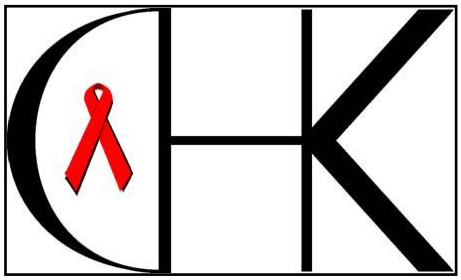
DOCTORAL DISSERTATION

Aging and co-morbidity among HIV-infected individuals.



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Denne afhandling er den 27. februar 2017 af Akademisk Råd, Det Sundhedsvidenskabelige Fakultet, Syddansk Universitet antaget til forsvar for den medicinske doktorgrad.

Ole Skøtt

h.a.dec.

Forsvaret finder sted den 4. maj, 2017 kl. 14.00 i auditoriet WP25, J.B. Winsløwsvej 25, 5000 Odense C.

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ISBN: 978-87-93496-46-0

PREFACE

This thesis is based on research performed during the period 2006-2015 while I was employed at the Department of Infectious Diseases at Odense University Hospital. As the scientific work would not have been possible without the data from the Danish HIV Cohort Study, I am indebted to the founder as well as the steering committee and all the departments that continue to deliver invaluable data to the cohort.

Above all, I feel sincerely privileged to have had the possibility to work with 3 admirable researchers: Niels Obel, Court Pedersen and Jan Gertstoft. With their impressive scientific insight, expertise in the HIV field and ability to complement each other and inspire me, their guidance and constructive criticism have always been highly rated.

I am grateful to all my co-authors that contributed to the research projects.

Thanks to all previous and current colleagues.

And finally I want to express my gratitude to my husband (Christian) and 3 boys (Emil, Peter and Carl Theodor) for their encouragement and tremendous patience with me.

Line Dahlerup Rasmussen, Odense, May 2016.

PUBLICATIONS

This thesis is based on the following papers:

I. Risk of cerebrovascular events in persons with and without HIV: A Danish nationwide population-based cohort study. Rasmussen LD, Engsig F, Christensen H, Gerstoft J, Kronborg G, Pedersen C, Obel N. AIDS. 2011; 25: 1637-46.

II. HIV and risk of venous thromboembolism: a Danish nationwide population-based cohort study. Rasmussen LD, Dybdal M, Gerstoft J, Kronborg G, Larsen C, Pedersen C, Pedersen G, Jensen J, Pedersen L, Sørensen HT, Obel N. HIV Med. 2011; 12: 202-10. Epub 2010.

III. Myocardial infarction among Danish HIV-infected individuals: Population attributable fractions associated with smoking. Rasmussen LD, Helleberg M, May MT, Afzal S, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Nordestgaard BG, Obel N. Clin Infect Dis. 2015. May 1;60(9):1415-23

IV. Utilization of psychotropic drugs prescribed to persons with and without HIV; A Danish natinwide population-based cohort study. Rasmussen LD, Obel D, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Obel N. HIV Med. 2014: 15:458-69. Epub 2014.

V. Statin therapy and mortality in HIV-infected individuals; A Danish nationwide population-based cohort study. Rasmussen LD, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Obel N. PLOS One 2013 8:e52828.;Epub 2013.

VI. Risk of cataract surgery in HIV-infected individuals: A Danish nationwide population-based cohort study. Rasmussen LD, Kessel L, Molander LD, Pedersen C, Gerstoft J, Kronborg G, Obel N. Clin Infect Dis. 2011; 53: 1156-63. Epub 2011

VII. Time trends in risk of severe age-related diseases among persons with and without HIV infection; A Danish nationwide population-based cohort study. Rasmussen LD, May MT, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Obel N. Lancet HIV 2015:DOI:10.1016/S2352-3018(15)00077-6.

* The unpublished edition of paper III was included in my PhD thesis in a version that differed substantially from the published version. The dataset has been changed, and the methods, results and discussion extensively changed in the current edition".

ABBREVIATIONS

ADMA: asymmetric dimethyl arginine
AIDS: Acquired Immunodeficiency Syndrome
aHR: adjusted Hazard Ratio
aIRR: adjusted Incidence Rate Ratio
aMRR: adjusted Mortality Rate Ratio
aMRR: adjusted Mortality Rate Ratio
ANRS: Agence Nationale de Recherche sur le Sida
ATHENA: AIDS Therapy Evaluation in the Netherlands national observational
cART: combination AntiRetroviral Therapy
CASCADE: Concerted Action on SeroConversion to AIDS and Death in Europe
CI: Confidence Interval
CMV: CytoMegaloVirus
CVD: CardioVascular Disease
D:A:D: Data collection on Adverse effects of antiretroviral Drugs
DDD: Defined Daily Dose
DHCS: Danish HIV Cohort Study
DM: Diabetes Mellitus
FDA: American Food and Drug Association
ESC/EAS: European Society of Cardiology and European Atherosclerosis Society
FIRST: Flexible Initial Retrovirus Suppressive Therapies trial,
HAART: Highly Active AntiRetroviral Therapy
HIV: Human Immunodeficiency Virus
HCV: Hepatitis C Virus
HOPS: HIV Outpatient Study
HR: Hazard Rate
IDU: Injection drug abuse
hsCRP: high sensitive C Reactive Protein
IL-6: InterLeukin-6
IR: Incidence Rate
IRR: Incidence Rate Ratio
JUPITER: Justification for the Use of Statins in Primary Prevention
LPS: LipoPolySaccharide
MI: Myocardial Infarction
MR: Mortality Rate
MRR: Mortality Rate Ratio
NNRTI: Non-Nucleoside Reverse Transcriptase inhibitor
NRTI: Nucleoside Reverse Transcriptase Inhibitor
PAF: Population Attributable Fraction
PAI-1: Plasminogen Activator Inhibitor 1
PI: Protease inhibitor
PIN: Personal Identification Number
RCT: Randomized Controlled Trial
SMART: Strategic Management of Antiretroviral Therapy Trial
SATURN: Stopping Atherosclerosis and Treating Unhealthuy Bone with Rosuvastatin in HIVtrial
suPAR: soluble urokinase Plasminogen Activator Receptor
sVCAM: soluble Vascular CellAdhesion Molecule1
sICAM: soluble Intercellular Adhesion Molecule 1
TNF-α: Tumor Necrosis Factor-α
VACS-VC: Veterans Aging Cohort Study-Virtual Cohort
VTE: Venous thromboembolism
vWF: von Willebrand Factor

TABLE OF CONTENT

INTRODUCTION

NATURAL HISTORY OF HUMAN IMMUNODEFICIENCY VIRUS AND AQUIRED IMMUNO DEFICIENCY VIRUS

Thirty-five years has elapsed since Acquired Immunodeficiency Syndrome (AIDS) was discovered in USA in the beginning of the 1981 with a cluster of young homosexual men with Kaposi's Sarcoma and/or Pneumocystis Pneumonia [1-4]. The first European [5, 6] and Danish cases [7-10] appeared shortly after the cases in America, although a Danish female surgeon returning from Zaire in 1976 with an AIDS-like syndrome, was later retrospectively diagnosed with AIDS [11]. These cases were followed by impressive epidemiological research of symptoms, signs, AIDS manifestations, characteristics of behaviour as well as an intense search for the cause of this destructive illness.

In 1983 the novel retrovirus, later known as Human Immunodeficiency Virus (HIV), was isolated and recognized as the causative agent [12-14]. Since then, HIV sequences have been identified in serum obtained in 1959 from a man from Congo and in a biopsy from 1960 [15, 16]. And, relaxed molecular clock analyses and other statistical approaches have dated the time at which HIV-1 group M started to diverge in humans to the beginning of the 20^{th} century [16],and recently placed the 1920s Kinshasa, Demographic Republic of Congo, as the focus of early transmission [17].

ANTIRETROVIRAL THERAPY

In 1986, mono-therapy with the first nucleoside reverse transcriptase inhibitor (NRTI), zidovudine, was introduced [18, 19]. Later other NRTIs arrived *(didanosine, lamivudine, stavudine and zalcitabine)*, and in 1994, dual NRTI-therapy was found to be more effective in slowing disease progression than mono-therapy [20-23]. Saquinavir, which was the first protease inhibitor (PI) approved for treatment, was approved by the American Food and Drug Association (FDA) in late 1995 [24-26].

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

In 1996 it was discovered that a combination of three antiretroviral drugs *(initially a "backbone" of two NRTIs and a PI)* was superior to dual therapy and had dramatic effects regarding viral suppression and gradual immune reconstitution [27-32]. This led to the introduction of Highly Active Antiretroviral Therapy (HAART), also named combination Antiretroviral Therapy (cART). Since then, HAART has had a profound impact on the HIV-associated prognoses including a declining AIDS-associated morbidity and mortality - thus extending the life expectancy of HIV-infected individuals [33-37]. During the last 20 years, the numbers of new drugs, targets and treatment modalities have largely emerged. "Boosted" PI-regimens *(~ concomitantly administered ritonavir)* have gradually replaced the "un-boosted" regimens, and non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimens have become the dominant first line treatment [38].

New drugs such as integrase inhibitors and entry inhibitors *(fusion inhibitors and co-receptor inhibitors)* have further arrived [39]. Importantly, the regimens have become more effective, better tolerated and the pill burden has generally decreased. As HIV-infected individuals now live longer, the management of HAART-associated toxicities as well as age-related problems *(including risk reduction and preventive care)* have therefore become of increasing importance.

HAART ASSOCIATED TOXICITIES:

Initially, the urgent need for new drugs lead to an accelerated and less constrained approval of new drugs that was based on short term trials [40]. Due to this, long-term effects and rare toxicities appeared and still appear after the approval of antiretroviral drugs, although, the level of severe toxicities has largely decreased with the introduction of newer drugs. Over the years, several short-term and long-term side-effects have been associated with antiretroviral drugs including anemia, bone marrow depression, hyperlactataemia, renal-, liver and mitochondrial toxicities *(mainly hepatic steatosis, peripheral neuropathy and peripheral lipoatrophy),* osteopenia, hypersensitivity reactions and rash, neuropsychiatric symptoms and metabolic alterations *(hyperlipidemia, impaired glucose tolerance and insulin resistance and maybe hypertension)* [40-56]. Some of these side-effects have led to major considerations concerning associated risks of end-organ diseases.

RISK OF MYOCARDIAL INFACTION

Spanning the past 15 years, risk of myocardial infarction (MI), ischemic heart disease and cardiovascular disease (CVD) in association with HIV and HAART has been the focus of intensive debate, extensive research and a considerable body of literature. Still, results are not consistent and the drivers of the association are not completely understood. Given the overall young age of the HIV population, the slow development of atherosclerosis, and thereby low absolute risk of MI, the sample size needed to detect a difference in risk has largely been prohibitive for establishing reliable estimates in the early years of the HAART era. Nevertheless, the major part of studies that examined risk of subclinical atherosclerosis as a surrogate measure of CVD, suggested an increased risk of CVD in association with a positive HIV status [57-74]. With time, many HIV cohorts have established the power to reach more credible estimates. Hence, based on results of several observational studies, HIV-infected individuals are believed to have a 1.5-2-fold increased risk of MI compared to the general population [75-94]. The proposed cause may rely on a number of known and unknown factors including a higher prevalence of conventional risk factors, exposure to potential toxic antiretroviral drugs and HIV infection in itself.

OTHER VASCULAR DISEASE

Despite less attention, HIV appears to exert a range of effects on other vascular diseases such as venous thromboembolism (VTE) and cerebrovascular

disease. VTE, defined as deep venous thrombosis and/or pulmonary embolism, is generally not considered an atherosclerotic disease; however, VTE and CVD share common risk factors [95-101]. Still, well performed studies with valid results on risk of cerebrovascular events and VTE in association with HIV and HAART are sparse and the contributing factors and potentially shared mechanisms are not fully elucidated.

POTENTIAL RISK FACTORS ASSOCIATED WITH MI, CVD AND OTHER AGE-RELATED DIASEASES

In the early part of the HAART era the main focus associated with the higher risk of MI and CVD was exposure to HAART including some individual antiretroviral agents with toxic potential. However, in year 2006 the results of the Strategic Management of Antiretroviral Therapy (SMART) trial [102] revealed that continuous exposure to HAART as opposed to drug sparing regimens reduced the risk of death as well as the risk of CVD. These results supported the hypothesis that risk of MI was partly mediated by long-term effects of HIV infection including degree of immunodeficiency and immune reconstitution on HAART, altered coagulation, and effects of chronic immune activation and persistent inflammation [103]. This was further extended by a hypothesis of HIV-induced accelerated aging as the reason for the overall increased risk of age-related conditions in the HIV-infected population [103]. However, the impact of the above mentioned factors seems unclear. Also, it seems imperative that we take into consideration the presence and impact of behavioural- and life-style associated factors, conventional risk factors and concurrent co-morbidity, among people living with HIV [85, 104-112]. Many of these factors may increase the risk of not only MI and CVD, but also contribute to the overall burden of age-related diseases among HIV-infected individuals. Therefore, it seems important to establish a higher awareness of important differences between individuals with and without HIV infection. As of these differences, it remains complicated to disentangle individual effects and relative contributions of potentially associated factors on risk of CVD and other age-related diseases.

THERAPEUTIC POTENTIALS BESIDE HAART

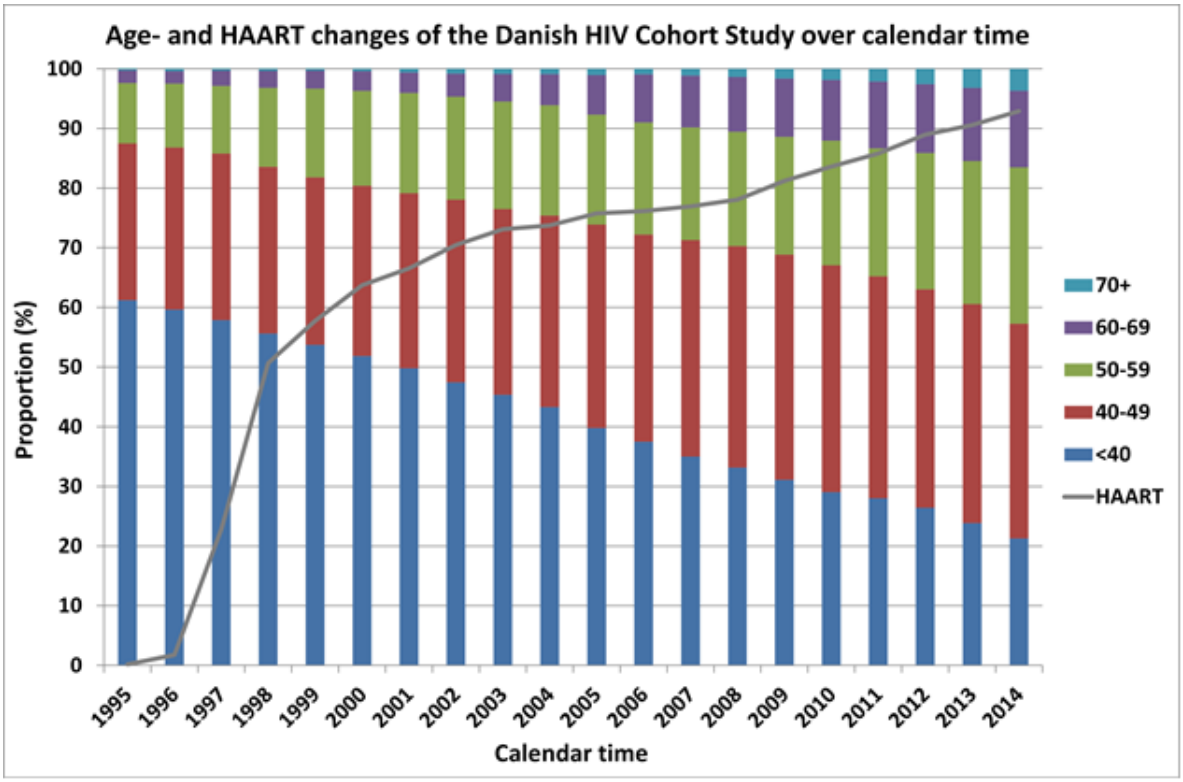
As HIV–induced inflammatory changes and immunologic effects may affect the endothelium and coagulation pathways, treatments that target pathways of immune activation and inflammation could be of interest. As an example, statin therapy might attenuate inflammation [113-118] thus potentially exerting beneficial effects on mortality in HIV-infected individuals beyond the known protective effects on risk of CVD. The potential role of statins in HIV infection has therefore been extensively debated during later years.

THE AGING HIV POPULATION

Globally seen the population living with HIV is aging [119, 120]. Also in Denmark, the percentage of the HIV-infected population being 50 years or older has increased considerably since the mid-nineties[121].

The knowledge of the long-term implications of aging with HIV is limited. As older age confer an increased propensity of age-related diseases and an associated high utilization of multiple non-antiretroviral drugs new challenges may emerge for people living with HIV as well as health care providers. On the contrary, the evidence of accelerated or premature aging, an overall poorly defined hypothesis that nevertheless has entangled the public media, still needs to be established.

Figure 1: Age-changes of the Danish HIV-population over calendar time



OBJECTIVES

The objectives of the thesis were:

1. To examine the risk of cerebrovascular events in HIV-infected individuals compared with the risk in the general population (STUDY 1).

2. To examine the impact of proven risk factors, risk factors often associated with HIV infection (e.g. IDU), immunodeficiency, use of antiretroviral drugs and HIV infection per se on risk of cerebrovascular events (STUDY 1).

3. To examine the risk of cerebrovascular events in parents of HIV-infected individuals to evaluate the impact of family-related risk factors (STUDY 1).

4. To examine the risk of VTE in HIV-infected individuals compared with the risk in the general population (STUDY 2).

5. To examine the impact of low CD4 cell count and HAART on the risk of VTE in HIV-infected individuals, as well as the risk posed by IDU (STUDY 2).

6. To estimate the risk of MI associated with smoking in HIV-infected individuals compared to the background population (STUDY 3)

7. To calculate the population attributable fraction (PAF) of MI associated with smoking in the Danish HIV-infected population and a matched population control cohort (STUDY 3).

8. To investigate longitudinal trends (by age, calendar year and time after HIV) in utilization of psychotropic drugs in HIV-infected individuals compared to an age and gender matched comparison cohort (STUDY 4).

9. To estimate the impact of HAART and efavirenz on utilization of psychotropic drugs (STUDY 4)

10. To use the utilization rate ratio (URR) as an estimate of the relative risk (RR) of psychiatric conditions (STUDY 4)

11. To estimate the impact of statin use on the risk of all-cause mortality in general as well as before and after a diagnosis of CVD, chronic kidney disease or DM (STUDY 5)

12. To determine the risk of cataract surgery in HIV-infected individuals compared with a population-based comparison cohort (STUDY 6)

13. To estimate the impact posed by predisposing ocular disease, low CD4 cell count and HAART in general as well as the effect of the following antiviral drugs: abacavir, tenofovir, PI and NNRTIs on risk of cataract surgery (STUDY 6)

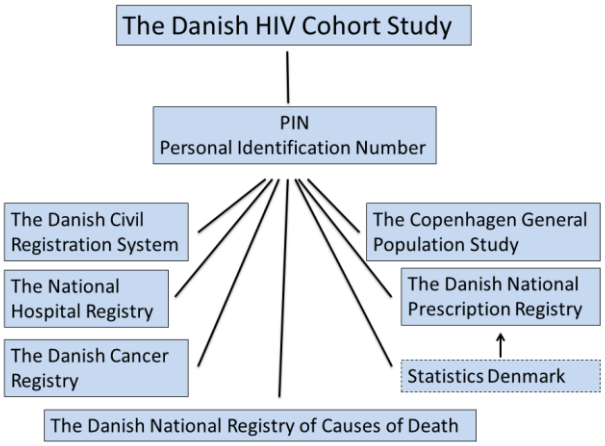
14. To investigate age and time trends (time since HIV diagnosis, HAART initiation and calendar time) in risk of severe age-related diseases among HIV-infected individuals and an age and gender matched comparison cohort (STUDY 7).

DATA SOURCES AND METHODOLOGICAL CONSIDERATIONS

a. DATA SOURCES

The studies included in this thesis were based on data from the Danish HIV Cohort Study (DHCS) and data from other high quality registries (Figure 2). These registries are further described in the individual papers (1-7).

Figure 2: Registries used in study 1-7



DHCS is an open, nationwide, prospective, population-based cohort study of HIV-infected individuals [122, 123]. It was initiated in 1998 as the HIV cohort in Western Denmark [124] and further expanded in 2004 to cover all Danish HIV-infected individuals treated at Danish hospitals since 1 January 1995. Individuals are included at the latter of 1 January 1995, date of HIV diagnosis or immigration. Consequently, DHCS covers prevalent HIV cases as of 1 January 1995 and when immigrated to Denmark after the HIV diagnosis. All other cases are incident.

At birth or upon immigration, all individuals in Denmark are assigned a unique 10-digit personal identification number (PIN - *also named the civil registration number*). The PIN enables linkage with national healthcare registries as illustrated in figure 2. This is of invaluable importance for Danish epidemiological research as it enables a high degree of individual-level tracking during follow-up and further keeps loss to-follow at a minimum. Consequently, the overall validity of the data is very high [125]. Although DHCS include almost all Danish HIV-infected individuals, the weakness of the cohort is by far the limited size of the population, which limits the power or statistical precision of the analyses and increases the risk of ***type 2 errors***. In contrast, the major strengths of the cohort is the high data quality, the well-defined region, the population based design, the long observation period and the almost complete follow-up. Furthermore, as a result of the PIN, siblings and parents of the HIV-infected individuals as well as the population controls can be identified, hence being subject of unique and powerful study designs.

b. STUDY DESIGN

A ***cohort*** is a group of people that share a common feature or condition *(i.e. birth during the same year ~ birth cohort, inhabitants in the same town ~ e.g. Framingham cohort or having the same disease*

~e.g. HIV cohort). The cohort is further described by the geographical area under study that can be more or less well-defined *(i.e. hospital, town, county, region or country).* A ***cohort study*** is often referred to as an ***observational study***; however, an observational study could also imply a ***case-control***- or a ***cross sectional study*** design. Whereas the ***randomized controlled trial*** (RCT) is an experimental trial, the observational study is a non-randomized non-experimental study. As a result, the observational study represents populations and practices in the real world setting (~ ***population based design***).

A ***cohort study*** is a study that follows a cohort over a period of time [125]. The cohort can be ***open*** *(~ ongoing inclusion)* or ***closed*** *(~ inclusion through a shorter period)*. Furthermore, inclusion may start at 1) diagnosis of the diseases, 2) treatment initiation, 3) a specific calendar year, or 4) at a specific age. The chosen start defines whether ***incident*** (1-2) or ***prevalent*** (3-4) cases are observed. The start further defines the underlying ***timeline/axis***. As prevalent cases may carry a ***healthy survivor effect***, it is important to distinguish between incident and prevalent cases and if possible to avoid the latter.

The ***observation time*** is the time that the cohort is observed. It is defined from inclusion till date of the outcome, emigration, loss to follow, death or the end of the observation period. In a classical cohort study the effect of an ***exposure*** on rate of a given ***outcome*** is observed over time. All studies included in this thesis are observational cohort studies. The majority of the studies are analytical studies that aim to test a predefined null hypothesis. As several problems may affect the direction, magnitude and precision of the observed association between the exposure and outcome in analytical observational studies, several important methodological problems and considerations have been addressed below.

c. ERRORS, BIAS, AND CONFOUNDING

In observational studies, the risk of errors, bias and confounding must always be evaluated.

Random errors are the statistical probability of observing a result that is merely a result of ***chance***. It does not influence the results in a particular direction, but affects the precision. Its magnitude is statistically presented as a p-value and a confidence interval (CI) [126].

The ***p-value*** is the probability that the findings would be as observed or even more extreme, if the null hypothesis was true *(~ the probability of rejecting a true null hypothesis)* [126, 127]. The ***confidence interval*** (CI) express the potential magnitude of chance events illustrated with an interval that, with the certainty of the illustrated percentage *(normally 95%)*, holds the true value of the measured association. If the test was run 100 times, the interval would include the true value 95% of the runs. The width of the CI quantifies the

precision or strength of the estimated association [126, 127].

A ***bias*** is a ***systematic error*** that affects the estimate of the association between the exposure and the outcome with the risk of wrong conclusions [127]. Bias can be divided in selection and information bias.

Selection bias refers to the exposure and the way individuals, with or without the exposure, are selected or recruited for the study. A selection bias may arise if a systematically difference, other than the exposure, are introduced in the study *(e.g. allocation bias)*, and this difference is associated with the outcome thus distorting the relationship between the exposure and the outcome [126, 127].

As described above, DHCS contains data on all individuals treated at Danish HIV centers and almost all individuals diagnosed with HIV in Denmark since1995. Although, the population controls have not been HIV-tested, the low prevalence of HIV in Denmark *(approximately 0.1%)* [128], the long follow-up and the selection of controls in the end of the period, make the potential impact of this problem small. DHCS do not capture HIV-infected individuals that have not yet been HIV tested. This may include late presenters or individuals with poor health seeking behaviour that may not resemblance the individuals included in the cohort. However, as the consultation in the clinics and the distribution of antiretroviral therapy is free of charge and restricted to the HIV centers in Denmark, the validity of HIV status is generally considered to be high and higher than in most other HIV cohorts abroad.

Of importance, the validity of an exposure might be threatened by large amounts of ***missing data***, especially if the missing data is not random. Some variables included in DHCS *(e.g. smoking-status)* are not complete. As individuals with and without this exposure may differ, use of e.g. smoking status as the main exposure *(i.e. study 3[129])* could be associated with a risk of selection bias. This, and the importance of a control group that resembles that of the HIV-infected population, is further discussed when debating the results of study 3[129] in the discussion section.

Survival analysis is a powerful method to analyse censored data from cohort studies; however, censoring ***(right and left censoring)*** must be ***non-informative*** or ***independent*** of the outcome *(i.e. the censored individual should resemble those individuals still in the risk set)* [130]. Violation of this assumption may introduce selection bias. As an example, the inclusion of prevalent HIV cases in DHCS, that are left censored, might be associated with the selection of more healthy individuals (***survival benefit***). Nevertheless, as the cohort is on-going and the time of follow-up is long, we expect that the effect might be diluted over time. Problems with ***informative right censoring*** are further discussed in study 5 [131] and in the discussion section of the thesis.

Finally, ***immortal time-bias*** [132], which has been further discussed in study 5 [131], can be described as the use of a fixed base-line exposure status, although the true exposure is not initiated until after follow-up has begun [133]. As the exposed individuals are provided with time in which they cannot die until the true exposure starts *(i.e. immortal person-time),* the effect of the exposure on e.g. risk of death might be substantially underestimated [133]. On the other hand, the risk associated with the unexposed group might be overestimated. Importantly, in survival analysis an individual must be at risk during all exposure time, why the exposure is based on allocation of person-time of follow-up rather than persons *(i.e. a dynamic population experiencing different time in each exposure category)* [125].

Information bias refers to the way the information of the outcome, exposure or confounding variables are gathered or measured [126]. One type of information bias is ***recall bias***. As most data in DHCS are drawn from patient files or retrieved electronically from laboratory data files, most information bias are avoided. Still, some data *(i.e. smoking, alcohol status and mode of HIV transmission)* depend on information from the individuals, thereby being prone to information bias. Furthermore, the potential methodological limitations associated with the use of diagnostic codes from hospital and death registries, must be underlined, as these codes carry a highly varying validity [134, 135]. Concerning HIV diagnosis and use of antiretroviral treatment, these data are validated by research nurses performing journal reviews at the individual centres. Furthermore, the daily leader of DHCS, Professor, Dr. Med. Niels Obel, has incorporated cross-checking and validation algorithms into the database in order to catch errors. As an example, all viral loads performed at the microbiological departments in Denmark, are every year cross-matched with the database, to see if potential HIV-infected individuals are missing.

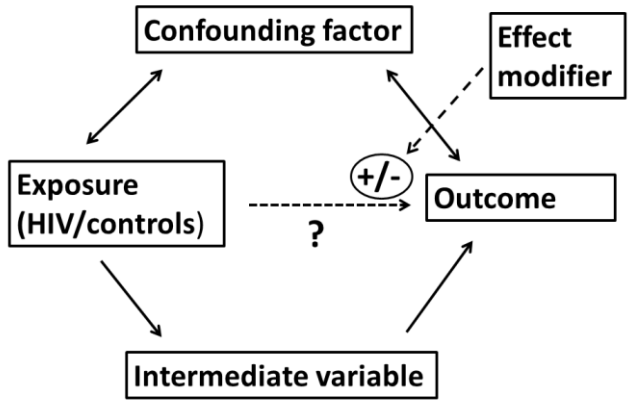
Bias in the classification of the outcome or explanatory variables can be described as non-differential or differential. ***Non-differential misclassification*** is random errors in the classification, in which 1) misclassification of the exposure is unaffected of the outcome, 2) misclassification of the outcome is unaffected of the exposure status, and 3) misclassification of the explanatory variables is unaffected of both the status of exposure and outcome [125, 127, 136]. If the misclassification is not random, and differs according to the status of the exposure, the misclassification is described as ***differential*** [136]. Non-differential misclassification tends to bias the association toward the null hypothesis, whereas differential misclassification can lead to large variations in both directions [127, 136]. As an example, we assume that any potential coding errors are unaffected by the exposure status, hence, occurring with an equal frequency in the groups. However, when comparing risk of a disease

between the general population and a population with a chronic disease such as HIV, that are monitored closely by regular visits by a hospital physician, risk of ***surveillance bias*** cannot be excluded and may to some extent represent differential misclassification. As HIV is mainly seen in young men that infrequently visits a general practitioner, this further points at the risk of surveillance bias.

Confounding is the influence of explanatory variables that are related to both the exposure status and the outcome *(e.g. differences in characteristics between individuals +/- exposure)*, but not on the causal pathway (~ ***intermediate variable/mediator***) [126, 127] (Figure 3). If confounding is not well-handled, the observed effect of the exposure on the rate of the outcome might actually be explained or produced by the confounding factor. This is further discussed in study 3[129]. As an example, we cannot presume that HIV infection and HAART exposure are the only differences that may exist between HIV-infected and non-HIV-infected individuals. On the contrary, these two groups differ on a number of factors, including demographics *(age, gender distribution, varying ethnicity)*, socio-economic and educational status, conventional CVD risk factors, risk taking behavior *(e.g. smoking, alcohol and other substance abuse)*, sexual preference, presence of comorbidity *(e.g. co-infections and psychiatric conditions)*, isolation, and degree of medical surveillance. As these factors are often risk factors for the outcome under study, they may present obstacles in order to disentangle true effects from confounding.

Several strategies associated with the study design or the analytical strategies can be used to prevent or reduce confounding.

Figure 3: Relationship between exposure, outcome, confounding factor, intermediate variable and effects modifier



First:

1) ***matching*** on key variables of importance for the outcome *(e.g. age and gender)* may strengthen the design; however, selection of an appropriate control group that matches all the above mentioned variables seems hardly possible and may in itself induce selection bias.

Additional methods include:

2) ***exclusion*** *(e.g. excluding individuals with prior occurrence of the disease under observation),*

3) ***restriction*** *(e.g. sensitivity analyses restricting the dataset to only males, only Danish born, non-immigrants, only incident cases etc.),*

4) ***stratification*** *(i.e. individuals are divided into subsets (strata) with the same characteristics that are analyzed separately)*, and
5) ***adjusting*** *(e.g. adjusting for several confounders with the use of a regression models/multivariate analyses)*.
Above all, these methods require that the variables are known and measured [126, 127].

Residual confounding is the distortion that remains after controlling for confounding. Residual confounding may arise when variables are measured less well [137]. As an example, smoking frequency *(cigarettes per day)* and duration *(years)*, age at initiation and time since quitting would probably be a better measure of the dose-response effect of the smoking exposure than the categorical baseline measure *(current/previous/never-smoker)* used in study 3 [129]; however, such analyses are complicated and the variables are often not available.

Finally, when performing a cohort study it is important to determine the presence of potential bias or confounding, to consider the possible magnitude and direction and essentially to try to minimize these [127]. Above all it is essential to be aware that the randomization, which is performed in large RCTs, is expected to remove all confounding factors *(measured, unmeasured and residual confounding factors)*. In contrast, unmeasured and residual confounding can never be excluded in observational studies. Hence, careful interpretation of results must always be applied.

f. MODELLING

Of importance the ***selection of relevant explanatory variables*** for a multivariate regression model should always be based on clinical knowledge. In order to achieve the minimal set of variables needed, the choice may further be guided by several methods including change in estimate *(i.e. the estimated exposure effect changes more than 10%)* [138, 139], forward or backwards selection[138], or forcing the variables into the model according to clinical relevance. Several reasons may lead to omission of important variables from a model *(e.g. lack of data or lack of clinical knowledge)*. However, this may lead to an ***under-fitted*** model with the risk of inducing ***type II errors*** *(i.e. the analysis may erroneously fail to reject a false null hypothesis)* [140, 141]. On the contrary, inclusion of a large number of variables may result in an ***over-fitted model***, hence increasing the risk of ***type I errors*** *(i.e. the result may erroneously reject a true null hypothesis)* [138, 139]. Usually 10 events per covariate are considered to be a minimum requirement in order to secure stable estimates of the relative risk [140, 141]. As cohort studies are important in analyses of rare events, the weakness of DHCS is the relative small size of the population, which further restricts the number of explanatory variables that can be included in the regression models. As an example a high number of variables were included in study 5 [131], hence overruling the

above mentioned rule. However, in these analyses, we wanted to force the same set of variables into the analyses as those used by Moore et al [142]. Importantly, restricting the analyses to age and gender, which remain the most important variables in risk analyses of morbidity and mortality, did not change the estimates considerably. ***Propensity score analyses***, in which multiple covariates are combined into a single score, can be useful in studies with limited numbers of events relative to the number of potential confounders [143]; however, this requires fixed baseline variables, which was not the case in our study [131].

In a regression model, the effect of the exposure on the risk of the outcome is considered to be independent of the level of the included variables. If this is not the case ***statistical interaction*** or ***effect modification*** is present (Figure 3) [127, 144]. As an example, HIV infection modified the relationship of smoking on risk of MI in study 3 [129]. Furthermore, presence of co-morbidity modified the relationship of statin on all-cause death in study 5 [131]. If effect modification is present, the effect measures must be presented either with the inclusion of an interaction term *(cross product term)* or stratified on the involved factors, as it could otherwise lead to biased results. Although, true effect modification is based on causality and therefore may have biological, clinical or public health relevance, the causal effect and the unbiased size of the effects might not always be easy to establish [127]. As discussed in study 3 [129], the effect of smoking may represent an underlying differences in other risk factors associated with being a smoker including risk behavior and social disparities and do not apply a strict causal association. Nevertheless, a biologic interaction between HIV and smoking that makes HIV-infected individuals more susceptible to injury related to smoking cannot be excluded.

d. CONFOUNDING BY INDICATION AND CHANELLING BIAS

The designs used to analyze adverse effects *(or unexpected beneficial effects)* associated with drugs remain subject of debate. RCTs are generally designed to evaluate the effect of a given intervention and are considered the gold standard. However, due to the limited number of patients, short duration of follow-up, stringent protocols that may deviate from general practice, and restrictive entry criteria with selection of healthy non-old males with no risk behavior or concurrent disease, RCTs illustrate an idealized setting according to practices and outcomes that may not be generalizable [126]. Furthermore, RCTs are often underpowered to evaluate the risk of rare and unexpected events [145, 146].

The major limitation of ***observational studies*** is that no matter how large they are, they lack the ability to attribute causality and thereby establish definitive answers [145]. Nevertheless, observational studies may provide important and essential information that may not be available in RCTs. In some

situations RCTs are not economically or logistically feasible *(e.g. too large sample sizes required as discussed in paper 5[131])* or not ethical to perform. In these cases, well-designed observational studies of a high quality are invaluable [147, 148]. The strength of observational studies relies on the large numbers of highly diverse individuals representing populations and practices in the real world setting. Additionally, the long duration of follow-up, which enables the researcher to observe rare and long-term outcomes, and finally the possibility to establish temporal relationships between the exposure and the outcome is of importance. However, the interpretation must be done with caution, especially when evaluating rare effects of drugs. In RCTs the strength rely on the random allocation of individuals to a given exposure/treatment regimen. This is not the case in observational studies, where many known and unknown factors may have affected the allocation thus making these studies prone to confounding by indication or channeling bias [146, 149].

Confounding by indication is described as characteristics of the individual that have made the physician prescribe or not prescribe a certain drug [126, 133, 137, 150]. However, this decision is based on a broad spectrum of patient-physician characteristics, that may be measured *(e.g. the indication, severity of the disease and co-morbidity)* or unmeasured/unknown *(e.g. health seeking behavior, socio-economic and educational status, risk behavior).* These characteristics might be associated with an increased or decreased risk of the outcome of interest why lack of controlling for these characteristics may lead to flawed conclusions. This has been further discussed in study 5 [131].

Channeling bias *(i.e. allocation/selection bias)* is associated with the characteristics of the prescribed drug. Channeling bias may arise when drugs with similar therapeutic indication but claimed advantages or disadvantages are channeled to or away from individuals with pre-existing morbidity Thereby the drugs are preferentially prescribed to groups of patients systematically different according to their baseline prognosis [133, 137, 145, 146]. As an example, abacavir was previously the preferred drug *(compared to tenofovir)* for individuals with high CVD risk due to perceived metabolic and renal safety. This potential channeling of drugs has been suspected to have affected the results of an increased risk of MI associated with abacavir *(further discussed in study 1[151]).* Problems may also arise when comparing drugs from first line and second line regimen, as second line drugs are mainly reserved for individuals who have failed on first line drugs (***confounding by severity***), or for whom the first line regimen was inappropriate [146]. As a result of both confounding by indication and channeling bias, an imbalance in the distribution of covariates may confound the observed impact of a treatment [145, 146].

Channeling bias and confounding by indication are difficult to avert and often not amenable to standard

solutions. Nevertheless, robust, well-designed population-based studies with comparable groups according to relevant characteristics, use of multivariable adjustment, time-updated exposures and subgroup stratification, and furthermore considering the underlying mechanism when designing the study, are essential and may correct for known *(but not unknown)* imbalances[152]. Other statistical techniques include the use of the ***Propensity Score*** *(i.e. the conditional probability of exposure to a treatment given observed covariates)* [143, 153] which can be used for matching, stratification and controlling for confounders [143, 145, 153, 154]. Another model is the ***Inverse Probability of Treatment Weightin***g *(i.e. creates a pseudo population in which the association between covariates and treatment is removed by weighting each observation by the inverse probability of receiving the actual treatment)* [155]. Nevertheless, these models are not without flaws and limitations. Both models require large sample sizes and only recorded variables can be included. In contrast, bias from unmeasured confounding cannot be corrected [143, 145]. Additionally, information regarding predictors of the outcome is lost [153]. The accuracy of the propensity score is of outmost importance and requires all relevant predictors of treatment to be adequately captured [143, 145, 153]. Furthermore, these models only handle fixed baseline covariates. Finally, a review of the application of the propensity score from 2006, showed that there was little evidence that these methods yielded substantially different estimates compared with conventional multivariable models, why it should not automatically be regarded as preferable [154].

e. PROBLEMS ASSOCIATED WITH ANALYZING EFFECTS OF DRUGS

In a simple design (~ RCT) the exposure of interest is constant (fixed) throughout follow-up, in which individuals can be assigned to either an exposed or an un-exposed group. However, this is seldom the case in the real world setting where drug-exposure changes over time. As a result the exposure must be included as a time-updated variable in order to avoid immortal time-bias, as previously discussed. In extension, some drugs require an induction period *(lagging time)* or accumulation of the drug to a sufficient level prior to an effect; however, the exact induction period or amount of exposure is seldom known. Hence, exposures are often simplified to estimates of current, cumulative or average exposure [125]. For drugs with a potential cumulative effect, it is extremely difficult to disentangle underlying effects of an increasing age, increasing time with HIV exposure and later calendar time (***collinearity***). Furthermore, the allocation of exposure time when actually at risk might be complicated for exposures that may change category several times during follow-up. First of all, changes in medication might be associated with warning signs of the outcome under observation. Second, effects of the drug may extend beyond drug cessation. As a result, ***reversed causation*** might be a risk *(i.e. the true causative*

drug might have been withdrawn or switched before the outcome occurs). Generally drug exposure can be analyzed ***as prescribed*** or by ***an intent-to-continue-treatment approach***. Nevertheless, when a drug is analyzed as intention to treatment approach, it is important to be aware of the risk of diluting an effect of drugs only used for a short period *(i.e. drugs used in the beginning of the HAART era)*. Essentially, this would bias the results towards the null-hypothesis (~ ***type 2 error***).

f. DRUG UTILIZATION STUDIES AND ASSOCIATED PROBLEMS

Data from the Danish National Prescription Registry can be used when analysing non-HAART drugs. Generally, these data are considered to be highly valid [156]. Nevertheless, this registry does not capture over the counter medicine, in-hospital use of drugs, and drugs paid and delivered by the hospitals *(e.g. chemotherapeutics, biologic drugs, HAART etc.)*. As such the actual use might be under-enumerated. However, unless these shortcomings are expected to differ between groups, the relative use should not be affected.

In study 4 [157], we used the redemption of drugs and the quantity of the drug dispensed as a measure of the drug utilization. Nevertheless, the quantity of the drug dispensed may differ according to the indication, the individual and the disease severity. Furthermore, the prevalence of the drug may be affected by competing risk by death in the HIV-infected cohort, thereby leading to an underestimation of the actual prevalence. However, if drug utilization is measured by the active amount of the ingredient *(i.e. grams)*, drugs with a low potency will account for a larger fraction of the total than drugs with high potency[158]. Counting tablets might be a different solution; however, the number of tablets may not reflect the variation in strength. and finally using the number of prescriptions could also be misleading[158]. As a result, we estimated the drug utilization in study 4 [157] given the defined daily dose (DDD) per 1,000 person-days. The DDD is defined as "the assumed average maintenance dose per day for a drug used for its main indication in adults" [158]. The DDD is a unit of measurement approved by WHO [158]. However, as the DDD is a technical drug use metric, it may not always reflect the formulations most often used, and may not necessarily reflect the recommended or prescribed dose. As such, the DDD reflect the global dosage irrespective of genetic variations of drug metabolism and is as such a rough estimate of consumption and not an exact picture of the actual use. Nevertheless, the DDD enables the researcher to assess trends in drug consumption and thereby comparison between populations and groups [158] and importantly give a signal of irrational drug use.

g. STATISTICAL METHODS

The measures of frequency and effect used in cohort studies are most often the incidence or the prevalence. The incidence rate (IR) or mortality rate

(MR) and the excess IR or excess MR are used as estimates of the ***absolute risk*** and ***absolute excess risk***, whereas the incidence rate ratio (IRR, or Hazard Ratio ~HR, adjusted ~ aIRR or aHR) or mortality rate ratio (MRR, adjusted aMRR) are used as a measure of the ***relative risk***. A 1:1 relationship between the rate and the risk is assumed, although this may not always be true. The statistical methods used to compute these measures include either the Cox Proportional Hazard Regression or Poisson Regression analysis. Whereas an explanation of these models and associated limitation is beyond the scope of this thesis, a few additional statistical methods and associated problems will be discussed below.

Competing risk may arise when the outcome is preceded by competing causes that prevent the individuals from experiencing the outcome of interest [149]. In survival analysis, all individuals must have a risk of experiencing the outcome if observed till the end, otherwise competing risk is present [159]. Whereas the rate is unaffected by competing causes, the risk is affected by both the rate of the outcome and the rate of the competing cause. If the rate of the competing cause is high, the risk of the outcome might be biased if the competing risk is not taken into account. Hence, the 1:1 relationship between the rate and the risk is lost. Furthermore, if the exposure has strong associations with competing causes *(diseases or death)* this may further affect the relative risk of the outcome. The only outcome with no potential competing risks is overall mortality. If competing risk is present, the ***cumulative incidence function*** can be used to estimate the risk of the outcome, recognizing the defined conditions as competing risk [159, 160]. The Fine and Gray model [161] can be used to estimate the sub-distributional hazard regression considering effects of competing risk. However, 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 [159, 160]. As a result, further interpretation is difficult.

The Standardization method can be used in order to remove the distortion caused by different age and gender distributions over time. Considering the design, direct standardization or indirect standardization can be applied. As an example, the direct standardization enables the researcher to estimate the hypothetical IR that would have occurred over years if the crude age-specific rates were present in a population whose age and gender distribution was that of the standard population *(i.e. a weighted average of the stratum specific rates with the weights taken from the standard population)* [162]. In contrast the indirect standardization enables the researcher, that does not have a control cohort or only have aggregated data to estimate a measure of the relative risk based on the expected rate calculated given the stratum specific rates from the standard population[162]. When using direct standardization, an internal or external standard can be applied. When using

several time lines in a Poisson regression model, the effect of one time line might act in collinearity with other time lines *(e.g. time with HIV, HAART and increasing age)*. Using age-standardized analyses may establish age-independent effects of time.

The Population attributable fraction (PAF) is an estimate of the fraction of a given outcome that on a population level is associated or attributable to a given risk factor.The PAF depends on the prevalence *(e.g. the proportion of individuals being ever smokers)* and the impact of the estimated risk factor in the population *(e.g. the relative risk of MI associated with smoking)*. Nevertheless, the estimate depends on the composition of the population. As a result, direct extrapolation between populations, cohorts and countries is not possible. Importantly, the PAF assumes causality between the exposure and the outcome. Therefore, it must be excluded that the effect may be a result of confounding rather than biology. This is further discussed in study 3 [129]. Above all, the true fraction of the risk of an outcome that could be prevented, if the potential risk factors were eliminated, can only be truly established in case of causality.

DISCUSSION

RISK OF CEREBROVASCULAR DISEASE

The major part of pre-HAART studies of stroke or cerebrovascular disease among HIV-infected individuals are autopsy studies [163-167], case-reports and early observational studies hampered by small sample size [168-173] and inclusion of young individuals with advanced HIV infection and associated opportunistic infections or cancer [168, 169, 171, 173]. Altogether, this contributes to difficulties in generalizing the results to the HIV population as of today. Still, despite several additional limitations *(e.g. insufficient confounder control and lack of HIV-negative population controls)*, the vast majority of the pre-HAART studies favours an increased risk in association with a positive HIV status [83, 168-178] and only very few studies reached other conclusions [165, 179].

With focus on the HAART-era, we *(study 1)* examined the risk of incident cerebrovascular events among 5,031 HIV-infected individuals and 45,279 age and gender matched population controls [151]. As cerebral co-morbidity *(defined as cerebral tumor, cancer, lymphoma or metastasis, non-HIV-associated or HIV-associated cerebral infections, or AIDS dementia)* may mimic or induce cerebrovascular events, we excluded individuals with prior cerebral co-morbidity and censored individuals developing cerebral co-morbidity during follow-up 30 days prior to such occurrence. Of note, we found that HIV-infected individuals with no injection drug abuse (IDU) had a 1.6-fold increased risk of cerebrovascular events relative to the population controls whereas an almost four times higher relative risk was observed among IDUs (HIV+ vs controls) (Table 1).

Table 1: Relative risk of cerebrovascular events (HIV+ vs controls)

Outcome:	Non-IDUHIV+ vs controls IRR (95% CI) Adjusted	IDU HIV+ vs controls IRR (95% CI) Adjusted
Cerebrovascular events	1.60 (1.30-1.95)	3.94 (2.16-7.16)
Subarachnoid haemorrhage	1.72 (0.80-3.69)	4.41 (0.81-24.09)
Intracerebral haemorrhage	1.47 (0.67-3.20)	35.62 (4.04-314.17)
Cerebral infarction	1.63 (1.10-2.41)	1.90 (0.50-7.19)
Unspecified stroke	1.54 (1.08-2.18)	4.62 (1.63-13.11)
Transient ischemic attack	1.64 (1.10-2.44)	1.19 (0.15-9.67)

Abbreviations: IRR: Incidence Rate Ratio; HIV+: HIV-infected individuals; IDU: Intravenous drug abuse

As, cerebrovascular events was a composite endpoint (*defined as non-traumatic subarachnoid hemorrhage, intracerebral hemorrhage, cerebral infarction, unspecified stroke, transient ischemic attack and stroke sequels)* consisting of diagnosis that to some extent relied on different etiologies, the gain in power could carry a risk of dilution of the observed effect. Nevertheless, for the non-IDU HIV-infected individuals, estimates of almost similar impact were found for the specific outcomes. In contrast, risk of cerebrovascular events among the IDU HIV-infected individuals, mainly relied on a substantially increased risk of subarachnoid hemorrhage, intracerebral hemorrhage and unspecified stroke. Generally, the lack of clinical assessment of the outcomes may have led to some misclassification; however, this is presumed to be non-differential.

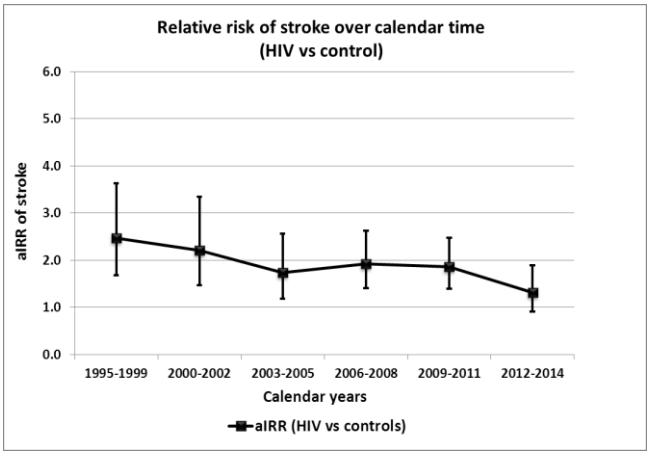
In accordance with our results, Vinikoor et al [180] used data from a large HIV cohort in North Carolina (2,515 HIV+) and reported an approximately 1.5-fold increased risk of ischemic stroke. Although, all endpoints in this study were adjudicated by three clinicians, the IR was compared to published results from the North Carolina site of the Atherosclerosis Risk in Communities Cohort, thereby limiting further confounder control. We *(study 1)* matched the population controls on key variables, stratified the analyses by the presence of IDU, and further analyzed the risk for individuals both with and without CVD risk factors. Still, we did not control for conventional CVD risk factors including smoking due to lack of sufficient data on these variables [151]. This could explain the more attenuated HIV-associated risk of ischemic stroke, found by Chow et al using Boston Research Patient Data Registry (4,308 HIV+) *(aIRR: 1.21; 95 % CI: 1.01-1.46)* [181], by Marcus et al using the Kaiser Permanente Northern and Southern California cohort (24,768 HIV+) *(aIRR: 1.4; 95% CI: 1.2-1.7)* [182] and by Sico et al using the Veterans Aging Cohort Study-Virtual Cohort (VACS-VC) (25,434 HIV+) *(aIRR: 1.17; 95% CI: 1.01-1.36)* [183]. Although, these results were adjusted for CVD risk factors, most variables were based on ICD-9 codes and language processing tools [181-183] why some factors *(e.g. IDU)* were poorly accounted for [182, 183] or not included in the models [181].

Whereas most studies have focused on risk of ischemic stroke, only few studies have examined the risk of intracranial hemorrhage among HIV-infected individuals. We *(study 1)* found a substantially increased risk of intracerebral hemorrhage among HIV-infected individuals with IDU *(aIRR: 35.62; 95 CI: 4.04-314.17)*; however, this ratio was based on a limited number of endpoints, and hence very large confidence interval *(~ low precision)*. A trend towards a substantially lower risk was observed for the HIV-infected individuals with no IDU compared to controls *(aIRR: 1.47; 95% CI: 0.67-3.20) (study 1)* [151]. Durand et al [184] used data from a Canadian administrative database (7,053 HIV+) and found a more than three times higher risk of intracerebral hemorrhage among HIV-infected

individuals *(aIRR: 3.28; 95% CI: 1.75-6.12)*. The risk was further reduced to a factor 2 *(aIRR: 1.99; 95% CI: 0.92-4.31)* in the absence of AIDS defining conditions. Consistent results of an HIV-associated risk of intracranial hemorrhage among HIV-infected individuals of the same magnitude *(aIRR: 1.85; 95% CI: 1.37-2.47)*, has been reported in a recent study by Chow et al [185] using data from the Partners HealthCare System Research Patient Data Registry (4,251 HIV+).

In a large study by Ovbiagele et al [186], data on all stroke admissions were obtained from all states within the United States that contributed to the Nationwide Inpatient Sample during 1997-2006. In this period, the study observed a 60.5% relative rise in ischemic stroke admissions among HIV-infected individuals *(~ 43% increase, when taking the 40% increase of the HIV population over the same period into account)*. In contrast, a 7.2 % relative reduction in overall stroke admissions was observed in the same period in the United States. However, as the study estimated the weighted proportion of stroke admissions that occurred in persons with and without HIV-infection, it could potentially be flawed by the underlying change in demographic of the HIV population. In contrast, Marcus et al [182] found stroke rates that approached each other with time (HIV vs controls). We obtained similar results in study 7 *[121]*, of which the relative risk of stroke declined with calendar time; hence arguing against such alarming results (Figure 4).

Figure 4: Relative risk of stroke with time



CONCLUSION

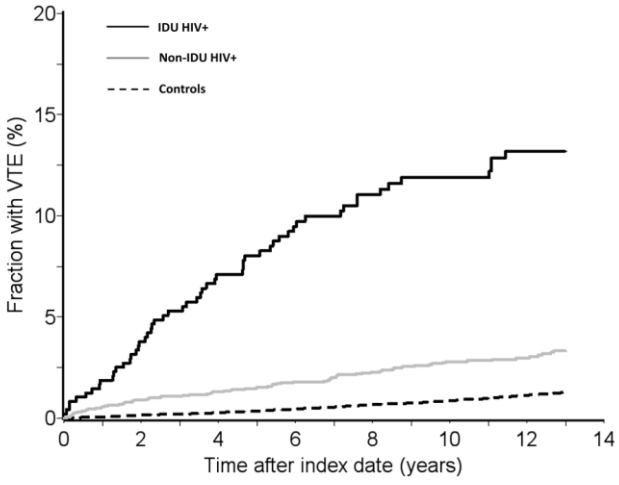
Studies both from the pre-HAART and HAART-era indicate an increased risk of cerebrovascular disease among HIV-infected individuals compared to the general population, and several mechanisms have been suggested as the possible cause [184, 185, 187]. Although, some mechanisms differ between ischemic and hemorrhagic stroke, several factors seem to overlap including, cerebral comorbidity, immunodeficiency, and risk behavior including illicit drug use [184, 188] and heavy alcohol consumption [182, 184, 189, 190]. HIV-infected individuals have a high prevalence of many traditional CVD risk factors [85, 105-112]; however, this alone does not seem to explain the increased risk of cerebrovascular disease. Therefore, other factors must be accounted for in order to isolate the role of HIV and HAART.

RISK OF VTE

Although, an approximately 2-10 fold increased risk of VTE among HIV infected individuals have been suggested, these finding relies on studies from the pre-/early HAART era [191-200]. Only few studies have examined the risk of VTE in HIV-infected individuals in the more established HAART era [201-210] and the majority of these studies are limited by small sample size, poor quality, and lack of population controls for comparison.

With data from DHCS spanning the years 1995-2007, we investigated the risk of VTE among 4,333 HIV-infected individuals and 43,330 age and gender matched population controls *(study 2)*[206]. As IDU is a strong risk factor for VTE [211, 212], all analyses were stratified by IDU (Figure 5).

Figure 5: Cumulated risk of VTE among HIV-infected individuals (IDU and non-IDU) and controls



Abbreviations: VTE: Venous thromboembolism; index date: last of: January 1995 or HIV diagnosis

We further applied previously developed models to analyse the results as provoked *(presence of malignancy, fracture, surgery, trauma or pregnancy)* and unprovoked VTE [96, 97], and adjusted the analyses for important factors including comorbidity. We *(study 2)* found an almost 15 times higher risk of VTE *(95% CI: 7.36-30.34)* among HIV-infected individuals with IDU compared with population controls. In contrast, a much lower, but still almost 4-fold increased risk *(aIRR: 3.84; 95% CI: 2.99-4.93)* was observed for HIV-infected individuals with no IDU compared to population controls (Figure 5). In accordance with our results, a 4-fold increased risk of deep VTE has also been found in a retrospective study of 465 HIV-infected individuals *(IR: 377 per 100.000 PYR)* from the Naval Medical Centre in California [202]; however, this relative risk was based on comparison with published estimates from the general population *(males of the same age)* and hence lack of confounder control. Of importance, we could not adjust for smoking status. However, although smoking is an established risk factor for atherosclerotic disease, the impact of smoking on risk of VTE seems much less clear than that of MI [99, 213]. Therefore, we do not presume that this have largely flawed our results [206].

Many of the early studies have focussed on a variety of acquired coagulopathies contributing to an overall hypercoagulable state or endothelial dysfunction as the reason for the increased risk of

VTE among HIV-infected individuals [214-216]. However, the pathogenesis of VTE, that in the general population appears to be multifactorial, may also be related to factors of HIV disease progression, HAART exposure or a disproportionally high prevalence of traditional risk factors, risk behaviour and provoking factors.

Concerning HIV-infected individuals with no IDU, we *(study 2)* found a more than 5-fold increased risk of provoked VTE and a more than 3-fold increased risk of unprovoked VTE compared to the population controls (Table 2) [206]. In extension of these results, a study by Jansen et al [217] found that pregnant HIV-infected women had a 120-fold increased risk of VTE compared to non-pregnant HIV-infected controls and a 157-fold higher risk of VTE compared to pregnant women with no HIV infection. Unfortunately, these results are based on very few cases *(annual IR: 313 per 1000 PYR; 95% CI: 65-915)* and the estimated relative risk further unadjusted for potential confounders.

A small nested case-control study *(45 VTE cases and 180 controls)* by Modi et al [210] recently pointed out that, prior surgery was associated with a more than 13 times higher odds of VTE for people living with HIV *(95% CI: 1.56-111.41)*. However, this study did not clarify if this provoking factor conferred a comparable or higher risk of VTE than that of the general population.

As HIV-infected individuals have been found to have a higher risk of smoking-related and virus associated cancer compared to the general population [218], this constitutes a very reasonable contributor to the increased risk of provoked VTE.

Table 2: Risk of VTE (HIV+ vs controls)

aIRR (95% CI)	
Non-IDU HIV+ vs controls	**IDU HIV+ vs controls**
All	
3.84 (2.99-4.93)	14.95 (7.36-30.34)
Unprovoked VTE	
3.42 (2.58-4.54)	12.66 (6.03-26.59)
Provoked VTE	
5.51 (3.29-9.23)	9.38 (1.61-54.50)

Abbreviations: VTE: venous thromboembolism; IDU: injection drug user; HIV+: HIV-infected individuals

Concerning unprovoked VTE, current use of antipsychotics has previously been associated with risk of VTE [219, 220]. As illustrated in study 4 [157], HIV-infected individuals have a higher utilization of antipsychotics than the general population. Although we adjusted for psychiatric diagnoses *(as a surrogate for the use of neuroleptic drugs)*, we cannot exclude that some of the increased risk of unprovoked VTE and overall VTE, may stem from a higher utilization of antipsychotics in this population. Furthermore, potential misclassification of IDU could account for some, but not all of the higher risk of unprovoked VTE in this group.

CONCLUSION

Although, IDU is a major risk factor for VTE - also among HIV-infected individuals, it seems remarkable, that an almost 4-fold increased risk of VTE for HIV-infected individuals with no IDU, has received so little attention compared to the 1.5-2-fold increased risk of MI observed in the same population. Although, VTE is provoked by many of the same factors that induce VTE in the general population, other factors could also be at stake.

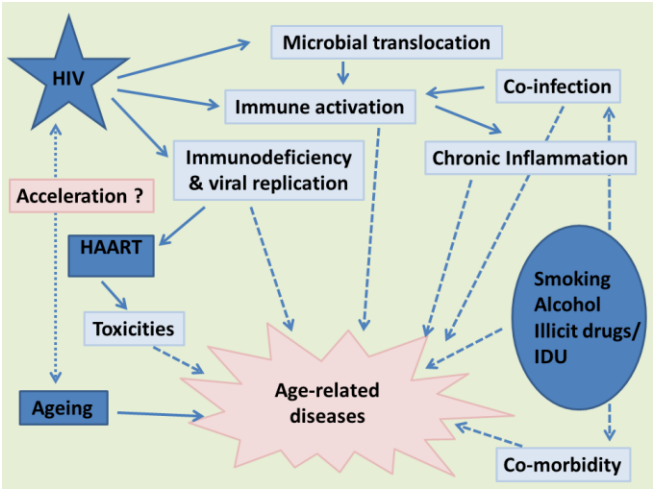
The overall burden of infections and other HIV-related morbidity have declined with the introduction of HAART. However HIV-infected individuals are largely aging and generally have a higher burden of age-related diseases thereby increasing the overall risk of hospitalizations. Prevention of risk factors should therefore be actively sought in order to avoid both provoked and unprovoked VTE.

TRADITIONAL AND UNTRADITIONAL RISK FACTORS

Spanning the past decade, the impact of conventional risk factors, and associations with HIV infection and HAART exposure on risk of CVD has been largely investigated. Above all, conventional risk factors are more prevalent among people living with HIV than among the general population [85, 105-112] thus likely being a major driver. Some antiretroviral drugs, mainly PIs and older NRTIs, are associated with metabolic complications including glucose alterations *(hyperglycemia, impaired glucose tolerance and peripheral insulin resistance)*, lipid abnormalities *(hypertriglyceridemia and hypercholesterolemia)*, fat redistribution syndromes *(lipoatrophy and hyperlipotrophy)* and maybe hypertension [55, 56, 221, 222]. These metabolic toxicities are potential risk factors for developing serious morbidity including diabetes mellitus (DM), MI and CVD, whereas VTE does not seem to share the same risk factors [96, 97]. Still, similar biological triggers could be responsible for activating coagulation and inflammatory pathways, as previously discussed [95, 96].

During later years an improved metabolic profile has been observed in some studies [111, 223]. This seems to be a result of increased availability of newer and more potent drugs with less toxicity, guidelines indicating use of more cardio-neutral HAART regimens [224], and initiation of primary prophylaxis *(e.g. use of statins)*. However, as these factors tend to accumulate with age, clinicians must keep on focusing on these parameters; yet, this is regardless of HIV status. By comparison, risk behavior including smoking, IDU and other concurrent co-morbidity still remains a large problem for the HIV-infected population adding to the complexity of the interplay of risk factors for CVD and other age-related diseases (Figure 6). A complete discussion of all risk factors is beyond the scope of this thesis. Therefore, the main focus of this thesis will be the presence and to some extent impact of risk behavior and concurrent comorbidity, as well as the impact of HIV, HAART and aging.

Figure 6: Potential risk factors of CVD and other age-related diseases



SMOKING:

Globally, smoking is a leading cause of morbidity and mortality [225-227]. Also among HIV-infected individuals, smoking have been found to have profound health implications [129, 218, 228-234]. With a prevalence ranging between 40-85%, smoking is 2-3 time more prevalent in the HIV-infected population than in the general population, and the average number of pack-years is presumed to largely exceed that of the general population [235-239]. Smoking therefore constitutes a major threat for the HIV-infected individual as well as for the public health burden.

Of importance, smoking may constitute a confounder in observational studies (HIV+ vs. controls) [85, 86]. Still, sufficient controlling for smoking is often prohibited by the lack of such data. Therefore, some of the previous conclusions considering health risks among HIV-infected individuals could be flawed. Recent evidence has shown that the impact of smoking on risk of health outcomes for the HIV-infected population is not only substantial, but also larger than that of the general population [129, 218, 233]. Helleberg et al [233] found that current vs. never smoking was associated with a 4.4-fold risk of all-cause mortality and a 5.3-fold risk of non-AIDS death. In this study, more than 60% of deaths among HIV-infected individuals were attributable to smoking and the number of life-years lost due to smoking exceeded that of life-years lost due to HIV itself [233]. Major impact of smoking on risk of death has also been observed in other HIV cohorts [229, 230, 240]. Although, a recent study found no association between renal function and smoking [241], a large number of diseases and multi-morbidity, a decreased quality of life and maybe a poor immunologic response to HAART seems to be highly affected by smoking [218, 228-232, 234, 242]

Concerning risk of MI and CVD, smoking is among the strongest contributors [243]. We (*Study 3*) [129] investigated risk of MI among 3,251 HIV-infected individuals and 13,004 age and gender matched population controls from the Copenhagen General Population Study. We found that the impact of smoking on risk of MI was not only large but also further potentiated by the HIV infection. And, whereas a positive HIV status was associated with an increased MI risk among current or previous smokers, never smokers had the same risk of MI (HIV+ vs controls) [132] (Table 3).

Table 3: Risk of MI (HIV vs controls)

Stratified by smoking status	aIRR (95 % CI)
Controls, never smokers	Ref.
HIV+, never smokers	1.01 (0.41-2.54)
Controls, previous smokers	Ref.
HIV+, previous smokers	1.78 (0.75-4.24)
Controls, current smokers	Ref.
HIV+, current smokers	2.83 (1.71-4.70)

Abbreviations: IRR: Incidence Rate Ratio, 95% CI: Confidence Interval.

These results differ from those presented by the large VACS-VC cohort [92], in which risk of MI was increased by a factor 1.48 *(95% CI: 1.27-1.72)* when adjusting for risk factors, comorbidity and substance use, and seemingly increased to a factor 1.75 *(95% CI: 1.27-2.42)* when restricting data to never smokers (HIV+ vs controls). In a more recent study from the VACS-VC [244], risk of MI was 2-fold increased *(95% CI: 1.0-3.9)* when restricting data to those with no major CVD risk factors (HIV+ vs controls). However, past smoking was not considered a major risk factor. We do acknowledge, that the difference in results may rely on lack of power in our study *(study 3)* [129]. An editorial by John Gill and Dominique Costagliola [289], that discussed the results of our study *(study 3)* [132], further suggested that the 14.2%, for whom we had no smoking data, may have introduced selection bias. We acknowledge that individuals with and without data on smoking status did differ on certain variables as illustrated in table 4.

Table 4: Characteristics of HIV-infected individuals with and without smoking data

	HIV+ with smoking data	**HIV+ no smoking data**
No. of individuals[1]	3,233	532
Male[1]	2,548 (78.8)	446 (83.8)
Age at baseline (years) [2]	44.6 (40-52.5)	44.3 (40.0-52.8)
Danish born[1]	2,323 (71.9)	370 (69.5)
Route of transmission		
MSM[1]	1,645 (50.9)	228 (42.9)
Heterosexual[1]	1,392 (43.0)	232 (43.6)
Hepatitis C[1]	302 (6.2)	46 (8.6)
HIV before year 1995[1]	1,021 (31.6)	163 (30.6)
Years since HIV diagnosis[2]	7.5 (2.6-13.1)	2.4 (0.0-8.4)
AIDS at baseline[1]	701 (21.7)	89 (16.7)
CD4 cell count at baseline (cells/µL)[1]	480 (310-674)	310 (120-500)
Viral load<400 copies/mL at baseline[1]	2,296 (71.3)	170 (35.1)
HAART at baseline[1]	2,497 (77.2)	210 (39.5)
Total observation time (person-years)	18,263	2,058
Observation time (years)[2]	5.8 (3.2-7.3)	2.8 (0.9-5.2)
Death before year 2004[1]	18 (6.3)	161 (64.9)
Death between year 2004-2006[1]	24 (8.3)	50 (20.2)
Death after year 2006[1]	244 (84.7)	37 (14.9)
Emigration[1]	71 (2.2)	47 (8.8)
Loss to follow-up[1]	3 (0.1)	6 (1.1)
Myocardial infarctions[1]	95 (2.9)	11 (2.1)
Incidence Rate [3]	5.2 (4.3-6.4)	5.3 (3.0-9.7)

Abbreviations: HIV+: HIV-infected individuals; HAART: highly active antiretroviral therapy, MSM: men who have sex with men .[1] number (%), [2] median (interquartile range; IQR) [3] (95 % Confidence Interval; CI).

Of note, a larger percentage of the individuals with no data on smoking died during observation, and death largely occurred before year 2004/2006 and shortly after the HIV diagnosis. Still, we do not believe that these differences introduced large bias *(e.g. an almost similar incidence rate was observed, table 4)*. Hence, as argued in the paper the study population mainly represented the HIV-infected population and the setting as of today.

The aforementioned editorial [245] further suspected that the difference in residency of the two cohorts might have flawed the results. As the controls were all residents in greater Copenhagen, a higher risk of MI could be suspected in this cohort compared to the expected risk in the general population, thereby underestimating the effect of HIV on risk of MI among never smoking individuals. As discussed in *paper 3* [129], the two cohorts may differ according to ethnicity, socioeconomic status, educational level, sexual orientation, abuse and health-seeking behavior.

However, as the controls in our study had volunteered to participate in a health study, thereby participating in outpatient visits and interviews on lifestyle, we believe that they rather represent a healthier subset of the general population. Therefore risk of MI, as well as the impact of smoking, could be slightly underestimated among the controls and vice versa. However, including an individually matched control cohort randomly selected from the entire Danish general population, did not show any large differences in incidence rate of MI compared to using population controls from Copenhagen General Population Study (Table 5). Therefore, it seems crucial to consider that the results might actually illustrate the lack of excess risk associated with being HIV positive among never smoking individuals. In extension, the results does not leave a large excess risk for other factors such as chronic immune activation and sustained inflammation, as well as potential effects of antiretroviral drugs to exert their impact on risk of MI.

Table 5: Risk of myocardial infarction among 3,233 HIV-infected individuals and 32,330 individually age and gender matched general population controls (1:10)

	Events (n)	PYR	IR/1,000 PYR (95 % CI)
Population controls, all	440	201,235	2.19 (1.99-2.40)
Controls from CGPS, all	125	63,128	1.98 (1.66-2.36)
HIV+, all	95	18,263	5.20 (4.25-6.36)

Abbreviations: HIV+: HIV-infected individuals; PYR: Person-years of follow-up; IR: Incidence Rate, 95% CI: Confidence Interval. CGPS: Copenhagen General Population Study

In order to see if our results *(study 3)* could be extrapolated to risk of CVD in general, we investigated risk of stroke among non-IDU HIV-infected individuals compared to a matched population comparison cohort from the Copenhagen General Population Study. In contrast to our expectations, we found an almost 3 fold increased risk of stroke among never smoking HIV-infected individuals compared to never smoking controls, whereas no difference in risk among current smokers (HIV+ vs controls) was observed (Table 6). Although, this may be a result of confounding due to unknown factors or factors that we cannot sufficiently account for *(i.e. hypertension, very high alcohol consumption etc.)*, we cannot exclude that smoking may have less impact on risk of stroke than risk of MI. Although, the relationship between smoking and risk of cerebrovascular disease has been less clear than that of smoking and coronary arterial disease, results from the Framingham study [246] showed that smoking conferred a significant impact on risk of stroke, although the impact was not as powerful as that of hypertension. In extension, results of the INTERSTROKE study [247, 248] established that current smoking was associated with a 2-fold increased risk of ischemic

Table 6: Risk of stroke among HIV-infected individuals and controls with smoking status and no IDU

	Events (n)	**PYR**	**IR/1,000 PYR (95 % CI)**	**Adjusted* IRR (95 % CI)**
Controls, all	116	24,356.1	4.76 (3.97-5.71)	Ref (1)
HIV+, all	64	8,364.2	7.65 (5.99-9.78)	1.67 (1.23-2.27)
Impact of HIV status stratified on smoking status				
Controls, never smokers	26	9,434.2	2.76 (1.88-4.05)	Ref.
HIV+, never smokers	23	2,641.9	8.71 (5.79-13.10)	2.87 (1.63-5.04)
Controls, previous smokers	43	8,962.7	5.36 (4.04-7.11)	Ref.
HIV+, previous smokers	13	1,778.6	7.31 (4.24-12.59)	1.46 (0.79-2.70)
Controls, current smokers	42	5,959.2	7.05 (5.21-9.54)	Ref.
HIV+, current smokers	28	3,943.6	7.10 (4.90-10.28)	1.05 (0.65-1.70)
Impact of smoking status stratified on HIV status				
HIV+, never smokers	23	2,641.9	8.71 (5.79-13.10)	Ref.
HIV+, previous smokers	13	1,778.6	7.31 (4.24-12.59)	0.76 (0.38-1.51)
HIV+, current smokers	28	3,943.6	7.10 (4.90-10.28)	0.84 (0.48-1.48)
Controls, never smokers	26	9,4434.2	2.76 (1.88-4.05)	Ref.
Controls, previous smokers	48	8,962.7	5.36 (4.04-7.11)	1.59 (0.98-2.56)
Controls, current smokers	42	5,959.2	7.05 (5.21-9.54)	2.17 (1.33-3.55)

Abbreviations: HIV+: HIV-infected individuals; PY: Person-years of follow-up; IR: Incidence Rate, IRR: Incidence Rate Ratio, 95% CI: Confidence Interval.*Adjusted for gender, age (time updated), calendar year (time-updated).

Table 7: Study methods and sensitivity analyses

Study methods:	**Sensitivity analyses:**
Use of similar definitions of stroke as that used by the Copenhagen General Population Study (ICD8: 430-438, ICD10: I60-I68, G45)	1) using similar definitions as in *study 1* [151] 2) changing outcome to: only ischaemic cerebrovascular disease, only ischaemic stroke and only haemorrhagic stroke
Individuals with cerebral comorbidity were not excluded excluded/censored	3) excluding individuals with cerebral comorbidity prior to inclusion and censoring those developing cerebral comorbidity during the observation time
	4) excluding non-Caucasians 5) excluding individuals with Hepatitis C 6) excluding individuals with transmission mode other than homosexual and heterosexual (i.e. coagulation disorders) 7) taking the CD4 cell count and treatment status into account

and intracerebral hemorrhagic stroke *(95% CI: 1.75-2.51)* in the general population. As we have no reason to believe that smoking is a risk factor for the general population and a protecting factor for people living with HIV concerning risk of stroke, we reviewed patient files and performed several sensitivity analyses to account for potential confounders or bias (Table 7). However, despite rigorous approaches none of these investigations resolved the potential confounding factor or an as-yet unanticipated cause.

In order to investigate to what extent smoking could explain the increased risk of MI in the HIV-infected population we (*study 3*) examined the PAF. We found that almost 3 in 4 MIs among HIV-infected individuals were associated with ever smoking compared with only 1 in 4 MIs among population controls. And more importantly, the estimated benefit of smoking cessation in the HIV-infected population (*PAF: 42%; 95% CI: 21-57%)* was the double of that of the comparison cohort *(PAF: 21%; 95% CI: 12-28%).* In the aforementioned editorial [245] , John Gill and Dominique Costagliola argued that it seemed unlikely that the PAF of smoking was as high as we found. However, as we argued in the paper, our results may largely rely on the detrimental effects of tobacco; yet, it may also partly reflect differences in behavior *(e.g. high consumption of alcohol, illicit drug use and collateral psychiatric disorders)* and social disparities *(e.g. low socioeconomic and educational levels)* associated with smoking-status [231, 232, 249, 250]. Essentially, our study reinforces and expands prior knowledge [251] by underscoring the link between smoking and risk of MI and the potentially larger contribution of smoking cessation for the HIV-infected population. Not only are these findings of interest for physicians treating HIV-infected individuals, they may also guide the prioritization of interventions; hence being of importance for the public health.

CONCLUSION:

Already in 1994, Chaisson et al [252] realized that smoking was associated with important causes of morbidity and mortality in HIV-infected individuals. He further stated *"In the past, some clinicians treating HIV-seropositive patients did not emphasize the importance of smoking cessation because it was assumed that the long-term health consequences of smoking were unimportant in patients whose longevity would be seriously compromised by AIDS. It is now clear that stopping smoking can provide health benefits*".

Today the life-expectancy of well-treated HIV-infected individuals approaches that of the general population [36, 253, 254]. Therefore, it seems essential to finally "focus on the silent elephant in the room", as described in the aforementioned editorial [245]. Smoking cessation seems to offer a large potential for reducing morbidity and mortality and bring immense health benefits among smokers.

PSYCHIATRIC CONDITIONS AND UTILISATION OF PSYCHOTROPIC DRUGS

HIV has been associated with a high rate of many psychiatric conditions [255-261]. In 2001, Bing et al [255] reported that almost half of the HIV-infected population examined screened positive for at least one psychiatric disorder. Similar results have been found by others [260], and depression seems to be the most prevalent disorder [255, 256, 262]. However, a recent study [261] identified a 4-fold increased risk of schizophrenia *(IRR: 4.09; 95% CI: 2.73-5.83)* and a 7-fold increased risk of acute psychosis *(IRR: 7.15; 95%CI: 4.45-10.8)* in association with HIV. Therefore, psychiatric conditions such as anxiety, insomnia, depression and psychosis, and hence the utilization of psychotropic drugs, may add up problems, negatively impact HV care [263] and potentially increase the risk of morbidity and mortality [261].

Although, psychiatric conditions have been described to be underdiagnosed among individuals with HIV infection, screening for psychiatric condition by non-psychiatrists may overestimate the true picture. To get an impression of the actual size of the problem, we therefore used the utilization of psychotropic drugs as a proxy for the presence of psychiatric conditions (*Study 4*) [157]. Despite a shorter time of observation *(median observation time: 7.71 vs. 11.13 median years)*, we found that HIV-infected individuals with no IDU or hepatitis C virus (HCV) had a higher prevalence of psychotropic drug (Table 8). In addition, a higher utilization rate *(DDD/person-days)* of psychotropic drugs was observed in association with HIV infection (Table 8).

Table 8: Prevalence of psychotropic drugs used

	Prevalence		URR
Ever used:	**HIV+**	**Controls**	**(HIV+ vs controls)**
Psychotropic drugs	54.5%	29.2 %	-
Antipsychotics	9.6 %	5.5 %	1.13
Anxiolytics	26.9 %	14.1 %	1.76
Hypnotics and sedatives	37.8%	14.3 %	4.42
Antidepressants	28.0 %	16.0 %	2.28

Abbreviations: Utilization Rate Ratio (URR)

Despite large differences in study designs, the results of a poster presented by Schwarz et al [264, 265] at the 15th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV showed highly similar results *(HIV+, no IDU: hypnotics/sedatives, OR: 4.00, 95% CI: 1.94-8.25; antidepressants, OR: 2.52; 95% CI: 1.70-3.76).* As we assumed that the URR may act as a proxy for the relative risk of disorders, this indicates that mood and anxiety disorders, including depression and insomnia, are indeed common among people living with HIV.

With data from the Agence Nationale de Recherche sur le Sida (ANRS) CO3 Aquitaine Cohort (3,863 HIV+), Bonnet et al [266] found a reduction in psychiatric events between year 2000 and 2004

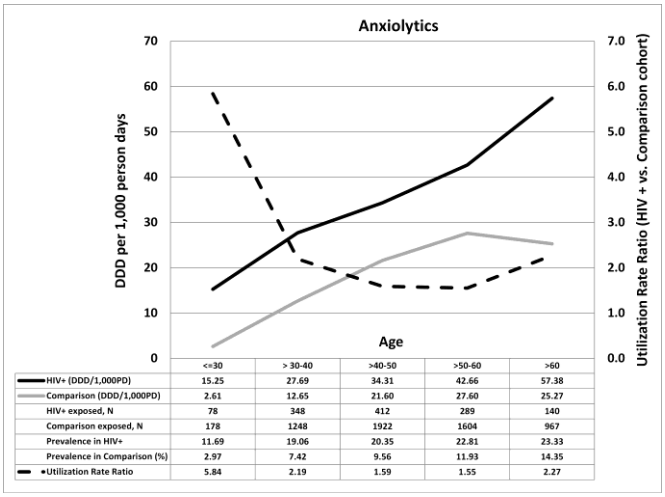
(26 to 14 per 1000 PYR, p: 0.003). In contrast , we *(study 4)* [157] found an increasing utilization of antipsychotics and antidepressants over the years. Our findings are consistent with the utilization in the background population, the trend found in other countries [267-271] and the expanded indications for these drugs.

Although, utilization of anxiolytics, hypnotics and sedatives decreased with calendar time, it increased with increasing age (Figure 6). Due to the addictive potential of these drugs, this may emphasize an increasing problem in the aging HIV-infected population, why alternative drugs must be implemented. With data from the VACS-VC, Goulet et al [272] found a higher risk of psychiatric disorders among HIV-infected veterans older than 50 years compared to non-infected veterans of the same age.

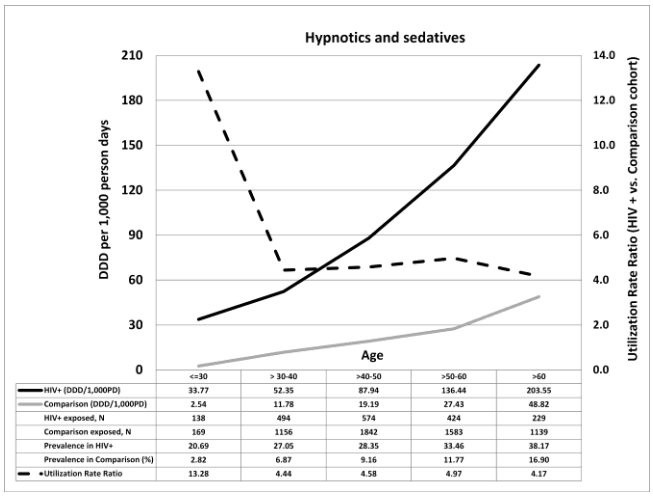
We *(study 4)* also found an increasing utilization of antidepressants with increasing age [157], which is consistent with the results of the utilization of antidepressants in the Scandinavian countries [273], and the results reported by Gutierrez et al [259], of an increasing risk of depression with higher age in HIV-infected individuals.

Our study (*study 4*) [157] illustrated a significant drop in utilization of anxiolytics, and hypnotics and sedatives in the late 90'ties, which could indicate a potential improvement in the physical and psychosocial wellbeing associated with HAART; however, consistent with the results of Vitiello et al [274], we observed no major change in utilization of psychotropic drugs in association with HAART exposure [157].

Figure 6: Utilization and URR of anxiolytics and hypnotics by age.



	<=30	> 30-40	>40-50	>50-60	>60
HIV+ (DDD/1,000PD)	15.25	27.69	34.31	42.66	57.38
Comparison (DDD/1,000PD)	2.61	12.65	21.60	27.60	25.27
HIV+ exposed, N	78	348	412	289	140
Comparison exposed, N	178	1248	1922	1604	967
Prevalence in HIV+	11.69	19.06	20.35	22.81	23.33
Prevalence in Comparison (%)	2.97	7.42	9.56	11.93	14.35
•Utilization Rate Ratio	5.84	2.19	1.59	1.55	2.27



	<=30	> 30-40	>40-50	>50-60	>60
HIV+ (DDD/1,000PD)	33.77	52.35	87.94	136.44	203.55
Comparison (DDD/1,000PD)	2.54	11.78	19.19	27.43	48.82
HIV+ exposed, N	138	494	574	424	229
Comparison exposed, N	169	1156	1842	1583	1139
Prevalence in HIV+	20.69	27.05	28.35	33.46	38.17
Prevalence in Comparison (%)	2.82	6.87	9.16	11.77	16.90
•Utilization Rate Ratio	13.28	4.44	4.58	4.97	4.17

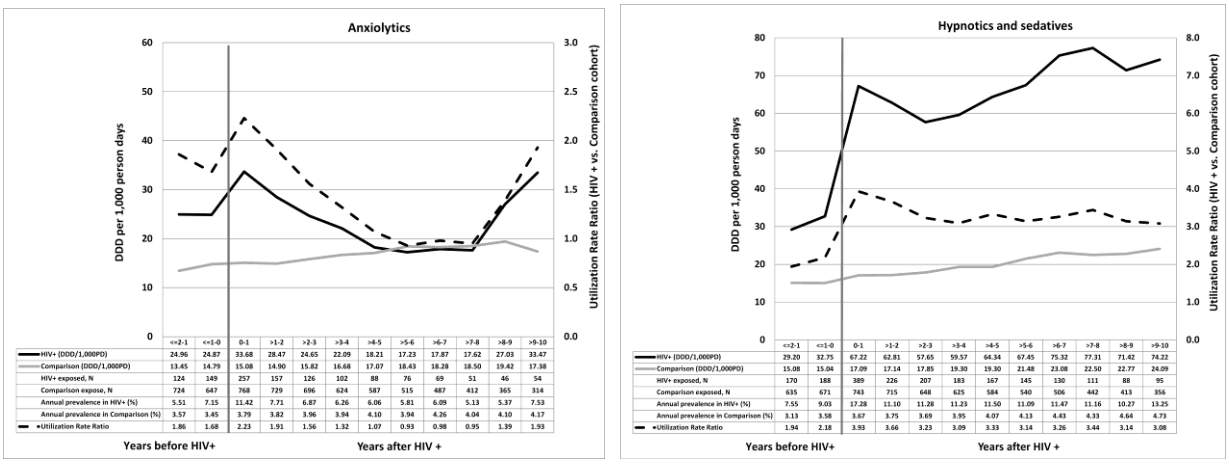
Abbreviations: DDD: Defined daily dose; PD: Person days; Utilization: DDD/1,000 person days; URR: Utilization rate ratio; HIV+: HIV-infected cohort; Comp.: Comparison cohort.

Efavirenz has been associated with a risk of neuropsychiatric symptoms [275-282]. A recent cross protocol analysis of four RCTs [283] observed a 2.3-fold increased risk of suicidality *(aIRR: 2.28; 95%CI 1.27-4.10)* and a 2.6-fold increased risk of attempted or completed suicide *(aIRR 2.58; 95% CI: 0.94-7.06)* in association with efavirenz. The largest effect was seen in the initial 24 weeks *(aIRR: 3.69; 95% CI: 1.41-9.63)* followed by a non-statistically significant trend *(aIRR: 1.54; 95% CI: 0.71-3.34)*. Contrary to our expectations, exposure to efavirenz was 1) not associated with a higher utilization of psychotropic drugs in our study (*study 4*) [157], 2) not associated with increased rates of depression in a study from the Swiss HIV cohort Study [262], 3) and not associated with a higher risk of acute psychosis in a study by Helleberg et al [261]. In the aforementioned study [283], other factors associated with suicidality included IDU, documented psychiatric history or recent pre-study psychoactive medication. We *(Study 4)* excluded IDUs, defined exposure to efavirenz as ever being exposed, and could not identify in-hospital psychotropic medication, hence potentially underestimating the rates slightly. Furthermore, awareness of efavirenz associated psychological side effects may have led to *channeling bias.* Still, in HIV-infected individuals with no IDU *(study 4)* efarenz did not seem to exert a large threat.

RISK BEHAVIOR AND CONCURRENT COMORBIDITY:

Apart from a higher prevalence of smoking, psychiatric conditions and a high utilization rate of psychotropic drugs with addictive potential, HIV-infected individuals have a high prevalence of other risk behavior including heavy alcohol use, illicit drug use and associated HCV co-infection [284-287]. These characteristics frequently co-occur, especially in association with lower socio-economic and educational status [260, 288-292, 293] and may predate the HIV infection [286, 294] (Figure 7).

Figure 7: Use of anxiolytics, and hypnotics and sedatives before (-2) and up to 10 years after HIV infection.



Abbreviations: DDD: Defined daily dose; PD: Person days; Utilization: DDD/1,000 person days; URR: Utilization rate ratio.

Helleberg et al [261] recently observed an almost two times higher risk of being diagnosed with HIV among individuals diagnosed with schizophrenia compared to individuals with no such diagnosis. However, this was only statistically significant for people with a history of substance use disorders, which further highlight the complex interplay of these conditions and characteristics.

Of note, Obel et al [294] found that HIV-infected individuals on successful HAART, who had no co-morbidity, alcohol or drug abuse, had an almost identical risk of death as that of a well-matched comparison cohort with no HIV infection. This implies that the distribution of risk factors and concurrent co-morbidity may represent important differences for individuals with and without HIV hence presenting a challenge in risk analyses (HIV vs controls).

Both the presence of HCV [295-297] and that of psychosocial stressors [298-300] have been suggested to be associated with an increased risk of CVD. The prevalence of obesity, DM and metabolic syndrome, and risk of CVD among individuals with serious mental illness, seems to exceed that of the general population with no mental diseases [301-310]. Treatment for some psychiatric conditions *(e.g. depression)* seems to positively affect the risk of CVD [300], whereas consumption of other psychotropic drugs *(e.g. atypical antipsychotic drugs)*are associated with metabolic abnormalities [303, 307, 308, 311, 312]. Finally, presence of unhealthy lifestyle and risky behavior associated with psychiatric conditions [310, 313] may also partly explain these findings.

The patterns of which HIV infection, conventional risk factors, risk behavior and concurrent co-morbidity interact appear complex and further include factors such as suboptimal health seeking behavior, differences in ethnicity, sexual orientation and low socio-economic and educational status, of which the latter is a well-established risk factor for CVD [314-318].

Using the highest educational achievement as a proxy for socio-economic position, Legarth et al [319] recently demonstrated a higher mortality rate among HIV-infected individuals with low vs high educational attainment, and the excess risk mainly stemmed from smoking and alcohol-related mortality. A similar trend was observed for the general population; however, the excess risk was larger for the HIV-infected population *(aIRR: 3.6; 95%CI: 1.5-8.9)* than that of the general population *(aIRR:. 2.0; 95% CI: 1.2-3.4)*. This further stresses the fact that many lifestyle related factors seem highly correlated with socio-economical and educational factors, and may further explain the observed increased risk of morbidity and mortality in the families of HIV-infected individuals [104, 320, 321].

CONCLUSION

The need to modify risk behavior, and treat concurrent co-morbidity is of great importance in the care of HIV-infected individuals. As the characteristics associated with being a smoker include alcohol and drug abuse, psychiatric co-morbidity *(e.g. depression, anxiety and emotional distress)*, low socio-economic and educational status, and potentially low perceived risk, these factors may represent large barriers that make smoking cessation highly problematic. Consequently, these factors also need to be addressed in smoking cessation programs and actively pursued in modern HIV care. Depression and other psychiatric conditions should be identified and treated as these conditions may negatively impact HIV care. Furthermore, prescription of drugs with addictive potential and risk of drug-drug interactions should be highly minimised in this population.

HAART EXPOSURE

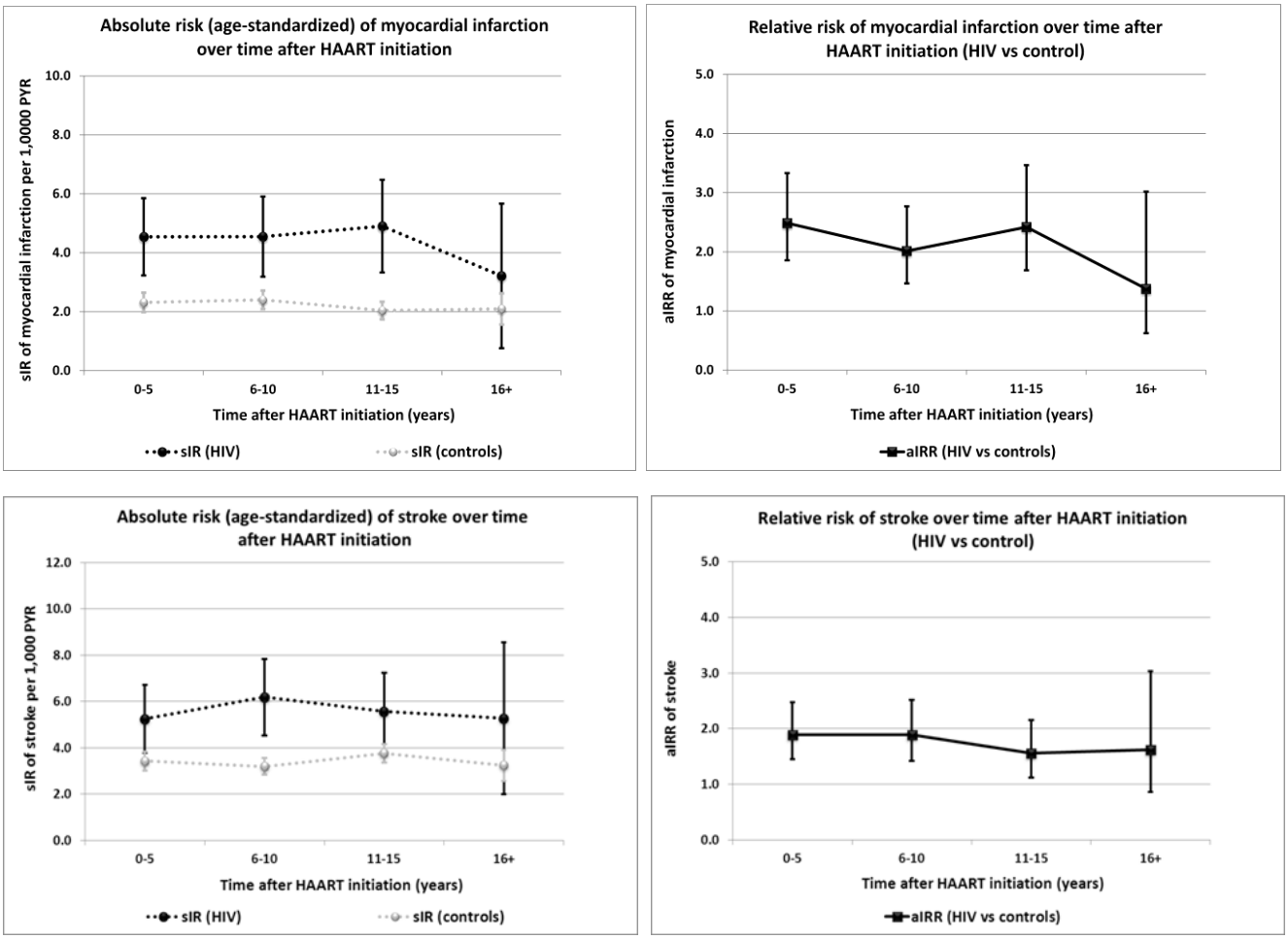
The antiretroviral drugs suspected to be involved with the increased risk of MI have mainly included cumulative exposure to "older" PIs, partly due to, but also beyond that conferred by lipid perturbations [87, 89, 90, 94, 322]. Furthermore, a potential association with current or recent exposure to abacavir has been suggested [91, 94, 323-329], and lately an association with cumulating exposure to abacavir has also been proposed [330, 331]. Clinical trials have failed to confirm the latter association which has further prompted extensive research; however, the association still remains subject of debate [89, 147, 330, 332-345].

Concerning risk of cerebrovascular events, the overall role of HAART exposure seems less clear [83, 151, 175, 177, 180-182, 189, 323, 346]. We (*study 1*) [151] examined the risk of cerebrovascular disease among 5,031 HIV-infected individuals and found no association between exposure to HAART and risk of cerebrovascular events *[(HAART with CD4 ≤ 200cells/μL: aIRR: 1.17; 95% CI: 0.50-2.75); (HAART with CD4 > 200 cells/μL: aIRR: 0.80; 95 % CI: 0.47-1.36)]*. In contrast, the Data collection on Adverse effects of Antiretroviral Drugs (D:A:D) study [83] analyzed the risk of the composite endpoint CVD *(i.e. MI and stroke)* and found a 26% increased risk *(95% CI: 1.14-1.38)* per additional year of HAART exposure; however, the majority of the endpoints were due to MI *(only 38 strokes)*. Furthermore, later observations from the D:A:D study [323], in which these endpoints were analyzed individually, illustrated discrepancies between associations of antiretroviral drugs between MI and ischemic stroke. Accordingly, this illustrates problems associated with the use of composite endpoints and subsequent extrapolations to the individual endpoints.

Furthermore, if the suggested 26% risk of CVD per additional year of HAART exposure would have come true, it would have led to age-independent alarming results with time. Initially such dramatic or accelerated effect was not supported in the analysis of MI from the Danish HIV Cohort Study [86]. However, it appears reassuring that we *(study 7)* [121], with more than 15 years of follow-up on HAART exposure did not find a cumulative increased risk *(age-standardized absolute or relative risk)* of neither MI nor stroke with time on HAART or calendar time (Figure 8). This further illustrates the difficulties in disentangling effects associated with cumulative drug-exposure from underlying effects of age, calendar time and time with HIV infection.

Importantly, protective effects of HAART on risk of ischemic stroke have also been found. As an example, a cohort study from Boston by Chow et al (4,308 HIV+) [181] observed that longer duration of exposure to HAART was associated with a 20% reduction in risk of ischemic stroke *(aIRR: 0.80; 95% CI: 0.73-0.88)*. Also, a small Spanish case-

Figure 8: Age-standardized absolute (sIR) and relative risk (aIRR) of MI and stroke with time after HAART initiation



Abbreviations: HIV: HIV-infected individuals; PYR: Person-years of follow-up; IR: Incidence Rate, sIR: Standardized Incidence Rate (standard: age and gender distribution (PYR) year 10-12 after HIV transmission); IRR: Incidence Rate Ratio, aIRR: adjusted Incidence Rate Ratio; 95% CI: Confidence Interval.

control study by Corral et al (25 cases and 100 controls)[189] observed a 3% lower odds of ischemic stroke per additional month of HAART exposure *(aOR: 0.97; 95% CI: 0.96-0.99)*. We (*study 1*) [151] observed an almost inverse relationship associated with the CD4 cell count *(further discussed below)*, hence stressing the potential association between HAART-associated immune restoration and viral suppression and associate reduction in risk.

Results concerning associations with individual antiretroviral drug-classes or drugs and risk of cerebrovascular events have also yielded conflicting results [83, 151, 175, 177, 181, 182, 189, 346, 347]. A large American study by Bozzette et al (36,766 HIV+) [175] observed no relation with

exposure to or duration of PI, NRTI or NNRTI and risk of the composite endpoint CVD. Similar results in association with ischemic stroke were observed by some [182], whereas others [181] observed a protective effect of *ever* using *(but not cumulative use of)* NNRTI *(aIRR: 0.38; 95% CI: 0.19-0.76)*. In line with prior results from the D:A:D study on risk of MI, more recent data [261] have shown a 6% increased risk of the composite endpoint of ischemic stroke and sudden death *(95% CI: 1.01-1.11)* in association with cumulative exposure to PI per year of exposure.

Finally, associations with abacavir deserve further attention. In contrast to the results of the D:A:D study performed by Sabin et al [323], that found no association between abacavir exposure and risk of stroke, we observed a 1.7-fold increased risk of cerebrovascular events *(95% CI: 1.03-2.68)* in association with first time exposure to abacavir (*study 1*) [151]. As the D:A:D study [323] was mainly performed to examine risk of MI, methods and results concerning stroke was only briefly mentioned, thereby making further comparison problematic. Nevertheless, a sub-analysis performed in our study (*study 1*) [151] highlighted that the risk of cerebrovascular events remained high even after first cessation of abacavir, hence indicating underlying *channeling bias.* In line with the D:A:D study [323], results from the Veterans Administration database [332] could not replicate an association between either current or cumulative exposure to abacavir and risk of cerebrovascular events. On the contrary, an association between abacavir exposure and risk of the composite endpoints CVD has been established in a post-hoc analysis by the SMART study [324] as well as in observational studies by other study groups [325, 327]. Nevertheless, the results of a higher CVD risk could be carried by an underlying effect of MI. Overall, these results underscore the problems with analyzing effects of drugs in observational studies, that due to lack of randomization, are prone to *channeling bias* and *confounding by indication*, and further carry a risk of *residual* and *unmeasured confounding* *(further discussed in the methodological considerations section)*. However, the overall healthier population and shorter time of follow-up used in RCTs, makes it difficult to detect rare outcomes and thereby almost impossible to detect differences in such risks [339]. Some studies have tried to ameliorate these shortcomings by pooling data from several RCTs. However, as the studies are often based on different design and set-up, this is also not without problems. Although, the changes in prescribing behavior that the initial and following studies have brought about, this issue remains very confusing for the acting clinician, and may ultimately never be resolved [345].

Concerning risk of VTE, exposure to HAART, particularly PIs, have previously been implicated as a risk factor; however, the data are limited, the results conflicting and generally based on data from

the pre/early HAART-era, and the study designs are simple including poor statistical analyses and generally lack of important confounder control [193, 195, 197, 199-201, 204, 210].

We *(study 2)*[206] observed an almost 2-fold increased risk of VTE among non-IDU HIV-infected individuals *(aIRR: 1.93; 95% CI: 1.00-3.72)* in association with HAART; however, sub-group analyzes were not performed. In contrast to HIV-infected individuals with no IDU, HAART-exposure did not seem to confer a risk among HIV-infected IDUs *(study 2)* [206]. This is presumed to be due to a selection of a healthier subset of the IDUs whom is exposed to HAART. To our knowledge, the existing literature and associate quality is poor, why further studies are needed in order to elucidate this question any further.

CONCLUSION

During the past 10-15 years, studies have raised concerns about the adverse effect of HAART on a large number of non-AIDS associated diseases including cardio- and cerebrovascular disease and VTE. Seemingly, the results of MI cannot be extrapolated to cerebrovascular disease and VTE, for which the effect of HAART, if any, seems less clear and needs further investigation. However, with utilization of an increasing number of new antiretroviral drugs the level of metabolic toxicity has declined. Continuous as compared to intermittent exposure to HAART, has been proved to reduce the risk of CVD by the SMART trial [105]. Also, the results of a large well-designed, multicontinental randomized study, known as the Strategic Timing of Antiretroviral Therapy trial (START) [348] has recently been published. This study showed that immediate HAART *(CD4 cell count ≥ 500cells/μL)* vs. deferred HAART *(CD4 cell count ≤350 cells/μL)* provided a 57% relative reduction of the composite endpoint serious AIDS-related events, non AIDS-related events and death *(HR: 0.43; 95% CI: 0.30-0.62)* and a 39% relative reduction in serious non-AIDS-related events *(HR: 0.61; 95% CI: 0.38-0.97)*. In contrast, no significant association with risk of CVD was found *(HR: 0.84; 95% CI: 0.39-1.81)*, although this could be a result of low power as of early termination of the study. As a result of these two RCTs, the timespan from HIV diagnosis to HAART initiation will decline. Although, some drugs should still be avoided in HIV-infected individuals with a high CVD-risk, the overall effect of HAART still seems to overstate any minor risks of toxicities.

RISK ASSOCIATED WITH HIV PER SE

Given the hypothesizes that HAART may lead to a higher risk of non-AIDS associated morbidity, less use of HAART could potentially reverse this risk. As a result, the SMART trial [102] randomized 5,472 HIV-infected individuals with a CD4 cell counts above 350 cells/μL to continuous *(viral suppression arm)* or intermittent HAART *(drug conservation arm).* In the drug conservation arm, HAART was *(re)* initiated when the CD4 cell count was below 250 cells/μL and stopped or deferred when the CD4 cell count was above 350 cells/μL. Surprisingly, interruptions of HAART with deferral until immunosuppression lead to higher rates of all-cause mortality *(aHR 1.8; 95% CI:1.2-2.9)*, serious opportunistic disease *(aHR: 6.6; 95% CI: 1.5-29.1)*, a composite endpoint of major CVD, renal or liver disease *(aHR: 1.7; 95% CI: 1.1-2.5)* and the individual endpoints major fatal or nonfatal CVD *(aHR: 1.6; 95% CI: 1.0-2.5)*, renal disease *(aHR: 4.5; 95% CI: 1.0-20.9)*, and liver disease *(aHR: 1.4; 95% CI: 0.6-3.8)* [102, 349]. A restriction of the analyses to HAART-naive individuals and individuals who had been off HAART for more than 6 month further increased the risk of the composite endpoint of CVD, renal or liver disease and non-AIDS defining cancers *(aHR: 7.02; 95% CI: 1.57-31.4)* [350]. These findings underscored the potential risk associated with the virus itself concerning risk of MI, CVD and other major age-related non-AIDS associated diseases as well as associated immunologic or inflammatory changes.

IMMUNODEFICIENCY

Still, in contrast to the strong association between immunodeficiency and risk of AIDS-associated morbidity and mortality, an association between immunodeficiency and non-AIDS morbidity remain less clear. With data from the American Flexible Initial Retrovirus Suppressive Therapies (FIRST) trial (1,397 HIV+), Baker et al [351] reported that the risk of the composite endpoint non-AIDS diseases declined with a higher *latest* CD4 cell count on HAART *(CD4+ < 200cells/μL, 200-350cells/μL and > 350 cells/μL: aIRR: 2.1, 1.7 and 0.7)*. A similar trends was observed for the individual non-AIDS associated diseases; however, lack of power limited the precision of the estimates [351]. Also, recent results from the AIDS Therapy Evaluation in the Netherlands national observational (ATHENA) cohort (12,800 HIV+) [352] have shown a similar trend.

Assessing the potential association with CD4 cell count and risk of morbidity and mortality, Phillips et al [353] compared the unpublished and published results of the SMART trial, the D:A:D study, the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) and the FIRST study. Data restricted to HAART-naïve individuals *(D:A:D and CASCADE)* showed a strong tendency *(unadjusted)* for a higher rate of death from any cause as well as non-AIDS associated causes with lower CD4 cell counts [353]. Additionally, data from all 4 studies showed an almost consistent

association of immunodeficiency with risk of liver disease, non-AIDS malignancy, and kidney disease, whereas risk of CVD and CVD mortality seemed less consistent.

A number of other studies have examined the impact of immunodeficiency on a variety of non-AIDS diseases, including CVD; yet, the results have been highly divergent, possibly due to differences in methods including definitions, cutoffs and CD4 cell count metrics, underlying treatment status, quality and completeness of the used measures, varying study populations, outcome definitions and varying confounder control. Moreover, risk of surveillance bias in those with the lowest CD4 cell counts cannot be excluded.

To sum up, a low *baseline* CD4 cell count has been associated with a higher risk of MI, CVD, stroke, increased coronary age and a higher prevalence ratio of carotid lesions in several studies [183, 333, 354-356].

Three small studies [60, 357, 358] have found an association between a low *nadir* CD4 cell count and several measures of preclinical arteriosclerosis. Also, results of the French Hospital database on HIV [359], data from two large tertiary care hospitals in Boston, Massachusetts [333], and data from the Kaiser Permanente Southern and Northern California health plans [93] have observed a lower MI risk with higher nadir CD4cell count. But, no association between nadir CD4 cell count and risk of MI, CVD or stroke was found by the D:A:D study [87, 360] or the CASCADE study [361].

Also, no association with the *duration* of or *cumulative* exposure to a low CD4 cell count and risk of CVD has been observed by the CASCADE and the D:A:D study [360, 361].

In a case-control study nested in the HIV Outpatient (HOPS) Study [354], a lower *latest* CD4 cell count *(CD4 < 350 cells/μL vs ≥ 500 cells/μL)* was independently associated with a 3.1-fold increased risk of CVD *(95% CI: 1.95-4.84)*. Similar results has been found for major coronary or other arterial diseases by the French ANRS CO8 APROCO-COPILOTE study [362] whereas several other studies found no association with latest CD4 cell count and CVD or associated death [266, 349, 361], or only an association of fatal CVD that became insignificant when lagging the CD4 cell count with 6 months [363].

Concerning risk of stroke or cerebrovascular disease several studies have observed an increased risk in association with a lower *recent* CD4 cell count [151, 172, 180, 182, 360, 364, 365].We *(study 1)* [151] found that immunodeficiency (*defined as the time with a CD4 cell count <200 vs ≥ 200 cells/μL)* before initiation of HAART more than doubled the risk cerebrovascular events, whereas no such association was detected after initiation of HAART (Table 9). Consistent with our results, Marcus et al [182] found a 3.2-fold higher risk of ischemic stroke *(95% CI: 2.3-4.4)* among HIV-infected

individuals with a *recent* CD4 cell count below 200 cells/μL compared with HIV-negative individuals from the Kaiser Permanente Nothern and Southern California Cohort. Also, the D:A:D study-group [360] showed a 2.3-fold *(95%CI: 1.29-3.94*) and a 1.6-fold *(95% CI: 1.03-2.59)* increased risk of stroke in association with a *latest* CD4 cell count below 100 cells/μL and between100-199 cells/μL, respectively.

Table 9: Impact of immunodeficiency on risk of cerebrovascular disease in HIV+

	aIRR (95%CI)
Time exposed to:	**Non-IDU HIV+**
CD4 > 200 cells/μl, no HAART	1 (ref)
CD4 ≤ 200 cells/μl, no HAART	2.26 (1.05-4.86)
CD4 ≤ 200 cells/ μl, HAART	1.17 (0.50-2.75)
CD4 > 200 cells/ μl, HAART	0.80 (0.47-1.36)

Abbreviations: aIRR: adjusted incidence rate ratio; ref: reference; HIV+: HIV-infected individuals; non-IDU: non injection drug abuse

Essentially some of the observed effect of immunodeficiency could be due to misclassification of cerebral co-morbidity, although, thorough analysis to avoid this problem had been performed in our study *(study 1)*[151] and the DAD study [360]. Furthermore, as immunodeficiency seems associated with higher levels of inflammatory markers and immune activation, it is difficult to establish the potential key player.

Finally, a recent study showed that a major decline in CD4 cell count among virally suppressed individuals, was associated with a concomitant drop in CD8 cells, CD3 cells and total lymphocytes, and a marked increased risk of CVD, cancer and death within 6 months of the decline [366]. The authors stated that, due to the short time interval, a lowered CD4 cell count could act as a surrogate marker of chronic inflammation and thus acting as a consequence rather than a potential cause of disease and mortality.

Concerning risk of VTE, a small nested case-control study of HIV-infected individuals by Modi et at [210] observed a 4.5 times higher odds of VTE *(95% CI: 1.52-13.37)* among HIV-infected individuals with a recent CD4 cell count below 200 cells/μL. Consistent results of a higher risk of VTE in association with immunodeficiency have also been established in other studies, however, most of these results were based on very simple statistical analysis [197, 202, 204, 208]. In contrast, a large multicentre study *(42,935 HIV+)* by Sullivan et al [195] could not establish such an association. Still, presence of opportunistic infections and presence of cytomegalovirus (CMV) was associated with a 1.5 times higher odds of VTE *(95% CI: 1.1-2.2)* a 1.9 times higher odds of VTE *(95% CI: 1.2-2.9)*, respectively. An association with opportunistic infections and AIDS in general have also been proposed by others [191, 196, 197, 205, 208]. Although, we *(study 2)* [206] found a trend towards a higher risk of VTE in the time with a CD4 cell count below 200 cells/μL, lack of power might have prevented us from producing statistically significant

results in all but unprovoked VTE among HIV-infected IDUs (Table 10).

Table 10: Impact of immunodeficiency on risk of VTE in HIV+

aIRR (95% CI) CD4 < 200 vs ≥ 200 cells/µl	
Non-IDU HIV+	**IDU HIV+**
All VTE	
1.53 (0.83-2.84)	2.18 (0.97-4.86)
Unprovoked VTE	
1.68 (0.84-3.36)	2.83 (1.23-6.49)
Provoked VTE	
1.14 (0.30-4.35)	NA

Abbreviations: VTE: Venous thromboembolism; aIRR: adjusted incidence rate ratio; HIV+: HIV-infected individuals; IDU: injection drug abuse; NA: Not applicable

Of note, the level of many coagulation factors of HIV-infected individuals have been proposed to correlate with the level of immunodeficiency or to be secondary to opportunistic infections [197, 205, 215, 216, 367]. However, for risk of VTE, immunodeficiency or opportunistic infections could also be a marker of a higher degree of immobilisation which is a well-known provoking factor [197].

CONCLUSION

These data further add to the complexity of the interplay of risk factors concerning vascular diseases and other age-related diseases. As a consequence of the direct correlation of factors laying on the same time-axis such as the duration of HIV, cumulative exposure to HAART and increasing age *(multi-collinearity)*, or the interrelationship of viral load, CD4 cell count and changes in inflammatory markers or markers of immune activation, it is hard to determine the actual key players and moreover ensure lack of confounding. Despite inconsistent results of the impact of immunodeficiency on risk of MI, CVD, and cerebrovascular events, there is growing evidence that other non-AIDS associated events such as liver and renal disease and potentially non-AIDS cancers as well as death due to non-AIDS events are associated with the degree and duration of immunodeficiency [351, 353, 361, 363, 368-370]. Furthermore, immunological control, as indicated by continuous HAART in the SMART study, appears to be highly important [102]. Finally, as indicated in prior studies of elite controllers [68, 371], and the results of the recent START study [348], in which a significant benefit was associated with early initiation of HAART regardless of CD4 cell count, additional factors might be at stake.

SUSTAINED LOW LEVEL INFLAMMATION

It is well recognized that the immune system plays a significant role in the development of atherosclerosis [372-374] and a number of markers of increased inflammation and altered coagulation have been independently related to risk of CVD and mortality in the general population [375-382]. The overall level of inflammatory markers *(including high sensitive C reactive protein (hsCRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α))* and markers of coagulation and fibrinolysis *(including D-dimer and fibrinogen)*, have been found to be higher among HIV-infected individuals compared to the general population with no HIV infection [112, 383-386]. Furthermore, the level of biomarkers is persistently higher in association with untreated HIV [112, 387], presumably due to the contribution of HIV replication and immune depletion [388]. HAART exposure seems to reduce the overall level of biomarkers [112, 387, 389-392]; but, despite HAART-induced viral suppression, the level of these markers fail to normalize [112, 385, 387, 393].

For the HIV-infected population, the level of several biomarkers *(mainly hsCRP, IL-6, D-dimer, fibrinogen, Plasminogen Activator Inhibitor 1(PAI-1) and soluble urokinase plasminogen activator receptor (suPAR))* seems to be associated with both risk of MI, CVD and other age-related diseases [394-402], HIV disease progression [402-405] and all-cause mortality [405-409]. Also, higher levels of markers of coagulation and endothelial activation *(e.g. soluble vascular cell adhesion molecule 1 (sVCAM), soluble intercellular adhesion molecule 1 (sICAM) and von Willebrand factor (vWF), and asymmetric dimethyl arginine (ADMA) that contributes to impaired endothelial function by inhibition of nitric oxide synthase)* have consistently been found among HIV-infected individuals compared to that of non-HIV-infected controls, and these markers have been shown to be associated with HIV replication, inflammation and subclinical atherosclerosis in some studies [72, 384, 392, 410, 411].

CHRONIC IMMUNE ACTIVATION

High levels of immune activation among HIV-infected individuals has previously been associated with HIV disease progression and mortality [383, 406, 407, 409, 412-415]. As chronic immune activation persist even among virally suppressed HIV-infected individuals on HAART, chronic immune activation has been suggested to play a central role in the development of atherosclerosis and potentially other age-related morbidity [73, 371, 415-418]. Yet, the mechanisms associated with chronic immune activation and associated risks, as well as the possible interplay with other risk factors, are not fully established.

Ongoing low level HIV replication in gut associated lymphatic tissue or reservoirs hidden from

antiretroviral therapy, asymptomatic CMV or HCV replication, and microbial translocation has been suggested to contribute to chronic immune activation [418-421]. The latter is described by a rapid depletion of CD4+ T cells in the gut associated lymphoid tissue that leads to a breakdown of the immunological and structural intestinal barrier in the gastrointestinal tract which again leads to entry of microbial products such as lipopolysaccharide (LPS) [417, 422]. As with immune activation, microbial translocation seems to be greater with more advanced HIV infection [417, 423], and seems to decrease upon HAART exposure [417, 424].

Extensive research have examined associations between several markers of immune activation inflammation and markers of endovascular dysfunction, subclinical atherosclerosis and risk of serious age-related diseases [70, 73, 352, 423, 425-440]. The results of these studies appear relatively divergent. Still, chronic immune activation and sustained low level inflammation is generally assumed to play a key role in risk of CVD and other serious age-related diseases, and to be associated with increased frailty and early or accelerated aging [103] *(further discussed later in the discussion)*.

CONCLUSION

Chronic immune activation and sustained low level inflammation has been suggested to explain a major part of the increase risk of age-related disease in HIV-infected individuals. HIV-infected individuals have higher levels of a number of biomarkers that further suggest ongoing chronic immune activation and sustained low level inflammation, even when on HAART. However, despite the tremendous amount of research in this area, the evidence concerning these biomarkers is, with the exception of the SMART trial [398, 407], mainly based on small cross-sectional studies that are unable to evaluate time-trends. Furthermore, the causal relationship seems somewhat inconsistent and reversed causality cannot be excluded. Therefore, it is unknown whether these biomarkers actually act as targets of intervention in which a reduction in biomarkers would lead to a reduction in morbidity or mortality.

ARE STATIN THE NEW WONDER DRUG?

As markers of immune activation, inflammation and coagulation appears to be associated with HIV disease progression, AIDS-associated and non-AIDS associated diseases as well as all-cause mortality, it is important to identify the role of adjuvant therapies that may target and attenuate the level of these parameters. Hence, the possible effects of statin therapy on the level of immune activation and inflammation have been examined [114, 116, 441-445].

Studies from the general population have generally shown reductions in inflammation markers in association with statin therapy independent of baseline levels of or reductions in lipid levels [446-448]. Similar results have been observed in most studies of HIV-infected individuals [118, 442, 449]. In the Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV (SATURN) trial [444, 445], HIV-infected individuals on stable HAART *(no known CVD or DM, normal levels of low density lipoprotein cholesterol, heightened levels of inflammation and immune activation)* were randomized to rosuvastatin versus placebo *(72 vs 75)* stratified by PI-based HAART regimens. Whereas no change in the level of activated T-cells was observed with 24 weeks of treatment, a significant decrease in markers of inflammation, and lymphocyte and monocyte activation was observed after 48 weeks of rosuvastatin treatment independent of the significant changes in lipid levels [445]. However, beside a modest increase in bone-mineral density [450], no clinical benefit of any potential improvements of immune activation has yet been found.

Of importance, statin therapy reduces the risk of CVD despite normal cholesterol levels, and reduces the risk of stroke, although, hyperlipidemia is not considered an important risk factor for stroke [446-448]. In the Justification for the Use of Statins in Primary Prevention (JUPITER) trial [448], 17,802 healthy men with elevated levels of hsCRP but no hyperlipidemia were randomized to rosuvastatin versus placebo. This study found a substantial reduction in all-cause mortality *(aMRR: 0.80; 95% CI: 0.67-0.97)* besides the well-known reduction in major CVD events. Consistent results was recently found by the Cochrane Collaboration when examining the effect of statin therapy for primary prevention [451], and has previously been found in association with secondary prevention [452]; however, in the latter meta-analysis, statin therapy was not associated with non-vascular causes of death *(aRR: 0.95, 95%CI 0.91-1.01)*[452]. Irrespective of the latter result, this has prompted extensive research of the potential beneficial and cholesterol independent effects of statin therapy.

Epidemiological studies from the general population have suggested a multitude of clinical effects including reduced morbidity and all-cause and

cause specific mortality [453-463]. Although, some of these studies have large, representative and ethnically diverse populations, a large number of endpoint [462, 463], and inclusion of special analyses to handle confounding by indication [463], this task is difficult if not impossible in non-RCT studies. In general, small negative studies are largely absent and very few results of large RCTs with hard clinical endpoints are present [464-468].

In 2011, Moore et al [142] found that, in virally suppressed HIV-infected individuals on HAART, statin therapy was associated with a substantial 3-fold reduction in all-cause mortality (*aMRR:0.33; 95% CI: 0.14-0.76)*. As briefly touched upon before, and further discussed in the methodological section, cohort studies are always challenged by confounding by indication when examining effects of drugs. However, the study by Moore et al [142] had also several other methodological problems that might have biased the results. In study 5 [131] , we attempted to confirm the results by Moore et al by using similar methods but avoiding important bias [131].

First, when using survival analysis, censoring must be independent of the outcome [130]. As Moore et al [142], censored time at virological failure, which is associated with a higher risk of mortality, the model was violated by *informative censoring*, hence, introducing *selection bias*. We applied two models: A) with censoring at virological failure *(VL > 500 copies/ml)*, and B) with no censoring at virological failure. In our results of model A, we were unable to reproduce the highly significant finding by Moore et al *(Model A: aMRR 0.75; 95% CI: 0.33-1.68)*. We cannot neglect the fact that our study could be underpowered to detect a significant association *(type 2 error)*; however, considering the impact size observed in the study by Moore et al this seems less likely. When we applied model B, no difference in mortality in association with statin therapy was found *(Model B: aMRR 1.17; 95% CI: 0.66-2.07)*.

Next, individuals treated with statins are expected to have an a priori increased CVD risk profile. In the European Society of Cardiology and European Atherosclerosis Society (ESC/EAS) guidelines for the management of dyslipidaemia [469], individuals with known CVD, DM or chronic kidney disease was considered CVD risk equivalents that needed active management of all risk factors, including statin therapy, independent of other factors. In contrast to the study by Moore et al [142], that did not take co-morbidity into account, we included comorbidity as defined by the presence of CVD or risk equivalents during the entire follow-up period. Furthermore, an interaction term between co-morbidity and statin therapy was included in order to take *confounding by indication* into account. These analyses showed a tendency toward a reduced mortality in individuals with a diagnosis of comorbidity, which is in straight line with prior knowledge of secondary prevention. However, we could not detect any difference in mortality in the

time with no comorbidity *[(+ censoring: aMRR: 1.12; 95% CI: 0.34-3.62), (-censoring: aMRR: 0.90; 95% CI: 0.28-2.88)]*. Above all, this implies, that the impact of statin on individuals with no comorbidity seems small or absent.

Similar result of the effect of statin therapy as primary prevention on risk of all-cause death has recently been published in a nested case-control study by Lang et al [470] *(aMRR: 0.86; 95% CI: 0.34-2.19)*. Although, several methods were used to overcome confounding by indication *(propensity scores and inverse probability of treatment,)* statin therapy was unfortunately defined at a fixed time point *(~ prescription of statin within 3 months prior to index date, Y/N)*.

Finally, the model used in our study *(study 5)* [131] may in its current form be slightly *over-fit (further described in the methodological section).* However; a more parsimonious model showed similar results. We believe we have improved the study upon the earlier analyses. Furthermore, a larger study by Overton et al [471], that also applied complex statistical models *(inverse probability of treatment and inverse probability of censoring weighting ~ marginal structural modelling)*, to avoid *confounding by indication* and *informative censoring* showed consistent results of no reductions in non-accidental mortality *(adjusted and weighted MRR: 0.82; 95% CI: 0.32-2.10).*

Overton et al [471] further examined risk of non-AIDS defining events in association with statin therapy and only found a significant reduction in non-AIDS-defining malignancies *(aIRR: 0.43; 95% CI: 0.19-0.94)*. A study by Chao et al [472] further yielded a statistically significantly 45% lower risk of non-Hodgkin lymphoma in association with ever versus never statin use *(aIRR: 0.55; 95%CI: 0.31-0.95)*. In accordance with these results, Galli et al [473], reported a 55% reduction in risk of cancer associated with exposure to statin therapy. However, as individuals who initiated statin therapy at some time during follow-up were considered statin users from the start of the study, the results might be flawed by *immortal time-bias (further discussed in the methodological section).* The study used the Fine and Gray model to compute the *sub-distributional hazard ratio* [161] in order to take competing risk by death into account. Although the choice of model is correct, the produced estimates has no resemblance with the hazard ratio in a Cox Proportional Hazard Regression analysis, and only describes the ordering and not a numerical value for the ratio *(further discussed in the methodological section)* [159, 160]. Therefore, the results can only state that statin therapy is associated with a lower risk, but not a 55% reduction in cancer occurrence *(sub-distributional hazard ratio: 0.45; 95% CI: 0.17-0.71)*.

Recently, two large RCTs of the association between statin therapy and sepsis-associated acute respiratory distress syndrome [474] and exacerbation of chronic obstructive pulmonary disease [475] was published in New England

Journal of Medicine. As no therapeutically benefit was apparent in any of these studies, both trials were terminated before time by the data and safety monitoring boards [476]. Although, such studies are expensive, they are needed to identify true associations of statin therapy, hence bridging the gap.

CONCLUSION:

The evidence for a statin-associated protective effect that extend beyond CVD prevention seems inadequate both for the HIV-infected and the general population. Recent studies among HIV-infected individuals mainly illustrate that it is extremely difficult, if not impossible, to evaluate effects of treatments in observational studies. Nevertheless, when trying, basic methodological problems must be avoided, as further stressed in our paper *(study 5)* [131]. Extensive evidence, provided by several large clinical trials, have demonstrated the effect of statins on primary and secondary prevention of cardio- and cerebrovascular disease, as well as mortality due to coronary artery disease in non-HIV-infected individuals [451, 452, 477-481]. However, the RCT based evidence to support the use of statins outside the recommendations in non-HIV as well as HIV-infected individuals still seems very weak. And, although these drugs are rather safe and tolerable, the concomitant use of antiretroviral drugs, mainly PIs, are associated with a risk of drug-drug interaction [482]. Finally, the potential benefits do not seem to outweigh the potential risk of DM [448, 483-485], although, results of the latter are highly conflicting [486, 487].

"PREMATURE OR ACCELRATED AGING"

As, HIV-induced chronic immune activation and persistent low-grade inflammation has been suggested to have a detrimental impact on the risk of age-related diseases [398], it has been hypothesized that the HIV-infected population may sustain premature or accelerated ageing [103, 488]. The deterioration of the immune system over time, which accompanies ageing in humans and is associated with increased susceptibility to common infections, neoplasms, age-related diseases, and decreased antibody response, is known as immune senescence [489]. Some of the alterations of the immune system that arise during HIV infection seems to be similar to the changes found during normal aging not affected by HIV, and differs from that of non-HIV-infected individuals of the same age *(e.g. accumulation of highly differentiated T-cells, primarily CD8+ effector cells, reduced/limited T-cell proliferative capacity, shortened telomere length and structural changes of the telomeres)* [120, 489-494]. Although, these findings have mainly been based on cross sectional studies, they have largely been used to support the hypothesis of accelerated aging. As persistent infections like CMV infection has been proposed to play a role in activation driven replicative exhaustion of T-cell compartments and shortened telomere length [489, 495], chronic immune activation and inflammation has been proposed to speed up the ageing process [120]. Finally, HIV infection has been associated with an earlier and increased presence of a *frailty-related phenotype* or *syndrome* that include myopathy, loss of muscle mass and weight, fatigue, functional impairments, cognitive dysfunction and motor abnormalities [496, 497].

Although, the "accelerated ageing" hypothesis as the reason for an increased risk of age-related diseases may seem vague and poorly defined, it has been repeatedly expressed in both academic papers and the popular press, and potentially spread fear in the HIV-infected population. However, as discussed in an editorial by Martin and Volberding [498], no consensus definition exist on accelerated aging. "So, what do clinicians and researchers mean when they claim HIV causes premature aging?"

Is "accelerated aging" defined by:

1) a higher risk of age-related diseases (HIV+ vs general population)?

2) a lower median age at diagnosis?

3) a higher relative risk of age-related diseases at all ages/in the younger ages/ increasing with age?

4) an age-independent increasing (absolute and relative) risk of age-related diseases with time?

1) A higher risk of age-related diseases (HIV+ vs general population)

First, we wanted to put pressure on the hypothesis by examining the risk of an age-related disease *(HIV+ vs. general population)* that had no apparent association with HIV or HAART *(Study 6)* [499]. As cataracts is highly associated with age [500], but not apparently associated with HIV or HAART exposure, we used cataract surgery *(~ cataracts)* as a surrogate marker of biological ageing. We identified 5,315 HIV-infected individuals from DHCS and 53,150 age- and gender-matched controls from the general population and investigated the relative risk of cataract surgery *(HIV vs controls)*. Contrary to our expectation, the main finding of the paper was, an almost two-fold increased risk of cataract surgery *(aIRR: 1.87; 95% CI: 1.50-2.33)* among HIV-infected individuals relative to the controls [499]. Although, a small South African case-control study by Pathai et al [501] found no statistically significant difference in lens density *(~ indicator of cataracts)* in association with HIV, results similar to ours was recently presented by Kempen et al [502]. However, the study design and applied methods largely differed between the three studies (Table 11).
As we found a higher relative risk of cataract surgery prior to the occurrence of predisposing ocular diseases, a higher occurrence of such conditions could not solely explain the observed increased risk of cataract surgery. The observed association between HAART exposure and risk of cataracts [499] could rely on differences in HAART induced insulin resistance; yet, an effect of HAART induced immune recovery uveitis and associated risk of cataracts [503], cannot be excluded.

We found that immunodeficiency was strongly associated with risk of cataract surgery *(CD4 cell count < 200 cells/μL: before HAART initiation: aIRR 3.11; 95% CI: 1.26-7.63, after HAART initiation aIRR 4.74; 95% CI: 2.60-8.62)*; however, the association relied on relatively few events. Pathai et al [501] established that a nadir but not current CD4 cell count < 200 cells/μL on HAART was associated with a higher risk of lens opacities. And finally, Kempen et al [502] found no association with neither nadir nor current CD4 cell count < 50cells/μL (Table 11).
Although, our results [499] are affected by uncertainty as to time elapsed from cataracts diagnosis to surgery, we, as opposed to the study by Pathai et al [501], had access to high quality longitudinal data with respect to events, antiretroviral treatment and immune status. Less than 90% completed at least 1 follow-up visit in the study by Kempen et al [502], hence limiting the completeness of data. Furthermore, the short duration of observation in this study may have limited the power to detect an association, hence compromising the results [502].

Table 11: Study design of the 3 compared studies

Athors:	Rasmussen et al, 2011	Kempen et al, 2014	Pathai et al, 2013
Study type:	Nationwide population-based longitudinal cohort study (Danish HIV Cohort Study)	Cohort study (Longitudinal Study of Ocular Complications of AIDS cohort (LSOCA))	Case-control study, HIV-infected individuals recruited from a community-based HIV-treatment center
Study period:	January 1995- August 2010, Denmark	September 1998-March 2008, USA	2011 Cape Town, South Africa
Study population:	5,315 HIV+; 53150 controls ≥ 16 years (individually matched on age and gender matched)	1,599 HIV-infected individuals (~ 3175 eyes) ≥ 13 years (all prior AIDS diagnosis. 53% had a nadir CD4 cell count < 50cells/µL.) * Population from the incident par of the study	242 HIV+ and 248 HIV- ≥30 years (Frequence matched by 5 year age intervals and gender)
Exclusion criteria:	- previous cataract surgery at inclusion	- intraoccular opportunistic infections at inclusion or during follow-up - Previous cataract at inclusion	- Diabetes mellitus - Ocular symptoms or pathology other than impaired vision at inclusion
Outcome:	Time to cataract surgery (Register-based)	Time to cataract (~lens opacities or prior surgery) (Complete ophthalmologic examination)	Cataract (~increased lens density) (Complete ophthalmologic examination)
Follow-up time:	7.9 years (IQR: 3.3-13.6)	4.6 years (IQR: 0.37-9.12)	None
Results:	IR: 2.07/1,000 PYR (95% CI: 1.68-2.54) aIRR: 1.87 (95%CI: 1.50-2.32)	IR: 3.7/1,000eye-years (95% CI: 2.6-5.3) aOR: 1.84 (95% CI: 1.006-3.36) comparison with LALES cohort (methods not described)	 aOR: 1.42; 95% CI: 0.67-3.03
Impact of ocular disease:	Risk prior to ocular disease: aIRR: 1.60 (95% CI: 1.24-2.06)	Main drivers: Age and ocular diseases	-
Impact of: **- Low CD4 cell count** **- HAART**	HIV vs controls: CD4 ≤ 200 before HAART: aIRR: 3.11 (95% CI: 1.26-7.63) CD4 ≤ 200 after HAART: aIRR: 4.74 (95%CI: 2.60-8.62) CD4 > 200 after HAART: aIRR: 1.87 (95%CI 1.46-2.39)	Current CD4+ < 50 cells/µL vz ≥ 50 cells/µL: aIRR: 1.64 (0.77-3.45) HAART vs no HAART: aIRR: 0.54 (0.26-1.11)	Trend towards increased risk in association with HAART and nadir CD4 cell count < 200cells/µ No association with: - current CD4 cell count, - CD4 > 200 and HAART/no HAART - HAART duration
Limitations:	Surveilance bias (HIV+) Uncertainty as to time elapsed from diagnosis to surgery. No adjustment for steroid use, DM, smoking (X1)	Recruited when presenting with eye symptoms! Surveilance bias – quarterly visits at eyedoctor Only 89% completed at least 1 follow-up visit. Moderate duration of observation. Power? No comparison cohort included. Use of "Los Angeles Latino Eye Study (LALES)" for comparison with general population. No adjustments for (X1)	Power!

Finally, we have previously shown the major impact of smoking on risk of MI among HIV-infected individuals compared to controls [129]. As smoking has been associated with risk of cataracts [504], the overall difference in results of the three studies could rely on lack of adjustment for smoking status in study 6 [499] and the study by Kempen et al [502]. Nevertheless, the lower prevalence of smoking and alcohol consumption among the HIV-infected individuals when compared to the controls in the study by Pathai et al [501] may suggest potential misclassification.

We recently performed a sensitivity analysis on our data-set in order to identify whether the difference in risk of cataract surgery was due to difference in smoking prevalence (Table 12). However, no major impact of smoking status on risk of cataract surgery among HIV-infected individuals was found.

Again we must emphasize that the large difference in risk behavior, viral co-morbidity, exposure to immunodeficiency and exposure to several antiretroviral drugs, may complicate comparison of HIV-infected individuals and individuals from the general population. Therefore, the existing data on relative risk of age-related diseases does not allow us to neither support nor falsify the "accelerated aging" hypothesis; however, a recent study that examined the risk of benign prostate hypertrophy, which is also highly associated with age, found no association with a positive HIV status[505].

Table 12: Risk of cataract surgery among HIV-infected individuals with smoking status and age and gender matched population controls (no smoking status).

	Events (n)	PYR	IR/1,000 PY (95 % CI)	Adjusted* IRR (95 % CI)
Controls, all	430	385,518	1.12 (1.01-1.23)	Ref.[a]
HIV+, all	77	38,187	2.02 (1.6-2.521)	1.84 (1.44-2.34)
Impact of smoking status stratified on HIV status				
HIV+, never smokers	30	12,196	2.46 (1.72-3.52)	Ref.
HIV+, previous smokers	20	6,481	3.09 (1.99-4.78)	1.02 (0.58-1.80)
HIV+, current smokers	27	19,510	1.38 (0.95-2.02)	0.65 (0.39-1.11)

Abbreviations: HIV+: HIV-infected individuals; PYR: Person-years of follow-up; IR: Incidence Rate, IRR: Incidence Rate Ratio, 95% CI: Confidence Interval

[a]Adjusted for age split at 30,35, 40, 45, 50, 55, 60 and 65, gender and calendar year split at 3, 6, 9 and 12 years after 1 January 1995.

2) A lower median age at diagnosis?

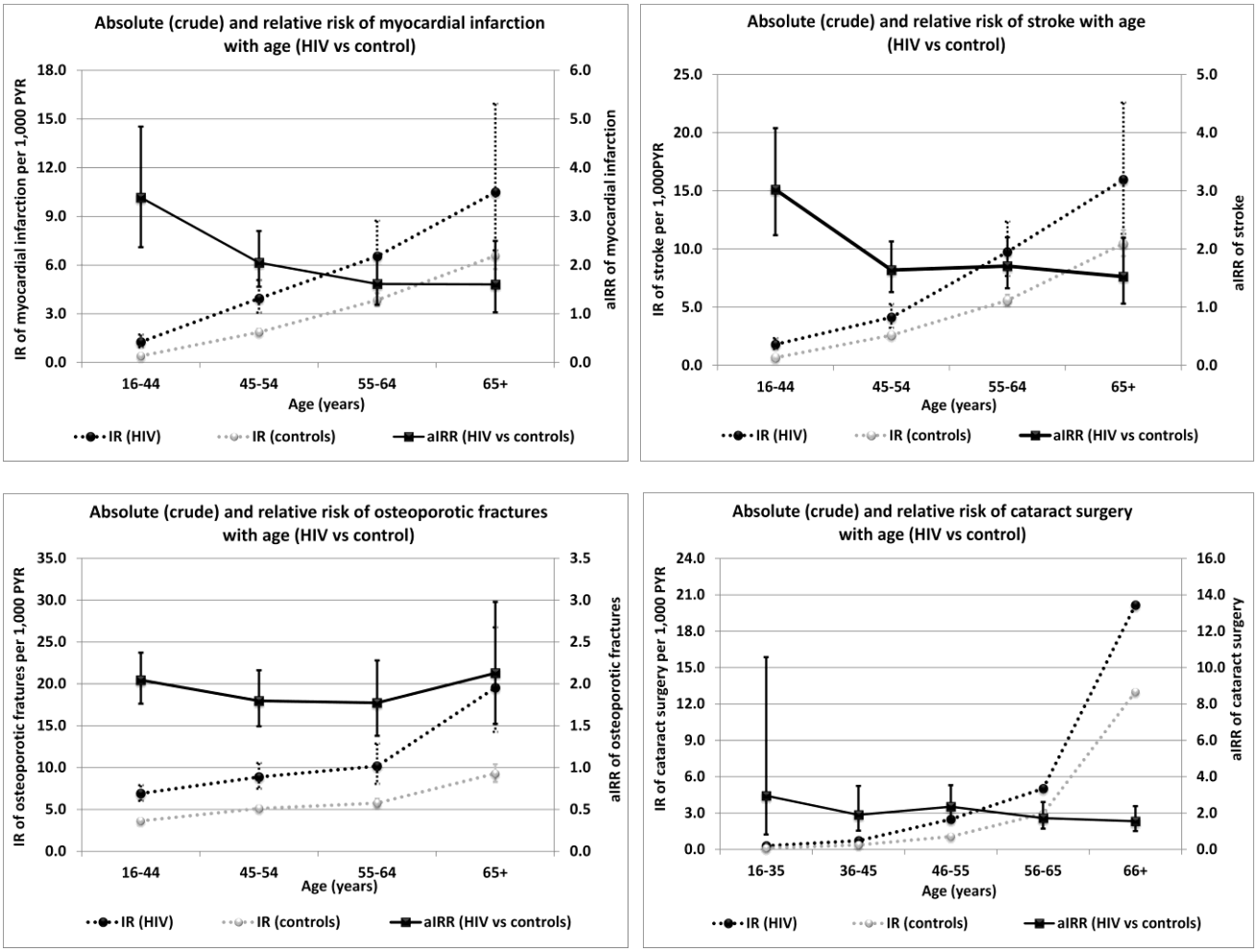
Several studies have reported a lower median age at diagnoses of age-related disease or at the presence of multi-morbidity for the HIV-infected population compared to the general population [120, 506, 507]. As an example, a previous study by Lorgis et al [507] identified 277,303 individuals (HIV prevalence ~ 0.22%) hospitalized with an MI over a 5-year period. This large study found that MI occurred at a much lower age among HIV-infected individuals than population controls *(HIV: 50±10 years vs. non-HIV: 68.3 ±14.9years)* [507]. However, as HIV-infected individuals have a high prevalence of many known risk factors [107], any detected difference in median age at event (HIV+ vs general population) may alone reflect earlier and larger exposure to these risk factors *(e.g. smoking)*, earlier diagnosis due to increased medical surveillance, or as previously shown for cancer, MI and end-stage renal disease [508, 509], be a result of the large differences in the age-distribution between the HIV-infected population and the general population. These age-differences are a result of observing a population with a disease *(i.e. HIV infection)* that most often presents itself in the younger age-groups and a population whom are at a higher risk of death *(competing risk)* . Hence, the truncated age-distribution prohibits observation of diseases that mainly occur at older ages *(~ lower proportion of observation time by older individuals - HIV vs controls)* [508]. If the difference in age-distribution is ignored, as illustrated in several examples by the important papers by Shiels et al [508] and Althoff et al [509], large difference in median age at diagnosis of disease might appear. After accounting for these differences in age-distribution, no clinical significant difference in median age at MI, cancer or end-stage renal disease remained between HIV-infected individuals and the general population [508, 509]. Altogether, these findings illustrate important methodological pitfalls concerning differences in time at risk. And, above all, the paper does not support premature aging as a cause of the diseases under investigation.

3) A higher relative risk of age-related diseases at all ages/in the younger ages/ increasing with age?

Due to the increasing prevalence of older HIV-infected individuals, the absolute risk of age-related diseases is with no doubt increasing with age. It remains less clear how the relative risk will evolve with increasing age. Most studies agree on a high relative risk in the young population [85, 510], whereas both higher [85] and lower [510] relative risks (HIV+ vs controls) for the older age-groups has been demonstrated. In study 7 [121] we examined the risk of 9 major age-related diseases *(i.e. diagnosis of or death from MI, stroke, virus associated -, smoking-related and "other" cancer, severe neurocognitive deficit ~dementia like disorders , chronic kidney disease, chronic liver disease, and osteoporotic fractures)* among 5,897 HIV-infected individuals and 53,073 age-and gender matched individuals from the general

population. Whereas the absolute risk of all 9 age-related diseases increased with age, we found a decreasing relative risk with age for all diseases but osteoporotic fracture (Figure 9); hence, no sign of a cumulative increasing relative risk with age was found. Similar results have also been found for risk of cataract surgery (Figure 9) *(Study 6)* [499]. It is important to emphasize that the high relative risk (HIV+ vs controls) in the younger age groups reflects a minor increase in a low absolute risk of age-related diseases in the young population. In contrast, the observed reduction in relative risk (HIV vs controls) with age might to some extent, but not exclusively, be a result of competing risk by death in the older HIV-infected population.

Figure 9: Absolute and relative risk of myocardial infarction, stroke, osteoporotic fracture and cataract surgery with increasing age



Abbreviations: HIV: HIV-infected individuals; PYR: Person-years of follow-up; IR: Incidence Rate, IRR: Incidence Rate Ratio, aIRR: adjusted Incidence Rate Ratio; 95% CI: Confidence Interval.

4) An age-independent increasing (absolute and relative) risk of age-related diseases with time

We cannot rule out that chronic immune activation and persistent inflammation may partly explain some of the excess risk of age-related diseases. However, if HIV-induced immune activation or chronic low level inflammation exerts any cumulative effect on risk of age-related diseases, we would expect the risk of age-related diseases to increase over time *(HIV, HAART and calendar time)* independent of age. In order to take age out of the equation, we therefore examined time trends in the absolute age-standardized risk and the relative risk (HIV+ vs controls) of age-related diseases (*Study7*). We found a stable or slightly reduced age-standardized and relative risk of CVD *(MI and stroke)*, cancer *(virus associate, smoking-related and "other")* and neurocognitive disorders with time after HIV diagnosis and HAART initiation. Although, the age-standardized risk of chronic kidney disease increased with time after HAART, no substantially increased risk was observed with time after HIV diagnosis. The opposite was observed for risk of chronic liver disease, whereas the age-standardized and relative risk of osteoporotic fractures increased with time after both HIV diagnosis and HAART initiation. This indicates that in the HAART-era the cumulative impact of HIV induced immune activation and chronic low level inflammation on risk of age-related diseases is limited; hence, refuting the hypothesis of accelerated aging being a major problem. Finally, we found *(study 7)* a stable or decreasing trend in the absolute age-standardized and relative risk of major age-related diseases except chronic kidney disease with calendar time. This is in straight line with results of a declining risk for MI and stroke by Klein et al [511, 512] and declining risk of death due to CVD and other age-related disease from the D:A:D study [527]. Although, this to some extent might be a result of secondary prevention, early detection and treatment, this does not support any idea of an age-independent increasing risk of age-related disease.

CONCLUSION

HAART has improved survival of the HIV-infected population resulting in a significant shift in the demographics of the HIV-infected population in the developed world. As, HIV-infected individuals are at a greater risk than the general population for several age-related diseases including cataracts these conditions seems to be a larger threat for the well-treated HIV-infected individual as of today than HIV-associated complications. Until now the absolute risk of age-related diseases, has remained low given the young age of the HIV-infected population. Yet, as these diseases are strongly associated with age, the absolute risk will increase with increasing age. However, there seems to be no indication of a lower median age at diagnosis, a cumulative increasing relative risk with age or an age-independent increasing risk with time.

GENERAL CONCLUSIONS AND HEALTH IMPLICATIONS

Today, HIV is one of the most extensively studied pathogens and, not only has the prognosis of HIV infection largely improved due to HAART - a large number of drugs are also available. The overall mortality has decreased and non-AIDS associated diseases now account for the majority of deaths. HIV-infected individuals have been found to have a higher risk of a number of age-associated conditions, including CVD. The mechanisms accounting for this has been immensely discussed and includes persistent immune dysfunction, inflammation, chronic immune activation and treatment toxicity. Yet, social factors, lifestyle, risk taking behavior and associated co-morbidity, seems to account for a substantial and potentially preventable part. Despite risk of toxicity and long-term complications, the risk-benefit ratio of HAART is unequivocally favorable with a substantial survival benefit. And, although some antiretroviral drugs may potentially increase the risk of some age-associated diseases, the SMART study indicated that continuous HAART may reduce the risk of not only AIDS-associated conditions and death, but also the risk of non-AIDS associated conditions. The reason may be associated with reductions in markers of inflammation, viral replication and improved immune function. Furthermore, given the results of the START study, treatment will in the coming years be initiated much earlier than it was a decade ago and thereby potentially prevent irreversible immune dysfunction.

As people living with HIV are now living to an older age, the focus on management of age-related diseases, multi-morbidity and poly-pharmacy will largely increase in the coming years. Generally, polypharmacy may further increase the risk of adverse drug events, drug interactions, inappropriate medication use, delirium, falls, fractures and poor adherence [513]. Hence, this may compound the burden and complexity of HIV management and increase the health care utilizations and costs. As such, it seems essential to attempt to reduce modifiable risk factors by encouraging lifestyle modifications and if needed implement therapeutically interventions. Antiretroviral drugs should be selected carefully and according to the individual's risk profile. And finally, rational pharmacotherapy should be engaged by carefully weighing benefits and risks when initiating new non-antiretroviral drugs in order to reduce the risks of drug-drug interactions and maintain compliance with HAART.

Finally, it is widely assumed that, the increased risk of age-related diseases among HIV-infected individuals, are associated with accelerated aging. However, there seems to be no evidence supporting this weak hypothesis. On a number of points, risk estimates of the HIV-infected population approaches the risk of the general population. Therefore, it is highly important that we do not make this population more ill than they are.

PERSPECTIVES

In light of our findings, important research questions arise.

As HIV-infected individuals have an increased risk of many age-related diseases and potentially multi-morbidity, use of non-antiretroviral drugs are expected to increase with increasing age of the population. Clearly the need for HAART does increase the total number of drugs; however, it is unclear whether the concurrent use of non-antiretroviral drugs is significantly more prevalent than that of the general population. Hence, longitudinal studies evaluating the risk of both multi-morbidity and poly-pharmacy among the HIV-infected population compared to a well-matched control cohort from the general population is needed to evaluate the size of these problems.

SUMARY (ENGLISH)

The prognosis of HIV-infected individuals has improved profoundly since the introduction of HAART with reductions in both HIV-associated morbidity and mortality. This has expanded the life expectancy of HIV-infected individual that is now approaching that of the general population - especially for those without co-morbidity, risk behavior or abuse. Overall, causes of death have shifted from AIDS-associated to non-AIDS-associated causes. Furthermore, studies from the past 10-15 years have shown that HIV-infected individuals have an increased risk of non-AIDS-associated age-related diseases when compared to the general population of the same age. The cause seems multifactorial and is still not fully established.

Risk of cardiovascular disease has been one of the major focus areas; however, we established that the risk of other vascular diseases was also largely increased and highly associated with drug abuse. We found that HIV-infected individuals with no injection drug abuse had a 1.6 times higher risk of cerebrovascular disease and a 3.8 times higher risk of venous thromboembolism than the general population, whereas the risk was 4-fold and almost 15 fold increased for the same conditions among HIV-infected injection drug abusers, respectively. In extension, we established that the impact of smoking on risk of myocardial infarction was not only large but seemed to be further associated with the HIV infection. We found that almost 3 in 4 MIs among HIV-infected individuals were associated with ever smoking compared with only 1 in 4 MIs among population controls, and that the estimated benefit of smoking cessation in the HIV-infected population was the double of that in the general population. All together risk behaviour seems to be a large driver of the excess risk of vascular disease.

To further investigate the presence and degree of other risk behaviour and co-morbidity, we examined the utilisation of psychotropic drugs over time both as a measure of psychiatric disorders as well as a measure of drug intake prior to and after the HIV diagnosis. Our results showed that HIV-infected individuals had a much larger utilisation of psychotropic drugs than the general population. This was mainly, but not only, carried by HIV-infected individuals with an injection drug use. Our results further illustrated a larger presence of psychiatric disorders (e.g. depression, anxiety and emotional distress) among the HIV-infected population than the general population, as well as a higher utilisation of drugs with addictive potential already prior to the HIV diagnosis. This further highlights the need to modify risk behavior, and identify and treat concurrent co-morbidity in order not to negatively impact HIV care.

During later years, and especially after the results of the SMART trial, the focus on HAART exposure on risk of non-AIDS associated diseases has largely been diminished. In contrast, extensive research has

sought to investigate the impact of chronic inflammation and sustained immune activation on risk of these diseases. Furthermore, studies have tried to identify adjuvant therapies that may target and attenuate the level of inflammation and immune activation. Due to the anti-inflammatory properties of statins, these drugs have been identified as potential wonder drugs, and some observational studies of HIV-infected individuals have established extensive reductions in mortality associated with statin therapy. However, most of these studies are associated with major methodological issues. Moreover, when attempting to confirm such extensive statin-associated reduction in mortality for the HIV-infected population but avoiding important bias in study design, we established no such association.

Chronic inflammation, sustained immune activation and "accelerated aging" have generally been believed to be of major importance for the risk of age-related diseases. As the hypothesis of accelerated aging seems poorly defined and not supported by solid evidence, we wanted to further investigate this issue. First, we investigated risk of cataract surgery, as a measure of cataracts, as this disease is highly associated with age, but not apparently associated with HIV or HAART exposure. We found an increased risk of cataracts among HIV-infected individuals compared to the general population; however, cataract surgery was highly associated with immunodeficiency. Therefore, this data did not allow us to neither support nor falsify the hypothesis.

Next, we examined the absolute age-standardized and relative risk of 9 major age-related diseases among HIV-infected individuals compared to the general population. This study supported that aging confer an increased absolute risk of age-related diseases. Consequently, the prevalence of major age-related diseases will increase with an increasing median age of the HIV population. On the contrary, we found no indication of an increasing relative risk with age, or an age-independent increasing absolute or relative risk with calendar time or time since the HIV diagnosis or HAART-initiation. This indicates that in the HAART-era the cumulative impact of HIV- induced immune activation and chronic low level inflammation on risk of age-related diseases is limited; hence, refuting the hypothesis of "accelerated aging" being a major problem.

SUMARY (DANISH)

Introduktionen af HAART i midt 90'erne har haft en helt afgørende betydning for HIV smittede personers prognose grundet en betydelig reduktion af både den HIV-associerede sygelighed og dødelighed. Som et resultat af dette lever HIV smittede personer stort set lige så længe som ikke-HIV smittede personer – især hvis de ikke har anden ko-morbiditet, risikoadfærd eller misbrug. Overordnet set er dødsårsagerne gået fra primært at være associerede med AIDS til nu i højere grad ikke at være associerede med AIDS. Endvidere har de sidste 10-15 års forskning vist, at HIV smittede personer har en øget risiko for ikke-AIDS associeret alders-relateret sygdom, når man sammenligner med risikoen hos baggrundsbefolkningen. Årsagen til dette lader til at være multifaktoriel og de enkelte faktorer er stadig ikke fuldstændig kortlagt.

Fokus har især været rette mod risikoen for kardiovaskulær sygdom. Vores resultater viser dog, at risikoen for andre vaskulære sygdomme også er betydelig øget og stærkt associeret med et intravenøst (i.v.) stofmisbrug. Vi fandt, at HIV smittede personer uden et i.v. misbrug, havde en 1,6 gange større risiko for cerebrovaskulær sygdom og en 3,8 gange øget risiko for venøse tromboembolier end baggrundsbefolkningen. Hos HIV smittede i.v. misbrugere var denne risiko til gengæld 4 gange og næsten 15 gange øget mht. de respektive sygdomme. Yderligere fandt vi, at rygning spiller en stor og umiddelbart større rolle for risikoen for myokardieinfarkt hos den HIV smittede befolkning end hos baggrundsbefolkningen. Vores resultater viste, at op mod 3 ud af 4 myokardie infarkter hos HIV smittede personer var associerede med rygning sammenlignet med kun 1 ud 4 hos baggrundsbefolkningen. Endvidere var den estimerede fordel af et rygestop dobbelt så stor hos den HIV smittede befolkning som hos baggrunds befolkning. Samlet set lader det til, at den HIV-associerede ekstra risiko for vaskulær sydom i høj grad er drevet af risikoadfærd.

For i højere grad at kortlægge graden af risikoadfærd og ko-morbiditet undersøgte vi forbruget af psykofarmaka over tid, både som et mål for prævalensen af psykiatriske sygdomme såvel som et mål for indtaget af psykofarmaka før og efter en HIV diagnose. Vores resultater viste, at HIV smittede personer havde et meget større indtag af psykofarmaka end baggrundspopulationen. Dette var til dels, men ikke udelukkende, associeret med et i.v. misbrug. Yderligere illustrerede vores resultater en større grad af psykiatrisk sygdom (eks. depression, angst og emotionelt stress) samt et højere indtag af psykofarmaka med afhængighedsskabende potentiale, allerede inden HIV diagnosen var stillet, blandt HIV smittede personer end hos baggrundsbefolkningen. Samlet set understøtter vores resultater behovet for at modificere risiko adfærd samt at identificere og behandle ko-morbiditet for på den måde at undgå en overordnet set negativ indflydelse af disse faktorer på den samlede HIV behandling.

Over de senere år, og især efter resultaterne af SMART studiet blev publiceret, er fokus på sammenhængen mellem HAART og risikoen for ikke-AIDS-associeret sygdom blevet reduceret. Til gengæld har de senere års forskning fokuseret på betydningen af kronisk immun aktivering samt en underliggende lav grad af vedvarende inflammation på risikoen for ikke-AIDS associeret sygdom. Endvidere har adskillige studier forsøgt at identificere en behandling som kan reducere niveauet af inflammation og immunaktivering. I kraft af statins antiinflammatoriske evne har det været udråbt som et potentielt vidunder lægemiddel. Nogle kohorte studier af HIV smittede personer har endvidere påvist omfattende reduktioner i dødelighed associeret med statin behandling. De fleste af disse studier har dog været associeret med store metodologiske problemer. Vi forsøgte derfor at genfinde en sådan reduktion i dødelighed men samtidig undgå vigtige bias mht. studie design. Men, vi var ikke i stand til at genfinde denne fordelagtige association.

Samlet set er kronisk immun aktivering, vedvarende inflammation og "accelereret aldring" vurderet til at være af stor betydning for risikoen for ikke AIDS-associeret alders-relateret sygdom. På trods af dette er hypotesen omkring "accelereret aldring" overordnet set dårligt defineret og ikke understøttet af solid evidens. Det var derfor et af vores mål, at vi ville forsøge at komme dette emne nærmere. I første omgang undersøgte vi risikoen for katarakt operation som et mål for katarakt, da denne sygdom er stærkt associeret med alder men ikke umiddelbart associeret med HIV eller HAART. Vores resultater viste at HIV smittede personer havde en øget risiko for katarakt sammenlignet med baggrundsbefolkningen, men at et lavt immunforsvar lod til at være en vigtig medvirkende årsag. Disse resultater tillod derfor hverken at støtte op om eller at afvise hypotesen om "accelereret aldring".

Som det næste undersøgte vi den absolutte alders-standardiserede og relative risiko for 9 større alders-relaterede sygdomme blandt HIV smittede personer sammenlignet med baggrundspopulationen. Studiet støttede op om det faktum, at aldring øger den absolutte risiko for alders-relateret sygdom. En konsekvens af dette er, at prævalensen af disse sygdomme vil stige med kalendertiden i kraft af en underliggende stigning i prævalensen af ældre HIV smittede personer. Til gengæld fandt vi ingen tegn på, at den relative risiko for alders-relateret sygdom steg med alderen, eller at den alders-uafhængige absolutte eller relativer risiko steg med kalendertiden, tiden efter HIV diagnosen eller tiden efter opstart af HAART. Samlet set indicerer dette, at, i HAART-æraen, er betydningen af en kumuleret effekt af HIV-induceret kronisk immun aktivering eller en lav grad af vedvarende inflammation på risikoen for alders-relateret sygdom begrænset. Vores studie afviser derfor hypotesen om, at accelereret aldring skulle være et stort problem for den HIV smittede befolkning.

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