Publications

The thesis is based on the following articles:


Abbreviations

AIDS: acquired immune deficiency syndrome
ARV: antiretroviral drug
CDC: Centers for Disease Control and Prevention
CD4: cluster of differentiation 4
CD8: cluster of differentiation 8
CI: confidence interval
CMV: cytomegalo virus
CPR number: central person registration number
DHCS: the Danish HIV Cohort Study
HAART: highly active antiretroviral therapy
HCV: hepatitis C virus
HIV: human immunodeficiency virus
HPV: human papilloma virus
IDU: injection drug use
INSTI: integrase strand transfer inhibitor
IQR: interquartile range
IR: incidence rate
IRR: incidence rate ratio
LTFU: loss to follow-up
MR: mortality rate
MRR: mortality rate ratio
MSM: men who have sex with men
NRTI: nucleos(t)ide reverse transcriptase inhibitor
NNRTI: non-nucleoside reverse transcriptase inhibitor
PI: protease inhibitor
PY: person-years
UK: United Kingdom
US: United States of America
VL: viral load
Preface

This thesis was carried out in the period 2011-2014 while I was employed at the Institute of International Health, Immunology and Microbiology at University of Copenhagen and at the Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet. The scientific work is based on data from the Danish HIV Cohort Study and the Antiretroviral Therapy Cohort Collaboration (ART-CC).

I am greatly indebted to Niels Obel and Jan Gerstoft who introduced me to epidemiological research. Their tremendous enthusiasm, guidance and support have always inspired and encouraged me and without them this work would not have been possible. I would also like to give special thanks to Ib Christian Bygbjerg for tireless support, inspiration, motivation and fruitful discussions. Special thanks to Peter Skinhøj for housing me at the Department of Infectious Diseases at Rigshospitalet during the work and for moral support. I would like to thank Gitte Kronborg, Carsten Schade Larsen, Court Pedersen, Gitte Pedersen, Lars Nørregaard Nielsen, Janne Jensen, Alex Laursen and study nurses who tirelessly have collected data for the HIV Cohort Study and for their inputs to the work. I would like to thank my colleagues at the office for inspiration, discussions, motivation and lots of laughs. Thanks to Margaret May, Suzanne Ingle and Jonathan Sterne from ART-CC, to Børge Nordestgaard and Shoaib Afzal from the Copenhagen General Population Study and to Tavs Qvist, Susan Cowan and Christian Graugaard for excellent collaboration. Thanks to Dorte Schiødt for all the help with practical issues. It has been a true privilege to work with the Danish HIV Cohort Study and with so many dedicated and intelligent colleagues.

Marie Helleberg, Copenhagen January 2015
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INTRODUCTION
With the introduction of highly active antiretroviral therapy (HAART) in 1996 the prognosis of HIV-infected individuals has improved tremendously (1;2). In settings with widespread access to HAART, rates of AIDS and death have decreased markedly (3). Now AIDS-related illnesses primarily occur within the first year of HIV diagnosis among individuals who are diagnosed late and have advanced immune deficiency at time of diagnosis (4). Subgroups of patients with poor adherence to care and treatment are also at significantly increased risk of progression to AIDS (5), but in the HIV-infected population as a whole, the disease burden has shifted from AIDS-related to non-AIDS related morbidity (6) and the proportion of deaths caused by non-AIDS-related disease has surpassed the proportion of deaths from AIDS (figure 1) (2;7).

Figure 1: Proportions of deaths caused by AIDS-related and non-AIDS-related disease among HIV-infected individuals in Denmark, 1995-2009

Like in the background population, cardiovascular disease and cancer have become the most common causes of death among HIV-infected individuals (2;7). In this transition the overall disease burden has decreased, but due to the increasing age of the HIV-infected population (figure 2), the incidence of non-AIDS related disease is increasing (8).

All though HAART has resulted in dramatic improvements in the prognosis of HIV-infected individuals there may also be some untoward effects. Prolonged exposure to antiretroviral drugs (ARVs) may be associated with toxicities, which can affect the long-term prognosis (9).

In spite of access to HAART the HIV-infected population still experience increased morbidity and mortality compared to the background population (9). The mechanisms behind this increase in morbidity and mortality have been extensively studied but the relative impact of immune dysfunction and lifestyle for the prognosis of HIV-infected individuals is controversial. Identification of factors associated with poor outcomes and quantification of the relative impact of these factors on the population-level could be of benefit in prioritizing resources and targeting interventions.

Figure 2: Age distribution of the HIV-infected population in Denmark 1995-2013

THE DANISH HIV COHORT STUDY
The Danish HIV Cohort Study, initiated in 1998, is a nationwide study, which include all HIV-patients who have attended care in one of the HIV care centres in Denmark at least once (10). Data from 1995-1997 were collected retrospectively. Due to the population-based, nationwide design, the longitudinal follow-up through more than eighteen years, and the ability to link to national registries, the Danish HIV Cohort Study offers unique opportunities to study the long-term prognosis as well as changes in risk factors and associated outcomes for the HIV-infected population in Denmark since the early era of HAART.

Data are collected systematically, updated yearly and include demographics, AIDS-related events, HAART and co-infections (hepatitis B & C, CMV and toxoplasmosis). Data on height, weight, blood pressure and smoking status are available for the majority of enrolled study subjects. Results of laboratory analyses (haematology, biochemistry, CD4 and CD8 counts) are extracted from electronic databases. Due to the nationwide design with inclusion of all individuals enrolled in HIV clinics in Denmark, results are generalizable to the HIV-infected population in the country, which is in contrast to a number of other cohorts which recruit only certain risk groups or patients who are followed in larger clinics. The Danish 10-digit civil registration number allows linkage to nationwide registries, which ensures complete information on vital status, emigrations, hospital admissions, outpatient visits etc. even for individuals who are lost to follow-up from the HIV clinics. This is a major advantage because individuals
lost to follow-up often have a worse prognosis than those who are retained in care. Thus analyses censoring individuals upon loss to follow-up are prone to be biased. Furthermore the nationwide registries offer the opportunity to compare HIV-infected individuals with matched controls from the background population. Limitations include the somewhat modest number of study participants which makes detailed modelling of data on rare clinical outcomes difficult. Data on behavioural and lifestyle related factors, apart from injection drug use and smoking are not available. However data on education, occupation, income etc. can be obtained through linkage to nationwide registries. The Danish HIV Cohort Study takes part in large multinational collaboration cohort studies (COHERE and ART-CC). Randomized clinical trials are needed to compare efficacy of HAART regimens without the risk of confounding e.g. bias by indication, but observational studies significantly adds to the knowledge gained by these trials, because they often better reflect “the real world” with less selected study populations and less intensive monitoring (11). These factors are likely to influence the estimates of risk of treatment failure, resistance and toxicity. Additionally, observational studies offer data from larger populations with extended follow-up time, enabling detection of rare events.

OBJECTIVES
This thesis is based on observational studies of HIV-infected individuals followed in the Danish HIV Cohort Study. The aim of the work was to examine factors of importance for the long-term prognosis of the HIV-infected population in the HAART era. Specifically, to assess changes of HIV virulence, achievements of HIV care and to study associations between HIV-related and lifestyle-related factors and morbidity and mortality among HIV-infected individuals. An additional study, based on data from a large multinational HIV cohort, ART-CC, aimed to confirm and expand findings of a study from the Danish HIV Cohort.

HIV VIRULENCE IN THE HAART ERA
In untreated HIV-infection both factors related to the virus and the host influence disease progression. The viral load peaks during primary infection and then reaches a rather stable level, or set point, and only increase slightly for several years (12;13). The viral set point is correlated to the rate of CD4 decline and disease progression in untreated individuals and with risk of transmission (14;15). The set point is influenced by a number of factors, including viral replicative fitness and the immune response of the host (16;17). The viral set point has been shown to be heritable through transmission from one individual to another and it is estimated that up to 33% of the variation in viral set point is attributable to the viral genome (18). Factors related to the function of both the innate and the adaptive immune responses are also of importance. Human leukocyte antigen alleles, concordance between human leukocyte antigen alleles in the transmission couple and polymorphisms in toll-like receptor alleles explain some of the variation in viral set point (19).

HIV virulence is defined by the time from infection to development of AIDS and death. The progression of HIV-related disease is halted by treatment, and in the HAART era the virulence of HIV has to be assessed using surrogate markers such as the viral set point and rate of CD4 decline prior to treatment initiation. In two studies from the late HAART era, individuals with primary or early HIV infection were randomized to receive immediate or deferred HAART. Among individuals randomized to the deferred treatment arm surprisingly high rates of HIV progression were observed (20;21). One of these trials was halted prematurely because the progression of HIV was faster than anticipated based on results of previous studies (21). It was speculated that this faster rate of progression could be due to an increase in HIV virulence. The virulence of HIV may evolve over time due to changes in factors which influence selection pressures. During evolution viral strains with intermediate virulence might have had an advantage for onward transmission because of slower disease progression and increased survival of the host resulting in a longer period with potential for onward transmissions (22). Theoretically HAART could affect the evolution of HIV, by exerting selective pressure, favouring transmission of more or less virulent HIV strains (23). With widespread use of HAART the balance might shift such that strains which cause very high viral loads in the early stages of disease may be more likely to be transmitted than strains, which cause low grade vi- raeemia and have a more protracted course. On a population basis this could fuel the epidemic as strains with higher virulence would increase risk of transmission from individuals who are yet undiagnosed and therefore not on treatment. On the other hand it has also been suggested that the use of HAART has diminished the virulence of HIV because individuals with low CD4 counts are treated earlier than those with slow disease progression, thereby the reducing the time where transmission could occur among those infected by more virulent strains (23).

Several studies have assessed changes in HIV virulence over time, but have reached different conclusions. Some studies have shown increases, while others have found decreases or no change in virulence (24-29). There may be several explanations for these conflicting findings. Treatment guidelines have changed over time, favoring earlier treatment initiation. This could confound analyses because of informed censoring or due to a non-linear slope of CD4 decline during progression of untreated HIV-infection (30). The rate of CD4 decline has
been shown to differ between HIV-subtypes (31). Furthermore the prevalence of transmitted drug resistance has changed since 1996 (32,33), and many drug resistance mutations viral affect fitness. Variations in the prevalence of transmitted drug resistance, composition of HIV-subtypes or of demographic characteristics of the HIV-infected population over time may therefore affect results of analyses of changes in virulence. Selection bias in recruitment into cohorts may also confound analyses. This could be the case if there have been changes in the likelihood of including fast progressors into cohorts of individuals with chronic HIV-infection or in inclusion of individuals without symptoms during acute HIV into seroconverter cohorts (34). Analyzing data from the Danish HIV Cohort Study we found no evidence of evolution in HIV virulence in the period 1995 to 2010 [I]. Thus an increase in virulence does not seem to explain the disappointing lack of decline in HIV incidence despite the increase in proportion of HIV-patients who are virally suppressed on HAART; other explanations must be sought. The marked increases in incidences of syphilis, lymphogranuloma venereum and gonorrhea suggests that behavioral factors, e.g. changes in attitudes regarding condomless sex, combined with a rather high proportion of HIV-infected who are unaware of being infected, contribute significantly to the ongoing epidemic (35-38).

An increase in virulence of HIV is unlikely to have a large impact on risk of morbidity in a treated population unless a high virulence would translate into an increase in proportion of individuals who have low CD4 counts at time of diagnosis, as a low nadir CD4 is associated with both short and long-term prognosis (39;40).

**Engagement in HIV care**

A major goal of HIV care is achieve continuous viral suppression in order to prevent progression to AIDS and death and to reduce onward transmission of HIV. In Denmark the health care system is well organized and treatment is free of charge. These factors are likely to explain some of the successes of HIV care. Once HIV patients are enrolled in care the majority succeeds in achieving viral suppression (41). The AIDS-related mortality has declined markedly in Denmark and elsewhere since the introduction of HAART. However, AIDS-related deaths still occur due to late diagnosis of HIV or poor adherence to treatment. An alarmingly high number of people living with HIV are diagnosed late and have severe immune deficiency at time of diagnosis (42). These “late presenters” have a high short-term risk of AIDS and death (42-44). Those who survive the first years of treatment have increased risk of poor immunological recovery and on the long term, increased risk of non-AIDS related morbidity and mortality (45;46).

**HAART effectively reduces risk of transmission from mother to child as well as sexual transmission (47-50). The majority of HIV-infected individuals enrolled in care in Denmark are virally suppressed and the proportion of patients with viral suppression has increased markedly since the pre- and early HAART era (figure 3) (41). It is therefore somewhat disappointing that the incidence of new HIV diagnoses in Denmark has been stable since 1995 (42;42). This may be explained by the presumably rather large proportion of individuals living with HIV who are unaware of being infected. Indeed, if the published estimates of the proportion of HIV-infected people in Europe, who are undiagnosed holds true (51), late/lack of diagnosis formed the largest gap in the continuum of HIV care in Denmark in 2010 (Figure 4). Clearly, efforts are needed to improve timeliness in HIV diagnosis.

In the US Centers for Disease Control and prevention (CDC) have recommended routine HIV screening of all adolescents and adults aged 13-64 upon presentation at a health care setting since 2006 (52). It has been estimated that universal HIV screening is cost-effective in settings where the proportion of undiagnosed HIV is >0.1% (53;54). The proportion with undiagnosed HIV in the general adult population in Denmark is presumably considerably lower than this threshold. Therefore HIV testing strategies, targeted at high risk groups, are needed. Risk groups in the Danish setting include men who have sex with men (MSM), immigrants from high-endemic areas and their partners, expatriates returning from high-endemic areas and injection drug users.
Approximately 45% of HIV-infected individuals in Denmark are MSM (10) and the incidence of new HIV diagnoses has increased since year 2000 in this risk group, while it is stable or decline slightly in other risk groups (figure 5). Many people still consider HIV and homosexuality stigmatising, which may be one of the barriers for HIV testing among MSM (55). Other barriers include inconvenience due to location or opening hours of health facilities, which offer HIV-testing, and anxiety while waiting for the result of the test (56). To address these issues the non-governmental organisation STOP AIDS initiated a community-based project in 2007 targeted at MSM. The project, named Checkpoint, aimed to improve access to and uptake of HIV testing, by reaching the group of MSM who traditionally are less likely to seek the established health care settings. During the first five years of the project, 3% (37) of users were diagnosed with HIV. They accounted for 11% of all MSM newly diagnosed with HIV in Copenhagen in 2008-2012. We could ascertain that 36 of the 37 newly diagnosed men were successfully linked to care, received HAART and achieved viral suppression [II]. During 2012 the project was expanded by implementing a mobile unit for on-site testing at sex venues such as saunas and during community events. This strategy proved to be effective. In 2013 the number of men newly diagnosed with HIV through Checkpoint almost tripled.

The project also offered extended counselling on prevention of sexually transmitted diseases, testing for syphilis and hepatitis B vaccination. The project was cost-effective, according to generally accepted estimates (57;58), and importantly, proved that HIV testing in community-based settings does not hamper linkage to care, as has previously been raised as a considerable concern. The project has now joined forces with Cross Over, a project targeted at immigrants, who may have difficulties in navigating in the Danish health care system. Additional interventions to improve timely diagnosis in this group, which has the highest risk of late presentation, are needed (42).

In Denmark it is mandatory to report new HIV diagnoses to the national surveillance system, but until 2013 these reports were anonymized, thus it has been difficult to assess the proportion that is successfully linked to care soon after diagnosis. Future studies on achievements in linking newly diagnosed HIV-infected individuals to care are warranted.

In the United States (US) lack of health insurance has been identified as a major barrier for engagement in care and the socially marginalized people have disproportionately high rates of loss to follow-up and poor outcomes (59;60). The extended social security system in Denmark may facilitate higher rates of engagement and retention in care. In the Danish setting, the overall risk of loss to follow-up from HIV care is low, but injection drug users and persons of foreign origin are at increased risk of being lost to follow-up [III]. Rates of loss to follow-up are higher among young people, those who do not have severe immune deficiency and those who have not yet initiated HAART. The risk of loss to follow-up decline with time engaged in care. The vast majority of those lost to follow-up return to care and more than half return within the first year after being lost to follow-up (defined as 365 days without contact to HIV care). The CD4 count drops median 50-70 cells per year with absence from care, which is similar to the median rate of CD4 decline among treatment naive HIV-infected individuals [II](61). Mortality rates double after loss to follow-up. Almost a third of those who return to care have CD4<200 cells/µL upon return and mortality rates are markedly increased the first six months following return to care, reflecting that some individuals only return when they are severely ill.

**Figure 4:** The continuum of HIV care in Denmark, 2010

![Figure 4](image)

**Figure 5:** Number of newly diagnosed HIV-patients in Denmark per year in the period 1996-2011

![Figure 5](image)
This finding highlights the importance of interventions to retain patients in care. Qualitative and interventional studies are needed to identify cost-effective interventions that effectively prevent attrition and improve adherence rates.

In studies from other resource-replete setting rates of loss to follow-up have been somewhat higher (62-64). Low socio-economic status and poor education have been associated with increased risk of loss to follow-up (59;65). Inability to determine whether the patients transferred and attended HIV care in other clinics not included in the study may have resulted in overestimation of rates of loss to follow-up in some studies. The Danish HIV Cohort Study has the advantage of including all HIV care centres in Denmark as well as access to data on all viral loads measured in the country. Furthermore, data on vital status on all study participants are available through the civil registration system and thus precise estimates of mortality rates after loss to follow-up can be determined.

GUIDELINES FOR HAART INITIATION
Guidelines for when to initiate HAART have changed over time favouring earlier treatment initiation. Data from observational studies indicating that clinical outcomes are better when treatment is initiated at high CD4 counts are accumulating (66;67), but data from randomized trials with clinical endpoints are lacking. Findings from a randomized study showing that early treatment initiation reduce transmission of HIV in heterosexual discordant couples and reduce morbidity in settings with a high prevalence of tuberculosis were recently published (68). In 2013 WHO changed guidelines and raised the recommended cut-off of CD4 count for when to initiate HAART from <350 to <500 cells/µL. Current US guidelines recommend HAART to all HIV-infected individuals, irrespective of CD4 count. Danish guidelines recommend that treatment is initiated at CD4 <350 cells/µL. Treatment should be initiated at higher CD4 counts in pregnant women and individuals with risk factors for rapid disease progression, and offered to those who wish treatment to reduce the risk of HIV-transmission to their partner (69). The hesitance to recommend HAART to all HIV-infected individuals is partly due to concern about long-term toxicity of ARVs. Inconvenience and costs are other factors that need to be balanced against potential individual health benefits, which have not yet been confirmed in randomized trials.

TOXICITY AND DURABILITY OF HAART
There have been a rapid development of antiretroviral drugs (ARVs) and at present time there are more than 20 drugs approved for the treatment of HIV-1. Newer drugs have somewhat better efficacy and a higher barrier for resistance development, but it is especially the tolerability and simplicity of treatment that has improved significantly.

The first nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) that were developed and used widely in the early HAART era have largely been abandoned due to side effects, such as lipodystrophy, neuropathy, lactic acidosis, anaemia, nausea and vomiting.

The current Danish guidelines, as well as many international guidelines for HIV treatment, recommend first line regimens to include a backbone of two NRTIs and a third ARV, which can be either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a boosted PI or an integrase strand transfer inhibitor (INSTI) (69-71). Most of the modern HAART regimens are well tolerated by the majority of patients. However, the two first line NRTIs, abacavir and tenofovir, have been associated with cardiovascular and renal toxicity, respectively. It is well documented that tenofovir can cause tubular dysfunction and mild to moderate renal impairment, but data on rates of end stage renal disease are limited (72;73). Due to the infrequency of these events, the risk of end-stage renal disease associated with tenofovir cannot be studied in randomized trials, but has been assessed in observational studies. In the Danish HIV Cohort Study we found a substantially increased risk of end-stage renal disease among HIV-infected individuals compared to the background population. There was no association between tenofovir and end-stage renal disease (74) all though a previous study from the same cohort showed associations between tenofovir and reduction in estimated glomerular filtration rate (75). Similar results were found in studies from the D:A:D study group, which included a larger population of HIV-infected individuals in Europe. In these studies tenofovir was associated with mild to moderate renal impairment (76) but no association was found with severe renal failure or end-stage renal disease, which occurred in only 0.4% of the study population (77). Both in the Danish HIV Cohort Study and in the D:A:D study it was found that tenofovir was discontinued in most HIV-patients when the renal function declined. Only few patients who progressed to end-stage renal disease remained on treatment with tenofovir (77;78). After discontinuation of tenofovir, renal function improves in most, but not all patients (76;79).

In 2008 the D:A:D study group published results of a large study showing that recent exposure to abacavir was associated with an almost two-fold increased risk of myocardial infarction both among individuals with a low and those with a high predicted 10-year risk of coronary heart disease (prediction based on traditional risk factors) (80). This study received a lot of attention and several other observational studies followed. Some studies similarly found an association between abacavir and cardiovascular disease (81-83), whereas other did not (84-86). No randomized trial has confirmed this
finding (87). It has been debated whether the discrepancies are caused by confounding (i.e., channelling bias) in the observational studies or if the results of randomized trials are hampered by selected study populations and limited follow-up time. A meta-analysis, conducted by FDA, including 26 randomized trials and 9,868 HIV-infected individuals, found no association between abacavir and myocardial infarction (88). Studies from the Danish HIV Cohort found that abacavir was associated with an approximately two-fold increase in risk of myocardial infarction and cerebrovascular events (83;89). In another unpublished study from the Danish HIV Cohort we found a considerable excess incidence rate of myocardial infarction associated with abacavir among smokers. The incidence rate of myocardial infarction was low among non-smokers, regardless of exposure to abacavir and there was no significant excess risk associated with abacavir among non-smokers (Table 1). These results are in line with findings from the D:A:D study, where the absolute risk associated with abacavir was limited in persons with a low predicted 10-year risk of coronary heart disease (excess IR 1.9 versus 16.6/1,000 PY among individuals with low versus high predicted 10-year risk). In the Danish HIV Cohort the population attributable risk of myocardial infarction associated with abacavir was only 3% in absence of smoking, whereas the population attributable risk associated with smoking among HIV-infected individuals on any HAART regimen was 72%.

It is not only the NRTIs which have been associated with toxicity. Cumulative exposure to PIIs has been associated with cardiovascular disease in observational studies (90). Short term toxicity of NNRTIs includes rash, hypersensitivity reactions and hepatitis (91;92). Efavirenz can cause neurological side-effects both after short- and long-term exposure (93;94). The INSTIs have few side-effects and their use is likely to increase after the introduction of INSTIs that allow once-daily dosing and have a higher barrier for development of resistance (95).

With the availability of ARVs with few side effects, achieving adherence to HAART has become considerably easier. In addition to the improvements in tolerability, the newer HAART regimens offer simplicity with reduced pill burden and once daily dosing. Currently there are four approved single tablet regimens on the European market. It has been shown that simplicity of the HAART regimen is associated with improved adherence, but when the HAART regimen consists of <5 tablets per day, the pill burden does not seem to be associated with rates of virological suppression (96). In a study from the Danish HIV Cohort we found no increase in risk of treatment failure or development of resistance after HIV-infected individuals treated with a single tablet regimen were systematically switched to a multi-tablet regimen for economic reasons (97).

### Table 1: incidence rates and incidence rate ratios of myocardial infarction among HIV patients on combination antiretroviral therapy stratified by smoking status and exposure to abacavir.

<table>
<thead>
<tr>
<th>Events (n)</th>
<th>PY</th>
<th>IR/1000 PY (95%CI)</th>
<th>Excess IR/1000 PY (95% CI)</th>
<th>Unadjusted IRR (95% CI)</th>
<th>Adjusted* IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abacavir, all</td>
<td>19</td>
<td>7,266</td>
<td>2.61 (1.67-4.10)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Abacavir, all</td>
<td>37</td>
<td>5,286</td>
<td>7.00 (5.07-9.66)</td>
<td>4.38 (1.84-6.93)</td>
<td>2.68 (1.54-4.65)</td>
</tr>
<tr>
<td>No abacavir, never-smokers</td>
<td>2</td>
<td>2,403</td>
<td>0.83 (0.21-3.33)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Abacavir, never-smokers</td>
<td>3</td>
<td>2,010</td>
<td>1.49 (0.48-4.63)</td>
<td>0.66 (-1.39-2.71)</td>
<td>1.79 (0.30-10.73)</td>
</tr>
<tr>
<td>No abacavir, previous smokers</td>
<td>6</td>
<td>1,529</td>
<td>3.92 (1.76-8.73)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Abacavir, previous smokers</td>
<td>9</td>
<td>1,057</td>
<td>8.52 (4.43-16.37)</td>
<td>4.59 (-1.79-10.98)</td>
<td>2.17 (0.77-6.10)</td>
</tr>
<tr>
<td>Abacavir, current smokers</td>
<td>25</td>
<td>2,219</td>
<td>11.27 (7.61-16.67)</td>
<td>7.97 (3.14-12.79)</td>
<td>3.41 (1.80-6.94)</td>
</tr>
<tr>
<td>No abacavir, never-smokers</td>
<td>2</td>
<td>2,403</td>
<td>0.83 (0.21-3.33)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Abacavir, never-smokers</td>
<td>3</td>
<td>2,010</td>
<td>1.49 (0.48-4.63)</td>
<td>0.66 (-1.39-2.71)</td>
<td>1.79 (0.30-10.73)</td>
</tr>
<tr>
<td>No abacavir, current smokers</td>
<td>11</td>
<td>3,333</td>
<td>3.30 (1.83-5.96)</td>
<td>2.47 (0.20-4.73)</td>
<td>3.97 (0.88-17.89)</td>
</tr>
<tr>
<td>Abacavir, current smokers</td>
<td>25</td>
<td>2,219</td>
<td>11.27 (7.61-16.67)</td>
<td>10.43 (5.87-15.00)</td>
<td>13.54 (3.21-57.14)</td>
</tr>
</tbody>
</table>

**Abbreviations:** PY: Person-years of follow up; IR: Incidence Rate, IRR: Incidence Rate Ratio, 95% CI: Confidence Interval.

*Adjusted for gender, age (time updated), calendar year of index date and time on antiretroviral therapy.
With the improvements in potency, safety profile and ease of administration of HAART, one could expect that the rate of treatment modifications would decrease. However, previous studies have failed to show increases in the durability of HAART regimens (98;99). These studies have focused on the first year after treatment initiation and analysed time to first switch or discontinuation. In the Danish HIV Cohort Study we analysed rates of first and of multiple treatment modifications in the period 1997 to 2009 [IV]. Similar to previous studies we found that rates of treatment modifications were high the first year after HAART initiation and rates of first treatment modification did not decrease in the study period, while rates of multiple treatment modifications decreased markedly. Reasons for treatment modifications changed during the study period. Switch of treatment due to virological failure was rare in the most recent years. Rates of switch due to toxicity also decreased, while modifications with the aim of treatment simplification increased. Changing trends in the use of different ARVs in the study period were associated with emerging evidence of long-term toxicity and changes in guidelines. These findings have been confirmed in a larger study from the Antiretroviral Cohort Collaboration (100).

Whether high rates of treatment modifications should be considered unfavourable can be debated. Different reasons for switch of ARVs are not of equal importance. Virological failure with development of resistance can have detrimental consequences, while switch to another regimen with equal virological efficacy but fewer side effects rarely causes major problems. Switches to simpler and better tolerated regimens may be advantageous; however multiple treatment modifications might indicate poor efficacy or tolerability of the ARVs. Treatment modifications will often be associated with costs and inconvenience due to the need for additional monitoring after the switch. If the HAART regimen is complex there is also a risk that switch of its components will lead to misunderstanding and errors in treatment.

CLINICAL SIGNIFICANCE OF CD4 CHANGES AFTER HAART
CD4 counts decline during untreated infection, but after initiation of HAART the CD4 count increases towards normal levels in the majority of HIV-infected individuals (101) [VII]. In individuals with low CD4 counts at HAART initiation the increase can continue for more than seven years. The CD4 count is a robust and well-validated marker of risk of AIDS and death in untreated HIV-infected individuals, and it also carries some prognostic information among individuals who are virally suppressed on HAART (102;103). Individuals with CD4 <200 cells/µL at time of HIV diagnosis have an approximately 6-fold higher risk of death the following year compared to those who have higher CD4 counts at time of HIV diagnosis (42). AIDS defining events are rare after prolonged viral suppression (103) and the need for monitoring of CD4 counts in well-treated individuals who are stable on HAART has been questioned (104;105).

Data on associations between low CD4 counts and increased risk of non-AIDS related morbidity and mortality among virally suppressed HIV-patients are accumulating (45;46;108). Several CD4 metrics can predict the risk of adverse outcomes among HIV-infected individuals. While the pre-HAART CD4 count is a strong predictor of risk of AIDS and death during the first year of treatment, CD4 gain and most recent CD4 count are associated with increased risk of cardiovascular, renal and liver disease, cancer and death, and these CD4 metrics are stronger predictors for the long-term prognosis of HIV-infected individuals on HAART [V], (46;106;109;110).

A number of risk factors for poor CD4 gain have been identified. High age and HIV-related factors such as immune activation, low level of HIV-RNA and long duration of immune deficiency prior to HAART are associated with increased risk of poor CD4 gain (106;107).

Figure 6: Crude mortality rates by CD4 gain during the first two years of HAART, stratified by pre-HAART CD4 count (cells/µL).

Whether the associations between poor CD4 gain/low CD4 counts and poor prognosis reflect a causal relationship, is a result of confounding or can be explained by reverse causation is not clear. It is possible that immune deficiency cause inflammation and impaired immune surveillance among those with low CD4 counts which increase the risk of non-AIDS related morbidity, e.g. cardiovascular disease and cancer. The fact that HIV-negative patients with inflammatory diseases and transplant recipients, are at increased risk
of cardiovascular disease and cancer supports the hypothesis of a causal relationship between immune dysfunction and risk of non-AIDS related morbidity (111-114).

Alternatively the associations between poor CD4 gain/low most recent CD4 count and increased morbidity and mortality could be explained by reverse causation due to redistribution of lymphocytes from blood to inflamed tissues or impaired bone marrow and thymic function due to subclinical disease (115;116). The latter hypothesis is supported by the finding that poor CD4 gain is associated with poor prognosis even among individuals who do not have very low CD4 counts at HAART initiation (figure 6). These hypotheses are not mutually exclusive and it is likely that more than one mechanism is at play.

Comorbidities and socioeconomic factors do not seem to confound the estimates of associations between CD4 gain and poor outcome. We found no association between CD4 gain and comorbidity, smoking or income among virally suppressed individuals (table 2) and the estimate of increase in risk of death associated with poor CD4 gain changed only marginally after adjustment for these parameters (MRR 2.3 versus MRR 2.6 for individuals with poor versus adequate CD4 gain).

Table 2: Risk factors for poor CD4 gain

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>IRR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 5 years)</td>
<td>1.03 (1.01-1.06)</td>
</tr>
<tr>
<td>VL (per log₁₀)⁰</td>
<td>0.68 (0.55-0.84)</td>
</tr>
<tr>
<td>CD4 &lt;200 cells/µL⁰</td>
<td>0.57 (0.34-0.95)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.97 (0.41-2.31)</td>
</tr>
<tr>
<td>Co-morbidity</td>
<td>1.04 (0.60-1.80)</td>
</tr>
<tr>
<td>Income (per 10.000 DKK/year)</td>
<td>0.99 (0.98-1.01)</td>
</tr>
</tbody>
</table>

Poor CD4 gain defined as CD4 increase <100 cells/µL the first two years of HAART.
⁰Adjusted for gender, origin and route of transmission
⁰At time of HAART initiation

Intriguingly, parents of HIV-patients with poor CD4 gain have increased mortality compared to parents of HIV-patients with adequate CD4 gain [V] (Figure 7). Socio-economic factors may explain this finding, but the estimate of increase in mortality associated with poor CD4 gain does not change markedly after adjustment for occupation or income (unpublished data) - neither in analyses of HIV patients nor in analyses of their parents.

Genetic factors may influence immune regulation and impact the prognosis of HIV-positive as well as HIV-negative individuals. Polymorphisms in genes coding for interleukin 7 and interleukin 10 have been associated with trajectories of CD4 counts and mortality in the HIV-infected population (117-120) and with excess inflammation and mortality in HIV-negative populations (121;122).

It has previously been shown that lymphocytes infiltrate atherosclerotic plaques as well as malignant tumours. Lymphopenia is often present in HIV-negative patients with unstable angina pectoris, myocardial infarction or heart failure, and low lymphocyte counts.
predict ischemic events and death among patients with acute coronary syndromes (123-125). Lymphocytes infiltrate solid, malignant tumors and a low lymphocyte count prior to chemo- or radiotherapy is associated with disseminated disease and adverse prognosis (126). To test the hypothesis that a significant CD4 decline in virally suppressed HIV-infected individuals could reflect redistribution of lymphocytes from blood to inflamed tissues we examined the risk of cardiovascular disease, cancer and death after CD4 decline. We found that the risk of these outcomes was markedly increased within six month after a significant CD4 decline [VI]. The CD8 count dropped concomitantly with the CD4 count and the CD4:CD8 ratio was constant. These findings suggest that clinicians should be vigilant when HIV-patients experience a marked CD4 decline or drop in lymphocyte count, which is not explained by uncontrolled HIV replication, medicine or known co-morbidities. In these cases examination for occult disease may be needed. Future studies are needed to determine if the association is specific for CD4 decline or if a decline in total lymphocyte counts similarly is associated with poor outcome among HIV-infected as well as uninfected individuals.

COURSE AND CLINICAL SIGNIFICANCE OF CD8 COUNTS
Trajectories of CD4 counts in HIV-infected individuals have been examined in a large number of studies, while the course of CD8 counts during long-term antiretroviral therapy is less well described. In acute HIV infection there is a brief period with lymphopenia, after which, CD8 counts increase markedly (127). CD8 counts remain elevated during untreated infection. All subsets of CD8 cells are elevated, but the proportion of naïve cells is decreased while proportions of highly differentiated and replication senescent cells are increased (128-130). CD4 cell depletion and increases in CD8 counts both contribute to inversion of the CD4:CD8 ratio during untreated HIV-infection (127).

In a study from the Danish HIV Cohort we found that total CD8 counts change only little after HAART initiation and remain elevated at a level approximately 2-fold higher than in the background population even after ten years of HAART [VII]. Total CD8 counts correlate with immune activation and the continuous elevation indicates that immunological perturbations persist even among individuals who are virally suppressed with normalized CD4 count after HAART (131). A low CD4:CD8 ratio in virally suppressed individuals is associated with elevated immune activation, immunosenescence and with increased risk of non-AIDS related morbidity and mortality (129). Whether CD8 counts are associated with clinical outcomes independently of CD4 counts is poorly elucidated. Analysing data from the Danish HIV cohort we found that low CD8 counts at start of HAART are associated with early AIDS-related mortality. Low CD8 counts may reflect advanced HIV-infection with T cell depletion or redistribution of lymphocytes from blood to inflamed tissues. Very high CD8 counts and CD8 cell activation at start of HAART are associated with poor CD4 gain [VII], (132,133) and markedly elevated CD8 counts after long-term HAART are associated with increased non-AIDS related mortality. The CD4 count is a robust predictor of risk of morbidity and mortality among HIV-infected individuals. The association between lower CD4 counts and increased morbidity persists even among individuals with CD4 counts >500 cells/µL (103). Low CD4 counts are associated with increased mortality both before and after HAART, all though the strength of the association diminishes after HAART. In contrast, associations between CD8 counts and mortality are only modest; the association between CD8 counts and mortality rates is U-shaped and change with time on HAART (figure 8). Moderately elevated CD8 counts do not seem to be associated with an adverse prognosis [VII]. The association between very high CD8 counts after long-term HAART and increased mortality may reflect detrimental effects of continuous immune activation, but whether the significant elevation of CD8 counts and the associated non-AIDS-related mortality is attributed to HIV-related factors is not known. Potential associations between elevated CD8 counts, immune activation and cardiovascular disease and cancers in virally suppressed individuals needs to be examined in adequately powered studies with long-term follow-up and clinical endpoints.

Figure 8: Crude mortality rates by CD8 counts, stratified by time after HAART initiation
IMMUNE ACTIVATION AND INFLAMMATION VERSUS LIFESTYLE

HIV-replication in untreated infection causes immune perturbations characterized by inflammation and immune activation with increased T cell turnover and high levels of circulating cytokines and coagulation activation markers (134;135). The immune activation decreases dramatically after initiation of HAART, but in the majority of HIV-infected individuals levels continue to be increased compared to the background population (136). Some data indicate that individuals who initiate HAART during acute HIV-infection have lower levels of inflammation and immune activation after long-term therapy compared to those who initiate treatment during chronic infection (137;138). Co-infections, such as CMV, HBV and HCV may contribute to the increased levels of inflammation markers in the HIV-infected population (139). Lifestyle-related factors, e.g. smoking behaviour may also add to the increase in levels of inflammation markers (140).

High levels of inflammation markers, such as interleukine-6, high sensitivity CRP and D-dimer have been associated with increased mortality in the HIV-positive as well as the HIV-negative population (141;142). Immune activation correlates with HIV viral load and risk of AIDS (143), but associations with risk of non-AIDS related clinical endpoints are not well defined. The perturbations of T cell subsets observed in HIV-infection have similarities to those seen in the elderly HIV-negative population and it has been claimed that immune activation is associated with accelerated aging (144), but there is a lack of clinical evidence to support this hypothesis. All though cardiovascular disease, liver disease, renal impairment and cancer occur at higher rates among HIV-infected individuals on HAART than in the background population, there are no clinical data confirming the hypothesis that this increased disease burden is caused by HIV-induced immune activation and inflammation. It is possible that lifestyle and behavioural factors, such as smoking, alcohol and substance abuse, poor diet, lack of exercise and other risk taking behaviour, cause a substantial part of the excess morbidity in HIV-infected population.

Based on the hypothesis that inflammation and immune activation explain the increased risk of non-AIDS related morbidity and mortality among HIV-infected individuals, treatment intensification and immune modulating drugs have been suggested as potential approaches to improve the prognosis of HIV-patients. However, so far studies have failed to show clinical benefit of these interventions (145-148). Efforts aiming at reducing the risk of cardiovascular disease, cancer and liver-related disease, by addressing unhealthy lifestyle-associated factors (e.g. smoking, alcohol and substance abuse), may have a larger impact (149).

HIV-INFECTION AND CARDIOVASCULAR DISEASE

Uncontrolled HIV-replication prior to HAART initiation or during treatment interruptions amplifies proatherogenic mechanisms and is associated with increased risk of cardiovascular disease (150-152). The incidence of cardiovascular disease among HIV-infected individuals on HAART is approximately two-fold higher than in the background population (153), (Figure 9A), but the estimate is lower when controlling for traditional risk factors (154). Cross sectional studies with surrogate endpoints have suggested that HIV-induced immune activation and inflammation are asso-

Figure 9: Kaplan-Meier curves showing cumulative probability of myocardial infarction among HIV-infected individuals compared to a matched control group from the background population, overall (A) and stratified by smoking status (B)

A

Black: HIV-infected individuals; grey: background population

B

Black: HIV-infected individuals; grey: background population;
Full line: ever smokers, broken line: never smokers
ciated with risk of cardiovascular disease, even among individuals who are virally suppressed on HAART (155;156). It has been argued that inflammation and immune activation causes accelerated aging in HIV-infected individuals (144). In a study from the D:A:D study group, the increase in risk of cardiovascular disease among HIV-infected individuals did not seem to be explained by accelerated aging (157). In another study from the same group immune deficiency was not found to be an important risk factor for cardiovascular disease either (158). Other potential explanations for the increased risk of cardiovascular disease in the HIV-infected population include toxicity of HAART and higher prevalence of unfavourable lifestyle-related factors such as smoking and substance abuse, e.g. cocaine. Dyslipidaemia is common among individuals on HAART and is a well-recognized risk factor among HIV-positive as well as HIV-negative persons. As described above, cumulative exposure to PIs and current exposure to abacavir have been associated with risk of cardiovascular disease (80;90). Even though exposure to efavirenz can cause increased levels of cholesterol, there is no evidence that efavirenz is associated with cardiovascular disease.

In the HIV-negative population, smoking is one of the strongest risk factors for cardiovascular disease. We estimated the impact of smoking on risk of myocardial infarction in the HIV-infected population in Denmark (159). As expected, smoking was strongly associated with risk of myocardial infarction and the population attributable fraction of myocardial infarctions associated with smoking was 72%. Strikingly, non-smoking HIV-infected individuals did not have higher risk of myocardial infarction than matched controls from the background population (IRR 1.01 (95%CI 0.40-2.54), Figure 9B), arguing against a major role for HIV-induced inflammation in absence of smoking. On the other hand the risk associated with current smoking was larger among HIV-infected individuals compared to the background population (IRR 6.15 (95% CI: 3.04-12.44) versus 2.18 (95% CI: 1.41-3.38)). We were not able to determine if this higher risk associated with smoking was explained by differences in amount of smoking or by interactions between smoking and HIV. The association between smoking and risk of myocardial infarction may not be entirely causal. It is likely that smoking is associated with other unhealthy behaviours, (i.e. use of cocaine) which may confound the observed association.

HIV-INFECTION AND CANCER

Untreated HIV-infected individuals with low CD4 counts are at high risk of developing certain types of cancer. Kaposi’s Sarcoma, non-Hodgkin lymphoma, primary CNS lymphoma and cervical cancer are AIDS-defining, but other types of cancer, such as anal cancer also occur at a substantially higher rate among HIV-infected individuals compared to the background population. Cancer incidences among HIV-infected individuals have changed since the introduction of HAART with a significant decrease in rates of AIDS-related cancers, especially of Kaposi’s sarcoma and non-Hodgkin lymphoma (160;161), and an increase in rates of non-AIDS-related cancers (162). The latter can presumably be attributed to increasing age of the HIV-infected population (163). In settings with widespread access to HAART the overall risk of cancer is two-three fold higher in HIV-infected compared to HIV-negative persons [VIII]. While the risk of AIDS-defining cancers are strongly associated with viral replication and immune deficiency (164;165), the impact of immune dysfunction on risk of non-AIDS-defining cancer among individuals on HAART has been debated (162;166). Among well treated HIV-infected individuals lifestyle related factors, such as smoking, may play an important role.

The classification of cancers as AIDS-defining or non-AIDS defining is somewhat arbitrary, and it may be more relevant from a biological point of view to classify cancers as infection-related or infection-unrelated. HIV-infected individuals are at significantly increased risk of cancers related to viral infections [VIII], (167;168). The risks of cervical and anal cancers are substantially higher in this population (169-171). Behavioural factors as well as immune deficiency may contribute to this increase in risk. Both cancers are strongly associated with infection with human papilloma virus (HPV). Low CD4 counts are associated with increased incidence and reduced clearance rates of HPV infection. Our finding that the incidence of anal cancer is significantly higher among HIV-infected MSM compared to HIV-infected heterosexual men points towards an important impact of behavioural factors (170;172). It has previously been shown that risks of both anal and cervical cancer are increased among smokers (173;174). More frequent screening of the HIV-infected population could potentially contribute to higher rates of diagnoses in this group. However, in a study from the Danish HIV Cohort we found that few HIV-infected women were adherent to the HIV-specific guidelines for screening for cervical cancer and HIV-infected women were not screened more often than the background population (175). Lung cancer and head-and-neck cancers are among the most common non-AIDS-related cancers in the HIV-infected population and their incidences are two-three fold higher than in the background population. Parents of HIV-infected individuals also have higher risk of lung cancer and head-and-neck cancer compared to parents of HIV-negative individuals (176;177), and although it cannot be ruled out that genetic factors explain the association, this finding indicate that socioeconomic and behavioural factors are of importance for the high risk of these cancers in the HIV-infected population. Both lung cancer and head-and-neck cancers are
strongly related to smoking. Some types of head and neck cancers are related to HPV infection (178), which may explain why low CD4 counts have been associated with increased risk of these cancers. Several studies have shown that the risk of lung cancer is increased among HIV-infected individuals even after controlling for smoking exposure. However, whether there is an association between low CD4 counts and risk of lung cancer is not clear (179).

In the Danish HIV Cohort study we found that for cancers overall the attributable fraction associated with smoking is considerable (approximately 27%) [VIII]. The increase in risk of cancer associated with smoking is larger among HIV-infected individuals compared to the background population [VIII]. The likely explanation is that HIV-infected individuals start smoking at a younger age and smoke more, but based on current data it cannot be ruled out that smoking and HIV-induced immune dysfunction have synergistic untoward effects.

Cancers which are not related to infections or smoking, do not seem to occur at higher rates in the HIV-infected population [VIII](180) and the risk of some hormone-dependent cancers, such as breast, uterine corpus and prostate cancer, may even be lower among HIV-infected individuals compared to the background population (181;182).

HIV replication is associated with increased risk of AIDS defining cancers, but not of non-AIDS defining cancers (164). Whether inflammation can increase the risk of cancer is debated. Biomarkers of inflammation have been associated with risk of cancer in HIV-positive as well as HIV-negative populations (183;184), but evidence of a causal relationship is lacking and the associations may be explained by confounding or reverse causation. In the SMART study, which was a randomized study of treatment interruptions versus continuous HAART, no association between treatment interruption and risk of non-AIDS cancer was found (164), which argues against a causal relationship between inflammation and risk of cancer. The association between immune activation and lymphoma in HIV-infection as well as in autoimmune disease is well documented (185-187). Immune deficiency seems to be an important risk factor for development of at least some types of cancer. The CD4 count is a strong predictor of risk of cancers related to viral infections and head-and-neck cancer (165), but may not be associated with other cancers that are not related to infections. As in any observational study there is risk of confounding in analyses of associations between low CD4 counts and risk of cancer. To disentangle the impact of immune deficiency on risk of cancer among HIV-infected individuals, standardized incidence rates of cancer among HIV-infected individuals have been compared to those of transplant recipients who are immunosuppressed, but differ in regard to lifestyle from the HIV-infected population (188). In these studies the risks of cancers related to infections and of lung cancer were increased in both groups. Similar results were found in another study examining the risk associated with immune deficiency in an HIV-negative population, where SIRs of cancers were compared in a population of renal transplant patients before renal replacement therapy, during dialysis and after transplantation (189). This study showed increases in relative risk of cancers related to viral infections as well as of lung cancer after transplantation.

In conclusion, there is evidence that behaviour and lifestyle have a significant impact on risk of cancer in the HIV-infected population. Immune deficiency continues to play a role for some cancer types, even in the era of HAART. The effects of inflammation and immune activation on cancers that are not related to infections seem to be limited, and the impact of these factors on the overall burden of cancer in the HIV-infected population on HAART may be smaller than the impact of factors related to lifestyle.

THE IMPACT OF SMOKING ON MORTALITY AMONG HIV-INFECTED INDIVIDUALS

The mortality of HIV-infected individuals is high the first year after HIV diagnosis. The majority of early deaths are AIDS-related and occur among individuals who are diagnosed late with advanced immune deficiency (190). More than one year after HIV-diagnosis the mortality and proportion of AIDS related deaths decrease significantly and cardiovascular disease and cancer are the most common causes of death in HIV-infected individuals who are not injection drug users (2,7;190). The life expectancy of well-treated HIV-infected individuals without alcohol or substance abuse is similar to that of the background population (191). The majority of HIV-patients in Denmark are on HAART and virally suppressed (41), and thus risk factors that have the largest impact among HIV-negative individuals may also be the most important risk factors in the HIV-positive population. It is estimated that smoking is the leading risk factor for disease burden in the western world and that tobacco kills nearly 6 million people each year (192). The prevalence of smoking is considerably higher among HIV-infected individuals compared to the background population. Almost 50% of the HIV infected population in Denmark are current smokers compared to approximately 25% in the background population (193).

We assessed the impact of smoking among HIV-infected individuals in the Danish HIV Cohort Study [IX]. The study population was followed from the latest of date of assessment of smoking status or one year after HIV diagnosis. Unsurprisingly we found that smoking was associated with markedly increased mortality and that current smokers had higher mortality than previous smokers. These findings are similar to previous studies conducted in the HAART era (194-
197). However, the striking finding of the study was that smoking was a stronger risk factor for death than both a history of AIDS (MRR 1.5 (95%CI 1.1-2.0) versus 4.4 (95%CI 3.0-6.7)) and baseline CD4 <200 cells/µL (MRR 2.9 (95%CI 2.0-4.3)) and that the loss of life years associated with smoking was larger than that associated with HIV-related factors (12 versus 5 years). We estimated that HIV-infected smokers loose approximately 12 years of life in association with smoking. The corresponding number in the Danish background population has been estimated to be approximately 5 years for moderate smokers and 9 years for heavy smokers (198). To assess the impact of smoking on mortality not only on the individual level, but also on the population level we estimated the population attributable risk and found that approximately 60% of deaths in the non-IDU HIV-infected population with long-term engagement in care was associated with smoking. This estimate was almost twice as high as in the background population [IX].

The finding that smoking seems to be associated with a larger loss of life years than HIV-related factors is not specific for the HIV-infected population in Denmark. We reached the same conclusion when studying a much larger population of HIV-infected individuals enrolled in the multinational Antiretroviral Therapy Cohort Collaboration study (ART-CC) [X]. In this study we found that smoking was associated with a loss of approximately 8 years of life. Data on current versus previous smoking were not available for all participating cohorts which may explain the somewhat lower estimate of loss of life years associated with smoking. Differences in methods for estimating life expectancies between the two studies and differences in lag time between HIV diagnosis and ascertainment of smoking status between the study populations may also explain why estimates of loss of life years varied between the two studies.

HIV-infected current smokers have excess risk of death from cardiovascular disease, cancer, liver disease and AIDS [IX, X]. Both the relative risk and the absolute risk associated with smoking are larger for non-AIDS related compared to AIDS related deaths.

The relative risk of death associated with smoking is larger for HIV-infected compared to HIV-negative individuals. Again we are unable to determine if this increase in relative risk is due to detrimental interactions between HIV-infection and smoking or if it is related to differences in quantitative exposure to smoking. The relatively high estimate of loss of life years in the HIV-infected population may indicate that the former hypothesis is true. However, the risk of death associated with smoking does not differ between individuals with a history of AIDS or baseline CD4 <200 compared to those with no history of AIDS and baseline CD4 ≥200 cells/µL, arguing against a hypothesis of interactions between smoking and immune deficiency [X].

The excess risk of death associated with smoking increase markedly with age. Thus as the HIV-infected population is aging we can expect an increase in smoking-related mortality the coming years unless interventions to argument smoking cessation are prioritized. In the background population a causal relationship between smoking and increased morbidity and mortality is assumed. Causality cannot be inferred on the basis of observational studies, and the associations between smoking and mortality, we observed in the two studies of HIV-infected populations, are unlikely to be explained by the causal effects of smoking alone. It is most probable that smoking is associated with other unhealthy lifestyle-related factors. However, this does not change the conclusion that lifestyle seems to have a major impact on the prognosis of HIV-infected individuals with long-term engagement in care, and that the impact of lifestyle is likely to be larger than that of residual HIV-induced immune activation and inflammation in a well-treated population.

Conclusions and perspectives

In the era of HAART the morbidity and mortality among HIV-infected individuals has decreased tremendously but is still increased compared to the background population. The relatively large proportion of HIV-infected individuals who are diagnosed late and those with poor adherence to treatment are at high risk of opportunistic infections and death. Thus interventions to improve timely HIV-diagnosis should be prioritized. In Denmark the majority of HIV-patients are retained in care and adherent to treatment, but there are subgroups, mainly among immigrants and drug users, with high rates of loss to follow-up. Innovative strategies to reach these individuals are needed. The majority of HIV-infected individuals engaged in care are well-treated and their life expectancy approaches that of the background population. However the incidence of non-AIDS related morbidity, primarily cardiovascular disease and cancer, is higher than in the background population. While a large number of studies have shown that markers of inflammation are elevated and that levels of immune activation are increased even after long-term treatment, the clinical implications of these findings are not clear. Lifestyle-related factors seem to explain a large part of the increase in morbidity among HIV-infected individuals. The present work shows that in the treated HIV-infected population the attributable fractions of myocardial infarctions, cancers and death associated with smoking are substantial. This raises the question of whether interventions to facilitate cessation of smoking and other unhealthy lifestyle-related risks should be integrated in HIV-care.
Summary

The prognosis of HIV-infected individuals has improved tremendously after the introduction of highly active antiretroviral therapy (HAART), but morbidity and mortality is still increased in this group compared to the background population. Both factors related to HIV care, HAART, immunological recovery and lifestyle are of importance for the prognosis of HIV-infected individuals. Late diagnosis is associated with a high risk of death within the following year, but while there have been significant improvements in efficacy, simplicity and tolerability of HAART, there have been very limited progress regarding reducing rates of late diagnosis of HIV. However, community-based testing facilities introduced in recent years may change this trend. Generally most HIV-patients in Denmark attend regular follow-up and are well treated, but there are some subgroups such as injection drug users and immigrants who have higher rates of loss to follow-up from HIV care. Mortality rates are significantly increased among those who have not attended follow-up for more than a year.

All though HAART has proven very efficacious in reverting immune deficiency, the T cell distribution and function may not normalize completely despite several years of treatment. The CD4 count is a strong predictor of risk of AIDS and death in untreated HIV-infected individuals. Among HIV-patients, who are virally suppressed on HAART and have little risk of developing AIDS-related disease, low CD4 counts are associated with increased risk of non-AIDS-related morbidity such as cardiovascular disease and cancer. The majority of patients have an adequate immune response to HAART, but, for reasons that are not fully understood, some patients have poor CD4 gain despite effective HAART. Poor CD4 gain after HAART is associated with increased morbidity and mortality even in individuals who are not severely immune deficient at HAART initiation. Genetic traits may influence the immunological recovery.

A marked drop in CD4 count is associated with a substantial increase in morbidity and mortality the following six months and may be a marker of undiagnosed illness. While the CD4 count normalize in the majority of HIV patients after long-term HAART, the CD8 count continues to be elevated. Low CD8 counts at HAART initiation are associated with increased risk of early HIV-related mortality. Very high CD8 counts are associated with poor CD4 gain and with increased long-term non-AIDS related mortality. CD8 counts that are moderately elevated compared to levels in the background population, may reflect immune activation, but do not seem to be associated with an adverse prognosis.

With improved survival and aging of the HIV infected population, lifestyle and co-morbidities have become of increasing importance for the life expectancy of HIV infected individuals. There is a well-documented increased risk of cardiovascular disease and cancer among HIV-infected individuals. Immune dysfunction despite HAART may play a role, but lifestyle-related factors, such as smoking account for a significant part of the increased risk. Among individuals with long-term engagement in care the loss of life years associated with smoking and accompanying lifestyle-related factors seems to be larger than that associated with HIV-related factors.

In conclusion multiple factors are of importance for the prognosis of HIV-patients. To achieve optimal outcome for the HIV-infected population success is needed in all stages of HIV care from timely diagnosis to retention in care and provision of effective and well-tolerated treatment before significant immune dysfunction ensues. In settings where the majority of HIV-infected individuals are well treated the impact of lifestyle-related factors may have surpassed that of factors related to the HIV-infection. A multifaceted strategy addressing both HIV-specific and lifestyle-related factors is needed to further improve the outcome for the HIV-infected population.
**Dansk resumé**

Prognosen for HIV smittede er bedret betydeligt efter indførelsen af kombinations antiretroviral behandling (HAART), men sygelighed og dødelighed er stadig højere blandt HIV patienter end i baggrundsbefolkningen. Faktorer relateret til organiseringen af behandling af HIV smittede, HAART, immunologisk rekonstitu- 

efion efter start på HAART og livsstil har betydning for prognosen for HIV smittede. Sen diagnose er associeret med høj dødelighed det følgende år, men mens der er sket betydelige forbedringer hvad angår effekt, bivirk-

ninger og enkelhed af HAART, har der været en man-
gel på fremskridt med hensyn til at mindske andelen af 

HIV smittede, der bliver diagnosticeret sent. Indenfor 

den seneste år er der startet projekter med tilbud om 

HIV-testing udenfor det etablerede sundhedsstelsel.

De fleste HIV patienter i Danmark følger de regelmæs-

sige kontroller og er velbehandelede, man blandt nogle 
grupper, især stofmisbrugere og immigranter, er der en 

større andel som udeligner fra kontroller. Dødeligheden 

er betydeligt forstærket blandt dem som ikke har været 

et HIV testet i mere end et år. Immunforsvaret bedres gradvis efter start på HAART, men fordelingen af subtyper af T-cellers og T-cell funktionen normaliseres ikke fuldstændig hos alle 

trods adskilte års behandling. CD4 celleralet er en stærk 

prediktor for risikoen for AIDS og død blandt unbe-

handlede, HIV smittede personer. HIV patienter som er 

viral supraprimerede på behandling har kun meget lille 

risiko for at udvikle AIDS, men dem der har et lavt 

CD4 celleralet trods viral suppression er i øget risiko for 

hjertekarsygdom og cancer. De fleste patienter har et 

adækvat immunrespons på antiretroviral behandling, 

men af grunde som ikke er helt afklarede, er der patienter, 

som ikke opnår sufficiens stigning i CD4 celleralet trods effektiv HAART. Manglende CD4 stigning efter 

HAART er associeret med øget sygelighed og dødelig-

hed, selv blandt personer som ikke er svært immunsup- 

primerede ved behandlingsstart. Genetiske faktorer kan 

måske påvirke det immunologiske behandlingsrespons.

Et markant fald i CD4 tal, trods viral suppression, er 

forbundet med betydeligt øget risiko for sygdom og 

død indenfor seks måneder, og kunne være en markør 

for udiagnosticeret sygdom.

CD4 celleralet normaliseres hos flertallet af HIV pat-

ienter efter langvarig HAART, men CD8 celleralet er 

hos de fleste vedvarende forhøjet. Et lavt CD8 celleralet 

ved start på HAART er associeret med øget risiko for 

tidlig HIV relatert død. Efter lang tids HAART er et 

svært forhøjet CD8 celleralet associeret med øget non-

AIDS relatert dødelighed. CD8 celleralet, der er mode-

rat forhøjede i forhold til baggrundsbefolkningen, kun-

ne reflektere immunaktivering, men synes ikke at være 

associeret med en dårlig prognose.

I takt med den øgede overlevelse og dermed højere 

alter af de HIV-inficerede population har livsstil og 

komorbiditet fået større betydning for den forventede 

overlevelse for den HIV smittede. Det er veldokumenteret 

at HIV patienter har øget risiko for hjertekarsygdom og 

cancer. Immunologisk dysfunktion trods HAART spil-

ler måske en rolle, men livsstilsrelaterede faktorer, som 

rygning, synes at forklare en betydelig del af den øgede 

risiko. Blandt HIV smittede, som har været i behand-

ling i mindst et år, er tabet af leveår som er relateret till 

rygning og assurerede livsstilsfaktorer større end tabet 

af leveår der er relateret til HIV.

Konklusion: For at opnå bedst mulig prognose for den 

HIV smittede population må man have fokus på en 
række faktorer, herunder tidlig diagnose, fri og lige 

adgang til effektive og veltålte antiretroviral stoffer 

for at opstår betydelig immunologisk dysfunktion 

samt sikre komplians til opfølgning og behandling. I 

Danmark og andre vestlige lande hvor hovedparten af 

HIV patienter er velbehandelede, hvad angår HIV, 

synes rygning og relaterede livsstilsfaktorer at have større 

betydning for sygelighed og forventet levealder end 

faktorer relateret til HIV infektionen. En multifacetteret 

strategi, der adresserer både HIV specifikke og livs-

stilsrelaterede faktorer er nødvendig for at forbedre 

prognosen for den HIV smittede population.
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