Cardiovascular Adverse Effects of Antiretroviral Therapy

PH. D. THESIS

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CONTENTS

This Ph.D.-thesis is based on three publications from the D:A:D study.

I. *Friis-Møller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, Pradier C, Morfeldt L, Mateu S, Law M, El-Sadr W, De Wit S, Sabin CA, Phillips AN, and Lundgren JD for the D:A:D study group.*

Cardiovascular risk factors in HIV patients – association with antiretroviral therapy Results from the D:A:D Study. AIDS 2003; 17(8)

II. *Law M, Friis-Møller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, Pradier C, Morfeldt L, Calvo G, El-Sadr W, De Wit S, Sabin C, and Lundgren JD for the D:A:D Study Group.*

Modelling the three year risk of myocardial infarction among participants in the D:A:D study. Hiv Medicine 2003; 4(1):1-10.

III. *The D:A:D Study group. Writing committee: Friis-Møller N, Sabin C, Weber R, d'Arminio Monforte A, El-Sadr W, Reiss P, Thiébaut R, Morfeldt L, De Wit S, Pradier C, Calvo G, Law M, Kirk O, Phillips AN, and Lundgren JD.*

Combination antiretroviral therapy and the risk of myocardial infarction. Results from the D:A:D Study. NEJM 2003; 349(21):1993-2003.

This thesis was submitted to the Faculty of Health Sciences of the University of Copenhagen January 6th 2004, and recommended for public defence by June 9th 2004.

The defence takes place November 12th 2004.

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This thesis will be published as an original article in the Danish Medical Bulletin.

PREFACE

The scientific work underlying this Ph.D.-thesis was conducted from April 2000 through December 2003 during my employment as clinical research associate at Copenhagen HIV Programme (CHIP), Hvidovre University Hospital, Denmark, and study coordinator for the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study – a position that I continue to hold and cherish. When I joined the group in 2000, the coordinating function immediately entailed collaboration with a large number of experts within the field of HIV epidemiology in an international setting. I feel very fortunate to have been offered this challenging and enjoyable job, at a time when my professional experience was primarily clinical, and research a yearned for aspiration.

First of all, I am immensely grateful to my supervisors, *Jens D. Lundgren* and *Ole Kirk*, for sharing their considerable scientific skills and guiding me through this absorbing project. Their vast experience, competence, patience and stamina have been crucial, and it has been a very educational and rewarding experience to collaborate with them both.

In particular, I am indebted to *Jens*, my primary supervisor and also the architect and chair of the D:A:D study. His impressive insight, marvellous energy and genuine scientific curiosity remain a constant inspiration. I had very limited scientific skills when I joined the group, and it has been a privilege to be coached and educated by such a capacity within the field.

Caroline Sabin and *Andrew Phillips* at the Royal Free Hospital in London, and *Matthew Law* at the National Centre in HIV Epidemiology and Clinical Research, Sydney, are all greatly thanked for their pleasant collaboration and excellent statistical analysis work. I am also immensely grateful for all the advice, supervision and input to analyses plans and articles that they have provided.

It has been a truly inspirational experience to collaborate with the members of the Steering Committee of the D:A:D study. This group of highly skilled researchers have all contributed collaboratively to the achievements of the study, and offered precious scientific guidance. I wish to express my sincere gratitude to each and every member of the Committee, to all co-authors, and I convey my thanks to all colleagues and collaborators in the D:A:D study. The enthusiasm, professionalism and teamwork on all parties has been a great experience.

Everyone at CHIP are greatly acknowledged for their support and dedication, in particular data-manager *Allen Sawitz*, who is devoting his skills in data handling on the D:A:D study and has been of great assistance, and treasured colleagues *Ole Kirk, Ulrik Bak Dragsted, Hans-Henrik Larsen, Jannik Helweg-Larsen*, and *Thomas Benfield* who have been forerunners or travelled on parallel tracks in CHIP, shared office and/or experience and with whom I've had many amusing scientific and non-scientific discussions.

Furthermore, I would also like to express my gratitude to *Jens Ole Nielsen* for housing me at the Department of Infectious Diseases in the first years of the project.

Anette Sjøl, department of Cardiology at Frederiksberg Hospital, is greatly thanked for offering precious assistance and expertise in the evaluation of cardiovascular disease entities, and I am grateful for our many fruitful discussions.

The teachers at the Medical Faculty, Copenhagen University, deserve much credit for their excellent Ph.D. courses – in particular I am grateful for the statistical courses.

Finally, I would like to thank my friends and family for putting up with me and supporting me, when I have sometimes been too occupied with my work.

Throughout the work on this Ph.D. thesis, my affiliation to the Faculty of Health Sciences, University of Copenhagen, was paid by a grant from the H:S (Hovedstadens Sygehus fællesskab). Travel and registration grants were donated by the Glasgow Conference (2000), the Conference of Retroviruses and Opportunistic Infections (2003), and by the Lipodystrophy Conference (2003). Niels and Desiree Ydes foundation provided a grant for a period of tranquillity in the infamous Golfe Juan, France, to finalize this thesis.

Copenhagen, October 2004,
Nina Friis-Møller

ABBREVIATIONS

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CART	Combination Antiretroviral Therapy
$CD4^+$ cell	CD4 receptor positive T lymphocyte cell
CHIP	Copenhagen HIV Programme
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
EMEA	European Agency for the Evaluation of Medicinal Products
HAART	Highly active antiretroviral therapy
HDL	Serum high density lipoprotein-cholesterol
HIV-1	Human immunodeficiency virus type 1
IQR	Inter-quartile range
MI	Myocardial infarction
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PI	Protease inhibitor
PY	Person Years
RR	Relative Rate
SAS	Statistical Analysis System
TC	Total serum-cholesterol
TG	Serum-triglycerides

INTRODUCTION

The use of combination antiretroviral therapy – a combination of three or more drugs from one or more antiretroviral drug classes (nucleoside reverse transcriptase inhibitors, NRTIs, protease inhibitors, PIs, and non-nucleoside reverse transcriptase inhibitors, NNRTIs) – for the treatment of HIV-1 infection has been abundant in the industrialised countries since 1996. The dramatic effects of antiretroviral combination therapy on reducing HIV-related morbidity and mortality are well described.[1-4]

Initially, the therapy was hypothesised to not only improve clinical conditions and survival of HIV-infected patients, but even to eradicate the virus and cure HIV infection. It was soon realized, however, that viral eradication was not possible with combination antiretroviral therapy.[5] Consequently, the current concept of antiretroviral therapy is based on a lifelong treatment, and long-term side effects of antiretroviral therapy have therefore become increasingly important.

The toxicity and tolerability of combination antiretroviral therapy influences the choice of components and time of starting therapy. Toxicities are frequent (50-75%,[6-10]) and as long-term benefits rely on almost complete adherence[11;12], the success of the treatment is highly dependent on the regimens tolerability. Obviously, severe morbidities as a consequence of the drugs should be limited as far as possible. A recent study showed that the frequency of severe toxicities (grade IV) had exceeded that of AIDS defining events in populations treated with combination antiretroviral therapy[13], thus emphasising that an optimal balance between effect and harm has not yet been achieved.

Due to the severe prognosis of untreated HIV-infection, the introduction of new antiretroviral drugs to clinical use has in general had a precipitated marketing procedure. Thus, by the time of introducing the drugs in clinical practice, the registered side effects are those recorded from pivotal phase III trials, the duration of which rarely exceeds 48 weeks. In addition to the short duration of follow-up, the number of patients included in these studies is relatively small and selected (sub-populations generally under-represented), and the possibility to identify rare adverse events therefore limited. Other side effects are only observed after longer exposure. Based on the relative short history of the drugs, it is only in recent years and based on observations from daily clinical practice or from observational studies that data pertaining to long-term toxicity has become available.

Among the various toxicities of combination antiretroviral therapy, a number of metabolic complications have been identified, including dyslipidemia, insulin resistance and diabetes mellitus; metabolic changes that are likely to increase the risk of cardiovascular disease (CVD). However, whether and how soon these antiretroviral therapy-induced abnormalities might result in a clinically detectable increased risk of CVD has been the object of much controversy.

In the late 1990ies a number of case reports and small case series were published on premature coronary heart disease (CHD) in young PI treated HIV infected persons.[14-18] A meta-analysis of the immediate risk of myocardial infarction (MI) in randomised trials comparing PI and non-PI containing regimens, demonstrated no significant differences between the regimens, but suffered from insufficient power due to few observed cases of MI.[19;20]

To address these concerns, a multi-cohort collaboration was formed in 1999 to conduct the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study. The primary objective of the study was to determine whether exposure to combination antiretroviral therapy was associated with the risk of MI.

Support for the study is given by the 'Oversight Committee for The Evaluation of Metabolic Complications of HAART', a committee with representation from the European Agency for the Evaluation of Medicinal Products (EMEA) and pharmaceutical industry producing ART.

OBJECTIVES

The objectives of this thesis were to, based on data from the D:A:D study:

i) describe the prevalence of risk factors for cardiovascular disease (CVD) in HIV-infected patients at baseline in D:A:D (*I; baseline analyses*)
ii) describe the association of antiretroviral drug regimens with CVD risk factors (*I*)
iii) estimate the predicted risk of MI (*II; modelling analyses*)
iv) describe the observed risk of MI in HIV-infected patients, and assess a possible association with combination antiretroviral therapy (*III; primary analyses*)

METHODS

STUDY DESIGN

Cohort collaboration

The D:A:D study is an observational study formed by the collaboration of eleven previously established HIV cohorts[6;21-30] (table 1), that committed to comply with specific requirements for the data collection, data structure, monitoring and quality assurance measures as outlined in the protocol for the D:A:D study. The coordinating office, staffed by the principal investigator, the study coordinator and the central data-manager, is localized at the Copenhagen HIV Programme at Hvidovre Hospital, and the central statistician affiliated to the coordinating office resides at the Royal Free Hospital in London.

The coordinating office has the overall responsibility of the D:A:D study including the formation of the central database, coordination and collection of data from each of the cohorts in to the central database, cleaning of the data, oversight with cohort/site per-

formance, review and query of event forms, preparation of study material including data collection forms, manuals and protocols, and the preparation of drafts of public communications from the study. The coordinating office organizes Steering Committee meetings, and it is centrally involved in drafting and executing analyses plans in collaboration with the Steering Committee and statisticians.

The principal investigator of D:A:D, principal investigators from each of the participating cohorts, a lead statistician and representation from the Oversight Committee form the Steering Committee of the D:A:D study. The Steering Committee approves all analytic proposals prior to their execution and has the overall responsibility for the scientific conduct of the study. This committee convenes quarterly by teleconference or face-to-face meetings.

Data-managers from all cohorts collaborate to ensure a uniform data structure (details of which are outlined in a Standard Operation Procedures for data-management).

The collaboration is unique by its substantial size and in the undertaking of extensive harmonization across cohorts to achieve a concerted prospective data collection; amongst others it has also entailed the development of a protocol for standardization of data exchange for use in this and other cohort collaborations (HICDEP; more information available at: www.cphiv.dk).

Design

The study was designed to permit the detection of a twofold increase in the incidence of MI with increasing exposure to combination antiretroviral therapy. A total of at least 100 new cases of MI were required to give the study sufficient power to detect such an increase in risk (two-sided type I error, 5 percent; power, 90 percent). The incidence of MI was assumed to be 3.3 per 1000 person-years, and hence the study had to collect at least 30,000 person-years of follow-up data before the primary objective could be examined (*III*).

The Steering Committee was blinded to the number of primary endpoints until this goal had been achieved.

Study population

A total of 23,468 HIV infected patients participate (table 1); patients are followed at 188 clinics in 21 countries situated in Europe, USA and Australia (*I, III*).

Eligible patients were all under active follow-up at the time of initiation of the D:A:D protocol, irrespective of antiretroviral treatment status. Patients were enrolled into D:A:D consecutively as they were seen in the clinic from the time the study was implemented in each of the participating cohorts. The first cohorts started to include patients in December 1999, and all patients were included prior to April 1st 2001.

Table 1. Demographic characteristics of the total D:A:D study population at baseline

Cohort [reference]	AHOD[29]	ATHENA[28]	Aquitaine[30]	BASS[26]	Brussels[24]	CPCRA[22]	EuroSida[21]	HivBIVUS[25]	ICONA[6]	Nice[23]	SHCS[27]	DAD
Country	Australia	Netherlands	France	Spain	Belgium	USA	Europe and Israel	Sweden	Italy	France	Switzerland	Total
Number of patients (%)	706 (3.0)	2861 (12.2)	1984 (8.5)	688 (2.9)	1097 (4.7)	2497 (10.6)	5090 (21.7)	968 (4.1)	2539 (10.8)	1077 (4.6)	3961 (16.9)	23468 (100.0)
Female (%)	4.1	14.1	25.1	24.9	43.7	20.0	21.5	19.7	31.7	29.9	29.6	24.1
Age (years; median, IQR)	40 (35-47)	42 (37-49)	39 (35-45)	38 (35-43)	37 (32-43)	40 (34-46)	39 (34-46)	41 (35-48)	36 (32-40)	38 (34-43)	39 (34-45)	39 (34-45)
HIV acquisition (%)												
Homosexual	87.5	67.7	41.2	33.7	23.6	55.7	48.3	59.1	19.9	26.7	36.9	45.0
Heterosexual	6.7	21.0	26.9	25.0	52.5	NA	24.1	26.1	37.0	32.6	33.7	26.2
Intravenous drug use	2.1	4.5	21.8	38.5	6.2	10.7	19.6	9.2	38.7	30.0	25.6	19.5
Previous AIDS (%)	22.7	34.0	23.8	33.7	18.3	23.2	34.1	19.6	12.9	27.5	25.4	26.2
CD4 count cells/μL; median, IQR)	500 (324-680)	450 (290-630)	429 (273-606)	496 (308-706)	435 (273-622)	265 (64-468)	358 (220-520)	480 (320-660)	522 (339-728)	428 (276-619)	426 (269-616)	418 (255-612
HIV-1 RNA log; median, range)	<2.7 (<2.7-5.9)	<2.7 (<2.7-6.3)	<2.7 (<2.7-6.5)	<2.7 (<2.7-6.3)	<2.7 (<2.7-5.9)	4.8 (<2.7-6.9)	<2.7 (<2.7-6.3)	<2.7 (<2.7-5.9)	3.0 (<2.7-6.8)	<2.7 (<2.7-5.9)	<2.7 (<2.7-6.9)	<2.7 (<2.7-6.9
Antiretroviral therapy naive %)	9.5	0.4	9.7	0.6	23.3	66.2	4.0	13.6	40.2	8.1	14.3	18.3

NA: Not available

Data collected

At enrolment and at least every 8 months thereafter standardised data collection forms are completed at the clinics providing information concerning antiretroviral therapy, clinical course, HIV RNA, CD4 count, risk factors for, history and incident cases of diabetes and CVD (MI, stroke, invasive cardiovascular procedures; detailed list of variables in *I,III*). All collected information was transformed into a standardised format and merged into a central data-set.

Endpoints

The primary endpoint in the D:A:D study was MI. All new cases of MI were reported immediately to the study coordinating office for central validation and for coding according to procedures applied in the World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project.[31-33] All endpoints had to fulfil the criteria for a definite, possible, or unclassifiable MI and were categorized as nonfatal (survival for at least 28 days) or fatal. The classification was based on an established algorithm[34] that included cardiac pain, cardiac enzyme levels, electrocardiographic readings, and in cases of death, autopsy results if available.

Since the development of the algorithm applied in the MONICA study, a new more sensitive diagnostic test, serum-troponin, has become available and the diagnostic criteria for MI has been revised.[35] As the endpoint definition in the D:A:D study was defined prior to this consensus report, and further in order to enable comparison with results from other studies of HIV-infected and -uninfected persons, it was decided to maintain the earlier definition. Information on serum-troponin was collected and elevated troponin levels were considered to have the same significance in the algorithm as elevated cardiac enzymes. Nonfatal MIs not associated with clinical symptoms (silent MIs) were not included.

An external cardiologist with expertise from the MONICA project reviewed the documentation of primary endpoints, and finally an endpoint committee evaluated the coding and classification and were consulted for assistance with diagnostically complicated cases.

The D:A:D database

The central database is created based on annual mergers of datasets from the 11 participating cohorts. At each merger this dataset is subsequently combined with a database containing information on validated endpoints.

The described baseline dataset (*I, II)* included information on 17,852 patients from 9 of the 11 cohorts; the two remaining cohorts supplied baseline characteristics later for a total of 23,468 patients enrolled in the D:A:D study.

The dataset contains broad information on CVD risk factors, the assessment of which have only recently become incorporated in daily clinical practice in HIV clinics. Therefore the proportion of missing data on some of these items is relatively high, with marked inter-cohort variation (*I,III*). It is possible that there may be a selection process for the screening for CVD risk factors in participating clinics, potentially related to the ART regimens used. However, although the proportion of missing data differed between therapy groups, there was no tendency for the proportion of missing data to be lower in the group of patients naïve to ART (reference group in *I*; *data not shown*).

The following numbers of measurements were available for analysis by the time of the third merger: 82,619 total cholesterol, 82,341 triglycerides, 39,795 high-density lipoprotein (HDL) cholesterol, 50,241 blood pressure measurements. The available HIV related data included 103,351 CD4 counts and 111,227 measurements of HIV RNA.

Follow-up

An a priori criterion for cohorts participating in D:A:D was the ability to maintain a loss to follow-up rate below 10% annually. This criterion has been followed carefully, and the overall annual loss-to follow-up rate has been kept at less than 5% (*III*).

The median time between patient visits to clinics (based on CD4 determinations) during prospective follow-up was 3 months.

Independent Advisory Panel

In order to be able to evaluate and announce if the study accumulated data necessitating a premature public disclosure of results, an Independent Advisory Panel was created, formed by specialists in cardiovascular and HIV epidemiology who were not otherwise involved in the D:A:D study. When the study had passed halfway of projected follow-up, this panel reviewed an interim report presenting endpoints and follow-up data, and concluded that premature disclosure was not indicated.

Quality assurance

The training of study and medical personnel for each cohort and at each site was performed before the initiation of the study. Site monitoring was conducted annually and included a review of source documents for all the reported endpoints, all cases of death and an audit of the case notes for a random sample of 10 percent of the remaining patients.

The quality of the data was also secured by extensive data checking and cleaning procedures put in place by the data-manager group, with revalidation and checking by the study coordinator and central statistician.

STATISTICAL METHODS

The analyses conducted on the baseline dataset (n=17.852; *I-II*) includes a cross-sectional descriptive analysis of the D:A:D cohort at baseline; an analysis of the prevalence of CVD risk factors; an analysis of the association of antiretroviral drug regimens with CVD risk factors; and an estimation of the predicted risk of MI.

The primary analyses conducted on follow-up data (n=23.468; *III*) describe the incidence of MI, and assesses the association with combination antiretroviral therapy (*III*).

The statistical analyses were conceived and conducted in collaboration with/ or by associated statisticians. Details of the analyses are provided in the papers (*I-III*). Briefly, the statistical analyses applied are standard methods for analyses of observational data:

Descriptive

Cross-sectional analyses described the prevalence of CVD risk factors at baseline (*I*). CVD risk factors were assessed as dichotomous categorical variables, where the cut off levels chosen were conservative estimates of 'high risk' based on levels used for risk scoring in the background population.[36-39] The prevalence of single risk factors was calculated for the proportions of patients, for whom data were available. Univariable chi-squared and Kruskal Wallis tests were used to compare categorical and continuous variables according to current ART use.

Logistic Regression

Logistic regression models were used to assess associations of CVD risk factors with ART (*I*). These models also included demographic, clinical and laboratory parameters that were significantly associated with the risk factor assessed at a level of $p<0.05$ in univariable models.

The dependent variable in logistic regression is binary (the above mentioned cut-offs were used for continuous variables). The independent variables were either categorical or continuous. Continuous variables were included untransformed or transformed (e.g. $\log_2$ transformed) according to their distribution and fit of the model. The independent categorical variables were fitted by creating dummy variables that took on different values for the different levels of the variable in question. The estimate in univariable and multivariable logistic regression models is the Odds Ratio (OR).

Cardiovascular Risk Prediction

Conventional cardiovascular risk equations were applied to baseline data to predict the three-year risk of MI (*II*). These risk equations were derived from the Framingham study[40], and are based on a parametric statistical model controlling for multiple CVD risk factors.

Three-year risk of MI was estimated for each individual. Current ART status at baseline grouped patients, and mean predicted three-year risks were calculated accordingly. Best estimates were obtained by applying the risk equations directly, with upper and lower limits corresponding to worst case and optimistic case scenarios (*II*). Predicted numbers of events were adjusted for the different baseline cardiovascular event rates in different countries based on WHO MI mortality rates.[41]

In order to provide a crude comparison of benefit versus harm associated with combination antiretroviral therapy use, the three-year risks of AIDS or death were also estimated based on a prognostic scoring system for patients receiving ART[42], and on estimated AIDS rates in untreated people with HIV for those patients not on ART or if they were to cease ART.[43]

Survival Analyses

In the analyses of incidence rates of MI (*III*), Poisson regression analyses were used. Poisson regression analyses have advantages over other survival analyses in that the proportional hazards criterion (i.e. that the risk hazard of two subjects is assumed to be constant over time) does *not* have to be fulfilled and repeated outcomes can be assessed. The Poisson model is important for estimating changes over time in incidence. The estimate from univariable and multivariable Poisson models is the relative rate (RR).

Different Poisson models were fitted. The underlying time scale was follow-up time in D:A:D – from baseline to the minimum of time of event, time of death, February 1st 2002 or last visit to an HIV clinic + 6 months, and the models assessed the relation between the incidence of MI and combination antiretroviral therapy exposure.

The primary model was adjusted for demographic factors (age, body-mass index, race, presence or absence of a family history of coronary heart disease, smoking status, and sex), mode of HIV-1 transmission, cohort, and presence or absence of prior cardiovascular disease before enrolment. Age was considered as a time-updated, continuous variable. All the other variables were treated as fixed categorical variables.

Missing Values

Missing values were treated in various ways. In baseline and primary analyses, specific categories were generated for missing data to allow for all individuals (*III*), or all individuals with complete data on the outcome (0/1; *I*), to be included in the analyses.

In order to derive risk estimates for all individuals in the modelling analyses (*II*), missing covariates were imputed. Imputation of missing binary covariates (smoker, diabetic) were imputed for an individual using the sex and country specific mean from the D:A:D data. Missing continuous covariates (systolic and diastolic blood pressure, serum triglycerides, total cholesterol and HDL cholesterol) were subsequently imputed using linear regression, where the independ-

ent variables included country, gender, age, ART, smoking- and diabetes-status.

Model Assumptions

Generally, the variables included in the final multivariable models were those that were independently ($p<0.05$) associated with the outcome in univariable models (*I*), those selected based on an a priori analyses plan (*I,III*), or those prerequisite to the model (*II*). Several important assumptions are implicit to the multivariable models and needs to be validated by assessing the fit of the model: The effects of the covariates are assumed to be additive and linear on the log scale, and interactions between covariates should be investigated.

Distribution plots, linear models, and the fitting of interaction terms tested model assumptions. These assessments entailed that some variables were included in separate models due to interaction, or that variables were transformed for a better fit.

Sensitivity Analyses

Sensitivity analyses were conducted to assess whether associations in the primary models could be reproduced if the assumptions were changed; i.e. an assessment of the robustness of the primary analyses. All analyses were checked by a number of sensitivity analyses, including such as (i) only including patients with complete data (*I,II*), (ii) only assessing lipid parameters in patients with fasting values (*I*), (iii) by various age assumptions (*II*), (iv) basing estimates on diastolic rather than systolic blood pressure (*II*), (v) changing the censoring date (*III*), (vi) including recurrent events (*III*), (vii) excluding patients with a prior cardiovascular event (*III*), (viii) excluding unclassifiable or possible endpoints, (ix) adjusting for calendar time (*III*).

All analyses were performed using Statistical Analysis System (SAS) version 6.12 or 8.2 (SAS Institute Inc, Cary, NC).

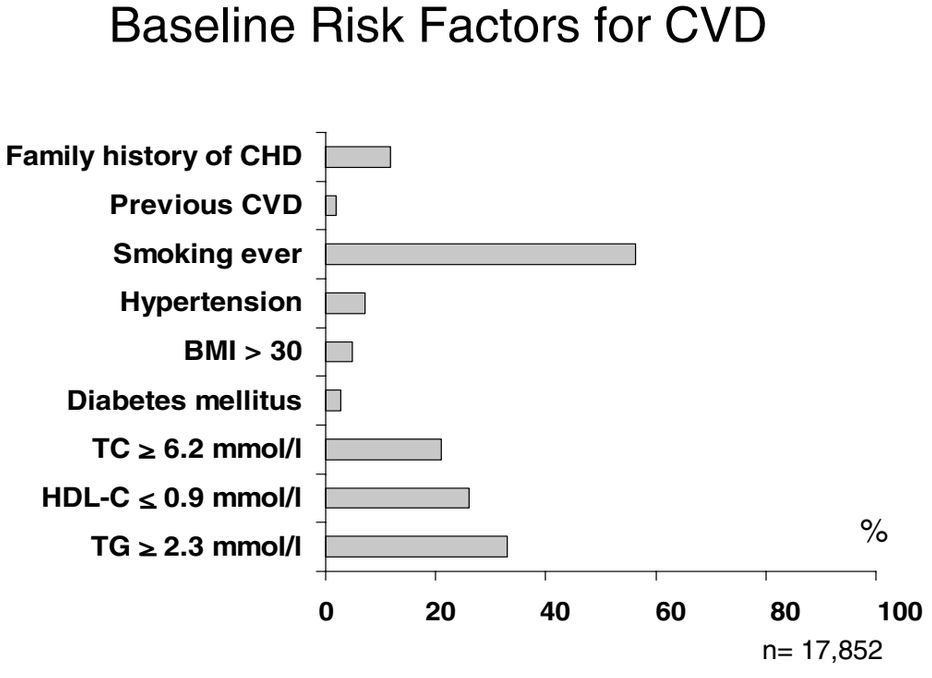
RESULTS

CVD RISK FACTORS AT BASELINE IN D:A:D

Cardiovascular risk factors were prevalent in the study population at time of enrolment in D:A:D (*I*; figure 1). Almost 25% of the study population were at an age where there is an appreciable risk of cardiovascular disease (male>45, female>55 years), with those receiving a PI and/or a NNRTI tending to be older. 11.4% had a family history of coronary heart disease with no significant difference between the antiretroviral therapy groups. 1.4% had a previous history of CVD, with the highest prevalence in the group of patients receiving a regimen containing both PI and NNRTI (1.9%). More than half of the study population were current cigarette smokers, with the highest prevalence among the patients either naïve to or not currently receiving antiretroviral therapy.

Hypertension was observed in 8% of the study popu-

Figure 1



lation, and was most prevalent among patients receiving all three drug-classes. However, after adjusting for other factors which univariably were associated with the presence of hypertension, this association disappeared.

The overall prevalence of diabetes was 2.5%. After adjustment for other factors, current therapy with a regimen containing NNRTI or NNRTI/PI was independently associated with the presence of diabetes (OR 1.89 (95% CI 1.15-3.10) and 1.97 (1.14-3.39)). The presence of lipodystrophy was associated with an increased risk of diabetes (OR 1.34 (1.17-1.54)).

Dyslipidemia was common with 22.2% having elevated total cholesterol (TC) above 6.2 mmol/L, 33.8% elevated triglycerides (TG) above 2.3 mmol/L and 25.7 % decreased HDL cholesterol below 0.9 mmol/L. The observed prevalence of elevated TC and elevated TG was highest in patients receiving all three drug-classes combined (44.1% and 54.3%). This difference remained after adjustment for other factors.

Among patients who currently or previously were exposed to antiretroviral therapy, level of immunofunction was independently associated with elevated total cholesterol. The association with CD4-count was present within each antiretroviral therapy regimen group, and the highest risk of elevated cholesterol was among patients with preserved or regained immunity, and equally the association with antiretroviral therapy was observed within each CD4 count stratum (figure 2a).

In all antiretroviral therapy groups, and also in the group of antiretroviral therapy naïve patients, higher HIV viral load was associated with a decreased risk of elevated total cholesterol (figure 2b).

Clinical signs of lipodystrophy, longer exposure times to NNRTI and PI's, and older age were all also associated with elevated total cholesterol level.

Figure 2 a

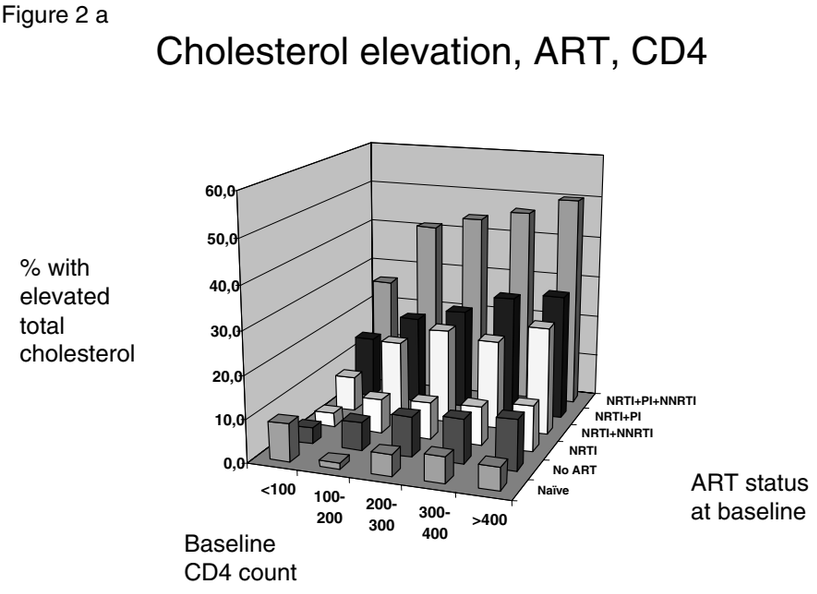
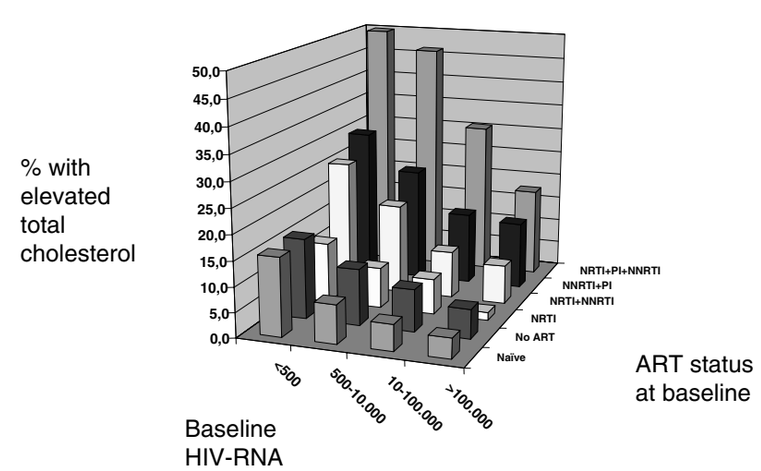


Figure 2 b



PREDICTED RISK OF MI

Based on the CVD risk profile at enrolment to D:A:D, the overall three-year risk of MI was estimated to be 0.72% (optimistic case to worst case scenario 0.35% to 1.12%). The highest risk was predicted for men, 0.92 % (0.47% to 1.42%) versus 0.07% (0.05% to 0.19%) in women.

The three-year risk of MI was estimated to increase from 0.30% (0.20% to 0.38%) in ART naive patients to 1.07% (0.43% to 1.77%) in patients receiving ART from all three drug classes. More than half of the MIs were projected to occur among the 16% of the individuals aged 50 or more.

To balance these results on adverse effects against the benefits of ART, the predicted three-year risk of AIDS or death was calculated. This was in the range 6.2 % to 11.1% in patients receiving ART if they continued treatment, and 22.5% to 29.4% if they were to cease ART.

In various sensitivity analyses, the estimated three-year MI risk was very similar to the overall best estimates (0.72%). Furthermore, the increasing estimated risk of MI with use of combination antiretroviral therapy was a consistent finding.

An extrapolation of these estimates over a ten-year period from baseline is shown in figure 3. The risk of MI gradually increases over time as a result of increasing age. Discrepancies in MI risk between untreated patients and patients receiving ART also continue to increase over the period. However, the cumulative 10-year risk of MI is estimated to be under 4.5%, even in the group of patients receiving all three classes of ART drugs, indicating that the risk of MI is unlikely to be a dominant cause of morbidity.

OBSERVED RISK OF MI

The total number of person-years (PY) of prospective follow-up available for the *primary analyses (III)* was 36,199 (median individual follow-up time 1.6 [interquartile range: 1.3 to 1.9] years). During this time, 126 patients developed a MI, providing an overall incidence of 3.5 (95% CI 2.9-4.1) per 1000 PY (4.2 (3.4-4.9) in men and 1.4 (0.7-2.4) in women). The incidence of MI increased gradually with longer exposure to combination antiretroviral therapy (figure 4). Among patients not exposed to therapy, the incidence of MI was lower than for any of the treated groups.

When fitting time exposed to combination antiretroviral therapy as a continuous variable, the RR per year of exposure was 1.26 (95% CI 1.12-1.41) after adjustment for demographic risk factors.

Other factors, which were also associated with MI, were: older age (RR per 5 years: 1.38 (1.26-1.50)), current or ex-smoking status (2.17 (1.30-3.62)), history of cardiovascular disease (5.84 (3.51-9.72)) and male gender (1.99 (1.04-3.79)), but not family history of coronary heart disease (1.18(0.64-2.17)). The primary model also included cohort, race, body mass index and HIV transmission group, but none of these variables were independently associated with the occurrence of MI in adjusted analyses. Intentionally, the model did not include variables such as plasma lipid concentrations, which could potentially be involved in the underlying mechanism.

Additional individual models assessed the influence of metabolic and HIV related factors. These models included all the variables in the primary model, and tested the new introduced factors separately. Total serum cholesterol level, serum triglycerides and diabetes mellitus were all also significantly associated with an increased rate of MI, whereas lipodystrophy was not, and hypertension only when fitted as time-updated. Preliminary assessments of the effects on the association of MI with exposure to antiretroviral combination therapy after adjustment for these metabolic factors suggested that the association diminished after adjustment for either total cholesterol or triglycerides, whereas the other factors did not affect the association.

DISCUSSION

The advantages of observational studies include the ability to assess multiple outcomes, to estimate incidence, and to identify the time sequence of events. Large observational cohorts allow for the study of rare outcomes.

There are some general methodologies, which are conceptual for he interpretation of observational data, and which we have applied for the D:A:D study: Importantly, the data collection should be obtained from a representative cohort. The hypothetical background for the particular analyses should be crystallised in the analyses plan, drafted prior to the exploration of the data, detailing which associations will be explored based on biological rationale and/or preliminary findings in other studies. The analytical approach is paramount, including multivariable models adjusting for relevant covariates known or expected to be associated with the outcome. Finally, the associations observed needs to be evaluated in the context of existing evidence from other sources.

This discussion focuses on placing our findings in the context of updated evidence, and draw a line from the description of risk factor profiles (*I*) to predicted and observed risk of MI (*II, III*). Finally, the strength and limitations of our study design will be discussed in more detail. The discussion includes a crude comparison of the observed impact of individual CVD risk factors on the risk of MI (*III*) with that expected based on modelling analyses (*II*) or from the literature. The latter is complicated by the fact that the absolute and relative impact of individual risk factors depends on the population in which it has been assessed, the definitions applied for the predictors and outcomes, respectively, and on the concomitant adjustment for other risk factors.

Figure 3. Best estimate of cumulative risk of MI by ART regimen to 10 years

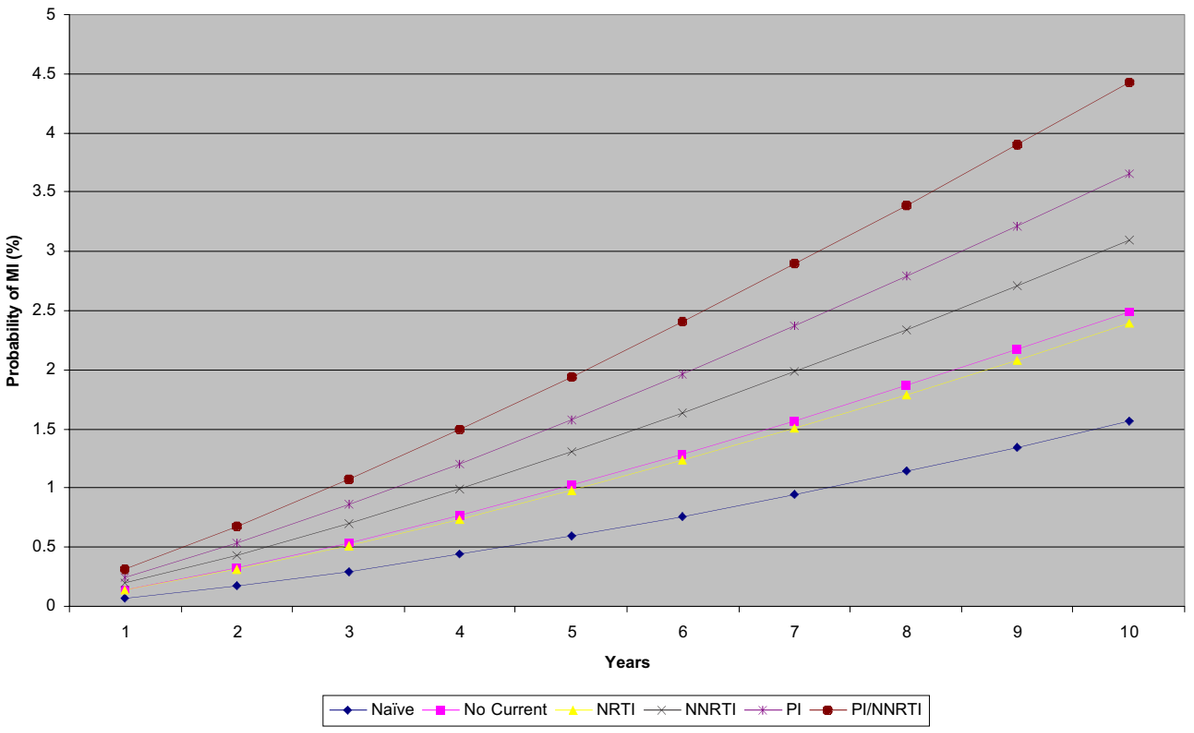
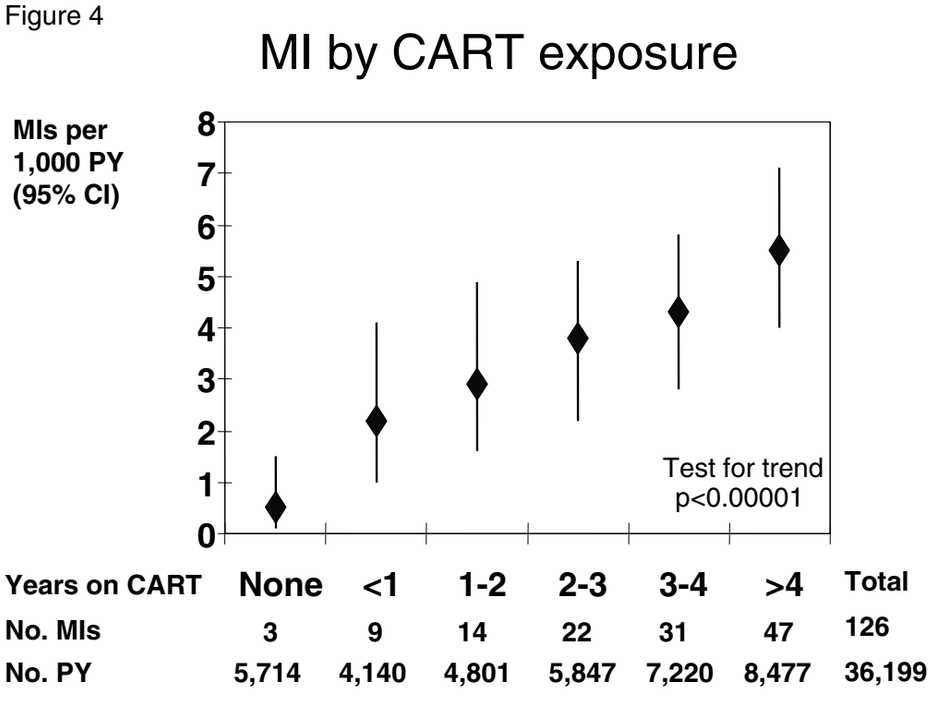


Figure 4



Years on CART	None	<1	1-2	2-3	3-4	>4	Total
No. MIs	3	9	14	22	31	47	126
No. PY	5,714	4,140	4,801	5,847	7,220	8,477	36,199

CVD RISK FACTORS

The analyses of CVD factors at baseline were cross sectional (*I*). Although we have reported relationships between specific treatment combinations and CVD risk factors, the cross-sectional nature of this study prevents us from establishing a causal relationship between the various regimens and the assessed risk factors. For example, we cannot exclude the possibility that dyslipidemia occurred prior to exposure to ART. However, as knowledge about a possible relationship between ART and CVD risk is relatively recent, it is unlikely that this has influenced the choice of ART regimen to any large extent or that this could explain the differences in risk profiles observed at baseline.

Dyslipidemia

Most PIs increase plasma levels of both total and low density lipoprotein (LDL) cholesterol, as well as levels of triglycerides but the extent of dyslipidemia varies greatly within the PI drug class[44-46]. Drugs from the NNRTI drug class has been found to increase total cholesterol and HDL cholesterol plasma levels[47;48], the latter effect potentially carrying a CVD risk reduction[49;50]. Certain drugs from the NRTI drug class can induce high triglyceride levels.

The baseline analyses (*I*) demonstrated that dyslipidemia was most prevalent among HIV-infected patients receiving combination antiretroviral therapy, in particular for regimens containing all three drug-classes. Subjects who had discontinued ART had similar TC levels as naïve, suggesting a reversible drug effect on total cholesterol level.

Dyslipidemia was most strongly correlated with antiretroviral regimens currently being used, and less with a history of previous exposure to the different drug classes. This finding corresponds with reports, in which the dyslipidemia associated with PI's occurred shortly after beginning therapy[4;51] and stabilized if the regimen remained unchanged. It is also consistent with studies which have shown that switch from PI to NRTI-only regimens is associated with attenuation of dyslipidemia within a short period of time[48;52;53] (however, at the risk of inferior viral suppression[53;54]).

Among patients who currently or previously were exposed to antiretroviral therapy, level of immunodeficiency and plasma HIV RNA were independently associated with elevated total cholesterol. The effect of antiretroviral therapy was observed within each CD4 count stratum, which indicates that the effect of antiretroviral substances cannot solely be explained by a reversal to 'normal' pre-disease cholesterol levels as a result of improved cellular immunity. This is in contrast to a recent study of seroconverters in the MACS cohort[55], which concluded that increases in serum cholesterol levels after HAART initiation probably represented a return to pre-infection levels. However, this study was small (n=50), and it did not analyse different HAART regimens separately.

The level of HDL-cholesterol, although to a lesser extent, likewise increased with more preserved cellular immunity and more suppressed HIV viral load, consistent with observations in the pre-HAART era.[56] There was little association with ART, except for higher levels in NNRTI containing regimens, consistent with findings in other studies.[47;48] In the MACS study,[55] HDL cholesterol dropped after seroconversion but remained low after ART initiation. It is possible that HDL may be dependent on other inflammatory parameters related to the chronic infection, which are not reverted by therapy. Further, the inverse relationship of HDL cholesterol with triglyceride levels (that increases with ART), due to lipid transport mechanisms, may be involved.

The effect of lipid levels on the risk of MI in the primary analyses (*III*) was very similar to that observed in studies if the background population.[57-59] The role of triglycerides in predicting cardiovascular disease has been the focus of much controversy; in D:A:D the predictive value was very similar to the effect of triglycerides in the MRFIT trial and in patients with the metabolic syndrome.[58;60]

Insulin Resistance

Insulin resistance and type 2 diabetes mellitus are also significantly associated with combination antiretroviral therapy.[61-65] In D:A:D, diabetes mellitus was observed in less than 3% of patients at baseline (*I,III*), whereas smaller studies using oral glucose tolerance test (OGTT) identified diabetes in approximately 8-10 % of patients taking PI-based combination antiretroviral therapy.[62;65] All studies have consistently reported that diabetes or abnormal glucose tolerance was significantly associated with current use of PI therapy.

In the setting of our observational study, the prevalence of diabetes is likely to be underestimated – in particular at baseline, when the recorded cases of diabetes were primarily those in whom anti-diabetic therapy was indicated. In the prospective part of the data-collection new cases of diabetes are reported as secondary endpoints (the diagnosis based on fasting plasma glucose measurements).[66] Increasing awareness of this treatment complication in recent years has lead to an increased screening for this entity, which in itself is likely to boost the incidence of diabetes over follow-up in D:A:D. Future analyses of diabetes as an outcome in our study will be challenged by this.

The direct effects of PIs on glucose metabolism appear to differ between the individual drugs. Indinavir has been shown to increase insulin resistance in non-HIV-infected adults after a single dose, whereas nelfinavir does not affect insulin sensitivity in vivo.[67;68] NRTIs have not been shown to affect glucose metabolism directly, but NRTIs are strongly associated with insulin resistance in patients with lipoatrophy.[69;70]

The presence of diabetes at baseline predicted the risk of MI in the primary analyses of D:A:D (*III*). The effect was comparable to that reported in studies of the background population.[57;71;72]

Lipodystrophy

The fat redistribution observed in association with use of anti-HIV agents is clinically seen as peripheral loss of subcutaneous fat (lipoatrophy), and central visceral fat accumulation. The prevalence, depending on definition, assessment, and patient cohort, has been reported to be between 20 and 80% (*I*,[61;73;74]). The diagnosis of lipodystrophy in clinical practice is generally subjective and relies on patients' reports and experience of the physician, and it is only recently that a case definition has been established.[75]

Lipoatrophy is associated with insulin resistance[69;70;76;77], and central fat accumulation may also exacerbate insulin resistance, as it is seen in the metabolic syndrome. In D:A:D and other studies, the presence of lipodystrophy was associated with several of the CVD risk factors discussed above, including dyslipidemia, hypertension and diabetes (*I*,[61;78]).

It was therefore surprising that lipodystrophy was not significantly associated with MI in D:A:D (*III*). Several factors may influence our ability to reliably assess the influence of lipodystrophy on risk of MI. First, the assessment of lipodystrophy was imprecise due to lack of standardisation. Secondly, as not all cohorts collected information distinguishing peripheral fat loss and central fat accumulation, it was not possible to perform an analysis separating these phenotypes, potentially representing different risk for CVD.[79] In addition, a univariable tendency towards a positive association shifted to negative after controlling for other factors (*III*), which implies that there was a confounding effect of some of these other factors.

Additional follow-up will allow us to reassess this in the future.

Hypertension

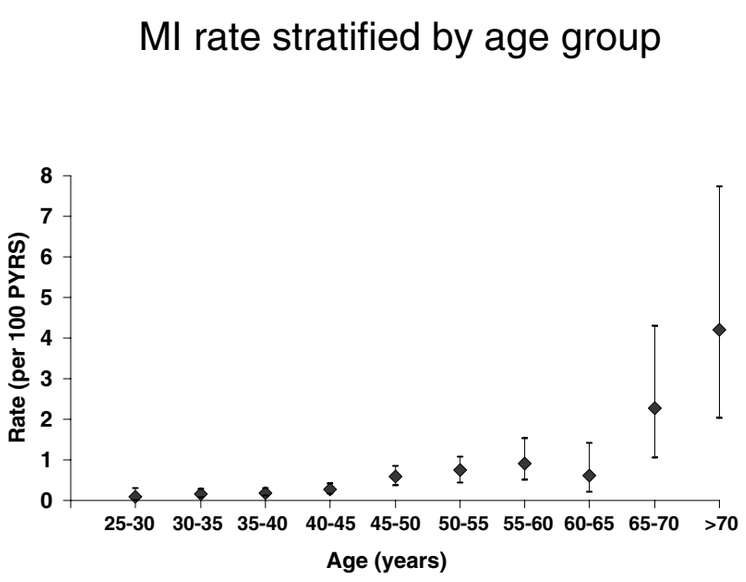
Data on the prevalence of hypertension in HIV patients are limited. A few studies have reported an increased prevalence of hypertension in PI treated patients[80;81] or in conjunction with lipodystrophy (*I*,[78]), whereas others did not observe an increased therapy related risk.[82;83] In our study, the associations between antiretroviral drug regimens and hypertension in univariable logistic models were no longer present after adjustment for other factors associated with hypertension. Thus, our data do not support a concern that HIV treatment per se is likely to induce hypertension (*I*). When assessed as an explanatory variable in the primary analyses, and fitted as time-updated, the presence of hypertension was associated with an increased risk of MI (*III*), at an estimate comparable to the risk conferred by hypertension in the background population.[57;60;84-86]

Age and gender

Age is one of the main risk factors for cardiovascular disease in the background population.[87;88] At similar ages the risk is considerably less in women, which is probably due to inherent gender differences as well as differences in exposure to traditional risk factors including smoking.

The median age for most HIV infected study populations, including that of the D:A:D study, is 40 years. Thus the a priori risk of CHD is currently relatively low in the majority of patients, but the proportion at risk will increase as the population ages. In D:A:D, an increasing incidence of MI was observed with increasing age, very similar to the age effect in the background population (*III*, figure 5). The adjusted estimate for age was a 38% relative increase in risk of MI per 5 years older; the median age among those who developed an MI was 48 years (range 25-82) – i.e. an age distribution not dissimilar to the predicted (*II*).

Figure 5



Currier et al. recently reported a particularly increased risk in young HIV patients compared to HIV negative controls[89], whereas older HIV patients in that study had a lower risk of CHD than matched negative controls; this finding warrants further investigation and confirmation from other studies.

Although in studies reporting on clinical CVD endpoints in HIV-infected patients, the incidence has been highest among men (*III*,[90]), it seems that women are at a particularly increased risk compared to age matched HIV-uninfected controls.[91] In the primary analyses of the D:A:D study, we observed a 3-fold higher incidence of MI in men compared to women (2-fold after adjustment for other factors) – i.e. a gender difference substantially less than the predicted 10-fold (*II*). This was driven by a larger than predicted risk in women (incidence 1.4 per 1000 PY; predicted 3-year risk of MI 0.07%). However, the number of MIs in women was small (n=12), rendering the incidence estimate imprecise.

Some studies have described more pronounced ART induced dyslipidemia in women.[91;92] (This may be related to higher PI through levels in women, which has been demonstrated also after controlling for body-mass index[93]). At baseline in D:A:D, female gender was independently associated with an increased risk of elevated total cholesterol, but a decreased risk of elevated triglycerides or of low HDL cholesterol (*I*; *data not shown*), however detailed analyses within the separate ART regimens were not conducted.

Nevertheless, if the discrepancy between predicted and observed gender difference is true (*II,III*), this would imply that there is a particularly increased risk in women which is unrelated to the factors included in the Framingham model (or that a difference in gender specific susceptibility to CVD risk factors in HIV patients cannot be predicted accurately by the Framingham model). Additional follow-up will allow us to explore this further.

Smoking

The prevalence of cigarette smoking was high in D:A:D, with more than half of the population being current smokers (*I*), being in line with consistent findings of higher rates of cigarette smoking in HIV infected populations than in uninfected age and gender matched background populations.[79;91;94;95] In the French APROCO-MC study the estimated attributable risk of coronary heart disease due to smoking was estimated to be 65% and 29% for HIV-1-infected men and women, respectively.[91] Smoking was also an important predictor of MI in the primary analyses of D:A:D (*III*) of a size comparable to what is expected for smoking to contribute to an increased risk of MI in HIV-uninfected.[59;60;88;96]

Co-infections

The possible role of infectious agents, both bacteria and viruses, in the pathogenesis of atherosclerosis and coronary heart disease has been intensely investigated, but largely with negative outcome.[97-101] Some studies suggest that there may be an association with the total pathogen burden and risk of CVD.[102-104]

It is possible that the total pathogen burden influences the overall risk of athrosclerosis in HIV infected patients; many patients have experienced an opportunistic infection prior to starting therapy (*I*,[105]), and hepatitis C and B co-infections are prevalent.[106-108] At the same time, hepatitis C infection is also associated with lower levels of total cholesterol irrespective of stage of liver disease[109], and the influence of hepatitis co-infection on CVD risk could theoretically be in any direction. To date there are limited data on clinical endpoints.[110] In D:A:D, prior AIDS defining illnesses were not associated with the risk of MI (*III*). The data-collection has recently expanded to include hepatitis serology, thus the impact of HCV and HBV co-infection on risk of MI can be assessed in the future.

HIV infection as a risk factor for cardiovascular disease

It has been hypothesized that factors associated with the HIV infection per se, HIV viral load and immunodeficiency, may also influence the progression rate of atherosclerosis. There is currently little available data to support or refute this assumption. Some studies have described a higher rate of CHD in HIV-infected patients than in matched HIV-uninfected controls [89;95]; however due to different risk factor profiles in the HIV infected population (*I*,[79;91]) which the abovementioned studies were unable to control for, it is not clear whether the observed increased risk in these studies was due to HIV, ART, co-infections, increased prevalence of smoking or other traditional risk factors. Preliminary data from the D:A:D study did not suggest any association between HIV-infection and atherosclerosis (*III*).

In the baseline analyses we observed that CD4 count and HIV viral load were independent predictors of elevated cholesterol, and it could be speculated that these markers might influence the risk of MI indirectly via this mechanism. However this was not the case (*III*). It may be that immunofunction and viral load exerts several opposite effects on the risk of MI via different mechanisms (e.g. that lower CD4 count and higher viral load per se, or via inflammatory or haemostatic markers would infer an increased risk which is counteracted by the effect on cholesterol – all hypothetically) – concealing or equalizing any impact on clinical manifestations. Also, we cannot rule out that the baseline analyses of this association was influenced by residual confounding, and thus that the described association of immunofunction and viral load, respectively, with the risk of elevated cholesterol might be associated – although we controlled for this – with cumulative prior ART exposure.

Other risk factors

Other factors known to influence the risk of cardiovascular disease in the background population includes country, ethnicity, genetic factors, physical activity, alcohol and substance abuse.

In D:A:D, information on country and ethnicity are available, and analyses controls for these (*II,III*). The data-collection also includes information on treatments for hypertension, diabetes and dyslipidemia, and some cohorts collect data on the use of steroids. Due to the risk of confounding by indication, and as the multivariable models are at this stage vulnerable for allowing to many explanatory variables, data pertaining to interventions for dyslipidemia were not included in the primary analyses (*III*; these data will in due course be assessed in separate analyses). The definitions of hypertension and diabetes, respectively, included the use of drugs to treat these disease entities (*III*).

Data related to other of the abovementioned factors are not collected as in most clinics they are not part of a standard medical record, and for some parameters – such as alcohol and substance abuse – cannot be reliably obtained.

Adjusting for HIV transmission mode may capture some of the effect of substance abuse associated with intravenous drug use, however other illicit drugs may be more prevalent among homosexual men – including such as cocaine, amphetamine and steroids (testosterone, growth hormone). (Of note, steroids are also used legitimately as experimental intervention to treat lipodystrophy or for the treatment of testosterone deficiency). Many of these drugs are known to influence the risk of MI, but the information provided to the treating physician/ hospital file is generally sparse. In case of admission for MI – or other ischemic disease – a history of these drugs will usually be obtained. Indeed information on use of some of these substances has also been provided in a few D:A:D MI event reports. But given that it is not possible to collect the data systematically for all, we cannot control for this exposure in the analyses. We have not excluded these endpoints from analyses, as that would introduce information bias.

It is assumed that these unmeasured factors are distributed in a random manner unrelated to ART use and thus are not expected to materially bias the results of the primary analyses (*III*; i.e. directed towards the null-value of effect).

A number of emerging CVD risk factors have been identified in the background population including C-reactive protein and homocysteine, the relevance and mechanisms of which are currently being investigated.[111]

Although it would be interesting to assess the predictive value of these factors in HIV-infection, this is beyond the scope of the D:A:D study.

PREDICTING MI RISK

Prediction models applied to the HIV infected population

The clinical significance of the changes in markers of cardiovascular risk that occur in patients on antiretroviral therapy is a central question, which is not simple to evaluate. Hypothetically, the medically induced metabolic changes might not infer comparable risk as similar levels occurring un-induced[112;113], or there might be a time lag between the development of those changes and the occurrence of new clinical events – similarly to the time lag occurring for the reverse situation at intervention to reduce risk factors.[114] In addition, patients have an inherent background risk of cardiovascular disease independent of the effects of antiretroviral therapy, related to their age, gender, ethnicity etc.

One way to try to decipher the expected increase in risk that could be attributable to therapy, in the anticipation of clinical endpoint studies, was to apply CVD prediction models developed for the background population (*II*).

Several factors may influence the applicability of the Framingham risk predictions in various populations, including differences in end-point definition, regional differences, secular trends in the incidence of coronary heart disease[114;115], and more recently changes in diagnostic tests and criteria for the diagnosis of MI.[35] The Framingham score has been validated for other populations[116;117], although more recent studies have found a tendency for over-prediction.[91;118-120]

The extent to which these risk equations apply to people with HIV in general, or the extent to which changes in cardiovascular risk factors that occur during ART immediately confer increased risks of the same magnitude predicted by these models is currently unknown. Furthermore, although we attempted to allow for different background rates of MI in different countries based on WHO MI mortality rates, there may be racial, geographical and temporal factors that we were not able to allow for. Thus it is with these reservations that the results of our modelling work should be viewed (*II*).

In general the published risk modelling studies suggest a somewhat higher risk of CVD among people being treated for HIV infection as compared to HIV infected not on therapy (*II*,[94;121]), and compared to HIV uninfected controls.[79;91;94]

Even though the risk models used may not be appropriate for an HIV population, the consistent finding of higher risk in treated people indicate that the drugs are exerting some effect – which has now also been observed in several studies on clinical endpoints (*discussed in the following section*). The interesting question is the quantification of the induced risk and evaluation of synergetic effects with other risk factors in HIV-infected patients.

Although not directly comparable, the observed incidence of MI in D:A:D (0.35 per 100 PY – i.e. corresponding to a 3-year risk of 1.1%) is within the range of the predicted risk (0.72% (optimistic case to worst

case scenario 0.35% to 1.12%), and closest to the upper limit estimate. This would imply that indeed the metabolic changes inferred by ART are clinically relevant, and it is possible that markers of the metabolic syndrome or insulin resistance may be relevant for CVD risk assessment in these patients (*II,III*).

Interestingly, the hypothesised and estimated time-lag (lower limit estimate in *II*) was not observed (figure 4;*III*). On the contrary the steepest increase in risk was observed shortly after initiation combination antiretroviral therapy. This early increase also exceeds the expected increase from applying the Framingham equation directly (*II*), and is thus particularly intriguing. An investigation of the putative underlying mechanisms is a primary focus in the future of the D:A:D study.

The predictors identified in the primary analyses (*III*) corresponds well to the predictors included in the Framingham score, although a direct comparison of predictions with observations is required before more firm conclusions can be drawn.

RISK OF CHD IN HIV

Evidence from studies of clinical endpoints

An overview over published studies of clinical cardiovascular disease endpoints in HIV infected patients is provided in table 2 (a & b).

Initial case reports of myocardial disease in young HIV-infected patients who had therapy-associated increases in lipids raised awareness of this issue.[14-18] The early retrospective studies provided conflicting evidence regarding the causal relationship between ART and cardiovascular disease.[95;122;123] Recent publications – including ours – more consistently points to an increased risk with ART (*III*,[90;124-126]), although not all are in agreement.[89;127;128] Of note, the studies differ in numerous ways with regards to objective, design, exposure variables and endpoint definitions, rendering direct comparison impossible (table 2). Unfortunately this also hampers the possibility to combine datasets for a joined analysis, or to conduct a meta-analysis.

A study at Kaiser Permanente suggested that the rate of hospitalization for coronary heart events in HIV-infected patients was greater than that seen in seronegative patients, but did not identify an association with specific ART drug classes.[95] However, a recent update suggested that the rate of admission increased with longer periods of exposure to PI, although the analysis was based on few events and not tested for significance[128] (table 2). A study from the Centers for Disease Control and Prevention (CDC) HIV Outpatient Study (HOPS) cohort concluded that use of PIs was associated with increased risk of cardiovascular events[124]; this study has the advantage of a prospective design, but is weakened by the lack of data on duration of ART exposure, long time between patient visits and few endpoints. In the French hospital database study an increased risk of MI was observed in patients exposed to PIs, and the effect was proportional to the duration of antiretroviral therapy (particularly if >18 months).[90]

It should be noted that although there was an increased risk of cardiovascular disease in these studies, the absolute number of cardiovascular events was small and those that occurred did so primarily in patients with other risk factors for coronary disease.

Data from the Veteran Affairs medical centres were analysed to evaluate the importance of cardiovascular disease as a cause of morbidity and mortality in patients with HIV infection.[127] This large retrospective study did not identify an increased risk of cardiovascular disease after exposure to combination antiretroviral therapy for up to 4-5 years. Of note, in that study the endpoint was the composite of admissions to and/or deaths from all types of cardiovascular disease. Identification of the endpoints was based on ICD codes, and no source verification was performed. Further, changes in admission policies in the Veterans Administration system have changed over time including decreasing admission of patients suspected for cardiovascular disease entities in more recent years, which may have influenced their results.

Generally, a weakness of the retrospective studies is the possibility of ascertainment bias, due to the retrospective design and as clinical awareness of the potential for antiretroviral therapy to induce MI occurred within the time period studied.

In the D:A:D study, although the absolute risk of MI was low – incidence 3.5 per 1000 person-years – exposure to combination antiretroviral therapy was associated with a 26 percent relative increase in the rate of MI per year of exposure during the first four to six years of use. This finding is plausible based on the adverse metabolic changes induced by combination antiretroviral therapy that are known risk factors for cardiovascular disease. It is also consistent with the results of modelling predictions, other clinical endpoint studies, and with the findings from studies of surrogate markers such as carotid intima media thickness.[129-131]

Table 2 (a) Cardiovascular Risk in HIV - Clinical Endpoint Studies

Ref#	Study/Principal Investigator	Study Design	Exposure assessed	Endpoints	HIV-uninfected controls	Analysis	CVD risk factors assessed/adjusted for?	Calendar period
89	California Medicaid/Currier	Retrospective analysis; Administrative database	ART / HIV+ versus HIV-	Composite CHD (ICD9 codes)	Yes	Incidence rate approach; log-linear regression	sparse§	1994-2000
///	D:A:D Study/ Lundgren	Prospective observational study	Combination Antiretroviral therapy	Acute MI (validated)	No	Incidence rate approach; Poisson regression	Yes/Yes	1999-2002
90	French hospital database on HIV (FHDH)/ Mary-Krause	Retrospective analysis of data obtained from FHDH	PI therapy	MI (ICD10 codes)	No (comparison with age matched French general male population)	Incidence rate approach; Calculation of standard moribity rate (SMR)	only age	1996-1999
126	HIV Insite Database/ Iloeje	Retrospective analysis; prospectively collected database	PI therapy	Composite CVD event (MI, angina, CAD, PCA/CABG, stroke, TIA, PVD)	No	Incidence rate approach; Cox models	Yes/Yes	1996-2002
124	HOPS/ Holmberg	Prospective observational cohort	PI therapy	Composite CVD (MI, angina, CVA) - review of patient files	No	Incidence rate approach; Cox models	Yes/Yes	1993-2002
128	Kaiser Permanente/ Klein	Retrospective analysis; Administrative database	PI and other ART / HIV+ versus HIV-	Composite CHD (ICD 9 codes)	Yes	Incidence rate approach; Cox models	Yes/only age	1996 - 2002
125	Maryland Clinical Cohort/ Moore	Retrospective analysis	ART	Composite CHD (MI,unstable angina) and CeVD (ischemic stroke, TIA)	No	Nested case control study; Conditional regression analysis	Yes/No	1996 -
20	Meta-analysis of Randomized Clinical Trials/ Coplan	Retrospective analysis; 30 Phase II/III industry sponsored double-blind, randomized studies	PI and NRTI	MI (from investigator reports)	No	MI rate per 1000 PY; Relative Risk (RR) for MI in patients taking PI vs NRTI only	Yes/No (randomised)	1996-1999
127	Veteran's Administration/ Bozzette	Retrospective analysis; Administrative database	ART	Composite CVD (admissions for and/or death from CVD or CeVD; ICD9-10 codes)	No	Incidence rates; Kaplan-Meier curves; time to event modelling; patient level regression models	sparse§	1993-2001

§: Only from ICD9-10 codes
CeVD: Cerebrovascular Disease
CVD: Cardiovascular Disease
CHD: Coronary Heart Disease

Table 2 (b) Cardiovascular Risk in HIV - Clinical Endpoint Studies

Ref#	Study/Principal Investigator	Gender (% female)	Age (mean) or median [IQR]	ART duration (median mths ART/PI)	# Patients (#PY)	# Endpoints (rate per 1000 PY)	Key Results [95% CI]
89	California Medicaid/Currier	27.3	70% <45 in HIV+; 55% <45 in HIV-	NA	28,513 (71,286)	1360 CHD (19)	Increased risk of CHD in young HIV+ compared to matched HIV-; Adjusted RR of CHD 2.06 (p<0.0001) for ART use in young HIV+.
///	D:A:D Study/ Lundgren	24.1	39 [34-45]	34/19	23,468 (36,199)	126 MI (3.5)	Adjusted RR of MI 1.26 [1.12-1.41] per year of CART exposure
90	French hospital database on HIV (FHDH)/ Mary-Krause	0	37.7 (±9.1) for non MI; 41.9 (±8.2) for MI	34/25	34,976 (88,029)	60 MI (0.7)	HR for MI 2.56 [1.03, 6.34] for PI versus no-PI
126	HIV Insite Database/ Iloeje	13.3	38 [18-88]	NA	6,711 (NA)	NA CVD (16) in PI / NA (5) in non-PI	Adjusted HR for MI: 2.0 [1.0-4.1] for PI versus no-PI
124	HOPS/ Holmberg	18	(42.6)	NA	5672 (17,712)	21 MI (1.42) in PI/ 2 (0.46) in non-PI	Adjusted HR for MI: 6.51 [0.89-47.80] for PI versus no-PI
128	Kaiser Permanente/ Klein	0	not provided	NA/47*	4,408 (18,792) HIV+ ; 39,425 (211,221) HIV- patients	62 MI in HIV+ (3.8) / 605 in HIV- controls (2.6)	Higher rate of MI in HIV+ than HIV- controls; no clear association with PI
125	Maryland Clinical Cohort/ Moore	42 and 32	46 and 41 yrs	NA	2671 (7.330)	43 CHD (5.9) 37 CeVD (5.0)	CHD/CVD risk associated with PI and d4T use
20	Meta-analysis of Randomized Clinical Trials/ Coplan	8 to 18	(37)	NA/12	10986 (11651)	19 MI (1.6)	RR of MI 1.69 [0.54-7.48] for PI versus NRTI-only
127	Veteran's Administration/ Bozzette	1.9	71% 35-55; 17% <35	17/16*	36766 (121,936)	1764 admissions for CVD (17 in 1995, 9 in 2001)	No increase in admission or death from CVD with increasing ART exposure

*: among exposed
NA: Not applicable
CeVD: Cerebrovascular Disease
CVD: Cardiovascular Disease
CHD: Coronary Heart Disease

STRENGTHS AND LIMITATIONS OF D:A:D

Design

A major advantage of prospective cohorts, as opposed to retrospective studies of automated administrative databases or registries, is that specific treatment and covariate information can be more reliably ascertained.

The D:A:D study has the advantage of a prospective design and of being a closed cohort, without the potential bias of continual patient enrolment. The study has been designed with adequate size and power to address the objective, and it has implemented extensive quality assurance measures including the prospective validation of all endpoints. Compared to most of the other studies, D:A:D has a greater duration of antiretroviral therapy, more patients receiving combination antiretroviral therapy, and far more complete data on cardiovascular risk factors.

The limitations are mainly related to the observational design of the study, as there are inherent biases and confounding associated with this design. Some of these have been addressed in the above (*information bias, residual confounding, confounding by indication)*. Other includes *selection bias,* which means that there are features that differs between exposed an unexposed, which may influence the outcome. The consecutive enrolment of patients from the participating centres attempted to ensure the inclusion of a representative subset of patients followed at the specific centres. However selection bias may be present as exposure or non-exposure to combination antiretroviral therapy relies on the stage of the underlying HIV-disease; although we think we have been able to take this into account in the multivariable models, there may be differences that we have not been able to adjust for. Further, *unmeasured* or *residual confounding* cannot be excluded – i.e. there are factors that we have not collected and therefore have not been able to adjust for (unmeasured confounding), and categorisation of variables in to categories that are to broad may have introduced residual confounding.

Another important possible source of bias is *survival bias,* which could be introduced if there was a difference in risk of MI among those who remained under follow-up as compared to those who were lost to follow-up. This could also be introduced if – since the analyses includes exposure also from prior to entry in D:A:D – there was a preferential loss-to follow-up prior to enrolment in D:A:D of patients with longer exposure to combination antiretroviral therapy and a concomitant lower risk of MI. However, although we are unable to assess this directly, it seems very unlikely. With regards to the prospective data-collection, the risk of survival bias is limited by the low rate of loss-to follow-up. This has also been enforced by various approaches in the individual cohorts, by contacting patients who do not appear for consecutive scheduled visits, or contacting other sources for a control of the vital status.

In addition, many measurements are not always conducted in a uniform manner, and there is a relatively high proportion of missing data. However, a number of sensitivity analyses were conducted, including such that assessed the influence of fasting status and completeness of the data, which generally confirmed the findings of primary analyses (*I-III*).

With regards to the study design, there are several inherent limitations to the cross-sectional design of the baseline analyses (*I*); in particular this design is poor for the identification of causal relationships, but can merely identify associations. A better understanding of the direction of causality can be obtained from longitudinal studies, where the exposure and outcome variables are assessed prospectively at several time points. However only randomized clinical trials can definitively determine causality.[132]

The prospective part of the D:A:D study allowed for reliable and objective assessment of exposures and outcomes, and multivariable analyses allowed for adjustment for possible confounders. In order to more directly assess possible causal mechanisms, we conducted a number of exploratory analyses. These analyses identified variables that might mediate the effect of combination antiretroviral therapy, and indirectly confirmed our assumptions in that these variables predicted the risk of MI and at the same time diminished the effect exerted by combination antiretroviral therapy. Taken in aggregate with biological plausibility and confirmation from other studies, this adds to the perception of a causal association.

The lack of a control group is perhaps the main limitation. Clearly, because of ethical concerns it is not possible to withhold ART in HIV infected patients meeting the criteria for initiating therapy. Thus, a comparison of the observed incidence of cardiovascular disease in an exposed population and an unexposed population at a similar stage of HIV infection cannot be performed. An alternative approach would be to consider a historical control group from the pre-HAART era; however, as discussed in the above, this would introduce the potential for bias in the ascertainment of MIs.

A comparison to a comparable cohort of HIV-negative persons would be of interest from a public health perspective, but is beyond the scope of the D:A:D study and would be quite difficult to do. As the D:A:D study population is diverse, originating from many countries around the world, with a different socio-demographic composition than most cohorts of non-HIV infected persons (e.g. large proportions of intravenous drug users and homosexual men) and with different patterns of traditional risk factors for cardiovascular disease, it would be virtually impossible to find a cohort of HIV-uninfected subjects with comparable CVD risk factor profiles. An indirect way of conducting such a comparison is to assess the fit of prediction models developed for the background population, i.e. a direct comparison of predicted estimates with the observed incidence. Such analyses are currently in progress.

Endpoints

A problematic issue for the study of outcomes is the lack of consensus definitions. Within the HIV research field an example to this effect is the newly recognised lipodystrophy syndrome[61;74;133], for which a case definition was only recently established in a large-scale international study.[75]

In the study of cardiovascular disease, choosing a well-defined single endpoint – in this case MI – has several benefits over using composite and less precise endpoints. A well-defined endpoint can be reliably assessed by means of established diagnostic criteria; it can be externally validated, and further aids comparison with findings in other studies. In addition, a narrow endpoint definition is particularly useful for the evaluation of specific putative mechanism of disease.

Composite endpoints primarily have the advantage of increasing the power, providing the opportunity to use smaller study populations or shorter follow-up to assess the outcome. However the interpretation of composite endpoints may be limited by biases related to differential associations of exposures and individual outcomes.[134;135] Endpoints based on admission codes or death certificate codes are, unless ascertained, generally less reliable.[136;137]

CONCLUSION AND PERSPECTIVES

In summary, we observed a high prevalence of multiple risk factors for cardiovascular disease in HIV-infected patients, particularly among patients currently receiving an antiretroviral therapy regimen containing all three drug-classes. Of concern, patients receiving combination antiretroviral therapy were also slightly older, more likely to be male, and more likely to be diagnosed with lipodystrophy, thus describing clustering of risk factors.

In models predicting the risk of MI, this translated to a predicted overall 3-year risk at population level in the range of 1%, with the highest risk in patients receiving regimens containing both PI and NNRTI. Subsequent analyses of observed clinical endpoints found the incidence of MI to be 3.5 per 1000 person-years of follow-up, and described a relative 26% increase in risk per year of exposure to combination antiretroviral therapy. Exploratory analyses confirmed assumptions that the metabolic changes induced by antiretroviral therapy are likely to mediate – at least part of – this increased risk. The observed increase in risk occurred soon after initiation of combination antiretroviral therapy, suggesting that the medically induced metabolic changes – likely in conjunction with yet unidentified drug factors – translate into risk of clinical disease without any substantial time-lag.

It is important to balance the possible increased risk of CVD against the proven benefits of combination antiretroviral therapy. The overall absolute risk of MI is modest and vastly outweighed by the known benefits of combination antiretroviral therapy in terms of the reductions in HIV-associated mortality and morbidity. However, atherosclerosis may take decades to progress to a clinically detectable degree, and thus further follow-up of our cohort is necessary to determine whether a substantial absolute increase in therapy-related CVD will emerge.

The key research question to be solved in the future is which mechanisms that are driving the associated risk of MI with combination antiretroviral therapy. As the association appears to be biphasic, with an initial rapid increase over the first year of exposure and a second more gradual phase (*III*, figure 4), there may well be different mechanisms at play that temporarily mediates the increased risk.

Potential explanations include changes in metabolic, immunological and virological factors induced by the drugs, although a drug effect per see (irrespective of its effects) should also be considered. A better understanding of the relative contribution of indirect effects of drugs on e.g. metabolic parameters from direct drug effects on the risk of CVD has important public health and individual patient management implications.

The fact that many studies have reported relationships with PI-based ART regimens may be related to the longer follow-up of patients exposed to PIs than those exposed to other types of combination antiretroviral therapy. Moreoever, dyslipidaemia and diabetes have both been observed in those using drugs from the nucleoside and non-nucleoside reverse transcriptase inhibitor drug classes,[70;138] and thus these drugs may also contribute to the risk of MI.

In D:A:D, we are unable, at the present time, to examine the risk associated with individual drug classes or drugs. Since the NNRTIs first became available after the introduction of the PIs, only a small number of patients in D:A:D have been treated only with the combination of NRTI and NNRTI (ie. without prior PI exposure). However, this treatment combination is currently standard-of-care. Among the 19% of the cohort who were treatment naïve at entry, we might expect a certain proportion to start treatment during follow-up. In addition, the study is currently enrolling a second cohort of patients. An evaluation of whether exposure to the current standard-of-care treatment is associated with a similar level of risk of CVD as those treatment combinations started in the early HAART era is warranted, and will require considerably more follow-up.

Based on our findings of a substantial role for conventional risk factors in the observed increase in MI in patients on combination antiretroviral therapy, clinicians should carefully monitor the risk of cardiovascular disease in their patients receiving combination antiretroviral therapy. A recent study from the US demonstrated that the rate of use of lipid-lowering agents increased more than sixfold from 1996 to mid-2000 in patients receiving PIs.[139] However, the benefits of such drugs on cardiovascular disease in those with HIV infection specifically and on drug-induced dyslipidaemia in general have not been quantified. Furthermore, a possible unfavourable effect of an increased tablet burden on adherence and subsequent

virological control of HIV cannot be ruled out. Such data are however pivotal to establish the benefit:risk ratio, which is the evidence-based foundation for the identification of patients for whom such supplementary therapy is indicated.

As the D:A:D and other studies continue to accumulate follow-up time, our ability to describe the exact nature of the relationship between combination antiretroviral therapy and cardiovascular disease, and to describe the extent to which this relationship can be explained by metabolic changes, will increase. Extended follow-up of these cohorts will also allow us to determine whether there are any differences between drug classes. The current evidence, however, support recommendations for patients on combination antiretroviral therapy to modify lifestyle risk factors associated with cardiovascular disease.

SUMMARY

This ph.d. thesis includes 3 published articles and a summary, and is based on work conducted in the period 2000-2003 during my employment as study coordinator for the D:A:D study at the Copenhagen HIV Programme, Hvidovre Hospital.

The purpose of the thesis was, based on data from the D:A:D study (the Data-collection on Adverse events of anti-HIV Drugs), a multinational cohort study of 23,468 HIV infected patients, to describe the prevalence of risk factors for cardiovascular disease in HIV infected patients, the possible association of antiretroviral therapy with such risk factors, and to examine a possible association between antiretroviral combination therapy and the risk of coronary heart disease.

The use of combination antiretroviral therapy for the treatment of HIV-infection has become abundant in the industrialized countries since it was introduced in the mid-1990ies. Several of the drugs can induce metabolic adverse effects, including a raise in cholesterol and triglycerides and the development of diabetes, which confers potential risk for cardiovascular disease.

There is a high prevalence of cardiovascular disease risk factors in HIV-infected, both of such that can be associated to the antiretroviral therapy, and such that are unrelated (e.g. cigarette smoking). There is a clustering of risk factors among patients receiving regimens containing drugs from all three antiretroviral drug classes.

The HIV infected population is relatively young, 40 years on average, and the absolute risk of cardiovascular disease therefore relatively low (we observed an incidence of 3.5 per 1000 person-years of follow-up). We found that combination antiretroviral therapy was associated with a relative 26% increase in risk of myocardial infarction per year of exposure, and preliminary analyses suggest that this is largely mediated via metabolic changes. The observed risk was very similar to that predicted from the Framingham risk score, suggesting that such models can be applied in the setting of HIV infection and antiretroviral therapy, where the metabolic changes are partly or entirely medically induced.

These analyses are based on data from observational studies, why causality cannot be determined definitively. Nevertheless, the prospective study design, the extensive quality assurance measures, the carefully analysed data including multiple sensitivity analyses, the biological plausibility and confirmation from other studies comforts us on the reliability of the reported associations.

Additional follow-up is warranted before guidelines on possible medical intervention can be made, but based on the current evidence in this area clinicians are encouraged to carefully monitor the risk of cardiovascular disease in their patients receiving combination antiretroviral therapy, and to modify lifestyle risk factors where indicated.

DANISH SUMMARY

Denne ph.d. afhandling omfatter 3 publicerede artikler og en sammenfatning, og er baseret på arbejde udført i perioden 2000-2003, under min ansættelse som projekt- koordinator af D:A:D studiet ved Copenhagen HIV Programme, Hvidovre Hospital.

Formålet med afhandlingen var på baggrund af data fra D:A:D studiet (data indsamling af bivirkninger til antiretroviral behandling), en multinational kohorteundersøgelse af 23.468 HIV inficerede patienter, at beskrive forekomsten af risikofaktorer for hjertekarsygdom hos HIV inficerede patienter, sammenhængen mellem antiretroviral behandling og sådanne risikofaktorer, og at undersøge en mulig sammenhæng mellem den antiretrovirale kombinations behandling og risiko for hjertekarsygdom.

Brug af antiretroviral kombinations terapi til behandling af HIV-infektion er blevet udbredt i den vestlige verden siden den blev introduceret i midten af 1990'erne. Flere af præparaterne har en række metaboliske bivirkninger, herunder stigning af kolesterol og triglycerider og udvikling af insulin resistens, der udgør potentielle risiko faktorer for hjertekarsygdom.

Der er en høj prævalens af risikofaktorer for hjertekarsygdom blandt HIV-smittede, herunder både sådanne der kan relateres til den antiretrovirale behandling og risikofaktorer der er uafhængige heraf (som fx rygning). Der er en ophobning af risikofaktorer blandt patienter der er i behandling med kombinations terapi, specielt for sådanne kombinationer der indeholder præparater fra alle 3 stofklasser.

Populationen af HIV-smittede er forholdsvist ung, 40 år i gennemsnit, og den absolutte risiko for hjertekar sygdom derfor relativt lav (vi observerede en incidens af myokardie infarkt på 3.5 per 1000 personårs opfølgning). Vi fandt at kombinations behandlingen var forbundet med en relativ øgning i risikoen for myokardie infarkt på 26% per års behandling, og foreløbige analyser tyder på at dette i væsentlig grad er medieret via de metaboliske forandringer. Den observerede risiko lå tæt på den forventede risiko vi havde estimeret ved brug af Framingham prædiktions modellen, hvilket kunne tyde på at sådanne modeller kan anvendes også ved HIV infektion, hvor de metaboliske forandringer helt eller delvist er medikamentelt inducerede.

Yderligere opfølgning er nødvendig før retningslinier for evt. medikamentel intervention kan udstikkes, men den aktuelle evidens på området tilskynder til at HIV smittede patienter i kombinationsbehandling generelt udredes for risikofaktorer for hjertekarsygdom, og at livsstils faktorer justeres lege artis.

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