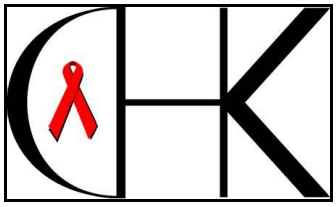
Risk of cardiovascular disease & diabetes mellitus in Danish HIV-infected individuals.

PH.D. THESIS



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This PhD Thesis has been accepted for the defence of the medical PhD by the Faculty of Health Sciences, University of Southern Denmark. The defence will take place June 18, 2014 at Odense University Hospital, WP. 15, St. Auditorium, Denmark

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PREFACE:

The scientific work underlying this thesis was conducted between the summer 2006 and February 2014; yet, consisted of shorter and longer periods of work separated by clinical work and maternity leave with my three boys. Still, the main part of the work was performed between 2010-2014 during my employment as PhD student at the Department of Infectious Diseases, Odense University Hospital and Clinical Institute, University of Southern Denmark.

First I want to express my deepest gratitude to my two supervisors Court Pedersen and Niels Obel for their huge support, optimism and enthusiasm, exceptional expertise and continuous inspiration.

As I started to work with Niels Obel already shortly after my graduation as MD, my research skills were limited. Niels Obel thus introduced me to research, clinical epidemiology, data-managing and statistics. During these years, he has had a tremendous patience with me and continuously passed on an impressive energy and joy with the work. To this date, he still keeps on inspiring me and challenging me both personally and scientifically.

Court Pedersen has with his large experience and great insight in the HIV area been able to inspire and motivate me. I am grateful for his guidance and constructive criticism, his constant support and encouragement in the day to day environment, his great help with the logistics and economically problems, and his guidance when choosing the academically paths in an often complicated and unknown environment.

Furthermore, I am immensely grateful to Jan Gerstoft, who constantly has improved the quality of my work. His scientific insight, impressive knowledge and expertise in the HIV area and ability to focus on the clinical relevance of the results has, with no doubt increased the scientific level of the research. His support has indeed been highly appreciated and his comments highly rated.

I would like to express my gratitude to all co-authors for their assistance an expertise: Gitte Kronborg, Carsten Schade Larsen, Gitte Pedersen, Janne Jensen, Lars Omland, Elisabeth R Mathiesen, Shoaib Afzal and Børge G Nordestgaard.

Thanks to all collaborators and centres in the Danish HIV Cohort Study for making this thesis possible. And not least thanks to the research nurses in Odense (Lene, Nete and Anita) who have always been ready to lend a hand.

To the previous and current colleagues at the PhD office in Odense (Belinda, Dennis, Michala, Stig and Mai) and in Copenhagen (Lars, Frederik, Casper, Rebecca, Marie, Kirsten, Frederikke and Tavs) I want to express my gratitude for inspiring discussions, an ever welcoming environment when entering the research office at Rigshospitalet and importantly a great working atmosphere spiced with large amounts of irony.

Lastly, I want to thank family and friends for putting up with me, and to my husband Christian and our three boys (Emil, Peter and Carl Theodor) for being in my life.

Line Dahlerup Rasmussen, February 2014.

PUBLICATIONS

This thesis is based on the following papers:

I. Mortality after myocardial infarction in HIV-infected patients who have initiated HAART.
Rasmussen LD, Gerstoft J, Kronborg G, Larsen CS, Pedersen G, Obel N. AIDS 2007, 21: 873-5

II. Risk of myocardial infarction in parents of HIV-infected individuals: A population-based Cohort Study.
Rasmussen LD, Omland L, Pedersen C, Gerstoft J, Kronborg G, Jensen J, Obel N.
BMC Infect Dis. 2010; 10: 169

III. Risk of Diabetes Mellitus in Persons with and without HIV; A Danish Nationwide Population-Based Cohort Study. Rasmussen LD, Mathiesen ER, Kronborg G, Pedersen C, Gerstoft J, Obel N.
PLoS One. 2012; 7: e44575. Epub 2012.

IV. Myocardial infarction among Danish HIV-infected individuals: Population attributable fractions associated with smoking and abacavir. Rasmussen LD*, Helleberg M*, Afzal S, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Nordestgaard BG, Obel N.
*Shared first authorship - Submitted at Antiretroviral Therapy January 2014.

ABBREVIATIONS

ACS: Acute coronary syndrome

AIDS: Acquired immunodeficiency syndrome

aIRR: adjusted Incidence rate ratio

aMRR: adjusted Mortality rate ratio

aMRR: adjusted Mortality rate ratio

aOR: adjusted Odds ratio

ASD: Adult Spectrum of Disease study

ATC: Anatomical therapeutic chemical classification

CAC: Coronary artery calcium score

CAD: Coronary artery disease

CDC: Centre for Disease Control and prevention

CD4: Cluster of differentiation 4

CHD: Coronary heart disease

CVD: Cardiovascular disease

D:A:D: Data collection on Adverse effects of antiretroviral Drugs

DCRS: Danish Civil Registration System

DHCS: Danish HIV Cohort Study

DM: Diabetes mellitus

FDA: American Food and Drug Association

FMD: Flow-mediated vasodilation

FHDH: French Hospital Database on HIV

GLUT-4: Glucose transporter 4

HAART: Highly active antiretroviral therapy

HIV: Human immunodeficiency virus

HCV: Hepatitis c virus

HDL: High density lipoprotein

HOPS: HIV Outpatient Study (HOPS)

IDU: Injection drug use

IMT: Intima-media thickness

IR: Incidence rate

IRR: Incidence rate ratio

IQR: Interquartile range

LDL: Low density lipoprotein

MACS: the Multicenter AIDS Cohort Study

MI: Myocardial infarction

MR: Mortality rate

MRR: Mortality rate ratio

MSM: Men who have sex with men

NNRTI: Non-nucleoside reverse transcriptase inhibitor

NRTI: Nucleoside reverse transcriptase inhibitor

OR: Odds ratio

PAF: Population attributable fraction

PAR: Population attributable risk

PI: Protease inhibitor

PR: Prevalence ratio

PYR or PY: Person-years at risk

RAMQ: Canadian Administrative databases

RR: Relative risk

RCT: Randomized controlled trial

SNP: Single nucleotide polymorphism

TC: Total cholesterol

TG: Triglyceride

VL: Viral load (HIV RNA)

TABLE OF CONTENT

INTRODUCTION

ANTIRETROVIRAL THERAPY AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

Shortly after the introduction of antiretroviral protease inhibitors (PIs) in 1995, treatment of Human Immunodeficiency Virus (HIV) with a combination of three antiretroviral drugs was found to be superior to the initial mono-therapy with nucleoside reverse transcriptase inhibitors (NRTIs), introduced in 1986, and the dual NRTI-therapy, introduced in 1994 [1-6]. From the beginning, it was clear that this new treatment approach marked an important and great step forward, and it rapidly became known as highly active antiretroviral therapy (HAART). Since then, HAART has led to a profound impact on the HIV-associated prognoses with a declining morbidity and mortality associated with Acquired Immunodeficiency Syndrome (AIDS), that generally has led to a notable extension of life expectancy for individuals living with HIV infection [7-12]. However, due to this prolonged survival, HAART-associated toxicities have become increasingly important. Some of the long term adverse effects, mainly the HAART-associated metabolic alteration, have led to major considerations concerning associated risks, why an understanding of the long-term safety of antiretroviral drugs has become increasingly important.

HAART ASSOCIATED METABOLIC TOXICITIES

INSULIN RESISTANCE, ALTERED GLUCOSE TOLLERANCE AND DIABETES MELLITUS

Already in 1997 the American food and drug association (FDA) issued a warning on the diabetogenic effects of PIs [13-16]. Since then, PIs have been associated with risk of glucose alterations including hyperglycaemia, impaired glucose tolerance, peripheral insulin resistance and potentially development of DM [17-24], which altogether seems to be induced by mechanisms including an inhibition of the activity of the glucose transporter 4 (GLUT-4) [25]. The main PIs of focus have been indinavir [26-29] and ritonavir [30-34]. In contrast, less diabetogenic adverse effects have been described for the newer PIs such as amprenavir and atazanavir [33-35]. Exposure to NRTIs, mainly the thymidine analogues stavudine and zidovudine [36-39], and possibly didanosine [38, 40-42] and lamivudine [36], have also been associated with an increased risk of insulin resistance, potentially due to drug induced mitochondrial dysfunction [39, 43]. Despite the extensive literature on metabolic alterations in HIV-infected individuals, no consensus regarding risk of DM has been reached. Prevalence estimates of DM are lower than measures of insulin resistance and impaired glucose tolerance. Moreover, a higher prevalence or risk of DM relative to the general population has been inconsistently reported [24, 44-63].

HYPERLIPIDAEMIA

As with several other infections [64-68], HIV infection has been associated with lipid abnormalities including reductions in high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol (TC), and elevations of triglyceride (TG) [66-70]. After initiation of HAART, TC and LDL but not HDL

seem to increase to pre-HIV levels [68]. Conversely, some antiretroviral drugs and drug combinations seem associated with a more atherogenic profile [22, 68, 71-74]. Notably, PIs have been associated with elevations in LDL, TC and TG [21, 22, 68, 71, 75-78], but the degree of dyslipidaemia varies highly within the drug class. In general, ritonavir [30, 74, 79, 80] and potentially ritonavir "boosted" PIs have been associated with a poor lipid profile [71, 74, 81, 82]. Regimens including thymidine analogues (NRTI), such as stavudine [73, 83], also result in higher lipid levels. Although, the NNRTIs efavirenz [71, 74, 84], and to a much lower degree nevirapine [71, 74, 85-87], may be associated with increased lipid levels, they are also associated with a substantial increase in HDL levels [71, 74, 85, 87] which generally may result in what is considered a beneficial lipid profile change (lower TC/HDL-ratio) concerning the cardiovascular risk [88, 89].

FAT REDISTRIBUTION

Fat redistribution syndromes known as lipoatrophy (peripheral/subcutaneous fat loss due to mitochondrial toxicity) and lipohypertrophy (central visceral fat accumulation) has mainly been reported in association with older NRTIs, especially stavudine and zidovudine [38, 90-100], and to a lesser degree PIs [86, 90, 91, 101-104]. Although less consistent, associations with the NNRTI efavirenz has also been described [92, 98, 105, 106]. In spite of potential adverse changes in the waist-to-hip ratio, fat redistribution syndromes have been shown to contribute to glucose alterations independent of changes in body mass index (BMI) [107].

HYPERTENSION

Several studies have shown associations between lipoatrophy, hyperlipidaemia and insulin resistance and/or DM, collectively known as lipodystrophy [108-112]. These metabolic disorders appear to be closely connected with hypertension in HIV-infected individuals [112-116]. Whereas HAART-associated hyperlipidaemia, fat redistribution syndromes and insulin resistance are well established toxicities, only vague, if any, associations of HAART exposure and risk of hypertension have been reported and results of associations with treatment duration, drug classes or specific drug regimens generally appear controversial [116-122].

Altogether, these metabolic toxicities are potential risk factors for developing serious long-term toxicities such as DM, myocardial infarction (MI) or cardiovascular disease (CVD); however, the risk of these conditions, and potential impact of certain antiretroviral drugs and other potential risk factors, is not fully established. As DM is a risk factor for CVD [46], and CVD is the leading cause of morbidity and mortality for individuals with DM [123], it seems important to investigate the magnitude of the problem in the HIV-infected population.

RISK OF CARDIOVASCULAR DISEASE

During 1998-2000, several case stories and small retrospective case series reported the development of premature coronary artery disease in HIV-infected individuals exposed to HAART, mainly PIs [124-133]. Although, a sub-analysis of 30 phase II/III RCTs of the effect of PI-only regimens, NRTI-only regimens or PI + NRTI regimens found no increased risk of MI in association with PIs (IRR: 1.69; 95% CI: 0.54-7.48), MI was not the main outcome of these studies. In addition, the average duration of PI exposure was short and the overall number of MIs small (~ low MI rate), thus making the study underpowered to detect any difference in MI rate [134]. As such, this issue led to great concerns of an HIV or HAART induced accelerated atherogenesis. In more than a decade, several studies have thus examined the potential risk of MI and cardiovascular disease (CVD) in association with HIV and HAART.

SUBCLINICAL ATHEROSCLEROSIS

As the development of atherosclerosis is rather slow and the event rate is low, several studies have investigated early markers of atherosclerosis. The major part of these studies [135-151], but not all [152-157], showed a higher prevalence of subclinical atherosclerosis in HIV-infected individuals compared to HIV-negative controls, as indicated by 1) an increased (carotid) intima-media thickness (IMT), 2) presence of calcified or non-calcified plaques detected by ultrasound or computed tomography coronary angiography, 3) an elevated coronary artery calcium (CAC) score, or 4) an impaired brachial artery flow-mediated vasodilation (FMD) assessed by ultrasound. However, despite the potential dynamic of such parameters [158] these studies were mainly of a cross-sectional nature, and the results of an association with the use of PI were largely inconsistent [135-139, 141, 142, 145, 146, 152, 155, 157, 159-167]. Furthermore, conventional risk factors was in some studies the only independent factor associated with subclinical atherosclerosis [136, 141, 152, 168].

RISK OF MYOCARDIAL INFACTION IN ASSOCIATION WITH HAART

Although, increasing rates of MI have been shown in some studies [169],a large study (36,766 individuals) from the Veterans Affairs database [170] found a declining incidence rate (IR) of cardiovascular- and cerebrovascular disease during 1995-2001 (IR: 1.7 to 0.9 per 100 person-years). Furthermore, no relation with either exposure to or duration of exposure to PI, NRTI or NNRTI and risk of cardio- or cerebrovascular disease was observed [170]. Still, several observational studies have found high rates of MI as well as acute coronary syndrome (ACS), ischemic heart disease, coronary artery disease (CAD), coronary heart disease (CHD) and CVD in association with HIV and HAART. Although, some observational studies had no comparison cohort [169, 171-174] and others compared HAART associated event rates with that of naïve individuals [170, 175] or individuals on PI-regimens with that of non-PI regimens [86, 176-179], the greater part of the studies with a HIV-negative control group [49, 52, 180-183] have agreed that HIV-infected

individuals have a 1.5-2-fold increased risk of MI relative to uninfected controls, whereas the relative contribution of HIV vs. HAART seems less clear. Importantly, these studies had highly varying size, power, exposure definition (HAART naïve/experienced), outcome definitions, outcome validations as well as large differences in setting and cohorts. Moreover, due to lack of sufficient data on smoking status [49, 170, 175, 179-181, 183], smoking could potentially have confounded these studies.

MYOCARDIAL INFACTION IN ASSOCIATION WITH ANTIRETROVIRAL DRUGS

In 2003, the Data-collection on Adverse effects of antiretroviral Drugs (D:A:D) study, a large (36,199 person-years) international collaboration of 11 cohorts in 21 countries, stated that the risk of MI increased progressively with a 26% relative increase in risk of MI per additional year of HAART exposure (95% CI: 1.12-1.41) during the initial 4-6 years [112]. Similar results were obtained when the D:A:D study used a composite endpoint of cardio-and cerebrovascular disease events [184]. Such dramatic or accelerated effect was not supported in an analysis from the Danish HIV Cohort Study [181] Nonetheless, the difference could be due to differences in the composition of drug regimens, demographic data and study design. Yet, major limitations in the two studies included lack of outcome validation and adjustments for smoking in the study from the Danish HIV Cohort Study [181] and inclusion of individuals with prior MI , inclusion of a high number of prevalent HAART users (74.5%), large percentages of missing data and lack of a comparison cohort in the D:A:D study [112]. As the cumulative exposure of a drug lies on the same time scale as an increasing age and increasing time with HIV, it seems impossible to exclude residual confounding. The best way to avoid such confounding seems thus to be the use of a well matched comparison cohort or standardized measure.

PIs have been the main drug-group of focus for many years due to its potential to induce metabolic side-effects. Yet, as described above the Veterans Affairs Database [170] and two other observational studies [44 , 185] have failed to reveal an adverse association between PI exposure and risk of MI, CHD, or cardio- and cerebrovascular disease; however, the latter two studies might have been underpowered to detect a difference [44, 185]. In contrast, results of an Italian open-label, observational trial [86] and several other observational studies including the HIV Outpatient Study (HOPS), HIV Insight Database, the French Hospital Database on HIV (FHDH), the Adult Spectrum of Disease Study (ASD) and 2 Canadian Administrative Databases (RAMQ) [176-180, 183], have supported a PI associated increased risk of MI, which in some studies seemed to increase with the length of PI exposure [177] [180]. In extension, the D:A:D study [186] in 2007, established that the cumulative exposure to PI increased the risk of MI with a relative rate of 1.16 (95% CI: 1.10-1.23) per additional year of exposure to PI, which was partly explained by dyslipidaemia. In a more recent D:A:D study [187], mainly the PIs indinavir and lopinavir/ritonavir have been incriminated, whereas no association with saqinavir or nelfinavir was found. These results

corresponded well with the more recent results from the FDHD (nested case-control study) [188] (OR: 1.15 per year of PI exposure; 95% CI: 1.06-1.26), although, this study found that the increased risk was associated with cumulative exposure to any PIs except saquinavir.

As the PI-associated MI risk found by the D:A:D study group was reduced after controlling for exposure to NRTIs, NRTIs was proposed to contribute to the increased risk of MI. In 2008, the D:A:D study [189] thus examined MI risk in association with NRTI exposure. This study found that current/recent exposure to the purine analogue abacavir (guanosine derivate) was associated with a 90% increased risk of MI [189]. Although small differences in change of lipids have been observed in association with abacavir as opposed to tenofovir [190-194], no difference in the TC/HDL-ratio has been found [191, 192, 194]. Thus, due to the generally perceived metabolic safety of abacavir [195], these findings were highly unexpected. As abacavir has been one of the two most used NRTIs (abacavir and tenofovir) to initiate HAART, this has been highly debated. However, the evidence on this subject as well as a possible explanation for this association is still not evident.
Besides the association with abacavir, an association with the purine analogue didanosin (adenosine derivate) (aIRR: 1.89; 95%CI: 1.47-2.45) was also found [189]; however, due to the extensive toxicity profile of this drug, it is generally not recommended for use anymore. Concerning other NRTIs, neither current/recent (aIRR: 1.14; 95% CI: 0.85-1.53) or cumulative (aIRR: 1.04; 95% CI: 0.91-1.18) exposure to tenofovir has been found to be associated with risk of MI [183, 187]. Also, no association with zidovudine, and stavudine (thymidine derivates) or lamivudine, zalcitabine and emtricitabine (cytosine derivates) has been found [187, 189]. Durand et al [183] recently found an increased risk of MI in association with any exposure to efavirenz and recent exposure to delavirdine; however, no MI risk associated with exposure to NNRTIs (current/recent, cumulative) have been found by others [170, 186-189].

OTHER FACTORS ASSOCIATED WITH RISK OF CVD

Although, prior observational studies indicate that the higher risk of MI in HIV-infected individuals is due to drug toxicities associated with some antiretroviral drugs, the SMART trial [196] in 2006 reported that continuous HAART as opposed to drug sparing regimens (CD4 count guided HAART) not only reduce the risk of death, but also the risk of MI. Subsequently, the mechanisms that drive the association seem to rely on complex associations of known and unknown factors, including both the virus (immunodeficiency and viral replication) and the treatment, as well as characteristics associated with an HIV positive status such as ethnicity, socioeconomic factors, behavioural and life-style associated factors and potentially risk taking behaviour (IDU, high risk sexual behaviour etc.). Moreover, a high prevalence of conventional CVD risk factors, such as smoking [44, 45, 47, 49, 112, 197-200], presence of concurrent co-morbid diseases including hepatitis C virus (HCV) and psychiatric conditions, as well as a high degree of surveillance could

therefore be a marker for a population at risk of CVD. Finally, chronic immune activation, persistent inflammation and accelerated aging have been proposed as potential candidates on the pathway [201]; however, in general the impact of the above mentioned factors seems unclear.

OBJECTIVES

The objectives of this PhD-thesis were:

1. To investigate the risk of DM in HIV-infected individuals compared to that of the general population (STUDY 1).
2. To evaluate the impact of age, body mass index (BMI), lipoatrophy, HAART and specific antiretroviral drugs on risk of DM in HIV-infected individuals (STUDY 1).
3. To establish whether the increased risk of myocardial infarction (MI) in HIV-infected individuals partly reflects an increased risk of MI in their families (STUDY 2).
4. To examine whether smoking has a higher impact on risk of MI among HIV-positive than the HIV-negative individuals (STUDY 3)
5. To evaluate if abacavir exposure potentiate the impact of smoking on risk of MI (STUDY 3)
6. To estimate to what extend factors associated with smoking and abacavir exposure can explain the increased risk of MI in the HIV-infected population (STUDY 3).
7. To estimate the fraction of the risk of MI that could be prevented, if the potential risk factors (smoking and abacavir) were eliminated (STUDY 3).
8. To establish the post-MI mortality in HAART-exposed HIV-infected individuals and to compare mortality with that of individuals with a MI without HIV infection and HIV-infected individuals without an MI (STUDY 4).

METHODS AND METODOLOGICAL CONSIDERATIONS

HIV IN DENMARK (STUDY SETTING)

Individuals who are tested HIV positive at a general practitioner or at a Danish hospital are referred to a department of infectious diseases and anonymously reported to the "Statens Serum Institute", from where the epidemic is under surveillance. Individuals diagnosed with HIV, whom at least once has attended a department of infectious diseases, are further registered in the Danish HIV Cohort Study. These individuals are given the opportunity to be followed in the outpatient department. Treatment of HIV infection is restricted to eight specialized centres (Departments of Infectious Diseases at 1) Copenhagen University Hospital, Rigshospitalet, 2) Hvidovre Hospital, 3) Odense University Hospital, 4) Aarhus University Hospital, Skejby, 5) Aalborg University Hospital, 6) Herning Hospital, 7) Hillerød Hospital and 8) as a new centre Roskilde Hospital - Kolding Hospital, which was previously an HIV centre, is now covered by Odense University Hospital). At these centres, HIV-infected individuals are seen on an outpatient basis at intended intervals of 12 weeks that, for individuals with well-controlled infection, has been extended to 24 weeks during the last years. HAART and treatment for opportunistic infections are provided free-of-charge. HAART is prescribed according to national guidelines from *The Danish Society of Infectious Diseases* [202, 203], that in the study period were presence of one of the following: acute HIV infection, AIDS defining disease or HIV-related disease, pregnancy, CD4 cell count < 300 cells/µl until mid-2008, and ≤ 350 cells/µl thereafter, and until January 2001, a plasma HIV-RNA >100,000 copies/ml. Compared to other countries, especially the USA, the Danish HIV care is well organized, and treatment failures and loss to follow-up are considered to be rare [204, 205].

DATA SOURCES

This PhD thesis is based on data from the Danish HIV Cohort Study and data from other high quality registries - all linked with the use of the personal identification number (PIN - also named the civil registration number).

The Danish HIV Cohort Study (DHCS)

DHCS is a nationwide, prospective, population-based cohort study [206] initiated in 1998 as the HIV cohort in Western Denmark [207]. In 2004 it was expanded to cover all Danish HIV-infected individuals treated at Danish hospitals, and through review of the medical files, data back to 1 January 1995 were retrieved and entered into the database. DHCS is an ongoing cohort (~ ***open cohort***). DHCS covers ***prevalent*** HIV cases as of 1 January 1995, as individuals diagnosed with HIV prior to this date are not included until 1 January 1995 (i.e. ***delayed entry***/***left censoring***). There may therefore be a healthy survivor effect in the pre-1995 population. Nevertheless, after a few years, this effect is expected to be diluted, where upon the cohort is ***incident*** for non-immigrants, but prevalent for immigrants. Importantly, immigrants cannot be included from

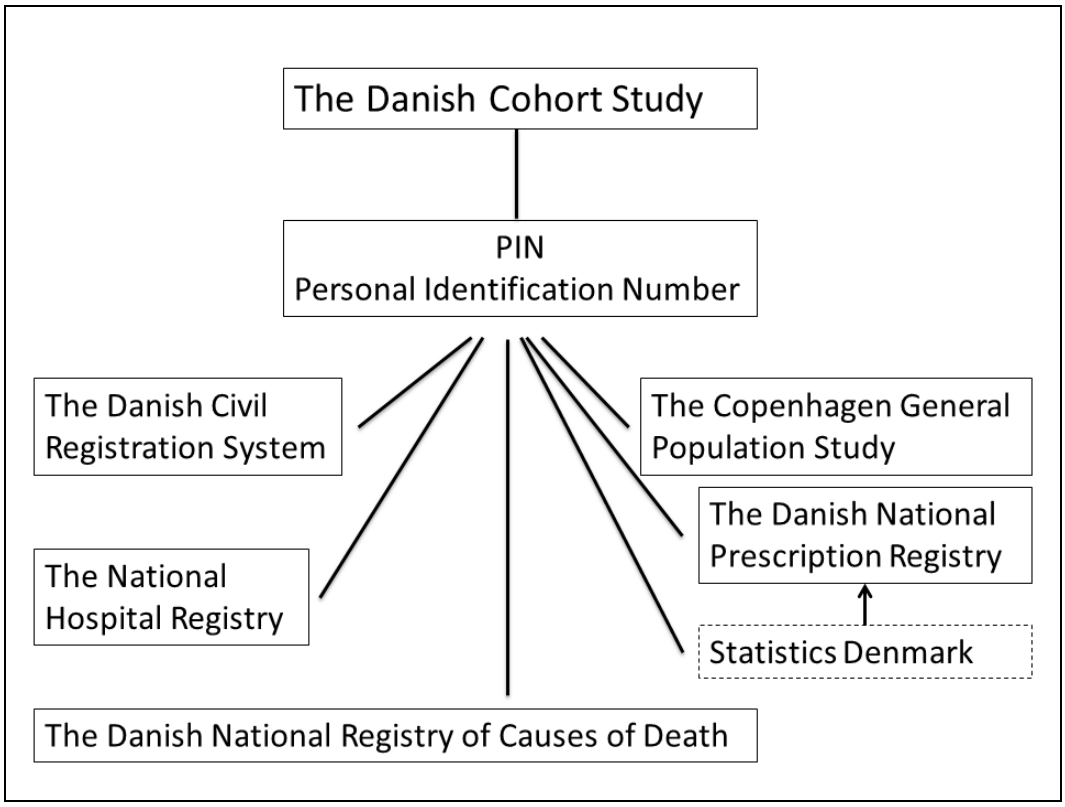
the date of their HIV-diagnoses if this date was prior to immigration, as this would cause ***immortal time-bias*** (i.e. survival benefit, as they cannot die or be censored before entering the cohort).

In general, HIV-infected individuals are identified by notification from physicians, review of hospital records as well as cross-checking of laboratory CD4 cell counts (CD4+ counts) and HIV-RNA viral loads (VL). The registry contains characteristics such as gender, date of birth, time and place of infection and mode of infection. Additionally, serological status regarding hepatitis B and C virus (HCV), cytomegalovirus and toxoplasmosis are included. Standardized data collection forms are completed at the clinics by trained research nurses and typed into the registry once a year. The yearly update of the registry include demographics, vital status, development of opportunistic infections or other AIDS defining illness, pregnancies and outcome of births, information on HAART and laboratory data. As HAART is only provided in the 8 HIV centres, data on HAART initiation and change in antiretroviral therapy (dates and specific drugs) is considered complete. CD4+ count and VL are extracted electronically from laboratory data files. Data on smoking and alcohol have been collected prospectively since 2004 and was obtained retrospectively from medical files for patients who died or emigrated prior to that year. By using serial numbers as opposed to the PIN, the identity of individuals in the database is kept anonymous for researchers. DHCS is a collaborative effort by the 8 HIV centres that due to a representative from each centre, whom is included in the steering committee, helps with ensuring a high level of data quality. Initially, on-site audits were performed once a year to verify the completeness and accuracy of a random sample of the records. Since then the chair and daily leader of the cohort, Professor, DMSci Niels Obel has incorporated cross-checking and validation algorithms into the database in order to identify potential errors, suspicious data or missing data. Such reports are sent to the HIV centres once a year for clarification and correction. This ensures continues data quality. The weakness of DHCS is the relative small size of the HIV-infected population compared to other HIV cohorts including the FHDH, HOPS, HIV Insight, the Veterans Affairs facilities, Eurosida and D:A:D, as smaller numbers of individuals under study may be associated with a risk of a low number of observed events, which in general may limit the power or statistical ***precision*** of the analysis (i.e. wide 95% confidence intervals (CI)) and increase the risk of ***over-fitted models*** (i.e. low number of events per covariate) and ***type2 errors***. On the other hand, the major strengths is the high data quality, the ***nationwide population-based design*** (i.e. a well-defined region minimizes selection bias), the long observation period, the almost ***complete follow-up*** (i.e. a substantial amount of loss to follow may threat the validity), and the inclusion of incident HIV-infected individuals and incident HAART users for all HIV-infected non-immigrants after 1995, that all together makes a powerful design.

Table 1. Type 1 and type 2 errors

Type I errors	The result may erroneously reject the null hypothesis (False positive)
Type II errors	The analysis may erroneously fail to reject a false null hypothesis potentially due to lack of power to detect the association of the exposure and outcome (False negative)

Figure 1. Included registries and linkage



The Danish Civil Registration System (DCRS)

1) Vital status, residency, and migration data for all Danish residents (death, emigration, loss to follow) [208-210],

The Danish National Hospital Registry (DNHR)

1) Discharge diagnoses from non-psychiatric hospitals - classified by the *International Classification of Diseases* [8th revision (ICD-8) and 10th revision (ICD-10)] [211, 212].

The Danish National Registry of Causes of Death (DNRCD)

1) Death certificates, specifying up to 4 diagnoses, according to the ICD codes [213].

The Danish National Prescription Registry (DNPR)

1) Redeemed prescriptions dispensed at Danish community pharmacies – classified by drug name and Anatomical Therapeutic Chemical Classification (ATC codes) [214, 215].

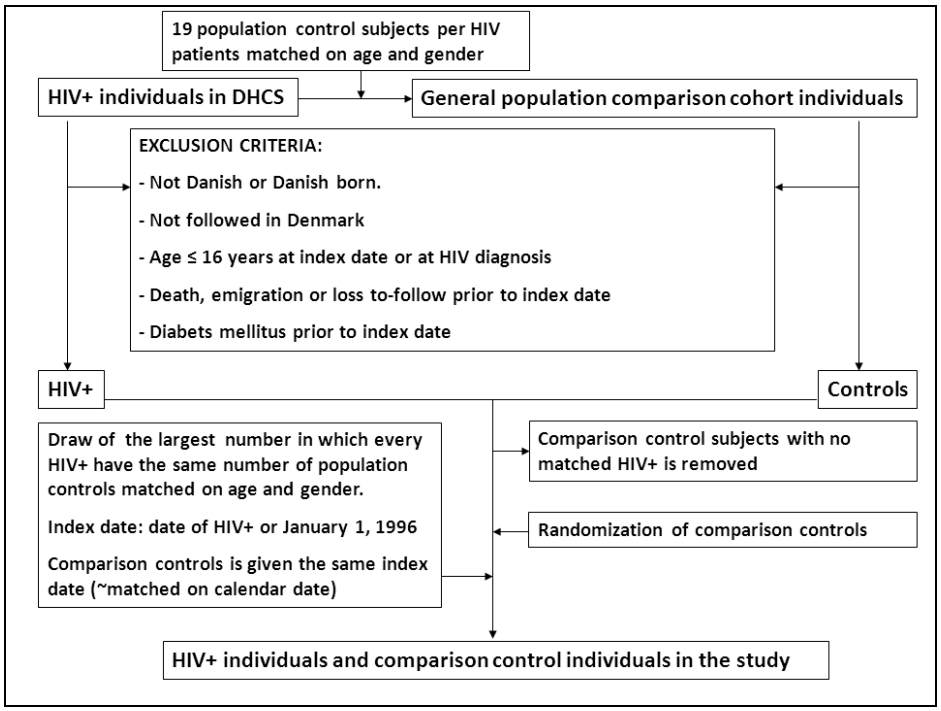
The Copenhagen General Population Study (CGPS)

1) A prospective cohort study initiated in 2003 and still recruiting individuals randomly selected from greater Copenhagen [216, 217].

(The latter 5 registries have been further described in the 4 papers)

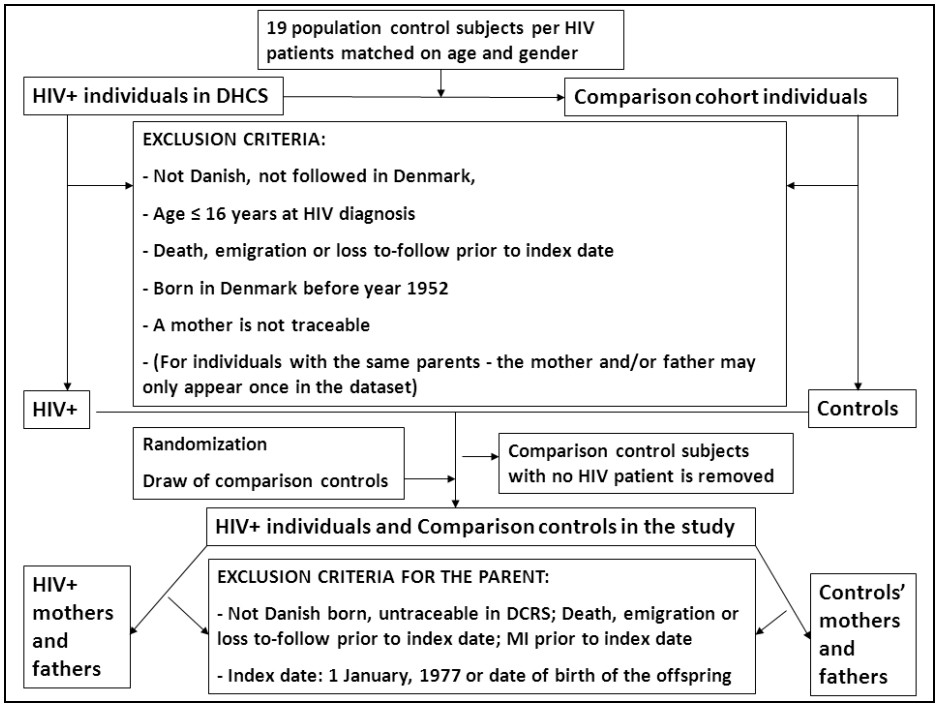
SAMPLING AND STUDY DESIGN

STUDY 1



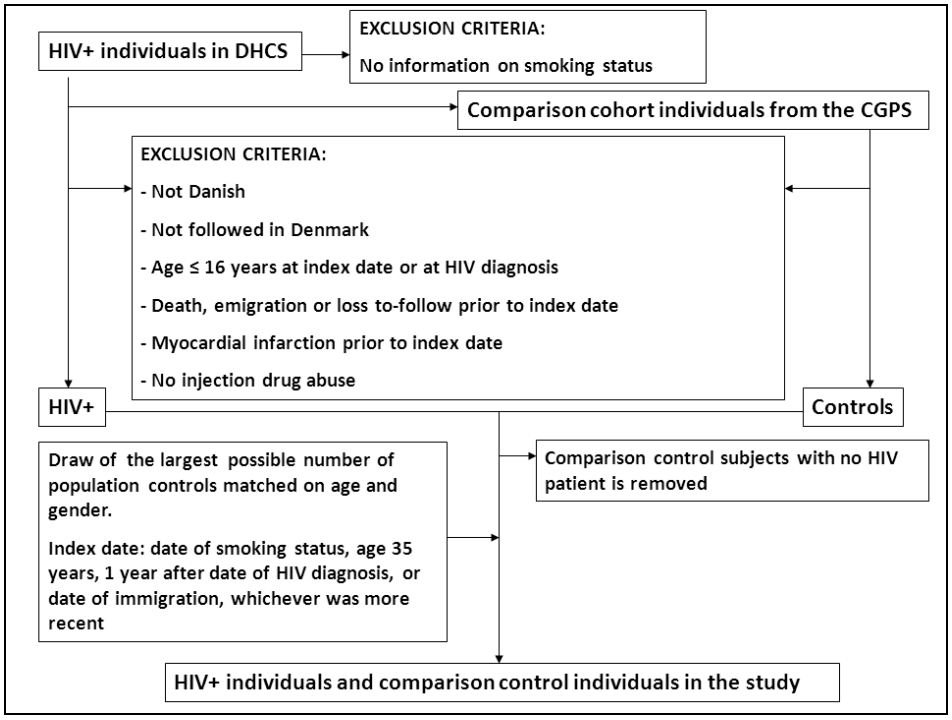
Study period: 1996-31 December, 2009.

STUDY 2



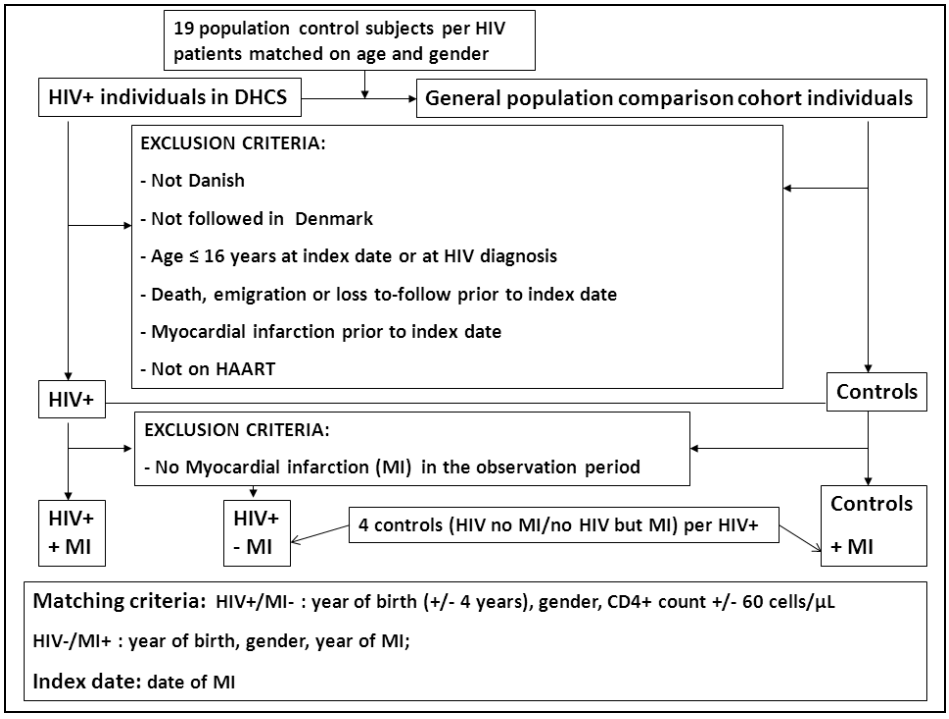
Study period: 1977-1 June, 2008.

STUDY 3



Study period: 1995-1 September, 2010.

STUDY 4



Study Period: 1995-31 December, 2004

EXPOSURE

In all 4 studies the main exposure of interest was **HIV status (HIV+/control).** Although, all HIV-infected individuals in the cohort are in fact HIV-positive, and all HIV-positive individuals in contact with the Danish hospital system are covered, the population controls are random samples from the background population, and have thus not been HIV-tested. Although, the prevalence of HIV in Denmark is low (approximately 0.1%) [218], we assumed that very few (less than 0.1%) individuals in the control group could be HIV positive (undiagnosed) without knowing it, as the observation under study furthermore is quite long. Accordingly, this should not exert any major impact on our findings.

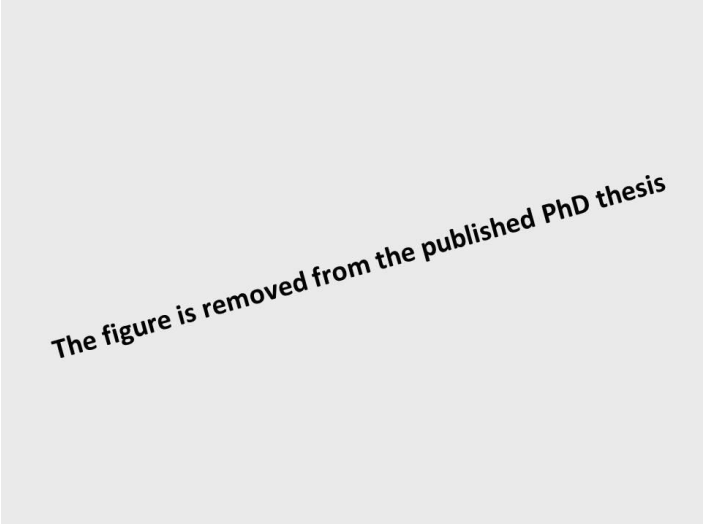
Other exposures of interest are further described below:

Study 1: **HAART status (HAART naïve/HAART experienced)** and exposure to certain antiretroviral drugs or drug groups **(PIs and NRTIs)** was retrieved from DHCS. As the distribution of antiretroviral therapy is free of charge and restricted to 8 centres, the validity of this data is quite high, and potential ***selection*** and ***information bias (recall bias***) are thus kept to a minimum. In our analyses, we analysed exposure to certain antiretroviral drugs as an intent-to-continue-treatment approach, why the effect associated with the drugs used for short time in the beginning of the HAART era might have been diluted and ***reversed causation*** cannot be excluded (further described below). Moreover, as the studies were based on observational data and thus not randomized, we cannot exclude ***confounding by indication*** or ***channelling bias***. Confounding by indication can be described as patient-physician characteristics including the indication, severity of the disease, comorbidity, health seeking behaviour, socio-economic and educational status, smoking status or e.g. alcohol intake etc., that all-together have made the physician prescribe/or not prescribe the specific drug. However, these characteristics may increase or decrease the risk of the event, thus confounding the actual effect of the drug itself [219-222]. Channelling bias (***allocation/selection bias***) is to a higher degree associated with characteristics of the drug itself and may arise when choosing between two drugs with similar therapeutic indication (e.g. tenofovir and abacavir), but claimed advantages or disadvantages of one of the drugs that may channel the choice away from e.g. patients with pre-existing morbidity. As such the one drug is preferentially prescribed to groups of patients systematically different according to their baseline prognosis [221-224]. Other exposures included **age, body mass index (BMI) and lipodystrophy.** Data on weight and height closest to baseline was used to estimate the BMI (defined as weight in kilograms divided by height in meters squared (BMI =kg/m^2). BMI was grouped by underweight: BMI: < 18.5; normal weight: BMI: 18.5-24.9; overweight: BMI: 18.5-24.9; obesity: BMI: > 30.0. For some individuals (29.6%), a BMI could not be estimated. As such, specific categories were generated for missing data to allow for all individuals to be included in the analyses. Lipodystrophy was extracted from DHCS as the first date an HIV-infected individual presented with lipoatrophy and introduced as a time-up dated exposure. Unfortunately, lipoatrophy was registered on account of the patients self-report and the clinician's opinion without

standardized assessments such as DEXA, MRI or CT scans. We did not include lipohypertrophy due to difficulties in differentiating it from abdominal obesity.

Study 2: **HIV status (HIV+/control) of the offspring of parents** were the main exposure of interest. These individuals were sampled using DCRS, of which the validity is quite high [213]. However, the registry is not complete concerning maternal and paternal status for individuals born prior to year 1960 and 1970, respectively. Still, among Danish born individuals, the percentage with a link to a mother increased rapidly in the beginning of the 1950'ies.

Figure 2. The percentage of persons born in Denmark with information on maternal identity by gender.



Pedersen CB. Dan Med Bull 2006; 53: 441-9.

We found that after year 1952 registration of the majority of mothers of HIV-infected individuals and population controls were identifiable in DCRS. Although, less than 10% of the mothers could not be traced from DCRS after that date, we presume that the bias this may introduce is equivalent in the two populations. To avoid immortal time-bias, parents could not be included in the analysis before the start of the Danish National Hospital Registry (January 1 1977) or date of birth of the offspring (the latest).

Study 3: The **population controls** were a sample **from CGPS**. Although, the validity of the data in this cohort is quite high, the cohort consists of voluntary participants in a health study of Danish individuals living in Copenhagen. We are thus aware that this sample may not necessarily represent a sample of the general Danish population, and may furthermore differ from the HIV-infected population on a number of variables including ethnicity, socioeconomic status, educational level, sexual orientation, abuse and health-

seeking behavior. **Baseline smoking status** (never, previous, current and never/ever smoking) and **current abacavir** exposure was furthermore used as exposure. We excluded individuals in whom data on smoking status could only be achieved from journal reviews or was missing (28 %) and defined smoking status as never, previous or current smoking and as never or ever smoking. As a clear dose-effect relationship of smoking and MI exists, exposure described by the intensity, duration and time since quitting could potentially have been a better measure of the exposure, but, such data was not available. Data on abacavir treatment was retrieved from DHCS. Abacavir exposure was included as current, why a person could initiate and end the treatment more than once during the study period (time-updated).

Study 4: We used discharge diagnoses retrieved from DNPR (ICD-8 and ICD-10 codes) to identify the presence/absence of an **MI diagnoses** to define the exposure. Associated problems are further described below.

OUTCOME

In the evaluation of the primary outcome in the 4 studies, we estimated the time to 3 different predefined outcomes measures (DM, MI and all cause death).

To identify **DM diagnoses** in *study 1*, we used hospital registry-based discharge diagnoses and diagnoses from outpatient departments (ICD-8 and ICD-10 codes from DNHR). As ICD codes are not valid for identification of DM, we also included data on redeemed prescriptions of anti-diabetic drugs (using the Anatomical Therapeutic Chemical Classification (ATC codes) from DNPR) as a surrogate marker of a DM diagnosis. As it seems unlikely that anti-diabetic drugs have been obtained from sources not captured by the database (e.g. the internet), these data are considered to have a high validity [215]. However, we could not distinguish whether anti-diabetic drugs had been used to treat DM, or potentially insulin resistance or fat redistribution. Moreover, as the DM diagnosis is considered a ***soft endpoint*** as compared to MI (***hard endpoint)***, we are well aware that the true DM incidence could be underestimated, as a type 2 DM diagnosis can be delayed for several years. Estimates of the true IR can only be reached through regular fasting glucose measurements for both groups. Moreover, lack of registration during first line of treatment (life style modifications) carries the risk of ***reversed causality*** (the true causative drug might have been withdrawn or switched before registration of a DM diagnosis or redemption of an anti-diabetic drug).

To identify **MI diagnoses** in *study2* and *3* (and to identify the exposure in *study 4*), we used hospital registry-based discharge diagnoses (ICD-8 and ICD-10 codes from DNHR). It is essential to be aware of the risk of misclassification that this method carries. This is associated with the risk of 1) incorrect diagnoses (including pre-hospital death), 2) incorrect coding of an MI as a different diagnosis, and 3) incorrect coding of a

different disease as an MI diagnosis. However, validation papers have previously shown, that using discharge diagnosis to identify MIs, is valid (high predictive value and high sensitivity) [225, 226]. Of importance, we used no emergency diagnoses or diagnoses coded as *obs. pro.*

Table 2. Sensitivity, specificity, positive predictive value, negative predictive value

	True status:			
Test status:	**+**	**-**	**Total:**	
+	**TP**	**FP**	**TP+FP= Test +**	***PPV:* TP/Test +**
-	**FN**	**TN**	**FN+TN= Test -**	***NPV:* TN/Test -**
Total:	**TP+FN = ILL**	**FP+TN = NOT ILL**		
	***Sensitivity:* TP/ILL**	***Specificity:* TN/NOT ILL**		

TP: True positive; FN: False Negative; FP: False positive; TN: True negative; *PPV: Positive predictive value; NPV: Negative predictive value. (The PPV and the NPV is dependent on the prevalence of the disease, whereas the sensitivity and the specificity are not)

In *study 2* some individuals might have had an MI diagnosed before study inclusion, as the DNHR was not initiated until January 1977 and as first and subsequent episodes of MI cannot be distinguish from the DNHR. Nevertheless, as the study subjects were young at inclusion and with only small differences in age, the proportion of post MIs misclassified as primary MI would presumably not differ between groups.

To identify **death from any cause** in *study 4*, we used data on vital status as registered in DCRS. These data are considered highly valid [209, 210]. In a descriptive sub-analysis, cause of death was examined for the HIV-infected group who had experienced an MI by the use of data from DHCS and DNRCD. In comparison with data from DCRS, data on the cause of death are generally considered to be less valid as the quality of the data relies upon the quality of the physicians' notification, classification and coding of the presumed cause of death in DNRCD [213].

The main limitation in our outcomes were the lack of clinical validation (journal reviews), which mainly relied on to the size of the control population. However, of importance we used the same source of data to ascertain the outcome measures for all study subjects in all studies. As such, we presume that any misclassification may occur with an equal frequency in the groups, thus being ***non-differential misclassification*** (i.e. unaffected of the exposure status) [227, 228]. Finally, we cannot exclude that HIV-infected individuals might be over-diagnosed due to regular follow-up at a physician compared to young men from the general population (i.e. ***surveillance bias***).

CONFOUNDER CONTROL AND MODEL FIT

Confounding is defined as the influence of a variable that is related to the exposure status and the outcome, but not on the causal pathway (***intermediate variable/step***) [219, 227, 228]. As an example smoking is a confounder when examining the risk of MI in association with HIV or HAART as it is a risk factor of MI and is more prevalent among HIV-infected individuals than the background population but not caused by the HIV infection itself. Although, hyperlipidaemia is also more prevalent among HIV-infected individuals, this factor is an intermediate variable, as both HIV and HAART may lead to changes in lipid status that again may lead to a higher risk of MI. Hyperlipidaemia thus lays on the causal pathway between HIV/HAART and MI. The same could also apply for DM and potentially hypertension. If confounding is not well-handled, the observed effect of the exposure might actually be the effect of the confounder. In an RCT the randomization should potentially remove all measured and unmeasured/unknown confounding. In observational studies, ***unmeasured*** and ***residual confounding (***i.e. variables measured less well e.g. never-, previous or current smoking leaves residual confounding as neither age at initiation of smoking, duration of or amount of smoking is taken into account [222, 228]) can never be excluded.

Fitting a multivariable model may be associated with several problems, as inclusion of too many covariates may introduce bias (***over-fitting***) [229, 230]. On the other hand, inclusion of too few covariates may lead to an unresolved question. In the 4 studies, covariates included for confounder control were assessed and included if they 1) deemed clinical relevance (forced into the model) or/and if 2) the estimated exposure effect changed by more than 10% (***change-in-estimate***) [229, 230]. We did not use ***backwards or forward elimination/selection*** of covariates [229].

Study 1: Age (time-updated variable in 5 year intervals (categorical)), gender, calendar year (time-update variable, split at 3, 5, 8, 11 years after 1 January 1996 (categorical)), IDU/HCV status*, CD4+ count > 200cels/µL after HAART initiation (time-updated)*.

(The analysis were restricted to Danish born individuals (surrogate measure for ethnicity) in order to avoid confounding posed by differences in ethnicity between groups (HIV-infected/comparison cohort). This may cause some misclassification as 1) all individuals not born in Denmark are considered non-Caucasian and 2) children of non-Caucasian immigrants are considered Caucasian if they are born in Denmark. However, as the Danish population is rather homogenous [211] with regard to the racial distribution of individuals born in Denmark (mainly Caucasians) and the HIV-infected population born in Denmark consist of 97.5% Caucasians, we assume this approach exclude major bias associated with race. Although, the dataset was not restricted to non-Caucasians, it made the HIV-infected and the control populations under study more comparable. In some studies [56, 231-234], but not all [36, 55, 235-237] HCV infection has been associated

with risk of DM. Our data did not support such an association (results not shown in the paper). Hence, further restriction was not performed.

Study 2: Age (continuous), year of birth (10 year intervals (categorical))

(Analyses were restricted to parents of Danish born individuals, born after 1952 and in whom at least the mother was traceable. The latter restriction was included, to make a simpler and more stringent design; i.e. families, in which a mother is not traceable, may differ substantially from families with an identifiable mother)

(Analyses were stratified by mode of transmission)

Study 3: Age (time-updated variable in 5 year intervals (included as continuous)), gender and calendar year of index date, time on HAART (continuous)*

(Analyses were restricted to non-IDUs to avoid confounding by risk behaviour and comorbidities associated with IDU).

Study 4: AIDS-defining events prior to index date, CD4+ count < 200 /≥ 200 cells/μL, HCV status and co-morbidity (Charlson's comorbidity index).

(The asterisk defines analyses in which only HIV-infected individuals were included).

Covariates not described as time-updated were includes as fixed/baseline covariates. Variables included as continuous covariates for-filled the ***assumption of linearity*** (the event rate - increases exponentially with a linear increase in the covariate, e.g. age) [238]. The joint effects of exposure and covariates were considered multiplicative on the intensity (incidence rate) ~ additive on the log risk scale (***additivity assumption*** ~ lack of ***interaction terms***) [238]. Hence, clinical important and plausible interaction terms, also described as ***effect modifiers*** (cross product term between exposure and covariates and between different covariates – estimated by the ***likelihood ratio test***) were carefully chosen and further examined [229, 230].

VARIABLES THAT NEED FUTHER DEFINITION

AIDS prior to index date, used in *study 4*, was defined according to the presence of one or more AIDS defining conditions as defined by the American Centre for Disease Control and prevention (CDC) [239].

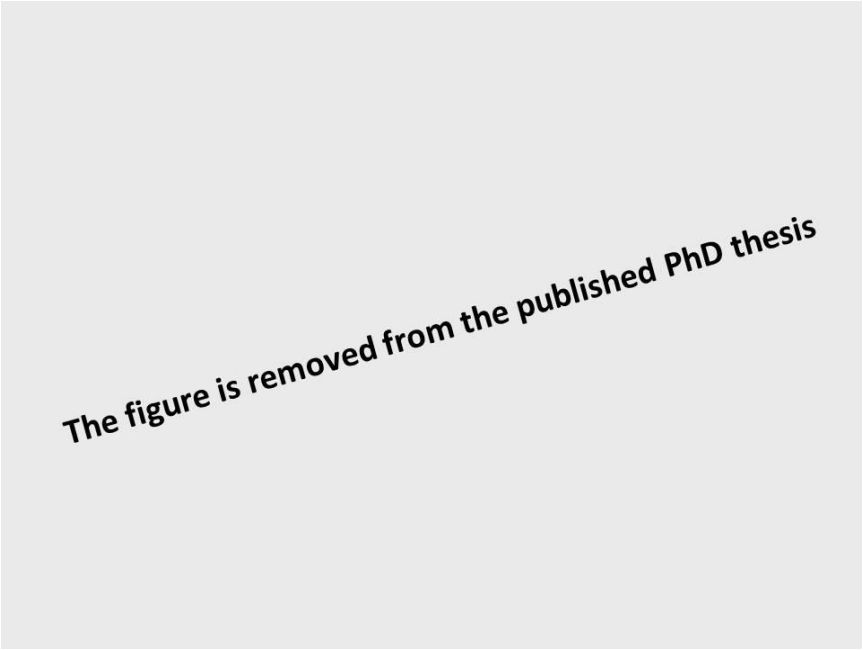
Mode of transmission, further defined as males who have sex with males (MSM), heterosexuals, IDU (including MSM with IDU), blood-transfusion (including haemophiliacs), perinatal and other or unknown mode of HIV-transmission, was retrieved from DHCS. This information is provided at inclusion into the cohort thus being a fixed variable in our dataset. Nevertheless, some individuals with an active IDU may have been misclassified as MSM, heterosexuals or unknown/others, whereas the opposite seems less likely. In *study 2*, we controlled the analyses for the presence of IDU and/or HCV, of which the latter was used as a surrogate marker of misclassified IDU. In *study 3*, we restricted our population by the absence of IDU. In

study 1, mode of transmission of the offspring was used to stratify the results of the parents by and as such used as a surrogate marker of socioeconomic status.

HCV status, used in *study 2* and *4*, was defined by data from DHCS, in which a HCV positive status was defined by the presence of at least 1 positive HCV antibody test or a positive HCV RNA test. We did not have HCV status for the comparison cohort. The estimated prevalence of HCV in the general population is approximately 0.38% [240]. Hence, risk of including HCV+ population controls, was considered small and not associated with large bias.

Charlson's Comorbidity Index [241], used in *study 4*, is a weighted index score developed in 1987 to assess the burden (number and seriousness) of comorbid conditions according to 19 categories of comorbidity (ICD-8 and ICD-10) [242, 243]. Each category is given a weight (1, 2, 3 and 6) based on the adjusted relative risk of 1-year mortality. The score increase with the level of comorbidity and the associated severity why the sum is an indicator of disease burden, and a strong predictor of mortality. We applied a score from data of non-HIV diagnoses prior to index date. Due to the improved prognosis associated with AIDS, and thus need for reassessment of the weights of AIDS (6 points) [244], we did not include AIDS in the applied score. We defined three co-morbidity levels according to the Charlson's index (low ~ 0, medium ~ 1-2; high ~ >2).

Figure 3. Diseases included in the Charlson's comorbidity index



(Charlson ME; J Chron Dis. 1987; 40: 373-383)

The score is limited by lack of inclusion from the secondary healthcare system. Furthermore, only comorbidity registered at baseline are included in the model. As the time of follow-up in the Danish HIV cohort is long and the median age at index date is low, baseline comorbidity may not represent the true difference in comorbidity between groups over time. In addition, due to advances in therapy for a number of the diseases in the score, including AIDS, the weights may not necessarily be up-to-date with reality. Furthermore, change in prevalence of these conditions due to change in admission practices, coding practices or real changes in prevalence of diseases as well as change in survival due to improved treatment could in general call for a revised edition. However, at the present a better model is not available.

MISSING DATA

As described previously, data on smoking-status was incomplete or missing for 28 % of HIV-infected individuals in *study3*. As smoking status was required to define the exposure, exclusion was required. In the same way individuals who could not be retrieved from DCRS were excluded in *study 2*..

In *study 1*, a body mass index was used for controlling. As such, specific categories were generated for missing data to allow for all individuals to be included in the analyses. None of the variables with missing data seemed to be missing completely at random, why methods of ***multiple imputation*** [245, 246] was not applied. For variables measured at every visit (weight, CD4+ count, VL), we used the principle of last measurement carried forward or backwards. Furthermore, a range of up to 1 month or 1 year (+/-) within the range of the specific data of interest was used.

STATISTICAL METHODS

For all 4 studies, ***survival analysis***, that deals with censored follow-up data, was applied to test the ***null hypotheses*** (i.e. that the exposure had no impact on the outcome). An assumption for the use of survival analysis is that ***censoring (both right and left censoring)*** must be ***non-informative*** or ***independent*** (censoring must not be associated with the outcome and the censored individual should resemble those individuals still in the risk set) [238]. In *study 1-4*, the main underlying time scale was ***time since index date*** (HIV infection/HAART/MI-diagnosis). In *study 1*, two additional time scales were applied (calendar years and age).

To compare baseline estimates between groups in *study 4*, we used chi-square test for categorical variables and Kruskal-Wallis test for continuous variables. For both tests a ***p-value* < 0.05** was considered statistically significant ***(i.e.*** the probability that the findings would be as observed or even more extreme, if the null hypothesis was true or the probability of rejecting the null hypothesis although it was true) [219, 228].

To analyse the data we used several statistical models. In all 4 studies, we computed **incidence rates (IR)** and **mortality rates (MR)** expressed as the number of events per 1,000 person years (PYR). In *study 3*, we estimated the **excess IR** and used the exact Poisson method to compute 95% Confidence Interval (CI) assuming that the reference IRs were fixed [228].

In *study 4* the **Kaplan Meier estimator** was used to construct a survival curve. This method was possible, as all-cause death has no ***competing risks***. In survival analysis, all individuals in the risk set must have a risk of experiencing the outcome if observed till the end with no censoring [247]. If this is not the case (e.g. the outcome is preceded by other events or death, in which the 1:1 relationship between the rate and the risk is lost), competing risk is present and other methods must be applied [247]. As a result, the **cumulative incidence function** was used in *study 1-3 t*o estimate the risk of DM and MI, recognizing all-cause death (*study 1* and *2*) and death from non-MI causes (*study 3*) as a competing risk [247, 248]. We did not use the Fine and Gray model [249] to estimate the sub-distributional hazard regression, as the ***sub-distributional hazard ratio*** has no resemblance with the hazard ratio, and only describes the ordering and not a numerical value for the ratio, and thus makes further interpretation difficult [247, 248].

To compute ***incidence rate ratios (IRR)*** and ***mortality rate ratios (MRR)***, and ***95% CIs*** (i.e. the magnitude of chance events illustrated with an interval that, with the certainty of the illustrated percentage, holds the true value of the measured association [219, 227, 228]), in the 4 studies, two different methods were applied. ***Cox Proportional hazard regression model*** was used in *study 2* and *4* and ***Poisson regression*** was used in *study 1* and *3*. For the Cox proportional hazard regression the time scale (has only 1) is usually time since baseline which leads to a decreasing risk set with time [238]. In contrast, Poisson regression can have several time scales e.g. time since baseline as well as age and calendar year (the risk set may increase with time). Whereas the Poisson regression model is a parametric model, the Cox regression model is a semi-parametric model, of which the baseline hazard is left unspecified [238, 250]. The Cox regression estimates the ***hazard ratio*** (ratio between two hazard rates); however, in all 4 studies it has been described as IRR and MRR. Above all, the Cox regression has a distributional assumption ~ the ***proportional hazard (PH) assumption*** [230, 238, 250]. Essentially, the PH assumption requires that the effect of the explanatory variables on the outcome is independent of time (equidistant) which means that the hazard ratio is constant over time [238, 250]. Nevertheless, if the assumption is violated, several methods can be applied. In *study 4*, the mortality rate ratio was not proportional over time. The violation is well illustrated as the lines on the Kaplan Meier curve cross each other, although this is not a formal way to examine for PH violation. As such, a "split" in the time-scale after 90 days was included in the model. If the effect of the variable on the outcome is changing over time, a ***time-dependent or time-varying variable*** can be included (e.g. the effect of the variable < 1 year and ≥ 1 year ~ ***extended Cox model***) [238]. A time-dependent variable may not be confused with a ***time-updated variable***, which, as opposed to a fixed variable, is a variable that may change its status

with time. The effect of a time-updated variable is nevertheless considered to be stable over time. Next, the ***stratified Cox regression*** model is another way of allowing for control by ***stratification*** of a variable that does not satisfy the PH assumption. The latter model as opposed to the normal Cox regression model, allow different baselines for different strata [238]. The Poisson regression model assumes an equal mean-variance relationship and ***piecewise constant intensity*** (~ incidence rate) ***within time intervals*** but not constant PH. However, if the intensity is constant, PH is constant whereas the opposite not necessarily may be true [238]. In *study 2*, this assumption was not satisfied, as the IRR, due to non-constant IRs, varied over calendar years. Consequently, a split in the calendar scale was inserted.

In *study 3* we estimated the **population attributable fraction (PAF)**, which is the fraction of the incidence of a disease (MI) in the population (exposed vs. unexposed) that is attributable to the exposure (e.g. smoking). As such, the PAF describes the fraction of the risk of MI that could be prevented, if the potential risk factor (e.g. smoking) were eliminated. The PAF has a value in identifying potential priority areas (policy and prevention/intervention) in terms of public health burden [251]. Still, whereas the PAF does not address the probability of causation, it assumes causality between the exposure and the outcome [252]. In addition, the estimates used to calculate the PAF, must not be confounded or include effect modification [251]. Although, our results to some degree relies on the detrimental effects of tobacco, it also reflect differences in behaviour, comorbidity and social disparities (low socioeconomic and educational levels) associated with smoking-status [253]. In extension, no strong biological cause or etiological assumption exists that explains the MI risk associated with abacavir, and both confounding by indication as well as channelling bias has been highly discussed in association with abacavir and risk of MI. Accordingly, the PAF associated with abacavir must be interpreted with caution. As the estimated PAF depends on the prevalence and the impact of the estimated risk factor in the population, it is important to be aware that the PAF cannot directly be extrapolated between populations, cohorts and countries [251].

To assess whether our results could be reproduced if the assumptions were changed (robustness of the findings), **sensitivity analyses** were performed.

APPROVALS AND PERMISSIONS

Data from DNHR, DNRCD and DNPR were obtained with approval from The Danish National Board of Health. The studies were approved by the Danish Data Protection Agency (journal no 2008-41-1781). Ethics approval and individual consent are not required by Danish legislation governing this type of research (no involvement of direct patient contact). The CGPS (used in study 2) were approved by a Danish Ethical Committee (H-KF-01-144/01) and all individuals from CGPS provided written informed consent.

MAIN RESULTS

STUDY 1

Baseline characteristic

3,540 Danish born HIV-infected individuals were identified from DHCS and 14,160 Danish born comparison cohort individuals from DCRS.

	Danish born	
	HIV-infected individuals (N =3,540)	**Comparison cohort individuals (N=14,160)**
Age at index date, years*	38.7 (32.2-46.6)	38.7 (32.2-46.6)
Male gender ‡	2,977 (84.1)	11,908 (84.1)
Caucasian race ‡	3,451 (97.5)	-
Body mass index (BMI) at index date:		
Underweight (BMI: < 18.5) ‡	199 (5.6)	-
Normal weight (BMI: 18.5-24.9) ‡	1,651 (46.6)	-
Overweight (BMI: 25.0-29.9) ‡	539 (15.2)	-
Obesity (BMI: > 30.0) ‡	104 (2.9)	-
Missing data on BMI ‡	1,047 (29.6)	-
HCV co-infection or IDU ‡	677 (19.1)	-
Lipoatrophy prior to DM diagnosis ‡	21 (20.0)	-

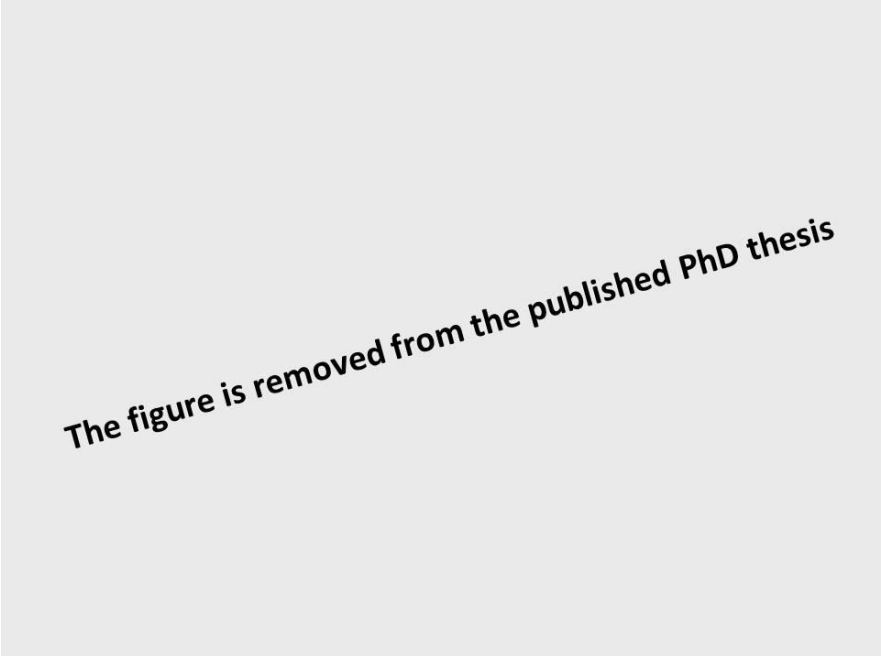
* Median, interquartile range (IQR); ‡ Number (N, %); HCV: Hepatitis C virus; IDU: intravenous drug abuse.

Follow-up

The study gave rise to a total of 28,342 person-years of follow-up (PYR) and 105 (3.0%) incident cases of DM in the HIV-infected population (IR: 3.70; 95% CI: 3.06-4.49) and 136,365.5 PYR and 528 (3.7%) DM cases in the comparison cohort (IR: 3.87; 95% CI: 3.56-4.22).

Cumulated incidence

The cumulated incidence of DM in the HIV-infected and non-HIV-infected population:



Relative rate of DM

	DM	PYR	IRR of DM	
			Unadjusted IRR (95% CI)	**Adjusted IRR (95% CI)**
Controls	528	136,366	Ref (1)	Ref (1)
HIV+ cohort	105	28,342	0.96 (0.78-1.18)	1.02 (0.83-1.26)
Calendar effect:				
1996 – 1998				
Controls	29	20,992	Ref (1)	Ref (1)
HIV+ cohort	18	4,768	2.73 (1.52-4.92)	2.83 (1.57-5.09)
1999 – 2010				
Controls	499	115,374	Ref (1)	Ref (1)
HIV+ cohort	87	23,574	0.85 (0.68-1.079	0.90 (0.72-1.13)

PYR: Person-years of follow-up

Impact of HAART on rate of DM

	DM	PYR	IRR of DM	
			Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)
1996 - 1998				
Non-HAART				
Controls	16	13,223	Ref (1)	Ref (1)
HIV+ cohort	8	2,904	2.28 (0.97-5.32)	2.40 (1.03-5.62)
HAART				
Controls	13	7,768	Ref (1)	Ref (1)
HIV+ cohort	10	1,864	3.21 (1.41-7.31)	3.24 (1.42-7.39)
1999 - 2010				
Non-HAART				
Controls	122	30,221	Ref (1)	Ref (1)
HIV+ cohort	7	5,028	0.34 (0.16-0.74)	0.45 (0.21-0.96)
HAART				
Controls	377	85,153	Ref (1)	Ref (1)
HIV+ cohort	80	18,546	0.97 (0.77-1.24)	1.00 (0.79-1.28)

Impact of age, BMI and lipoatrophy

HIV–infected individuals showed a higher risk of DM with older age and higher BMI. A diagnosis of lipoatrophy was associated with a 2.3-fold risk of DM (aIRR: 2.30; 95%CI: 1.39-3.80).

Impact of PI's and NRTI's

Initiation of stavudine (adjusted IRR: 1.81; 95%CI:1.19-2.75) and saquinavir (adjusted IRR: 1.53; 95%CI: 1.01-2.34) was linked with a higher risk of DM. Moreover, use of indinavir (adjusted IRR: 1.38; 95%CI: 0.91-2.11) and didanosine (adjusted IRR: 1.52; 95%CI: 0.99-2.34) showed a trend towards a higher DM-risk. Nelfinavir, atazanavir, ritonavir +/-lopinavir and PI in general, as well as zidovudine, abacavir, tenofovir and lamivudine did not increase the risk of DM. Cumulative use of HAART was not associated with a higher DM-risk.

STUDY 2

Baseline characteristics

In total 2,269 HIV-infected individuals and 9,076 matched population controls were included in the analyses.

	HIV-infected individuals (N=2,269)	**Control subjects (N=9,076)**
Males ‡	1,895 (83.5)	7,580 (83.5)
Route of infection:		
MSM ‡	1,205 (53.1)	
Heterosexual ‡	555 (24.5)	
IDU ‡	336 (14.8)	
Other/unknown ‡	173 (7.6)	

‡: Number (N, %); MSM: males who have sex with males; IDU: Injection drug abuse

Furthermore, 2,269 mothers and 2,022 fathers of HIV-infected individuals and 9,076 mothers and 8,460 fathers of control subjects were identified.

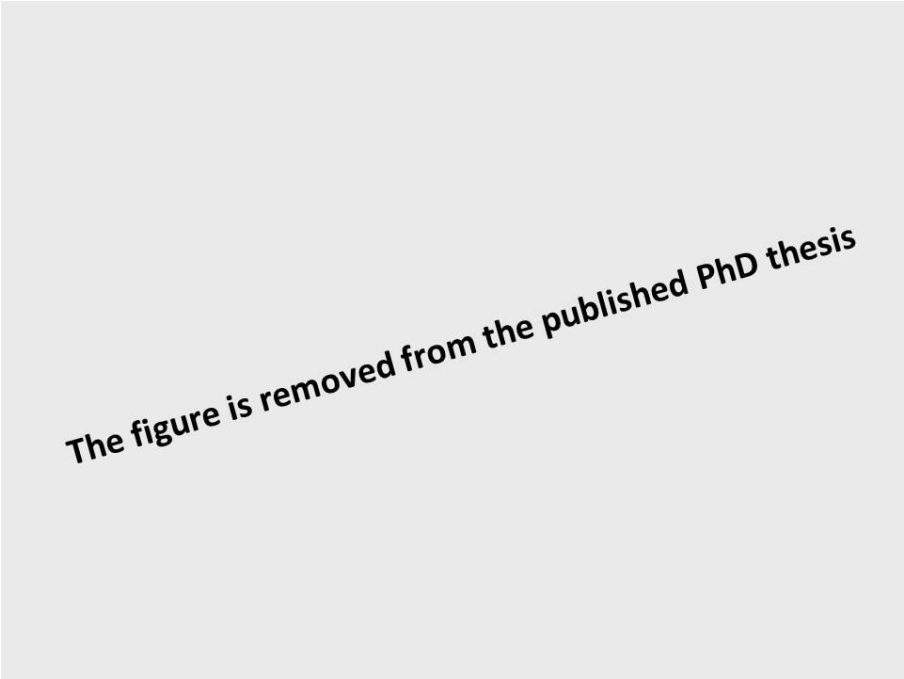
	Mothers of		**Fathers of**	
	HIV-infected individuals (N=2,269)	**Control subjects** (N=9,076)	**HIV-infected individuals** (N=2,022)	**Control subjects** (N=8,460)
Age at index date, years*	38.3 (32.3-44.9)	38.7 (32.4-45.3)	41.5 (34.6-49.1)	41.9 (34.6-49.2)
Number in whom observation time started after 1. January 1977 ‡	197 (7.9)	699 (7.7)	200 (8.8)	773 (8.1)
Emigration during follow-up *	19(0.8)	35 (0.4)	18 (0.9)	44 (0.5)
Loss to follow-up *	0 (0.0)	1 (0.0)	1 (0.0)	3 (0.0)

‡: Number (N, %); *: Number (N, %)

Follow-up

The study gave rise to a total of 61,900 and 49,443 person-years of follow-up (PYR) and 129 (5.7) and 257 (12.7) MIs for the mothers and fathers of HIV-infected individuals, and 256.033 and 213.883 PYR and 434 (4.8) and 1,085 (12.8) MIs for the mothers and fathers of controls, respectively. The overall risk of MI was 2.08/1,000 PYR (95% CI: 1.75-2.48) versus 1.70/1,000 PYR (95% CI: 1.54-1.86) for mothers of HIV-infected individuals and mothers of control subjects and 5.20/1,000 PYR (95% CI: 4.60-5.87) versus 5.07/1,000 PYR (95% CI: 4.78-5.38) for fathers of HIV-infected individuals and fathers of control subjects.

Cumulated incidence of MI in parents of HIV-infected individuals and population controls



Relative rate of MI in parents of HIV-infected individuals and population controls

	Mothers:		Fathers:	
	Unadjusted IRR (95% CI)	**Adjusted IRR (95% CI)**	**Unadjusted IRR (95% CI)**	**Adjusted IRR (95% CI)**
All parents	1.25 (1.03-1.52)	1.31 (1.08-1.60)	1.03 (0.90-1.18)	1.04 (0.901.19)
Parents of MSM	1.11 (0.84-1.48)	1.10 (0.82-1.47)	0.98 (0.81-1.18)	0.94 (0.78-1.13)
Parents of heterosexual	1.46 (0.99-2.15)	1.59 (1.07-2.35)	1.02 (0.77-1.35)	1.07 (0.81-1.41)
Parents of IDU	1.31 (0.82-2.08)	1.63 (1.02-2.60)	1.31 (0.94 -1.83)	1.42 (1.01-2.00)

IRR: Incidence rate ratio; 95% CI: Confidence Interval;
MSM: Males who have sex with males; IDU: Injection drug abuse

STUDY 3

Baseline characteristics

In total, 2,869 HIV-infected individuals and 10,129 matched population controls were included in the analyses.

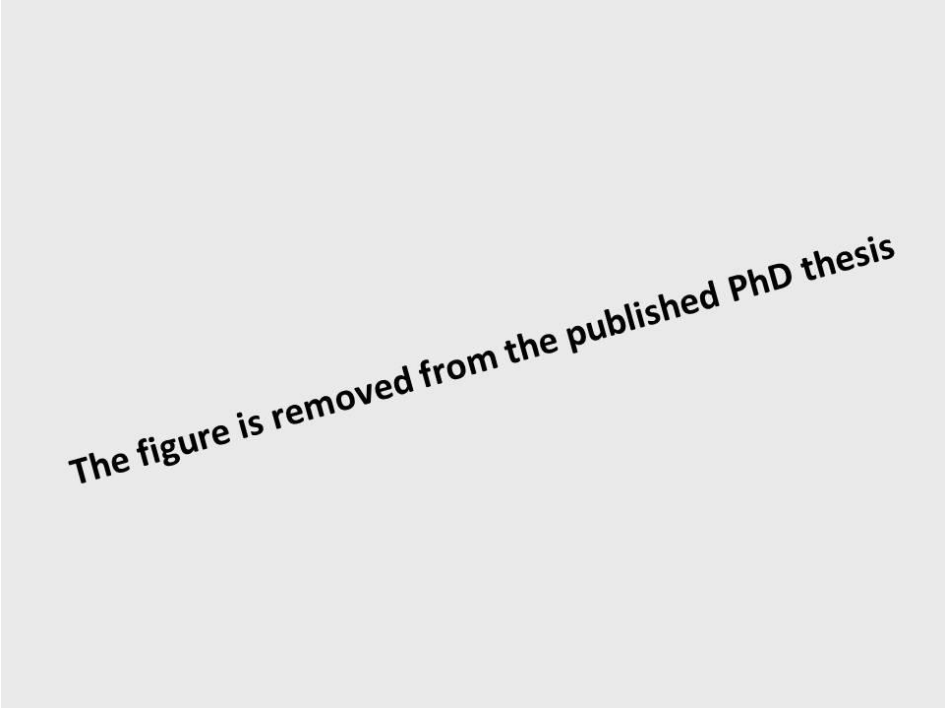
	HIV patients (N= 2,869)			Population controls (N = 10,129)		
	Smoking status			Smoking status		
	Current (47.3%)	Previous (17.6%)	Never (35.1%)	Current (20.7%)	Previous (32.5%)	Never (46.8%)
No. of individuals ‡	1,357	505	1,007	2,092	3,293	4,744
Male‡	1,161 (85.6)	402 (79.6)	668 (66.3)	1,648 (78.8)	2,481 (75.3)	3,620 (76.3)
Age at baseline*	43 (38-50)	45 (38-54)	42 (37-52)	45 (39-53)	46 (39-55)	42 (37-49)
Danish born ‡	1,073 (79.2)	387 (76.6)	639 (63.5)	2,092 (100)	3,293 (100)	4,744 (100)
AIDS at baseline‡	301 (22.2)	112 (22.2)	219 (21.8)			
TG, mmol/L *	1.6 (1.1-2.6)	1.7 (1.1-2.8)	1.5 (1.0-2.4)	1.7 (1.1-2.6)	1.6 (1.0-2.3)	1.4 (1.0-2.1)
TC, mmol/L *	5.2 (4.4-6.1)	5.3 (4.5-6.4)	5.2 (4.4-6.1)	5.6 (4.9-6.3)	5.5 (4.8-6.2)	5.3 (4.7-6.0)
BMI*	22.3 (20.3-25.0)	22.2 (20.1-24.4)	23.2 (20.9-25.9)	26.2 (24.0-28.7)	25.6 (23.3-28.4)	25.6 (23.5-28.2)
Hypertension ‡	74 (5.5)	35 (6.9)	81 (8.0)	126 (6.0)	187 (5.7)	158 (3.3)
DM ‡	46 (3.4)	23 (4.6)	54 (5.4)	66 (3.2)	83 (2.5)	81 (1.7)

‡: Number (N, %); * median, interquartile range (IQR); TG: Triglyceride; TC: Total cholesterol; BMI: Body mass index

Follow-up

The study gave rise to a total of 13,870 person-years of follow-up (PY) and 60 (2.1%) incident MIs for the HIV cohort and 35,105 PY and 133 (1.3%) incident MIs for the comparison cohort.

Cumulated incidence of MI stratified by HIV- and smoking-status



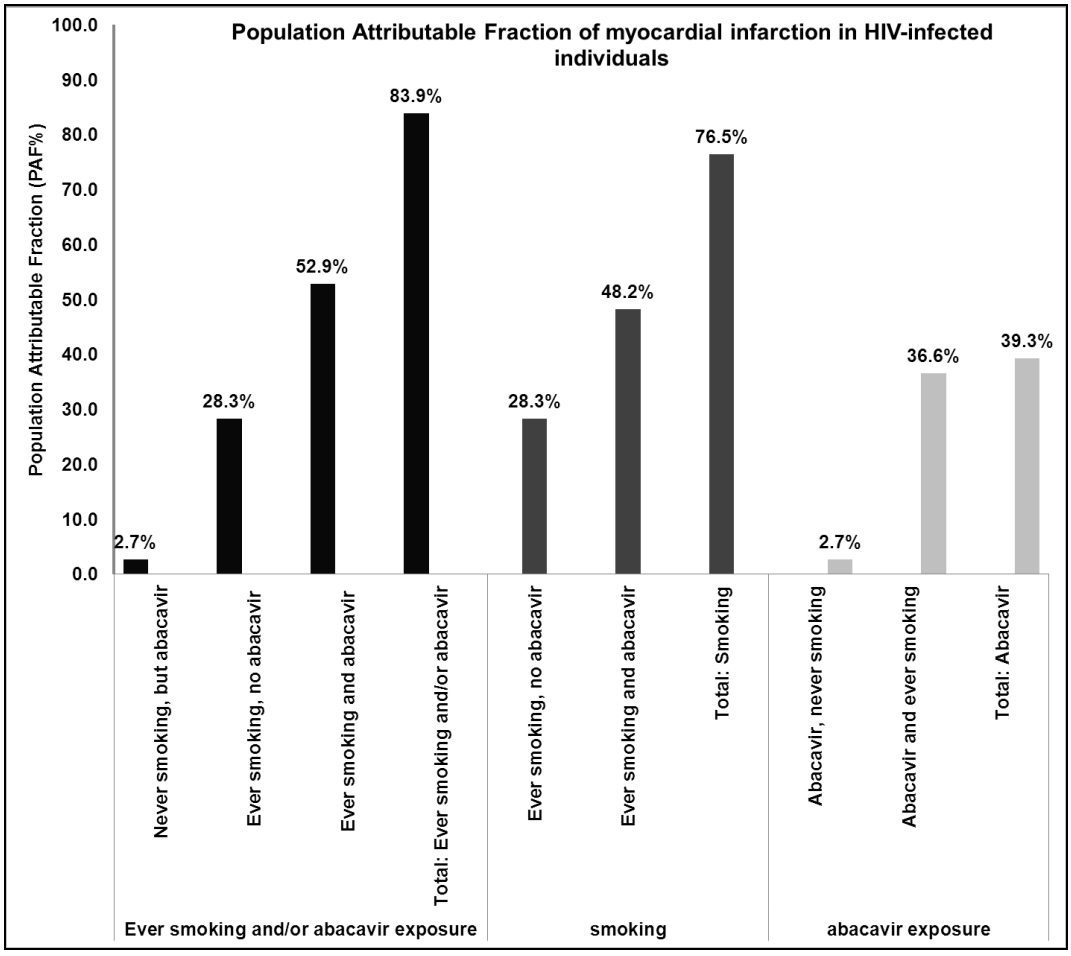
Association of smoking status and risk of MI

In never-smokers HIV was not associated with an increased risk of MI (aIRR: 1.14 95% CI: 0.38-3.46). Previous and current smoking was associated with a higher risk of MI in HIV-infected individuals relative to population controls [(aIRR: 2.32; 95% CI: 1.04-5.13)) and (aIRR: 1.79; 95% CI: 1.05-3.04)]. The absolute and relative risk of MI associated with ever smoker was higher in HIV-infected than non-HIV-infected individuals.

Association of smoking status and exposure to abacavir and risk of MI

In HIV-infected current smokers abacavir exposure was associated with a 3.6 times higher risk of MI (aIRR: 3.63; 95% CI: 1.77-7.43). In never-smoking HIV-infected individuals, current abacavir exposure had no major impact on risk of MI (never-smokers aIRR: 1.74; 0.29-10.47). Current smoking and current abacavir exposure compared to none of these exposures increased the risk of MI more than the additive effect of the two exposures (IRR: 13.67; 95% CI: 3.23-57.98) indicating a potential synergistic effect of smoking and abacavir.

Population Attributable Fraction (PAF%)



STUDY 4
Baseline characteristics

In total, 44 HIV-infected individuals with an MI after HAART-initiation (HIV+/MI+), 176 HIV-infected individuals with no MI after HAART-initiation (HIV+/MI-) and 176 non-HIV-infected individuals were identified (HIV-/MI+) from DHCS and DCRS.

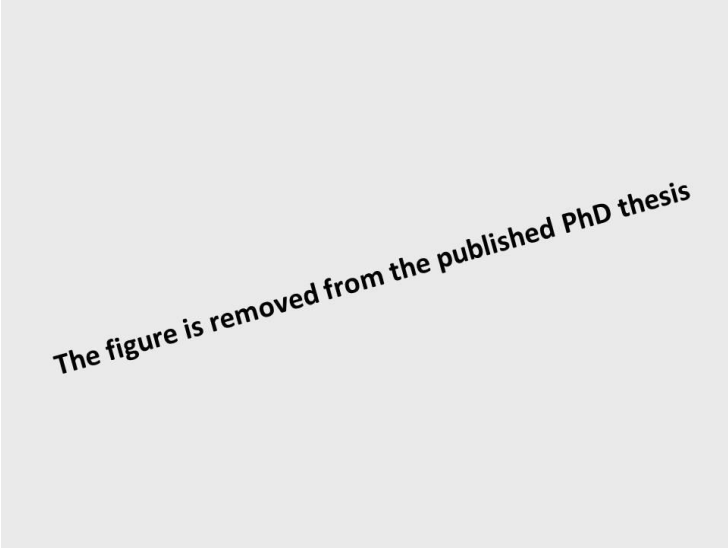
	HIV+/MI+ (N = 44)	**MI+ (HIV-) (N = 176)**	**HIV+ (MI-) (N = 176)**	**P-values**
Males ‡	41 (93.2)	164 (93.2)	164 (93.2)	1.000
Age*	49.8 (41.5-58.8)	50.4 (41.4-58.8)	49.8 (42.1-58.0)	0.907
Race:				0.595
Caucasians ‡	40 (90.9)		155 (88.1)	
Country of origin:				1.000
Danish ‡	37 (84.1)		148 (84.1)	
Infection mode:				0.270
MSM ‡	31 (70.5)		100 (56.8)	
IDU ‡			10 (5.7)	
Heterosexual contact ‡	10 (22.7)		56 (31.8)	
Other/unknown ‡	3 (6.8)		10 (5.7)	
HIV characteristics:				
HIV+ before January 1, 1995 ‡	28 (63.6)		113 (64.2)	0.944
Time from HIV-positive to MI / index date *	7.7 (2.8-14.1)		8.3 (4.1-13.1)	0.782
Time from HAART to MI /index date*	2. 9 (1.8-5.9)		3.3 (1.5-5.0)	0.530
CD4 count*	314 (199-524)		310 (200-522)	0.950
HCV status ‡	2 (4.5)		19 (10.8)	0.263
AIDS ‡	21 (47.7)		54 (30.7)	0.033
Co-morbidity (Charlson's index):				0.015
0 co-morbid diagnoses ‡	27 (61.4)	137 (77.8)	129 (73.3)	
≤ 2 co-morbid diagnoses ‡	16 (36.4)	37 (21.0)	36 (20.5)	
≥ 3 co-morbid diagnoses ‡	1 (2.3)	2 (1.1)	11 (6.3)	

*: median (IQR: interquartile range) ‡: Number (N, %)
HAART: highly active antiretroviral therapy, MSM: men who have sex with men; IDU: Injection drug abuse, HCV; Hepatitis C, PYR: Person-years of follow-up
(Associations between 2 categorical variables were tested using chi-square. All tests were 2-tailed and a p-value < 0.05 was considered statistically significant. Associations between 2 continuous variables were tested using Kruskal-Wallis test)

Follow-up

The study gave rise to a total of 1103 person-years of follow-up (HIV+/MI+: 102 years, HIV-/MI+: 504.4 and HIV+/MI-: 497.3) and 11 (25%), 18 (10.3%), and 23 (13.1%) deaths for the HIV+/MI+, HIV-/MI+ and HIV+/MI- groups, respectively.

Kaplan Meier curve of time from MI/index date till death in 1) HIV+/MI+, 2) HIV-/MI+ and 3) HIV+/MI-.



Post MI rate of death

	Mortality Rate Ratio (MRR)			
	HIV+/MI+ vs. MI+*		HIV+/MI+ vs. HIV+**	
	Unadjusted MRR (95% CI)	Adjusted MRR (95% CI)	Unadjusted MRR (95% CI)	Adjusted MRR (95% CI)
0—90 days post-MI	1.5 (0.5-4.1)	1.3 (0.5-3.6)	10.7 (2.1-54.9)	11.7 (1.8-78.2)
≥ 90 days post MI	7.4 (2.1-26.3)	4.5 (1.2-16.3)	1.4 (0.6-3.5)	1.6 (0.6-4.3)

*Adjusted for Charlson's comorbidity index
** Adjusted for Charlson's comorbidity index, AIDS prior to the MI, HCV status and CD4+ count.

Cause of death

Eleven patients died in the HIV/MI-group; 4 due to heart diseases and 5 because of AIDS defining diseases. None of the patients in the HIV-group died due to heart problems.

DISCUSSION

RISK OF DIABETES

During time, highly varying DM rates have been reported in association with HIV and HAART ranging from 4.4 per 1000 person-years in the Swiss HIC cohort Study [55] and 5.7 per 1,000 person-years in the D:A:D study [53] to 14.1 per 1,000 person-years in the French APROCO-COPILOTE cohort study [236] and 17 (HAART naïve) and 47 (HAART experienced) per 1,000 person-years in the American multicentre AIDS cohort study (MACS) [254]. We (study 1) found a much lower rate of DM for our HIV-infected population and matched population controls. Yet, as non-Caucasian race has been associated with insulin resistance and DM in individuals with HIV [36, 53, 55, 63], we restricted our population to Danish-born individuals as a surrogate measure of Caucasian race. This restriction, along with other factors might have led to the lower rate of DM in our study. Nevertheless, measures of IR might vary by cohort and by country (USA vs. countries in Europe) due to differences in risk factors including age, gender, race, BMI, HCV status, socio-economic status, calendar year and experience towards antiretroviral drugs prior to and in the early-HAART era. Moreover, differing outcome definitions, may lead to differing estimates. As a matched design using relative measures may control for most of these factors, these estimates may to a higher degree illustrated the impact of HIV and HAART per se and may thus be extrapolated between populations, cohorts and countries.

CALENDAR EFFECT AND ASSOCIATIONS WITH HIV AND HAART

The prevalence of diabetes among hospitalized HIV-infected individuals has been described to increase compared to earlier calendar years and compared to the background population in USA [255]. Importantly, we did not find any increased risk of DM in the present HIV era (after 1998). In contrast, we found an almost 3-fold increased risk of DM was found for the years 1996-1998. This increased risk relied on a higher risk both prior to and after HAART initiation. However, drugs with potential diabetogenic effects including some of the early antiretroviral was used in the pre-HAART era. Furthermore, as hyperglycaemia can be induced by drugs used in the treatment of HIV-associated illness including pentamidine isothinate, trimethoprim-sulfamethoxazole, corticosteroids, dapsone, isoniazid, rifampicin and ganciclovir, these drugs could occasionally cause DM [256, 257]. Results of the American MACS have suggested that chronic HIV infection in itself may contribute to glucose abnormalities [36]. Also an association with immunosuppression has been shown, but the results remain inconsistent [36, 55-57, 61, 258-260]. Moreover, as with risk of MI and all-cause mortality [261, 262] an association with systemic inflammation (higher levels of inflammatory markers) and risk of DM has been found both for the general population [263-265] and in HIV-infected individuals on HAART [266]. This could indicate an association with immune activation and sustained inflammation. As individuals in the early years of our study, may have experience long episodes of immunodeficiency and as such represent a population more severely affected by the HIV infection than most HIV-infected individuals today, this may explain some of the observed risk.

Moreover, to investigated whether the high rate of DM in the HIV-infected population relative to the population controls in the early calendar years could be explained by ***systematic errors (bias)*** including ***differential misclassification*** (the misclassification differs according to the exposure status) of the observed individuals we searched the cohort for trends in gender, date of HIV-infection, date of HAART initiation, use of antiretroviral drugs (PIs and NRTIs), treatment centres, CD4+ counts, AIDS defining diagnosis, lipoatrophy, diagnostic codes (DNHR) and potential trends in anti-diabetic drugs used (DNPR) (results not included in the paper). However, no errors seemed to be able to explain the observed results.

IMPACT OF AGE AND BODY MASS INDEX

Consistent with the results of others [48, 53, 55-57, 61, 63, 236], we found that the risk of DM increased with older age and higher BMI indicating that traditional risk factors apply in the same way in HIV-infected individuals as for the background population. Unfortunately, we could not estimate BMI for the general population, which may have affected our estimates of a lower risk of DM in the HAART-naïve HIV-infected population relative to the population controls after 1998. Although, impotence associated with DM could be a protective factor of risk taking sexual behaviour and thus risk of contracting HIV, we do not presume that HIV may protect against contracting DM. We believe that the results could be a consequence of a lower BMI in the period prior to initiating HAART for the HIV-infected population compared to the population controls. As such, lack of confounder control might have led to an underestimation of the impact of HIV infection per se. Nevertheless, Butt et al [56] also found a lower risk (prevalence) of DM in association with HIV even after adjusting for BMI and other confounders (aOR: 0.84; 95% CI: 0.72-0.97) .

In the pre- and early-HAART-era, HIV-infected individuals generally had a low BMI, were cachectic or developed AIDS-associated wasting syndrome [267, 268]. In the HAART-era, several studies have reported a lower BMI and a lower prevalence of obesity for HIV-infected individuals mainly in individuals naïve to HAART compared to non-HIV-infected individuals [45, 52, 56, 57, 197, 198, 200, 269-273]. Yet, on effective HAART the BMI tend to normalize/increase, due to improved nutritional and immunological status [68, 269, 270, 274, 275]. Extending that, a lower baseline CD4+ count and higher VL has been shown to be strongly associated with a positive gain in BMI after initiating HAART [275]. Although, this may reflect a return-to-health phenomenon, overweight, and associated risks, may become an increasing problem in the HIV-infected population as it is in the background population [276, 277]. As an increasing prevalence of obesity is associated with an increasing risk of a large number of diseases including insulin resistance, DM and CVD, this could potentially explain the findings of an increasing prevalence of DM over time found by Glass et [198] and Koutis et al [255]; still, a mediating effect of aging can also not be excluded. As an estimated 50% of people living with HIV in the United States will be 50 years or older by year 2015 [278, 279], DM is expected to increase in the HIV-infected population. Nevertheless, we found no indication that the risk in the years after 1998 increased more than that of the general population of the same age. In other

words, it seems important to monitor and prevent overweight and obesity in the HIV-infected population, whereas screening for DM should be provided in accordance with guidelines for the general population.

ASSOCIATIONS WITH ANTIRETROVIRAL DRUGS

HAART has generally been associated with risk of glucose alterations and an up to 4-fold higher rates of DM [36, 53, 56, 254] relative to non-HIV-infected individuals have been described. In the years after 1998, we found a higher rate of DM in the HAART period relative to the non-HAART period. Nevertheless, relative to the comparison cohort of the same age and gender and followed over the same calendar years, no difference in DM rates was observed. As such this may illustrate some of the difficulties that may exist when analyzing risk outcomes associated with exposure to drugs. Furthermore, risk associated with drugs may change with calendar years due to change in age, behavior, use of antiretroviral drugs with potential toxicity, as well as switch of drugs due to awareness of associated toxicity (change in guidelines for the preferred HAART regimens).

In further analyses, we did not find any association with newer antiretroviral drugs including abacavir and tenofovir, which is in line with prior results of the D:A:D study [53]. We did not examine the risk associated with any NNRTIs. Although, recent research has shown a potential association with efavirenz and risk of DM in two African studies [258, 280], this needs confirmation in larger longitudinal studies. We found that exposure to some of the older antiretroviral drugs (indinavir, saquinavir, stavudine and didanosine) were associated with a higher risk of incident DM, which especially for the NRTIs stavudine and didanosine [53, 55, 236, 260] and the PIs [57, 60], mainly indinavir [53, 55, 236, 258, 260], but in one other study also saquinavir [57] have been suggested by others. Nevertheless, zidovudine [53, 60], lamivudine [36, 54] and ritonavir [254], as well as specific drug combinations [55] have also previously been incriminated. As the use of antiretroviral drugs have changed over the years, indinavir, saquinavir, stavudine and didanosine are not used in modern HAART-regimens, why the associated risk has no larger impact on the HIV-infected population in the western world as of today. Yet, for resource limited settings, such as Africa, these antiretroviral drugs are largely used and may thus illustrate alarming results.

ASSOCIATIONS WITH LIPOATROPHY

Fat redistribution syndromes have generally been associated with older antiretroviral drugs, mainly the thymidine analogues stavudine and zidovudine [38, 90-100]. These drugs have generally been thought to contribute indirectly to glucose alterations through NRTI induced changes in body fat [107]. Although, glucose alteration, including DM, have also been seen in the absence of such change [21, 37, 53-55], this could explain the 2-fold increased risk of DM (aIRR: 2.30; 95% CI:1.39-3.80) that we and others [236] have observed in association with lipoatrophy.

CARDIOVASCULAR DISEASE RISK ASSOCIATED WITH DIABETES MELLITUS

CVD risk factors, that in general seems prevalent in the HIV-infected population, may cluster, thus carrying a risk of the metabolic syndrome, that has several features in common with the lipodystrophy syndrome [281, 282]. The metabolic syndrome appears to be highly prevalent among HIV-infected individuals [282]; however, a recent study has shown that its presence does not seem to increase the risk of CVD over and above the risk conferred by the components of the syndrome separately [281], which indicate the lack of an interaction between the individuals factors of the syndrome. As described previously CVD is the leading cause of morbidity and mortality for individuals with DM [123] and DM is a well-known risk factor for CVD. However, a DM diagnosis may not confer the same impact on risk of MI as for the general population. This might be illustrated with the estimated population attributable risk for CVD in association with DM which in a study by Calvo-Sanchez was 6.57% for the HIV-infected population compared to 17.24% for the general population [283]

RISK OF CARDIOVACULAR DISEASE

GENTIC AND ENVIRONMENTAL FACTORS ASSOCIATED WITH RISK OF MYOCARDIAL INFARCTION

It is well established that a parental history of MI is an independent predictor of future MI among the general population [284], whereas the hereditary component of MI or CVD among HIV-infected individuals is less well examined. Many of the previous studies on MI did not have access to data on parental risk of MI [49, 170, 175-177, 179-181]. Even in the D:A:D-study, 37% had missing data on this variable [46]. Yet, in some studies, a high prevalence (30-37%) of this risk factor has been reported [47, 283]. A study from the general population found that in young individuals with an MI (≤ 45 years) both smoking and family history were the most common risk factors [285]. In extension, a recent study by Calvo-Sanchez et al [283] found that having a family history of CVD led to a 7.7-fold risk of CVD.

To explore the genetic component and evaluate 23 common single nucleotide polymorphism (SNPs) with known coronary artery disease (CAD) associations a recent study genotyped 1,875 HIV-infected individuals from 24 observational studies [286]. Consistent with studies of the uninfected general population [287], an unfavourable genetic risk score was found to be associated with a 1.47 times higher risk of CAD (aOR: 1.47; 95% CI: 1.05-2.04) [286]. This risk did not change after adjustment for family history of CAD and as such contributed to CAD to a similar degree as family history [286]. However, contrary to our predictions, Chow et al [284] found that the genotype score in those with and without a parental history were similar. Furthermore, nothing indicates that a high genotype score is associated with risk of HIV.

Whereas the D:A:D study and studies from the general population define a family history as a first-degree relative with MI before age 50 [112], other studies lack a definition on this variable. Yet, a wider definition

on family history may be associated other factors of interest. A family history of MI may reflect genetic factors as well as racial, ethnic, cultural, environmental, and socio-economic, psychosocial, behavioural and lifestyle related factors that may be shared in families [284, 288]. Such factors may not only affect the individual's risk of disease, but also the way of acting upon illness as well as the health seeking behaviour in general. Additionally, the clinician's attitude, belief and thus delivery of health care services may also be affected. In our study (STUDY 2) we did not examine whether a family history increase the risk of MI, as this seems to be well-established. On the contrary, we investigated the risk of MI in parents of HIV-infected individuals and parents of a well-matched comparison cohort in order to see if an association existed between risk of MI and risk of having a child with HIV. We found that mothers of HIV-infected individuals had a higher risk of MI (IRR: 1.31; 1.08-1.60) than the respective mothers of the controls [289]. This was primarily confined to mothers of HIV-infected off-springs that was registered with IDU or heterosexual mode of infection. In fathers of HIV-infected individuals risk of MI was only increased if the offspring was infected by IDU (IRR: 1.42; 95% CI 1.01-2.00) [289]. Although risk of cerebrovascular events was not increased among parents of HIV-infected individuals [290], the incidence of lifestyle associated cancers such as lung-, head and neck cancer have also been shown to be increased among parents of HIV-infected individuals [291]. In addition, previous studies on HIV and HCV co-infected individuals found that their siblings died at a much higher rate (MRR: 4.23; 95%CI 1.10-1.85) than sibling of controls [292]. These results support that family related factors and risk taking behaviour associated with HIV, IDU and HCV infection, influence the risk of morbidity and mortality in this population, and thus may affect the mechanism behind the higher risk of MI. A major fraction of the increased risk of MI observed in HIV-infected individuals may therefore stem from non-HIV and non-HAART related factors. Surprisingly, a poor CD4 response after initiating HAART, which is associated with activation of CD8+ T-cells and elevated markers of inflammation, was not only associated with increased mortality among HIV-infected individuals independent of smoking, alcohol and concurrent comorbidity, but also increased mortality among their parents [293]. As immune activation and persistent inflammation seems to play an important role in the risk of MI and CVD in general, genetic factors associated with immune recovery could also play a part.

RISK OF MYOCARDIAL INFARCTION IN ASSOCIATION WITH SMOKING

Smoking is one of the largest contributors to MI worldwide [294] and possibly one of the predominant risk factor associated with MI among HIV-infected individuals [51, 295, 296]. Smoking is highly prevalent in the HIV-infected population(40-70%), with rates of 2-3-fold that of the general population and a high average number of pack-years [45, 47, 49, 51, 112, 171, 186, 198, 283, 297-299]. As such, smoking may lead to profound health implications for the HIV-infected population [253, 300-304]. In a recent study by Helleberg et al [304], smoking was associated with a 4.4 fold-fold risk of all-cause mortality and a 5.3-fold

risk of non-AIDS death. Furthermore, the number of life-years lost due to smoking in the HIV-infected population exceeded that of life-years lost due to HIV itself [304].

Although, the Danish HIV Cohort Study has access to data on smoking status, no such data is accessible for the general population. We therefore (STUDY 3) used population controls from the CCPS and found that current smoking as opposed to never smoking, increased the risk of MI more than 6-fold (aIRR: 6.24; 95%CI:2.44-15.92) in association with HIV infection, whereas the risk was only 3-fold increased among the population controls (aIRR: 3.18;95% CI: 1.71-5. 93) (STUDY 3). Several other HIV cohorts have found lower estimates than us (2-4 fold rates of MI) [46, 52, 177, 186, 283, 305, 306]. The discrepancies may rely in the definition of smoking (ever/never or current, previous, never), differences in pack-years of tobacco exposure, differences in outcome definition (MI, CVD etc.), as well as confounder control. We did not adjust the results according to dyslipidaemia, hypertension or diabetes, as these risk factors may act on the causal pathway from HIV, HAART, smoking or abacavir to MI. Restriction or stratification could have been a possible solution in a larger study. Nevertheless, lack of reliable data and power limited the performance of such analyses. The observed higher estimates associated with current vs. never smoking compared to results of other studies could thus be a result of not adjusting for these variables. Our results may therefore represent an effect of smoking as well as being a smoker which may reflect differences in behaviour, comorbidity and social disparities (low socioeconomic and educational levels) [253].

As described in the introduction, HAART, or some antiretroviral drugs, seems to be associated with an 1.5-2 fold increased risk of MI in HIV-infected individuals relative to uninfected controls [49, 52, 180-183]. Triant et al [49] and Freiberg et al [52], found that this relative risk was independent of smoking status ((never-smokers: HIV IR: 7.08% vs. non-HIV IR: 2.64%, $p< 0.0001$) and (never-smokers: HIV+ vs. non-HIV: aIRR: 1.75; 95%: 1.27-2.42), respectively). In contrast, we found that the risk was dependent on smoking status. As such, HIV-infected individuals who had never smoked had no increased risk of MI in our study (aIRR: 1.14; 95% CI: 0.38-3.46). Due to the small size of our study, we cannot exclude the risk of a type 2 error (false negative). Yet, as we analysed risk of MI in never-smoking HIV-infected individuals using data from a nationwide cohort, and detected only very few MIs, it seems crucial to consider that these results might actually illustrate the lack of excess risk associated with being HIV positive, among never smoking individuals.

POPULATION ATTRIBUTABLE FRACTION ASSOCIATED WITH SMOKING

The higher prevalence of CVD risk factors do not fully explain the increased risk of MI among HIV-infected individual; yet, they exert an important modifiable or even preventable risk [49, 186, 306]. Interventions to reduce these modifiable risk factors should thus be actively pursued. However, in a society with scarce resources, it seems of utmost importance that the money is used were they will contribute the most. Although many of the conventional risk factors seem highly prevalent, their contribution to MI in general and

compared with that of the general population could be small, as illustrated in the matched case-control study (data from 1997-2009) by Calvo-Sánchez et al [283]. In this study [283] the estimated population attributable risk (PAR) for developing acute coronary syndrome (ACS) was almost twice as high for smoking (54.35% vs. 30.58%) in HIV-infected individuals compared to non-HIV-infected individuals, whereas the PAR for DM (6.57% vs. 17.24%) and hypertension (9.07% vs. 38.81%), despite a high prevalence of these risk factors, was much lower for the HIV-infected population than that of the non-HIV-infected population. The PAR for DM (6.57%) and hypertension (9.07%) in the HIV-infected population was much lower than the PAR for smoking (54.35%) in the HIV-infected population. Whereas, smoking accounted for 54.35% of all ACS in the HIV-infected population, the combined PAR of all three risk factors only accounted for 60%. In contrast, the combined PAR (68.75%) in the non-HIV-infected population was more than the double of the PAR for smoking (30.58%) [283].

We found (STUDY 3) that more than 75% of MIs in the Danish HIV-infected population was associated with ever smoking, which is larger than that described by Saves et al [45] (65% for men and 29% for women), and Calvo-Sanchez et al [283] (PAF: 54.35%) and much larger than that found by Lifson et al [305](current vs non-smokers PAF: 25.3% and current vs. previous smokers PAF: 17.4%). Still, as previously described, the PAF is difficult to extrapolate between populations and regions. As such the PAF are specific to the certain population and may not be representative of other HIV-infected populations.

The observed association with smoking may represent the detrimental effects of tobacco in which the PAF would illustrate the fraction of the risk of MI that is attributable to smoking and thus could be prevented, if smoking were eliminated; however, as mentioned above, an underlying difference in behaviour, comorbidity and social disparities might also exist [307] and as such represent an effect of being a smoker rather than the causal effect of tobacco alone. Nonetheless, as smoking is associated with increased rates of not only atherosclerotic disease, but also chronic pulmonary disease, non-AIDS-associated cancers, decreased bone mineral density, periodontal disease, oral and anal infection with human papilloma virus and potentially cancers related to human papilloma virus, bacterial pneumonia, several AIDS defining conditions and death in HIV-infected individuals as well as the quality of life and maybe the immunologic response to HAART [253, 300-305], smoking may pose a much bigger threat for the HIV-infected population than it might have done in the early epidemic. As a high consumption of alcohol, illicit drug use and collateral psychiatric disorders as well as a lower socio-economic status is highly associated with smoking in the HIV-infected population [303, 307-309], smoking cessation programs may face numerous barriers prior to success. Special designed interventions that effectively reduce the prevalence of smoking should thus be highly prioritized and integrated in modern HIV care, and may even be the intervention with the greatest impact on morbidity and mortality in general [304, 310]. Still, other underlying comorbidities and unconventional risk factors should also be addressed.

ABACAVIR AND RISK OF CARDIOVASCULAR DISEASE

The unexpected finding of a 90% increased risk of MI (95% CI: 1.47-2.45) in association with current/recent use (< 6months) of abacavir independent of cardiovascular risk factors was initially presented by the D:A:D study group in 2008 at the 15th Conference on Retroviruses and Opportunistic Infections (CROI)[311] and later published in the Lancet [189]. Consistent results were subsequently reproduced in a post-hoc analysis by the SMART study [312] for both MI (19 events; aIRR: 4.25; 95% CI: 1.39-13.0), and the composite endpoints minor CVD (58 events; aIRR: 2.70; 95% CI: 1.51-4.83), major CVD (70 events; aIRR: 1.80; 95% CI: 1.04-3.11) and expanded CVD (112 events; aIRR: 1.91; 95% CI: 1.25-2.92). The Australian STEAL study [313], an RCT examining 357 HIV-infected individuals already on HAART switching to a fixed dose of a regimen including tenofovir or abacavir, found that tenofovir was associated with a lower rate of CVD than abacavir (1 vs. 8 events; HR: 0.12; 95% CI: 0.02-0.98). However, the power of the STEAL study, and to some extend the SMART study, was limited given the shorter duration of follow-up and the low number of events. Many subsequent observational studies have supported an abacavir associated higher risk of MI [183, 314, 315] and risk of cerebrovascular events[290]. The latter association was in contrast to the results of the DAD study [189]; however, due to the poor presentation of these results in the D:A:D study[189], further discussion of study differences is problematic.

We further extended these findings (STUDY 3) with the results of a nonstatistically significant 1.74-fold risk of MI in association with current abacavir exposure among never smokers which parallels the findings by the D:A:D-study [189]. Yet, in current smokers concurrent abacavir exposure was associated with a 3.6 times higher risk of MI, which extend the findings of the SMART study [312] of a higher risk of MI associated with current use of abacavir in those with an underlying increased risk of CVD.

Two observational studies from the French Hospital Database [188] and the Veterans Administration database [316] could not replicate an association between either current/recent or cumulative exposure to abacavir and risk of MI, CVD or stroke when stratifying the results by use of cocaine or IDU [188], or adjusting the analyses for chronic kidney disease [316]. Also, Triant et al found no such association with abacavir exposure [317]. GlaxoSmithKline pooled the data of 52 RCTs undertaken in the period 1995-2006 (14,174 naïve individuals) and found no increased risk of MI in association with abacavir exposure [318, 319]; however, only 12 of the included trials were randomized for abacavir vs. control. In addition, PI therapy in the control arm might have confounded the results [319]. Consistent results were also presented in a small meta-analysis (6RCTs) by Ribaudo et al [320], a large comprehensive meta-analysis (28 RCTs) by Cruciani et al [321], and a large meta-analysis (26 RCTs) performed by the American FDA [322]. Nevertheless, contrasting results has recently been published in a meta-analysis including both RCTs and observational studies [62]. And to make it even more confusing the Swiss HIV Cohort Study recently presented an unpublished abstract at the 7thInternational AIDS Society conference in Kuala Lumpur [323]of no risk associated with cumulative (aIRR: 1.04; 95%CI: 0.99-1.09) or current abacavir exposure (aIRR: 0.42;

95%CI: 0.25-0.69). However, any abacavir use within the last 1-6 moths was associated with a more than 3-fold risk of CVD (aIRR: 3.36; 95% CI: 2.04-5.53), why it was judged that the current risk of CVD cumulated with abacavir exposure over the past 6-24months [323].

The discrepancies between studies may rely on a number of differences between studies (design, set-up, analytical approach). Given the selected healthier population with well-controlled HIV disease and the shorter time of follow-up used in RCTs (usually 24- and 48- weeks), a potentially low rate of MI would be expected. A difference in MI rate could therefore be almost impossible to detect [324]. Furthermore, MI or CVD was not the primary outcome of the RCTs. This may have influenced the completeness of the reporting of such adverse events. Although, many HIV cohorts produce large representative samples with long follow-up, which makes them highly efficient in evaluating the risk of rare events, the observational design for analyses of drug effects is not without problems. Lack of randomization of abacavir, presence of channelling bias (i.e. preferred use of abacavir for individuals with high CVD risk due to perceived metabolic and renal safety) and confounding by factors such as smoking, dyslipidaemia, DM, kidney function or use of cocaine and IDU, has been thoroughly discussed [188, 316, 324-327]. Renal dysfunction is known to channel the choice of drug away from tenofovir and towards abacavir in order to avoid additional nephrotoxicity [316, 328]. However, although slightly attenuated, the risk of CVD in association with abacavir exposure still remained (aIRR: 1.48; 95% CI: 1.08-2.04) when Choi et al [315] adjusted their analyses for kidney disease. Although, we restricted our analyses to individuals with no IDU, and stratified our results on smoking status, we cannot exclude confounding due to chronic kidney disease. However, a later performed sub-group analysis by the D:A:D study-group, showed that accounting for glomerular filtration rate in their analyses did not change their results to a considerable extent [329].

As abacavir has been used both as a first- and a second line regimen (salvage regimen) this could represent additional problems. Nonetheless, a study from the DHCS [314] found an increased risk of MI in association with abacavir exposure for individuals initiating abacavir within 2 years of HAART initiation as opposed to after 2 years. Yet, the estimate was higher in the group who initiated abacavir after more than 2 years, thus representing differences in characteristics e.g. adherence problems or drug toxicity, and thus confounding by indication or channelling bias [314]. Nevertheless, application of the propensity score matching method did not change the estimates to a considerable extend [314].

As the risk of MI in association with abacavir disappeared after drug cessation (up to 6 month after) in the original D:A:D study, a rapid potentially inflammatory mechanism was proposed [189, 324]. Nonetheless, such reversible association has not been established in all studies [290, 314, 323]. Finally, it has been noted that studies showing a significant association with abacavir exposure predominantly consisted of HAART-experienced individuals as opposed to the predominantly HAART naïve individuals included in RCTs [316]. As such, difference in VL and CD4+ count, immune activation and inflammation markers between studies for and against an association could also lead to differences in results [330].

PLAUSIBLE BIOLOGICAL MECHANISM OF THE ABACAVIR ASSOCIATED RISK OF MI

Due to the conflicting results of abacavir exposure and risk of CVD, a plausible causal mechanism has been sought. As abacavir is associated with the risk of a hypersensitivity reaction in individuals with HLA B*5701, which may represent pro-inflammatory properties, a more protracted, but similar mechanism has been suggested to be involved in the development of CVD [283, 331, 332]. In addition, altered platelet reactivity has been implicated as an underlying mechanism [333, 334]. We (STUDY 3) observed a potential synergistic effect of smoking and abacavir exposure as the combination of both exposure conferred a risk higher than the addition of the risk conferred by the individual components . As smoking may affect the platelet reactivity [335, 336], such results could support a plausible explanation of a clinically significant interaction. However, as we could not distinguish the effect of tobacco and the effect of being a smoker, this needs further investigation. Other explanations of a possible abacavir associated mechanism include the possible link between abacavir and a lower arterial FMD acting as a measure of impaired endothelial function (arterial stiffness) [337]. Nevertheless, in contrast to the results of a small study by Hsue et al [337], a recent study by Wohl et al [338] found no difference in FMD in individuals who had randomly been allocated to abacavir or tenofovir. Despite only moderate changes in cholesterol with abacavir exposure, a small study by Sinn et al [339] found that 4 weeks after a switch from abacavir to tenofovir, the augmentation index and the Framingham score was significantly reduced mainly driven by a lower TC (0.8mmol/L, p: 0.002). In addition, some studies have found higher levels of TG in association with abacavir compared to tenofovir [190, 338]; however, higher levels of TG has previously been established to have small if any effect on risk of MI in HIV-infected individuals [340].

Further mechanisms, including associations with T-cell activation and endothelial leukocyte adhesion, oxidative stress, apoptosis as well biomarkers of inflammation, coagulation and endothelial function associated with risk of CVD have yielded conflicting results [166, 192, 312, 338, 341, 342`, 343-351]. As such, conclusions regarding a causal relationship cannot be drawn, why the subject remains unresolved and highly confusing for the acting clinician.

POPULATION ATTRIBUTABLE FRACTION ASSOCIATED WITH ABACAVIR

However, it is worth emphasizing that contrary to the D:A:D study [189], that found a marginally significant interaction with low and medium/high CVD risk, the SMART study [312] showed that the excess risk of CVD associated with abacavir exposure increased with the level of underlying risk factors (>= 5 risk factors ~ aIRR: 3.06; no risk factors ~ aIRR: 1.34). In concordance, we found (STUDY 3) that the association of abacavir exposure on risk of MI was highly dependent on smoking-status with a stepwise increased risk of MI. As we cannot assume causality between abacavir and risk of MI, we cannot determine whether a switch in choice of drug away from abacavir would indeed result in a reduction in MI risk. Still, our results indicated that 36.6 % of the MI's were associated with abacavir exposure in HIV-infected ever smokers,

whereas the association in never smokers was small (2.7%). These findings further confirm the calculations based on the Framingham equation model made by Kowalska et al [352], that estimated that if all 50-year-old HIV-infected individuals, with no other risk factors for MI than smoking, terminated smoking, the number of MIs attributed to abacavir exposure would decline with time by 83.9%. This implicate, that the potential excess risk associated with abacavir is most marked among individuals with a high CVD risk, in whom it seems reasonable to avoid this drug, whereas the risk seems negligible in those with a low CVD risk [352].

RISK OF MYOCARDIAL INFARCTION IN AN AGING HIV-IFECTED POPULATION

Until now the absolute risk of MI, has remained low given the young age of the HIV-infected population. Yet, given the expanded survival of HIV-infected individuals, and hence progressively aging population, the absolute risk will substantially increase, as age remains the strongest risk factor for CVD. Recently, it was shown that HIV-infected individuals with an age of 65 years or older had an almost 6 times higher risk of MI, an almost 18 times higher risk of stroke , and an almost 4 times higher risk of DM relative to HIV-infected individuals younger than 50 years [353]. However, the excess risk compared to the background population, including age-stratified IRR, could not be evaluated. As such, the evidence of a potentially accelerated risk of MI or CVD, still seems limited [138, 354]. Nevertheless, a recent study [355] showed a significant interaction between HIV, age and smoking on cIMT, suggesting that HIV potentially modifies the relationship of age and smoking on cIMT, thus emphasizing the need to modify risk associated behaviour.

PROGNOSIS OF MYOCARDIAL INFARCTION

As previously described, the D:A:D study in 2003 stated that the risk of MI increased progressively with a 26% relative increase in risk of MI per additional year of HAART exposure [112]. If HAART was to induce a fast progressing atherosclerosis that would proceed after an MI diagnosis, this could have devastating consequences for the post-MI prognosis. However, unpublished data from the EuroSIDA on trends over calendar time presented in a paper by Phillips et al [356] indicated that the start of the HAART era was actually accompanied with a decline in death from non-AIDS causes. Moreover, the D:A:D study group [199] in 2008 found that despite increasing age and thus deteriorations in CVD risk profile since 1999, MI rates have remained relatively stable. This could be due to a more aggressive targeted approach towards risk factors as well as use of or switch to more lipid friendly HAART regimens [199]. However, as the D:A:D study, as opposed to the Danish HIV cohort study, is as closed cohort, and individuals are censored at date of MI, they could not exclude that these results were not due to an artificial reduction in the incidence of MI, due to drop out from the risk set of high risk individuals (~ healthier population left in the dataset). In extension, a recent study by Helleberg et al [357] showed that although HIV-infected individuals with no

IDU had a 2.4 times higher mortality rate (MR) of ischemic heart disease and a 2.6 times higher MR of stroke compared to a well-matched comparison cohort, the age-standardized mortality rates (MR) associated with cardiovascular death did not change substantially from 1995-2008 [357]. Also, Bonnet et al [358] found stable rates of CVD between year 2000 and 2004.

POST-MYOCARDIAL INFARCTION MORTALITY

We (STUDY 4) [359] compared the rate of death in HIV-infected individuals who had experienced an MI on HAART with that in 1) non-HIV-infected individuals with an MI and 2) HIV-infected individuals with no MI. We evaluated rate of death in two periods (short-term: 0-3 months, long-term: > 3months) due to violation of the proportional hazard assumption as well as predetermined time-split of clinical interest. We [359] observed that a diagnosis of MI was associated with a 10.3 times higher short-term mortality in HIV-infected individuals (time < 3 months; 95% CI: 2.1-54.9), whereas no difference in the long-term mortality was observed (time ≥ 3months: aMRR: 1.4 ; 95% CI: 0.6 - 3.5). Likewise, an HIV diagnosis did not change the short-term mortality among individuals with an MI diagnosed (time < 3 months: aMRR: 1.5; 95% CI: 0.5 -4.1); however, the long-term mortality increased with a factor 7.4 (time ≥ 3months; 95% CI: 2.1 - 26.3). In spite of a relatively short observation period and a low numbers of deaths, this do not indicate that HIV leads to a more aggressive post-MI prognosis [359]. Although, an MI diagnosis may increase the mortality in HIV-infected individuals, we found no indication that an HIV diagnosis increased mortality in individuals with an MI diagnosis beyond what was expected from the addition of the risks of the two diseases.

We could not evaluate in-hospital mortality and as such evaluated the individuals who had survived until date of discharge. Yet, a small study by Matetzky et al [360] found a rather benign in-hospital course, with no deaths or re-stenosis. Also, Ambrose et al [361] and Escaut et al [171] found a low in-hospital mortality; however, neither of these studies had a control group for comparison. In contrast, a large American study by Pearce et al [362], observed a higher in-hospital mortality in HIV-infected individuals with an MI compared to non-HIV-infected MI individuals, also after adjustments for a substantial lower rate of MI procedures (aIRR: 1.38; 95% CI: 1.1-1.87) [362].Yet, substance abuse was not included in the model, and controlling for smoking and dyslipidaemia seemed limited by potential underreporting in the HIV-infected group compared to the non-HIV-infected group (prevalence of smoking: 25% vs. 30% and dyslipidaemia: 25% vs. 42%) thus potentially explaining some of the excess mortality observed. In contrast, a French study by Lorgis et al [273] used the national administrative database and reported a similar in-hospital (3.1% vs. 2.1%, P: 0.168) and 1-year post-MI mortality (1.4% vs. 1.7%, P: 0.642) for HIV-infected and non-HIV-infected MI individuals matched on age, gender and type of MI. Of note, 27% of the study population (the percentage of HIV/non-HIV not described) were loss to follow. This may have biased the long-term results, especially as the study reported fractions instead of relative rates (survival analysis) with censoring at loss-to follow.

CONCLUSIONS

HIV-infected individuals are at increased risk of MI and potentially other vascular diseases. Yet, in spite of a large number of studies on this issue, research concerning the potential causes and mechanisms are still ongoing. However, treatment toxicity as well as persistent immune dysfunction, chronic immune activation, sustained inflammation, and potentially accelerated aging seems to play a part. Yet, as shown in the present study, traditional risk factors, including smoking and other risk-taking behaviour and potentially presence of concurrent diseases may actually account for an even larger and quite important part of the CVD risk, as this, theoretically spoken, is modifiable.

Despite risk of long-term complications, HAART still provides substantial survival benefit for the HIV-infected population. In extension, substantial evidence indicate that, although some antiretroviral drugs may increase the risk of CVD, continuous HAART in general may indeed reduce the risk of not only AIDS-associated conditions and death, but also non-AIDS associated conditions like CVD. The reason may be associated with reductions in inflammation markers, viral replication and improved immune function; however, this still needs further exploration. Finally, there is no indication that the survival benefit provided by HAART is negated by a HAART-induced accelerated post-MI-prognosis.

In these years the scarce resources have become a reality. Hence, prioritization of health resources is of outmost importance. As such, it seems prudent to attempt to reduce modifiable risk factors. Physicians should encourage therapeutic lifestyle interventions including smoking cessation and intervention in order to avoid a sedentary lifestyle with risk of obesity. Furthermore, careful selection of antiretroviral drugs should be applied according to the underlying CVD risk profile.

PERSPECTIVES

Whether the HIV-infected population ages prematurely remains to be established. However, an aging HIV-infected population is a reality not only for the prosperous countries. An aging HIV population will lead to new challenges and problems [278, 279], such as multi-morbidity[50, 353] and poly-pharmacy (multiple drug-regimens, adherence problems, drug interactions and toxicities). As such, strategies to overcome problems associated with multi-morbidity and poly-pharmacy should be further explored. In general, efforts are needed to bring down the prevalence of CVD risk factors and improve the quality of life for the individual living with HIV. The cost effectiveness of including focused counselling towards smoking, alcohol, illicit drug abuse, diet and exercise as well as focus on the psychological health of people living with a chronic illness needs further exploration.

ENGLISH SUMMARY

This PhD thesis is composed of four articles and a review. The work leading to the thesis began in 2006, at a time when cardiovascular research in association with human immunodeficiency virus (HIV) and highly active antiretroviral therapy (HAART) was at its highest. After years of struggling with keeping HIV-infected individuals alive, the impressive effect of HAART had led to increased lifespans why HAART-associated toxicities including glucose alterations, dyslipidaemia and alterations in body fat, became increasingly important. Concerns were thus raised of the risk of diabetes mellitus (DM) and myocardial infarction (MI), and the impact of associated risk factors including antiretroviral drugs. Moreover, a potential devastating prognosis was proposed with accelerated atherosclerosis even after experiencing an MI. We therefore aimed to address topics that have been of particular interest among HIV researchers and clinicians including risk of DM and MI, and associated risk factors. We used the large, exceptional Danish health care registry system including the Danish HIV Cohort Study, a comprehensive nationwide observational clinical database of HIV-infected individuals.

In accordance with most other studies, our results supported a strong association with traditional risk factors for DM including older age and higher BMI. Presence of lipoatrophy further increased the risk of DM with a factor 2. We established that native individuals living with HIV had a higher rate of DM in the first years after introduction of HAART (1996-1998) relative to native population controls irrespective of HAART exposure. However, importantly risk of DM in the HIV era after 1998 was not increased. Still, a linkage with the use of indinavir, saquinavir, stavudine and didanosine and risk of DM was found. Nevertheless, as these drugs are not used in modern HAART-regimens in the western world, the associated risk is mainly a problem in resource limited settings.

Previous reports have established that the presence of HIV infection and HAART exposure is associated with a 1.5-2-fold higher rate of MI. In contrast, the mechanisms that drive the association are widely debated, and seem to include several unknown factors.

It is well-established that cumulative exposure towards protease inhibitors is associated with a higher risk of MI. In contrast, a potential association with current abacavir exposure seem less clear, as no causal relationship has been established, and as channelling bias and significant confounding remains an obstacle for the interpretation of established results. Of note, we found a higher risk of MI in mothers of HIV-infected off-springs compared with mothers of population controls, indicating that beside potential toxic effects of the antiretroviral drugs, characteristics associated with an HIV positive status including socio-economic, behavioural and life-style associated factors and potentially risk taking behaviour, may also have a large impact on previously established risk estimates.

The HIV-infected population has been described to have a high prevalence of conventional CVD risk factors, mainly smoking; yet, many MI risk estimates could be confounded by this factor. Of note, we found that the risk of MI was highly dependent on smoking status. In contrast to previous studies, our results indicated that

HIV-infected individuals who had never smoked had no increased risk of MI compared with the general population. Conversely, current smoking increased risk of MI with a factor 1.8. In accordance, with the SMART study that showed that the cardiovascular disease risk associated with abacavir exposure increased with the level of underlying risk factors, we found that the risk of MI associated with abacavir increased with the underlying level of smoking. Furthermore, risk of MI in current smokers exposed to abacavir had a more than 13 times higher risk of MI than HIV-infected individuals with none of these exposures, indicating a possible synergistic relationship of these two factors.

It seems prudent to reduce the risk of MI in this population, in order not to counteract the long term beneficial effects of HAART. However, as resources are limited, it is important to use the most cost-effective interventions. We thus calculated the population attributable fraction (PAF) of MIs associated smoking and abacavir.

We found that more than 75% of MIs in the Danish HIV-infected population was associated with ever smoking and more than 80% were associated with smoking and/or abacavir exposure. In contrast, the PAF associated with abacavir exposure in the absence of smoking was negligible.

Finally, we found that there was no indication that an MI diagnosis should increase the risk of death more than what is expected given the presence of both diagnoses. We believe that these findings will be of interest for physicians treating HIV-infected individuals and is of importance for the public health by providing some guidance in the prioritization of interventions.

DANSK RESUMÉ

Ph.d. afhandlingen indeholder 4 originalarbejder og en oversigt. Da arbejdet på denne ph.d. påbegyndtes i 2006 var forskning inden for kardiovaskulær sygdom i relation til human immundefekt virus (HIV) og højaktiv antiretroviral behandling (HAART) på sit højeste. Efter i flere år at have kæmpet for at holde HIV smittede patienter i live, havde den imponerede effekt af HAART medført et betydeligt fald i dødeligheden og dermed en forlænget levetid. I forlængelse af dette fik bivirkninger til HAART inklusiv påvirket glukose metabolisme, dyslipidaemi og ændringer i fordelingen af kropsfedt en større betydning og den potentielle risiko for udvikling af diabetes mellitus (DM) og akut myokardie infarkt (AMI) medførte derfor udtalt bekymring. Den formodede prognose blev endvidere spået til at være yderst aggressiv på baggrund af en accelereret arteriosklerose også efter et overstået AMI.

Formålet med ph.d.en var derfor at undersøge kerneområde med speciel interesse for HIV specialister og forskere inden for området heriblandt risikoen for DM og AMI og associerede risikofaktorer så som effekten af antiretrovirale lægemidler. Studierne bygger primært på data fra det Danske HIV kohorte studie, et stort landsdækkende population-baseret register over danske HIV smittede patienter.

I tråd med resultater fra andre studier fandt vi at en ændring i de traditioneller risikofaktorer for udviklingen af DM inklusiv en højere alder og et højere body mass index havde samme effekt på risikoen for DM som i baggrundspopulationen. Tilstedeværelsen af lipoatrofi medførte endvidere en 2 gange større risiko for DM. Vi fandt at dansk fødte HIV positive patienter havde en næsten 3 gange højere risiko for AMI i de første år efter introduktionen af HAART (1996-1998) sammenlignet med dansk fødte populationskontroller i samme periode. I modsætning hertil var den efterfølgende periode, og således den nuværende HIV æra, ikke forbundet med nogen øget risiko for DM. På trods af dette viste enkelte antiretrovirale lægemidler (indinavir, saquinavir, stavudin og didanosin) tegn på en associeret risiko øgning for DM. I kraft af at disse lægemidler sjældent benyttes i den vestlige verdens moderne HAART regimer har disse resultater primært betydning for lande, som på baggrund af få ressourcer, stadig benytter sig af ældre antiretrovirale lægemidler med betydelige bivirkninger.

Tidligere studier har vist, at en positive HIV status og behandling med HAART er forbundet med 1,5-2 gange større risiko for AMI. Til gengæld er årsagerne til denne øgede risiko fortsat stærkt debatteret. Vores resultater viste en øget risiko for AMI hos mødre til HIV positive patienter set i forhold til mødre af populationskontroller, hvilket indikerer at andre kendte og ukendte faktorer også spiller ind heriblandt socioøkonomiske så vel som adfærds og livsstils relaterede faktorer samt en potentielt set risiko betonet adfærd.

Det er veletableret at den kumulerede eksponering for protease inhibitorer er forbundet med en øget risiko for AMI. En potentiel association med abacavir lader dog til at være langt mere problematisk, da den kausale sammenhæng er ukendt og channelling bias samt betydelig confounding har været en hæmsko for fortolkning af de etablerede resultater. På tråds af at HIV smittede patienter generelt set har en højere

prævalens af almindelige kardiovaskulære risikofaktorer, især rygning, er en betydelig andel af tidligere studier påvirket af manglende kontrollering for rygestatus. I modsætning til flere andre studier fandt vi at risikoen for AMI hos ikke-rygende HIV positive patienter var på samme niveau som den ikke rygende baggrundsbefolkning. Nuværende rygning var til gengæld forbundet med en 1.8 gange øget risiko for AMI. I tråd med resultater fra SMART studiet, fandt vi at den abacavir associerede risiko for AMI steg med graden af rygestatus. Risikoen for AMI hos rygende HIV positive patienter med samtidig eksponering for abacavir steg med mere end en faktor 13 sammenlignet med ikke rygende HIV positive patient udsat for behandlingsregimer uden abacavir. Et sådan resultat indikerer et potentielt set underliggende synergistisk forhold.

For ikke at udligne den positive effekt af HAART er det vigtigt at forebygge og potentielt set reducerer risikoen for AMI i den HIV smittede population. I kraft af begrænsede ressourcer, er det dog yderst nødvendig at pengene benyttes der hvor de bidrager med den største effekt. Vi beregnede derfor den populations associerede fraktion (PAF) af AMI tilfældene som var associeret med rygning eller abacavir. Vi fandt at rygning var relateret til mere end 75 % af de AMI tilfælde som diagnosticeres hos danske HIV smittede patienter hvorimod rygning og abacavir var relateret til mere end 80 %. I modsætning hertil var bidraget af abacavir forsvindende lille hos HIV positive patienter som ikke røg.

På trods af en spået dårlig prognose for HIV positive patienter som har udviklet et AMI, fandt vi at dødeligheden ikke var større en hvad vi havde forventet givet summen af de to diagnoser. Generelt set mener vi at vores resultater har betydning for både den behandlende læge i HIV ambulatoriet såvel som i led med den politiske prioritering af interventioner.

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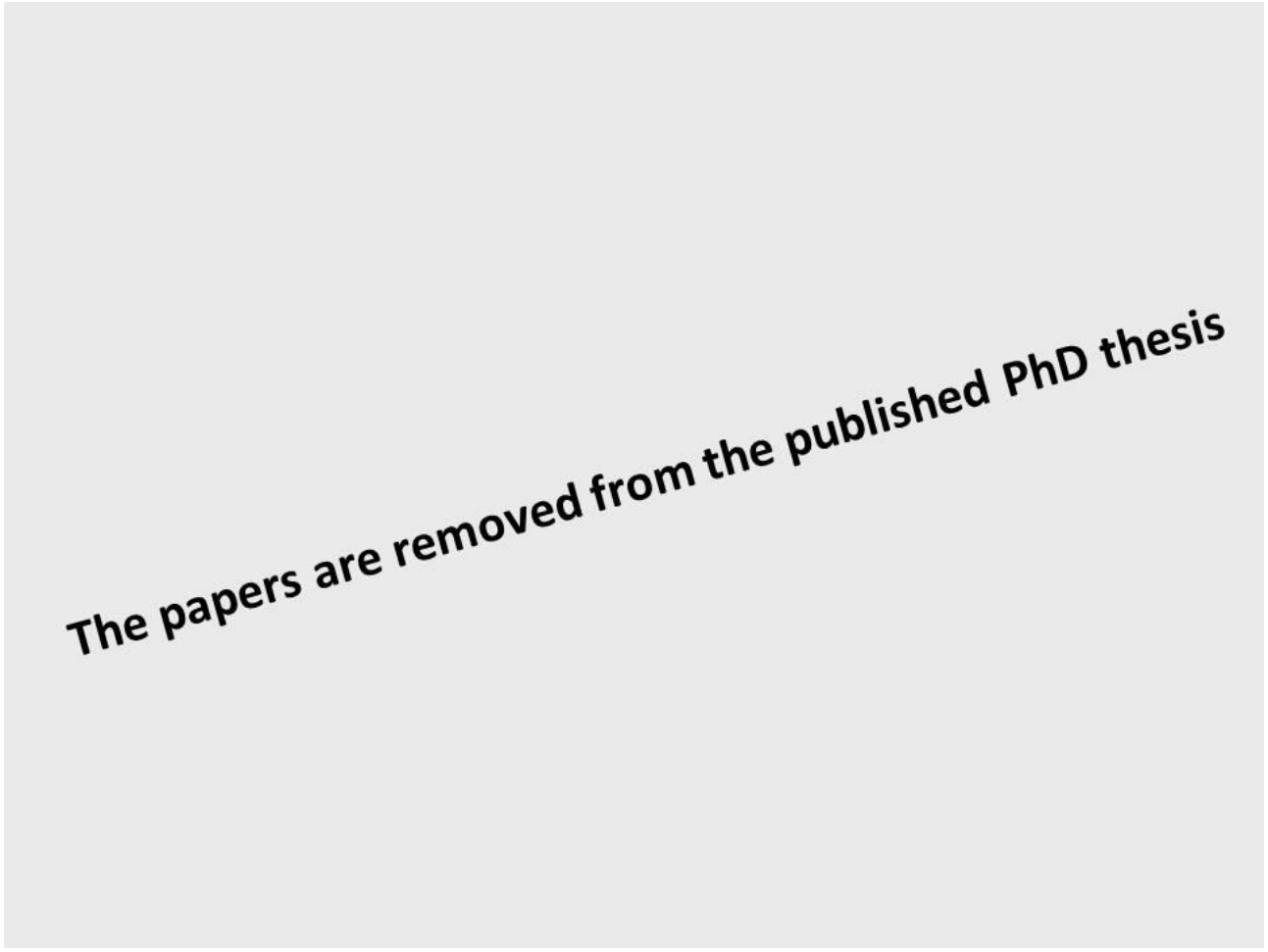
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