virological control (228–230). Getting full control requires a tailored ART combination that suits the individual in terms of virus susceptibility, side effects, and pill burden. Despite such “technical optimization” with regard to efficacy, however, some individuals experience temporary or permanent viraemia.

We do not always know the causes of these single or repeated episodes of detectable viral load, but we need to know their clinical relevance and role as predictors of long-term effectiveness. Many other predictors of poor outcome are present before starting therapy and include both biological and biochemical markers such as low CD4+ cell count, high viral load, malnutrition, and anaemia (77,219,231–233); and social determinants such as substance use (234), low socioeconomic status (235), mental disorders, and distress (236,237). Once a person has started ART, additional and valuable information is obtained from the virological response during the first 6 months, where increased viral load is associated with higher risk of death (238,239).

In paper IV, we went one step further and looked at whether virological control during the post-primary treatment period (7-18 months after ART initiation) was related to long-term clinical and paraclinical outcome. We found a clear difference in prognosis between persons with virological suppression 100% of the time (Group 1), persons with virological suppression part of the time (Group 2), and persons with no virological suppression at all during the 12-month period (Group 3). Whereas 89% of persons in Group 1 would be alive and virologically suppressed 6 years later (7.5 years after starting ART), this would be true for just 71% in Group 2 and 43% on Group 3 (Table 2).

Even the subgroup with virological suppression 75-99% of the time had a 2.17 times higher risk of death than the fully suppressed Group 1 (95% confidence interval 1.31-3.61). In all three groups, CD4+ cell counts continued to rise for 7.5 years, mostly so in Group 1. Later published studies similarly found that the number of episodes with viral rebound >500 copies/mL was inversely related to CD4+ cell count increase (240), and that the percentage of time with virological suppression was inversely related to future risk of virological failure (241).
Table 2
6-year prognosis from 1.5 to 7.5 years after ART initiation, according to level of viral suppression during the preceding 12-months. Group 1=fully suppressed, Group 2=partly suppressed, Group 3=not suppressed. (Source: paper IV)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cumulative survival from 0 to 72 months after baseline (percent) †</th>
<th>95% CI</th>
<th>Percent with VL&lt;400 copies/ml 72 months after baseline</th>
<th>95% CI</th>
<th>Percent alive and with VL&lt;400 copies/ml 72 months after baseline</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td>(90 - 94)</td>
<td>96</td>
<td>89</td>
<td>(87 - 90)††</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>(82 - 89)</td>
<td>83</td>
<td>71</td>
<td>(68 - 74)††</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>(71 - 81)</td>
<td>57</td>
<td>43</td>
<td>(40 - 46)††</td>
<td></td>
</tr>
</tbody>
</table>

Baseline: 18 months after HAART initiation
VL: viral load
†: Kaplan-Meier survival estimates
††: 95% CI estimated as [95% CI for survival] * [percent undetectable at 72 months]

7.5 Conclusion
Fortunately, the prevalence of TCF seems to have stabilized in Denmark (paper III) and in other settings (242). Hence, the concerns expressed 10 years ago that the majority of persons with HIV might exhaust their treatment options due to accumulation of multi-drug class failure have been somewhat allayed, but the risk remains relevant in many settings (243). With an estimated annual risk of TCF of 0.5-1.5% (227,242), the cumulative risk in children (244) and other HIV-infected persons with a long life ahead of them is far from negligible. Further, we must be aware that measured prevalences can be influenced downward by newly infected persons entering the population, i.e. increasing the denominator, and by increased mortality in persons with TCF, i.e. decreasing the numerator (paper VI) (226,245). Not only is comprehensive virological failure associated with increased risk of death (paper VI), a worsened long-time prognosis is also seen even after modest viraemia (paper IV). Many persons experience viraemia because they find it hard to adhere to treatment, and they often require more intensive support from the health care system or from peer groups, family or friends (187). Teams providing care for persons with HIV should keep in mind that “partial virological responders” compose a group at high risk of future failure and death and who should be given increased attention and support. Despite the positive trends, continued investments in development of new antiretroviral drugs will be required to ensure future treatment options for all persons with HIV.
8 DRUG RESISTANCE

8.1 Background

8.1.1 Emergence of drug resistance

HIV mutates heavily, and thus has the potential to mutate into new strains that are resistant towards ART. The wild type virus phenotype is the most fit and will therefore remain the dominant sub type in persons who are infected with this type, as long as they are not exposed to antiretroviral drugs. When treating with ART, though, the virus can escape pressure from the antiretroviral drugs by emergence and proliferation of new mutations that are resistant towards the given treatment (246). It is therefore ultimately important to suppress viral replication completely (81), thereby avoiding the vicious cycle of ongoing replication and subsequent emergence of drug-resistant virus.

8.1.2 Relation between ART and drug resistance

Whether an ART combination is effective in the individual depends on both behavioural and biological factors. A multitude of factors influence adherence in the individual (195,237,247–250), and each drug has specific pharmacokinetic and pharmacodynamic properties (214,251–253). Each drug selects for up to several specific mutations (252), and some mutations confer cross-class resistance (254) to other drugs within the same class. Even though it is possible to fully suppress viral load for years and thereby avoid emergence of drug resistance, longer time on ART is invariably associated with increased risk of drug resistance both in the individual (255) and at the population level (5,256) (paper V). The clinical implications of individual mutations vary (257,258), but the accumulation of multiple resistance mutations towards several drugs and drug classes is associated with poor prognosis (7,259–262) (paper VII).

8.1.3 Relation between viral load and HIV transmission

Whether exposure to HIV results in HIV transmission depends on factors such as properties of the virus, properties of the recipient's immune system, genetics (e.g., the CCR5Δ32 mutation) (263), mode of exposure, and the amount of virus that enters the recipient (264). Evidence from early observational studies supported the theory that transmission was markedly diminished with low viral load (67,70,265,266), but only recently has it been confirmed in randomized trials that the risk of sexual HIV transmission from partners on ART with fully suppressed viral load is extremely low, both through heterosexual (86) and homosexual (267,268) intercourse.

8.1.4 Public debate

Before these game-changing RCTs, it was vividly discussed which kind of advice should be given to discordant couples comprising an HIV-infected person and an HIV-negative partner. In the so-called “Swiss Statement” from 2008 (269,270), the Swiss public health authorities publicly stated: “HIV positive individuals do not risk transmitting HIV to an HIV negative partner if the person has had undetectable HIV in the blood for at least 6 months has adhered strictly to his/her antiretroviral regimen, and is free of any other sexually transmitted infections”. The statement was heavily criticized by a range of international bodies (271) for being over-concluding on available evidence.
While the above dispute predominantly concerned the transmission risk from individual to individual and the associated advice on HIV prevention, experts also discussed and studied the potential population effect of more and more persons being on ART, and the associated lower viral load in the population. The number of persons diagnosed with HIV was increasing as a result of rising or stable incidence combined with longer survival. Could it be, though, that the positive effect of better treatment coverage would counterweight this trend and thereby would result in lower population viral load, ultimately reducing transmission?

### 8.2 Time trends in drug resistance development (paper V)

With an ever-larger cumulated time on ART in the population, and an increasing number of persons no longer on first-line treatment, there was fear that this would translate into an increasing incidence of new resistance mutations (272). On the other hand, the continuous advent of new drugs less likely to induce resistance could pull the trends in the other direction, as could a natural “saturation” of mutations occurring in the individual during the first months or years of treatment. Many studies reported scaringly high prevalences of drug resistance (273–276) and increasing time trends (277), but these studies were often cross-sectional in design and based on data collected from convenience-sampling. Thus, drawing conclusions on the incidence of new mutations was not possible.

As HIV genotyping started to become a more frequent procedure, large resistance databases became a valuable tool to estimate not just prevalence, but also incidence of new mutations. We used DHCS and DHSD to create a nationwide, Danish data set where genotypic test results were combined with clinical and paraclinical data. By applying strict criteria to when a new mutation was detected in an individual, related to when this individual had experienced virological failure, and with which ART regimen, we were able to estimate time trends in the incidence of new mutations (paper V).

We found decreasing population-based incidence rates of drug resistance acquisition during 1999-2005 for all three drug classes (Figure 4). Later studies from Italy and Switzerland have reported a decline in the prevalence of resistance-conferring mutations (215,278–280), and a very recent pan-European analysis found not only a moderate decline in the prevalence of resistance, but also a steep drop from 31% in 2000 to 1% in 2008 of persons who had exhausted available drug options (281). Even though most studies report declining trends, there may be geographical areas and subgroups in which the forecast is less optimistic. A recent Spanish study in children with HIV reported rising prevalence of resistance mutations for all three major drug classes (282), while a study from the United Kingdom found a 17% 8-year cumulative risk of any mutation, although the cumulative risk of a PI mutation among those who started ART with a ritonavir-boosted PI regimen was only 7% (283).
Figure 4
Declining incidence rates of new drug resistance mutations from 1999 to 2005 for the three major drug classes. PI=protease inhibitor. NRTI=nucleoside reverse transcriptase inhibitor. NNRTI=non-NRTI. (Adapted from paper V)

8.3 Time trends in transmission of drug-resistant HIV (paper VI)
A worst-case scenario of the population effect of introducing ART would be if drug resistance induced by antiretroviral drug pressure was widely transmitted from person to person and thereby increasing the proportion of persons newly infected with HIV who had limited treatment options already from the beginning. This concern grew along with the wide expansion of ART availability, changing thresholds for ART initiation putting more people on therapy, and the longer cumulated time on ART in the populations. Indeed, early studies found higher than 20% prevalence of transmitted drug resistance (TDR) on both sides of the Atlantic Ocean (284–288), increasing over time, and with multidrug-resistance prevalence up to 10.2% (289). Another issue adding further concern were reports of changes in risk behaviour: the general opinion that HIV was becoming a treatable disease meant that persons without HIV were less worried about protecting themselves; and the improved health among persons with HIV was leading to a more active life and sex life (290). Pulling in the other direction, towards less transmission of drug-resistant virus, was the
growing scientific evidence that persons with fully suppressed HIV replication are de facto non-transmitters (67, 265, 266), and the fact that drug-resistant viral strains are less fit and therefore possibly less likely to be transmitted (246, 261, 291, 292). Indeed, a number of studies reporting declining or stable levels of TDR were published in the early 2000s (293–297).

Based on the availability of comprehensive data on ART regimens and viral load in our cohort of Danish persons with HIV we were able to estimate temporal changes in the prevalence of persons with HIV who could potentially transmit drug-resistant virus (paper VI). We found a decrease from 1997 to 2004 in the prevalence of potential transmitters of drug-resistant HIV (Figure 5), brought about by successful re-suppression of viral load in potential transmitters as well as a decline in the incidence of drug resistance.

**Figure 5**

(Source: paper VI)
Later studies from different parts of the world have expressed diverging trends: a decline and stabilisation of TDR (298,299) and a projected stabilisation of persons with extensive triple-class failure and viral load >50 copies/mL (300) in the UK, stable TDR in France (301), and an increase in TDR in Canada (302) driven by NRTI and NNRTI resistance. Recent reports from Sub-Saharan Africa, the Dominican Republic, and other part of Latin America (303–306) indicate emergence of TDR in locations where viral load testing and resistance monitoring are not routine practices.

8.4 Consequences of harbouring drug resistance mutations (paper VII)

Virological failure, drug resistance, and mortality are intertwined. Virological failure increases the emergence of drug-resistant mutations, mutations decrease the chance of virological re-suppression (307–311) and immunological recovery (312), and the result of this vicious cycle is increased risk of death (222,259,260,262,313).

Our results should be interpreted together with a range of related findings: increased mortality in patients with multidrug-resistance (314–316); specific individual mutations related to the prognosis (317); and the link between viral load suppression, increased CD4+ cell count (318) and reduced clinical progression (319) after TCF. The prognosis after TCF has improved during later years, and recent studies point towards the introduction of new antiretroviral drugs having been the prime driver (245,320). If a patient experiences virological failure, a resistance test is used to guide the clinician in choosing a different and efficacious drug regimen. If no drug resistance is found, the clinical team will have confidence in attempting to improve adherence on the current treatment regimen. Such a drug-conservation strategy is often preferred, because a regimen change could mean one less treatment option for future use, and because second-line regimens are often more costly than first-line combinations. Unfortunately, in areas with no easy access to drug resistance tests, the clinical decision has to be made as a qualified guess, predominantly based on HIV RNA measurements. Testing for drug resistance was not yet routine in the early years of combination ART (and still is not in most low- and middle-income countries). More knowledge was therefore needed concerning the association between observed virological failure and existence of drug resistance, and on the association between drug resistance and death in persons with virological failure.

In Denmark, drug resistance testing had long been routine every time a patient experienced virological failure, so the testing pattern was less influenced by convenience sampling seen in many previous studies. Combined with the nationwide nature of the cohort, DHCS therefore provided a very good setting for assessing both the prevalence of resistance mutations in patients with virological failure, as well as the influence of these on mortality (paper VII). In our population of persons with triple-class virological failure, 88% had resistance mutations towards at least one drug class, and 61% had resistance mutations towards all three major drug classes. A high overall number of mutations as well as three individual mutations, one from each drug class, were independent predictors of death. The relationship between resistance mutations and death seemed to be mediated by low CD4+ cell count (Table 3).
Table 3

Mortality after triple-class virological failure according to number and pattern of drug-resistance mutations. (Adapted from paper VII)

<table>
<thead>
<tr>
<th>Mortality after TCF</th>
<th>Adjusted for CD4 cell count at baseline*</th>
<th>Adjusted for time-updated CD4 cell count*</th>
<th>Adjusted for CD4 cell count at baseline and influential individual mutations*</th>
<th>Adjusted for time-updated CD4 cell count and influential individual mutations*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRR  95% CI P</td>
<td>MRR  95% CI P</td>
<td>MRR  95% CI P</td>
<td>MRR  95% CI P</td>
</tr>
<tr>
<td>Number of mutations</td>
<td>&lt;=8  1.0 - -</td>
<td>1.0 - -</td>
<td>1.0 - -</td>
<td>1.0 - -</td>
</tr>
<tr>
<td></td>
<td>&gt;=9  2.3 1.1-4.8 0.020</td>
<td>1.4 0.7-2.9 0.348</td>
<td>0.9 0.4-2.3 0.896</td>
<td>0.8 0.3-1.9 0.556</td>
</tr>
<tr>
<td>CD4 count at baseline</td>
<td>&gt;200 1.0 - -</td>
<td>- - -</td>
<td>1.0 - -</td>
<td>- - -</td>
</tr>
<tr>
<td></td>
<td>50-200 3.6 1.5-8.8 0.005</td>
<td>- - -</td>
<td>3.4 1.4-8.4 0.008</td>
<td>- - -</td>
</tr>
<tr>
<td></td>
<td>&lt;50  8.6 3.0-24.7 &lt;0.001</td>
<td>- - -</td>
<td>10.5 3.6-30.5 &lt;0.001</td>
<td>- - -</td>
</tr>
<tr>
<td>CD4 count, time-updated</td>
<td>&gt;200 - - -</td>
<td>1.0 - -</td>
<td>- - -</td>
<td>1.0 - -</td>
</tr>
<tr>
<td></td>
<td>50-200 - - -</td>
<td>3.1 1.2-8.0 0.020</td>
<td>- - -</td>
<td>3.0 1.1-7.9 0.026</td>
</tr>
<tr>
<td></td>
<td>&lt;50 - - -</td>
<td>9.9 3.9-25.4 &lt;0.001</td>
<td>- - -</td>
<td>9.6 3.7-25.1 &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>T215Y - - -</td>
<td>- - -</td>
<td>3.0 1.3-7.4 0.014</td>
<td>3.0 1.2-7.0 0.014</td>
</tr>
<tr>
<td></td>
<td>G190A/S - - -</td>
<td>- - -</td>
<td>2.7 1.2-6.4 0.023</td>
<td>1.7 0.7-3.9 0.228</td>
</tr>
<tr>
<td></td>
<td>V82F/A/T/S - - -</td>
<td>- - -</td>
<td>1.1 0.5-2.7 0.760</td>
<td>0.8 0.4-1.9 0.656</td>
</tr>
</tbody>
</table>

Cox regression analysis of time to all-cause death after triple-class virological failure (TCF). *Further adjusted for log(10) viral load (VL) and age at baseline, gender, year of TCF, and being antiretroviral therapy (ART)-naive at initiation of highly active ART. CI, confidence interval; MRR, mortality rate ratio.

8.5 Conclusion

The fear of a vicious cycle of virological failure → drug resistance → transmission of resistance → virological failure remains relevant in 2014. Local epidemics are not yet under control (321), and while the positive reports are examples of what can be achieved in privileged settings; the negative reports remind us of how much needs to be done to get every corner of the epidemic under control. Our findings in papers V and VI underline the crucial importance of always having an effective treatment option available for the patient with drug-resistant virus. Access to viral load testing and resistance monitoring needs to be expanded to allow every person with HIV initiate ART with an efficacious drug combination, detect virological failure in time, and change to the best possible second or third line regimens when needed.
9 LIFE EXPECTANCY

9.1 Background

9.1.1 Improved HIV management

The decade around the millennium saw a tremendous improvement in HIV management. Drugs and drug regimens became more efficacious and effective, they were less likely to induce resistance if a pill or two were forgotten, they had fewer side effects, and one tablet a day drug combinations became available (200,202,203,213,216). Overall, it was becoming easier to live with HIV, in particular for those who were not infected with drug-resistant virus and who were to receive ART for the first time. Not only had the drugs become better, medical staff was also learning how best to use the available drugs and monitoring tests. Further, being an HIV patient in a state-of-the-art clinic meant access to psychosocial support such as counselling and peer groups, important ingredients in the package offered to persons with HIV (187).

9.1.2 Reduced mortality

Positive reports were emerging, comparing mortality rates in successfully treated HIV-infected persons with those of the general population. Mortality rate ratios were as low as 3-10 and thus approaching those of other chronic diseases (39,219,322,323), and in a subgroup of the Swiss HIV Cohort Study the excess mortality rate was found to be below just 5 deaths per 1,000 persons per year (324). A computer model estimated that patients in the Collaborations in HIV Outcomes Research/US (CHORUS) cohort had a median survival from diagnosis to death of 20.4 years (325), about twice as long as in untreated patients.

9.1.3 The need to plan for the future

As persons with HIV were now clearly surviving longer in countries where HIV treatment was available (220), affordable and accessible, many started to ask the question of how long they might expect to live. Many persons with HIV were banned from privileges which most persons take for granted such as obtaining mortgage, life insurance or even health insurance, because HIV was still largely seen as a disease which would only allow you few years of survival. Other important decisions such as having a child or saving up for retirement pension might also become relevant and necessary if death was no longer near coming.

There was a strong desire among persons with HIV to know their life expectancy, but many experts considered available knowledge too uncertain to try to answer this question. The first highly effective ART combination regimens had been introduced in 1996-1997 (11), so any predictions would be based on less than 10 years of experience. There were many unanswered questions: How long would people be able to adhere to treatment? Would any effective treatment eventually lead to virological failure? Would mutations emerge, either being a result of the failing treatment or even be the primary cause of failure? Would the speed of new and more effective drugs arriving on the market be able to keep up with the accumulation of drug mutations in the population? While it was likely that we would continue to see individual success stories, might one dare hope that this would translate into substantial improvement on the population level?
9.2 Long-term survival (paper VIII)

9.2.1 Measuring survival

Whatever method used to estimate survival in a population, it has shortcomings. A cohort life table created from direct observation of mortality in a very large cohort followed for a very long time will allow for accurate estimates of both median survival time (the time until half of the studied population has died) and life expectancy (the arithmetic mean of the survival times (326)) in that cohort. Due to the many years of data collection involved in this approach, the measurements would likely be outdated and therefore not useful to predict survival in a current cohort. Another approach is to use a period life table, whereby age-specific mortality observed in a cohort over a shorter period of time is assumed to apply to an individual over a life span (327). The clear disadvantage of this approach when studying the effect of ART on population survival is that we estimate survival way beyond the current maximum experience with ART. A third approach is to use mathematical computer models. These models, based on cohort data, can apply different assumptions to the effect of uncertain and potentially influential factors such as future effectiveness of ART and drug-resistance development, but their reliability is highly dependent on the quality of the data that are driving the assumptions that go into the model.

9.2.2 Survival in persons with and without HIV

In Paper VIII, we used period life table methods to estimate age-specific mortality rates and median survival for the entire DHCS cohort in different time periods, compared with a cohort from the general population. The previously described advantages of DHCS being nationwide and with very few persons lost to follow-up allowed us to interpret the results as a largely unbiased picture of the impact of a cross-healthcare system effort to manage HIV. We found a clear improvement in survival over time from the period before availability of highly effective combination ART (1995-1996), to the period immediately thereafter (1997-1999), with further improvements to the period after 2000 when highly effective ART had become established across the health care system. Most importantly, we estimated that a 25-year old person with HIV had a 50 percent chance of surviving another 32.5 years (Figure 6) if he/she was under care in the period 2000-2005.

In DHCS, HCV coinfection is correlated to injecting drug use (IDU) and is a marker of family-related increased risk of death (328,329). During the period 2000-2005, persons with HCV represented 16% of the cohort. The median survival for a 25-year old HIV/HCV coinfected person was just 20 years (Table 4), whereas for a person with HIV but without HCV infection the median survival was 39 years, only 12.2 years less than in the matched general population cohort. We saw no signs of increased mortality with increasing years on ART, nor with increasing time after diagnosis. Thus, although we had less than 10 years observation of any individual, we carefully assumed that our results were a valid estimate of long-term survival.
9.2.3 Current status

Not surprisingly, studies estimating life expectancy and mortality have been booming in later years. The gap between persons with HIV and the general population is consistently reported to have declined over time, shown in single- and multi-cohort studies from China (330), the United States (76), and across Europe(76,331). The difference in life expectancy between persons with HIV and the general population reported in recent studies range from 7 to 18 years (332–337). In selected subpopulations for example non-IDUs or persons with good CD4+ cell response to ART or high CD4+ cell count at ART initiation, life expectancy approaches (338), equals(333,339–342) or in some cases even exceeds (332) that of the general population. Recent review studies come to the conclusion that the life expectancy for persons with HIV equals that of the general population “under ideal circumstances” (343–346). A recent Australian study showed no signs of increasing standardized mortality ratios (SMRs) in persons who had been on ART for up to 15 years (347),
compared to the general population. Further, in those countries most affected by HIV, we are even 
beginning to see the overall population impact. In Kwazulu-Natal in South Africa, life expectancy 
increased from 49.2 years in 2003 to 60.1 years in 2011, predominantly due to the introduction and 
expansion of ART (348).

**Table 4**

*Mortality rates and median survival at age 25 years for persons in DHCS, stratified according to 
“time on ART”, “time after diagnosis”, “observation period”, and HCV status. (Adapted from paper 
VIII)*

<table>
<thead>
<tr>
<th></th>
<th>Persons with HIV</th>
<th>Persons with HIV observed 2000-2005 only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PYR</td>
<td>events</td>
</tr>
<tr>
<td>All</td>
<td>2274</td>
<td>570</td>
</tr>
<tr>
<td>HAART period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART</td>
<td>8271</td>
<td>537</td>
</tr>
<tr>
<td>1st year of HAART</td>
<td>2905</td>
<td>124</td>
</tr>
<tr>
<td>2nd-3rd year of HAART</td>
<td>4534</td>
<td>121</td>
</tr>
<tr>
<td>4th-5th year of HAART</td>
<td>3570</td>
<td>92</td>
</tr>
<tr>
<td>6th year of HAART onwards</td>
<td>3754</td>
<td>90</td>
</tr>
<tr>
<td>Time since diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st-2nd years after diagnosis</td>
<td>3426</td>
<td>169</td>
</tr>
<tr>
<td>3rd-4th years after diagnosis</td>
<td>3419</td>
<td>133</td>
</tr>
<tr>
<td>5th-6th years after diagnosis</td>
<td>3130</td>
<td>110</td>
</tr>
<tr>
<td>7th-8th years after diagnosis</td>
<td>2789</td>
<td>117</td>
</tr>
<tr>
<td>9th-10th years after diagnosis</td>
<td>2414</td>
<td>129</td>
</tr>
<tr>
<td>Hepatitis C status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV-positive</td>
<td>4149</td>
<td>246</td>
</tr>
<tr>
<td>HCV-negative</td>
<td>10535</td>
<td>724</td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed 95-99</td>
<td>3242</td>
<td>402</td>
</tr>
<tr>
<td>Observed 91-99</td>
<td>5927</td>
<td>222</td>
</tr>
<tr>
<td>Observed 01-05</td>
<td>1364</td>
<td>346</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus, PYR: person-years at risk, MR: mortality rate, HCV: hepatitis C virus, HAART: highly active antiretroviral therapy

### 9.3 Conclusion

The uncertainty and fear about the durability of the HIV response at the population level is 
gradually changing towards a positive feeling for the future. Our study (paper VIII) was one of the 
first to put an age to the life expectancy, and with several subsequent, similar reports from other 
corners of the world, it has become evident that the overall prognosis for persons with HIV is 
moving rapidly in the right direction.
10 COMORBIDITY

10.1 Background

10.1.1 AIDS-related comorbidity

Comorbidity in persons with HIV has always been in focus, simply because the clinical manifestation of HIV infection occurs through emergence of infections and other diseases as the virus gradually breaks down the immune system. Unusually high prevalence of Pneumocystis Carinii pneumonia and Kaposi Sarcoma (349,350) were some of the first observations which lead to characterization of AIDS (351) and later discovery of human immunodeficiency-related virus (HIV) (352). As most AIDS-defining conditions are associated with different levels of immune deterioration, the pattern of comorbidity changes when the CD4+ cell count decreases (353). ART-induced immune recovery not only lowers the incidence of AIDS, it also results in many of the AIDS-defining conditions waning or disappearing as the CD4+ cell count restores; this has been observed even for the malignancy Kaposi sarcoma (354–357).

10.1.2 Non-AIDS related comorbidity

The pattern of non-AIDS comorbidity is equally complex; the epidemiology of the comorbidity is far from fully explored and understood, and the pattern of comorbidity changes from one cohort to another. Some comorbid conditions are present before the person acquires HIV, and some diseases emerge after the HIV infection. In scientific studies, diseases and conditions occurring after the person has been diagnosed with HIV are sometimes called “non-AIDS events”. These events can be related to HIV, or they might be no different from those observed in a similar non-HIV population with regard to aetiology and incidence rate.

Several non-AIDS diseases are more common in persons with HIV compared to the general population (358–361). A recent review focusing on anal cancer, Hodgkin’s lymphoma, hepatocellular carcinoma, and lung cancer found two-fold increased rates of these four cancer types (362). Highly increased risk of anal cancer was also found in studies from Denmark (363), and the United States (364), the latter additionally finding a 21-fold increased rate of vaginal cancer, as well as moderately increased rates of melanoma, oropharyngeal, colorectal and renal cancer. A meta-analysis studied cancers in 444,172 persons with HIV and performed a similar analysis for 31,977 transplant patients to control for possible confounding by immune suppression. This study found increased standardized incidence ratios for lung cancer, leukaemia, renal cancer, oesophagus cancer and stomach cancer (365). A study among US Veterans showed a doubled risk of end-stage renal disease for HIV-infected compared with uninfected among black persons (366), and other studies have shown increased risk of cardiovascular disease(CVD) (367)(368) and liver disease (369) compared to the general population.

Despite their label, the prevalence of many “non-AIDS” diseases is inversely related to CD4+ cell count. This includes liver diseases (369), non-AIDS malignancies (358,370), renal diseases (358,371), and bacterial non-AIDS infections (372–375). An exception is perhaps CVD (358,376).
which doesn’t seem to follow this pattern. In some cohorts, non-AIDS morbidity has decreased over time as the CD4+ cell levels in the population has increased (377,378).

10.2 Mechanisms by which non-AIDS conditions could be related to HIV

There are numerous possible ways by which non-AIDS related conditions and diseases could be higher in persons with HIV than in persons without HIV (358): **Common risk:** Some comorbidities share risk factors and transmission mode with HIV, for example sexually transmitted diseases, HCV, IDU overdose, and human papilloma virus (HPV)-associated cancers. **Immune activation:** HIV induces alterations in the immune and inflammatory systems and activates the coagulation system (379–383). The immune activation leads to increased lymphocyte turnover which might be linked to cancer (384,385), in particular those types which might be associated with infections (361). These include Chlamydia pneumoniae and lung cancer (386); Helicobacter Pylori and gastric cancer (387); HPV and anal, cervical, and oropharyngeal cancer (388); HBV/HCV and liver cancer (389); and Ebstein-Barr virus and Hodgkin Lymphoma (390). Immune activation might also be related to CVD (391), faster progression of liver disease in persons co-infected with HBV and HCV (392,393), and renal disease such as HIV associated nephropathy (HIVAN) and immune complex glomerulonephritis (394). **Age:** With persons with HIV now living longer, they acquire the same age-related diseases as persons without HIV, for example cancer, neurocognitive impairment, diabetes (395), CVD, and chronic obstructive pulmonary disease. These have to be recognised by the HIV physician, but they also pose new challenges of multi-drug pharmacotherapy and potential drug interactions (396,397). **ART:** Lipodystrophia, hyperlipidemia, CVD, and renal disease are all related to specific antiretroviral drugs (398–407), and antiretroviral therapy might also play a role in the premature frailty observed in persons with HIV(408–410). **Lifestyle and social conditions:** Smoking and rough living conditions are all more common among groups of persons with HIV (411,412), associated with increased risk of for example lung diseases, violent injuries and psychiatric illness (413). **Chronic disease:** As HIV is now a chronic disease, we might see conditions found more frequently among persons with chronic diseases such as depression(414–416).

10.3 Comorbidity and survival (paper IX)

10.3.1 Measuring comorbidity and causes of death

As mortality has declined towards that of the general population, the pattern of diseases and conditions causing death have changed (221,417–421). Fewer persons with HIV now die from AIDS, and while this has led to an increase in the relative contribution of non-AIDS causes of deaths (Paper VIII) (Table 5), several studies report a simultaneous decline in absolute mortality from non-AIDS deaths (419,422,423). The association between high CD4+ cell count and lower risk of death also seems consistent across different causes of both AIDS deaths and non-AIDS deaths (358,424,425).

While comorbidity is indeed related to mortality, there is no direct relationship between causes of death and the incidence and prevalence of different comorbidities. Some non-AIDS diseases might not be fatal and therefore never be registered on a death certificate, and others might be overtaken by other non-AIDS events causing immediate death. Thus, diseases associated with shorter time
between occurrence and death will more frequently be registered as causes of death, for example in a person with a deadly disease such as cancer who dies from a myocardial infarction. A person might be registered as dying from an acute event, even if an underlying chronic disease is causing this, for example a person with diabetes who dies from a stroke caused by diabetic angiopathy. Different practices in coding of deaths from one hospital to another might also lead to quite different results, or the validity of causes of death, which are often quickly registered by the physician who happens to be on-call when the person dies, can be rather low. This is of course improved if the standardized coding of deaths (CODE) is used (426,427).

Table 5

Cause-specific mortality in DHCS. (Source: paper VIII)

<table>
<thead>
<tr>
<th>Mortality rates according to cause of death and calendar period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of death</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Observed 95-96</td>
</tr>
<tr>
<td>All causes</td>
</tr>
<tr>
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The above-mentioned “pitfalls” make analyses of cause-specific survival complicated, because of the many “competing risks” involved. Competing risk statistical models do exist (428), but exploring the relative contribution of comorbidity on mortality is best done by computing relative survival. The relative survival method is a standard approach that can be applied to any disease (429,430). Study groups are stratified into different modes of exposure (disease versus no disease) and all-cause survival and mortality rates are compared between the two groups.

10.3.2 Survival according to comorbidity present before HIV acquisition

In paper IX, we used relative survival methods to explore the impact of comorbidity acquired before HIV acquisition on mortality. Access to morbidity records going back 10-20 years before persons in our cohort were diagnosed with HIV enabled us to compare strata with or without comorbidity. Further, access to good survival data in a matched general population enabled us to estimate and adjust for background mortality. We identified comorbidity acquired before HIV as at least one point
on the Charlson Comorbidity Index (CCI) score at the time of HIV diagnosis. We found that persons with HIV, compared to the general population, had more CCI comorbidity (11.3% vs 8.0%). All-cause mortality increased with higher CCI score. In an adjusted analysis, having a CCI score of at least one was associated with a 1.84 times increased risk of death, compared a CCI score of zero. We also found that any CCI comorbidity had more impact on mortality in persons with HIV than in persons without HIV, with 34.1%-58.8% of the excess mortality caused by this interaction effect.

Figure 7

Visual presentation of the total mortality in persons with HIV and the relative contribution of background mortality (13.6%), HCV coinfection and CCI (31.5%), and HIV (55%). (Source: paper IX)

Finally, we estimated the size of the proportion of deaths in DHCS could be attributed to comorbidity acquired before HIV or to comorbidity otherwise not on the causal pathway between HIV infection and death. For this analysis, we included background mortality as well as mortality due to CCI score and HCV coinfection. We first calculated the population attributable risk (PAR) of comorbidity (HCV and CCI) in our cohort. The \( PAR = (IR_{\text{pop}} - IR_{\text{unexp}}) / IR_{\text{pop}} \) if a given population had been unexposed. All-cause mortality in the
population with HIV was 2.09% per year. Among those persons unexposed to HCV (HCV=0) and other comorbidity (CCI=0), all-cause mortality was 1.43%. This gives a PAR of 0.32 (0.66/2.09). Thus, if all persons with in our HIV cohort had been without HCV and CCI comorbidity, 32% of deaths would have been avoided. We now calculated the excess risk (ER) of HIV, compared to the general population, in persons free of HCV and CCI comorbidity. The ER is the difference between the incidence rate in the exposed population, and the incidence rate in the unexposed population: \( ER = IR_{\text{exp}} - IR_{\text{unexp}} \). All-cause mortality in the population with HIV (HCV=0, CCI=0) was 1.43% per year. Among matched general population controls (HCV=0, CCI=0), all-cause mortality was 0.28%. Thus, the ER of being infected with HIV is 1.15% per year. Our final calculation estimated that 32% of the observed mortality in our cohort was due to HCV and CCI comorbidity, 55% (1.15/2.09) was due to HIV, and the remaining 13% (0.28/2.09) corresponded to the background mortality in the population (Figure 7).

### 10.4 Conclusion

The results in paper IX can help us focus and set ambition levels for future efforts to reduce mortality in persons with HIV (344,431). Our findings confirm that persons acquiring HIV differ at large from the general population, and that we cannot expect overall mortality rates to reach the level of the general population. Instead, we need to identify groups with increased, preventable risk of death. The origin, aetiology, and consequences of each comorbid condition should be explored and characterised, both at the population level and in the individual; including behavioural, social and biological root causes.

### 11 PERSPECTIVES

#### 11.1 Status of the epidemic

This work has given a brief overview of the immense improvements in morbidity and mortality for persons infected with HIV during the first 30 years of the epidemic - with particular focus on how the nine papers included in this thesis might have played a role. The “road to success” for the individual starts with early diagnosis, followed by early enrolment into a health care system that provides high quality HIV care at all levels. Timely initiation of effective ART is essential, and continued monitoring of viral load is the key to avoiding the development of drug resistance and ensuring the shift to appropriate second-line regimens when needed. If the person is fortunate to stay free of other diseases, a successful trip down this road will be rewarded with a life expectancy that is very close to that of a comparable person who is not infected with HIV. This scenario has become reality for many persons in high-income countries, but many groups and individuals have not yet experienced the full benefit (321). Further, although we have also seen immense improvements in countries where the epidemic has hit the hardest (348), only 45% of those living with HIV in sub-Saharan Africa in 2013 knew their status, only 39% received ART, and only 29% had fully suppressed viral load (321).

The good news is that the overall feeling for the future in the HIV community is optimistic. UNAIDS has dared to set ambitious, although not unrealistic targets for the future: a 90% reduction in new
infections, a 90% reduction in stigma and discrimination, a 90% reduction in AIDS-related deaths; and an end to the AIDS epidemic as a public health threat by 2030 (321,432). Achieving these goals will require a concerted effort from the HIV community, including targeted research to gain new knowledge as well as a strong focus on scaling up implementation of existing solutions.

11.2 Future opportunities

11.2.1 Integrated solutions

The successful HIV response is causing fewer new infections, lowering mortality, and reducing AIDS-related discrimination, while other diseases are climbing up the ladder as important contributors to the global burden of disease (433,434). The days of AIDS exceptionalism are over (435,436), and HIV needs to find its natural place as a chronic disease. More than ever do we need stronger health systems that can provide HIV care and management integrated with that of other diseases in horizontal programmes (437,438). Although the feasibility of integrated care models has been shown in some settings (439–441), more implementation science is required to identify solutions that are scalable and sustainable.

11.2.2 Timely diagnosis

An unacceptably large gap exists between the number of persons living with HIV, and the proportion of those who have yet been diagnosed (321). To fill the gap, countries need detailed knowledge on the demographics of their local epidemic (345). Which groups and geographical areas have high prevalence of persons with HIV, and how can they be reached? “Know your epidemic” was a slogan originally used to describe the need to tailor HIV prevention efforts (83,85), but it is equally relevant (84) when it comes to finding those persons who are unaware of their positive HIV status. Several current initiatives support this challenge: The European HIDES Study aims to develop a model for implementation of targeted HIV testing in clinical settings (149), WHO and UNAIDS have produced guidelines on how countries can use available data for a more effective response to their local HIV epidemic (84), and the systematic collection and compilation of detailed global epidemiological data by UNAIDS provides an invaluable backbone for many of these efforts (442). Ultimately, each country needs to develop its own HIV detection strategy, optimally suited to the local epidemic.

11.2.3 Improved care

Today’s increased life expectancy in persons with HIV calls for improved understanding of aging and comorbidity. There is a growing need for age-specific guidelines (345), for clinicians across specialties who can provide care for an aging population with multiple morbidities (358), and for health systems that can deliver the continuity of care required by older people (443,444). As comorbidity patterns have changed, there is also a growing need to identify groups and individuals with increased, preventable risk of death and disease. We must re-evaluate screening programmes for cancer and other diseases to assess their cost and effectiveness in HIV populations (359,445), and put greater focus on promoting healthy lifestyle (345). Better understanding of the drivers behind non-AIDS comorbidity will allow us to develop and prioritize interventions that are targeted to a specific clinic, cohort, or population.
Despite the undisputable success of ART, a number of questions pertinent to HIV treatment and care remain to be answered. It is currently debated whether individuals will benefit from starting ART at a higher level than the most commonly used CD4+ cell count threshold of 350 cells/µL (89,446). Current evidence is largely based on observational studies (447) and should therefore be interpreted with caution due to the inherent risk of confounding by indication in this type of study. The ongoing Strategic Timing of AntiRetroviral Treatment (START) trial (448) is enrolling study subjects with CD4+ cell count above 500 cells/µL who are randomized to start ART immediately or defer initiation until the CD4+ cell count falls below 350 cells/µL. The START trial should be able to provide new and valuable knowledge on the subject. We also need more knowledge on how to control the chronic immune activation and inflammation which seems to play an important role in the changing patterns of comorbidity (449,450).

Human, technical, and financial resources in health systems are not equally distributed, and many local HIV epidemics are not yet under control (321). Being a key component of effective HIV care, access to viral load testing and drug resistance monitoring needs to be expanded to allow every person with HIV to initiate ART with an efficacious drug combination, detect virological failure in time, and change to the best possible second or third line regimens when needed. If we continue to improve and implement new models of HIV care delivery, mortality can decline even further. These models should be based on an inclusive and individualized approach to patient management, taking into account the biological and psychosocial properties of each person with HIV.

11.2.4 New therapies

The world must not stop investing in the development of new therapies. New antiretroviral drugs will be essential to ensure continued availability of effective treatment options for those who have failed one or more drug combinations and are therefore accumulating multi-drug resistant virus. The scientific community should also continue to look for ultimate solutions such as a preventive vaccine (451) or an HIV cure (15–18). The allogeneic bone marrow transplantation from a donor homozygous for CCR5Δ32 to the so-called “Berlin Patient” was the proof-of-concept that HIV eradication is technically possible (263), and the most promising current methods being investigated are gene therapy (452) or purging of viral reservoirs(453,454).

11.2.5 Prevention

HIV prevention is outside the scope of this thesis, but deserves a few lines due to the recent advances in effective prevention strategies, the dual effect of ART as prevention and treatment at the same time, and the role of prevention as the ultimate goal to eliminate HIV. The immense amount of HIV prevention research has revealed a range of effective behavioural, biomedical, and structural interventions. Combination prevention combines these interventions in tailored programmes, and is hoped to have a considerable impact on reducing new infections in the coming years (455,456). More simple in its approach is treatment as prevention, which in the most radical version relies on a population-based test-and-treat-all strategy to reduce the population viral load and thereby the incidence of new infections (88,457–459). Upcoming results of cluster-randomized trials will be awaited with excitement (97,105–108,110,111).
CONCLUSION

This thesis attempted to map the “road to success”, i.e., the road leading to increased survival of individuals and populations with HIV, ultimately to levels approaching those of the general population. Each of the nine studies explored and discussed some of the many bumps and other challenges that one needs to navigate in order to proceed safely down this road. Through a massive global effort for decades, HIV research has taken a giant leap to get this far. The studies in this thesis have each paid their modest contribution to show how crucially important it is to be diagnosed in time, to have access to a well-functioning health system, and to keep free of comorbidity both before and after acquiring HIV. After many years of struggle and despair, the HIV response is on the right track. Thanks to enormous advances in prevention and treatment, we are now looking towards a promising future. With a continued, relentless effort from every corner of the HIV community, we will be able to continue rapid expansion and implementation of current and new knowledge to benefit persons with HIV, persons at risk of acquiring HIV, and global public health.

SUMMARY

The work on this thesis began in 2003 when the global HIV epidemic was out of control. A minority of persons with HIV were benefitting fully from the recently introduced highly efficacious antiretroviral therapy (ART) combinations. Among the global challenges were lack of access to good health care, drug toxicity, and emergence of drug-resistant virus. It was unknown how long the drugs could maintain their efficacy in the individual even if administered as intended, and there was a fear that the increased drug pressure would increase the prevalence of drug resistance, subsequently leading to transmission of resistant virus from one individual to another, and thereby waning the treatment options available. Hence, we were far from the ideal conditions where an HIV-infected individual gets to know immediately that he/she is infected, has access to specialized medical and social support, receives a drug combination which effectively suppresses the virus and has no side effects, and is free of comorbid conditions both before and after he/she gets infected. The nine papers on which this thesis is based each aimed to provide new knowledge to aspects of the above.

Late diagnosis and late presentation to clinical care continue to be major barriers to improved HIV management. We used nationwide hospital registries to explore the potential for an indicator disease-based HIV testing strategy. A range of conditions that were manifestations of the HIV infection itself were found to be associated with highly increased risk of HIV diagnosis during the coming year, but less so 3 to 5 years later. Other conditions were associated with an almost constant 5-year long increased risk of being diagnosed with HIV because they share behavioural risk factors with HIV, making them indicators of not only current HIV but also of future HIV acquisition. Hence, indicator condition-based testing should be adapted to the local epidemic and could be a valuable addition to the existing detection practice.

Once diagnosed, getting the full benefit of modern HIV care requires access to a good health care system. We compared temporal trends in quality and quantity of ART introduction in Denmark and Greenland. Despite similar levels of health worker education and economic resources, ART
implementation and mortality decline in Greenland lacked several years behind Denmark. The study reminded us that although economy may be a prerequisite for implementing an effective HIV care system, it is certainly not all it takes.

The nationwide nature of the Danish HIV Cohort Study also allowed us to study a number of time trends at the population level. Despite what was feared, we found that the prevalence of triple-drug class virological failure (TCF) seemed to have stabilized after 2000; that the incidence rates of drug resistance acquisition were decreasing during 1999-2005; and that the prevalence of potential transmitters of drug-resistant HIV decreased during 1997-2004. We also looked at some of the consequences of virological failure and drug resistance and found that even modest levels of viraemia were associated with a high risk of future failure and death, and that in persons who have experienced TCF, the number and pattern of resistance mutations were independent predictors of death. Hence, despite the overall positive trends in virological failure and drug-resistance development at the population level, our findings underline the crucial importance of always having an effective treatment option available for the individual patient with drug-resistant virus.

As mortality was declining for persons with access to ART and good HIV care, it became important to know how long persons with HIV could expect to live compared to the general population. We projected long-term survival and found that a 25-year old person with HIV and without hepatitis C virus (HCV) coinfection had a 50 percent chance of surviving another 39 years, only 12.2 years less than a person in a matched general population cohort would survive.

With improved survival and declining HIV-related comorbidity, non-HIV related comorbidity became a more visible contributor to the health status of persons with HIV. We assessed the impact of non-HIV related comorbidity acquired before the person became infected with HIV. We found that 32% of the observed mortality in our cohort was due to HCV and comorbidities measured by the Charlson Comorbidity Index, 13% corresponded to the background mortality in the population, and that only 55% of the mortality could be attributed to HIV. Our findings confirmed that persons acquiring HIV differ at large from the general population, and that we should not expect overall mortality rates in populations with HIV to reach the levels in the general population.

This thesis attempted to map some of the many challenges on the road towards increased survival of individuals and populations with HIV up to a level, which today in many settings is close to that of the general population. The studies in this thesis have each paid their modest contribution to show how crucially important it is to be diagnosed in time, to have access to a well-functioning health system, and to keep free of comorbidity both before and after acquiring HIV. After many years of struggle and despair, and thanks to enormous advances in prevention and treatment, we are now looking towards a promising future.
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Morbidity and Risk of Subsequent Diagnosis of HIV: A Population Based Case Control Study Identifying Indicator Diseases for HIV Infection

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Abstract

Background: Early identification of persons with undiagnosed HIV infection is an important health care issue. We examined associations between diseases diagnosed in hospitals and risk of subsequent HIV diagnosis.

Methods: In this population-based case control study, cases were persons with incident HIV infection diagnosed in Denmark between 1 January 1995 and 1 June 2008. Risk-set sampling was used to identify 19 age- and gender-matched population controls for each HIV case, using the HIV diagnosis date as the index date for both cases and controls. Prior hospital diagnoses obtained from Danish medical databases were first categorized into 22 major disease categories (excluding AIDS-defining diseases except tuberculosis) and then subdivided into 161 subcategories, allowing us to examine specific diseases as potential HIV indicators by conditional logistic regression.

Results: The study included 2,036 HIV cases and 35,718 controls. Persons with the following disease categories had a high risk of HIV diagnosis during the subsequent 5-year period: sexually transmitted infections and viral hepatitis (adjusted odds ratio [aOR] = 12.3, 95% CI: 9.60–15.7), hematological diseases (aOR = 4.28, 3.13–5.85), lower respiratory tract infections (aOR = 3.98, 3.14–5.04), CNS infections (aOR = 3.44, 1.74–6.80), skin infections (aOR = 3.05, 2.47–3.75), other infections (aOR = 4.64, 3.89–5.54), and substance abuse (aOR = 2.60, 2.06–3.29). Several specific diseases were associated with aORs >20 including syphilis, hepatitis A, non “A” viral hepatitis, herpes zoster, candida infection, endocarditis, thrombocytopenia, and opioid abuse.

Conclusions: Targeted testing for HIV in patients diagnosed with diseases associated with HIV may lead to earlier treatment and thereby reduced morbidity, mortality and HIV transmission.

Introduction

Despite three decades of concerted effort, the HIV epidemic remains a tremendous public health challenge in both low- and high-income countries [1,2]. Many individuals newly diagnosed with HIV present at a late stage of the disease with severe immune depletion, resulting in delayed initiation of antiretroviral therapy (ART) which worsens their prognosis [3] and increases further transmission of HIV [4,5,6]. Recently, the European Center for Disease Control and Prevention’s Dublin Declaration Progress Report 2010 concluded that “the rates of late diagnosis remain unacceptably high” in Europe and Central Asia [7]. Thus, intensified HIV testing and treatment has been advocated to lower the prevalence of undiagnosed HIV infection and to control the HIV epidemic [8,9].

The U.S. Centers for Disease Control and Prevention (CDC) recommend routine HIV testing for all persons under age 65 who come into contact with the health care system [10]. While this approach may be cost-effective in areas with high HIV prevalence [11] and does prolong life expectancy [3], alternative approaches such as targeted HIV testing may be more appropriate in other settings with lower HIV prevalence or different health care systems [2].

Targeted HIV testing based on risk groups (e.g. injecting drug users [IDU] [12], presence of AIDS-defining illnesses, or coming from a high-HIV prevalence country is practised in most
European countries [13]. However, many opportunities for HIV testing are missed by the health care system prior to HIV diagnosis [14]. In 2007, the pan-European initiative “HIV in Europe” recommended that targeted testing based on the presence of diseases associated with HIV, so-called indicator diseases [15], should be developed as an additional tool to guide targeted HIV testing. HIV indicator diseases may be a result of individual risk behavior or coexisting HIV infection. Several studies have identified indicator diseases within a narrow spectrum of conditions [16,17,18,19], but to date there has been no comprehensive study of HIV indicator diseases. In the absence of adequate data, guidance is based mainly on expert opinion [15]. There is substantial information regarding the prevalence of indicator diseases in the HIV-infected population. In contrast, the relative risk of HIV among patients with indicator diseases remains poorly described. The goal of this study was to delineate medical conditions that identify individuals at increased risk of subsequent HIV diagnosis.

Methods

Ethics statement

The Danish Data Protection Agency approved the establishment of the cohort and the linkage between the four registries in this study (J.no. 2008-41-1781). The study was not subject to approval by the ethics committee because data collection did not involve direct patient contact and all data was fully de-identified.

We conducted a population-based nested case control study among persons with and without an incident HIV diagnosis in Denmark. The adult population of Denmark is 4.3 million with an estimated HIV infection prevalence of 0.09% [20]. The Danish healthcare system provides free, tax-supported medical care for all residents, including antiretroviral treatment of HIV.

Data sources

The civil registration system (CRS) number, assigned at birth, uniquely identifies each person living in Denmark since 1968 and is used for personal identification in all Danish administrative and medical databases. We used this unique 10-digit CRS-number to link data among the following registries: The Danish HIV Cohort Study (DHCS) is a prospective, open, nationwide, population-based cohort of all HIV-infected individuals receiving care in Danish HIV clinics since 1 January 1995 [20]. The study is ongoing, with continuous enrolment of newly diagnosed patients. The Danish Civil Registration System records demographic information, vital status, and immigration and emigration dates for all Danish citizens beginning in 1967 [21]. The Danish National Registry of Patients (DNRP) contains information on all patients discharged from Danish hospitals since 1977 [22]. It includes diagnoses coded by the treating physician according to the International Classification of Diseases, 8th revision (ICD-8) up to the end of 1993 and according to the 10th revision (ICD-10) thereafter. The registry covers public as well as private hospitals and so constitutes a virtually complete population-based database. The Danish Cancer Registry (DCR) has registered all incident cancers in Denmark since 1943, classifying cancers registered after 1977 according to ICD-10 [23].

Study population

Cases. Cases were identified from DHCS and included all individuals who (I) were diagnosed with HIV between 1 January 1995 and 1 June 2008; (II) were at least 16 years of age on the date of HIV diagnosis; and (III) were living in Denmark for at least 5 years prior to HIV diagnosis. The index date for HIV cases was defined as the date of first positive HIV test as registered in the DHCS.

Population controls. Controls not diagnosed with HIV were identified from the CRS using incidence density sampling, which involves matching each case to a sample of those who are at risk at the time of case occurrence [24]. To ensure sufficient statistical power to detect differences in the occurrence of rare events, we sampled for each case 19 random age- and gender-matched population controls that were alive on the HIV diagnosis date of their respective case. The date of HIV diagnosis/sampling constituted the index date for both cases and controls. Controls who, according to the CRS, had not been living in Denmark for at least 5 years prior to the index date were excluded. Hence, all study subjects were living in Denmark during the 5-year period prior to their index date and therefore at risk of both exposure and outcome.

Identification of hospital diagnoses and grouping of disease categories

For all study subjects we extracted hospital diagnoses from outpatient contacts and hospital stays from the DNRP and DCR, up to the day prior to the index date. ICD-10 codes were the primary source for grouping diseases. We defined 22 disease categories of interest according to the type and anatomical location of the disease (Table S1). ICD-8 codes were translated to the corresponding ICD-10 disease categories and first-time diagnoses were assigned to the appropriate category. In addition, a total of 161 subcategories were created for the 22 disease categories, allowing us to examine specific diseases as potential HIV indicators (Table S1). Except for tuberculosis, we excluded AIDS-defining diseases, because their association with HIV infection is well established [25].

Statistical analyses

For cases and controls, we tabulated gender, age (16–39 years, 40–49 years, 50–59 years, and 60+ years), and hospital contact(s) in the 5-year period prior to the index date for each of the 22 disease categories (yes/no). For cases, the following variables were also included: race, most likely mode of HIV acquisition, presence of AIDS [25] at diagnosis, first CD4+ cell count, and HIV RNA measurement (within 180 days of HIV diagnosis). Frequencies and percentages were computed for all variables.

Conditional logistic regression analyses

For each of the 22 disease categories, conditional logistic regression analysis was used to estimate odds ratios (OR), which is an unbiased estimate of the incidence rate ratio (IRR) for subsequent HIV diagnosis [24]; unadjusted ORs as well as ORs adjusted for the remaining 21 disease categories were estimated. Observation data were subsequently stratified into three time periods prior to the index date (less than 1 year, 1–2 years, and 3–5 years) to explore changes in ORs in the years following a given disease or disease category. We used first-time diagnoses registered within each time stratum for a given disease/disease category. Adjusted ORs were calculated for each disease category and time period (adjusted for the remaining 21 disease categories). To identify specific HIV-indicator diseases, we explored risk estimates for the 161 specific subcategories within each of the 22 disease categories. We computed both unadjusted odds ratios for each subcategory and odds ratios adjusted for other diseases within the same disease category (Table S1). In all analyses, only first-time diagnoses for a given disease/disease category were utilized.
Results

We identified 2,363 individuals diagnosed with HIV between 1 January 1995 and 1 June 2008 and 38,684 controls. Of these, 327 cases and 2,966 controls were excluded because they immigrated to Denmark less than 5 years prior to their index date. Thus, a total of 2,036 cases and 35,718 controls were included resulting in an average of 17.5 controls per case.

In the 5 years prior to their index date, there were a total of 158,416 hospital contacts for both cases and controls, of which 34,520 represented a first-time diagnosis for one of the 22 disease categories. Table 1 shows characteristics of cases and controls on their index date. In the 5 years preceding the index date, 69.8% of cases (1,421 of 2,036 cases) and 53.6% of controls (19,148 of 35,718 controls) had at least one hospital contact for one of the 22 disease categories delineated below.

Disease category and risk of subsequent HIV diagnosis

Several disease categories were associated with an increased risk of HIV diagnosis during the following 5-year period (Figure 1). Persons diagnosed a disease in the category “sexually transmitted infections (STIs) and viral hepatitis” had the highest risk of subsequent HIV diagnosis (adjusted OR [aOR] = 12.3, 95% confidence interval [CI], 9.60–15.7). Also persons with infections (lower respiratory tract infections, CNS infections, skin infections, and other infections), hematological diseases, non-AIDS defining cancers, substance abuse, poisoning, ear, nose, and throat diseases, skin diseases, and gastrointestinal diseases had higher risk of HIV diagnosis during the following 5 years. Seven disease categories were not associated with subsequent HIV diagnostic eye diseases, kidney diseases, lung diseases, ischemic heart disease (IHD), non-IHD vascular diseases, neurological diseases, and trauma. A decreased risk of subsequent HIV diagnosis was found for persons with rheumatological diseases (aOR = 0.72, 95% CI: 0.62–0.85), non-diabetes endocrine diseases (aOR = 0.60, 95% CI: 0.42–0.86), and diabetes (aOR = 0.40, 95% CI: 0.23–0.69).

Time trends in risk of HIV diagnosis following hospital contact

Figure 2 shows the relative risk of subsequent HIV in 3 time periods: <1 year, 2–3 years, and 3–5 years after each of the 22 disease categories. The time trends can be categorized in four main groups: 1) no time trend (eye diseases, lung diseases, kidney diseases, IHD, neurological diseases, trauma, rheumatological diseases, non-diabetic endocrine diseases, and diabetes); 2) A highly increased risk of HIV in the first year after diagnosis and no or only a slightly increased risk thereafter (hematological diseases, non-AIDS malignancy, skin diseases, gastrointestinal diseases, and non-IHD vascular diseases); 3) gradually decreased risk of HIV over time (lower respiratory tract infections, CNS infections, skin infections, other infections, and ear, nose, and throat diseases); 4) persistently increased risk both in the short and the long term (STIs and viral hepatitis, substance abuse, and poisoning).

Specific HIV-indicator diseases

To determine whether any specific diseases were strong indicators of subsequent HIV diagnosis, we further divided the 22 disease categories into 161 subcategories. Table 2 reports risk estimates for subcategories associated with at least a 3-fold elevated risk of subsequent HIV diagnosis (all 161 subcategories shown in Table S2). We found strong associations between all groups of STIs and viral hepatitis and subsequent HIV diagnosis. Several other specific groups of infectious diseases, including meningitis, herpes zoster, endocarditis, and malaria, were closely associated with later HIV diagnosis. Among hematological diseases and cancers: thrombocytopenia, anemia, lymphadenitis, non-AIDS defining lymphomas, and secondary and unspecified malignant neoplasm of lymph nodes were associated with later HIV diagnosis. Genital cancers were not. In the group of gastrointestinal diseases, diseases of the oral cavity, liver diseases, and fissures/abscesses of the anal cavity were strongly associated

Table 1. Baseline characteristics of of HIV cases and HIV-uninfected controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 2,036)</th>
<th>Controls (n = 35,718)</th>
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<tr>
<td>Sex, n (%)</td>
<td></td>
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<tr>
<td>Female</td>
<td>380 (18.7)</td>
<td>6,555 (18.4)</td>
</tr>
<tr>
<td>Male</td>
<td>1,656 (81.3)</td>
<td>30,819 (81.6)</td>
</tr>
<tr>
<td>Age at diagnosis, n (%)</td>
<td></td>
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<tr>
<td>16–39 years</td>
<td>1,122 (55.1)</td>
<td>18,899 (52.9)</td>
</tr>
<tr>
<td>40–49 years</td>
<td>510 (25.1)</td>
<td>9,266 (25.9)</td>
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<tr>
<td>50–59 years</td>
<td>290 (14.2)</td>
<td>5,410 (15.2)</td>
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<tr>
<td>60+ years</td>
<td>114 (5.6)</td>
<td>2,143 (6.0)</td>
</tr>
<tr>
<td>Hospital contact, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,421 (69.8)</td>
<td>19,148 (53.6)</td>
</tr>
<tr>
<td>No</td>
<td>615 (30.2)</td>
<td>16,570 (46.4)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1,798 (88.7)</td>
<td>–</td>
</tr>
<tr>
<td>Black</td>
<td>119 (5.9)</td>
<td>–</td>
</tr>
<tr>
<td>Asian</td>
<td>55 (2.7)</td>
<td>–</td>
</tr>
<tr>
<td>Inuit</td>
<td>19 (0.9)</td>
<td>–</td>
</tr>
<tr>
<td>Other</td>
<td>36 (1.8)</td>
<td>–</td>
</tr>
<tr>
<td>Mode of HIV exposure, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>975 (47.9)</td>
<td>–</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>722 (35.5)</td>
<td>–</td>
</tr>
<tr>
<td>IDU</td>
<td>210 (10.3)</td>
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</tr>
<tr>
<td>Other</td>
<td>49 (2.4)</td>
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</tr>
<tr>
<td>Unknown</td>
<td>80 (3.9)</td>
<td>–</td>
</tr>
<tr>
<td>AIDS at diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,847 (90.7)</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>189 (9.3)</td>
<td>–</td>
</tr>
<tr>
<td>First CD4 count (cells/µL)</td>
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<td></td>
</tr>
<tr>
<td>&lt;350</td>
<td>999 (49.1)</td>
<td>–</td>
</tr>
<tr>
<td>≥350</td>
<td>829 (40.7)</td>
<td>–</td>
</tr>
<tr>
<td>missing</td>
<td>208 (10.2)</td>
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</tr>
<tr>
<td>First HIV RNA measurement (copies/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10⁵</td>
<td>343 (16.9)</td>
<td>–</td>
</tr>
<tr>
<td>&gt;10⁵ &amp; ≤10⁶</td>
<td>622 (30.6)</td>
<td>–</td>
</tr>
<tr>
<td>&gt;10⁷</td>
<td>660 (32.4)</td>
<td>–</td>
</tr>
<tr>
<td>missing</td>
<td>411 (20.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Baseline was defined as the index date which was the date of HIV diagnosis for cases and their corresponding controls (matched on the day the case was diagnosed with HIV).
*In the 5 years prior to the index date for at least one of the 22 disease categories in Table S1.
*Within 180 days of HIV diagnosis. The table shows data on HIV cases and HIV-uninfected controls with at least 5 years of continuous observation prior to the index date. MSM, men who have sex with men; IDU, injection drug use.
later HIV diagnosis. Among skin diseases, seborrheic dermatitis was associated with increased risk of HIV diagnosis. Ischemic heart disease, including myocardial infarction (aOR = 0.81, 95% CI: 0.39–1.69) and other cardiovascular diseases, were not associated with later HIV diagnosis, with the notable exception of thrombophlebitis (aOR = 5.29, 95% CI: 3.51–7.96).

Among fourteen individuals diagnosed with hepatitis A infection, 12 (85.7%) were men who had sex with men (MSM). Five (50%) of ten cases with malaria were non-Caucasian immigrants. In 62 of 68 cases (91.2%) with opioid abuse-related diagnoses, IDU was registered as the mode of HIV transmission.

First-time diagnoses for the 52 diseases in Table 2 represented 3,257 (1.9%) of all 138,416 hospital contacts registered in the 5 years prior to the index date. In the HIV cohort of 2,036 individuals, 782 (38.4%) had at least one hospital contact for an indicator disease, while this occurred for only 2,475 (6.9%) of 35,718 controls. For the 1,826 cases not reporting IDU as mode of HIV infection, 613 (34.1%) had a first-time hospital contact for diseases listed in Table 2.

**Discussion**

We found that HIV indicator diseases identified over one-third of all individuals who would become diagnosed with HIV in the subsequent 5-year period. Recognition of these HIV indicator diseases could aid healthcare personnel in identifying individuals at increased risk of undiagnosed HIV. The time-dependent association between the date of diagnosis of some disease categories and HIV diagnosis suggests that for several categories repeated (e.g., yearly) HIV testing is advisable.

To our knowledge, this is the first population-based study conducted to identify HIV indicator diseases across all disease categories. While some indicator diseases we identified were previously recognized [15,16,17,26], the size of our study population and completeness of hospitalization data allowed us to provide risk estimates with high statistical precision for the majority of disease groups. Our results thus can be used to guide strategies for targeted HIV testing in the hospital settings.
Our study also had some limitations. As in other observational HIV cohort studies, an unknown proportion of people with HIV may have died without being diagnosed with the condition. Thus, we may have underestimated the relative risk of subsequent HIV diagnosis after life-threatening conditions such as cancer [26]. Additionally, we had access only to hospital diagnoses, which may have caused us to underestimate the occurrence of diseases diagnosed outside the hospital system, such as syphilis, fungal skin infections, and herpes zoster. If patients at risk of HIV are more prone to be diagnosed in a hospital setting this will lead to a potential overestimation of the predictive value of some HIV predictor diseases if compared with those from a non-hospital setting. Another potential shortcoming is inaccuracies in diagnoses reported to national hospital databases. However, the positive predictive value of registry diagnoses (i.e. the proportion of subjects with a given registry diagnosis which is correct when compared to medical records) is generally high (70%–99%) [22,27]. Furthermore, risk of subsequent HIV diagnosis may be elevated if a given disease diagnosis increases the likelihood of HIV testing, regardless of its actual association with HIV infection. This phenomenon would produce a close association between the timing of the disease and HIV diagnoses. Although persons with some disease categories had increased risk of HIV in the first year thereafter (Figure 2), the increased risk of subsequent HIV diagnosis was also observed more than 1 year after the disease. Hence, this phenomenon could only have a moderate effect on the 5-year estimates. Finally, it should be noted that due to our use of hospital diagnoses, we were unable to identify clinical features that may further enhance the predictive value of given indicator diseases (e.g. “florid or hard to treat” fungal skin infection).

HIV indicator diseases can be grouped into three major types. First, disease manifestations of acute HIV infection (e.g. acute viral

### Disease category

<table>
<thead>
<tr>
<th>Disease category</th>
<th>aOR (95% CI) 3-5 y prior to i.d.</th>
<th>aOR (95% CI) &lt;1 y prior to i.d.</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>11.2 (7.74–16.2)</td>
<td>25.0 (16.5–37.7)</td>
<td></td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>1.56 (0.89–2.71)</td>
<td>10.5 (7.53–14.7)</td>
<td></td>
</tr>
<tr>
<td>CNS infections</td>
<td>1.86 (0.42–8.25)</td>
<td>5.25 (1.96–14.1)</td>
<td></td>
</tr>
<tr>
<td>Skin infections</td>
<td>3.96 (2.91–5.37)</td>
<td>4.81 (3.44–6.73)</td>
<td></td>
</tr>
<tr>
<td>Other infections</td>
<td>2.49 (1.81–3.43)</td>
<td>11.6 (8.94–15.0)</td>
<td></td>
</tr>
<tr>
<td>Hematological diseases and cancers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>2.05 (1.07–3.92)</td>
<td>8.80 (5.73–13.5)</td>
<td></td>
</tr>
<tr>
<td>Non-AIDS defining cancers</td>
<td>0.81 (0.38–1.71)</td>
<td>2.37 (1.50–3.74)</td>
<td></td>
</tr>
<tr>
<td>Substance abuse and poisoning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance abuse</td>
<td>3.33 (2.44–4.56)</td>
<td>3.07 (2.15–4.39)</td>
<td></td>
</tr>
<tr>
<td>Poisoning</td>
<td>3.37 (2.41–4.69)</td>
<td>3.65 (2.42–5.51)</td>
<td></td>
</tr>
<tr>
<td>Other disease categories</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear, nose, and throat diseases</td>
<td>1.87 (1.41–2.47)</td>
<td>2.46 (1.81–3.35)</td>
<td></td>
</tr>
<tr>
<td>Skin diseases</td>
<td>1.43 (0.93–2.20)</td>
<td>2.57 (1.70–3.89)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal diseases</td>
<td>1.18 (0.94–1.48)</td>
<td>2.08 (1.67–2.58)</td>
<td></td>
</tr>
<tr>
<td>Eye diseases</td>
<td>1.48 (1.01–2.19)</td>
<td>1.31 (0.82–2.11)</td>
<td></td>
</tr>
<tr>
<td>Lung diseases</td>
<td>1.11 (0.66–1.85)</td>
<td>1.61 (0.99–2.59)</td>
<td></td>
</tr>
<tr>
<td>Kidney diseases</td>
<td>1.21 (0.67–2.18)</td>
<td>0.93 (0.49–1.77)</td>
<td></td>
</tr>
<tr>
<td>Non-IHD vascular diseases</td>
<td>0.97 (0.69–1.36)</td>
<td>1.50 (1.12–2.01)</td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>0.82 (0.39–1.70)</td>
<td>1.14 (0.62–2.08)</td>
<td></td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>0.61 (0.37–1.01)</td>
<td>1.34 (0.90–1.98)</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>0.92 (0.81–1.04)</td>
<td>1.05 (0.91–1.21)</td>
<td></td>
</tr>
<tr>
<td>Rheumatological diseases</td>
<td>0.84 (0.67–1.05)</td>
<td>0.70 (0.54–0.91)</td>
<td></td>
</tr>
<tr>
<td>Non-diabetic endocrine diseases</td>
<td>0.69 (0.38–1.25)</td>
<td>0.78 (0.46–1.30)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.69 (0.35–1.35)</td>
<td>0.27 (0.11–0.66)</td>
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</tbody>
</table>

![Figure 2. Association between risk of subsequent HIV diagnosis and time of hospital contact. doi:10.1371/journal.pone.0032538.g002](image-url)
Table 2. Specific diseases (diagnosed in the 5 years before the index date) associated with at least 3-fold elevated risk of subsequent HIV diagnosis.

<table>
<thead>
<tr>
<th>Disease category</th>
<th>Specific disease</th>
<th>Cases n = 2,036</th>
<th>Controls n = 35,718</th>
<th>aOR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Syphilis</td>
<td>15</td>
<td>2</td>
<td>94.7 (20.9–429)</td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Hepatitis A</td>
<td>14</td>
<td>4</td>
<td>41.6 (11.7–148)</td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Non “A” viral hepatitis[^b^]</td>
<td>77</td>
<td>55</td>
<td>23.6 (16.5–33.7)</td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Anogenital herpes simplex</td>
<td>7</td>
<td>10</td>
<td>12.7 (4.65–34.8)</td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Condyloma</td>
<td>55</td>
<td>95</td>
<td>8.99 (6.32–12.8)</td>
</tr>
<tr>
<td>STIs and viral hepatitis</td>
<td>Other STIs</td>
<td>18</td>
<td>12</td>
<td>14.8 (6.35–34.6)</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>Unspecified pneumonia</td>
<td>126</td>
<td>290</td>
<td>7.56 (6.03–9.48)</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>Pneumococcal pneumonia</td>
<td>9</td>
<td>14</td>
<td>4.33 (1.63–11.5)</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>Influenza and viral pneumonia</td>
<td>9</td>
<td>43</td>
<td>3.21 (1.51–6.81)</td>
</tr>
<tr>
<td>CNS infections</td>
<td>Bacterial meningitis</td>
<td>5</td>
<td>9</td>
<td>14.7 (5.63–38.1)</td>
</tr>
<tr>
<td>CNS infections</td>
<td>Viral meningitis or encephalitis</td>
<td>9</td>
<td>25</td>
<td>6.33 (2.90–13.8)</td>
</tr>
<tr>
<td>CNS infections</td>
<td>Other CNS infections</td>
<td>9</td>
<td>10</td>
<td>5.51 (1.60–19.0)</td>
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<tr>
<td>Skin infections</td>
<td>Abscess, furuncle, carbuncle</td>
<td>137</td>
<td>409</td>
<td>5.15 (4.17–6.35)</td>
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<tr>
<td>Skin infections</td>
<td>Fungal skin infections</td>
<td>3</td>
<td>11</td>
<td>4.41 (1.18–16.5)</td>
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<td>Skin infections</td>
<td>Erysipelas</td>
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<td>76</td>
<td>3.92 (2.39–6.45)</td>
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<td>Skin infections</td>
<td>Other skin infections</td>
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<td>95</td>
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<td>Other infections</td>
<td>Herpes zoster</td>
<td>22</td>
<td>8</td>
<td>337 (143–79.6)</td>
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<tr>
<td>Other infections</td>
<td>Candida infection[^c^]</td>
<td>40</td>
<td>22</td>
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<td>Other infections</td>
<td>Endocarditis</td>
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<td>7</td>
<td>232 (8.71–61.9)</td>
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<td>Tuberculosis and other mycobacterial infections</td>
<td>24</td>
<td>21</td>
<td>15.2 (7.99–29.1)</td>
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<tr>
<td>Other infections</td>
<td>Malaria</td>
<td>10</td>
<td>12</td>
<td>9.53 (3.86–23.5)</td>
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<tr>
<td>Other infections</td>
<td>Mononucleosis</td>
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<td>19</td>
<td>8.64 (4.04–18.5)</td>
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<tr>
<td>Other infections</td>
<td>Lymphangitis</td>
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<td>8</td>
<td>7.88 (2.40–25.9)</td>
</tr>
<tr>
<td>Other infections</td>
<td>Unspecified viral illness</td>
<td>23</td>
<td>44</td>
<td>7.87 (4.56–13.6)</td>
</tr>
<tr>
<td>Other infections</td>
<td>Sepsis</td>
<td>23</td>
<td>34</td>
<td>4.90 (2.52–9.52)</td>
</tr>
<tr>
<td>Other infections</td>
<td>Infectious gastroenteritis</td>
<td>50</td>
<td>216</td>
<td>3.48 (2.49–4.87)</td>
</tr>
<tr>
<td>Other infections</td>
<td>Other types of infection</td>
<td>67</td>
<td>182</td>
<td>4.77 (3.46–6.56)</td>
</tr>
<tr>
<td>Hematological diseases and cancers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Thrombocytopenia</td>
<td>15</td>
<td>10</td>
<td>240 (10.5–54.7)</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Unspecified anemia</td>
<td>24</td>
<td>44</td>
<td>7.26 (4.19–12.6)</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Lymphoma</td>
<td>18</td>
<td>43</td>
<td>5.83 (3.22–10.5)</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Aplastic and other specified anemias</td>
<td>7</td>
<td>17</td>
<td>4.58 (2.38–8.79)</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Lymphadenitis</td>
<td>13</td>
<td>42</td>
<td>3.44 (1.42–8.30)</td>
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<td>Nutrition deficiency anemia</td>
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<td>27</td>
<td>3.11 (1.11–8.70)</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>Other hematological diseases</td>
<td>5</td>
<td>18</td>
<td>4.30 (1.54–12.0)</td>
</tr>
<tr>
<td>Non-AIDS defining cancers</td>
<td>Secondary and unspec. malignant neoplasm of lymph nodes</td>
<td>11</td>
<td>22</td>
<td>6.74 (3.14–14.5)</td>
</tr>
<tr>
<td>Substance abuse and poisoning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance abuse</td>
<td>Substance abuse opioids</td>
<td>69</td>
<td>17</td>
<td>43.5 (24.6–76.8)</td>
</tr>
<tr>
<td>Substance abuse</td>
<td>Substance abuse other</td>
<td>51</td>
<td>55</td>
<td>6.54 (4.07–10.5)</td>
</tr>
<tr>
<td>Drug poisoning</td>
<td>Narcotics and hallucinogens</td>
<td>47</td>
<td>43</td>
<td>112 (7.08–17.8)</td>
</tr>
<tr>
<td>Other disease categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ear, nose, and throat diseases</td>
<td>Other acute upper respiratory tract infection</td>
<td>10</td>
<td>30</td>
<td>5.02 (2.38–10.6)</td>
</tr>
<tr>
<td>Ear, nose, and throat diseases</td>
<td>Chronic disease of tonsils and adenoids</td>
<td>43</td>
<td>129</td>
<td>4.95 (3.45–7.09)</td>
</tr>
<tr>
<td>Skin diseases</td>
<td>Sebaceous dermatitis</td>
<td>9</td>
<td>8</td>
<td>11.8 (4.30–32.6)</td>
</tr>
<tr>
<td>Gastrointestinal diseases</td>
<td>Fissure/abscess of anal and rectal regions</td>
<td>50</td>
<td>202</td>
<td>4.35 (2.87–6.61)</td>
</tr>
<tr>
<td>Gastrointestinal diseases</td>
<td>Liver diseases</td>
<td>31</td>
<td>103</td>
<td>4.06 (2.27–7.25)</td>
</tr>
<tr>
<td>Gastrointestinal diseases</td>
<td>Disease of salivary glands, oral mucosa, tongue and lips</td>
<td>15</td>
<td>58</td>
<td>3.97 (2.88–5.48)</td>
</tr>
</tbody>
</table>
illness or lymphadenitis); second, diseases associated with coexisting HIV infection (e.g. herpes zoster or thrombocytopenia); and third, diseases associated with behavior that increases the risk of acquiring HIV (e.g. hepatitis and opioid abuse). The latter group is highlighted in Figure 2, which shows that disease categories related to individual risk behaviors (e.g. STIs and viral hepatitis) are associated with an elevated risk that remained constant over time. Other disease categories which may be related to coexisting HIV, such as respiratory infections, hematological diseases, and non-AIDS-defining cancers, showed a highly increased risk of HIV in the first year after the diagnosis. The close connection between the date of hospital contact and date of HIV diagnosis suggests that the hospital contacts led to subsequent HIV testing as part of the recommended medical work-up (e.g. following lymphoma diagnosis) [15]. Among specific HIV indicator diseases, acute hepatitis A virus (HAV) infection was associated with high risk of subsequent HIV diagnosis. The strong association between acute HAV infection and MSM suggests that HAV infection is a proxy for high-risk sexual behavior in our study population [28]. Our data also indicate that endocrine and rheumatological diseases (except infectious arthropathy) are associated with decreased risk of HIV which may be related to low-risk sexual behavior among individuals with these chronic diseases [29]. Factors associated with a specific behaviour or transmission risk (such as MSM, IDU, and migration) may represent potential confounders. Information on these risk factors were not available for controls and thus, could not be adjusted for in the analyses. However, the purpose of the present study was to identify indicator diseases regardless of their causal or non-causal relationship to HIV infection. Therefore, our estimates represent the risk of a given disease compared to a representative sample of a background population of mixed race and sexual orientation. Our study only included first-time diagnosis and we did not assess the effect of repetitively diagnoses (e.g. pneumonia). Repetitive diagnoses of indicator diseases in an individual is most likely associated with even higher risk of undiagnosed HIV, and thus should prompt immediate HIV testing.

Targeted HIV testing is practiced in many countries [2,13,15] and may be more cost-effective than universal HIV testing in low HIV prevalence regions like Denmark [30,31]. However, until now the lack of a thorough delineation of HIV indicator diseases has markedly reduced the efficacy of targeted HIV testing [2]. Another barrier to HIV screening is the acceptance of testing among patients [32]. While the acceptance of universal opt-out HIV testing among emergency department patients has varied greatly [33,34] and was as low as 24% in a recent trial [35], physician recommended HIV testing is more acceptable for most patients [32]. Thus, expanding targeted HIV testing using indicator diseases may be an agreeable approach for patients. In our study, HIV indicator diseases could potentially detect approximately two out of every five persons with HIV at an earlier stage. If the earlier diagnosis leads to earlier ART initiation, indicator disease-based HIV screening has the potential to reduce both HIV-related morbidity and HIV transmission [36,37,38]. This screening strategy should of course be added to the usual HIV screening initiatives to ensure un-delayed diagnosis of the remaining 60% of persons with HIV. Almost one-third of cases in our study had no hospital contacts in the 5 years prior to their HIV diagnosis. Therefore, national screening initiatives could aim to expand current non-hospital based strategies such as community outreach programs aimed at high-risk groups [e.g. sex workers and drug users] [39,40].

In conclusion, knowledge of HIV indicator diseases may optimize national HIV testing programs but the effectiveness of indicator disease-based HIV screening needs confirmation in clinical studies. Although the use of indicator diseases may enhance the identification of undiagnosed HIV-infected individuals, this strategy can only be supplementary to systematic HIV testing of risk groups identified through information about their behavioral risk-taking profiles.

### Supporting Information

**Table S1** International Classification of Diseases, version 8 and version 10 codes used for the analyses.

[XLS](http://www.plosone.org/article/file.xhtml?id=info%3Adoi%2F10.1371%2Fjournal.pone.0032538.S001)

**Table S2** Section 1 to 22 contains information and risk estimates on subcategorized disease groups for each of the 22 major disease categories. First-time diagnoses within each disease group were recorded if occurring less than 5 years prior to index date. Unadjusted odds ratio [OR] and adjusted odds ratio [aOR] for all other variables within the same disease category are shown with

### Table 2. Cont.

<table>
<thead>
<tr>
<th>Disease category</th>
<th>Specific disease</th>
<th>Cases n = 2,036</th>
<th>Controls n = 35,718</th>
<th>aOR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung diseases</td>
<td>Respiratory disease principally affecting the interstitium</td>
<td>7</td>
<td>13</td>
<td>9.22 (3.63–23.4)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>Lung abscess/empyema without pneumonia</td>
<td>5</td>
<td>11</td>
<td>6.42 (2.16–19.1)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>Pneumothorax</td>
<td>8</td>
<td>40</td>
<td>3.25 (1.98–5.35)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>Other lung diseases</td>
<td>21</td>
<td>95</td>
<td>3.00 (1.37–6.55)</td>
</tr>
<tr>
<td>Non-IHD vascular diseases</td>
<td>Thrombophlebitis</td>
<td>34</td>
<td>106</td>
<td>5.29 (3.51–7.96)</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>Facial nerve disorder</td>
<td>8</td>
<td>31</td>
<td>3.04 (1.46–6.35)</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>Polyneuropathy</td>
<td>9</td>
<td>47</td>
<td>4.52 (2.07–9.85)</td>
</tr>
<tr>
<td>Rheumatological diseases</td>
<td>Infectious arthropathy</td>
<td>14</td>
<td>75</td>
<td>3.18 (1.76–5.74)</td>
</tr>
</tbody>
</table>

*Adjusted for other diseases within the same disease category (Table S1).

*Diagnosis of non-A hepatitis coded by ICD-10 were evenly distributed between hepatitis B and C with no significant difference in their association to risk of subsequent HIV diagnosis.

*The subcategory *candida infection* primarily consisted of “oral candidiasis” but other types of candidiasis also occurred albeit at too low frequencies to be analyzed separately. Risk estimates for all 161 subcategories are shown in Table S2. aOR, adjusted odds ratio; CI, confidence interval; STIs, sexually transmitted infections; IHD, ischemic heart disease.

Acknowledgments
The authors thank the staff of their clinical departments for their continuous support and enthusiasm.

References

Author Contributions
Conceived and designed the experiments: OSS NL JG HTS NO. Performed the experiments: OSS NL NO. Analyzed the data: OSS NL. Contributed reagents/materials/analysis tools: OSS NL LO GK BR JG HTS NO. Wrote the paper: OSS NL LO GK BR JG HTS NO. Data collection and linkages were undertaken by: OSS LO GK BR JG NO.
Implementation and Effectiveness of Antiretroviral Therapy in Greenland

Nicolai Lohse,*† Karin Ladefoged,‡ and Niels Obel††

Analyses from the Danish HIV Cohort Study showed that, despite comparable economic means and general education of healthcare personnel, antiretroviral treatment of HIV in Greenland began later and has been implemented at a slower pace with lower therapeutic effectiveness than in Denmark. However, implementation and quality of care improved considerably in recent years.

Like in Western Europe, the first case of HIV was observed in Greenland in the mid-1980s (1), but the epidemic in this isolated polar country has evolved differently compared with other industrialized countries (2). In a previous study we showed that most patients were infected through heterosexual contact and were middle-aged at the time of diagnosis. Many patients belonged to a socially marginalized group characterized by low income, unemployment, and heavy drinking. Even though highly active antiretroviral therapy (HAART) is tax-supported and free, we found an overall mortality rate of 11% per year for patients given HAART during 1997–2003 (2). In a molecular epidemiologic study, we showed that HIV was introduced at least 9 times into Greenland, and that one of these introductions has given rise to a circulating epidemic that has included 76% of all infected persons (3). Recently, we found 28% prevalence of transmitted drug resistance, corresponding well with the impression of low drug adherence and high risk behavior (T.V. Madsen et al., unpub. data). Contributing to the disappointing results could be the vast geography with often long distances to healthcare facilities, the short supply of specialized physicians, and the composition of the HIV-infected population. In 2002 the overall responsibility for treating HIV patients was transferred to the Department of Internal Medicine at Dronning Ingrids Hospital, Greenland’s main hospital, located in the capital, Nuuk. The chief physician at that department takes care of HIV patients in Nuuk and supervises treatment of HIV patients in other areas. With access to data from all HIV-infected persons in Greenland and Denmark, we aimed to compare the Implementation and effectiveness of HAART during 1997–2007 in 3 areas: Nuuk; Greenland’s remote districts (all towns and settlements except Nuuk); and the Western European country of Denmark, the former colonial power with which Greenland still has tight economical, social, and constitutional bonds.

The Study

The population-based Danish HIV Cohort Study (DHCS) collects clinical and paraclinical data on all HIV-infected persons under care in Denmark and Greenland since 1995 (2,4), including antiretroviral treatment, HIV RNA (viral load), and date of death or emigration. Patients from DHCS were followed from first visit to an HIV clinic until date of death, emigration, or last visit to the clinic. To estimate viral loads and CD4 cell counts between measurements, we carried forward the last observed value. HAART was defined as combination antiretroviral treatment with at least 3 drugs, including at least 1 protease inhibitor (PI), or 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), or abacavir. On January 1 for each year of the study we estimated the proportion of patients receiving HAART, each antiretroviral drug class, and selected antiretroviral drugs. National guidelines in both countries have recommended HAART initiation at a CD4 cell count <300 cells/μL, a threshold that has not changed since 1997. Among patients who had never received HAART, we estimated the proportion with a CD4 cell count <300 cells/μL, and among patients who had begun a HAART regimen at least 90 days previously, we estimated the proportion with a viral load <400 copies/mL. Annual mortality rates (MR) in the HIV population were estimated by person-years analysis; Poisson regression was used to test for trends over time.

Conclusions

Among 124 HIV patients in Greenland, 98 (79%) were infected through heterosexual contact, 78 (63%) were male, 111 (90%) were Inuit, and 98 (79%) were infected in Greenland. The median age at diagnosis was 50 years (interquartile range [IQR] 40–57 years), and the median CD4 cell count at diagnosis was 350 cells/μL (IQR 220–530 cells/μL). Among 4,702 HIV patients in Denmark, 2,114 (45%) were infected through homosexual contact, 1,745 (37%) through heterosexual contact, and 537 (11%) through intravenous drug use; 3,542 (75%) were male; 3,723 (79%) were Caucasian, and 650 (14%) black African. Half, 2,370, 1

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1 The Danish HIV Cohort Study: Departments of Infectious Diseases at Copenhagen University Hospitals Rigshospitalet (J. Gerstoft, N. Obel) and Hvidovre (G. Kronborg), Odense University Hospital (C. Pedersen), Aarhus University Hospitals, Skejby (C.S. Larsen) and Aalborg (G. Pedersen), Herning Hospital (A.L. Laursen), Helsingør Hospital (B. Kvinesdal), and Kolding Hospital (A. Møller).
were infected in Denmark, 725 (15%) in Africa, and 1,046 (22%) unknown; the median age at diagnosis was 34 years (IQR 28–42). The median CD4 cell count at diagnosis was 284 cells/μL (IQR 108–490 cells/μL).

In Greenland only 3% had begun HAART on January 1, 1997, as opposed to 28% in Denmark (Figure 1). The proportion on HAART increased gradually up to 81% in 2006, but not until 2003 did the proportion in Greenland reach the level in Denmark. Further, as late as 2001, 96% of all treatment regimens in Greenland included an unboosted PI (26% in Denmark), and in 2002 only 7% were NNRTI based (40% in Denmark). At that time the International AIDS Society USA guidelines carefully encouraged the use of boosted PIs, and NNRTI-based regimens were considered an equally effective alternative to PI-based regimens (5). Only after 2002 did the pattern shift, and in 2006 it was similar to that in Denmark, with approximately half of the combinations being NNRTI based, the other half PI based, with ritonavir-boosted lopinavir used in 65% of all PI regimens on January 1. The newer PI atazanavir was used in only 9% of PI regimens in 2006 (28% in Denmark). NNRTI used in Greenland has almost exclusively been efavirenz, whereas in Denmark 24% of NNRTI use on January 1, 2006, was nevirapine. The difference between the curves “ever on HAART” and “currently on HAART” in Figure 1 reflects the number of persons currently interrupting their treatment; the proportion with interruption is higher in Greenland than in Denmark. Structured treatment interruptions have not been recommended in Greenland or Denmark, so these persons supposedly have interrupted their therapy because of compliance problems. There was no difference in the uptake of HAART between Nuuk and the remote districts in Greenland (data not shown).

Until 2002, >30% of patients not yet receiving HAART in Greenland had a CD4 cell count <300 cells/μL (Figure 2). In comparison, the proportion in Denmark has been <30% since 1998, with <5% having a CD4 cell count <200 cells/μL since 2001. Among patients ever starting a HAART regimen, the proportion with suppressed viral load in Greenland was <45% until 2003 but has increased to 73% in 2006 (Figure 2). Nuuk reached the 75% mark in 2004, whereas the increase in the remote districts started later and reached 69% in 2006. The proportions in Denmark were 62% in 1998, 81% in 2003, and 88% in 2006.

The overall mortality rate among HIV patients in Greenland decreased from 139 (95% confidence interval [CI] 81–239) per 1,000 person-years in 1995–1997 to 59 (95% CI 35–99) in 2004–2006, corresponding to a 9% decrease per year (mortality rate ratio [MRR] = 0.91, 95% CI 0.84–0.98, p = 0.014) (Table). The decrease was most marked in patients in Nuuk (MRR = 0.86, 95% CI 0.77–0.96, p = 0.006) and less in the districts (MRR = 0.96, 95% CI 0.86–1.08, p = 0.533).

Treatment of HIV patients in Greenland began at a later stage of disease and has been implemented at a slower pace with lower therapeutic effectiveness than in Denmark, despite comparable economic means, general education of healthcare personnel, and common therapeutic guidelines. From other studies we know that patient support and education improve adherence (6) and that guideline-recommended therapy is more likely to be chosen if the physician is specialized in HIV and has >20 HIV patients in care (7), regardless of whether this physician is a generalist or infectious disease specialist. We observed marked improvements in the choice of antiretroviral drug combinations and effectiveness of HAART from 2003 onwards. These advances coincided with the establishment of a dedicated...
team in Nuuk and were most pronounced in that city when compared with the remote districts. Even though this temporal association does not prove causation, the improvements are likely to be partly attributable to the increased focus on HIV in the capital. The MR among HIV patients in Nuuk in recent years was higher than that in Denmark, but part of this difference may be attributable to a high background mortality rate among HIV-uninfected persons in Greenland (2,8) and an older HIV-infected population. As previously reported, sexually active persons in Greenland undergo frequent HIV testing (2), and CD4 cell counts were high at diagnosis, ruling out late testing as a contributor to the high MR. In conclusion, healthcare systems in the sparsely populated and isolated polar areas may be less fit to take on state-of-the-art care and treatment for HIV or other diseases previously unknown in the area, and an extra effort from the such providers may be needed to maximize control of the disease.

This study was supported by unrestricted grants from the Greenland Health Science Foundation, the Greenland AIDS Foundation, and the Danish AIDS Foundation. N. Obel has received research funding from Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, GlaxoSmithKline, Abbott, Boehringer Ingelheim, Janssen-Cilag, and Swedish Orphan.

Dr Lohse is a senior researcher at the Department of Clinical Epidemiology, Århus University Hospital, and the Danish HIV Cohort Study, Copenhagen University Hospital, Rigshospitalet, Denmark. His primary research interests include the clinical epidemiology of HIV.

References


Table. Mortality rate per 1,000 person-years among HIV patients in Greenland and Denmark, 1995–2007*

<table>
<thead>
<tr>
<th>Year</th>
<th>Greenland</th>
<th></th>
<th>Denmark</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Nuuk</td>
<td>Remote districts</td>
<td></td>
</tr>
<tr>
<td>2001–2003</td>
<td>84 (50–141)</td>
<td>107 (58–199)</td>
<td>54 (20–144)</td>
<td>25 (22–28)</td>
</tr>
</tbody>
</table>

Mortality rate ratio change per year, 1995–2006

<table>
<thead>
<tr>
<th></th>
<th>Greenland</th>
<th></th>
<th>Denmark</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Nuuk</td>
<td>Remote districts</td>
<td></td>
</tr>
<tr>
<td>0.91 (0.84–0.98)</td>
<td>0.86 (0.77–0.96)</td>
<td>0.96 (0.86–1.08)</td>
<td>0.82 (0.80–0.84)</td>
<td></td>
</tr>
</tbody>
</table>

p value† 0.014 0.006 0.533 <0.001

*Ranges in parentheses are 95% confidence intervals.
†Test for trend.


Declining risk of triple-class antiretroviral drug failure in Danish HIV-infected individuals

Nicolai Lohse, Niels Obel, Gitte Kronborg, Alex Laursen, Court Pedersen, Carsten S. Larsen, Birgit Kvinesdal, Henrik Toft Sørensen and Jan Gerstoft

Objectives: To analyse the incidence, prevalence, and predictors for development of triple-class antiretroviral drug failure (TCF) in individuals infected with HIV.

Design: Population-based observational cohort study from 1 January 1995 to 31 December 2003, focusing on all 2722 recipients of highly active antiretroviral therapy (HAART) in Denmark.

Methods: We used person-years analysis, Kaplan–Meier survival curves and Cox regression analysis. TCF was defined as a minimum of 120 days with viral load > 1000 copies/ml on treatment with each of the three major drug classes.

Results: We observed 177 TCFs, yielding a crude incidence rate (IR) of 1.8 per 100 person-years [95% confidence interval (CI), 1.6–2.1]. Seven years after initiation of HAART, 17.2% (95% CI, 14.5–20.5) of antiretroviral (ART)-experienced patients, but only 7.0% (95% CI, 4.3–11.2) of ART-naive patients were estimated to have failed. After an initial rise, the IR from the third to the sixth year of HAART declined significantly for ART-experienced patients [incidence rate ratio (IRR), 0.80 per year (95% CI, 0.66–0.97); P = 0.022], and non-significantly for ART-naive patients [IRR, 0.79 per year (95% CI, 0.53–1.18); P = 0.255]. The IR for all patients being followed each year declined from 1997 to 2003 [IRR, 0.88 (95% CI, 0.81–0.96); P = 0.002]. The prevalence of TCF remained stable at less than 7% after 2000. Predictors of TCF at commencement of HAART were a CD4 cell count below 200, a previous AIDS-defining event, previous antiretroviral exposure, earlier year of HAART initiation, and young age.

Conclusions: The risk of TCF is declining in Denmark and the prevalence remains stable.


Keywords: HIV, AIDS, cohort studies, highly active antiretroviral therapy, treatment failure

Introduction

Highly active antiretroviral therapy (HAART) effectively suppresses viral load (VL) in individuals infected with HIV [1]. HIV-related morbidity and mortality have decreased to less than one-fifth since HAART was introduced in 1996–1997 [2–5]. However, several controlled trials with strict intention-to-treat designs have indicated failure rates of 20–55% during the first year of HAART [6–8], but also in observational cohort studies the number of patients on HAART found to have detectable VL has been substantial [9,10]. High failure...
rates are associated both with regimens that have low efficacy and with poor patient compliance (often due to side effects), resulting in development of drug resistance. A high prevalence of resistance has been documented in the HIV Cost and Services Utilization Study (HCSUS) [11], and mathematical models predict a 42% prevalence of resistant virus in San Francisco by 2005 [12]. When failure occurs during an initial regimen (recommended to include two drug classes), general practice is to include a drug class from the new drug class in the next regimen [13–16], since full or partial cross-resistance within a class is the rule. Thus patients who fail their second or third regimen often harbour multi-resistant viruses that are unresponsive to the three major drug classes: nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [17,18]. Not all patients with triple class failure (TCF) experience virological failure and progressive disease [19]; however the majority of patients whose disease progresses despite HAART belong to the TCF group and this group is expected to grow in coming years [20]. It would thus be of interest to analyse the incidence and prevalence of TCF, as the development in these parameters over time would give an indication of the proportion of patients who experience disease progression despite HAART. Recently the consequences of TCF have been delineated in a large study of 2488 patients with TCF [19], but little is known about the prevalence of TCF. The current study defines TCF according to detectable HIV-RNA despite treatment and does not include genotypic and phenotypic assays. We examine the incidence, prevalence, and predictors for TCF development in a cohort consisting of all Danish HIV-infected patients who received HAART.

Methods

The Danish HIV cohort study

The Danish HIV Cohort Study (which started as The HIV Cohort Study in Western Denmark) is a prospective, population-based cohort study of all HIV-positive individuals seen at Danish HIV clinics since 1 January 1995. Data collection and processing have been described in detail elsewhere [21,22]. The Danish health care system provides free tax-supported medical care and antiretroviral treatment to HIV-infected individuals. Treatment for HIV infection in Denmark is restricted to five departments of infectious diseases and four departments of internal medicine with one or more infectious disease specialists. The drugs are delivered to the patients directly from these hospital departments. HIV medicine cannot be prescribed by general practitioners or bought from Danish pharmacies. Patients treated at all HIV centres in Denmark have been enrolled in the cohort, and complete data on all patients seen in any of the centres since 1 January 1995 have been collected from patient files and entered into the database. As of 1 January 2004, the cohort consisted of 3974 patients, 2722 of whom received HAART. Use of the Danish 10-digit personal identification number enables the centres to avoid multiple registrations of the same patient, and to document whether patients lost to follow-up have died or moved outside the country. The database is regularly updated with information on prophylactic and antiretroviral treatment, development of opportunistic infections and other AIDS-defining illnesses, and laboratory values including VL, CD4 cell count and blood lipids. All patients who began HAART were eligible for the study. We had information on the date when the patient started antiretroviral monotherapy or dual treatment (ART).

Definitions

The following definitions were used for the purposes of this study.

HAART
At least three antiretroviral drugs, including at least one PI or NNRTI, or abacavir.

AIDS
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 [23,24].

Virological failure
Defined as a VL of more than 1000 copies/ml for a total of 120 days (not necessarily successive) while receiving treatment with a given drug class. Periods of treatment interruptions, whether doctor or patient initiated, did not count as time with failure. VL was considered as being more than 1000 copies/ml only in time periods between two consecutive viral load measurements above 1000 copies/ml. Failure of a drug class could occur when it was administered alone or as part of a multi-drug regimen [19].

Time of TCF
The date the patient met the failure requirements for three drug classes. VL and CD4 cell counts at initiation of HAART were recorded as the last available measurement before this date, or the following measurement after this date up to 14 (VL) or 90 (CD4) days after.

Unboosted PI-regimen
Defined as any antiretroviral regimen that contains one or more PIs but not ritonavir, or a regimen containing ritonavir as the only PI.

Statistical analysis
We calculated time from start of HAART (baseline) to triple-class failure and summarized failure over time. Patients were followed from initiation of HAART, and
were censored at death or at last visit to the clinic. We stratified data by calendar period and constructed Kaplan-Meier survival curves for each period. The use of a model with left truncation (delayed entry) [25,26] allowed us to estimate long-term, event-free survival from baseline (initiation of HAART), based on data from a short observation period (i.e. observation years 2001–2003). Log rank test was used to test for differences between curves. We estimated TCF incidence rates (IRs) using person-years analysis, stratified according to pre-baseline ART-experience, and either according to time since HAART initiation or according to calendar time. Point prevalence of TCF was estimated as the proportion of living patients with TCF among all patients receiving HAART on 1 July each year. Poisson regression was used to estimate trends in IRs and prevalence. We performed Cox’ regression analyses to find predictors for TCF, using time since initiation of HAART as the time scale, and time zero at baseline + 18 months to ensure a cohort truly at risk of an event (due to the conditions included in our event definition). The following four variables: pre-baseline ART-experience; pre-baseline AIDS-defining event; baseline CD4 cell count; and baseline VL were mutually examined for interaction by stratified analysis. Due to hereby observed variation in the risk estimates, the adjusted analysis was stratified into ART-naive and ART-experienced patients, whereas the other three variables were kept in the model. The following five variables were then entered into the adjusted model if they caused a 10% change in the point estimates of the effect of any of the three variables included in the model above: age, gender, mode of infection, race/ethnicity, and year of HAART initiation. In ART-experienced patients we additionally tested the variable: time since starting antiretroviral drugs. The proportional hazards assumption was checked using Schoenfeld’s residuals [27]. Seventy-three of 177 patients lacked measurement of VL at baseline. These patients were followed up in the same way as the other patients from time of initiating HAART, but they could not count any failure time until their first measured VL, as stated in our definition of failure. Seventy-two of these 73 patients started HAART before mid-1997. Viral loads were measured a median of 3.98 times a year [inter-quartile range (IQR), 3.3–4.7].

**Approvals and permissions**

The Danish Data Protection Agency approved the establishment of the cohort study. The study was not subject to approval by the ethics committee as the collection of data did not involve direct patient contact.

**Results**

**Incidence and prevalence of triple-class drug failure**

Of the 3974 patients in the cohort, 2722 started HAART, and 1092 were exposed to all three drug classes. We observed 177 events (TCF) among patients initiating HAART during a median observation period of 3.7 years (IQR, 1.7–5.4) following treatment initiation, yielding an incidence rate (IR) of 1.8 per 100 person-years at risk (PYR) [95% confidence interval (CI), 1.6–2.1]. The IR reached a maximum of 2.9 (95% CI, 2.2–3.8) and 3.0 (95% CI, 2.2–4.1) per 100 PYR in the third and fourth year after HAART initiation, thereafter declining to 1.2 (95% CI, 0.6–2.5) in the sixth year (Fig. 1) [trend third to sixth year: IRR, 0.84 per year (95% CI, 0.70–1.00); P = 0.047]. For patients exposed to ART before HAART, the overall IR was 2.9 per 100 PYR (95% CI, 2.4–3.4), reaching a maximum of 4.6 (95% CI, 3.3–6.4) in the fourth year following HAART initiation, declining to 1.4 (95% CI, 0.6–3.2) in the sixth year [trend from third to sixth year: IRR, 0.80 per year (95% CI, 0.66–0.97); P = 0.022]. For ART-naive patients, the overall IR was 0.8 per 100 PYR (95% CI, 0.6–1.1). After a rise to 1.6 (95% CI, 0.9–2.7) in the third year following HAART initiation, the IR declined with no significant trend [third to sixth year: IRR, 0.79 per year (95% CI, 0.53–1.18); P = 0.255].

The IR per calendar year, for patients being followed up that year, peaked in 2000 at 3.7 (95% CI, 2.9–4.8) with declines to 1.6 (95% CI, 1.1–2.3) in 2001; 0.7 (95% CI, 0.4–1.3) in 2002, and 0.4 (95% CI, 0.2–1.1) in 2003. There was a significant declining trend in the IR from 1997 to 2003 [IRR, 0.88 per year (95% CI, 0.81–0.96); P = 0.002].

Seven years after baseline, 12.4% (95% CI, 10.4–14.8) of all patients; 17.3% (95% CI, 14.5–20.5) of ART-experienced patients; and 7.0% (95% CI, 4.3–11.2) of ART-naive patients were estimated to have failed. When the observations were split into three observation periods

![Fig. 1. Incidence of triple-class drug failure for each year after initiation of highly active antiretroviral therapy](image-url)
A lower risk of TCF was seen after 2001 than in previous years, regardless of when patients initiated HAART (log rank test for all pair wise combinations: $P = 0.000$) (Fig. 2). For all patients observed in the 2001–2003 period, the estimated 7-year cumulative incidence proportion was 6.8% (95% CI, 4.8–9.6). For ART-naive patients, the estimated 7-year cumulative incidence proportion in 2001–2003 was only 5.5% (95% CI, 2.9–10.1), corresponding to an IR of 0.6 (95% CI, 0.4–1.0) per 100 PYR.

The point prevalence of patients with TCF among patients on HAART, measured at 1 July each year, increased from 1997 to 2001 [increase 1.60 per year (95% CI, 1.46–1.76); $P = 0.000$], with a non-significant decrease observed from 2001 to 2003 [increase 0.94 per year (95% CI, 0.83–1.07; $P = 0.356$)] (Fig. 3).

Patients with triple-class drug failure

Individuals who developed TCF had a lower CD4 cell count at baseline than other patients [median, 75 (IQR, 26–170) versus 190 (80–300) $\times 10^6$ cells/l]. A higher log$_{10}$VL at baseline was observed only in ART-experienced [median, 4.9 (IQR, 4.3–5.5) versus 4.2 (IQR, 3.0–5.0)], but not in ART-naive [median, 5.2 (IQR, 4.2–5.5) versus 5.0 (IQR, 4.5–5.7)] patients (Table 1). Patients who experienced TCF were also more likely to have had a previous AIDS-defining event (46 versus 23%), to be ART-experienced (79 versus 37%), they were younger [median age, 36.8 (IQR, 30.6–44.2) versus 38.7 (IQR, 32.7–46.5) years], and they had initiated HAART earlier (median, November 1996 versus August 1998). Patients with and without TCF did not differ with regard to gender, mode of acquisition, or race. The last drug class to fail was NNRTI in 94% of the patients and PI in 6% of the patients. Ninety-eight percent of the patients had been exposed to an unboosted PI.

Predictors for development of triple-class drug failure

In crude analyses, an earlier year of HAART initiation, having a high viral load or a CD4 count below 200 $\times 10^6$ cells/l at the time of HAART initiation, having had an AIDS-defining event, being ART-experienced, and being young were associated with a higher risk of TCF (Table 2). In the multivariate model, a high VL at the time of HAART initiation was a predictor in ART-experienced, but not in ART-naive patients (Table 2). The TCF risk among ART-naive patients who initiated HAART after 1999 was one-tenth of that faced by ART-naive patients with earlier HAART initiation, adjusted for other predictors.

Discussion

In this study, based on a complete nationwide cohort of 2722 HIV-patients who initiated HAART, we found a declining IR of TCF after 2001, and a stable prevalence in the period 2000–2003. Thus we observed no signs of increasing failure incidence rates over time in a population in which HAART was offered to all those who were at risk of clinical progression. The IR of TCF differed substantially according to ART exposure before HAART, both in terms of absolute risk and in timing. Among the ART-experienced, a peak was observed 3–4 years after HAART initiation. The length of time for the IR to reach the maximum stems from the TCF definition, in which the occurrence of an event requires exposure to three drug classes and a minimum time period for virological failure to occur.

The decline in IR from the fifth year of HAART onwards may have several explanations. First, patients with prior...
ART experience who fail their first HAART are often cross-class resistant to both NRTI and PI and are at high risk of failure in their second regimen. Assuming that these cross-class-resistant patients experience TCF in the first years after initiating HAART, the remaining patients being followed up will have a risk of failure more close to that observed among the ART-naive. Other patients with high risk of failure, due to low adherence, could potentially produce a similar peak. However, the absence of a clear peak among the treatment-naive patients suggests that pre-existing resistance from a former regimen (as opposed to behavioural factors) mainly drove early failures among patients with a pre-HAART ART experience. Furthermore, the fifth year of HAART is by definition after 2000; it is possible that failure was less likely in this period due to better regimens or better patient coaching. In fact, stratification by observation period did provide evidence that patients fared better in 2001–2003 than during earlier periods of HAART, unrelated to date of HAART initiation.

Mocroft et al. [28] have studied the risk of TCF in a pan-European cohort study. They found a 6-year risk of TCF of 21.4% among ART-experienced, and 11.2% among ART-naive individuals with overall IRs of 1.6 and 3.9 cases/100 PYR in the two groups, respectively, which is higher than that observed in our study. Several explanations may be given for the differences in the IR of TCF observed in the two studies. First the possibility that the IR of TCF is lower in Denmark than in other parts of Europe cannot be excluded. This could be related to structural health care issues such as a high caseload per HIV-treating physician [29], high representation of academic centres, or the mode of drug delivery. Second, the lower risk could be related to the infected population, although the present study failed to identify mode of transmission as a risk factor. Third, our study was a nationwide cohort including all patients with ART experience in Denmark, making it possible to minimize recruitment bias.

We used a rather lax definition of TCF, because this definition approaches our clinical impression of failure, and because the same definition was used in another study [19] showing poor prognostic outcome for these patients. The statistical analyses hinged on the definition of when a person became at risk of developing TCF. In common survival analysis an individual is arguably not at risk until after 119 days of failing the third drug class, but use of this...
definition would have excluded all but very high-risk patients. We therefore used a clinically more relevant time scale, starting when the patient was first exposed to HAART (and thereby potential drug failure), and we restricted the regression analyses to observations after 18 months, a reasonable time period to develop TCF. When the analyses were repeated using time of HAART initiation as the starting point, or restricted to patients exposed to three drug classes for a minimum of 120 days, we obtained similar significant results.

One striking observation is the decrease in failure rate according to year of HAART initiation; after controlling for confounding factors, initiating HAART during the early years carried a six-fold higher risk than initiating after 1999. There are a number of possible explanations. First, the strategy has changed from PI-based to NNRTI-based HAART over time. Second, although boosted PI therapy has been used from 1996 [30], it has not totally replaced unboosted PI treatment until after 2001 (data not shown). Studies suggest that boosted PIs are superior to unboosted PIs [7] as they produce higher drug levels, which in turn have been associated with better outcomes [31,32]. In this study, initiating therapy with an unboosted PI was associated significantly with TCF (data not shown). However, comparisons of drugs and regimens in cohort studies should be done with utmost caution, as biases are likely to be present. Third, the increasing number of drug combinations available for in-class substitutions could induce better individual patient tolerability. However the success of regimens containing NNRTIs, a drug class with very limited in-class substitution possibilities, suggests that this is not the sole reason. A fourth explanation for the improved outcome over time could be better patient coaching in more recent years, following recognition of adherence as the key factor for success of a regimen [33]. This observed decrease in failure rate corresponds with recent years’ advances in HIV treatment. These recent advances should be kept in mind when setting success rates (not too low) for HIV treatment rollout programs in resource-limited settings.

The strength of our study was the completeness of the Danish HIV Cohort. Although HIV-infected individuals who are not on antiretroviral treatment may visit their general practitioner without inclusion in the cohort, all patients seen in a public HIV clinic are captured. The fact that antiretroviral drugs are distributed only through these clinics (where they are provided free of charge) ensures that almost all Danish HIV patients receiving ART are included in the cohort, eliminating the risk of selection bias.

Our study had several limitations. First, we did not have resistance data. Triple-class drug failure was defined by previously established criteria [19], where virological failure of a given magnitude (VL > 1000 copies/ml)

### Table 2. Incidence rate ratios for development of triple-class drug failure estimated by univariate and multivariate Cox’s regression analysis.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Crude</th>
<th>ART-experienced</th>
<th>ART-naive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRR 95% CI P-value</td>
<td>IRR 95% CI P-value</td>
<td>IRR 95% CI P-value</td>
</tr>
<tr>
<td>IDU 0.84</td>
<td>0.46–1.56 0.584</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female 1.26</td>
<td>0.88–1.79 0.207</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Caucasian 0.74</td>
<td>0.50–1.08 0.119</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Art-naive at HAART 0.29</td>
<td>0.20–0.43 0.000</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age at HAART 0.73</td>
<td>0.62–0.86 0.000</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Male homosexual 0.81</td>
<td>0.59–1.11 0.194</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AIDS at HAART 2.78</td>
<td>2.03–3.80 0.000</td>
<td>1.6 1.1–2.4 0.010</td>
<td>2.0 0.9–4.5 0.084</td>
</tr>
</tbody>
</table>

CD4 cell count at initiation of HAART (cells $\times 10^{3}$/l)

| > 200 | 1 | 0.000 | 1 | 0.002 | 1 | 0.035 |
| 50–200 | 2.91 | 1.88–4.52 0.000 | 2.4 | 1.3–4.2 | 0.003 | 1.2 | 0.5–3.1 | 0.667 |
| < 50 | 5.44 | 3.47–8.54 0.000 | 2.9 | 1.6–5.4 | 0.001 | 3.0 | 1.1–7.9 | 0.032 |

VL at initiation of HAART (log$_10$ copies/ml)

| VL < 4 | 1 | 0.013 | 1 | 0.000 | 1 | 0.743 |
| VL 4–5 | 1.41 | 0.76–2.61 0.270 | 2.0 | 1.0–3.9 | 0.055 | 0.6 | 0.1–2.3 | 0.435 |
| VL > 5 | 2.02 | 1.14–3.56 0.016 | 3.5 | 1.8–6.8 | 0.000 | 0.6 | 0.2–2.2 | 0.446 |
| missing | 2.85 | 1.63–4.92 0.000 | 2.0 | 1.1–3.8 | 0.029 | 0.8 | 0.2–3.2 | 0.716 |

Year of HAART initiation

| ≤ 1996 | 1 | 0.000 | 1 | 0.031 | 1 | 0.004 |
| 1997–98 | 0.34 | 0.25–0.47 0.000 | 0.6 | 0.4–1.1 | 0.046 | 0.8 | 0.3–2.1 | 0.691 |
| 1999–2003 | 0.06 | 0.02–0.17 0.000 | 0.4 | 0.1–1.8 | 0.248 | 0.1 | 0.0–0.6 | 0.008 |

1 test for trend;
2 test for trend (missing viral load measurements omitted). ART, antiretroviral therapy; IRR, incidence rate ratio; CI, confidence interval; IDU, intravenous drug use; HAART, highly active antiretroviral therapy; VL, viral load.
during a given time period (120 days) was interpreted as a surrogate marker for drug failure. Resistance does not necessarily develop to all drugs in a regimen despite failure [7,34], and resistance might have been developed to all three major drug classes in patients not meeting our definition of TCF. Individuals with TCF did have difficulty attaining full viral suppression, however, as only 19% of viral load measurements after the time of TCF were below 400 copies/ml; this contrasts with 70% of VLs in other patients in the cohort after HAART initiation. Furthermore another study has revealed a high risk of death among patients with triple class failure [19]. Thus we do believe that the majority of disease progression-related morbidity and mortality would occur in patients within this group, although we cannot exclude that a few patients receiving HAART will experience disease progression without fulfilling our criteria for TCF, for example, due to intolerance to a whole class.

Second, we had no information about patient compliance. Some patients may not have taken any of the prescribed drugs, and may have fulfilled our criteria without developing drug resistance.

Third, although HIV-RNA assays were initiated in 1995, they only became available for all patients from mid-1997 on, and we may have missed some early HAART failures. We did not include failure prior to HAART in the analyses, but used prior ART experience as an explanatory variable. NNRTIs were not available in Denmark before 1997 so we were only concerned about PI or NRTI failure before 1997. However, 167 of our TCF-patients failed NNRTI as the last drug class, and another seven initiated therapy with HAART after mid-1997, leaving only three patients (1.7%) that could have been observed with TCF at an earlier date. Had any of these been picked up earlier it would only have strengthened our finding of a declining IR. Of the 40 patients who met the criteria for NNRTI-failure, but did not develop TCF, only seven (0.3% of all non-TCF) had received HAART before mid-1997. The temporal distribution of these NNRTI failures was similar to that for TCFs in general, thus they are unlikely to have biased our results. Only failure with the three major drug classes was investigated, as fusion inhibitors have been used in less than 15 patients in Denmark. Our study was carried out during a time when surveillance revealed a yearly prevalence of transmitted resistance in the country between 0 and 5% [35]. If transmitted resistance increases, the IR of TCF is likely to increase as well. On the other hand the low level of transmitted resistance could be a consequence of the low IR of TCF.

In conclusion, we found a declining IR of TCF in the Danish HIV-infected population. Importantly, the study provides evidence that high drug pressure on HIV at the population level does not inevitably result in increasing rates of drug failure.

References


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

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Background. Our objective was to examine whether virological control during the first 6–18 months after HAART initiation is a predictor for viral suppression, CD4+ cell count increase, and mortality in human immunodeficiency virus (HIV)—infected patients 18–90 months after initiation of highly active antiretroviral therapy (HAART).

Methods. We conducted a population-based observational cohort study in Denmark. Patients were divided into 3 groups, according to the proportion of time each patient had a detectable HIV RNA load (i.e., >400 copies/mL) during the 6–18 months after HAART initiation: 0% of the time interval (group 1), 1%–99% of the time interval (group 2), and 100% of the time interval (group 3). The proportion of patients with undetectable HIV RNA, CD4+ cell count changes, and mortality were examined by logistic, linear, and Cox regression analyses, respectively. We constructed cumulative mortality curves.

Results. We observed 2046 patients, for a total of 8898 person-years of follow-up that started at 18 months after HAART initiation. Mean CD4+ cell count increase rates during 72 months of follow-up were as follows: group 1, 3.3 × 106 cells/L per month (95% confidence interval [CI], 2.9–3.7 × 106 cells/L); group 2, 2.9 × 106 (95% CI, 2.5–3.3 × 106 cells/L); and group 3, 2.6 × 106 (95% CI, 2.0–3.3 × 106 cells/L). Survival at 72 months were as follows: group 1, 92.7% (95% CI, 90.5%–94.4%); group 2, 85.6% (95% CI, 81.1%–88.5%); and group 3, 76.1% (95% CI, 70.6%–80.7%). At 72 months, 96% of group 1, 83% of group 2, and 57% of group 3 had an HIV RNA load of <400 copies/mL (P<.01). Treatment interruption before baseline was a predictor of mortality in group 2 (adjusted rate ratio, 2.94; 95% CI, 1.75–4.92).

Conclusions. Viral suppression during the first 6–18 months after HAART initiation predicts viral suppression, CD4+ cell count progression, and survival at 72 months.

The introduction of HAART has dramatically changed the prognosis for patients with HIV infection in the Western world [1]. A low CD4+ cell count and a high HIV RNA load at the time of HAART initiation [2–5] predicts poor outcome with respect to progression to AIDS or death. Some studies have shown that viral load and CD4+ cell count retain their predictive value when measured after HAART initiation [6–11]. Current therapeutic guidelines recommend achieving an undetectable viral load, preferably <50 copies/mL and at least <400 copies/mL [12, 13]. Continuous viral suppression is associated with an increase in CD4+ cell count and low disease progression [14–16]. However, a considerable number of patients do not achieve these goals [17–21], or they experience viral load rebound to values >400 copies/mL after initial viral suppression. Such patients have a continuously or intermittently detectable viral load, mainly because of poor adherence to treat-

Received 8 June 2005; accepted 29 August 2005; electronically published 30 November 2005.

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Clinical Infectious Diseases 2006;42:136–44
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1658-4630/2006/4201-0022$15.00
ment [22, 23]. Previous prognostic studies have focused primarily on the first 6 months after HAART initiation as the "prognostic period" [24], but analysis of this period is often hampered by episodes of acute toxicity and changes in regimen, and viral load does not reach its nadir until the end of the period. After receiving HAART for 6 months, a patient's viral load should remain undetectable; in addition, episodes of acute toxicity should have passed, or the regimen should have been adjusted. At this time, although long-term toxicities have not emerged yet, HAART recipients have probably entered a period that is representative for the remaining lifelong treatment period. Our aim was to examine whether virological control during the first year of this period (6–18 months after HAART initiation) is a predictor for viral suppression, CD4+ cell increase, and mortality in HIV-infected patients 18–90 months after HAART initiation in a nation-wide, population-based cohort with complete follow-up, and to study the impact of baseline characteristics on these outcomes.

SUBJECTS AND METHODS

The Danish HIV Cohort Study
The Danish HIV Cohort Study is a prospective, nation-wide, population-based cohort study of all HIV-infected individuals treated in Danish HIV clinics since 1 January 1995. Study methods have been described in detail elsewhere [25, 26]. Treatment for HIV infection in Denmark is restricted to 8 specialized treatment centers. The Danish health care system provides free, tax-supported medical care for all residents, including antiretroviral treatment for HIV-infected individuals. As of 1 March 2005, 4084 adult patients were enrolled in the study. The cohort study is ongoing, with continuous enrollment of both newly diagnosed HIV-infected individuals and individuals with HIV infection who move into the country. Use of the Danish 10-digit personal identification number enables treatment centers to avoid multiple registrations of the same patient and allows tracking of deaths and losses to follow-up due to emigration. Updates are performed annually.

Study Population
Eligible for the study were patients who initiated HAART before 1 January 2002, had at least 1 viral load measurement within 6 months after HAART initiation, and were alive at 18 months after HAART initiation. Patients not known to have died and not seen in the clinic since 1 January 2004 were considered to be lost to follow-up.

Antiretroviral Therapy
HAART was defined as combination antiretroviral treatment with at least 3 drugs, including 1 protease inhibitor (PI), or 1 nonnucleoside reverse transcriptase inhibitor (NNRTI), or abacavir. Patients classified as “naive” had been unexposed to antiretroviral treatment as of 2 weeks before HAART initiation.

Treatment Interruption
Treatment interruption was defined as a period of at least 2 weeks in which the patient was not taking antiretroviral drugs, after receiving HAART for the first time.

CD4+ Cell Count and Viral Load Measurements
To determine CD4+ cell counts at time points between measurements, the last measured value was carried forward. To model viral load values at time points between measurements, each measured value was carried forward for 30 days or until the next measurement, whichever came first. The measured viral load was extrapolated back to 30 days after the previous measurement. A viral load was considered to be detectable at a value of at least 400 copies/mL. We used this model because a (high) viral load measured at a visit to the health care clinic often leads to revision of the antiretroviral treatment regimens and, therefore, subsequent changes in viral load.

Prognostic Groups
We calculated the proportion of time that each patient had a detectable viral load during the 1-year period extending from 6 to 18 months after HAART initiation. For analysis purposes, on the basis of this proportion, patients were divided into 3 subgroups: group 1 had detectable HIV RNA for 0% of the time interval, group 2 had detectable HIV RNA for 1%–99% of the time interval, and group 3 had detectable HIV RNA for 100% of the time interval. For some analyses, patients were divided into 6 "detectable viral load" subgroups: 0% (group 1), 1%–25% (group 2A), 26%–50% (group 2B), 51%–75% (group 2C), 76%–99% (group 2D), and 100% (group 3).

Statistical Analyses

Viral load. The prevalence of patients with an undetectable viral load was calculated at baseline (18 months after HAART initiation) and every 18 months thereafter. Ratios between groups calculated at 72 months were analyzed using a logistic regression model.

CD4+ cell count. CD4+ cell count increases were computed for each of 4 consecutive 18-month periods after baseline and for the whole 72-month period. Individual increases were calculated for all patients observed throughout each period. Differences in mean CD4+ cell increases between groups were examined using a linear regression model.

Mortality. We computed the amount of time from baseline to death or end of follow-up and constructed cumulative mortality curves. Patients were censored at the time of their last clinic visit. We performed Cox proportional hazards regression.
analyses to estimate mortality rate ratios (MRRs) and to adjust for covariates.

**Model building.** Variables entered into the regression models included patient group, injection drug use as the mode of infection, hepatitis C antibody status, antiretroviral exposure preceding HAART initiation, having an AIDS diagnosis before HAART initiation, CD4+ cell count at HAART initiation (used in Cox and logistic models) or at the beginning of each period (used in the linear model), white race, sex, age at HAART initiation, initiation of HAART before 1 January 1999, and known treatment interruption before baseline. Using the patient group as the predictor variable, all other variables causing a 10% change in the risk estimates were entered into the regression models one by one [27]. Because of variation in the risk estimates, patients with a known treatment interruption were analyzed separately in the Cox model.

**Prediction of survival with undetectable viral load.** To predict the chance of being alive with an undetectable viral load at 72 months after baseline, cumulative survival and confidence intervals were multiplied by the proportion of patients with an undetectable viral load at that time.

**RESULTS**

**Study population.** There were 2404 patients who had initiated HAART before 1 January 2002. The cumulative survival at 18 months after HAART initiation was 94.5% (95% CI, 93.5%–95.3%). There were 2046 patients who met the inclusion criteria at 18 months (baseline). Of the 358 patients who did not meet the inclusion criteria, 132 patients died before baseline, and 45 patients could not be observed for a full 18 months. Of these 45 patients, 28 left the country, and 17 had initiated HAART <18 months before their most recent clinic visit. An additional 181 patients who started HAART were excluded because their first viral load measurement occurred >6 months after HAART initiation. Of these 181 patients, 131 initiated HAART before mid-1997, when viral load measurements became routine for all patients. Of the 2046 patients, 1173 had undetectable viral loads throughout the whole period extending from 6 to 18 months after initiating HAART (group 1), 546 had detectable viral loads part of the time (group 2), and 327 had detectable viral loads throughout the whole period (group 3). The 2046 patients were observed for a total of 8898 person-years after baseline. Sixty-seven patients (3.2%) were lost to follow-up. Five hundred twenty-nine patients remained under observation at the beginning of the period 1173, 546, 327, 2046.

**Table 1. Number of patients who underwent follow-up at different periods after baseline, by patient group.**

<table>
<thead>
<tr>
<th>Variable, by observation period</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–18 months after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underwent observation at the beginning of the period</td>
<td>1173</td>
<td>546</td>
<td>327</td>
<td>2046</td>
</tr>
<tr>
<td>Censored</td>
<td>61</td>
<td>19</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Died</td>
<td>19</td>
<td>28</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td>Total observed during the period</td>
<td>1093</td>
<td>499</td>
<td>292</td>
<td>1884</td>
</tr>
<tr>
<td>18–36 months after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored</td>
<td>275</td>
<td>56</td>
<td>16</td>
<td>347</td>
</tr>
<tr>
<td>Died</td>
<td>20</td>
<td>24</td>
<td>23</td>
<td>67</td>
</tr>
<tr>
<td>Total observed during the period</td>
<td>798</td>
<td>419</td>
<td>253</td>
<td>1470</td>
</tr>
<tr>
<td>36–54 months after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored</td>
<td>235</td>
<td>77</td>
<td>35</td>
<td>347</td>
</tr>
<tr>
<td>Died</td>
<td>11</td>
<td>12</td>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td>Total observed during the period</td>
<td>552</td>
<td>330</td>
<td>204</td>
<td>1096</td>
</tr>
<tr>
<td>54–72 months after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored</td>
<td>312</td>
<td>143</td>
<td>80</td>
<td>535</td>
</tr>
<tr>
<td>Died</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Total observed during the period</td>
<td>231</td>
<td>182</td>
<td>116</td>
<td>529</td>
</tr>
</tbody>
</table>

**NOTE.** Baseline was 18 months after HAART initiation.

**Prediction of survival with undetectable viral load.** To predict the chance of being alive with an undetectable viral load at 72 months after baseline, cumulative survival and confidence intervals were multiplied by the proportion of patients with an undetectable viral load at that time.

**Progression of CD4+ cell counts.** The final adjusted linear regression model for CD4+ cell count increase included CD4+ cell count at the beginning of each period. The mean CD4+ cell count increased significantly for 4 consecutive 18-month periods in all groups. The crude mean CD4+ cell count increase during the 72-month period after baseline was 3.3 × 10^6 cells/L per month (95% CI, 2.9–3.7 × 10^6 cells/L per month) for group 1, 2.9 × 10^6 cells/L per month (95% CI, 2.5–3.3 × 10^6 cells/L per month) for group 2, and 2.6 × 10^6 cells/L per month (95% CI, 2.0–3.3 × 10^6 cells/L per month) for group 3 (table 3). The difference between groups in this 72-month period was significant in the adjusted model (P<.01 for individual group...
Table 2. Patient characteristics, by patients grouped according to the percentage of time each patient had a viral load $\geq 400$ copies/mL during the period 6–18 months after HAART initiation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 $(n = 1173)$</th>
<th>Group 2 $(n = 546)$</th>
<th>Group 3 $(n = 327)$</th>
<th>$P^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of time with a viral load $\geq 400$ copies/mL</td>
<td>0</td>
<td>1–99</td>
<td>100</td>
<td>...</td>
</tr>
<tr>
<td>Sex, % of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76</td>
<td>75</td>
<td>75</td>
<td>.758</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>25</td>
<td>25</td>
<td>...</td>
</tr>
<tr>
<td>Age at HAART initiation, median years (IQR)</td>
<td>39 (33–48)</td>
<td>37 (32–45)$^a$</td>
<td>37 (32–45)$^a$</td>
<td>.0005</td>
</tr>
<tr>
<td>CD4$^+$ cell count, median cells/mm (IQR)$^a$</td>
<td></td>
<td></td>
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<tr>
<td>At HAART initiation</td>
<td>209 (97–304)</td>
<td>169 (72–279)$^b$</td>
<td>140 (60–270)$^a$</td>
<td>.0005</td>
</tr>
<tr>
<td>At baseline</td>
<td>390 (242–530)$^c$</td>
<td>300 (190–440)$^c$</td>
<td>210 (120–340)$^c$</td>
<td>.0005</td>
</tr>
<tr>
<td>Viral load at HAART initiation, median log$_{10}$ copies/mL</td>
<td>4.6 (3.2–5.3)</td>
<td>4.6 (3.4–5.2)</td>
<td>4.7 (3.7–5.2)</td>
<td>.328</td>
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<td>Mode of infection, % of patients</td>
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<td>Hepatitis C coinfection, % of patients</td>
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<td>Previous antiretroviral exposure at HAART initiation, % of patients</td>
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<tr>
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<td>29</td>
<td>52</td>
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<td>Race, % of patients</td>
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<td>76</td>
<td>69</td>
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<td>Treatment interruption within first 18 months on HAART</td>
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<td>83</td>
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</tr>
<tr>
<td>Date of HAART initiation, % of patients</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 1 January 1999</td>
<td>53</td>
<td>73</td>
<td>84</td>
<td>.0005</td>
</tr>
<tr>
<td>After 1 January 1999</td>
<td>47</td>
<td>27</td>
<td>16</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Baseline was 18 months after initiation of HAART. IQR, interquartile range.

$^{a}$ $P < .0005$ compared with group 1, by Student’s t test.

$^{b}$ $P = .001$ compared with group 1, by Student’s t test.

$^{c}$ $P < .0005$ compared with each of the other 2 groups.

$^{d}$ Determined by $\chi^2$ test for binary variables; 1-way analysis of variance for continuous variables.

Comparisons. Figure 1 shows the progression of median CD4$^+$ cell count commencing from HAART initiation.

**Survival.** The cumulative 72-month survival from baseline was 87.7% (95% CI, 85.9%–89.3%) for all patients and 92.7% (95% CI, 90.5%–94.4%), 85.6% (95% CI, 82.1%–88.5%), and 76.1% (95% CI, 70.6%–80.7%) for groups 1, 2, and 3, respectively (figure 2). The final adjusted Cox regression model included age at HAART initiation. With group 1 as the reference group, group 2 had a crude MRR of 2.38 (95% CI, 1.68–3.36), and group 3 had crude MRR of 3.96 (95% CI, 2.81–5.60). The adjusted MRRs were as follows: group 2, 2.63 (95% CI, 1.86–3.72); and group 3, 4.53 (95% CI, 3.20–6.42). When group 2 was divided into 4 subgroups (i.e., for a total of 6 groups), according to the proportion of time each patient had a detectable viral load, the mortality curves suggested similar mortality rates for subgroups A and B and for subgroups C and D (figure 2). Therefore, we compared subgroups C and D (51%–99% detectable) with A and B (1%–50% detectable) and found an age-adjusted MRR of 1.61 (95% CI, 1.00–2.59; $P = .048$).

**Subgroup with treatment interruption.** The group 2 sub-
group that experienced treatment interruption for any reason during the first 18 months of HAART (n = 92) had a mortality rate of 6.87 deaths per 100 person-years at risk (95% CI, 4.57–10.34 deaths per 100 person-years). This mortality rate exceeded that for the group 2 subgroup without treatment interruption (adjusted MRR, 3.48; 95% CI, 2.10–5.79). It was also higher than that for group 3 patients without treatment interruption (adjusted MRR, 1.59; 95% CI, 0.97–2.60) but similar to that for group 3 patients with treatment interruption (adjusted MRR, 0.98; 95% CI, 0.52–1.87). The adjusted MRR of treatment interruption versus no treatment interruption within group 2 was at least 2.2 in all strata of the following variables: mode of infection (injection drug use vs. other), antiretroviral drug use preceding HAART initiation (naive vs. experienced), having an AIDS diagnosis before HAART initiation, having a CD4+ cell count at the time of HAART initiation (>100 vs. <100 cells/µL, and >200 vs. <200 cells/µL), sex, and date of HAART initiation (before vs. after 1 January 1999). Reasons for treatment interruption were noted for 85 of the 92 subjects. The most common reasons were compliance problems (30%), patient’s wish (25%), and drug intolerance (27%). “Doctor’s decision” was noted as a reason for only 6% of patients.

Causes of death. The causes of death were categorized as either HIV related (AIDS-defining conditions and bacterial infections), non–HIV related, or unknown. The prevalence of causes of death in group 1 were as follows: HIV related, 18%; non–HIV related, 58%; and unknown, 24%. The prevalence of causes of death in group 2 were as follows: HIV related, 35%; non–HIV related, 47%; and unknown, 18%. The prevalence of causes of death in group 3 were as follows: HIV related, 43%; non–HIV related, 40%; and unknown, 17%. The prevalence of causes of death among patients in group 2 without treatment interruption were as follows: HIV related, 31%; non–HIV related, 53%; and unknown, 16%. And prevalences of causes of death in group 2 with treatment interruption were as follows: HIV related, 43%; non–HIV related, 35%; and unknown, 22%.

Survival with undetectable viral load. In table 4, cumulative 72-month survival is combined with viral load measurements 72 months after baseline to predict the chance of being alive and virologically suppressed 90 months (7.5 years) after initiating HAART (conditioned on the patient being alive at baseline, 18 months [1.5 years] after initiating HAART). The probability of a successful outcome using these criteria ranged from 89% for group 1 (95% CI, 87%–90%) to 71% for group 2 (95% CI: 68%–74%), and 43% for group 3 (95% CI, 40%–46%).

DISCUSSION

In this study, we found the degree of postprimary virological suppression (6–18 months after HAART initiation) positively associated with CD4+ cell count increases, survival, and the chance of having a viral load <400 copies/mL at 90 months (7.5 years) after HAART initiation.

The strength of our study was the use of a population-based,
Table 3. Univariate and multivariate linear regression analysis of CD4+ cell count increases after baseline.

<table>
<thead>
<tr>
<th>Group variable, by time period</th>
<th>Crude</th>
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<th>Adjusted</th>
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<tr>
<td></td>
<td>Value</td>
<td>95% CI</td>
<td>P</td>
<td>Value</td>
<td>95% CI</td>
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<tr>
<td>0–18 months after baseline</td>
<td></td>
<td></td>
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<tr>
<td>Mean CD4+ increase per month, cells × 10^6/L</td>
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<td></td>
</tr>
<tr>
<td>Group 1 (constant)</td>
<td>4.7</td>
<td>4.1–5.2</td>
<td>&lt;.01a</td>
<td>8.4</td>
<td>7.6 to 9.3</td>
<td>&lt;.01b</td>
</tr>
<tr>
<td>Group 2 (added to group 1)</td>
<td>−1.8</td>
<td>−2.7 to −0.8</td>
<td>&lt;.01</td>
<td>−2.5</td>
<td>−3.4 to −1.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Group 3 (added to group 1)</td>
<td>−3.7</td>
<td>−4.8 to −2.6</td>
<td>&lt;.01</td>
<td>−5.2</td>
<td>−6.3 to −4.1</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>CD4+ cell count at 18 months</td>
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<tr>
<td>18–36 months after baseline</td>
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<td>Mean CD4+ increase per month, cells × 10^6/L</td>
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<tr>
<td>Group 1 (constant)</td>
<td>3.3</td>
<td>2.7–3.9</td>
<td>.27a</td>
<td>7.0</td>
<td>5.8 to 8.1</td>
<td>&lt;.01a</td>
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<td>−1.1 to 1.0</td>
<td>.96</td>
<td>−0.8</td>
<td>−1.9 to 0.3</td>
<td>.13</td>
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<td>−2.1 to 0.4</td>
<td>.20</td>
<td>−2.5</td>
<td>−3.8 to −1.2</td>
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<td>CD4+ cell count at 36 months</td>
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<tr>
<td>36–54 months after baseline</td>
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<tr>
<td>Mean CD4+ increase per month, cells × 10^6/L</td>
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<tr>
<td>Group 1 (constant)</td>
<td>2.3</td>
<td>1.6–3.1</td>
<td>.46a</td>
<td>5.7</td>
<td>4.3 to 7.1</td>
<td>.23a</td>
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<td>.11</td>
<td>0.3</td>
<td>−0.9 to 1.6</td>
<td>.60</td>
</tr>
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<td>−1.2 to 1.7</td>
<td>.76</td>
<td>−1.2</td>
<td>−2.7 to 0.3</td>
<td>.11</td>
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<td>CD4+ cell count at 54 months</td>
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<tr>
<td>54–72 months after baseline</td>
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<tr>
<td>Mean CD4+ increase per month, cells × 10^6/L</td>
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<tr>
<td>Group 1 (constant)</td>
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<td>1.4–3.7</td>
<td>.32a</td>
<td>7.2</td>
<td>5.3 to 9.0</td>
<td>.01b</td>
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<td>.04</td>
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<td>−4.0 to −0.7</td>
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<td>−2.1</td>
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<tr>
<td>0–72 months after baseline</td>
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<tr>
<td>Mean CD4+ increase per month, cells × 10^6/L</td>
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<tr>
<td>Group 1 (constant)</td>
<td>3.3</td>
<td>2.9–3.7</td>
<td>.04a</td>
<td>4.2</td>
<td>3.6 to 4.8</td>
<td>&lt;.01b</td>
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<td>−1.0 to 0.2</td>
<td>.16</td>
<td>−0.6</td>
<td>−1.2 to 0.0</td>
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<td>−1.3 to 0.0</td>
<td>.05</td>
<td>−1.0</td>
<td>−1.7 to −0.3</td>
<td>&lt;.01</td>
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<tr>
<td>CD4+ cell count at baseline</td>
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</tbody>
</table>

**NOTE.** Baseline was 18 months after HAART initiation. Baseline value is expressed as ×10^6 cells/L. For example, during the 52–72-month period after baseline, univariate analysis reveals that a patient in group 2 has an expected increase of 0.8 × 10^6 cells/L (2.5 × 10^6 cells/L − 1.7 × 10^6 cells/L). If the CD4+ cell count at 54 months is 400 × 10^6 cells/L, multivariate analysis shows that the expected increase per month is 1.74 × 10^6 cells/L (7.2 × 10^6 cells/L − 2.3 × 10^6 cells/L + [400 × −0.0078]).

*a* Test for trend.

nationwide cohort and the large number of patients who were observed for at least 72 months after baseline. Few patients were lost to follow-up, and most of the viral load data were entered electronically into the database. The study recorded planned treatment interruptions as well as patient-reported noncompliance, both obtained from the medical chart. Although the algorithm for modelling viral load between measurements may be questioned, we obtained similar results when we reanalyzed data by carrying forward the previous viral load measurement. Therefore, we believe that measurement bias is likely to be small and that such bias could be in either direction.

Of 2404 patients who had initiated HAART, 181 patients were excluded because their first viral load measurement occurred >6 months after HAART initiation. These 181 patients had a cumulative 72-month survival from baseline of 91% (95% CI, 85%–94%), which is similar to survival for patients included in the study. The potential selection bias introduced by the exclusion of this patient group is minor and could be in both directions. Not all patients were observed for the whole study period. Thus, the observed increase in CD4+ cell counts over time from baseline could be caused by patients with a decreasing CD4+ cell count ending follow-up early. However, only a
few patients died, few were lost to follow-up, and there was an even distribution over time of patients who were censored because they reached the end of their observation period. Furthermore, we analyzed individual CD4⁺ cell count changes rather than computed means at different time points. Therefore, we believe that the observed CD4⁺ cell count increases reflect an actual improvement in immunological status.

A treatment interruption during the first 18 months of HAART was a strong predictor of death in patients with partial virological suppression. Treatment interruption could be associated with other risk factors for death, such as end-stage AIDS, drug toxicities, or non–HIV-associated conditions related to poor adherence. However, the excess mortality observed in patients who had an episode of treatment interruption before baseline remained constant throughout the 6-year period after baseline, making it unlikely that the excess could be explained by end-stage AIDS or drug toxicities at the time of treatment interruption. A smaller proportion of patients died of non–HIV-related causes of death in the subgroup with treatment interruption than in the subgroup without treatment interruption, suggesting that non–HIV-associated conditions were not responsible for the excess mortality. Treatment interruptions were not advocated in Denmark during the study period, and, therefore, comparison with the outcomes of trials examining the effect of physician-guided structured treatment interruption [28] should be performed with caution.

Episodes of low-level viremia after full virological suppression have been associated with higher levels of viral replication,
drug resistance, impaired CD4+ cell count increases, and higher risk of subsequent sustained viral rebound [29–31], but other studies have used a number of different cutoff levels to define viremia, and the results have not been consistent. A recent study has shown that transient viremia in the range 50–200 copies/mL is due to random variation in the HIV RNA load around a steady state below this level [32], but, in another study, mathematical modelling has shown that these episodes are not evenly distributed among patients [33]. Our findings are in accordance with Easterbrook et al. [30], indicating strong clinical and paraclinical implications following even a short period of having an HIV RNA load of >400 copies/mL.

To our knowledge, this is the first study to take into account all available viral load measurements during a predetermined period to assess the prognostic value of intermittent viremia. Interestingly, we found that any degree of viremia involving a vial load of >400 copies/mL (groups 2A–D) was associated with higher mortality, compared with full virological suppression (group 1). The poor prognosis can be the result of a number of reasons. First, it could be associated with a drug-resistant virus emerging during the initial viremic period. Second, it could be associated with less strict virological targets in patients with competing comorbidity. Third, and what we believe is most likely, viremia and treatment interruption could be markers of poor adherence and even markers of non–HIV-related risk factors for death.

In conclusion, achieving an undetectable viral load is the primary treatment goal in daily clinical practice. Our study indicates that patients experiencing ≥1 viral load measurements >400 copies/mL at considerable higher risk of clinical progression than are patients with fully suppressed virus. Additional studies are needed to explore the underlying causes of this treatment failure. Physicians should be aware of the even worse prognosis for these patients following a treatment interruption and should focus on improving adherence by means of intensive patient coaching, individualizing drug regimens, and treatment of underlying comorbidity to avoid these interruptions.

Acknowledgments

Financial support. The Danish AIDS Foundation; Odense University Hospital (Odense, Denmark); Preben and Anna Simonsen’s Foundation; The Foundation of the Danish Association of Pharmacists; and Clinical Hospital (Odense, Denmark); Preben and Anna Simonsen’s Foundation; and Clinical Hospital (Odense, Denmark); Preben and Anna Simonsen’s Foundation; and Clinical Hospital (Odense, Denmark); Preben and Anna Simonsen’s Foundation; and Clinical Hospital (Odense, Denmark).

Potential conflicts of interest. N.O. has received unrestricted research grants from GlaxoSmithKline, Roche, Abbott, Merck Sharp & Dohme, and Boehringer Ingelheim. All other authors: no conflicts.

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Original article

The incidence rate of HIV type-1 drug resistance in patients on antiretroviral therapy: a nationwide population-based Danish cohort study 1999–2005

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2Department of Clinical Epidemiology, Århus University Hospital, Århus, Denmark
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Background: Newer antiretroviral treatment regimens for HIV carry a lower risk of inducing drug resistance mutations. We estimated changes in incidence rates (IRs) of new mutations in HIV-infected individuals receiving highly active antiretroviral therapy (HAART).

Methods: Population-based data were obtained from the Danish HIV Cohort Study and the Danish HIV Sequence Database. We included treatment-naive patients initiating HAART after December 1997 and computed time to first drug resistance mutation, identified as new mutations detected within 1 year after a 60-day period of treatment failure (HIV RNA > 1,000 copies/ml). We estimated annual IRs of new resistance mutations towards nucleoside reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs) and protease inhibitors (PI), and of new specific resistance mutations.

Results: A total of 1,829 individuals were observed for 7,294 person-years at risk (PYR). The IR of NRTI resistance decreased from 13.1 per 1,000 PYR (95% confidence interval [CI] 4.9–35.0) in 1999 to 3.7 (1.9–7.2) in 2004–2005 (test for trend P = 0.024). The IR of NNRTI resistance decreased from 15.4 (2.2–109.6) in 1999 to 7.9 (4.6–13.6) in 2004–2005 (P = 0.077). The IR of PI resistance decreased from 7.5 (1.4–21.8) in 1999 to 2.9 (0.7–11.4) in 2002–2003 (P = 0.148). The IRs were low for specific resistance mutations, except for M184V (IR 5.6 [4.0–7.9]) and K103N (IR 8.2 [5.6–12.0]).


Introduction

The presence of antiretroviral drug resistance mutations in individuals infected with HIV might hamper the effectiveness of antiretroviral treatment (ART) because the mutations reduce the chances of full viral suppression. The increasing use of ART in both high- and low-income settings could lead to an increase in incidence and prevalence of drug resistance. By contrast, newer highly active antiretroviral therapy (HAART) regimens for HIV treatment carry a lower risk of inducing drug resistance than older regimens [1]. Some studies have shown increasing prevalence of drug resistance in treatment-experienced populations [2–4], but there are recent reports from areas with high treatment coverage of decreasing prevalence of both resistance mutations [5,6], and of individuals who could potentially transmit drug resistance [7]. Whether the decreasing prevalence is accompanied by a similar trend in population-based incidence has not been shown. With access to all HIV RNA (viral load [VL]) measurements, genotypic drug resistance test results and treatment history in a nationwide population-based cohort of individuals with HIV type-1 (HIV-1), this study aimed to estimate changes over time in the incidence of antiretroviral drug resistance mutations among ART-naive patients initiating HAART.

Methods

Data sources
The Danish HIV Cohort Study (DHCS) is a prospective, nationwide, population-based cohort study of all
HIV-1-infected individuals treated in Danish HIV clinics since 1 January 1995 [8]. The study cohort is ongoing with continuous enrolment. The study data are updated annually and include details such as demographics, treatment information and measurements of VL and CD4+ T-cell counts. Data are collected from patient files and entered into the database annually. As antiretroviral medication is administered exclusively from nine departments of infectious diseases in Denmark, the database contains all patients on treatment.

The Danish HIV Sequence Database (DHSD) is a prospective, nationwide, population-based database of all genotypic resistance tests performed in Denmark after 31 December 1999. Genotypic resistance tests in the DHSD are performed on the initiative of the treating physician, mainly in patients with virological failure. Data from the DHCS are linked to the sequences in the DHSD by an identification number unique to each patient. No information was available on the patients’ genotypic resistance profile prior to drug initiation.

Study population
All ART-naive patients in the DHCS initiating HAART after 31 December 1997 and who had more than one HIV RNA (VL) measurement were included in the study.

Antiretroviral therapy
HAART was defined as a regimen of at least three antiretroviral drugs consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with either a non-NRTI (NNRTI), a protease inhibitor (PI) or abacavir, or in the absence of NRTIs, a combination of boosted lopinavir and efavirenz. The Danish national guidelines for initiating HAART have remained largely unchanged since 1997. Treatment initiation is recommended for patients with CD4+ T-cell counts <300 cells/µl, acute or symptomatic infection or pregnancy [9]. A VL>100,000 copies/ml was an indication for treatment initiation only until 2001.

Virological failure
Virological failure was defined as VL>1,000 copies/ml for a minimum of 60 days, while receiving treatment with a given drug class. Accordingly, periods with VL>1,000 copies/ml during a treatment interruption did not count as time with virological failure. VL was considered to be >1,000 copies/ml only during time periods between two consecutive VL measurements >1,000 copies/ml. The cutoff value of 1,000 copies/ml corresponds with the official guidelines of the ViroSeq™ Genotyping System version 2 by Abbott Diagnostics (Foster City, CA, USA).

Genotypic drug resistance
The sequences containing the reverse transcriptase and the protease gene collected for the DHSD were obtained through sequencing using the ViroSeq™ HIV-1 Genotyping System version 2 (Abbott Diagnostics). All sequences were analysed for the International AIDS Society (IAS)–USA 2007 primary drug resistance mutations [10] and only these mutations were included in the actual analysis.

Date of drug resistance acquisition
In accordance with the main purpose of the study – to look at incidence trends over time and not crude rates of resistance development – we applied strict criteria to define the date of drug resistance acquisition. This increased the specificity and minimized misclassification with regards to time of resistance development. We were aware that this could lead to an underestimation of the crude rate of resistance development. For each class of drugs, we linked the date when the first genotypic resistance mutation was detected to the most recent virological failure during treatment with the given class of drugs. If the virological failure had occurred no earlier than 365 days before the date of the detected genotypic resistance, the event date (date of drug resistance acquisition) was defined as day 30 (out of 60) with VL>1,000 copies/ml. Using this definition, the resistance mutations detected (for example, in the year 2000) could have a corresponding event date in 1999. If no virological failure had occurred during the 365 days before the first detected resistance mutation, the date of acquisition could not be defined and no event was registered. We only used the first performed genotypic resistance test after a virological failure. The 365-day period was increased to 730 days in a sensitivity analysis. Another sensitivity analysis defined the date of drug resistance acquisition as the day of resistance testing, dismissing the requirement of previous virological failure; thus, allowing us to use all available resistance test results.

Risk time
Time at risk for acquiring a mutation conferring resistance towards a drug class was defined as the cumulative time a patient was treated with a HAART regimen including at least one drug from that drug class. We computed time at risk from either the start of the HAART or 31 December 1998, whichever came last, until the first event (date of drug resistance acquisition), death, emigration or 31 December 2005. A patient could change from one risk set to another as he changed treatment regimen.

Incidence rates
Incidence rates (IRs) of acquiring drug resistance mutations were stratified according to calendar periods and were estimated in three ways: as the IR of the first new mutation within a drug class, as the IR of any...
new mutation within a drug class and as the IR of each specific mutation. For the second estimate, we counted all new resistance mutations within each drug class; thus, a person with more than one mutation within a drug class could have multiple events that all counted towards calculation of the IR. Trends over calendar time were analysed by Poisson regression. We used Stata statistical software version 9.2 (College Station, TX, USA) for all analyses.

Results

Patient characteristics

A total of 1,829 treatment-naive patients (1,275 men and 554 women) initiated HAART after 31 December 1997 and had >1 VL measurement. The median CD4+ T-cell count at the time of HAART initiation was 239 cells/µl (interquartile range [IQR] 119–350) and the median log_{10} VL was 4.0 copies/ml (IQR 2.6–5.1). Median follow-up time after HAART initiation was 3.8 years (IQR 1.7–5.7). A total of 317 individuals experienced virological failure while on treatment. During the 60 days of VL >1,000 defining failure, 316 had received NRTIs, 174 had received NNRTIs and 186 had received PIs. Of all the 1,829 study patients, 186 had received PIs. Of all the 1,829 study patients, 1,751 individuals exposed to NRTIs, 35 acquired at least one new drug resistance mutation to within a drug class and as the IR of each specific mutation. For the second estimate, we counted all new resistance mutations within each drug class; thus, a person with more than one mutation within a drug class could have multiple events that all counted towards calculation of the IR. Trends over calendar time were analysed by Poisson regression. We used Stata statistical software version 9.2 (College Station, TX, USA) for all analyses.

Incidence rates of first new drug resistance mutations within a drug class

Of the 1,731 individuals exposed to NRTIs, 35 acquired at least one new drug resistance mutation to this drug class during 1999–2005, yielding an IR of 5.9 per 1,000 person-years at risk (PYR; 95% confidence interval [CI] 4.3–8.3; Table 1). The IR decreased significantly throughout the study period from 13.1 per 1,000 PYR (95% CI 4.9–35.0) in 1999 to 3.7 per 1,000 PYR (95% CI 1.9–7.2) in 2004–2005 (test for trend P<0.024; Table 1). Of the 1,428 patients exposed to NNRTIs, 35 acquired at least one new NNRTI resistance mutation, yielding an overall IR of 10.6 per 1,000 PYR (95% CI 7.6–14.8). The IR decreased from 15.4 per 1,000 PYR (95% CI 2.2–109.6) in 1999 to 7.9 per 1,000 PYR (95% CI 4.6–13.6) in 2004–2005 (test for trend P=0.077). Of the 982 individuals exposed to PI, only 5 acquired at least one new primary (according to the IAS–USA [10]) PI resistance mutation during the study period. The overall IR of PI resistance was 2.0 per 1,000 PYR (95% CI 0.8–4.8) and decreased non-significantly from 7.5 per 1,000 PYR (95% CI 1.4–21.8) in 1999 to 2.9 per 1,000 PYR (95% CI 0.7–11.4) in 2002–2003 (test for trend P=0.148). When the allowed period between virological failure and resistance was increased from 1 to 2 years, we found slightly higher rates of resistance but similar trends of resistance rate changes over time for all three major drug classes. When previous virological failure was not taken into account, we found 2–3× higher crude IR rates but similar decreasing trends over time.

Incidence rates of any new drug resistance mutation within a drug class

The IR of any new NRTI-related mutation was 10.2 new mutations per 1,000 PYR (95% CI 5.95–10.38), whereas it was 14.2 per 1,000 PYR (95% CI 7.38–14.18) for any new NNRTI-related mutations and 2.8 per 1,000 PYR (95% CI 0.40–3.78) for any new PI-related mutation (Table 1). The IR for NNRTIs and NNRTIs decreased significantly between 1999 and 2005 (P<0.001 and P=0.017, respectively).

Incidence rates of specific drug resistance mutations

The most frequent new NRTI-related drug resistance mutation was M184VI, with an overall IR of 5.6 per 1,000 PYR (95% CI 4.0–7.8). The IR decreased from 9.8 per 1,000 PYR (95% CI 3.2–30.5) in 1999 to 3.7 per 1,000 PYR (95% CI 1.9–7.4) in 2004–2005 (test for trend P=0.049). K103N was the most frequent NNRTI-related drug resistance mutation, with an IR of 8.2 per 1,000 PYR (95% CI 5.6–12.0), decreasing over time from 15.4 per 1,000 PYR (95% CI 2.2–109.6) in 1999 to 6.1 per 1,000 PYR (95% CI 3.3–11.3) in 2004–2005 (test for trend P=0.270). D30N and L90M were the two most frequent PI-related drug resistance mutations, each occurring at an IR of 0.81 per 1,000 PYR (95% CI 0.2–3.2). The IR of other specific drug mutations are listed in Table 2.

Discussion

In this study, based on Danish nationwide data from individuals infected with HIV-1, we found decreasing population-based IR of drug resistance acquisition during 1999–2005 for all three major antiretroviral drug classes, although non-significantly for PIs. The most frequent mutations were M184V and K103N. To our knowledge, this study is the first to estimate resistance acquisition trends in a population where the total time on treatment prior to an event for all patients is counted in the denominator. The major strength of our study was the access to all HIV patients, VLs, treatment history and resistance tests performed within a geographical area.
The strict criteria applied to define the date of drug resistance acquisition increased the specificity and minimized misclassification with regards to time of resistance development. It also made the results more robust towards bias stemming from the changes of the testing criteria and improvements in resistance mutation detection assays over time. Patients with resistant virus who maintained a low VL might have passed unrecognized by our analysis because they did not have a resistance test performed or because they did not meet the criteria of virological failure; therefore, the crude rates of resistance mutation acquisition are likely to be underestimated, but the observed trends over time most likely reflect actual trends.

During the study period, resistance testing became increasingly sensitive and tests can now be performed in patients with low VL. Consequently, we saw an increase in the proportion of patients with genotypically-resistant HIV detected at low-level viraemia (data not shown), which might have increased the most recent IR, biasing our results towards underestimating the decrease over time. Increased prescription of resistance tests would bias the results in a similar fashion. The total number of resistance tests has decreased over the years because virological failure has become less common [9]; however, resistance tests have been used with increasing frequency and even in scenarios where non-compliance or drug holidays are likely reasons for virological failure. We do not, however, provide information on mutations accumulating during treatment of patients with repeated or chronic failures of therapy.

Table 1. Incidence rates of resistance mutations within each drug class

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<td>(3.4–10.0)</td>
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<td>13</td>
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<td>1</td>
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<td>0</td>
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<tr>
<td>Time at risk, ×1,000 person-years</td>
<td>2.5</td>
<td>0.3</td>
<td>0.8</td>
<td>0.7</td>
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<tr>
<td>IR (95% CI)a</td>
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<td>7.51</td>
<td>1.33</td>
<td>2.85</td>
<td>0</td>
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<td>(0.85–4.89)</td>
<td>(1.9–30.0)</td>
<td>(0.2–9.4)</td>
<td>(0.7–11.4)</td>
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<tr>
<td>Any new resistance mutation</td>
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<td>1</td>
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<td>Time at risk, ×1,000 person-years</td>
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<td>(0.3–10.2)</td>
<td>(0.3–9.7)</td>
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</table>

aNumber of individuals/(time at risk × 1,000 person-years).
bNumber of mutations/(time at risk × 1,000 person-years). CI, confidence interval; IR, incidence rate; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
Current guidelines recommend resistance testing when drug-exposed patients experience sustained increased VL [11], as well as prior to ART initiation. All HIV patients in Denmark are now tested at the time of diagnosis and we therefore know that the prevalence of transmitted drug resistance is as low as 4% (unpublished data) and has remained stable since year 2000. Only 45 individuals in Denmark have been infected with drug-resistant HIV after year 2000 and because these were assumedly treated with susceptible drug combinations according to their resistance profiles, it is unlikely that events in these patients have biased our results in any significant way.

The exposure times observed reflect the use of the different antiviral classes. Up to year 2000 the drug combination of choice in ART-naive patients was a PI plus two NRTIs, but already in 2002 NNRTIs had almost completely overtaken the role PIs. In Denmark, use of boosted PIs was routine treatment already in the late 90s, which might be why we find a very low incidence of PI mutations.

The IR of specific drug resistance mutations show which mutations develop first during HAART. The NRTI-related mutation M184V/I is selected by and causes high-level resistance to lamivudine [12], one of the core medications in the Danish treatment recommendations [13]. D30N is induced by nelfinavir, which confers resistance only to this drug [14]. By contrast, L90M causes a broad range of cross-resistance to the PI drug class [15]. A recent study by the UK collaborative group on HIV drug resistance and UK collaborative HIV cohort study found a decrease in the prevalence of PI resistance from 1998 to 2005, with L90M being the most prevalent PI resistance mutation, which is similar to our findings [6]. Also in accordance with our findings, this group and the UK CHIC Study Group [16] found that the risk of developing resistance was higher during NNRTI treatment than during treatment with PIs. In addition, a recent study by Riddler et al. [17] reported that HIV in patients on efavirenz treatment were at greater risk of developing drug resistance mutations than HIV in patients on boosted lopinavir. Similar conclusions were reached in a paper from the Swiss HIV Cohort Study [18].

Vercauteren et al. [19] estimated the magnitude of acquired resistance, categorized as multidrug resistance and full-class resistance. In accordance with our results, the authors found a decreasing incidence of antiretroviral drug resistance mutations over time, but did not exclude patients previously treated with mono or dual antiretroviral regimens from their study. Because all patients included in our study had been treated with HAART regimens only, the decrease cannot be ascribed to failure of earlier mono and dual treatment. Furthermore, different ways of calculating IR make the studies difficult to compare.

In conclusion, we found that the incidence of HIV resistance to antiretroviral drugs was decreasing in Denmark. We assume that this was driven by increased drug adherence, which has been a major focus for both patients and healthcare professionals. Newly developed antiretroviral drugs are more tolerable, can be taken once daily and also have altered resistance mutation patterns. A decreasing risk of developing resistance to antiretroviral drugs is of major public health importance and serves as an optimistic scenario for low-income countries that are currently scaling-up access to HAART.

Acknowledgements

The DHSD was created with grants from the Danish Agency for Science Technology and Innovation (reference number 271-06-0619).

Disclosure statement

The authors declare no competing interests.
References


Declining prevalence of HIV-infected individuals at risk of transmitting drug-resistant HIV in Denmark during 1997–2004

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Background: Transmission of drug-resistant HIV is a potential threat to the substantial clinical benefit of highly active antiretroviral therapy (HAART). To explore the background for the low rates of drug resistance transmission (2–5%) in our population, we estimated acquisition of HIV drug resistance and examined temporal trends in the prevalence of patients at risk of transmitting drug-resistant HIV.

Methods: The study population included all 4,025 patients from The Danish HIV Cohort Study seen during the period 1995–2004. Virological failure to a given drug class was defined as a viral load (VL) >1,000 copies/ml for 120 days while on a HAART regimen including that drug class. In addition, receiving nucleoside reverse transcriptase inhibitors (NRTIs) for 180 days before HAART counted as NRTI failure irrespective of VL. Having experienced failure was considered a proxy for harbouring drug-resistant virus in subsequent observation time. Patients with a current VL >1,000 copies/ml were considered at risk of transmitting HIV.

Results: We found a decrease from 1997 to 2004 in the prevalence of potential transmitters of drug-resistant HIV. The number of these patients with previous NRTI failure decreased from 429 (24% of all patients) in 1998 to 213 (8.0% of all patients) in 2004. Previous protease inhibitor (PI) failure peaked at 279 (14%) in 1999, declining to 142 (5.3%) in 2004. Previous NNRTI failure peaked at 121 patients (4.7%) in 2002, and occurred in 113 patients (4.2%) in 2004. Of all 686 potential transmitters in 2004, 31% had previously experienced NRTI failure, 21% PI failure, and 16% non-NRTI failure.

Conclusion: In the population of HIV-infected individuals in Denmark with complete follow-up, the number at risk of transmitting drug-resistant virus declined over time.

Introduction

While the consequences of resistance to anti-HIV drugs are often serious for individuals harbouring resistant virus, drug resistance also has significant public health implications. In particular, concerns have been raised that drug-resistant viruses can be transmitted and ultimately reverse the major clinical benefit of highly active antiretroviral therapy (HAART). A number of studies have estimated drug resistance in HIV populations treated with antiretroviral drugs (population resistance) or drug resistance in newly infected patients (transmitted resistance). Studies in the US and the UK have found a high and increasing prevalence of population resistance [1–4], and transmitted resistance in these countries is reported to be over 20% and rising [5–8]. In contrast, transmitted resistance is reported to have fallen to below 10% in Canada [9], Australia [10] and Amsterdam, the Netherlands [11], and to be stable in Europe [12] at a level just above 10%.

Transmission of drug-resistant HIV depends on a number of factors, including the proportion of patients on treatment, rates of acquiring resistance, and the relative fitness and transmissibility of the virus [3].
Despite the importance of the problem, accurate data on these risk factors are lacking. Surveillance in Denmark since 2000 has revealed a very low and stable prevalence of transmitted drug resistance (5% or less) [13], in a context of high treatment coverage. In order to gain insight into trends underlying this low transmitted resistance prevalence, we aimed to examine temporal changes in the prevalence of individuals at risk of transmitting drug-resistant HIV. Using data from a complete nationwide cohort of Danish HIV-infected individuals, we combined detailed information on individual treatment regimens and periods of incomplete virological suppression to estimate both acquisition of drug-resistant viruses and subsequent risk of transmitting them to other individuals.

Methods

The Danish HIV Cohort Study

The Danish HIV Cohort Study is a prospective, nationwide population-based cohort study of all HIV-positive individuals seen at Danish HIV clinics since 1 January 1995 [14,15]. The Danish healthcare system provides free tax-supported antiretroviral treatment and other medical care to these patients. Treatment for HIV infection occurs in only eight hospital clinics, which distribute HIV drugs directly to patients. HIV medicines cannot be prescribed by general practitioners or purchased from Danish pharmacies. Use of the Danish 10-digit personal identification number enables treatment facilities to avoid multiple registrations of the same patient, and to document whether patients lost to follow-up have died or moved outside the country. All patients who were seen in Danish HIV clinics up to 1 January 2004 were included in the study. Patients who had not been seen since 1 January 2003 and were not known to have died were considered lost to follow-up.

Antiretroviral therapy

HAART was defined as use of at least three antiretroviral drugs, including at least one protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI), or abacavir. Treatment interruptions longer than 14 days were documented, including temporary interruptions during infections and pregnancy. National guidelines for initiating HAART have remained almost unchanged since 1997 and have mainly been CD4-driven, with a CD4+ T-cell count of 300×10^6 cells/ml used as the threshold for treatment initiation. A minor guideline revision concerned HIV-RNA level: until 2001, HIV-RNA >100,000 copies/ml was considered an indication for treatment initiation, but since then there has been no upper HIV-RNA limit. However, this indication accounted for only a minor proportion of HAART initiations, as did the three additional indications: acute HIV infection, clinical symptoms and pregnancy. Treatment interruptions have not been recommended in Denmark.

Prolonged virological failure as a proxy for acquired drug resistance

During HAART, virological failure of a drug class (nucleoside reverse transcriptase inhibitor [NRTI], PI or NNRTI) was defined as an HIV RNA viral load (VL) of >1,000 copies/ml for a total of 120 days (not necessarily successive) while on treatment with that drug class. Hence, periods of treatment interruption did not count as time with virological failure. VL was considered to be >1,000 copies/ml only during time periods between two consecutive VL measurements >1,000 copies/ml. Failure of a drug class could occur when it was administered as monotherapy or as part of a multi-drug regimen [16,17]. In addition, administration of mono- or dual-drug therapy including NRTIs for 180 days prior to HAART counted as NRTI failure, irrespective of VL in this period. Cohort study data included information on the date when the patient started mono- or dual-NRTI therapy, as well as detailed information on drug combinations and timing of combination changes in the HAART regimen. A patient was considered to have ‘previous virological failure’ of a drug class at all follow-up times after meeting the definition of virological failure. Previous virological failure was considered a proxy for acquired drug resistance [18,19].

Population at risk of transmitting drug-resistant HIV

Patients with a current VL >1,000 copies/ml were considered potential transmitters of HIV. To define the ‘current VL,’ we computed VL values at time points between measurements [20]: each measured value was carried forward for 30 days or until the next measurement, whichever came first. The measured VL was extrapolated back to 30 days following the previous measurement. This model was used because detection of a high VL at a clinic visit often leads to revision of the antiretroviral treatment regimen, thereby producing a change in VL. Patients with previous virological failure of a given drug class were considered at risk of transmitting resistance to that class if a subsequent ‘current VL’ was >1,000 copies/ml.

Statistical analysis

The following point prevalences were computed on 1 January of each year during 1997–2004 among patients under observation on that day: (i) prevalence of patients with a current VL >1,000 copies/ml (denominators: [A] all patients, [B] patients who were receiving or had ever received HAART, [C] patients who had never received any antiretroviral treatment); (ii) prevalence of patients with an ongoing treatment interruption.
Risks of transmitting drug-resistant HIV

Trends in median CD4+ T-cell count and VL over time were examined. Viral load was 4.9 \texttimes 10^{11} \text{ copies/ml} from 4.6 in 1997 to 5.0 in 2004 (IQR: 4.1–5.4) copies/ml, and 38% had received mono- or dual-NRTI therapy prior to HAART initiation. During the years of the study, no change was observed in median CD4+ T-cell count at HAART initiation (P=0.40). However, there was a statistically significant increase in median \log_{10} VL from 4.6 in 1997 to 5.0 in 2004 (P=0.02). The proportion of patients receiving HAART increased gradually from 44% in 1997 to 65% in 1998 and then further to 82% in 2004. Out of the total number of patients, 4.6% were lost to follow-up and 2.3% of patients initiating HAART were lost to follow-up. Patient characteristics are shown in Table 1.

Prevalence of patients with a current VL >1,000 copies/ml
The proportion of all patients with a current VL >1,000 copies/ml decreased from 69% in 1997 to 26% in 2004. The relative change between 2000 and 2004 was 0.88 per year (95% confidence interval [CI]: 0.86–0.90). Additionally, among patients who were receiving or had ever received HAART, a steady decline was noted in the percentage of those with a current VL >1,000 copies/ml, for example, from 58% in 1997 to 14% in 2004, with a relative change between 2000 and 2004 of 0.84 per year (95% CI: 0.81–0.86). Among patients who had never received any antiretroviral treatment, the percentage with a current VL >1,000 copies/ml varied between 78% and 85%, with no trend.

Prevalence of patients with previous virological failure
The prevalence of patients ever receiving HAART who were currently interrupting their regimen on 1 January each year was 6.1% in 2004 and varied from 5.4% in 1998 to 7.8% in 1999.

Prevalence of patients with previous virological failure
The prevalence of previous NRTI failure was identical to the prevalence of previous ‘failure of any drug class’, because at the time of failing a PI or an NNRTI, all patients had either previously failed an NRTI (that is, as monotherapy), or they had an NRTI included in the regimen they received at the time of PI- or NNRTI-failure. Isolated first failures of a PI, a NNRTI, or a combination of the two did not occur. In 1997, 62% of all patients had a previous NRTI failure, and this percentage steadily decreased to 37% in 2004 (Figure 1). The relative change between 2000 and 2004 was 0.94 per year (95% CI: 0.92–0.96). Previous PI failure peaked at 19% in 2001 and decreased to 16% in 2004, with a relative change of 0.94 per year between 2000 and 2004 (95% CI: 0.92–0.97). Previous NNRTI failure increased steadily to 7.0% in 2004, with a relative change of 1.14 per year during the period 2000–2004 (95% CI: 1.08–1.20). In 2004, 998 patients had previously failed the NRTI drug class, 416 had previously failed the PI class, and 187 had previously failed the NNRTI class. Among patients who had never received mono- or dual-NRTI therapy, the prevalence of previous NRTI failure increased from 1997 to 2000 and stabilized at about 8% thereafter. The relative change between 2000 and 2004 was 1.00 per year (95% CI: 0.95–1.06; Figure 2). The prevalence of previous PI failure decreased after an initial rise, with a relative change of 0.93 per year between 2000 and 2004 (95% CI: 0.87–0.99), and the prevalence of previous NNRTI failure increased during that period with a relative change of 1.29 per year (95% CI: 1.16–1.44).

Prevalence of patients with previous virological failure and a current VL >1,000 copies/ml
The number of patients considered at risk of transmitting HIV because of a VL >1,000 copies/ml following NRTI failure decreased from 429 (24% of all cohort
patients) in 1998 to 213 (8.0% of all cohort patients) in 2004 (Figure 3). This represented a relative change in prevalence of 0.81 per year between 2000 and 2004 (95% CI: 0.78–0.84). The number with previous PI failure and a current VL >1,000 copies/ml peaked at 279 (14% of all cohort patients) in 1999 and declined to 142 (5.3% of all cohort patients) in 2004. This was a relative change in prevalence of 0.80 per year between 2000 and 2004 (95% CI: 0.77–0.84). The number of cohort patients experiencing a previous NNRTI failure and a current VL >1,000 copies/ml peaked at 121 (4.7% of all cohort patients) in 2002 and was 113 (4.2% of all cohort patients) in 2004; this corresponds to a relative change in prevalence of 1.04 per year between 2000 and 2004 (95% CI: 0.97–1.10). Figure 4 shows the number of patients at risk of transmitting drug-resistant HIV each year and their status 1 year later in terms of remaining at risk, achieving re-suppression, having died or being lost to follow-up. For each year from 1998 to 2003, fewer patients remained at risk compared with the year before, and fewer new patients entered the risk group. Of all 686 potential transmitters in 2004, 31% had previously experienced NRTI failure, 21% PI failure and 16% NNRTI failure (Figure 5). The median viral load was lower among the 213 patients with previous drug failure than among the other 473 patients [log_{10} VL=4.02, (IQR: 3.25–4.91) versus log_{10} VL=4.37 (IQR: 3.79–4.75); P<0.01]. Out of the 473 patients with no previous failure, 286 were antiretroviral therapy-naive. Patient characteristics for these two groups are detailed in Table 1.

Analyses restricted to homosexual men
On repeating all analyses for the 1,775 patients infected through male homosexual contact, we observed similar temporal trends in the prevalence of a current VL >1,000 copies/ml, treatment interruptions, previous virological failure and the potential to transmit drug-resistant HIV.

Discussion
This study, encompassing all HIV-infected individuals receiving care in Denmark, indicates a decrease in the

<p>| Table 1. Characteristics of all study patients and of patients at risk of transmitting HIV on 1 January 2004 |</p>
<table>
<thead>
<tr>
<th>All study patients</th>
<th>Patients with viral load &gt;1,000 copies/ml on 1 January 2004</th>
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<tr>
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<tr>
<td>Europe excluding Denmark</td>
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<td>Other</td>
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</tr>
<tr>
<td>Unknown</td>
<td>176</td>
</tr>
<tr>
<td>Log_{10} viral load, copies/ml</td>
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</tr>
<tr>
<td>Median</td>
<td>–</td>
</tr>
<tr>
<td>IQR</td>
<td>–</td>
</tr>
</tbody>
</table>

IDU, injection drug users; IQR, interquartile range; MSM, men having sex with men.
prevalence of potential transmitters of HIV, and in particular a decrease in the prevalence of potential transmitters of drug-resistant HIV between 1997 and 2004. The decrease stemmed from declining incidence (fewer patients failing their treatment), as well as successful re-suppression of viral load to below 1,000 copies/ml following failure. Mortality and loss to follow-up did not play a role.

Among patients unexposed to mono- or dual-NRTI therapy, we observed a steady prevalence of previous failure of ‘any drug class’ since 2000 (equal to NRTI failure). However, in the same time period there was a decrease in previous PI failure and an increase in previous NNRTI failure. The decline in previous PI failure corresponded temporally with the shift from non-boosted to ritonavir (RTV)-boosted PIs, for which development of resistance occurs slowly [21,22], and with the increased use of NNRTIs in initial regimens instead of PIs. The stable and low prevalence of resistance to ‘any drug class’ since 2000 corresponds well with HIV treatment advances in recent years, with emphasis on adherence as the key factor for success of a regimen [23].

Most studies attempting to estimate the prevalence of drug resistance use data from phenotypic [1,7,19,20] or genotypic resistance [2,5,9–12] tests. However, it is challenging to compare such test results from different time periods because the indication for testing has changed over time, and the interpretation of mutations has also changed. Furthermore, patients can harbour mutations undetected in a particular sample, thus introducing bias to the prevalence estimates. Several additional factors make it difficult to accurately estimate the prevalence of drug resistance based on resistance tests: the cohort from which the samples are taken is seldom representative of the HIV-infected population as a whole; 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.

The Danish HIV Cohort Study confers a number of research advantages. Because it includes almost all
patients in Denmark with a known HIV infection and has few losses to follow-up, it provides an ideal setting for measuring temporal trends. Frequent VL measurements for all patients and detailed data on antiretroviral therapy permit use of previous virological failure as a proxy for carrying drug-resistant virus. A recent study among patients with continuous detectable VL and no mutations at baseline, estimated the incidence rate of acquiring a new resistance mutation to be 3.11 mutations per year (highest among patients with a VL between 1,000 and 10,000 copies/ml) [18].

At the same time, a number of limitations must be noted. First, the chance of resistance mutations being present at the time when virological failure is recorded varies by drug and drug combination, and resistance may not be present towards both drugs when failing a two-drug combination. The 180 days of mono- or dual-NRTI therapy required before NRTI resistance is assumed may underestimate the true resistance to some drugs, for example, the M184V lamivudine resistance mutation. Additionally, acquiring PI resistance occurs slowly for a patient on a HAART regimen that includes a RTV-boosted PI, and resistance mutations may not be present at resistance tests performed shortly after failure [21,22]. We may therefore have overestimated the prevalence of PI resistance in later years. Furthermore, development of NNRTI resistance occurs quickly, leading to underestimation of the prevalence of NNRTI resistance. In later years the use of NNRTIs and RTV-boosted PIs has increased, and the use of mono- and dual-NRTI regimens has ceased. These three factors would inflate our observations of decreasing NRTI failure, of decreasing PI failure combined with increasing NNRTI failure among patients who received HAART as their initial antiretroviral regimen, and of similar trends in the prevalence of drug resistance transmitters. Finally, we may record virological failure in patients not taking their drugs at all, in which case resistance will not have emerged. The more tolerable regimens used during recent years may have improved...
compliance and therefore contributed to the observed decrease in failure.

It is important to note that our data did not account for transmitted resistance, potentially causing an underestimation of drug resistance in our population. This may contribute substantially to population resistance in populations with high transmitted resistance. However, such resistance was as low as 2% in Denmark in 2000 [13] and surveillance does not suggest it is currently rising (LB Jørgensen, unpublished data). Another issue is the pool of undiagnosed HIV-infected individuals in Denmark, estimated at around 1,000 individuals; we have assumed that most individuals in this group do not harbour drug-resistant virus, in light of the low level of transmitted drug resistance. Also, the possible reduced transmission capacity of resistant viral strains [3,24,25] may lead to overestimation of the number of drug resistance transmitters.

Furthermore, we did not have data on all factors that might influence the risk of HIV transmission, for example circumcision [26] or the presence of other sexually transmitted diseases [27]. We identified potential transmitters of HIV using a cut-off of 1,000 copies/ml, because transmission has been documented to be low in patients with VL <1000 copies/ml, and to increase with higher VL [28–31].

Thus, our methods may lead to misclassification of both transmitters and carriers of drug resistance; however, we believe that such misclassification is constant over time. Given our well-defined and almost complete study population, we are confident that the temporal trends estimated in this study mirror actual decreasing trends in potential transmitters of antiretroviral drug resistance.

Nevertheless, approximately one third of individuals in care with VL >1,000 copies/ml were estimated to be potential carriers of drug-resistant virus. Risk behaviour and its possible association with healthcare-seeking behaviour remain unknown factors contributing to the transmission rates. Recent
surveillance has revealed that since 2002 an increasing number of men having sex with men (MSM) are among those newly diagnosed with HIV infections [32], and there has been a concomitant epidemic of syphilis among the MSM group in Copenhagen [33]. Although the results of our analyses for MSM were similar to those for the study population as a whole, risky behaviours may be increasing in this group, both among individuals known and unknown to be infected with HIV.

Two studies have estimated the prevalence of population resistance in the United States [1,3]. In 2001 Blower et al. [3] predicted an increasing prevalence of drug resistance based on data from the first years of HAART. The decline found in our study accords with the improved treatment success on the population level in later years [16,20,34]. Richman et al. [1] detected 78% drug resistance in late 1998 in a US population in which most patients had previously received mono- or dual-NRTI therapy. Our study also found a high prevalence (60%) of potential drug resistance in 1997. The decline we documented since then may well be due to an increase in the denominator: mono- or dual-NRTI-naive patients receiving HAART as their first antiretroviral regimen, who are less likely to fail.

A recent study from the UK [4] pooled results from genotypic resistance tests over several years and combined them with registry data for patients receiving treatment, in order to estimate the prevalence of drug resistance among patients receiving antiretroviral therapy. Interestingly, the research team found a trend of initial increase followed by stabilization, most marked for PI resistance, together with continuously increasing NNRTI resistance. This is similar to our findings for mono- or dual-NRTI-naive patients. The drug resistance prevalence found in the UK study by the end of 2002 is higher than our estimates, not surprising for a population with a high prevalence of transmitted resistance (>20%) [5,8]. A recent cross-European study of transmitted drug resistance in 2,208 drug-naive patients found that 10.4% carried resistant HIV, with underlying increases in NNRTI mutations and decreases in PI mutations, and with large regional differences (0–23%) [12].

To our knowledge our study is the first to combine information on previous drug failure with a current VL.

Figure 4. Number of patients at risk of transmitting drug-resistant HIV on 1 January each year

Patients with previous virological failure [viral load (VL) >1,000 copies/ml for 120 days whilst on highly active antiretroviral therapy, or 180 days of mono- or dual-nucleoside reverse transcriptase inhibitor therapy] and a current VL >1,000 copies/ml. Each bar is divided according to the patients’ status one year later: still at risk (VL >1,000 copies/ml), re-suppressed (VL <1,000 copies/ml), dead or lost to follow-up.
in order to detect patients at risk of transmitting drug-resistant viral strains to others. The prevalence of previous drug failure tended to stabilize, while the prevalence of potential transmitters decreased due to successful viral re-suppression, in accordance with recent years’ therapeutic improvements. Declines in transmitted drug resistance found in the Netherlands [11] and Canada [9] may be signs of a similar reduction in potential transmitters in those areas. However, NNRTI failure in particular must be closely monitored in the future. The observed increase in failure of this drug class reflects current drug prescribing patterns, and the weak genetic barrier makes it more likely for drug resistance to develop to these drugs than to RTV-boosted PIs administered as part of a HAART regimen.

Among the population of Danish HIV-infected individuals, subject to intense and complete follow-up, we have found that the number of HIV-infected individuals at risk of transmitting drug-resistant virus is declining. These positive trends have come about despite heavy exposure to antiretroviral drugs and despite the continuous accumulation at the population level of patients with a history of virological failure. Continuation of these trends would mean preserving initial therapeutic options for the majority of newly infected individuals in the future. This would enable the HIV-infected population to fully benefit from new drugs on the market without fear of being ‘hit from behind’ by drug resistance carried on from less successful therapeutic strategies on previous occasions.

Acknowledgements

The study was supported by grants from The Danish AIDS Foundation; Odense University Hospital, Denmark; Preben and Anna Simonsen’s Foundation; The Foundation of the Danish Association of Pharmacists; and Clinical Institute, University of Southern Denmark.
References

Genotypic drug resistance and long-term mortality in patients with triple-class antiretroviral drug failure

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Objective: To examine the prevalence of drug-resistance-associated mutations in HIV patients with triple-drug class virological failure (TCF) and their association with long-term mortality.

Design: Population-based study from the Danish HIV Cohort Study (DHCS).

Methods: We included all patients in the DHCS who experienced TCF between January 1995 and November 2004, and we performed genotypic resistance tests for International AIDS Society (IAS)-USA primary mutations on virus from plasma samples taken around the date of TCF. We computed time to all-cause death from date of TCF. The relative risk of death according to the number of mutations and individual mutations was estimated by Cox regression analysis and adjusted for potential confounders.

Results: Resistance tests were done for 133 of the 179 patients who experienced TCF. The median number of resistance mutations was eight (interquartile range 2–10), and 81 (61%) patients had mutations conferring resistance towards all three major drug classes. In a regression model adjusted for CD4+ T-cell count, HIV RNA, year of TCF, age, gender and previous inferior antiretroviral therapy, harbouring ≥9 versus ≤8 mutations was associated with increased mortality (mortality rate ratio [MRR] 2.3 [95% confidence interval (CI) 1.1–4.8]), as were the individual mutations T215Y (MRR 3.4 [95% CI 1.6–7.0]), G190A/S (MRR 3.2 [95% CI 1.6–6.6]) and V82F/A/T/S (MRR 2.5 [95% CI 1.2–5.3]).

Conclusions: In HIV patients with TCF, the total number of genotypic resistance mutations and specific single mutations predicted mortality.

Introduction

Virological failure to all three major drug classes (nucleoside reverse transcriptase inhibitors [NRTIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs] and protease inhibitors [PIs]), that is, triple-class failure (TCF), in HIV patients is an important clinical problem and is associated with a poor prognosis [1,2], with CD4+ T-cell count and plasma HIV RNA (viral load [VL]) at time of TCF as independent prognostic factors for death [3]. Virological failure is associated with development of drug resistance [4]. Therefore, patients with TCF are likely to harbour multiple drug resistance mutations. A few studies have examined the association between resistance mutations and mortality in treatment-experienced patients [5–7]. Lucas et al. [7] found no association between the number of resistance mutations and mortality, whereas Zaccarelli et al. [6] found that mortality was associated with multiple-drug class-wide resistance. Most attempts to clarify the association between resistance mutations and clinical outcome have been hampered by convenience sampling (that is, including only patients in whom resistance testing was performed for reasons other than inclusion in the study), and no previous studies have examined the association between resistance mutations and clinical outcome in a population of patients with TCF. Therefore, the goals of the present study were to (i) examine the prevalence of mutations in patients with
TCF and (ii) examine how resistance mutations influence long-term mortality in TCF patients, while controlling for other prognostic factors.

Materials and methods

The Danish HIV Cohort Study (DHCS)

DHCS is a prospective, nationwide, population-based cohort study of all HIV-infected individuals treated in Danish HIV clinics since 1 January 1995 [8]. The study is ongoing, with continuous enrolment. HIV treatment in Denmark is restricted to eight specialized centres, and the Danish health care system provides free tax-supported medical care, including antiretroviral treatment. Updates of the study cohort are performed annually. Complete data on all patients seen in any of the centres since 1 January 1995 have been collected from patient files and entered into the DHCS database.

Virological failure

TCF was defined as in a previous study [1]. Virological failure was defined as a VL >1,000 copies/ml for a total of 120 days (not necessarily successive) while receiving treatment with a given class of drug. Periods of treatment interruptions, whether physician- or patient-initiated, were not counted as failure time. VL was defined as >1,000 copies/ml during the period between two consecutive VL measurements >1,000 copies/ml. Failure of a drug class could occur whether it was administered alone or as part of a multidrug regimen. The time that TCF occurred was the date the patient met the failure requirements for three drug classes. All DHCS patients that experienced TCF up to 1 November 2004 were eligible for the study.

Genotyping

We performed genotypic resistance tests for International Aids Society (IAS)-USA 2005 primary drug resistance mutations [9] on virus from plasma samples taken during a period 1 month before to 6 months after the date of TCF. We chose this interval to ensure that resistance mutations were also present on the date of TCF. On the basis of our dynamic definition of virological failure, the date of TCF typically occurs at a time between two dates of VL measuring. Consequently, samples were rarely available for that specific date.

Mortality data

Dates of death and migration were obtained from patient files and confirmed by the Civil Registration System [10]. Causes of death were registered in DHCS and were divided into HIV-related (AIDS-defining illnesses and bacterial infections), non-HIV-related (other causes) and unknown.

Statistical methods

Follow up began at the date of TCF onset (baseline) and continued until death, last clinic visit or 1 May 2006. We computed Kaplan–Meier survival curves for the patients according to the main study variables. The relative risk of death associated with individual mutations, and the number of mutations (that is, more than versus fewer than the median) were estimated by Cox proportional hazard analysis. The influence of the following covariates on mortality estimates was evaluated in bivariate models, and in a model including them all: CD4+ T-cell count at baseline (<50, 50–200 or >200 cells/μl) or time-updated CD4+ T-cell count, carrying forward the most recent observation; log_{10} VL at baseline (<4, 4–5, >5 log_{10} copies/ml); gender; age at baseline; being antiretroviral-drug-naive prior to initiating highly active antiretroviral therapy (HAART); and year of TCF onset. As proposed by others [11], the influence of individual mutations was not assessed if the mutation was present in <10% of the study population. The proportional hazards assumption was tested based on Schoenfeld residuals and was found to be appropriate. Interactions of key variables were examined by stratified analyses. Patient characteristics were compared with χ² test and Student’s t-test. A significance level of 0.05 was used for all analyses. Analyses were performed using Stata (College Station, TX, USA) statistical software, version 9.2.

Approvals and permissions

The study was approved by the Danish Data Protection Agency. Because the study did not entail any interaction with patients, it was not necessary to obtain patient consent or approval from the ethics committee.

Results

Study population

HAART was initiated in 2,797 DHCS patients during the study period. The median time of follow up after HAART initiation was 4.1 years (interquartile range [IQR] 1.9–6.3).

One-hundred and seventy-nine patients developed TCF. For 133 of those patients, a resistance test, done within the required interval surrounding the date of TCF, was available. Twenty-nine percent of the tests were done within 1 month of the date of TCF, and 74% were done within 3 months. Only one patient experienced TCF after 2001. At the time of TCF, the mean number of days with failure while on HAART for each drug class was 754 days for NRTIs, 167 days for NNRTIs and 688 days for PIs. Comparison of the 133 patients with and the 46 patients without an available resistance test revealed a significant difference in a single variable: number of
days with NNRTI failure (167 days versus 192 days, \(P = 0.01\)) (Table 1).

Resistance pattern
The 133 patients with an available resistance test had a mean of 6.3 mutations (median 8; IQR 2–10), including 3.6 NRTI-associated mutations, 1.3 NNRTI-associated mutations and 1.4 primary PI-associated mutations. Of those patients 127 (95%) had \(\geq 1\) drug resistance mutation. If only primary PI mutations were counted, the number of patients with \(\geq 1\) resistance mutation was 117 (88%). One-hundred and one (76%) of the patients had resistance mutations to \(\geq 2\) drug classes, and 81 (61%) had resistance mutations towards all three major drug classes. The frequency of individual resistance mutations is shown in Table 2. The six most frequent NRTI-associated mutations observed were at codons 215 (60%), 184 (50%), 41 (49%), 67 (40%), 210 (35%) and 219 (29%). The most frequent NNRTI-associated mutations were at codons 103 (53%) and 181 (25%), and the most frequent primary PI-associated mutations were at codons 90 (41%), 82 (33%), 46 (30%) and 84 (14%).

Mortality after TCF
The median time of follow up of the 179 patients after baseline was 4.3 years (IQR 2.8–5.5). The mortality rate after baseline was 70 (95% confidence interval [CI] 54–92) per 1,000 person-years (PYR), as opposed to an overall mortality in DHCS after initiation of HAART of 29 (95% CI 26–32) per 1,000 PYR. Patients in the upper half, harbouring \(\geq 9\) mutations, had increased mortality compared with patients harbouring \(\leq 8\) mutations (MRR 2.3; 95% CI 1.2–4.3) (Table 3; Figure 1). None of the other covariates

### Table 1. Patient characteristics

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<th>Patients with resistance test</th>
<th>Patients without resistance test</th>
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<tr>
<td>Caucasian</td>
<td>109 (82)</td>
<td>35 (76)</td>
<td>0.510†</td>
</tr>
<tr>
<td>Black African</td>
<td>20 (15)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (3)</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Year of TCF, (n) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>24 (18)</td>
<td>5 (11)</td>
<td>0.241†</td>
</tr>
<tr>
<td>1998</td>
<td>32 (24)</td>
<td>15 (33)</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>37 (28)</td>
<td>16 (35)</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>29 (22)</td>
<td>6 (13)</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>10 (8)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>CD4+ T-cell count at initiation of HAART, (\text{cells/\mu l})</td>
<td>80 (30–170)</td>
<td>100 (22–220)</td>
<td>0.363‡</td>
</tr>
<tr>
<td>CD4+ T-cell count at time of TCF, (\text{cells/\mu l})</td>
<td>194 (88–340)</td>
<td>180 (80–310)</td>
<td>0.643‡</td>
</tr>
<tr>
<td>Log(_{10}) VL at initiation of HAART, (\text{cells/\mu l})</td>
<td>5.2 (4.3–5.6)</td>
<td>5.1 (4.1–5.4)</td>
<td>0.553‡</td>
</tr>
<tr>
<td>Log(_{10}) VL at time of TCF, (\text{cells/\mu l})</td>
<td>4.3 (3.7–4.9)</td>
<td>3.9 (3.5–5.0)</td>
<td>0.376‡</td>
</tr>
<tr>
<td>Age at initiation of HAART, (\text{years})</td>
<td>36.8 (30.1–46.4)</td>
<td>38.9 (34.1–43.9)</td>
<td>0.734‡</td>
</tr>
<tr>
<td>Age at time of TCF, (\text{years})</td>
<td>40.0 (33.2–49.3)</td>
<td>42.8 (37.4–46.0)</td>
<td>0.609‡</td>
</tr>
<tr>
<td>Mean time with failure, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRTI</td>
<td>754</td>
<td>696</td>
<td>0.373‡</td>
</tr>
<tr>
<td>NNRTI</td>
<td>167</td>
<td>192</td>
<td>0.030²</td>
</tr>
<tr>
<td>PI</td>
<td>688</td>
<td>610</td>
<td>0.230²</td>
</tr>
</tbody>
</table>

*Comparison of patients with and without resistance test. †\(\chi^2\) test. Values given as median (interquartile range). ‡Student’s \(t\)-test. ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; IDU, intravenous drug users; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TCF, triple-class failure; VL, viral load.
examined changed the estimated regression coefficient more than 15%, and in the full adjusted model the MRR remained at 2.3 (95% CI 1.1–4.8).

Other prognostic factors for death were CD4+ T-cell count at baseline (50–200 versus >200, MRR 2.9 [95% CI 1.4–6.2]; <50 versus >200, MRR 7.5 [95% CI

Table 2. Mutations present at time of TCF and their association with mortality

<table>
<thead>
<tr>
<th>Position</th>
<th>Patients with mutation, n %</th>
<th>Distribution of individual mutations (n patients)</th>
<th>Cox regression analysis of time to all-cause death after TCF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MRR 95% CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>NRTI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T215C/E/I/V/IY</td>
<td>80 (60)</td>
<td>C (2), E (1), F (13), I (2), V (2), Y (60)</td>
<td>1.9</td>
</tr>
<tr>
<td>T215Y mutation only</td>
<td>60 (45)</td>
<td>Y</td>
<td>2.8</td>
</tr>
<tr>
<td>T215F mutation only</td>
<td>13 (10)</td>
<td>F</td>
<td>0.6</td>
</tr>
<tr>
<td>M184V/I</td>
<td>66 (50)</td>
<td>I (3), V (63)</td>
<td>0.8</td>
</tr>
<tr>
<td>M41L</td>
<td>65 (49)</td>
<td>L (65)</td>
<td>2.2</td>
</tr>
<tr>
<td>D67N</td>
<td>53 (40)</td>
<td>N (53)</td>
<td>1.0</td>
</tr>
<tr>
<td>L210W</td>
<td>47 (35)</td>
<td>W (47)</td>
<td>2.0</td>
</tr>
<tr>
<td>K219Q/E</td>
<td>38 (29)</td>
<td>E (16), Q (22)</td>
<td>0.7</td>
</tr>
<tr>
<td>K70R</td>
<td>33 (25)</td>
<td>R (33)</td>
<td>0.6</td>
</tr>
<tr>
<td>L74V</td>
<td>31 (23)</td>
<td>V (31)</td>
<td>1.5</td>
</tr>
<tr>
<td>V118I</td>
<td>25 (19)</td>
<td>I (25)</td>
<td>2.3</td>
</tr>
<tr>
<td>T69D/N</td>
<td>21 (16)</td>
<td>D (11), N (10)</td>
<td>1.0</td>
</tr>
<tr>
<td>E44D</td>
<td>18 (14)</td>
<td>D (18)</td>
<td>2.4</td>
</tr>
<tr>
<td>NRTI (MDR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A62V</td>
<td>2 (2)</td>
<td>V (2)</td>
<td>-</td>
</tr>
<tr>
<td>NNRTI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K103N</td>
<td>71 (53)</td>
<td>N (71)</td>
<td>0.8</td>
</tr>
<tr>
<td>Y181C/I</td>
<td>33 (25)</td>
<td>C (28), I (5)</td>
<td>2.0</td>
</tr>
<tr>
<td>G190A/S</td>
<td>26 (20)</td>
<td>A (21), S (5)</td>
<td>3.0</td>
</tr>
<tr>
<td>L100I</td>
<td>17 (13)</td>
<td>I (17)</td>
<td>1.2</td>
</tr>
<tr>
<td>V108I</td>
<td>12 (9)</td>
<td>I (12)</td>
<td>-</td>
</tr>
<tr>
<td>V106A/M</td>
<td>8 (6)</td>
<td>A (3), M (5)</td>
<td>-</td>
</tr>
<tr>
<td>Y188L/C/H</td>
<td>7 (5)</td>
<td>C (1), H (1), L (5)</td>
<td>-</td>
</tr>
<tr>
<td>P225H</td>
<td>3 (2)</td>
<td>H (3)</td>
<td>-</td>
</tr>
<tr>
<td>Primary PI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L90M</td>
<td>54 (41)</td>
<td>M (54)</td>
<td>1.0</td>
</tr>
<tr>
<td>V82F/A/T/S</td>
<td>44 (33)</td>
<td>A (30), F (4), S (3), T (7)</td>
<td>1.8</td>
</tr>
<tr>
<td>M46L/I</td>
<td>40 (30)</td>
<td>I (33), L (7)</td>
<td>1.1</td>
</tr>
<tr>
<td>I84V</td>
<td>18 (14)</td>
<td>V (18)</td>
<td>1.0</td>
</tr>
<tr>
<td>G48V</td>
<td>6 (5)</td>
<td>V (6)</td>
<td>-</td>
</tr>
<tr>
<td>D30N</td>
<td>6 (5)</td>
<td>N (6)</td>
<td>-</td>
</tr>
<tr>
<td>N88D/S</td>
<td>4 (3)</td>
<td>D (2), S (2)</td>
<td>-</td>
</tr>
<tr>
<td>L33F</td>
<td>3 (2)</td>
<td>F (3)</td>
<td>-</td>
</tr>
<tr>
<td>V32I</td>
<td>2 (2)</td>
<td>I (2)</td>
<td>-</td>
</tr>
<tr>
<td>I47V</td>
<td>2 (2)</td>
<td>V (2)</td>
<td>-</td>
</tr>
<tr>
<td>I50V/L</td>
<td>1 (1)</td>
<td>V (1)</td>
<td>-</td>
</tr>
<tr>
<td>No major resistance mutations</td>
<td>16 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One class</td>
<td>16 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two classes</td>
<td>20 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three classes</td>
<td>81 (61)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following International AIDS Society mutations were not detected in any of the study patients: K65R, Y115F, T69(SXX), V75I, F77L, F116Y, Q151M, M230L and P236L.

Mortality rate ratio (MRR) was estimated only for those mutations present in ≥10% of the study patients. *Adjusted for CD4+ T-cell count, log10VL, age at baseline, gender, year of triple class failure (TCF) and being antiretroviral therapy (ART)-naive at initiation of highly active ART. CI, confidence interval; MDR, multidrug resistance mutations; NNRTI, non-nucleoside reverse transcriptase inhibitor mutations; NRTI, nucleoside reverse transcriptase inhibitor mutations; PI, protease inhibitor mutations; VL, viral load.
3.5–16.1]), log_{10} VL at baseline (<4 versus 4–5, MRR 1.8 [95% CI 0.9–1.8]; <4 versus >5, MRR 3.6 [95% CI 1.7–7.5]), male gender (MRR 2.8 [95% CI 1.2–6.7]) and year of TCF (MRR 0.7 per additional year [95% CI 0.6–0.9]) (Table 3). In the adjusted model, only the number of mutations and CD4+ T-cell count at baseline (50–200 versus >200, MRR 3.6 [95% CI 1.5–8.8]; <50 versus >200, MRR 8.6 [95% CI 3.0–24.7]), remained significant predictors of death (Table 3). When CD4+ T-cell count was entered as a time-updated variable, however, the prognostic value of number of mutations at baseline became insignificant (≥9 versus ≤8 mutations, MRR 1.4 [95% CI 0.7–2.9]), while CD4+ T-cell count remained a predictor (50–200 versus >200, MRR 2.8 [95% CI 1.2–6.7]; <50 versus >5, MRR 3.6 [95% CI 1.7–7.5]).

Table 3. Mortality after TCF

<table>
<thead>
<tr>
<th>Number of mutations</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRR</td>
<td>95% CI</td>
</tr>
<tr>
<td>≤8</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>≥9</td>
<td>2.3</td>
<td>1.2–4.3</td>
</tr>
<tr>
<td>CD4+ T-cell count at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;200</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>50–200</td>
<td>2.9</td>
<td>1.4–6.2</td>
</tr>
<tr>
<td>&lt;50</td>
<td>7.5</td>
<td>3.5–16.1</td>
</tr>
<tr>
<td>Log_{10} VL at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>4–5</td>
<td>1.8</td>
<td>0.9–1.8</td>
</tr>
<tr>
<td>&gt;5</td>
<td>3.6</td>
<td>1.7–7.5</td>
</tr>
<tr>
<td>Year of TCF</td>
<td>0.7</td>
<td>0.6–0.9</td>
</tr>
<tr>
<td>Age at baseline (per year)</td>
<td>1.0</td>
<td>1.0–1.0</td>
</tr>
<tr>
<td>ART-naive at HAART initiation</td>
<td>0.8</td>
<td>0.4–1.6</td>
</tr>
<tr>
<td>Male gender</td>
<td>2.8</td>
<td>1.2–6.7</td>
</tr>
</tbody>
</table>

Cox regression analysis of time to all-cause death after triple-class failure (TCF). ART, antiretroviral therapy; CI, confidence interval; HAART, highly active antiretroviral therapy; MRR, mortality rate ratio; VL, viral load.

Figure 1. Kaplan-Meier survival curve. Time to all-cause death after triple-class virological failure (TCF), according to the number of genotypic drug resistance mutations at baseline (time of TCF)
MRR 3.1 [95% CI 1.2–8.0]; <50 versus >200, MRR 9.9 [95% CI 3.9–25.4]) (Table 4).

The following individual mutations were significantly associated with increased mortality in the adjusted model (Table 2): G190A/S, MRR 3.4 (95% CI 1.6–7.0); T215Y, MRR 3.2 (95% CI 1.6–6.6); and V82F/A/T/S, MRR 2.5 (95% CI 1.2–5.3). When these three mutations and number of mutations were included in the same regression model, the prognostic value of number of mutations (MRR 0.9 [95% CI 0.4–2.3]) and V82F/A/T/S (MRR 1.1 [95% CI 0.5–2.7]) decreased, whereas CD4+ T-cell count at baseline, T215Y and G190A/S remained marked and statistically significant prognostic factors for death (Table 4). Finally, when including time-updated CD4+ T-cell count instead of CD4+ T-cell count at baseline in that same model, only the T215Y mutation remained a prognostic factor for death (MRR 3.0 [95% CI 1.3–7.0]), along with the latest CD4+ T-cell count (50–200 versus >200, MRR 3.0 [95% CI 1.1–7.9]; <50 versus >200, MRR 9.6 [95% CI 3.7–25.1]) (Table 4). Substituting the thymidine analogue mutation (TAM)-1 pattern [12,13] (M41L+L210W+T215Y present at the same time) for T215Y in the model gave similar and significant results (data not shown). No mutations were significantly associated with decreased mortality, and we found no association between mortality and the number of drug classes with resistance mutations (data not shown).

Causes of death were 60% HIV-related, 30% non-HIV related and 10% unknown.

The above-mentioned Cox models included a large number of variables for a dataset with only 40 deaths. However, if the analyses were repeated without adjusting for log_{10} VL at baseline, age at baseline, gender, year of TCF and being antiretroviral-drug-naive before initiation of HAART, the resulting regression coefficients were of equal magnitude and equally statistically significant (data not shown). Intravenous drug use (IDU) was seen in only five persons (Table 1) and therefore not assessed as an independent risk factor. To explore the potential confounding effect, we added IDU to the four multivariate regression models, but observed no substantial change in regression coefficients or their precision (data not shown).

Discussion

In this population-based study we found that more than three of five HIV patients with TCF had resistance mutations to all three drug classes. Patients with a high number of drug resistance mutations had a 2.3-fold increased risk of death, independent of CD4+ T-cell count at time of TCF. Furthermore, some specific mutations were associated with increased mortality.

Our study had a number of strengths. For instance, the population-based design allowed us to identify all patients with TCF in Denmark with complete follow up and extensive covariate data. We were able to identify resistance test results on most patients near the date of TCF. Follow up after TCF was long-term, loss of follow-up was minimal, and the time-updated CD4+ T-cell count was included in the model.
up was minimal, and we evaluated a clear clinical endpoint: death.

Increased mortality in patients with greater numbers of resistance mutations could reflect a direct effect whereby resistance mutations cause lower susceptibility to antiviral drugs and lead to faster disease progression. The effect of the most recent (time-updated) CD4+ T-cell count on the relative risk estimates supports this theory, revealing an association between a high number of mutations at the time of TCF and subsequent low CD4+ T-cell count. However, the T215Y mutation, part of the TAM-1 pattern [12,13], was a predictor of death at all time-updated CD4+ T-cell count levels, indicating that the effect of mutations on mortality was indirect and exerted through mechanisms other than lowering of the CD4+ T-cell count. The T215Y mutation is frequently generated during non-suppressive mono- or dual-NRTI therapy, which was restricted to patients with advanced HIV disease before HAART was a standard HIV treatment in Denmark. Thus, the T215Y mutation might be a marker for patients who were already immunodeficient in the early 1990s and not only a marker for cross-resistance to the NRTI class of drug. The T215Y mutation is also a marker for the TAM-1 pattern, which is associated with more extensive cross-resistance [14], longer use of HAART [13] and greater relative fitness [16] than the TAM-2 pattern.

It is reassuring that, in our study of TCF, one mutation from each of the three drug classes was associated with mortality, independent of the total number of mutations. All three mutations are associated with a number of other mutations. For instance 215Y is associated with mutations in positions 41, 44, 67, 118 and 210 [20]; G190A/S is associated with mutations in position 181 of the reverse transcriptase gene [21]; and V82A is associated with mutations in positions 46 and 48 of the protease gene [22]. The G190A/S mutation [21] has been observed primarily during therapy with nevirapine, didanosine and zidovudine, an outdated combination; the V82F/A/T/S mutation confers resistance to regimens such as ritonavir and indinavir [23], as well as to ritonavir-boosted saquinavir [9]. Thus, all three mutations are associated with exposure to antiretroviral regimens rarely used anymore. In summary, mutations and mortality seemed to be associated with previous non-suppressive therapy, longer duration of HIV infection and more advanced early disease. Thus, the mutations could be proxies for unmeasured factors known to be associated with poor survival. Another factor could be low compliance to treatment regimens, which is associated with increased mortality through coexisting conditions like mental illness and substance abuse [17–19], and presumably also through non-compliance to other essential therapies. On the other hand the assumption that hard-core non-compliers failing with wild-type virus fared worst was not supported by our data. Other potential confounders could be inferior HIV care and/or treatment by inexperienced physicians, but this would be unlikely in our setting where treatment and care is free and limited to specialized clinics. Hence, these numerous identified or potential associations might all be contributory causes to the observed mortality, but no conclusion can be made from our data.

Drug-resistant HIV can be less fit than wild-type virus [24], and cross-sectional studies have found a U-shaped association between the number of resistance mutations and plasma concentrations of HIV RNA, suggesting that an increasing number of mutations will eventually overcome the reduced fitness that allows virological suppression at lower levels of resistance [11,25]. Twelve percent of our patients had no resistance mutations detected at the time of TCF. Some of these patients could be totally non-compliant, fulfilling our criteria for virological failure without ever taking drugs that would induce resistance mutations. In fact, the very low rate of transmitted resistance in Denmark [26,27] makes it likely that the majority of totally non-compliant patients will have no resistance mutations. In our follow-up study, these patients had plenty of time to improve compliance, which might explain why we did not find a similar U-shaped association between the number of mutations and mortality.

Previous studies of mutations and mortality [6,7,28] included only 14–19 patients with triple-class mutations, whereas in the present study 81 (61%) of the patients with confirmed TCF had major mutations to all three drug classes. Thus, our data suggest that the virological failure model [2,3] captures patients in whom viral resistance is an essential component of the failure.

Because we analysed a fairly large number of mutations (that is, 21) the observed effect of individual mutations might be a chance finding. However, the panel of mutations that we analysed were selected by the IAS-USA and on the basis of biological relevance [9], and we used a minimum prevalence criterion of 10% as suggested by others [11].

Our study has some further limitations. Firstly, resistance tests within the analytical window were available for only 133/179 patients with TCF. However, comparison of characteristics of the two groups revealed no significant differences, making major bias in selection unlikely. Secondly, the number of resistance mutations we detected is likely to be an underestimation, because particular mutations selected during previous antiretroviral regimens might go undetected when the test is performed without...
pressure on the mutation in question. However, the number of undetected mutations is assumed to be similar in all patients, because all were triple-drug-class-experienced and would, therefore, previously have been exposed to drugs not included in their regimen at the time of resistance testing. Thus, this information bias would probably not affect the association between mortality and the number of mutations. Thirdly, with only 40 deaths, the study was not large enough for more advanced analyses. For example, we could not analyse the effect of rare resistance mutation patterns or the effect of individual drug combination regimens in association with different resistance mutations. Nor did we analyse outcome in relation to the number of drugs to which the virus was sensitive because of frequent regimen switches and because being drug-resistant versus drug-sensitive in this scenario is often not an all-or-none phenomenon. Finally, the development and appearance of drug mutations depend on the drug combinations used and the order in which they are used. The vast majority of the failures occurred early in the study period, at a time when most PI regimens were unboosted, and our findings might not apply to patients experiencing TCF today, who would probably have a different treatment history. However, the prevalence and distribution of mutations in our study was comparable to that found in other studies [25].

We conclude that in patients with TCF the total number of genotypic resistance mutations and specific single mutations predict mortality and are associated with a further decline in CD4+ T-cell count. The majority of these mutations appear to have accumulated during suboptimal therapies in the 1990s. When TCF occurs, resistance is an important contributor to the increased mortality associated with the condition. We await the introduction of further drug classes to see whether this will eliminate failure-related mortality, or whether we will face resistance to five or six drug classes in the future.

Acknowledgements

The study was supported by grants from the Danish AIDS Foundation, Odense University Hospital, Preben and Anna Simonsen’s Foundation, the Foundation of the Danish Association of Pharmacists, the Danish Medical Research Council and the Clinical Institute of the University of Southern Denmark. No funding sources were involved in study design, data collection, analysis, report writing or the decision to submit the paper.

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Survival of Persons with and without HIV Infection in Denmark, 1995–2005

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Background: The expected survival of HIV-infected patients is of major public health interest.

Objective: To estimate survival time and age-specific mortality rates of an HIV-infected population compared with that of the general population.


Setting: All HIV-infected persons receiving care in Denmark from 1995 to 2005.

Patients: Each member of the nationwide Danish HIV Cohort Study was matched with as many as 99 persons from the general population according to sex, date of birth, and municipality of residence.

Measurements: The authors computed Kaplan–Meier life tables with age as the time scale to estimate survival from age 25 years. Patients with HIV infection and corresponding persons from the general population were observed from the date of the patient’s HIV diagnosis until death, emigration, or 1 May 2005.

Results: 3990 HIV-infected patients and 379 872 persons from the general population were included in the study, yielding 22 744 (median, 5.8 y/person) and 2 689 287 (median, 8.4 years/person) person-years of observation. Three percent of participants were lost to follow-up. From age 25 years, the median survival was 19.9 years (95% CI, 18.5 to 21.3) among patients with HIV infection and 51.1 years (CI, 50.9 to 51.5) among the general population. For HIV-infected patients, survival increased to 32.5 years (CI, 29.4 to 34.7) during the 2000 to 2005 period. In the subgroup that excluded persons with known hepatitis C coinfection (16%), median survival was 38.9 years (CI, 35.4 to 40.1) during this same period. The relative mortality rates for patients with HIV infection compared with those for the general population decreased with increasing age, whereas the excess mortality rate increased with increasing age.

Limitations: The observed mortality rates are assumed to apply beyond the current maximum observation time of 10 years.

Conclusions: The estimated median survival is more than 35 years for a young person diagnosed with HIV infection in the late highly active antiretroviral therapy era. However, an ongoing effort is still needed to further reduce mortality rates for these persons compared with the general population.


For author affiliations, see end of text.

Knowing the expected survival of HIV-infected patients is of major public health interest. Mortality rates have decreased substantially in recent years as a result of improved effectiveness of highly active antiretroviral therapy (HAART) (1). Studies comparing mortality rates for HIV-infected persons with age- and sex-specific mortality rates for the general population (2–5) have reported 3- to 10-fold increase in successfully treated patients. The relative mortality rate, however, is highly dependent on the age distribution of the study sample and does not in itself answer questions about survival. We therefore aimed to estimate median survival and age-specific mortality rates for an entire HIV-infected population compared with a cohort from the general population. Persons with HIV infection were followed from before initiation of HAART and included those with such predictors of lower survival as poor response to therapy, AIDS diagnosis, low CD4 count, high viral load, and poor adherence to treatment (6, 7). Linking data from the population-based Danish HIV Cohort Study (DHCS) (8) and the Danish Civil Registration System (CRS) (9, 10) allowed us to use product-limit methods that are analogous to the period life tables used by national authorities for estimating median survival (11).

METHODS

Study Sample

The DHCS is a prospective, nationwide, population-based cohort study of all HIV-infected persons treated in Danish HIV clinics since 1 January 1995 (8, 12). The study is ongoing, with continuous enrollment of both newly diagnosed residents and immigrants with existing HIV infection. Treatment for HIV infection in Denmark is restricted to 8 specialized centers, and the Danish health care system provides free tax-supported medical care, including antiretroviral treatment for HIV infection. The study databases are updated annually. Adult (≥16 years) DHCS participants with residency in Denmark were included at their first visit to an HIV clinic. The Civil Registration System (CRS) is a national registry of all Danish
residents; this registry contains information on date of birth; sex; address; date of migration; and date of death, if applicable (9). A 10-digit personal number (Central Person Registry [CPR] number), assigned at birth, uniquely identifies each person. The CRS is updated within a week of a person’s birth, address change, death, or emigration. Use of the CPR number enables treatment centers to avoid multiple registrations of the same patient and allows tracking of deaths and persons lost to follow-up due to emigration. Using the CRS records, we drew a random sample of persons from the general population and matched them to each HIV-infected patient according to sex and month of birth and residence in the same municipality as the patient on the date of diagnosis (Denmark has a population of approximately 5.3 million persons living in 270 municipalities). We aimed to sample 99 persons from the general population for each HIV-infected person according to sex and month of birth; sex; address; date of migration; and date of death, if applicable (9). A 10-digit personal number (Central Person Registry [CPR] number), assigned at birth, uniquely identifies each person. The CRS is updated within a week of a person’s birth, address change, death, or emigration. Use of the CPR number enables treatment centers to avoid multiple registrations of the same patient and allows tracking of deaths and persons lost to follow-up due to emigration. Using the CRS records, we drew a random sample of persons from the general population and matched them to each HIV-infected patient according to sex and month of birth and residence in the same municipality as the patient on the date of diagnosis (Denmark has a population of approximately 5.3 million persons living in 270 municipalities). We aimed to sample 99 persons from the general population for each HIV-infected person according to sex and month of birth and residence in the same municipality as the patient on the date of diagnosis (Denmark has a population of approximately 5.3 million persons living in 270 municipalities). We aimed to sample 99 persons from the general population for each HIV-infected person according to sex and month of birth and residence in the same municipality as the patient on the date of diagnosis (Denmark has a population of approximately 5.3 million persons living in 270 municipalities). We aimed to sample 99 persons from the general population for each HIV-infected person according to sex and month of birth and residence in the same municipality as the patient on the date of diagnosis (Denmark has a population of approximately 5.3 million persons living in 270 municipalities).
Approvals and Permissions
The Danish Data Protection Agency approved the establishment of the cohort study. The study was not subject to approval by the ethics committee because data collection did not involve direct patient contact.

Role of the Funding Sources
The Danish HIV Cohort study receives funding from the Danish AIDS Foundation, Odense University Hospital, Preben and Anna Simonsen’s Foundation, the Foundation of the Danish Association of Pharmacists, and the Clinical Institute at the University of Southern Denmark. The funding sources were not involved in the design, data collection, analysis, or writing of the study.

RESULTS

Study Sample
We included 3990 HIV-infected persons and 379 872 persons from the general population: The respective median observation time after age 25 years was 5.8 person-years (interquartile range, 2.2–9.9) and 8.4 (4.3–10.3) years, respectively (Table 1). One hundred twenty-one (3.0%) HIV-infected patients and 11 552 (3.0%) persons from the general population were lost to follow-up; of these, 107 (2.7%) patients with HIV infection and 10 234 (2.7%) persons from the general population emigrated. There were 2045 (51%) incident HIV cases diagnosed after 1 January 1995; 75% were observed within 31 days of diagnosis, and 95% came under observation within the first 181 days after diagnosis. After HAART was introduced in 1996, the prevalence of patients receiving this treatment gradually increased, surpassing 75% in 2002 to 2004. At any given time, fewer than 5% of HIV-infected patients were interrupting treatment. The number of patients under observation varied with age and was highest (range, 515 to 1004) for those who were 30 to 50 years of age (Table 2).

Survival from Age 25 Years
All participants were observed from age 25 years: HIV-infected persons had a median survival of 19.9 years (17.5 years for men and 24.2 years for women), whereas persons from the general population had a median survival of 51.1 years (50.8 years for men and 54.8 years for women) (Table 3). During the late HAART period (2000 to 2005), median survival of HIV-infected patients had increased to 32.5 years (32.1 years for men and 32.3 years for women) overall, and to 38.9 years (37.8 years for men and 40.1 years for women) after persons with known HCV infection were excluded (Figure).

Mortality Rates
The mortality rate was 43 per 1000 person-years (95% CI, 40 to 45) for HIV-infected persons and 4.7 per 1000 person-years (CI, 4.6 to 4.8) for the general population (Table 3). The highest mortality rate, 124 per 1000 person-years (CI, 112 to 137), was observed in the pre-HAART period (1995 to 1996). This rate decreased to 38 per 1000 person-years (CI, 33 to 43) in the early HAART period (1997 to 1999) and to 25 per 1000 person-years (CI, 23 to 28) in the late HAART period (2000 to 2005). In patients receiving HAART, the highest mortality rate of 48 per 1000 person-years (CI, 40 to 57) was observed during the first year of treatment but decreased to 27 per 1000 person-years (CI, 22 to 32) during the second and third years of HAART, to 26 per 1000 person-years (CI, 21 to 32) during the fourth and fifth years of HAART, and to 26 per 1000 person-years (CI, 21 to 31) from the sixth year onward. Mortality rates were even lower among patients treated during the late HAART period. Although mortality rates declined with calendar time, we found no change in mortality rates from the first to the tenth year after the diagnosis of HIV infection. In the late HAART period, the mortality rate was 26 per 1000 person-years (CI, 19 to 34) during the first 2 years after diagnosis, 17

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### Table 1. Characteristics of Study Participants*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with HIV Infection</th>
<th>General Population without HIV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persons, n</td>
<td>3990</td>
<td>379 872</td>
</tr>
<tr>
<td>Men, %</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>Median age at study entry (interquartile range), y</td>
<td>37.2 (31.0–44.8)</td>
<td>36.9 (30.9–44.6)</td>
</tr>
<tr>
<td>Median observation time (interquartile range), y</td>
<td>5.8 (2.2–9.9)</td>
<td>8.4 (4.3–10.3)</td>
</tr>
<tr>
<td>Incident cases (diagnosed after 1 January 1995), n (%)</td>
<td>2045 (51)</td>
<td>–</td>
</tr>
<tr>
<td>Entered the cohort within 31 days after diagnosis</td>
<td>1956 (76)</td>
<td>–</td>
</tr>
<tr>
<td>Entered the cohort within 181 days after diagnosis</td>
<td>1943 (95)</td>
<td>–</td>
</tr>
<tr>
<td>Most likely method of infection, n (%)</td>
<td>1863 (47)</td>
<td>–</td>
</tr>
<tr>
<td>Male homosexual activity</td>
<td>1377 (35)</td>
<td>–</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>480 (12)</td>
<td>–</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td>270 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3287 (82)</td>
<td>NA</td>
</tr>
<tr>
<td>Black</td>
<td>446 (11)</td>
<td>NA</td>
</tr>
<tr>
<td>Other</td>
<td>257 (6)</td>
<td>NA</td>
</tr>
<tr>
<td>Positive for hepatitis C infection</td>
<td>668 (17)</td>
<td>–</td>
</tr>
</tbody>
</table>

* HIV = human immunodeficiency virus; NA = not available.
Table 2. Age-Specific Mortality Rates*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>Patients with HIV infection</th>
<th>General population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under observation at the beginning of each age period, n</td>
<td>25–30 y</td>
<td>170</td>
<td>1045</td>
</tr>
<tr>
<td></td>
<td>&gt;30–35 y</td>
<td>566</td>
<td>60,322</td>
</tr>
<tr>
<td></td>
<td>&gt;35–40 y</td>
<td>959</td>
<td>107,786</td>
</tr>
<tr>
<td></td>
<td>&gt;40–45 y</td>
<td>950</td>
<td>112,343</td>
</tr>
<tr>
<td>PYR (in thousands), n</td>
<td>Patients with HIV infection</td>
<td>1.77</td>
<td>3.97</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>184</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>Patients with HIV infection</td>
<td>54</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>128</td>
<td>385</td>
</tr>
<tr>
<td>Events, n</td>
<td>Patients with HIV infection</td>
<td>128</td>
<td>385</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>184</td>
<td>432</td>
</tr>
<tr>
<td>Mortality rate for patients with HIV infection, per 1000 PYR</td>
<td>Patients with HIV infection</td>
<td>30.5 (23.4 to 39.9)</td>
<td>28.5 (23.7 to 34.3)</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>35.8 (30.8 to 41.4)</td>
<td>45.7 (39.7 to 52.7)</td>
</tr>
<tr>
<td>Mortality rate for general population, per 1000 PYR</td>
<td>Patients with HIV infection</td>
<td>0.7 (0.6 to 0.8)</td>
<td>0.9 (0.8 to 1.0)</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>1.3 (1.3 to 1.4)</td>
<td>2.6 (2.4 to 2.7)</td>
</tr>
<tr>
<td>Mortality rate ratio (patients vs. general population)</td>
<td>Patients with HIV infection</td>
<td>44.53 (32.0 to 61.9)</td>
<td>32.04 (25.9 to 39.7)</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>27.4 (23.1 to 32.4)</td>
<td>18.04 (15.4 to 21.1)</td>
</tr>
<tr>
<td>Excess mortality rate (patients vs. general population, per 1000 PYR)</td>
<td>Patients with HIV infection</td>
<td>29.8 (21.7 to 38.0)</td>
<td>27.6 (22.3 to 32.9)</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>34.4 (29.1 to 39.7)</td>
<td>43.2 (36.6 to 49.7)</td>
</tr>
<tr>
<td>Patients observed during the years 2000–2005</td>
<td>Patients with HIV infection, PYR (in thousands)</td>
<td>0.77</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for patients with HIV infection, per 1000 PYR</td>
<td>6.5 (2.7 to 15.6)</td>
<td>11.6 (7.7 to 17.5)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for general population, per 1000 PYR</td>
<td>0.6 (0.5 to 0.8)</td>
<td>0.8 (0.7 to 0.9)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate ratio (patients vs. general population)</td>
<td>8.88 (3.2 to 24.7)</td>
<td>15.14 (9.7 to 23.5)</td>
</tr>
<tr>
<td></td>
<td>Excess mortality rate (patients vs. general population, per 1000 PYR)</td>
<td>5.8 (1.6 to 11.5)</td>
<td>10.9 (6.1 to 15.6)</td>
</tr>
<tr>
<td>HCV-negative patients observed during the years 2000–2005</td>
<td>Patients with HIV infection, PYR (in thousands)</td>
<td>0.67</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for patients with HIV infection, per 1000 PYR</td>
<td>1.5 (0.2 to 10.6)</td>
<td>8.5 (5.0 to 14.3)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for general population, per 1000 PYR</td>
<td>0.6 (0.5 to 0.8)</td>
<td>0.8 (0.7 to 0.9)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate ratio (patients vs. general population)</td>
<td>2.6 (0.4 to 19.3)</td>
<td>10.7 (6.2 to 18.6)</td>
</tr>
<tr>
<td></td>
<td>Excess mortality rate (patients vs. general population, per 1000 PYR)</td>
<td>0.9 (–2.1 to 3.8)</td>
<td>7.7 (3.2 to 12.1)</td>
</tr>
<tr>
<td>HCV-positive patients observed during the years 2000–2005</td>
<td>Patients with HIV infection, PYR (in thousands)</td>
<td>0.10</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for patients with HIV infection, per 1000 PYR</td>
<td>38.5 (14.5 to 102.7)</td>
<td>28.2 (14.7 to 54.2)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate for general population, per 1000 PYR</td>
<td>0.8 (0.4 to 1.6)</td>
<td>0.7 (0.5 to 1.0)</td>
</tr>
<tr>
<td></td>
<td>Mortality rate ratio (patients vs. general population)</td>
<td>41.6 (10.8 to 160.9)</td>
<td>42.1 (19.2 to 92.6)</td>
</tr>
<tr>
<td></td>
<td>Excess mortality rate (patients vs. general population, per 1000 PYR)</td>
<td>37.8 (8.0 to 75.5)</td>
<td>27.5 (9.1 to 46.0)</td>
</tr>
</tbody>
</table>

* Values in parentheses are 95% CIs. HCV = hepatitis C virus; HIV = human immunodeficiency virus; NA = not available; PYR = person-years at risk.

The mortality rates for HIV-infected persons relative to those for the general population (mortality rate ratio) were highest in the younger age groups. The decrease in mortality rate ratio with age was driven by the natural age-dependent increase in mortality rates in the reference population. The mortality rate ratio decreased from 44.5 (CI, 32.0 to 61.9) for persons who were age 25 to 30 years to 3.4 (CI, 2.3 to 5.1) for those who were age 65 to 70 years. Among persons observed in the late HAART period, the mortality rate ratio varied from 15.1 (CI, 9.7 to 23.5) to 3.0 (CI, 2.0 to 4.6) for all HIV-infected patients. After HCV-positive persons were excluded, the mortality rate ratio during this period ranged from 11.5 (CI, 8.0 to 16.6) to 2.8 (CI, 2.0 to 4.0).

In contrast to the age-related decrease in mortality rate ratio, the excess mortality rate for HIV-infected patients compared with that for the general population was lowest in the younger age groups and increased with age. The
excess mortality rate during the late HAART period was not higher than 12.3 per 1000 person-years (CI, 7.7 to 16.9) among HCV-negative persons who were younger than 50 years but increased gradually with age to 53.8 per 1000 person-years (CI, 12.1 to 95.5) among persons who were 65 to 70 years. These figures were 2- to 4-fold higher if all patients and observation years were included (Table 2).

Causes of Death

The mortality rate for HIV-related death decreased from 71 per 1000 person-years (CI, 63 to 81) in the early HAART period to 7.0 per 1000 person-years (CI, 5.8 to 8.6) in the late HAART period, and non–HIV-related deaths decreased from 23 per 1000 person-years (CI, 18 to 29) to 9.4 per 1000 person-years (CI, 7.9 to 11.2) (Table 4). Thus, the proportion of known causes of death that were related to HIV infection decreased from 76% in 1995 to 1996, to 57% in 1997 to 1999, and to 43% in 2000 to 2005.

### Causes of Death

#### Table 2—Continued

<table>
<thead>
<tr>
<th>Age</th>
<th>&gt;45–50 y</th>
<th>&gt;50–55 y</th>
<th>&gt;55–60 y</th>
<th>&gt;60–65 y</th>
<th>&gt;65–70 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>727</td>
<td>517</td>
<td>373</td>
<td>224</td>
<td>77</td>
</tr>
<tr>
<td>Person-Years</td>
<td>90 759</td>
<td>65 254</td>
<td>45 872</td>
<td>27 170</td>
<td>11 023</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>3.07</td>
<td>2.26</td>
<td>1.53</td>
<td>0.67</td>
<td>0.28</td>
</tr>
<tr>
<td>Person-Years</td>
<td>386</td>
<td>280</td>
<td>185</td>
<td>86</td>
<td>40</td>
</tr>
<tr>
<td>Number</td>
<td>155</td>
<td>111</td>
<td>69</td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td>Person-Years</td>
<td>1795</td>
<td>2036</td>
<td>2031</td>
<td>1543</td>
<td>1169</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>50.5 (43.1 to 59.1)</td>
<td>49.2 (40.8 to 59.2)</td>
<td>45.2 (35.7 to 57.3)</td>
<td>73.4 (55.4 to 97.1)</td>
<td>93.1 (63.4 to 136.7)</td>
</tr>
<tr>
<td>Person-Years</td>
<td>4.7 (4.4 to 4.9)</td>
<td>7.3 (7.0 to 7.6)</td>
<td>11.0 (10.5 to 11.4)</td>
<td>18.0 (17.1 to 18.9)</td>
<td>29.1 (27.5 to 30.8)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>10.81 (9.1 to 12.8)</td>
<td>7.24 (6.0 to 8.8)</td>
<td>4.05 (3.2 to 5.2)</td>
<td>4.23 (3.2 to 5.6)</td>
<td>3.43 (2.3 to 5.1)</td>
</tr>
<tr>
<td>Person-Years</td>
<td>45.9 (37.9 to 53.8)</td>
<td>41.9 (32.7 to 51.0)</td>
<td>34.3 (23.6 to 45.0)</td>
<td>55.4 (34.8 to 75.9)</td>
<td>64.0 (28.2 to 99.8)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>1.93</td>
<td>1.40</td>
<td>1.14</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>Person-Years</td>
<td>32.2 (25.1 to 41.3)</td>
<td>33.5 (25.2 to 55.6)</td>
<td>34.3 (25.0 to 46.9)</td>
<td>49.3 (33.3 to 73.0)</td>
<td>81.3 (49.1 to 134.9)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>4.5 (4.3 to 4.8)</td>
<td>7.1 (6.7 to 7.5)</td>
<td>10.6 (10.0 to 11.1)</td>
<td>17.0 (16.1 to 18.1)</td>
<td>28.5 (26.6 to 30.5)</td>
</tr>
<tr>
<td>Person-Years</td>
<td>6.81 (5.2 to 8.9)</td>
<td>5.14 (3.8 to 7.0)</td>
<td>3.16 (2.3 to 4.4)</td>
<td>3.04 (2.0 to 4.6)</td>
<td>3.11 (1.9 to 5.2)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>27.6 (19.6 to 35.7)</td>
<td>26.4 (16.8 to 36.0)</td>
<td>23.7 (12.9 to 34.4)</td>
<td>32.3 (12.9 to 51.6)</td>
<td>52.8 (11.7 to 94.0)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>1.53</td>
<td>1.22</td>
<td>1.10</td>
<td>0.49</td>
<td>0.18</td>
</tr>
<tr>
<td>Person-Years</td>
<td>15.0 (10.0 to 22.6)</td>
<td>27.8 (19.9 to 39.0)</td>
<td>30.1 (21.4 to 42.4)</td>
<td>47.0 (31.3 to 70.8)</td>
<td>82.3 (49.6 to 136.5)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>4.6 (4.3 to 4.9)</td>
<td>7.0 (6.6 to 7.5)</td>
<td>10.6 (10.0 to 11.2)</td>
<td>17.0 (16.0 to 18.1)</td>
<td>28.5 (26.6 to 30.5)</td>
</tr>
<tr>
<td>Person-Years</td>
<td>3.0 (2.0 to 4.7)</td>
<td>4.3 (3.1 to 6.2)</td>
<td>2.8 (2.0 to 4.0)</td>
<td>3.0 (2.0 to 4.6)</td>
<td>3.1 (1.9 to 5.2)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>10.4 (4.3 to 16.6)</td>
<td>20.8 (11.4 to 30.2)</td>
<td>19.5 (9.2 to 29.8)</td>
<td>30.0 (10.8 to 49.3)</td>
<td>53.8 (12.1 to 95.5)</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>0.40</td>
<td>0.18</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Person-Years</td>
<td>98.5 (72.0 to 134.8)</td>
<td>71.3 (41.4 to 122.7)</td>
<td>141.3 (63.5 to 314.6)</td>
<td>109.9 (27.5 to 439.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>4.3 (3.8 to 4.9)</td>
<td>7.3 (6.4 to 8.3)</td>
<td>10.1 (8.0 to 12.8)</td>
<td>17.3 (13.0 to 23.0)</td>
<td>27.9 (20.4 to 38.0)</td>
</tr>
<tr>
<td>Person-Years</td>
<td>21.6 (15.2 to 30.8)</td>
<td>10.3 (5.7 to 18.8)</td>
<td>13.7 (5.3 to 35.5)</td>
<td>3.5 (0.5 to 26.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Rate (95% CI)</td>
<td>94.2 (63.3 to 125.1)</td>
<td>64.0 (25.2 to 102.8)</td>
<td>131.2 (18.1 to 244.3)</td>
<td>92.6 (59.8 to 244.9)</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Discussion

In this population-based cohort study, we estimate a median remaining lifetime of more than 35 years for a 25-year-old, HIV-positive person without HCV infection who received care in the twenty-first century. We expect this estimate to be robust because the study included all patients, regardless of such prognostic factors as CD4-positive cell count, HIV RNA, disease stage, history of AIDS, treatment adherence, or time receiving HAART. The increase in survival over time was attributable mainly to a decrease in HIV-related deaths. Despite the encouraging survival expectations, the study still shows large, age-dependent excess mortality rates in the HIV-infected cohort compared with the general population. The excess mortal-
ity rates increased with increasing age, whereas the relative mortality rates decreased.

Three previous studies have compared age- sex- and calendar-year–specific mortality rates of HIV-infected patients with those of the general population (2, 3, 14). In the Dutch ATHENA (AIDS Therapy Evaluation, the Netherlands Study Group) cohort (2), the overall mortality rate among HIV-infected patients was considerably lower (10.6 per 1000 person-years) than in our study; as in our study, the ATHENA cohort showed a pattern of decreasing relative mortality rates with increasing age but no increase in the excess mortality rate with age. However, the study sample was restricted to antiretroviral-naive patients who had survived the first 24 weeks of a HAART regimen. In contrast, we included all patients, both those who were diagnosed with advanced disease and did not survive for 24 weeks and those who did not yet meet the criteria for HAART, which allowed us to estimate survival in the total HIV population. In the Swiss HIV Cohort Study, Jaggy and colleagues (14) studied excess mortality rates in pa-

<table>
<thead>
<tr>
<th>Variable</th>
<th>PYR</th>
<th>Events</th>
<th>Mortality Rate per 1000 PYR (95% CI)</th>
<th>Median Survival after Age 25 Years (95% CI), y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Men</td>
</tr>
<tr>
<td>General population</td>
<td>2 689 287</td>
<td>12 565</td>
<td>4.7 (4.6–4.8)</td>
<td>5.5 (5.4–5.6)</td>
</tr>
<tr>
<td>Patients with HIV infection</td>
<td>22 744</td>
<td>970</td>
<td>43 (40–45)</td>
<td>47 (44–50)</td>
</tr>
<tr>
<td>HAART period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART</td>
<td>8271</td>
<td>537</td>
<td>65 (60–71)</td>
<td>75 (68–82)</td>
</tr>
<tr>
<td>1st year</td>
<td>2605</td>
<td>124</td>
<td>48 (40–57)</td>
<td>51 (42–62)</td>
</tr>
<tr>
<td>2nd–3rd year</td>
<td>4534</td>
<td>121</td>
<td>27 (22–32)</td>
<td>28 (23–35)</td>
</tr>
<tr>
<td>4th–5th year</td>
<td>3570</td>
<td>92</td>
<td>26 (21–32)</td>
<td>26 (21–33)</td>
</tr>
<tr>
<td>6th year onward</td>
<td>3764</td>
<td>96</td>
<td>26 (21–31)</td>
<td>27 (22–34)</td>
</tr>
<tr>
<td>Time since diagnosis</td>
<td>3436</td>
<td>159</td>
<td>46 (40–54)</td>
<td>53 (44–62)</td>
</tr>
<tr>
<td>1st–2nd years after diagnosis</td>
<td>3419</td>
<td>133</td>
<td>39 (33–46)</td>
<td>44 (36–53)</td>
</tr>
<tr>
<td>5th–6th years after diagnosis</td>
<td>3136</td>
<td>116</td>
<td>37 (31–44)</td>
<td>40 (33–50)</td>
</tr>
<tr>
<td>7th–8th years after diagnosis</td>
<td>2799</td>
<td>117</td>
<td>42 (35–50)</td>
<td>47 (39–58)</td>
</tr>
<tr>
<td>9th–10th years after diagnosis</td>
<td>2614</td>
<td>129</td>
<td>49 (42–59)</td>
<td>56 (46–67)</td>
</tr>
<tr>
<td>Hepatitis C status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4149</td>
<td>246</td>
<td>59 (52–67)</td>
<td>68 (59–78)</td>
</tr>
<tr>
<td>Negative</td>
<td>18 595</td>
<td>724</td>
<td>39 (36–42)</td>
<td>43 (40–46)</td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995–1996</td>
<td>3243</td>
<td>402</td>
<td>124 (112–137)</td>
<td>136 (122–151)</td>
</tr>
<tr>
<td>1997–1999</td>
<td>5857</td>
<td>222</td>
<td>38 (33–43)</td>
<td>41 (35–47)</td>
</tr>
<tr>
<td>2000–2005</td>
<td>13 644</td>
<td>346</td>
<td>25 (23–28)</td>
<td>27 (24–30)</td>
</tr>
<tr>
<td>Patients with HIV infection observed 2000–2005 only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART</td>
<td>2946</td>
<td>66</td>
<td>22 (18–29)</td>
<td>26 (20–34)</td>
</tr>
<tr>
<td>1st year</td>
<td>1073</td>
<td>46</td>
<td>43 (32–57)</td>
<td>50 (37–69)</td>
</tr>
<tr>
<td>2nd–3rd year</td>
<td>2464</td>
<td>57</td>
<td>23 (18–30)</td>
<td>23 (17–31)</td>
</tr>
<tr>
<td>4th–5th year</td>
<td>3398</td>
<td>81</td>
<td>24 (19–30)</td>
<td>24 (18–30)</td>
</tr>
<tr>
<td>6th year onward</td>
<td>3763</td>
<td>96</td>
<td>26 (21–31)</td>
<td>27 (22–34)</td>
</tr>
<tr>
<td>Time since diagnosis</td>
<td>1875</td>
<td>48</td>
<td>26 (19–34)</td>
<td>30 (22–40)</td>
</tr>
<tr>
<td>1st–2nd years after diagnosis</td>
<td>1901</td>
<td>32</td>
<td>17 (12–24)</td>
<td>17 (11–26)</td>
</tr>
<tr>
<td>5th–6th years after diagnosis</td>
<td>1786</td>
<td>32</td>
<td>18 (13–25)</td>
<td>16 (11–25)</td>
</tr>
<tr>
<td>7th–8th years after diagnosis</td>
<td>1630</td>
<td>34</td>
<td>21 (15–29)</td>
<td>23 (16–33)</td>
</tr>
<tr>
<td>9th–10th years after diagnosis</td>
<td>1439</td>
<td>24</td>
<td>17 (11–25)</td>
<td>18 (12–29)</td>
</tr>
<tr>
<td>Hepatitis C status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2245</td>
<td>127</td>
<td>57 (48–67)</td>
<td>59 (48–74)</td>
</tr>
<tr>
<td>Negative</td>
<td>11 399</td>
<td>219</td>
<td>19 (17–22)</td>
<td>22 (19–25)</td>
</tr>
</tbody>
</table>

* HAART = highly active antiretroviral therapy; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PYR = person-years at risk.
patients “successfully treated with HAART” (excess mortality rate, 3.1 to 8.0 [HCV-negative] and 20.5 to 25.9 [HCV-positive] per 1000 person-years) vs. “unsuccessfully treated with HAART” (excess mortality rate, 117.4 [HCV-negative] and 112.7 [HCV-positive] per 1000 person-years). These authors did not report the distribution of ages in their study groups or the age-specific excess mortality rates. In another analysis based on the Swiss HIV Cohort Study, Keiser and colleagues (3) found a decrease in the mortality rate from 130 per 1000 person-years during 1990 to 1995 to 30 per 1000 person-years during 1997 to 2001, and a concomitant decrease in standardized mortality rate ratio from 79.3 to 15.3. There was a 21% withdrawal rate in that study, and no information was available regarding participants’ age distributions. Because mortality rates among HIV-infected and noninfected persons are highly age-dependent, the age-specific mortality rates reported in our study allow transparency and easier comparison among studies and samples. Further, the 2 Swiss Cohort Study reports were based on data up to 2001 and did not incorporate the advances in treatment effectiveness obtained during the subsequent 4 years. Braithwaite and coworkers (15) used data from the Collaborations in HIV Outcomes Research/US (CHORUS) cohort (16) to develop a computer model that incorporated time-updated CD4-positive cell counts, viral load, adherence to treatment, and development of resistance; the estimated median survival from that model was 20.4 years for newly diagnosed patients. Compared with CHORUS, which is a clinic-based, multistate cohort study requiring informed consent from the patients, DHCS is geographically based with almost complete inclusion of HIV-infected patients in our area. Therefore, we may interpret our findings as a result of the total health care effort in our area.

The mortality rates for HIV-infected patients may be put into clinical perspective by comparison with mortality rates among patients with type 1 diabetes mellitus, another serious chronic disease of young adults. Laing and coworkers (17) estimated age- and sex-specific mortality rates for patients with type 1 diabetes (per 1000 person-years). They reported the following mortality rates for women and men, respectively: for age 30 to 39 years, 3.2 and 4.2; for age 40 to 49 years, 8.5 and 11.6; for age 50 to 59 years, 19.1 and 26.2; and for age 60 to 69 years, 44.6 and 63.2. These rates are slightly lower than the rates we found among persons without HCV infection during the late HAART era.

The strengths of our study are its population-based setting, minimal participants lost to follow-up, high quality of the death registration (that is, we are certain that all

### Table 4. Mortality Rates according to Cause of Death and Calendar Period*

<table>
<thead>
<tr>
<th>Observation Period</th>
<th>Cause of Death</th>
<th>PYR, n</th>
<th>Events, n</th>
<th>Mortality Rate per 1000 PYR (95% CI)</th>
<th>All-Cause Mortality Rate, %</th>
<th>Mortality Rate by Known Causes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1995–1996</strong></td>
<td>All causes</td>
<td>3243</td>
<td>402</td>
<td>124 (112–137)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV-related</td>
<td>3243</td>
<td>231</td>
<td>71.2 (62.6–81.0)</td>
<td>100</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Non–HIV-related</td>
<td>3243</td>
<td>75</td>
<td>23.1 (18.4–29.0)</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3243</td>
<td>96</td>
<td>29.6 (24.2–36.2)</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td><strong>1997–1999</strong></td>
<td>All causes</td>
<td>5857</td>
<td>222</td>
<td>37.9 (33.2–42.2)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV-related</td>
<td>5857</td>
<td>104</td>
<td>17.8 (14.7–21.5)</td>
<td>100</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Non–HIV-related</td>
<td>5857</td>
<td>80</td>
<td>13.7 (11.0–17.0)</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>5857</td>
<td>38</td>
<td>6.5 (4.7–8.9)</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td><strong>2000–2005</strong></td>
<td>All causes</td>
<td>13 644</td>
<td>346</td>
<td>25.4 (22.8–28.2)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV-related</td>
<td>13 644</td>
<td>96</td>
<td>7.0 (5.8–8.6)</td>
<td>100</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Non–HIV-related</td>
<td>13 644</td>
<td>128</td>
<td>9.4 (7.9–11.2)</td>
<td>100</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>13 644</td>
<td>122</td>
<td>8.9 (7.5–10.7)</td>
<td>100</td>
<td>35</td>
</tr>
</tbody>
</table>

* HIV = human immunodeficiency virus; PYR = person-years at risk.
dates were correct for all registered deaths), clearly defined
date of inclusion of all prevalent and incident cases of HIV
infection in Denmark (1 January 1995), and large propor-
tion of incident cases observed shortly after patients were
diagnosed with HIV infection, which allowed us to follow
up on most patients from before initiation of HAART.

Despite the advantages of good-quality data, our study
has limitations. First, survival predictions were based on
the assumption that the observed mortality rates also
would apply in subsequent years, whereas actual observa-
tion time of any individual patient was at most 10 years.
However, we found no increase in mortality rates through
the first 10 years of infection or increase in mortality rates
with increasing time receiving HAART, and we found a
decrease in mortality rates with increasing calendar period.
These findings agree with a previous study from DHCS, in
which we observed a decreasing incidence of triple-class
drug failure with successive calendar periods (18). Thus,
we did not see any signs of waning effectiveness of
HAART, which is currently debated and considered to be
a potential health threat because of multiclass drug failure,
accumulation of drug resistance, and long-term drug tox-
icities (19, 20) Although our predictions reach far beyond
the current experience with HIV and HAART, we saw no
signs that a 50-year-old patient who was infected several
years previously had a higher mortality rate than a recently
infected 50-year-old patient. Second, some eligible HIV-
infected persons may not have been included in the study.
Because dispensing antiretroviral drugs in Denmark is re-
stricted to HIV clinics and is free, many of the missed
patients are probably those who do not fulfill the criteria
for HAART and therefore have low mortality rates. This
would cause overestimation of mortality rates in the HIV-
infected cohort. In contrast, many patients may not seek
care despite being eligible for antiretroviral treatment and
may belong to a group with such comorbid conditions as
mental health disorders or addiction problems. This group
would have a higher risk for death, because of HIV infec-
tion and comorbid conditions, thus leading to an underes-
timation of the mortality rates in the HIV-infected cohort.
Third, the HIV-infected population is thought to differ
from the general population regarding socioeconomic and
behavioral factors (8, 21) and having acquired HIV infec-
tion probably indicates a tendency toward risk behaviors.
Studies have shown a higher frequency of smoking and
alcohol consumption among HIV-infected patients (22,
23). Matching the cohort of persons from the general pop-
ulation according to sex, age, and place of residence may
partly correct for these group differences, but any residual
confounding by lifestyle or comorbid conditions would
cause an overestimation of the observed excess mortality
rates because of HIV infection. Fourth, the results are in-
fluenced by the composition of the study sample. In our
cohort, and in most other HIV-infected populations, the
mortality rates differ in subgroups (defined by HCV coin-
feciton status, ethnicity, risk behaviors, and sex), and may
be influenced by the person’s position on different time
scales (age, time receiving HAART, and calendar time). To
explore and clarify the effect of these covariates, we pre-
sented mortality rates in selected time strata. The subgroup
with the best prognosis (persons without HCV coinfection)
comprised 84% of all persons observed in the late
HAART era and was therefore chosen as a clinically useful
reference group.

The survival projections in our study depend on con-
stant continuous treatment success beyond the 10 years of current
experience with HAART. Further, with the easy access to
HAART and HIV care in Denmark, our findings may
represent a best-case scenario. Not all subgroups of patients
have the same prognosis, and treatment must be individu-
alyzed according to actual risk estimates. Our study sug-
gests that most young persons with HIV infection can ex-
pect to survive for more than 35 years, but an ongoing
effort is still needed to further reduce mortality rates in
these persons.

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Århus, Denmark; Hvidovre University Hospital, Hvidovre, Denmark;
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Massachusetts.

Acknowledgments: The authors thank the staff of their clinical depart-
ments for their continuous support and enthusiasm.

Grant Support: The Danish HIV Cohort study receives funding from
the Danish AIDS Foundation, Odense University Hospital, Preben and
Anna Simonsen’s Foundation, the Foundation of the Danish Association
of Pharmacists, and the Clinical Institute at the University of Southern
Denmark.

Potential Financial Conflicts of Interest: Consultancies: J. Gerstoft
(Roche, Glaxo, Abbott, Boehringer Ingelheim, Merck Sharp & Dohme,
Swedish-Orphan Drugs); Honoraria: J. Gerstoft (Roche, Glaxo, Abbott,
Boehringer Ingelheim, MSD, Swedish-Orphan Drugs); Grants received:
N. Obel (Roche, Bristol-Meyers Squibb, Merck Sharp & Dohme,
GlaxoSmithKline, Abbott, Boehringer Ingelheim, Janssen-Cilag, Swed-
ish-Orphan Drugs).

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Final approval of the article: N. Lohse, A.-B.E. Hansen, G. Pedersen, G. Kronborg, J. Gerstoft, H.T. Sørensen, M. Væth, N. Obel.
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Obtaining of funding: N. Obel.
Administrative, technical, or logistic support: N. Lohse, N. Obel.
Collection and assembly of data: N. Lohse, A.-B.E. Hansen, G. Kronborg, J. Gerstoft, N. Obel.
Comorbidity Acquired Before HIV Diagnosis and Mortality in Persons Infected and Uninfected With HIV: A Danish Population-Based Cohort Study

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Background: We aimed to estimate the impact of comorbidity acquired before HIV diagnosis on mortality in individuals infected with HIV.

Methods: This cohort study compared 2 different cohorts. The prospective population-based nationwide observational Danish HIV Cohort Study was used to compare all adults diagnosed with HIV in Denmark from 1997 with a matched general population cohort. Comorbidity history was ascertained from the Danish National Patient Registry and vital statistics obtained from the Danish Civil Registration System. Cox regression was used to estimate the impact of Charlson comorbidity index (CCI) and hepatitis C virus coinfection on mortality, and population attributable risk was used to assess the proportional impact of comorbidity on mortality.

Results: CCI comorbidity was present before HIV diagnosis in 11.3% of 1638 persons with HIV, and in 8.0% of 156,506 persons in the general population. The risk for death in patients with HIV with at least 1 CCI point was 1.84 times higher than in those with no CCI points (adjusted mortality rate ratio, 95% confidence interval: 1.32 to 2.57). The annual risk of dying for patients with HIV vs general population with 0, 1, 2, and 3+ CCI points was 1.70% (1.44 to 2.00) vs 0.27% (0.26 to 0.28), 4.37% (3.01 to 6.32) vs 1.36% (1.26 to 1.47), 8.06% (4.94 to 13.16) vs 2.44% (2.22 to 2.68), and 10.15% (5.08 to 20.30) vs 5.84% (5.19 to 6.58), respectively. Comorbidity acquired before HIV, hepatitis C virus coinfection, and background mortality accounted for 45% of total mortality in the population infected with HIV.

Conclusions: Almost half of deaths in persons diagnosed with HIV in a health care setting with free access to highly active antiretroviral therapy stemmed from factors unrelated to HIV disease.

Key Words: HIV, comorbidity, mortality, cohort study, population based (J Acquir Immune Defic Syndr 2011;57:334–339)

INTRODUCTION

Mortality and morbidity among persons infected with HIV has decreased dramatically since the introduction of highly active antiretroviral therapy in 1996–1997. Despite this progress, mortality remains markedly higher than in uninfected persons. The proportion of all deaths among persons infected with HIV due to non-AIDS and noninfectious causes such as drug-induced toxicity, cardiovascular disease, renal disease, cancer, and HIV-induced morbidity other than AIDS has increased to as much as 46%. Several recent studies have focused on coexisting non-AIDS and noninfectious morbidity as prognostic factors in these patients but the impact of diseases acquired by patients before their HIV diagnosis remains poorly understood, with the exception of hepatitis C virus (HCV) infection. We aimed to estimate the impact of comorbidity acquired before HIV diagnosis on mortality in individuals infected with HIV.

METHODS

Study Type

This matched cohort study investigates the impact of non-HIV comorbidity on all-cause mortality in a cohort of persons infected with HIV compared with a cohort of persons from the general population.

Study Population

Our study population consisted of all persons who were diagnosed with HIV in Denmark between January 1, 1997, and
May 1, 2005; who were at least 16 years old; and who were Danish residents at the time of HIV diagnosis. These inclusion dates allowed for at least 2 years of observation time for all subjects. For each patient with HIV, we sampled general population controls, matched on sex and year and month of birth, who were alive and living in the same municipality as the patient on the patient’s diagnosis date. Denmark has a population of approximately 5.3 million persons living in 270 municipalities. We aimed to sample 99 controls for each individual infected with HIV, but because of a shortage of eligible controls in some municipalities, the mean number of controls per patient in the final general population cohort was 95.5.

Data Sources

Persons with HIV were identified from the Danish HIV Cohort Study (DHCS), which has established an open population-based cohort that includes all persons infected with HIV seen in 8 HIV clinics in Denmark since January 1, 1995.18,19 Denmark provides free, tax-supported health care including treatment for HIV. Antiretroviral drugs and specialist treatment are available exclusively through the HIV clinics, and persons diagnosed with HIV are referred directly to one of these clinics for care and treatment. It is estimated that more than 99% of patients accept this offer, so the cohort is virtually complete regarding incident cases of HIV in Denmark. The annually updated data of DHCS include antiretroviral treatment, development of AIDS-defining illnesses, CD4+ cell counts, HIV RNA [viral load (VL)], and deaths, in addition to sex, age, ethnicity, most likely mode of infection, HCV coinfection at time of diagnosis, and preexisting AIDS-defining illness.18 General population control subjects were sampled from the Danish Civil Registration System (DCRS), which has maintained information on all Danish residents since 1968, using unique personal identifiers (CPR numbers).20 The DCRS records date of birth, sex, place of residence, date of migration, and date of death.20 Its database is updated within a week of a person’s birth, address change, death, or emigration. Comorbidity before an HIV diagnosis was assessed both for persons with HIV and for general population controls through the Danish National Patient Registry (DNPR). This registry, established in 1977, covers all Danish hospitals and records all hospital admissions, diagnoses, and, since 1995, outpatient and emergency visits.21 The DNPR covers private and public hospitals. We used CPR numbers to link data between these registries.

Study Variables

Outcome Variable

Death from any cause was obtained through the DCRS.

Main Explanatory Variables

We computed a Charlson comorbidity index (CCI) score for all study subjects based on their complete hospital history in the DNPR. The CCI22 was developed to classify comorbid conditions that alter the risk for 1-year mortality after hospitalization in longitudinal studies. The CCI has been adapted and validated for use with hospital discharge data in International Classification of Diseases databases for the prediction of short- and long-term mortality.23 The following 19 disease categories are included in the CCI: myocardial infarction (1 point), congestive heart failure (1 point), peripheral vascular disease (1 point), cerebrovascular disease (1 point), dementia (1 point), chronic pulmonary disease (1 point), connective tissue disease (1 point), ulcer disease (1 point), mild liver disease (1 point), diabetes (1 point), hemiplegia (2 points), moderate to severe renal disease (2 points), diabetes with end-organ failure (2 points), any tumor (2 points), leukemia (2 points), lymphoma (2 points), moderate to severe liver disease (3 points), metastatic solid tumor (6 points), and AIDS (6 points). A CCI score was calculated for each patient infected with HIV as of the date of HIV diagnosis. For each patient’s general population control, we computed a CCI score as of the same date. The disease category “AIDS” was excluded in our computations because it was closely related to the main exposure in our study.

HCV infection is not included in the CCI score but is common in individuals infected with HIV due to shared routes of transmission. We thus included it as a study variable, assuming that HCV infection occurred at the same time or before HIV acquisition. Patients with at least 1 positive HCV antibody test or a positive HCV RNA test were considered HCV-positive; others were considered HCV-negative. HCV status was available for 98.0% of study patients infected with HIV. We did not have individual data on HCV infection among the general population controls mainly because HCV infection is often asymptomatic and therefore not diagnosed; also, it was not a separate diagnosis in the International Classification of Diseases, 8th Revision, which was used in the DNPR until 1993. As the estimated prevalence of HCV in Denmark is just 3 per 1000,24 all individuals in the general population cohort were considered HCV-negative in our analyses.

Other Explanatory Variables

Information on the sex (male/female), age (in years), ethnicity (white/yes/no), mode of infection (injecting drug use yes/no, men who have sex with men yes/no, heterosexual yes/no, blood transfusion yes/no, hemophilia yes/no, other yes/no, unknown yes/no), CD4+ T-cell count at time of diagnosis (median, interquartile range), VL at time of diagnosis (median, interquartile range), and HCV serostatus of the subjects was obtained from the DHCS.

Statistical Analyses

Basal Characteristics

Chi-square test was used to examine differences in distribution of characteristics between persons with HIV and the general population.

Survival Analysis

We linked all study subjects to the DCRS and computed observation times from their date of HIV diagnosis until death from any cause, migration, or June 1, 2007, whichever came first. We used Cox regression analysis to compute unadjusted hazard ratios as a measure of mortality rate ratios (MRRs) for patients with HIV diagnosed after January 1, 1997, comparing different covariate strata (model A). In addition to CCI (0 points vs ≥1 points), the following covariates were included:

$\text{CCI} = 1\text{point} + 2\text{points} + 3\text{points} + 4\text{points} + 5\text{points} + 6\text{points} + 7\text{points} + 8\text{points} + 9\text{points}$
in the analysis: CD4+ T-cell count at time of diagnosis, defined as the first measurement within 90 days after diagnosis (>200 cells/μL, 50–200 cells/μL, <50 cells/μL, or missing); age at time of diagnosis; history of injecting drug use; white race; sex; and log(VL) at time of diagnosis, defined as the first measurement within 90 days after diagnosis (<4 copies/mL, 4–5 copies/mL, >5 copies/mL, or missing). We estimated the MRRs in a multivariate model including all covariates (model B), and in adjusted models including CCI and one of each of the other covariates successively (models C and D). Model C examined the influence of each covariate on CCI score. Model D examined the influence of CCI score on each of the other covariates. To assess the potential bias introduced by immigrants with previous hospital admissions not registered in the DNPR, we carried out a sensitivity analysis restricting the population to individuals infected with HIV born in Denmark. Another sensitivity analysis excluded lymphoma from the CCI score because some lymphomas might have been AIDS-defining events.

**Interaction Between HIV and Comorbidity Effects**

Mortality rates (MRRs) were computed within 4 CCI strata (0, 1, 2, and ≥3 points), separately for patients and for general population controls. Kaplan–Meier curves were constructed, and log-rank test was used to compare survival within each stratum. For controls, observation time was computed starting from the date of HIV diagnosis of the corresponding HIV-infected person. To estimate the interaction between baseline comorbidity and HIV effects, defined as deviation of MRRs from an additive model,25 we assessed the effect measure modification on MR differences. The interaction between the prognostic effects of HIV and comorbidity was computed as the interaction risk (IAR)25 with a bootstrap 95% confidence interval (CI)26: IAR\textsubscript{pop,x} = (MR\textsubscript{hiv,x} − MR\textsubscript{hiv,0}) − (MR\textsubscript{pop,x} − MR\textsubscript{pop,0}), where IAR\textsubscript{pop,x} is the IAR difference for persons with HIV and CCI = X; MR\textsubscript{hiv,x} and MR\textsubscript{hiv,0} are the MRRs for patients with HIV with CCI = X (X can take the values 1, 2, or 3) and CCI = 0, respectively; and MR\textsubscript{pop,x} and MR\textsubscript{pop,0} are the MRRs for population controls with CCI = X and CCI = 0, respectively. The proportion of excess deaths in persons infected with HIV due to interaction within each comorbidity stratum CCI = X was IAR\textsubscript{pop,x}/(MR\textsubscript{hiv,x} − MR\textsubscript{hiv,0}) = 1 − (MR\textsubscript{pop,x} − MR\textsubscript{pop,0})/(MR\textsubscript{hiv,x} − MR\textsubscript{hiv,0}). A sensitivity analysis restricted the population to persons not coinfected with HCV.

**Population Attributable Risk**

The population attributable risk (PAR) of death due to CCI points in the population infected with HIV was calculated as follows:27 If MR\textsubscript{hiv,0} is the MRR in the population with HIV and MR\textsubscript{hiv,0}, then PAR is (MR\textsubscript{hiv} − MR\textsubscript{hiv,0})/MR\textsubscript{hiv}.

**RESULTS**

Among the 1638 patients with HIV diagnosed from January 1, 1997, to May 1, 2005, we observed 195 deaths in 9350 person-years of follow-up, yielding an MR of 2.09% (95% CI: 1.81 to 2.40) per year. In comparison, the 3676 deaths observed among 156,506 general population control cohort members followed for 939,560 person-years yielded an MR of 0.39% (95% CI: 0.38 to 0.40) per year. Morbidity was present before HIV diagnosis in 354 (21.6%) of 1638 patients with HIV, of whom 185 (11.3%) had acquired at least 1 CCI point and 211 (12.9%) were coinfected with HCV; morbidity was present in 12,531 (8.0%) of 156,506 general population controls (Table 1).

The most common CCI comorbidities were liver disease in 48 (2.9%) patients vs 793 (0.51%) general population controls, lymphoma in 11 (0.67%) patients vs 232 (0.15%) controls, and solid malignant tumors in 22 (1.34%) patients vs 1773 (1.13%) controls. Among 1638 patients with HIV, 155 (9.5%) did not have available CD4+ T-cell count data and 187 (11.4%) did not have available VL data at the time of diagnosis.

**Comorbidity as a Predictor of All-Cause Mortality in Patients With HIV**

The MRR for patients with HIV with at least 1 CCI point compared with patients with HIV with no CCI points was 3.27 (95% CI: 2.38 to 4.50; Table 2, model A). This estimate decreased after adjustment for other prognostic factors for death (Table 2, model C), but the CCI score remained a predictor of death in the fully adjusted model (MRR = 1.84, 95% CI: 1.32 to 2.57; Table 2, Model B). CCI score did not

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the Study Populations</th>
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<tbody>
<tr>
<td>Persons With HIV, n (%)</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>No. persons</td>
</tr>
<tr>
<td>Person-y at risk</td>
</tr>
<tr>
<td>End points (deaths)</td>
</tr>
<tr>
<td>Female sex</td>
</tr>
<tr>
<td>Median (IQR) age at HIV diagnosis (y)</td>
</tr>
<tr>
<td>White race</td>
</tr>
<tr>
<td>Most likely mode of infection</td>
</tr>
<tr>
<td>Heterosexual</td>
</tr>
<tr>
<td>Men who have sex with men</td>
</tr>
<tr>
<td>Injecting drug use</td>
</tr>
<tr>
<td>Blood transfusion</td>
</tr>
<tr>
<td>Hemophilia</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Median (IQR) CD4 at HIV diagnosis (cells/μL)</td>
</tr>
<tr>
<td>Median (IQR) log(VL) at HIV diagnosis (copies/mL)</td>
</tr>
<tr>
<td>HCV positive</td>
</tr>
<tr>
<td>CCI points at HIV diagnosis</td>
</tr>
<tr>
<td>≥3</td>
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<tr>
<td>≥2</td>
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<td>≥1</td>
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*The general population was matched on sex.
†The prevalence of HCV in Denmark is estimated at 0.3%.24
‡Based on estimated prevalence of HCV in Denmark of 0.3%.

CD4, CD4+ T-cell count; IQR, interquartile range; NA, not applicable/available.
substantially affect the MRR of other risk factors known to be associated with death in patients infected with HIV (Table 2, model D). In a sensitivity analysis in which lymphoma diagnoses—some of which are AIDS-defining—were excluded from the CCI score, we obtained similar MRR estimates (1.90, 95% CI: 1.36 to 2.66). In an analysis restricted to patients with HIV of Danish origin (69% of all patients), the effect of CCI score in the fully adjusted model decreased slightly to 1.74 (95% CI: 1.22 to 2.48).

**Interaction Between HIV and Comorbidity Effects on All-Cause Mortality**

MRs for persons with HIV compared with the general population within CCI strata were 1.70 per 100 person-years at risk (95% CI: 1.44 to 2.00) vs 0.27 per 100 person-years at risk (95% CI: 0.26 to 0.28) for CCI = 0; 4.37 (3.01 to 6.32) vs 1.36 (1.26 to 1.47) for CCI = 1; 8.06 (4.94 to 13.16) vs 2.44 (2.22 to 2.68) for CCI = 2; and 10.15 (5.08 to 20.30) vs 5.84 (5.19 to 6.58) for CCI = 3+. Survival curves are depicted in Figure 1. There was a high IAR on rate differences in all CCI strata, indicating sizeable interaction between HIV and comorbidity effects (ie, the joint effects of HIV and comorbidity exceeded the sum of their individual effects on mortality). Compared with patients with no CCI points, 58.8% of the excess deaths in patients with a CCI score of 1, 66.0% of the excess deaths in patients with a CCI score of 2, and 34.1% of the excess deaths in patients with a CCI score of 3 or higher resulted from this interaction (Table 3). Restricting the analyses to the subgroup not coinfected with HCV produced similar IAR estimates in the 3 CCI point strata.

**Mortality Due to Conditions Existing Before HIV Diagnosis**

The PAR of death due to comorbidity acquired before HIV diagnosis was 0.19, that due to HCV coinfection was 0.16, and that due to baseline comorbidity and HCV coinfection combined was 0.32. Thus, 32% of mortality in the Danish population with HIV could be attributed to comorbidity acquired before HIV diagnosis or to HCV coinfection. This is illustrated in Figure 2 as the relative difference between the mortality for all patients (MR = 2.09%)}
underestimation of comorbidity might have happened because of lack of information on diagnoses made in the primary care sector or because of the presence of mild diseases that would go undiagnosed. Relative overestimation of comorbidity might have happened in some high-risk groups later diagnosed with HIV, for example, injecting drug users or men who have sex with men. These groups might be in more frequent contact with the health care system, which would increase the chances of any disease being diagnosed and lead to information bias. In both cases, restricting diagnoses to those causing hospitalization have most likely reduced bias and strengthened our findings rather than weakening them. Another concern is that discharge diagnoses from hospital visits outside Denmark were not captured, so CCI scores could be underestimated in immigrants. However, a sensitivity analysis excluding patients of non-Danish origin only slightly changed the risk estimates for CCI score.

Few studies have looked at deaths due to conditions present before HIV diagnosis. Delpierre et al. found a 3.75 times higher risk for death in persons unemployed at the time of HIV diagnosis. Assuming that comorbidities are associated with unemployment, these results support our findings. HCV coinfection affects mortality both directly and indirectly through family-related risk factors. It is normally diagnosed during the initial screening of patients newly per year, 95% CI: 1.81 to 2.40) and that for HCV-negative patients with no CCI points (MR = 0.28% per year, 95% CI: 0.27 to 0.30) comprised 20% of the latter group, an estimated 45% (2.09 - 1.43 + 0.28/2.09) of total mortality in the population infected with HIV could not be ascribed to HIV itself, nor to toxicity from antiretroviral drugs.

### DISCUSSION

In this population-based study, we found that morbidity acquired before HIV diagnosis was an independent risk factor for death. Almost half of mortality in persons diagnosed with HIV in a health care setting with free access to highly active antiretroviral therapy stemmed from factors unrelated to the HIV disease or associated factors such as toxicity from antiretroviral drugs. Furthermore, comorbidity acquired before HIV diagnosis acted synergistically with HIV as a risk factor for death.

Our study had a number of strengths. First, the population-based setting of DHCS allowed us to minimize selection bias by including all patients infected with HIV residing in Denmark at the time of diagnosis. Second, the DCRS with its complete follow-up allowed us to generate a matched general population comparison cohort and to collect accurate information on time of death and emigration, which further minimized selection bias. Third, the DNPR provided access to information on all diseases treated in a hospital going back almost 20 years before the HIV diagnosis. Thus, information on morbidity before HIV diagnosis was prospectively collected and not influenced by recall bias. Finally, a unique personal identifier allowed us to link all registries.

Our study also had limitations. Persons may be infected with HIV for years before they are diagnosed; thus, a disease registered before HIV diagnosis may actually have been acquired after HIV infection and could be a consequence of HIV rather than a risk factor acquired before HIV. However, we believe this was a minor source of bias. Only diagnoses obtained before the hospital visit at which HIV was diagnosed were used in computing the CCI score, and no points were counted for HIV and/or AIDS diagnosis. The only AIDS-defining illness that may have contributed to the CCI score was lymphoma, and a sensitivity analysis excluding CCI points for lymphoma did not change any estimates or conclusions.

Underestimation of comorbidity might have happened because of lack of information on diagnoses made in the primary care sector or because of the presence of mild diseases that would go undiagnosed. Relative overestimation of comorbidity might have happened in some high-risk groups later diagnosed with HIV, for example, injecting drug users or men who have sex with men. These groups might be in more frequent contact with the health care system, which would increase the chances of any disease being diagnosed and lead to information bias. In both cases, restricting diagnoses to those causing hospitalization have most likely reduced bias and strengthened our findings rather than weakening them. Another concern is that discharge diagnoses from hospital visits outside Denmark were not captured, so CCI scores could be underestimated in immigrants. However, a sensitivity analysis excluding patients of non-Danish origin only slightly changed the risk estimates for CCI score.

![Mortality rates](image)

**FIGURE 2.** Visual presentation of the total mortality in persons with HIV and the relative contribution of background mortality (0.28/2.09 = 13.6%), HCV coinfection and CCI (2.09 - 1.43/2.09 = 31.5%), and HIV (1.43 - 0.28/2.09 = 55%).
diagnosed with HIV and most often assumed to be acquired at the same time as or before HIV through an identical route of transmission. No previous studies have quantified the proportion of mortality attributable to comorbidity acquired before HIV diagnosis.

Part of the excess mortality in persons with comorbidity acquired before HIV diagnosis may be due directly to the comorbidity itself. As we have shown previously for HIV/HCV-coinfected patients, the excess mortality may also be due to lifestyle and other family-related risk factors such as tobacco use, alcohol intake, drug abuse, and risk behaviors. Finally, the interaction between baseline comorbidity and HIV contributed considerably to the excess mortality. This could be purely biological but could also stem from suboptimal treatment occurring when physicians and patients face the complexity of HIV in combination with another serious disease. HCV coinfection did not seem to underlie the observed synergistic effects.

Depending on the distribution of age and mode of infection among persons infected with HIV, we would expect similar findings in other high-income settings. Illness and death in an HIV-infected population with access to up-to-date antiretroviral therapy can be lowered through earlier diagnosis, increased adherence, and optimization of antiretroviral regimens. Recent studies suggest, however, that relying solely on these measures may not suffice as patients get older and acquire age-related and possibly also drug toxicity–related diseases. Prevention of causes of non-AIDS non-infectious death is important but can decrease mortality only to a certain extent. The considerable burden conferred by diseases acquired before HIV diagnosis, found in more than 1 in 5 patients in this study, calls for a comprehensive approach to treatment and care. Involvement of a team of medical specialists is clearly needed. Still, additional mortality reduction may not always be possible.

In conclusion, persons infected with HIV in Denmark have a considerable burden of disease acquired before HIV diagnosis. HIV and its treatment seem to account directly for little more than half of the deaths in this population. In addition, HIV infection seems to increase the impact of comorbidities diagnosed before HIV diagnosis on risk for death. Further studies aiming to identify biological and sociocultural risk factors for comorbidity are required to increase our understanding of the complex interaction between HIV and diseases acquired before HIV.

REFERENCES


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