PhD thesis

Non-HIV determinants of outcome in Danish HIV-infected patients: Impact of study participation, body fat distribution and family-related factors.

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This thesis is based on the following papers

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II. Hansen AB, Lindegaard B, Obel N, Andersen O, Nielsen H, Gerstoft J. Pronounced lipoatrophy in HIV-infected men receiving HAART for more than 6 years compared with the background population. *HIV Med* 2006; 7(1):38-45.

III. Hansen AB, Gerstoft J, Kronborg G, Pedersen C, Sorensen HT, Obel N. Mortality in siblings of patients coinfected with HIV and hepatitis C virus. *J Infect Dis* 2007; 195(2):230-235.

IV. Hansen AB, Lohse N, Gerstoft J, Laursen A, Pedersen C, Sorensen HT, Obel N, Cause-specific excess mortality in siblings of patients coinfected with HIV and HCV. *Submitted*

Abbreviations

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral treatment
CD4	cluster of differentiation 4
CI	confidence interval
CPR	Centrale Person Register (Danish name of CRS)
CRS	the Danish Civil Registration System
DAPIS	the Danish Protease Inhibitor Study
DHCS	the Danish HIV Cohort Study
EMR	excess mortality rate
NHR	the Danish National Hospital Register
HAART	highly active antiretroviral therapy
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IDU	intravenous drug use
MR	mortality rate
MRR	mortality rate ratio
MSM	men who have sex with men
NNRTI	non-nucleoside analogue reverse transcriptase inhibitor
NRD	the National Registry of Deaths
NRTI	nucleoside analogue reverse transcriptase inhibitor
PI	protease inhibitor
PYR	person-years at risk
RCT	randomised controlled trial
RNA	ribonucleic acid
SQVhgc	saquinavir hard-gel capsule
VL	viral load

Contents

1. Introduction

1.1 The HIV epidemic

A quarter of a century has passed since the first cases of acquired immunodeficiency syndrome (AIDS) –later linked to the human immunodeficiency virus (HIV) (1) - were reported in the USA (2;3) and shortly thereafter in Europe (4). Since then, the HIV-epidemic has grown to be one of the most devastating pandemics in medical history. As of 2006, 40 million people are living with HIV and more than 25 millions have lost their lives to AIDS. Concurrently immense therapeutic progress has taken place. Most of the persons with HIV, however, live in regions with still inadequate access to effective HIV therapy. Less than 2% of newly infected persons are living in Northern America or Western Europe, and three quarters of all AIDS deaths in 2006 occurred in sub-Saharan Africa (5). In Denmark, the HIV incidence has been relatively stable over time with a median of approximately 230 new case per year yielding an annual incidence rate of 5 new HIV cases per 100,000 persons > 15 years (6;7). The mortality from the disease has fallen drastically to about 70 deaths per year, and the prevalence of HIV is gradually increasing. The main routes of transmission are homosexual (44%), heterosexual (36%) and intravenous drug use (11%) (6;7).

1.2 Therapeutic advances

In the late 1980´s the first nucleoside analogue reverse transcriptase inhibitor (NRTI) zidovudine was shown to prolong survival (8), but the effect did not last due to development of resistance mutations (9). Subsequently developed NRTIs were also without a durable effect, although sequencing or combination of two drugs did yield additional survival benefit (10;11).

The development of protease inhibitors (PIs), growing knowledge about the large turnover of HIV virions and CD4 cells, and the high mutation rate of HIV were the rationale for combining of several (typically three or four) antiretroviral drugs, creating the approach that became known as Highly Active Anti-Retroviral Therapy (HAART) (12;13) to suppress HIV replication effectively and for long periods. Shortly after HAART was introduced in the West in 1995-1996, large cohort studies reported dramatic decreases in morbidity and mortality (14;15).

The first HAART regimens consisted of a backbone of two NRTIs in combination with a PI, and also triple NRTIs treatment have been used (16). Current treatment guidelines (17) recommend regimens with an NRTI backbone combined with a non-nucleoside-analogue reverse transcriptase inhibitor (NNRTI) or with a boosted PI. Boosting refers to co-administration of low doses of ritonavir (a PI itself and a potent inhibitor of cytochrome P450 3A4) to increase bioavailability of the drug (18).

The ideal treatment goal is elimination of HIV and thereby elimination of transmission and of the excess morbidity and mortality associated with HIV-infection. In the absence of curative treatment of HIV [due to the existence of latent virus reservoirs (19)], the primary treatment goal is maximal

and durable suppression of viral replication resulting in lowering of the plasma viral load below the level of detection by current assays. Viral suppression enables recovery of CD4 cell count and the CD4-cell mediated immune responses, reducing thereby risk of opportunistic infections (20;21) and death.

The therapeutic advances have steadily improved the life expectancy of HIV patients. As we recently reported, the projected survival of a newly diagnosed 25-year-old patient is more than 35 years (22). Despite the encouraging survival expectations, the excess mortality of HIV-infected, patients compared with that of the general population, remains large, with an estimated difference of up to 15 years (22).

1.3 Limits of the long-term effectiveness of HAART

The introduction of HAART has radically changed prognosis, disease spectrum and quality of life for HIV-infected patients. In randomised studies comparing HAART regimens, severe non-AIDS adverse events have become more common than new AIDS-defining events (23) and exert similar impact on morbidity and mortality. In populations with access to HAART, causes of death not directly related to HIV-infection now account for more than half of recorded deaths (22;24).

The HAART regimens remain complex and require rigorous adherence to lifelong daily intake of three or more drugs. In addition, there are several other obstacles to lifelong therapeutic success (25;26):

1. Incomplete viral suppression (virological failure).

Viral suppression may be incomplete allowing (new) drug-resistance mutations to emerge (27). Virological failure may be caused by low potency of the HAART regimen due to low genetic barrier to resistance, or by an inadequate pharmacokinetic profile. Furthermore, resistance mutations may be present prior to HAART initiation either due to transmission of resistant strains or due to earlier mono- or dual NRTI-treatment. Finally, virological failure may be caused by poor adherence due to side effects or due to other patient-dependent factors (28).

2. Therapy- related adverse effects.

Complications of therapy bear substantially on the health of HIV-infected patients. Some adverse effects have been readily recognized because they are common and/or occur early in the course of therapy. These include the neuropsychiatric side effects of efavirenz, hypersensitivity reactions or rash associated with abacavir and NNRTIs, anaemia or nausea from zidovudine, or gastrointestinal side effects of many of the PIs (25). Associations of other complications, such as liver toxicity (29) or the HIV-associated lipodystrophy syndrome, with specific drugs or drug classes, immune status, comorbidities and lifestyle have been hard to pinpoint.

The HIV-associated lipodystrophy syndrome was described a few years after the introduction of HAART (30) as a combination of subcutaneous lipoatrophy and central fat accumulation associated with dyslipidaemia and insulin resistance (31). There have been large variations in reported prevalences ranging from 20 to 80%. The discrepancies can probably be ascribed to lack of a consensus definition (32), inclusion of patients with various lengths of HAART exposure, and the absence of reference values. But it has been estimated that approximately 50% of HIV outpatients have clinically apparent lipodystrophy (25;33). Lipodystrophy is disfiguring and potentially stigmatising and besides decreased quality of life it may cause poorer adherence to therapy (34). Furthermore, the dyslipidaemia and insulin resistance is associated with increase risk of cardiovascular disease (35). Since the first case reports, there have been enormous research efforts to understand the pathophysiology of HIV-associated lipodystrophy, which may be seen as overlapping syndromes related to different antiretroviral drugs rather than one entity (36).

3. Co-existing determinants of morbidity and mortality.
Individuals with good adherence to currently recommended HAART regimens are unlikely to have insufficient viral response or to develop resistance mutations (28;37). Consequently, other determinants of health outcome, *e.g.* comorbid hepatitis C (HCV) or hepatitis B infection, life-style risk factors, such as smoking and drug abuse, become increasingly important. These and other patient-related factors may contribute to the health disparities between HIV-infected and HIV-negative populations, yet it can be difficult to single out these factors. HCV coinfection is common among HIV-infected individuals, with coinfected patients having poorer prognosis than HIV/HCV-monoinfected patients. The worse prognosis is only partly explained by a higher frequency of liver-related deaths (38;39). HCV infection is closely linked to drug abuse and thus to a lifestyle associated with high mortality. It is thus still unclear what respective roles HCV-induced diminished response to HAART and patient-specific lifestyle factors play in the inferior prognosis of HIV/HCV-coinfected patients.

1.4 Measuring long-term outcome

In clinical epidemiology, efficacy (measured in RCTs) is the extent to which a specific intervention produces a beneficial result under ideal conditions, while effectiveness is a measure of the extent to which an intervention fulfills its objectives under routine circumstances (40). Measurements of efficacy obtained in randomised trials are merely short-term compared with the lifelong duration of HAART, and it is unclear whether beneficial results achieved in trials can be fully generalised into daily clinical practice. Therefore, observational studies are essential for assessing long-term impact of HAART complications, comorbidities and lifestyle-related factors. It is also important to know how the results from observational studies reflect those from randomised trials with regard to therapeutic outcome on the population level.

2. Aim of thesis

The thesis has the following aims:

1. To compare the efficacy of HAART in HIV-infected patients initiating treatment within a randomised controlled trial with the effectiveness of the same HAART regimens in an observational cohort study.

2. To establish the prevalence and severity of metabolic complications including body fat redistribution in HIV-infected men after long-term HAART treatment, as compared with the general population.

3. To examine whether, independently of HCV pathogenicity, HCV/HIV coinfection is a marker for familial risk factors affecting survival in HIV-infected patients.

3. General methodological and statistical considerations

In contrast to experimental studies (randomised controlled trials), the investigator does not assign exposure in observational (non-experimental) studies (41). This thesis is based on four observational studies (three prospective cohort studies and a cross-sectional study). This section addresses methodological issues pertaining specifically to the design of the four studies and generally to observational studies of HIV and its treatment.

3.1 Cohort studies

In clinical research a cohort is a group of individuals defined by the presence (or absence) of an exposure; the cohort is followed over time, during which the occurrence of one or more outcomes of interest (42) is measured. The key advantage of a cohort study is the ability to establish a temporal relationship between exposure and outcome, further incidence rates and thereby absolute risk can be established directly. Since individuals can be followed over long periods, cohort studies can provide information on a range of both short-term and long-term health effects of a single exposure.

An important issue for designing cohort studies is the selection of an appropriate comparison group. It can be internal comparisons whereby a cohort is assembled from members of a population in whom the predictor variable (exposure of interest) is measured and the outcome occurrence is measured during follow-up. An example of such study is the Danish HIV Cohort Study, in which occurrence of outcomes over time is compared between HIV patients who are positive or negative for hepatitis C antibody. The comparison group can also be a sample of external population controls in a double-cohort study (43). Often data from a registry is used to efficiently form a population-based external control group.

Analytic cohort studies typically test whether exposure to a given factor is associated with a health outcome. Characteristics associated with an increased risk of a specified outcome (often occurrence of disease) are called risk factors (40). Prognostic factors are studied in sick individuals and are conditions that are associated with a given outcome of a disease (44). Neither risk factors nor prognostic factors are necessarily causal factors; the terms risk marker or prognostic marker are sometimes used to specify when the factor is not a cause of the outcome.

3.2 Cross-sectional studies

In a cross-sectional study exposure and outcome are measured at the same point in time, thus precluding inferences about causality; with the exception of the cases where the exposure is a permanent trait, for example a genotype (42). Cross-sectional studies can be particularly useful and feasible in providing estimates of prevalence, prevalence ratios, or characteristics of diseases in specific groups of individuals.

3.3 Bias in epidemiologic studies

Bias is a systematic error in data collecting or interpretation that affects an estimate obtained in a study. Bias can be classified into three categories: selection bias, information bias and confounding (41).

Selection bias

Selection bias stems from the procedures used to select study subjects or from factors that influence study participation (41). In cohort studies, it may occur if groups of subjects characterised by unrepresentative relationship between exposure and outcome are selectively recruited into the study or are selectively lost to follow-up. A number of studies have shown that non-participants are likely to differ from participants with respect to motivation and attitudes towards health, or to risk factor profile (42). Losses to follow-up are one of the major sources of bias in cohort studies because they are often associated with both the exposure and the outcome of interest. In survival analyses, loss to follow-up that is associated with the outcome is called informative censoring.

The strategies to minimise selection bias are mainly applicable at the design stage of the study (table 1), but it may be possible to use previously collected data to assess any systematic differences between participants and non-participants (paper I) or between those who are lost to follow-up and the remainder of the cohort (paper II) (42). Alternatively, sensitivity analyses can be used to examine the potential effect of most extreme outcome in those who are lost to follow-up (44); analogue to "the missing equals failure" analysis often used in HIV research (45).

Information bias

Information bias stems from errors in measurements of study variables (misclassification). When misclassification of subjects for dichotomous exposure or outcome status is nondifferential (i.e. independent on the other study axis) the resulting estimate may be biased towards the absence of an association. Misclassification is differential when the proportion of error in classification of outcome status differs according to the exposure status or vice versa. Differential misclassification may cause either over- or underestimation of the association between exposure and outcome.
In DHCS many of the variables either are validated laboratory tests (e.g. CD4 cell count or viral load) or are defined by objective criteria (e.g. AIDS-defining diagnoses), whereby the potential for misclassification is reduced. Other variables, such as patient-reported HIV exposure category, may be subject to misclassification; for example HIV transmission by IDU or homosexual contact may be underreported due to fear of stigmatisation.

Confounding

A confounder is a factor associated with the exposure and the outcome of interest, that is not a causal intermediary between them (46). In observational studies of intended and unintended effects of antiretroviral medication, a special concern is confounding by indication, as individuals that are prescribed a particular drug may differ systematically from those not prescribed the drug with

respect to both measured and unmeasured confounders. A gold-standard method for removing both known and unknown confounding is randomisation of experimental studies, which becomes more effective as the sample size increases. Other methods to control for confounding are listed in table 1 and include matching of exposed and unexposed subjects on confounding characteristics, such as age or gender. The matched double-cohort design will be discussed in section 3.7.

Prevalent cohorts versus incident

Prevalent cohorts may be prone to length biased sampling due to selection of disproportionate numbers of long duration cases (40) or onset confounding (47): for example if a covariate (e.g. positive HCV serostatus) is associated with earlier calendar dates of infection, this covariate would appear associated with outcomes that are dependent on cumulative time since infection .

Table 1. Strategies to minimise bias

Type	**Selection bias**	**Information bias**	**Confounding**
Tool	Well-defined study base (e.g. geographical area) to reduce referral bias. Reduce non-participation. Reduce loss to follow up. Procedures to track subjects lost to follow-up. Use of incident cohorts.	Standardisation of the measurements Well defined criteria for exposure and outcome categories Identical data sources and data collection for the exposed and the unexposed.	Randomisation Matching Restriction/exclusion Stratification Standardisation Multivariate analyses and modelling

3.4 Outcome in epidemiologic studies of HIV

Clinical epidemiology focuses on health outcomes in humans e.g. death, disease, discomfort, disability and dissatisfaction, in short, the whole range of conditions that are important for patients (44). Outcomes are selected based on prior knowledge or hypotheses about the potential effects of exposure on the clinical course of the disease. Among HIV-infected patients outcomes of interest are often AIDS-defining diseases or death, laboratory markers of disease (CD4 cell count, viral load) or side effects of therapy e.g. lipodystrophy.

Prior to the introduction of HAART, primary endpoints in clinical trials of HIV patients were clinical endpoints (progression to AIDS or death). The decreasing rate of disease progression in the HAART era made the use of these hard end-points less feasible. Therefore both the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) approved the use of plasma viral load and CD4 cell count as surrogate markers (48) for approval of antiretroviral drugs. Viral load and CD4 cell count are long-established measures of risk of progression to AIDS (49). Recent findings confirm that the relationship between the viral load/ CD4 cell count and risk of clinical disease continues to hold true for newer antiretroviral drugs approved in trials lacking data on hard clinical endpoints (50). Even though CD4 cell count and viral load may not capture the full net effect of treatment (48;51), they gauge immediate treatment goals, predict disease progression and are measurable by standardised assays.

Death as outcome

Observational studies of overall mortality must be conducted with care as numerous confounding factors may affect mortality (52) and competing risks may complicate the interpretation of overall mortality as outcome. Mocroft *et al* (53) have discussed the problems of using all-cause mortality as an endpoint in evaluation of the effectiveness of antiretroviral treatment in subpopulations with many competing risks (e.g. individuals with IDU) after the proportion of non-HIV-related deaths has increased: Drug-injecting HIV-infected patients showed diminished response to HAART as measured by the composite endpoint of progression to AIDS or all-cause mortality. After restricting the endpoint definition to progression to AIDS or AIDS-related death, these patients did not show a poorer response to HAART. Mocroft therefore advocated that causes of death should be reported in epidemiologic studies of HIV (53). The minimum requirement could be to report the proportion of HIV-related and non-HIV related deaths (paper I). Classifying causes of death is, however, a more subjective process than establishing the fact of death (54). Categorisation may be difficult and dependent on the current knowledge of disease pathophysiology. In the Data collection and Adverse events of anti-HIV Drugs (DAD) study, deaths from causes generally referred to as non-HIV-related (including liver-related causes, non-AIDS malignancies, and heart disease) were also more likely to occur in persons with lower CD4 cell counts (55). Recently the Strategies for Management of Antiretroviral Therapy (SMART) study further challenged the conception of HIV-related morbidity and mortality. Compared with patients randomised to continuous HAART, patients randomised to CD4-count-guided structured treatment interruptions had not only more AIDS

events, but also more major cardiovascular, renal, and hepatic disease, and more deaths from causes other than opportunistic diseases (56).

3.5 Evaluating the outcome of antiretroviral therapy in observational studies

Observational studies are well suited (54) to study risk factors (39;57;58), diagnosis, prognosis (22;59)) and temporal trends (6;60). But when it comes to examining effects of therapeutic interventions, including antiretroviral medication, confounding by indication (41;54) becomes a problem. This type of confounding occur if those who take a drug differ from those who do not according to the medical indication for which the drug was prescribed (41). The decision to prescribe a drug is based on a mutual patient-physician agreement conditional on a range of factors including disease severity, previous treatment, health behaviour, expected adherence and physician's experience (41). Some of these potential confounding factors are difficult or impossible to measure, and therefore to control. The only way to fully control for these confounding problems is randomisation. Therefore in studies of intended effects of drugs, the randomised controlled trial and meta-analyses of randomised trial have been placed in the top of evidence (61;62). Recently several larger reports have shown that in most cases summary measures from observational studies agree with those obtained in randomised trials (61;63;64) and questioned this hierarchy of research designs (61;63). Still, observational studies and randomised trials may reach divergent conclusions, as in the case of hormone replacement therapy (HRT) and coronary heart disease (65;66). Although researchers may abstain from drawing comparative conclusions when they expect confounding by indication (67;68), they may not be able to predict its presence (69). Nevertheless, comparisons of outcomes from RCTs and those from observational cohort may supply valuable information for design of future studies. For example, scrutinising differences between RCTs and observational cohort studies pointed towards the problem of including prevalent HRT users, because the risk of coronary heart disease is the highest early in the course of the therapy.

Three treatment comparison from randomised controlled trials of antiretroviral therapy were emulated in three different cohorts by Phillips *et al* (70); in one of these nine comparisons the adjusted results from an observational cohort was in direct contrast to the trial result. For this comparison the treatment groups in the observational cohort were substantially unbalanced with respect to patient characteristics, leading the authors to attribute the discrepancy to residual confounding. Overall, the authors concluded that whenever an RCT is unfeasible for economic, practical or ethical reasons, well designed observational studies may be an acceptable alternative, especially when the results from several cohort studies are carefully considered: when differences between groups are not substantial (70); and when there is sufficient overlap between covariates measured in the treatment groups. To give a historical example, no randomised trial has compared first-line saquinavir hard-gel capsule (SQVhgc)-based HAART with other PI-based HAART regimens, but several cohort studies consistently reported inferior virological outcome of SQChgc (71;72). However, given the potency of currently recommended HAART regimens the potential differences in treatment effects are likely to be small, making the result susceptible to smaller bias

(73). For example, differences found in observational studies comparing nevirapine versus efavirenz as part of initial treatment (74;75) were not confirmed in a subsequent RCT (76). Thus, for comparison of antiretroviral regimens the main contributions of cohort studies may be documenting uncertainty and generation of hypotheses for future randomised trials (70).

After having addressed the question to what extent relative risks determined in RCTs are comparable with those obtained in observational cohort studies, a related but separate question remains. Namely, whether outcomes for patients enrolled in RCTs are better than for patients who are not enrolled in RCTs: i.e. what effect do entry criteria, type of patients included into trials, and trial participation itself have on the outcome (77). In antiretroviral therapy trials it is often assumed that these factors cause an overestimation of the drug effects when compared with what can be expected in daily clinical practice (77). Evidence from other therapeutic fields does not support this view, however (78-80). A number of studies have shown under-representation, in antiretroviral trials, of intravenous drug users, non-whites and economically disadvantaged patients (81-84); as a result, efficacy could be overestimated. To our knowledge no study has compared the responses to specific HAART regimens in an RCT with those observed in an observational cohort drawn from the same population and during the same time span as the trial participants.

Study of adverse effects

An average RCT often has insufficient number of patients and inadequate length of follow-up to observe rare or long-term adverse effects of drugs (85). Treatment with HAART is lifelong and has high benefit/risk ratio, while antiretroviral drugs are approved on the basis on relatively short trials that use surrogate outcomes. Together these factors necessitate observational studies to assess long-term drug safety. Observational studies are particularly useful for examining the impact of identified unintended effects (as well as intended effects) on a population level (paper II) (33). The DAD study is an example of a large observational cohort providing supplementary information on the impact of long-term side effects of HIV medication (29;35).

It is argued that observational studies are at their methodological best in exploring unexpected adverse effects (67;85) as the prescribing patterns remain unaffected by expected susceptibility to the adverse effect. But as a specific adverse effect becomes better known, confounding by observation becomes more likely, as the perceived risk of the adverse effect will affect physicians prescribing of the drug (85).

Studies of the association between adverse events and specific antiretroviral drugs are complicated by the co-administration of three or four drugs from different drug classes (table 2) and by frequent switches of one or more drugs. The new-user designs (86) restricting follow-up to patients that can be followed from initiation of antiretroviral therapy (87) may partly help to overcome some of these difficulties. The inclusion of prevalent HAART-users may confound observational studies of drug effects substantially because of 1) time-varying risks, with highest risk immediately after initiation

or after a long lag-time, 2) inability to adjust for baseline covariates that are affected by the drug, 3) use of recently licensed drugs by patients with previously insufficient treatment response, which may be a marker of advanced HIV disease or a long-term treatment experience, and 4) some drugs being used as second-line therapy following other drugs according to sequencing patterns. For instance, the Swiss HIV Cohort Study reported that current use of abacavir was associated with lipodystrophy, but it was impossible to examine whether the effect was seen because patients were switched to the abacavir from stavudine due to toxicity of the latter (88). Later RCTs have not demonstrated any contribution of abacavir use to lipodystrophy (89;90).

Table 2: Drugs licensed for treatment of HIV in Denmark as of January 2007.

Drug class:	Nucleos(t)ide-analogue reverse transcriptase inhibitors (NRTI)	Non-Nucleos(t)ide-analogue reverse transcriptase inhibitors (NNRTI)	Protease inhibitors (PI)	Fusion inhibitors
Generic name:	Abacavir Didanosine Emtricitabine Lamivudine Stavudine Zidovudine Tenofovir	Efavirenz Nevirapine	Atazanavir Fosamprenavir Indinavir Lopinavir Nelfinavir Ritonavir Saquinavir Tipranavir	Enfurvitide

3.6 Choice of modelling strategy

Multivariate analyses and modelling are important tools in clinical epidemiology, and the goal of the study should guide the choice of modelling strategy: In prediction modelling the goal is to select - from a large collection of candidate variables - a set of variables that best predicts the outcome; consequently all variables in such analyses are initially treated equally important. In risk-factor and explanatory prognostic models the goal is to establish the association between a risk factor (i.e. exposure or treatment variable) and the outcome; all variables other than the risk-factor under study are considered only as potential effect modifiers or confounders (to be adjusted for) of the association between the risk factor and the outcome (91). Paper I is a risk-factor study aiming to estimate the relative risk of treatment failure in individuals "exposed" to participation in an RCT. The change-in-estimate method, in which variables are included if they cause a pre-specified (often 10%) change in the exposure effect estimate, is tailored to achieve efficient confounder control in risk-factor studies (92;93) often preceded by stratified analyses to detect effect modification. The primary application of the change-in–estimate method is when there are too many potential confounders to be included in a full model.

3.7 Matched cohort studies

Definition and purpose

Matching refers to the selection of a reference group (controls in a case control study or unexposed subjects in a cohort study) that is similar to the subjects in the index group with respect to the distribution of one or more potentially confounding factors (46). Matching may potentially affect both validity and efficiency. Validity is here defined as the control of confounding factors (the extent to which any measured effect is unaffected by potential group differences on the confounding variables). Efficiency is here defined as the precision of the effect estimates, primarily in relation to number of subjects or person-time, but also in relation to the monetary or other costs of the study (94). Matching is often used as an effective way to prevent confounding by constitutional factors such as sex and age that are strong determinants of outcome, but are not likely to be on the causal pathway, and are also not targets of intervention (94). Close matching on continuous variables, such as age, may help to avoid residual confounding that may be present in stratified analysis or multivariate modelling (46). Matching can also be used to prevent confounding by factors that are difficult or impossible to measure, such as sibship, to control for a range of genetic and environmental factors, or matching by centre in multicentre studies (42).

Matching in cohort studies

Matched cohort studies are less represented than matched case-control studies in medical journals (95); and the issue of matching in cohort studies is often only cursory (if at all) covered in basic textbooks (43). Matching in cohort studies has different implications for validity and efficiency as defined above than matching in case-control studies. In a cohort study, selecting a sample of unexposed subjects that is matched to the exposed subjects on potential confounders removes the association between exposure and the matching factors and thereby confounding at baseline. Accordingly, the matching does not need to be accounted for in a stratified analysis to obtain an unbiased result estimate of effect. There are, nevertheless, other arguments for preserving the matching in the analyses. In a cohort study subjects may be followed over longer time periods, and while matching ensures balance of one or more confounding variables between the exposed and the unexposed individuals at baseline, it does not necessarily ensure balance as the study progresses and subjects are lost to follow up or succumb to competing risks. Unbalanced censoring in a matched cohort study can be addressed by either preserving the matching in (conditional) analyses or by controlling for the matching variables in the analyses.

The effect of matching on efficiency in a cohort study is more ambiguous. Constanza argues that matching on a confounder increases the precision (i.e. leads to narrower confidence intervals) while matching on a non-confounder can reduce precision in small samples (N<30) (94). Matching in cohort studies have been compared to the blocking in randomised studies (matched randomisation) to increase efficiency, but Rothman and Greenland point out that while blocking in a randomised study does not affect the covariate distribution in the study, matched selection of population controls may alter the covariate distribution of the entire cohort study, and argue therefore that efficiency may be also be decreased in certain situations (96). However, given large sample sizes of many cohort studies the issues related to efficiency may be more of theoretical than practical importance (94).

4. Data sources

4.1 Literature search

Since for the topics covered in this thesis no specific set of search terms was sufficiently sensitive and specific, several search strategies were used. PubMed (www.pubmed.gov) was searched regularly using combinations of relevant MESH (Medical Subject Heading) terms e.g. “HIV Infections”, “Randomized Controlled Trials”, “Cohort Studies”, “Comorbidity”, "HIV-Associated Lipodystrophy Syndrome", “Hepatitis C” and selected keywords e.g. “Charlson”, “observational”, “non-participation“, “trial effect”, “lipoatrophy”, “familial”, or “sibling”. Articles were also captured in the reference lists of relevant papers and using the “Related Links” feature in PubMed. In addition, frequent communication with supervisors and colleagues working with HIV care or clinical epidemiology, participation in international HIV conferences, and literature updates from professional websites such as www.medscape.com and www.clinicaloptions.com helped to stay updated with the most important articles in the rapidly expanding scientific HIV literature.

4.2 The Danish HIV Cohort Study (DHCS)

DHCS was initiated in 1998 as The HIV Cohort Study in Western Denmark (97), and extended in 2003 to a nationwide collaboration between the eight HIV treating centres in Denmark (6). The cohort includes all HIV-infected patients seen in any Danish HIV treatment centre after 1 January 1995. As of January 2007 the cohort includes 4744 patients above 15 years of age of whom 1105 have died and 235 have emigrated. The cohort is open and newly diagnosed or immigrating patients are enrolled whenever they present to the centres. According to clinical practice patients are seen in the clinics at three- to four-month intervals. Data are extracted annually from patient files at the centres, and entered into the database centrally under a serial number. Main data included are demographics, treatment history, laboratory values, death (date and cause) and clinical data on progression to AIDS. Laboratory data are mainly entered electronically (after being cross-linked with the serial number locally). Crosschecking and validation algorithms are incorporated into the database in order to prevent errors. In addition, 5-10 percent of records are monitored during annual visits to the participating clinics.

The strengths of the Danish HIV cohort

Although there is a wealth of observational HIV cohorts studies, among DHCS is medium sized (98), DHCS is suited for a number of specific aims. Firstly, DHCS is population-based (99) including all HIV patients in a defined geographical area. Further the unique identifier – the CPR-number (see below) - enables treatment centres to avoid multiple registrations of the same patient and allows tracking of deaths and losses to follow-up due to emigration. For the outcome of death, losses to follow-up occur only due to emigration, which substantially reduces the likelihood of selection bias. The population-based design enhances the ability to study population-based prevalences, incidences, and population trends after long-term use of HAART. Secondly, DHCS includes patients early in course of HIV-infection. If the diagnosis of HIV is regarded as the

initiating event DHSC closely approximates an incident cohort for patients diagnosed after 1995. When start of HAART is regarded as the initiating event (paper II), DHCS is an incident cohort (for patients initiating HAART in Denmark) and do not have the methodological problems of including prevalent users of HAART. Finally, the numerous Danish registries and the universal use of the CPR number enable comparisons with the general population (paper III), allow examination of clustering of disease or death in families (paper III and IV) and make possible (through cross linkage to other registries) the inclusion of covariates or outcomes that are not typically accessible in HIV observational cohorts, such as hospital discharge diagnoses (paper I), history of cancer, psychiatric diseases, causes of death, and socio-economic factors.

4.3 Danish registers

We used data from three Danish registers: The Danish Civil Registration System, The Danish National Hospital Register, and the National Registry of Deaths (NRD).

The Danish Civil Registration System (CRS)

CRS includes data on all persons who were alive and had permanent residence in Denmark on April 2, 1968 or later. Records, identifiable by the unique 10-digit personal number (CPR-number), include date of birth, gender, residency, migration, CPR-numbers of parents and spouses, and vital status. The CRS is updated within a day of a person's birth, address change, death, or emigration (100;101). The CPR-number is an invaluable tool enabling, via record-linkage among all Danish health registries, study of a variety of health outcomes on the population level (102;103) as well as studies of familial clustering of morbidity and mortality. The CRS does not contain direct information on CPR-numbers of siblings, but this information can easily be obtained using parental data. Of note, parental identity is not complete for the whole period covered by CRS: Among persons born in Denmark, the percentage of those with a link to the maternal CPR-number increased rapidly from 8.0% in 1950 to 98.7% in 1960 and 99.9% in 1970. The percentage of those with a link to the father increased from 7.6% in 1950 to 95.7% in 1960 and 99.2% in 1970 (101).

The Danish National Hospital Register (NHR)

The NHR was established in 1977 and comprises data on 99.4% of all discharges from Danish non-psychiatric hospitals. Since 1995 data on outpatients and emergency patients have been recorded as well. DHR includes dates of admission and discharge, the surgical procedures performed, and up to 20 discharge diagnoses classified according to the Danish version of International Classification of Diseases (ICD). The 8^{th} revision was used until end of 1993, the 10^{th} revision thereafter (104).

The National Registry of Deaths (NRD)

The NRD contains information from all Danish death certificates since 1943, coded according to the Danish version of International Classification of Diseases [ICD-8 from 1972 through 1993, and ICD-10 from 1994 through 2001] (105).

5 Methods

5.1 Selection of study subjects

Paper I

We used DHCS to identify all HAART-naïve patients who initiated indinavir, ritonavir, or saquinavir/ritonavir in combination with two NRTIs during the inclusion period of the Danish Protease Inhibitor Study (DAPIS: a national multicentre RCT comparing initial treatment with indinavir, ritonavir or saquinavir/ritonavir during 96 weeks). Patients who initiated indinavir, ritonavir, or saquinavir/ritonavir-based HAART as part of the randomised comparison within DAPIS were classified as trial participants. Patients who initiated these regimes in daily clinical practise were classified as non-trial participants. Thus, we could follow two groups of patients receiving the same HAART regimens during the same time period, who were discordant with respect to trial participation status.

Paper II

HIV-infected men receiving HAART for at least 6 years were recruited from the outpatient clinics at Rigshospitalet (Copenhagen, Denmark) and Aalborg Hospital (Aalborg, Denmark). HAART was defined as therapy including a minimum of three antiretroviral drugs. Patients were approached consecutively when they arrived for scheduled outpatient visits and asked for informed consent. For the reference group, healthy HIV-negative men from similar age groups were recruited among hospital staff and by adverts in local press. Men and women have very different body constitution, and because there were few women with long-term HAART experience we restricted the study to men.

Paper III and IV

Selection of siblings was carried out in three steps:

1. Selection of index patients:

From DHCS we identified all patients who were living in Denmark at time of HIV diagnosis and whose HCV antibody status was known. These were classified into HIV/HCV-coinfected patients (at least one serological test positive for HCV and/or with detectable HCV-RNA) and HIV/HCV-monoinfected patients (at least one negative serological test for HCV and no history of a positive test).

2. Selection of population controls:

Using CRS we identified up to 99 population controls for each HIV patient. Controls were matched to patients by gender, age, and municipality of residence at the date of HIV diagnosis.

3. Creation of three cohorts of siblings:

For both HIV patients and population controls, we identified all full siblings (i.e. siblings with the same mother and father) in CRS and created three cohorts of siblings: (1) siblings of HIV/HCV-coinfected patients, (2) siblings of HIV-monoinfected patients, and (3) siblings of population

controls. Thus a matched design was used to select siblings of population controls; however siblings were not directly matched on age, gender or municipality.

5.2 Statistical methods

Comparing baseline characteristics (paper I and III)

The distribution of individual characteristics between groups was compared using the χ^2 test for dichotomous variables, and the Mann-Whitney test or the Kruskal-Wallis test for continuous variables.

Study outcomes

Paper I: Viral load and CD4 count response were chosen as the outcomes, as these are well established surrogate endpoints in both RCTs and observational studies of antiretroviral therapy. We compared prevalence of undetectable viral and proportions with a CD4 count response after 96 and 240 weeks by logistic regression.

Paper I and III: Mortality was an outcome in studies I and III. We computed person-years of follow-up and constructed group-specific Kaplan-Meier survival curves. We used Cox's regression analyses to compute mortality rate ratios (MRRs) between groups. The proportional hazards assumptions were assessed graphically.

Paper IV: We calculated cause-specific excess mortality rates (EMR). The mortality rate in "exposed" persons (being a sibling of HIV/HCV-coinfected or HIV/HCV-monoinfected individuals) minus the mortality rate in non-exposed persons (siblings of population controls).

Paper II: This paper had a more descriptive approach. We computed Spearman's correlation coefficients between body composition and metabolic parameters in the HIV-infected group. Linear regression was performed to assess the effect of potential explanatory variables on body composition. The distribution of morphological and metabolic characteristics between HIV-infected and the control group was compared using the Mann-Whitney test.

Control of bias

We used a number of the strategies listed in table 1 or discussed above to optimise control of confounding. We used restriction in design (papers II and III), restriction in analyses (papers I and III), a matched double-cohort study for identical selection of siblings (paper III), stratification on matched sets (paper III) and multivariate analyses (papers I, II and III).

For model building in paper I, we repeated the crude analyses in separate datasets according to strata of important variables and when interaction was observed these variables were included in separate models. Thereafter we applied the change-in-estimate method (92;93). All variables

causing changes of more than 10% in the outcome effect estimates were retained in the final models. Type of initial protease-inhibitor was forced into all multivariate analyses.

To examine the magnitude of potential selection bias or informative censoring due to losses to follow-up, we compared study subjects with eligible non-participants (paper II), and compared last measured CD4 cell count in patients lost to follow-up among exposed and non-exposed (paper I).

Missing values
Paper I: Missing values were treated in several ways: for baseline viral loads a specific category was created for missing values to allow for all individuals to be included in the analyses. Viral loads and CD4 cell counts during follow-up they were grouped according to 12-week intervals. If no CD4 count was available for a 12-week interval the missing value was replaced by the value from the previous period (i.e. the principle of "last value carried forward"). Missing viral loads were regarded as detectable (>500 copies/ml); however when the viral load just before and after a missing viral load was undetectable that missing viral load was considered undetectable.

5.3 Ethical considerations

As there are no interventions assigned by investigators in observational studies, ethical considerations relate mainly to privacy issues (54) and to safety of applied diagnostic procedures. DHCS has been approved by the Danish Data Agency, and patient data are stored under a serial number to ensure that individual identity is anonymous in the database. DHCS has also been presented to the Danish Ethical Committee System, but since the collection of data does not involve direct patient contact the study was not subject to direct approval. The cross-linkage of data from the Danish Civil Registration System, the National Registry of Deaths (paper III) and the Danish National Hospital Register (paper I) was also approved by the Danish Data Agency. Study II was approved by the Regional Ethical Committee System.

6 Outcome of HAART in HIV-infected patients treated within and outside a randomised controlled trial: a nationwide Danish study (paper I)

6.1 Background

As discussed above it is unknown to what extent efficacy found in randomised trials of antiretroviral drugs can be translated into effectiveness in daily clinical practice. The setting of the DHCS, together with the Danish tradition of conducting multicentre RCTs provided a unique possibility to explore this research question in a population-based setting. We aimed to compare the outcome of HAART in HIV-infected patients initiating equivalent regimens within and outside an RCT.

6.2 Methods

From the Danish HIV Cohort Study we identified all patients initiating one of three protease-inhibitor-based (indinavir, ritonavir, or saquinavir/ritonavir) HAART regimens: 425 patients within the national randomised trial DAPIS and 677 patients outside the trial. We compared proportions of patients with undetectable viral load and CD4 count response after 96 and 240 weeks by logistic regression and compared mortality by Cox's regression analysis.

6.3 Main results

Viral load and CD4 cell count

Throughout the follow-up period patients initiating HAART within DAPIS (trial participants) were more likely than patients initiating HAART in daily clinical practice (non-participants) to have undetectable viral load and to achieve and maintain an immunological response.

At weeks 96 and 240, the adjusted odds ratios for having undetectable viral load among trial participants were 1.28 (95% CI: 0.94-1.74) and 1.70 (95% CI: 1.16-2.50), compared with non-participants. The corresponding adjusted odds ratios for having a ≥100 cells/µl CD4 cell count increase were 1.37 (95% CI: 1.03-1.82) and 1.52 (95% CI: 1.04-2.25). The only factor that moderated the odds ratio for virological and immunological success was the type of initial treatment arm.

Mortality

Among ART-naïve patients, we observed no difference in mortality between trial participants and non-participants, crude MRR=1.00 (95% CI: 0.62-1.62), adjusted MRR=1.01 (95% CI: 0.60-1.69). By contrast, among the ART-experienced group, we observed lower mortality among trial participants, crude MRR=0.46 (95% CI: 0.27-0.77) and adjusted MRR=0.60 (95% CI: (0.33-1.07). This decreased mortality among the ART-experienced trial participants was attributable to a decreased risk of both HIV-related death, crude MRR=0.58 (95% CI: 0.28-1.22) and non-HIV-related deaths, crude MRR=0.37 (95% CI: 0.17-0.78).

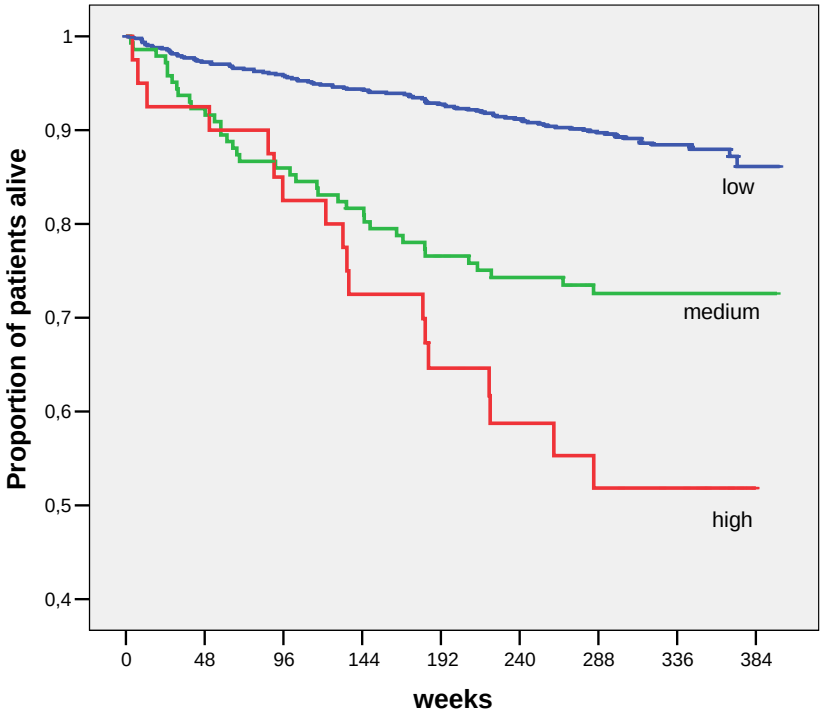
6.4 Comments

Charlsons comorbidity index

Importantly, we found no evidence that comorbidities contributed to the difference in outcome between trial participants and non-participants. The Charlson index (106) is a weighted index of the number and the seriousness of comorbid diseases and consists of 19 major disease categories including HIV/AIDS. The index was developed as an applicable method for classifying comorbid conditions that might alter the risk of mortality for use in longitudinal studies, and it has been widely adapted for use with hospital discharge data (107;108). We computed a modified Charlson comorbidity index based on non-HIV discharge diagnoses registered in NHR prior to baseline and defined three co-morbidity levels: low (score of 0); medium (score of 1-2); high (score of >2). Despite the widespread use in other patient groups, Charlson index has only been infrequently used in longitudinal HIV studies (109).

It is possible that comorbid diseases are coded differently in patients with and without HIV, but as the present study only included HIV patients, any bias introduced would be non-differential. Further, we examined mortality according to comorbidity level in a Cox's proportional hazards regression model.

Figure 1: Kaplan-Meier curves for time from initiation of HAART to death according to three comorbidity levels by the Charlson index.



Compared with patients with low comorbidity level, mortality was significantly higher in both patients with medium comorbidity level [crude MRR=2.68 (95% CI, 1.85-3.89)] and patients with high comorbidity level [crude MRR= 4.97 (95% CI, 3.01-8.21)]. The estimates were adjusted for pre-baseline ART experience, pre-baseline AIDS-defining event, baseline CD4 count and VL, HCV serostatus, mode of infection, gender, race, age and year of initiation of HAART using the change-in-estimate method. Although the estimates were modified by age and pre-baseline AIDS-defining events, comorbidity level remained an independent predictor of death: adjusted MRR= 1.96 (95% CI, 1.33-2.88) for medium versus low comorbidity level, and adjusted MRR= 3.76 (95% CI, 2.26-6.26) for high versus low level. These results support the applicability of Charlson index for control of confounding by comorbidity in HIV-infected patients. Of note, assessing how well the comorbidity index predicts mortality is only an indirect measure of how well the index can control for confounding. While the Charlson index is useful to assess existence and direction of confounding by comorbidity, residual confounding may still be present (110). In our study, adjusting the main analyses for comorbidity did not considerably affect the estimates, suggesting that any residual confounding is likely to be small.

Missing values and loss to follow-up

There is a variety of methods including imputation to deal with missing values, all of which may be confined with bias (111). We used relatively simple strategies. For viral loads a missing value was regarded detectable. In case measurements just before and just after the missing value were undetectable the missing viral load was considered undetectable. We assume this is a reasonable approximation of the "true" value. For CD4 cell counts we used the principle of last value carried forward. For isolated missing values it might be a fair approximation, but this method may overestimate the CD4 cell counts if values are consecutively missing because the patient did not present at the clinic for clinical control and dispensing of antiretroviral drugs.

As mentioned earlier, drop-outs due to loss to follow-up or death are likely to be informative. We observed more deaths among non-participants. As those with the highest viral load and lowest CD4 count are most likely to die before next measurement, this may have biased our results of virological and immunological response towards the null hypothesis. Among those lost to follow up we observed no difference in last measured CD4 cell count between trial participants and non-participants suggesting that losses to follow-up were a minor source of bias.

6.5 Discussion

Thanks to the minimal losses to follow-up and long follow-up time we could compare both short- and long-term outcome of specific HAART regimens in trial participants and the general HIV population. This enabled us to explore possible explanations for the better outcome among trial participants. The papers from Peppercorn (79) and Braunholz (78) provide a useful theoretical framework to analyse differences in outcomes within a trial may and in daily clinical practice. A better outcome could be a consequence of selection of trial participants by strict inclusion and exclusion criteria, or of the trial context (with better outcome in high-volume or specialized centres, or if recruiting clinicians tend to be better clinicians). The differences may be also due to confounding by differences in baseline characteristics associated with both the enrolment probability and the outcome. Further, there may be a true trial effect, where trial participants have better outcomes than similar patients treated off-protocol. Within this trial effect, there could be a protocol effect manifest in more explicit guidelines, a care effect, exerted by more nursing and extra follow-up in the trial, or a "Hawthorne effect" [the effect of being under study on the persons being studied (40)] with changes in patients´ or clinicians´ behaviour (e.g. patients have better adherence to treatment or clinicians have better adherence to guidelines) due to involvement in trial. Finally, the experimental treatment offered in the trial might be better than standard treatment.

DAPIS did not have strict entry criteria and adjustment for differences in baseline covariates and exclusion of non-participants from smaller centres that did not participate in DAPIS had little influence on the results. Our study provided, however, no evidence of a significant trial effect as the differences in outcome persisted up to three years after end of trial follow-up. We presume that the differences are mainly caused by unmeasured confounding. The same type of complex factors behind the mutual patient-physician decision that determine allocation of a specific treatment and cause confounding by indication may also have affected decisions to participate in DAPIS, causing unmeasured confounding (112). The higher mortality in ART-experienced non-participants was partly attributable to non-HIV related deaths, which seems counterintuitive given that the distribution of comorbidity was similar among trial-participants and non-participants (table 1 in paper I). Non-participants might have had more unmeasured risk factors, but as discussed above recent evidence indicates that also causes of death generally perceived as non-HIV related are more frequent in patients with lower CD4 cell counts.

Smith et al (113) examined 828 ART-naïve patients initiating HAART during a 8-year period either as part of an RCT or outside an RCT, regardless of therapeutic regimen. They reported much larger differences between efficacy in trials and effectiveness in daily clinical practice, with trial participants having an OR of 2.6 for attaining virological success within 48 weeks. However, they could not discern whether the difference was due to the HAART regimens used in RCTs.
In our study, proportionately more trial participants received the more potent saquinavir/ritonavir combination, but we adjusted for the imbalances in treatment arms between trial-participants and

non-participants. DAPIS had very few exclusion criteria and our results may not be comparable with the results of trials with strict inclusion criteria.

Concluding remarks

We found small but statistically significant differences in virological and immunological response favouring trial participants. ART-experienced trial-participants had a lower mortality than ART-experienced non-participants. The observed differences generally were small, defying the notion that encouraging results obtained in RCTs are unrealistic overestimations of what can be achieved in routine clinical practice. Similarly to recent publications from other therapeutic fields (80;114) we found no evidence of a specific trial effect.

7. Pronounced lipoatrophy in HIV-infected men receiving HAART for more than six years compared with the background population (paper II)

7.1 Background

When the HIV-associated lipodystrophy syndrome (consisting of subcutaneous lipoatrophy, central fat accumulation, dyslipidaemia and insulin resistance) was first recognized it was ascribed to the newly introduced protease-inhibitors (30;31) but subsequent RCTs evaluating replacement of the PI component with an NNRTI or a third NRTI could not demonstrate improvement of the morphologic features (115).

Attempts have also been made to develop a broad case definition of HIV-associated lipodystrophy (33). But it has been questioned whether this condition is a true fat redistribution syndrome, with central fat accumulation, or rather a selective loss of fat from some compartments (87;116), and the metabolic and morphological abnormalities are probably best described as overlapping syndromes each related to specific drugs or drug classes (36). Hereafter the term lipodystrophy will used when the text is referring to studies that have investigated lipodystrophy more broadly as the presence of peripheral lipoatrophy and/or central lipohypertrophy. The term lipoatrophy will be used specifically for subcutaneous lipoatrophy.

When the present study was initiated, data from both cross-sectional studies and prospective cohort studies (87;117-120) had indicated that duration of therapy and the choice of NRTI were risk factors for changes in body composition. Partly owing to the lack of a consensus definition, reported lipodystrophy prevalences varied from 10% to 80% (121). Therefore, we aimed to describe the long-term consequences of HAART therapy on body fat distribution by objective measurements.

7.2 Aims

The primary aim was to quantify the severity of body fat redistribution in HIV-infected men after long-term HAART exposure as compared with population controls.
Secondary aims were 1) to evaluate association between objective measurements of body fat distribution and the subjective definition of lipodystrophy in HIV-infected men, 2) to compare lipid and insulin measurements in the HIV-infected group with population values, and 3) to evaluate association between objective measurements of body fat distribution and metabolic parameters among the HIV-infected patients.

7.3 Methods

87 HIV-infected men who had received HAART for at least six years and 34 HIV negative men were recruited in a cross-sectional design. Whole-body composition was assessed by dual-energy X-ray absorptiometry (DEXA) (Norland XR). DEXA scanning measures fat mass, lean mass and bone mineral content in body regions of interest. The peripheral fat mass (arm and leg fat, limb fat

mass) is a good measure of subcutaneous fat mass, but DEXA scanning cannot discern the contribution of visceral fat and subcutaneous fat to the truncal fat mass. Similarly to the Lipodystrophy Case Definition Study (33) patients were classified as having clinical lipodystrophy if at least one of the eight defined body composition changes was rated as moderate or severe by both patient and clinician. Plasma samples were obtained after an overnight fast.

7.4 Main results

Compared with HIV-negative controls, the body composition of HIV-infected individuals was dominated by a substantial decrease in fat mass affecting peripheral as well as central sites. The largest relative decrease was seen for limb fat resulting in a higher trunk/limb fat ratio in the HIV-infected group. The fat depletion was most pronounced for patients exposed to stavudine, but also patients never exposed to stavudine had fat depletion when compared with population controls.

Table 3: Morphological parameters in HIV-infected individuals and controls

	HIV-infected, all N=87	HIV-infected, stavudine exposed N=47	HIV-infected, stavudine naive N=40	HIV-negative controls N=34
Total fat mass (kg)	12.3 (8.4-15.6)	10.0 (7.2-13.3)	14.0 (11.5-17.3)	19.2 (15.3-23.0)
Limb fat mass (kg)	4.3 (2.8-5.6)	3.3 (2.2-4.8)	4.9 (3.9-6.2)	7.9 (7.0-9.5)
Trunk fat mass (kg)	6.7 (4.6-9.7)	6.1 (4.1-7.9)	7.5 (5.8-10.3)	10.8 (7.6-12.7)
Trunk/limb fat ratio	1.7 (1.3-2.2)	1.9 (1.3-2.3)	1.5 (1.2-2.0)	1.2 (1.1-1.3)

Data are expressed as medians and interquartile ranges.

Sixty-two per cent of patients satisfied the clinical diagnosis of lipodystrophy by having moderate or severe body fat change in at least one site reported by both patient and clinician. Patients without a clinical diagnosis of lipodystrophy also had reduced peripheral and central fat mass, compared with population controls, but not an increased trunk/limb fat ratio.

HIV-infected individuals had increased triglycerides, increased p-insulin levels and reduced HDL-cholesterol levels compared with healthy controls. HIV patients had also higher total cholesterol and LDL-cholesterol levels than controls, but these differences did not reach statistical significance. All adverse metabolic parameters among the HIV-infected patients correlated with increased trunk/limb fat ratio; furthermore, insulin levels correlated positively with trunk fat mass.

7.5 Discussion

We demonstrated that nearly all patients treated with HAART for more that six years had substantial fat depletion at both peripheral and central sites. Thus HIV patients were not characterized by central fat accumulation. In contrast many studies assessing lipodystrophy based on patient reports and/or physical examination of changes in fat mass found high prevalence of central fat accumulation (33;119). Other studies of both men (32) and women (122) have found that subcutaneous lipoatrophy is the dominating fat abnormality in HAART-treated HIV-infected patients. In general, studies that used population controls and objective measurements found that lipoatrophy was the dominating feature distinguishing HIV patients from controls (32;122;123), although one study of women found more central fat in a subgroup of HIV-infected women as compared with controls (124). We found - like The Study of Fat Redistribution and Metabolic Change in HIV Infection (FRAM) study (32) - that also HIV-infected who did not fulfil criteria for a commonly used clinical diagnosis of lipodystrophy had fat depletion compared with population controls. The clinical diagnosis of lipodystrophy may underestimate lipoatrophy. Further, as fat loss is more pronounced peripherally it may appear clinically as central fat accumulation.

Prospective studies following patients from start of conventional HAART therapy have shown that after an initial period with fat gain due to health reconstitution the dominating feature is progressive peripheral fat loss (87;125). Conventional HAART therapy has consisted of two NRTIs of which one was a thymidine –analogue (stavudine or zidovudine) and a third drug. In our study, all patients but one were exposed to both protease-inhibitors and thymidine-analogues. Consequently our results are not generalisable to patients who received other ART combinations.

Actually a number of RCTs have specifically linked the two thymidine-analogues with the observed progressive fat loss. First a number of studies have demonstrated that in patients with severe lipoatrophy, replacement of stavudine or zidovudine with abacavir or tenofovir led to increases in limb fat mass (89;126). Studies of ART-naïve patients have confirmed this picture, patients randomised to stavudine- or zidovudine-containing HAART progressively loose subcutaneous fat while there is no evidence of fat loss in patients randomised to abacavir or tenofovir containing HAART (90;127;128). Consequently, the use of thymidine-analogues in first-line HAART is decreasing. The thymidine-analogues arguably exert mitochondrial toxicity through inhibition of mitochondrial polymerase-γ in fat tissue which causes depletion of mitochondrial DNA (25;129). This toxic effect causes influx of macrophages with inflammation (130;131) and apoptosis of fat cells. It is, however, not well explained why the toxicity specifically affects subcutaneous fat tissue.

It is well described that triglycerides, total and LDL cholesterol increase with initiation of HAART, and hypercholesterolaemia, triglyceridaemia and low HDL cholesterol are common in patients on antiretroviral therapy (132). The pathogenesis is complex and the magnitude of changes differs by drug class and by individual drugs within a drug class (121). Of note, a prospective study of seroconverters has demonstrated that HIV infection decreases triglycerides, LDL and HDL levels.

This suggest that the HAART-induced increase in lipids partly represents a return to preinfection levels (133). Given the multifactorial interaction between HIV infection, immune restoration, lifestyle, and both acute and chronic drug effects, our study was underpowered to the high number of correlations and study in detail of the association between fat distribution and metabolic parameters, and firm conclusions cannot be drawn. It was, however, a consistent finding that imbalance between truncal and peripheral fat (probably reflecting imbalance between subcutaneous and visceral fat) was associated with all adverse metabolic parameters among the HIV-infected patients.

8. Mortality in Siblings of Patients Coinfected with HIV and Hepatitis C Virus (paper III)

8.1 Background

Coinfection with HCV is common in HIV-infected patients due to overlapping routes of transmission, with 15-30% of HIV patients in the Western world being coinfected with HCV (134-136). The main route of HCV transmission is intravenous drug use (IDU). Contaminated blood products played a sad role in the 1980'es, while sexual and perinatal HCV transmission is rare compared with sexual and perinatal HIV transmission (137). Prevalence of HCV coinfection varies according to transmission patterns in the HIV population; approximately 16% of Danish HIV patients are coinfected with HCV (22).

The impact of HCV on the course of HIV infection

There is consistent evidence that HIV patients coinfected with HCV have a higher risk of liver-disease related deaths than HIV/HCV-monoinfected patients, but the influence of HCV on response to HAART and risk of liver-disease unrelated death is less clear. A number of studies, including a study from DHCS have found increased mortality from causes unrelated to liver disease (38;39;138;139), but other studies did not (140;141). It has been suggested that HCV exerts a direct pathogenic effect by compromising HAART-induced immune restoration (38;142;143) but it has been difficult to separate the direct pathogenic effects of HCV from indirect effects of associated risk factors.

We explored the hypothesis that HIV/HCV-coinfection is a marker of familial risk factors that promote premature death independently of the pathogenicity of HCV.

8.2 Methods

We compared mortality from age 20 years to age 50 years in 437 siblings of HIV/HCV-coinfected subjects, 1856 siblings of HIV/HCV-monoinfected subjects and 285,509 siblings of age and gender matched population controls by Cox's regression analyses.

8.3 Main results

Mortality was substantially higher in siblings of HIV/HCV-coinfected patients compared with either siblings of HIV-monoinfected patients [mortality rate ratio (MRR) =2.97 (95%CI, 1.98-4.45)], or with siblings of controls [MRR =4.23 (95% CI, 3.09-5.79)]. Siblings of HIV-monoinfected patients had slightly higher mortality [MRR = 1.43 (95% CI, 1.10-1.85)] than siblings of population controls. Restricting analyses to Danish-born siblings, youngest siblings, oldest siblings, or siblings of HIV patients without haemophilia did not change the conclusions. Similarly, results were not affected by adjusting for siblings' birth year or gender.

9. Cause-specific excess mortality in siblings of HIV/HCV-coinfected individuals (paper IV)

9.1 Background

To further explore the excess mortality shared among family-members of HIV/HCV-coinfected individuals we proceeded to compare cause-specific mortality among the three groups of siblings: siblings of HIV/HCV-coinfected individuals, siblings of HIV/HCV-monoinfected individuals and siblings of population controls. Due to the high parenteral transmissibility of the hepatitis C virus (HCV) we hypothesized that HCV-infection most often is a marker of relevant exposure e.g. intravenous drug use (IDU). Thus, we specifically aimed to estimate the impact of drug- and alcohol abuse on mortality in siblings of coinfected patients.

9.2. Methods

We identified causes of death for the sibling groups in the National Registry of Deaths and classified causes of death into five groups based on information on the death certificates:

1. Alcohol or drug abuse-related;
2. HIV (excluding those related to substance abuse);
3. Natural causes (excluding those related to HIV or substance abuse);
4. Unnatural causes (including deaths due to accidents, suicide, or homicide and excluding deaths related to substance abuse);
5. Unknown causes.

It has been shown that relying exclusively on underlying cause of death leads to underestimation of alcohol- and drug-related mortality (144-146). Therefore we used a combination of diagnoses for the underlying (main) and contributory causes of death (144) to capture deaths related to abuse of alcohol and drugs (including some prescription drugs) (see table 4). For all other causes we only used the underlying cause of death. Still, death certificate data are not entirely accurate (147), and potential differential misclassification could lead to underestimation of the contribution of substance abuse to the category of unnatural deaths (146;148).

For the three groups of siblings, we computed total and cause-specific mortality rates (MR) and calculated total and cause-specific excess mortality rates (EMR). The results are displayed in details in table 1 in paper IV.

Table 4:
International Classification of Diseases (ICD) diagnoses used to define deaths related to alcohol and drug abuse.

ICD-8 (until 1993)	ICD-10
291	F10-F19
303-304	G312
304	G621
5710	G721
5711	I426
5771	K292
N979-N980	K70
E8530	K860
E8540	T51
E860	X41-X42
E980 in combination with	X45
N9650, N9780, or N9790.	Y11-Y12
E950 in combination with	Y15
N9650, N9780, or N9790	X60-X64 in combination with T40.

9.3. Results

The all-cause EMR for siblings of HIV/HCV-co-infected individuals, compared with siblings of population controls, was 3.01 (95% CI, 1.55-4.46) per 1,000 PY. The excess mortality was mainly caused by substance abuse-related deaths [EMR = 2.23 (95% CI, 1.08-3.38) per 1,000 PYR], and to a lesser extent by unnatural deaths [EMR = 0.67 (95% CI, -0.05-1.35) per 1,000 PYR] and by deaths with HIV as the underlying cause [EMR = 0.38 (95% CI; -0.08-0.85) per 1,000 PYR]. No siblings of HIV/HCV-co-infected patients had acute or chronic HCV infection or other liver-related diagnosis as the underlying cause of death.

10. Discussion of the results from paper III and IV

We found substantially increased mortality in siblings of coinfected patients that might be caused be shared environmental (socioeconomic) or genetic factors. The effect could be mediated through shared susceptibility to high-risk behaviour such as substance-abuse or shared susceptibility to some infectious pathogens. Some studies have indicated that inherited factors or perinatal programming may contribute to predisposition to infectious diseases (149-151). The analyses of causes of death in paper IV revealed that the excess sibling mortality was largely explained by substance-abuse related deaths and other unnatural deaths. Thus we did not find support for the hypothesis that the excess mortality was caused by hepatitis C-infection or increased susceptibility to other infectious diseases.

We did not examine the environmental or genetic mechanisms underlying the clustering of substance abuse-related deaths in families of HIV/HCV-coinfected individuals. Adoption and twin studies have shown that in addition to family environment, there is a strong genetic contribution to the familial pre-disposition for substance abuse (152;153). The genetic contribution is complicated and acts through several pathways including, for example, its role in impulsive behaviour or other personality disorders (152;154). Our results lend further credence to the hypothesis that excess mortality in co-infected patients mainly stems from factors other than the pathogenicity of the HCV disease itself. Substance abuse and associated risk behaviors may directly increase mortality. Furthermore, suboptimal health-seeking behavior, poor adherence to medical treatment, drug- or alcohol-related comorbidity, or health care providers' inattention to HCV-infected patients may indirectly cause decreased survival.

The interaction between HIV and HCV

Although liver-disease related mortality is high among HIV patients coinfected with HCV, the majority of these patients die from causes unrelated to liver-disease. Among the 443 coinfected patients prospectively followed in a study from DHCS, 22% of the 112 deaths observed during the follow-up were definitively or probably liver-related (39). HIV infection negatively influences the clinical course of HCV, causing faster progression to cirrhosis (136), Treatment with HAART attenuates this effect (155) probably by increasing the CD4 cell count (156). Recent publications even suggest that coinfected patients with successful response to HAART have liver fibrosis scores (157) and fibrosis progression rates (158;159) similar to those of HCV monoinfected patients. The D:A:D study recently confirmed the strong association between immunodeficiency and elevated risk of liver-related deaths, an effect also seen in patients with CD4 cell counts above 200/µL (29). Older age and continued alcohol consumption among HCV-infected individuals are other important prognostic factors that increase HCV replication and accelerate fibrogenesis (160). However, information on alcohol intake is often inadequately recorded in HIV cohort studies and is therefore usually not considered when assessing influence of HIV on the course of HCV infection or vice versa. There are no data on alcohol intake in DHCS, but in a study from USA alcoholism was present in 30% of coinfected patients, confounded both mortality and the risk of progression to AIDS, and was an predictor of progression to AIDS or death (161). Finally, increased

hepatotoxicity or mitochondrial toxicity of ART, insulin resistance (162), neurocognitive dysfunction (163), and increased risk of cardiovascular disease (164) may all complicate the clinical course in HIV/HCVcoinfected patients.

Concluding remarks

The improved treatment response with pegylated interferon (165-168), considerations about the negative interactions between HIV and HCV, and the poorer prognosis in coinfected patients have attracted growing attention to anti-HCV treatment, with widening of indications (137;169;170). The negative influence of HIV on HCV is mainly seen in patients with immunosuppression and/or ongoing HIV replication, and the findings of negative interactions between HIV and HCV may be confounded by factors as substance abuse and related disorders. Further, less than half of the patients (27-44%) achieved sustained virological response in RCTs evaluating the combination of pegylated interferon and ribavirin (165-168), accompanied by high toxicity profile. This warrants an individualised approach to treatment, taking into account a range of factors (171), including status of liver disease, immune status, presence of other risk factors, and prevention of drug and alcohol use. HIV treatment strategies that prevent immunodeficiency are of high priority given the strong association between immunodeficiency and the risk of liver-related death. Research should focus on ways to identify patients in whom HCV treatment will prevent progression of liver fibrosis, and to further develop and validate non-invasive methods to evaluate inflammation and fibrosis (for example serum fibrosis markers and transient elastography). Methodologically, the familial clustering of substance-abuse associated issues underscores the need for adequate control groups, and/or extensive control of confounding, and careful evaluations of causes of death when effects of HCV are investigated in clinical cohorts.

Similarly, it can be difficult to pinpoint to what extent specific diseases or conditions are directly HIV- or HAART related or rather associated with modes of HIV-infection. For example, HIV-infected individuals have higher incidence of anal cancers than general population controls, which may be related primarily to higher prevalence of anal human papilloma virus infection among men who have sex with men (172;173). HIV-infected individuals may differ from the general population with regard to demographic (6) or socioeconomic factors and having acquired HIV may indicate tendency towards risk taking behaviour. To give another example, Danish HIV patients receiving HAART have higher incidence of myocardial infarction than general population controls matched on age, gender and residency (174). But smoking seems to be more frequent among HIV infected patients (175) which might contribute to the observed difference.

11. General conclusions and perspectives

Even with free access to antiretroviral treatment, HIV infection is associated with excess morbidity and mortality, with still approximately one third of deaths in Danish HIV patients directly related to the HIV infection (6;22). These deaths may be caused by late diagnosis and thereby delayed initiation of treatment, or they may be caused by emergence of resistance mutations with exhaustion of treatment options (176). Fortunately, recent works have documented decreasing incidence of triple-drug class failure, and many patients become virologically re-suppressed after a period of virological failure (60;177). This thesis provides insight into the impact of toxicities, comorbidities and associated life-style factors on the health of HIV-infected patients.

Long-term follow-up studies that continuously monitor known and emerging toxicities are crucial. Lipoatrophy that seemed inevitable few years ago is now avoidable, but it took surprisingly long from the first descriptions of HIV-associated lipodystrophy syndrome to identification of the link between thymidine-analogues and lipoatrophy as the dominating feature. Future delays in this process should be avoided by use of adequate study designs, e.g. new-users design restricting the analysis to persons under observation at the start of HAART (86), and by further development and implementation of methods that appropriately control for time-varying exposures that are both confounders and intermediates on the causal pathway (178-180), and methods that take confounding by indication into account (67;181-183). Use of properly matched general population controls can help to better discern whether specific diseases are truly HIV or HAART-related and occur with higher frequency among HIV-infected and/or HAART exposed individuals (174). The occurrence of diabetes, low energy fractures, and acute and chronic kidney failure in relation to both treatment with HAART and specific antiretroviral drugs are areas that need future clarification.

With the growing success of HAART, risk factors that are also determinants of health in the general population gain greater relative importance – and risk factors that are associated with modes of HIV transmission are likely to be more prevalent among HIV-infected individuals. Therefore, clinical databases need to scale up information on risk factors such as smoking, alcohol use, comorbidities and socioeconomic factors. In Denmark, the availability of data on many of these factors from linkable national registries is a great advantage.

Due to the importance of near-perfect drug adherence for the successful outcome of HAART, personal resources and health behaviour may have a strong impact on the course of HIV in HAART-treated individuals. Although the majority of Danish HIV patients are well-functioning (184), they are also living with a chronic disease. Depressive symptoms and major depression are common among them and have implications for long-term outcome (184;185). HCV-coinfection also identifies a vulnerable subgroup with familial clustering of substance abuse. Psychiatric disease in HIV patients is, however, a poorly described area (186) and risk factors for and prognostic importance of psychiatric disease in HIV-infected patients should be further addressed.

All relevant issues cannot be a clarified in single observational clinical cohorts. Multi-cohort collaborations and metaanalyses are needed to evaluate rare outcomes or rare exposures, and to follow sufficient numbers of patients from the time of seroconversion as exemplified by the COHERE (187) and CASCADE collaborations (188). There is also a continuous need of randomized controlled trials to evaluate not only HAART regimens, but also other interventions (189;190) and treatment strategies (56). One of the pending research questions is when to start HAART. Rationales for the current guidelines are changing, and decisions have been based on inferences from observational studies (191).

The greatest challenge, however, is to ensure access to HAART and to the research progresses achieved in the resource-rich part of the world to all those in need of therapy.

12. Summary

Growing evidence shows enduring and stable effect of highly active antiretroviral therapy (HAART) in HIV-infected persons. Despite the progress in the development of potent HAART regimens, HIV infection is still associated with considerably increased morbidity and mortality, even in areas with free access to treatment. We hypothesised that an increasing proportion of HIV-associated morbidity and mortality is attributable to side effects, to associated co-morbidities (including hepatitis C virus infection), and to life-style related risk factors. A further hypothesis was that randomised controlled trials (RCT) should be supplemented and complemented by well-performed observational studies in order to monitor and understand the full range of these associations in HIV-infected populations.

The thesis had the following aims

1. To compare the efficacy of HAART in an RCT with the effectiveness of HAART in an observational cohort study.
2. To establish the prevalence and severity of metabolic complications including body fat redistribution in HIV-infected men after long-term HAART treatment as compared with general population controls.
3. To examine whether HCV/HIV coinfection is a marker for familial risk factors that affect survival in HIV-infected patients independently of HCV pathogenicity.

RCTs are the reference standard for evaluating efficacy of interventions, including efficacy of antiretroviral therapy. However, in the field of antiretroviral therapy little is known about the extent to which beneficial treatment effects found in an RCT are also achievable in daily clinical practice. The setting of the Danish HIV Cohort Study (DHCS) provides a unique possibility to explore this research question. The Danish Protease-Inhibitor Study (DAPIS) was a national multicentre RCT recruiting patient from 1996 to January 2001 in order to compare three different protease-inhibitor-based HAART regimens within 96 weeks of follow-up, and at the same time we could identify from the DHSC all patients who were initiating the same regimens but were not recruited to an RCT. The population-based selection of patients into this cohort combined with minimal losses to follow-up and long follow-up time enabled examinations of short- and long-term outcomes after initiation of specific HAART regimens in trial participants, as compared with the general HIV population, and to explore possible explanations for any differences between trial participants and non-participants. At both short-term (96 weeks) and long-term follow-up (240 weeks) we found small but statistically significant differences in virological and immunological response favouring trial participants. For mono- or dual-antiretroviral therapy (ART)-experienced, but not for ART-naive patients, trial participants had a lower risk of death which was partly accounted for in adjusted analyses.
The observed differences may be mainly explained by unmeasured patient characteristics rather than by the clinical care in the trial, because the differences were undiminished after the end of follow-up in the RCT. Of importance, the differences were small implying that encouraging results

obtained in RCTs should give reason for similar expectations for patients in routine clinical practice.

Few years after the introduction of HAART, an HIV-associated lipodystrophy syndrome was described. The syndrome was thought to consist of peripheral lipoatrophy and central fat accumulation associated with dyslipidaemia and insulin resistance. We assessed regional fat distribution using dual-energy X-ray absorptiometry in 87 HIV-infected men who had received HAART for at least six years and in 34 HIV-negative men. Compared with controls, HIV-infected persons had reduced total fat mass (median 12.3 vs.19.2 kg, p<.001), limb fat mass (4.3 vs. 7.9 kg, p<.001), and trunk fat mass (6.7 vs. 10.8 kg, p<.001) and higher trunk/limb fat ratio (1.7 vs.1.2, p<.001). Patients without clinical lipodystrophy also had reduced amounts of limb and trunk fat. In conclusion, generalised lipoatrophy was the dominating feature present in the vast majority of male patients after long-term treatment with thymidine-analogue based HAART.

Co-infection with the hepatitis C virus (HCV), common in HIV-infected individuals, is a marker for poor prognosis. It is unclear whether the prognostic impact is caused directly by the viral disease, or whether it is an epiphenomenon of the infection. We hypothesised that HCV/HIV coinfection is a marker for familial risk factors that affect survival in HIV-infected patients independently of HCV pathogenicity. To examine this, we compared mortality in three groups: siblings of patients coinfected with HIV and HCV, siblings of HIV-monoinfected patients, and siblings of general population controls. Mortality was substantially higher in siblings of HIV/HCV-coinfected patients compared with either siblings of HIV-monoinfected patients [mortality rate ratio (MRR) =2.97 (95%CI, 1.98-4.45)], or with siblings of controls [MRR =4.23 (95% CI, 3.09-5.79)]. Siblings of HIV-monoinfected patients had slightly increased mortality [MRR = 1.43 (95% CI, 1.10-1.85)] relative to siblings of controls. The excess sibling mortality was largely explained by increased risk of substance-abuse related deaths and other unnatural deaths. Thus, HCV infection is a marker of familial factors that affect the survival of HIV-infected patients independently of HCV pathogenicity, and studies that fail to account for these factors may overestimate mortality attributable to HCV-infection.

The work of this thesis demonstrated the significant impact of external risk factors and side effects on the health of the HIV-infected population. These factors should be addresses in order to further improve the prognosis and quality of life of HIV-infected patients, and long-term follow-up studies that continuously monitor known and emerging toxicities are crucial.

13. Danish summary

Ph.d. afhandlingen består af fire videnskabelige artikler og en oversigt. Der er efterhånden god evidens for at moderne kombinationsbehandling (highly active antiretroviral therapi, HAART) af HIV-inficerede har vedvarende og stabil effekt. På trods af potente kombinationsbehandlinger er HIV-infektion dog fortsat associeret med betydelig øget morbiditet og mortalitet. Baggrunden for denne afhandling er hypotesen om, at en tiltagende proportion af den HIV associerede morbiditet og mortalitet kan tilskrives bivirkninger, komorbiditet inklusive kronisk hepatitis C virus (HCV) infektion og livsstilsrelaterede risikofaktorer. Randomiserede kontrollerede studier bør derfor suppleres af veldesignede observationelle studier for at monitorere og belyse betydningen af disse faktorer og deres samspil blandt HIV-inficerede.

Randomiserede kontrollerede studier er guldstandard, når effekten af medicinske interventioner skal evalueres. Vedrørende antiretroviral behandling er der begrænset viden om, i hvilken grad gavnlige behandlingseffekter fundet i randomiserede kliniske studier også kan opnås i daglig klinisk praksis. Formålet med studie I var at sammenligne effekten af HAART i et randomiseret kontrolleret studie (efficacy) med effekten i et observationelt kohortestudie (effectiveness). Det Danske Protease-Hæmmer Studie var et nationalt randomiseret multicenter studie designet til at sammenligne tre forskellige protease-hæmmer baserede HAART regimer over 96 ugers med inklusionsperiode fra 1996 til januar 2001. I Den Danske HIV Kohorte kunne vi identificere alle patienter, der startede et af de tre regimer i denne periode og sammenligne kortids- og langtidsresultater efter start af samme HAART regimer mellem studiedeltagere og patienter behandlet udenfor studie. Vi fandt små men statistisk signifikante forskelle på både virologiske og immunologiske parametre efter 96 og 240 ugers follow-up til fordel for studiedeltagere. Blandt patienter tidligere behandlet med et eller to antiretrovirale stoffer, men ikke blandt behandlingsnaive patienter, havde studiedeltagere mindre dødelighed. De observerede forskelle forblev i samme størrelsesorden op til tre år efter afslutning af de 96 ugers follow-up i det randomiserede studie. Forskellene kan derfor formentlig tilskrives umålt konfounding og ikke en bedre klinisk behandling i det randomiserede studie.

Få år efter indførelsen af HAART blev rapporteret om forekomst af HIV-associeret lipodystrofi. Syndromet blev initialt beskrevet som en kombination af perifer lipoatrofi og central lipohypertrofi. Formålet med studie II var at beskrive prævalens og sværhedsgrad af metaboliske komplikationer inklusive ændringer i fedtfordeling blandt HIV-inficerede mænd sammenlignet med baggrundsbefolkningen. Vi brugte helkrops DEXA-scanning til at bestemme regional fedtfordeling hos 87 HIV-inficerede mænd behandlet med HAART i mindst seks år og 34 HIV-negative mænd. Sammenlignet med kontroller havde HIV-inficerede mindre samlet fedt mængde (median 12,3 vs.19,2 kg, $p<0,001$), mindre perifert fedt (4,3 vs. 7,9 kg, $p<0,001$), mindre trunkalt fedt (6,7 vs. 10,8 kg, $p<0,001$) og større trunkal/perifer fedt ratio (1,7 vs.1,2, $p<0,001$). Også patienter uden klinisk lipodystrofi havde mindre fedt både perifert og trunkalt. Generaliseret lipoatrofi var således et dominerende træk hos flertallet af langtidsbehandlede HIV-inficerede.

Koinfektion med HCV er markør for dårligere prognose. Det er uafklaret om den prognostiske betydning er drevet af selve HCV infektionen eller af risikofaktorer associeret med HCV-infektion. Formålet med studie III og IV var at undersøge om HIV/HCV koinfektion er markør for familiære faktorer, der påvirker overlevelse blandt HIV-inficerede uafhængigt af HCVs patogenetiske egenskaber. For at undersøge dette sammenlignede vi dødelighed i tre grupper: søskende til HIV/HCV- koinficerede patienter, søskende til HIV-monoinficerede patienter og søskende til populations kontroller. Dødeligheden var betydeligt højere blandt søskende til HIV/HCV-koinficerede patienter sammenlignet med enten søskende til HIV-monoinficerede patienter [mortalitets rate ratio (MRR) =2,97 (95 % CI, 1,98-4,45)], eller med kontrolsøskende [MRR =4,23 (95 % CI, 3,09-5,79)]. Søskende til HIV-monoinficerede patienters havde en mindre overdødelighed [MRR = 1,43 (95 % CI, 1,10-1,85)] sammenlignet med kontrolsøskende. Overdødeligheden blandt søskende til HIV/HCV-koinficerede patienter var hovedsageligt forklaret af misbrugsrelaterede dødsfald og død af unaturlige årsager. HCV infektion er altså en markør for familierelaterede faktorer, der påvirker overlevelsen blandt HIV-inficerede uafhængigt af HCV patogenicitet.

Ph.d. afhandlingen påviser, at bivirkninger og eksterne risikofaktorer har stor indflydelse på sundhedstilstanden blandt HIV-inficerede. Disse faktorer bør adresseres for yderligere at forbedre prognose og livskvalitet blandt HIV-inficerede personer, og langtidsstudier bør fortsat monitorere kendte og nytilkomne bivirkninger.

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