



Assessing the risk and benefit of combination antiretroviral therapy for HIV-1 infection with a special focus on cause-specific mortality

PhD thesis

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This thesis is based on the four original manuscripts referred to by Roman numerals.

Paper I

Implementing the number needed to harm in clinical practice: Risk of myocardial infarction in HIV-1 infected patients treated with abacavir.

HIV Med 2010;11(3):200-208

Justyna D. Kowalska, Ole Kirk, Amanda Mocroft, Leif Høj, Nina Friis-Møller, Peter Reiss, Ian Weller and Jens D. Lundgren.

Paper II

The Coding Causes of Death in HIV (CoDe) Project - Initial results and evaluation of methodology.

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Justyna D. Kowalska, Nina Friis-Møller, Ole Kirk, Wendy Bannister, Amanda Mocroft, Caroline Sabin, Peter Reiss, John Gill, Charlotte Lewden, Andrew Phillips, Antonella d'Arminio Monforte, Matthew Law, Jonathan Sterne, Stephane De Wit and Jens D. Lundgren for the CoDe Working Group and the D:A:D Study Group.

Paper III

A standardized algorithm for determining the underlying cause of death in HIV infection as AIDS or non-AIDS related: Results from the EuroSIDA Study.

HIV Clinical Trials 2011;12(2):109-117

Justyna D. Kowalska, Amanda Mocroft, Bruno Ledergerber, Eric Florence, Matti Ristola, Josip Begovac, Helen Sambatakou, Court Pedersen, Jens D. Lundgren and Ole Kirk for the EuroSIDA Study Group.

Paper IV

Long-term exposure to combination antiretroviral therapy and risk of death from specific causes: no evidence for any previously unidentified increased risk due to antiretroviral therapy.

Manuscript

Justyna D. Kowalska, Joanne Reekie, Amanda Mocroft, Peter Reiss, Bruno Ledergerber, Jose Gatell, Antonella d'Arminio Monforte, Andrew Phillips, Jens D. Lundgren and Ole Kirk for the EuroSIDA Study Group.

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Preface

This work was conducted from March 2008 to May 2011 during my employment as a research fellow at Copenhagen HIV Programme (CHIP), the Faculty of Health Sciences, the University of Copenhagen.

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Finally, I would like to dedicate this thesis to my husband *Marcin* and son *Kuba*, two amazing guys who have endlessly supported me.

Justyna Dominika Kowalska
Copenhagen, May 2011

Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
cART	Combination Antiretroviral Therapy
CDC	Centers for Disease Control and Prevention
CD4	CD4+ lymphocyte T (T helper cell)
CHIP	Copenhagen HIV Programme
CI	Confidence Interval
CKD	Chronic kidney disease
ESLD	End-stage liver disease
ESRD	End-stage renal disease
CoDe	Coding Causes of Death in HIV
CRF	Case report form
CVD	Cardiovascular disease
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs study
ESPRIT	Evaluation of Subcutaneous Proleukin Randomized International Trial
EuroSIDA	European study of ‘Syndrome d’Immuno-Deficience Acquis’
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein cholesterol
HIV-1	Human Immunodeficiency Virus type 1
HIV RNA	HIV ribonucleic acid
IQR	Inter-quartile range
IR	Incidence rate
IRR	Incidence rate ratio
INSIGHT	International Network for Strategic Initiatives in Global HIV Trials
MI	Myocardial infarction
NADM	Non-AIDS defining malignancies
NCEP ATP	National Cholesterol Education Program Adult Treatment Panel
NNH	Number needed to harm
NNT	Number needed to treat
OR	Odds ratio
PYFU	Person years of follow-up
RCT	Randomized controlled trial
RR	Relative Risk
SMART	Strategies for Management of Anti-Retroviral Therapy study
START	Strategic Timing of Antiretroviral Therapy study

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Introduction

Combination antiretroviral therapy (cART) has dramatically improved prognosis of HIV-positive persons. The effect of cART is achieved by suppressing the HIV replication, thereby allowing the person's immune system to regenerate, which is reflected by an increasing CD4 lymphocyte count. This response leads to a markedly decreased risk of developing an AIDS event and of all cause mortality, which represents general cART benefit. Among the various causes of death, the decline in non-AIDS-related death has been less pronounced than the decline in AIDS-related death. Consequently, the proportion of non-AIDS-related deaths has substantially increased. Besides the traditional risk factors, residual effects of HIV-infection and adverse drug reactions from cART may contribute to the risk of such deaths. However, the incidence of cause-specific mortality in patients on cART, and especially its change with exposure to treatment, has not been studied sufficiently. Although the contribution of adverse cART reactions to all cause mortality is not likely to affect the overall net treatment benefit at a population level, it may translate into net harm for certain subgroups of persons. Rational use of any treatment needs to be weighed against its potential benefits and risks, yet scientific methods for such assessment are limited ¹.

Adding a harm perspective to cART benefit

Until now the harm and benefit of antiretroviral treatment, although encouraged ²⁻⁴, has not yet been linked in a meaningful way, especially in the context of the underlying pre-treatment risks. As discussed by Sharp et al. investigation of dependence of treatment effect on measured baseline characteristics of a patient is essential in defining who would benefit most and who least from medical intervention ⁵. The risk: benefit ratio for specific cART components and in relation to different endpoints is still unknown, despite that such information would

allow informed treatment choices in terms of undertaken risks and expected net benefit ^{3,6}.

Presenting results as relative risk (RR) is standard in observational studies ⁷, but may be difficult to translate into clinical practice. The number needed to harm (NNH), together with absolute risk increase (ARI), may reflect any adverse effect attributed to treatment better than RR in clinical terms ⁸⁻¹⁰. Both NNH and RR are measures that attempt to summarize two numbers (the risk of event with and without treatment). RR summarizes the relative increase in the risk of an event according to whether the patient receives a given treatment or not and the NNH indicates the number of patients that need to be treated to observe the adverse effect of a treatment in one additional patient. Although this approach was first proposed in 1988 ¹¹ it is still infrequently applied to describe risk of adverse events of treatment ^{12,13}, including in HIV studies. Currently NNH is calculated mostly for the results of RCTs and presented in summary papers as a single number describing the difference in adverse treatment effect between treatment and control groups ¹⁴⁻¹⁶. However, if used in this way it does not capture the effect of underlying risk variation in a trial population ¹⁷. Although that approach has been strongly suggested by CONSORT ¹⁸ we rarely see NNH recalculated for different underlying risks ^{19,20}.

Impact of cART on cause-specific mortality

Since 1996 when cART was introduced, prognosis for HIV-1 positive patients has dramatically improved. In the EuroSIDA study the mortality rate in 1998 has dropped to less than a fifth of that from 1995, which is mostly attributed to the wide cART utilization ²¹⁻²³. The incidence of all deaths has continued to decrease between the early (1996-1998) and late cART (1999-2004) eras across all CD4 count strata, yet the drop in death rate was no longer that spectacular ^{22,24}. Although survival continued to improve with newer forms of cART

^{25,26}, mortality of HIV-positive persons still remained higher than that of the general population ²⁷⁻³⁰ and similar to that of HIV-negative persons with serious chronic diseases e.g. insulin-treated diabetes ²³.

Simultaneously a substantial change in causes of death was observed: though AIDS-related death remained the most frequent, the proportion of non-AIDS-related causes continued to increase ³¹⁻³³. In the EuroSIDA study the proportion of non-HIV-related death was 18.8% in 1996 and 51.6% \geq 2002 ³¹. In comparison, in the HIV Outpatient Study (HOPS) the crude rate of non-AIDS deaths decreased from 0.91 per 100 PYFU in 1996 to 0.55 per 100 PYFU in 2004, but the proportion increased from 13.1% to 42.5%, respectively ³⁴. According to recent EuroSIDA findings, one third of patients were estimated to have died within first year from a non-AIDS event and developing a non-AIDS event during follow-up was associated with an approximately 7-fold higher risk of death ³⁵ (consistent with results from the SMART study ³⁶) indicating significant contribution of non-AIDS events to deaths.

As HIV-positive persons on cART live longer it is expected that they will experience an increasing contribution of non-AIDS-related causes to overall mortality ^{33,37}. However the spectrum of causes of death does not reflect that of the general population ³⁸⁻⁴¹. The Antiretroviral Therapy Cohort Collaboration (ART-CC) reported the majority of 1876 deaths observed between 1996 and 2006 by 13 HIV cohorts were due to non-AIDS defining malignancies (NADM), cardiovascular disease (CVD), liver disease or non-AIDS infections ⁴². Similar findings were presented from the CASCADE study that is a study of HIV-positive patients with known date of seroconversion ³³. The Mortalite study, registering causes of death of HIV-positive patients from all hospital wards and HIV networks in France, reported major changes in cause-specific mortality between year 2000 and 2005 with an increasing proportion of deaths due to NADM, liver disease, CVD and unknown causes along with

increasing heterogeneity of non-AIDS-related death causes in general ³².

Factors contributing to increase in non-AIDS-related deaths in patients on cART

Both residual effects of HIV infection, such as immunodeficiency or low level viral replication, and accumulating treatment toxicities may contribute to observed changes in causes of death. Recently it became more clear that death causes generally thought to be non-HIV-related are more likely to occur at lower CD4 count ⁴³⁻⁴⁵ which suggests that ongoing immunodeficiency plays a role in developing some of non-AIDS-related diseases. Immunodeficiency has been found to be an independent risk factor for both all-cause and non-AIDS deaths in SMART study analyses ⁴⁶. Findings from EuroSIDA and other studies show that low current CD4 count is associated with increased incidence of certain NADM ^{45,47-50}. In addition, in vitro studies presented poor control of oncogenic transformation associated with decreased immune function as a possible underlying pathomechanism ^{51,52}. Furthermore, the EuroSIDA, SMART and DAD studies reported an association between immunodeficiency and liver-related death ^{38, 46, 53, 54}, whereas no such association was found for CVD-related death ⁴⁶. The DAD study reported use of specific antiretrovirals, namely abacavir, didanosine, indinavir and ritonavir to be associated with increased risk of myocardial infarction, which suggests that treatment toxicities could play an important role in non-AIDS-related mortality ⁵⁵. The association between MI and abacavir was also substantiated by joint DAD-SMART analyses and other studies ⁵⁶⁻⁵⁸. Although ARVs are known to adversely affect lipid and glucose metabolism ⁵⁹ these data show that new potential mechanisms of toxicity should be taken into account ^{60,61}. Ongoing immune activation of inflammatory and coagulation pathways, still present in patients on effective cART, is discussed as a possible underlying pathomechanism for some non-AIDS

morbidities and increased levels of certain biomarkers linked to increased mortality⁶²⁻⁶⁶. Finally, despite the generally protective role of cART on HIV-related renal disease, increasing exposure to tenofovir, indinavir, atazanavir and lopinavir was associated with a higher incidence of chronic kidney disease (CKD) and its contribution to mortality in the long term is of general concern^{67, 68}.

This is a challenging area in HIV research as it is generally difficult to differentiate potential negative effects received from use of cART from those that are exacerbated or caused by HIV infection itself and which cART is correcting⁶⁹.

The role of cohorts in long-term investigation of net benefit of cART

While data on drug antiviral potency and short-term toxicities are reported by RCTs, adverse events that are rare, delayed in onset or unique to special populations are not likely to be identified by such studies⁷⁰. Since cART is a life-long treatment, short-term observations are not enough to confirm that a benefit observed during 24 to 48 weeks by an RCT is sustainable, hence the fundamental role of cohort studies^{71,72}. Unfortunately, the association between cause-specific mortality and time of exposure to cART is not sufficiently investigated, especially for longer-term exposure. Palella et al. reported longer time spent on cART to be associated with death from a non-AIDS cause⁷³, however this study did not investigate more specific non-AIDS causes and two thirds of patients had treatment exposure of less than four years. On the contrary the ART-CC collaboration showed no significant increase in any non-AIDS-related cause of death⁴², but these analyses were not adjusted for conventional risk factors or co-morbidity status.

An important limitation of cohorts is that information on non-AIDS-defining fatal and non-fatal outcomes was, and for some still is not routinely collected⁷⁴. Prospective cohort studies can significantly contribute to the knowledge surrounding treatment harm, if the

observed increasing heterogeneity in non-AIDS outcomes is captured. This requires collaborations between cohorts with a special focus on standardizing the approach for collecting and defining non-AIDS end-points^{32,75}.

Cause of death as an end-point in HIV studies

Survival is the most ultimate and intuitive outcome in any study as it directly reflects the prevention of disease and death⁷⁶. In HIV studies, surrogate markers such as CD4 cell count or HIV RNA viral load, were proven to not always correctly and adequately predict long-term intervention benefit^{77,78}. However, in addition to certification of a patient's death it is necessary to provide causal relation between disease/injury or intervention and the fatal outcome, or in other words, to identify the underlying cause of death. For example in the ESPRIT study about half of the primary events were deaths, 90% of them unrelated to HIV, far above what was assumed in the study design^{78,79}. Cause of death is the key factor to determine the clinical response to treatment marking the transition from one state of health to another⁸⁰, but as with all end-points it must have diagnostic certainty and sensitivity⁸¹. Far too many studies still use cause of death as assigned by sources which prove to be non-optimal i.e. death certificates or discharge hospital documentation^{82,83}. Although death certificates are useful for global health surveillance, they lack the accuracy and reproducibility required for scientific research. For example, Villar et al. identified that among 166 audited death certificates more than 70% were inaccurately completed with over 40% listing the mechanism of death without an underlying disease and 20% with improper causal sequencing of diseases⁸⁴. The additional limitation for death certificates is the usage of ICD-10 coding system, which limits the choice of HIV-related diseases to several general categories⁸⁵. Consequently, it is difficult to compare the data on cause-specific death between the studies as it is unlikely that the definitions, quantity and quality of data

collected and adjudication process are similar. As patients with HIV experience a much wider range of co-morbidities and with death becoming a rare end-point, it is increasingly important to develop standards enabling cross-study comparisons and trends over time to be monitored more easily ^{75,86}.

Rationale and objectives

Further investigation of the aspects underlined in the introduction to this thesis is of direct clinical implication, resulting in the determination of the optimal life-long therapy which at this point is the only available treatment strategy.

Therefore the objectives of this thesis are to:

1. Identify and test methods allowing for individual quantification of antiretroviral drug attributable harm and its relation to underlying risks (I)
 - a) Incorporate it into a tool allowing for quick and practical assessment
2. Investigate whether treatment benefit in terms of decrease in the risk of cause-specific mortality prolongs with follow-up and exposure to cART (II,III,IV)
 - a) Evaluate practical application of already available method for standardization of the underlying cause of death assessment, namely the CoDe project (II)
 - b) Develop a protocol unifying information on causes of death collected through a longitudinal observation in the EuroSIDA study (III)
 - c) Analyse trends for cause-specific death observed over time of exposure to cART in the EuroSIDA study (IV)

Methods

Number needed to harm

The NNH was calculated as the reciprocal of absolute risk increase (ARI), $1/\text{ARI}$, in accordance with standard methodology^{8,11}. As an example of an adverse event, we used the association between current or recent exposure to abacavir and increased rate of myocardial infarction (MI), recently reported by the D:A:D study group^{56,87}. The study reported an increased risk of MI, of RR 1.90, in patients on abacavir, which remained unchanged with longer exposure. The ARI was calculated as the difference between the risks of MI with and without treatment with abacavir (the latter being the underlying risk).

The NNH was therefore calculated as:

$$1/[(\text{underlying risk of MI} \times 1.9) - \text{underlying risk of MI}]$$

The underlying risk of MI was calculated with a parametric statistical model based on the Framingham equation⁸⁸ incorporated into the R statistical program (www.r-project.org/) to calculate the NNH for each underlying risk of MI and to create graphs relating NNH values to different risk components. For simplicity the RR of MI in patients on abacavir was assumed not to vary with increasing exposure to abacavir or according to the underlying risk of MI in our calculations.

Assumptions about the time of exposure

The Framingham equation is limited to predicting CVD risk over 4–12 years reflecting the characteristics of the Framingham Heart Study population⁸⁸. As the median follow-up in the D:A:D study was 5.1 years per person⁸⁷, we calculated the probability of an MI occurring within the next 5 years.

Relating NNH to underlying risk of MI and its components

To relate NNH to different components contributing to the underlying risk of MI, we performed a series of calculations with

different risk equation modifications, and profiles reflecting possible clinical interventions. Graphs were created for male gender and stratified into 4 groups according to smoking status and lipid profile. Using NCEP ATP III guidelines⁸⁹ and the first and third quartile lipid values from the D:A:D study^{59,90}, we defined thresholds for lipid profiles as:

- favourable: a total cholesterol <170 mg/dL (4.4 mmol/L), HDL > 60 mg/dL (1.5 mmol/L)
- unfavourable: a total cholesterol of 240 mg/dL (6.2 mmol/L), HDL of 35 mg/dL (0.9 mmol/L).

Within these groups, the NNH was plotted against age and systolic blood pressure (sBP), and for the latter a value of 120 mmHg was chosen.

Estimating uncertainty for NNH

To summarize the uncertainty associated with NNH, the 95% CI for the RR of MI (1.47, 2.45) reported by Sabin et al.⁸⁷ was incorporated in to the calculations.

Interpreting the results

All NNH values represent the number of patients who need to be treated with abacavir for 5 years to observe MI in one additional patient as a consequence of this treatment. For example if the underlying risk of MI is 5%, the ARI will be 4.5% (i.e. a 90% increase) and the NNH with abacavir will be 22. An ARI of 4.5% implies that using the drug over the next 5 years will increase this patient's risk of having an MI from 5 to 9.5%, and an NNH of 22 implies that if 22 patients with an estimated underlying risk of MI of 5% use abacavir over this same 5-year period, one additional patient may be expected to develop an MI which would not have occurred had this group of patients not used abacavir.

NNH values cannot be addressed with commonly defined limits for what represents an acceptable risk or not⁹¹. The general approach is: the higher the NNH, the better. One possible solution is to relate NNH to already recognized

high- or low-risk values ^{5,19,92}. For example, using the 10 and 20% cut-offs proposed in the NCEP/ATP III guidelines for assessing 10-year CHD risk ⁸⁹ we defined low-, medium- and high-risk groups with absolute risks of MI of <5, 5–10 and >10% over 5 years, respectively.

It is also important to relate treatment harm and benefit to the size of the effect that the treatment has. For instance, while initiating cART in a patient with a CD4 count below 200 cells/mm³ and serious risk of developing opportunistic infection we are willing to accept higher treatment harm, therefore lower NNH, than for a patient with CD4 cell count above 350 cells/mm³ ^{8,93}.

Furthermore, as the NNH values can be calculated for any chosen outcome they should always be interpreted in relation to this specific context ⁹⁴. For example, NNH to cause any bleeding requiring hospitalization in stroke survivors ⁹¹ was 467 for aspirin and 126 for warfarin, but for central nervous system bleeding alone NNH was 534 and 301, respectively.

The Coding Causes of Death in HIV (CoDe) Project Methodology

The CoDe project was launched in 2004 based on a joint agreement of scientific boards of several large cohort studies and ongoing RCTs that routinely collect data on causes of death. Through an initial pilot phase (CoDe Pilot), the CoDe CRF, Review CRF and guidelines were tested widely both at clinics taking part in the D:A:D study and externally. For the CoDe Pilot reviewers appointed by the CoDe Working Group tested the review process on a total of 80 cases from more than 20 clinics ⁹⁵. As a result, the documents were modified in order to ensure clarity of the guidelines and facilitate the collection of data and completion of the CRFs. A final version of the protocol and study documents were released in February 2005⁹⁶ and the project Working Group assigned to continue methods evaluation.

Copenhagen HIV Programme serves as a coordinating centre for the CoDe project and considerable part of this thesis was devoted to the coordination of the implementation of the CoDe project and evaluation of its practical application.

The CoDe project consists of:

- the protocol of collection and ascertainment of information on death circumstances and contributing factors
- a central adjudication process
- uniformed coding system (CoDe classification)

Data collection, ascertainment and quality assurance

The 4-page CoDe CRF consists of the following sections:

- Section 1. Background demographics
- Section 2. What data sources were available for the completion of this form
- Section 3. Risk factors
- Section 4. Co-morbidities
- Section 5. Cause of death
- Section 6. Post-mortem/autopsy
- Section 7. ART and laboratory values prior to death
- Section 8. Adverse effects to any type of medical treatment

Copies of autopsy reports are requested to be sent along with the CoDe CRF.

In Section 5, centres are asked to provide their judgment on the immediate and underlying condition that caused death, all HIV and non-HIV-related diagnoses the patient had at the time of death, and a short narrative summary of the events leading to death. The *Instructions for the completion of the CoDe Cause of Death form* are provided, although the CoDe form is intended to be largely self explanatory.

Office personnel at the coordinating Centre, including a specialist HIV clinician, assess the CoDe forms. Cases lacking information or stating unknown death circumstances are queried. During the querying process, centres receive feedback on the quality of the forms received, and further training is provided if necessary.

Central adjudication process

The final coding of cause of death is conducted through a central adjudication process performed independently by two randomly matched, external reviewers who remain anonymous to each other and who each follow specific guidelines. A total of 32 physician-reviewers from 22 European and one Australian clinic have been recruited ⁹⁷. Based on information reported on the CoDe CRF, reviewers assign one immediate, up to four contributing and one underlying cause of death. Briefly, the immediate cause is the disease/injury that directly leads to death; contributing causes are those that contribute to the fatal outcome; and the underlying cause is the disease/injury that initiated the train of morbid events leading directly or indirectly to death. This definition is used by investigators reporting from the centres as well.

Cause of death classification

For coding causes of death, 33 predefined categories are used ⁹⁶. Categories 1–19 include specific causes of death known to be common in HIV-positive populations, categories 20–30 are more general referring to organ or system of organs and, if none of the above is applicable “other,” “unclassifiable,” or “unknown” codes can be used. Reviewers are also asked to indicate their level of certainty to limit the “unknown” category and obtain more complete specific codes.

Both reviewers have to agree on the underlying cause of death. If there is an initial disagreement, an adjudication process is followed to reach consensus and the reviewers are able to view their co-reviewer’s coding and correspond online via a comment box. If reviewers decide that they cannot achieve consensus, the case is referred to an additional reviewer for final decision. The integrated work processes of the CoDe project are outlined in **Figure 1**.

CoDe method’s implementation

The method is suitable for both clinical trials and observational studies, and is publicly available at CHIP’s website (www.cphiv.dk). Currently, CoDe has been implemented by three observational studies (the D:A:D study ⁵², the EuroSIDA study ⁹⁸ and the HIV/TB Project ⁹⁹) and by the INSIGHT group ^{46,100,101}, for the collection and central adjudication of causes of death. In addition, the CoDe classification was used by ART-CC to describe data on causes of death provided by participating cohorts with different collection procedures ⁴².

Figure 1 . The integrated work processes of the CoDe project.

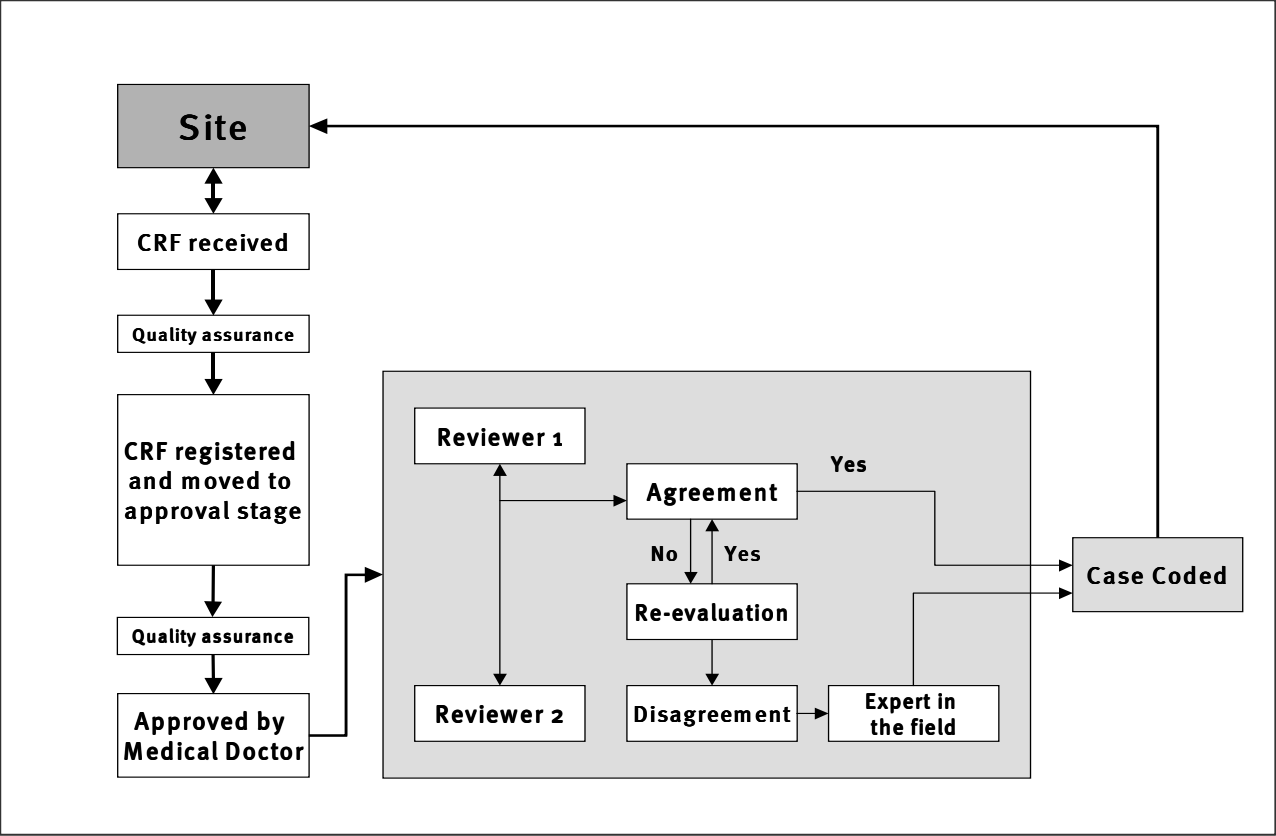
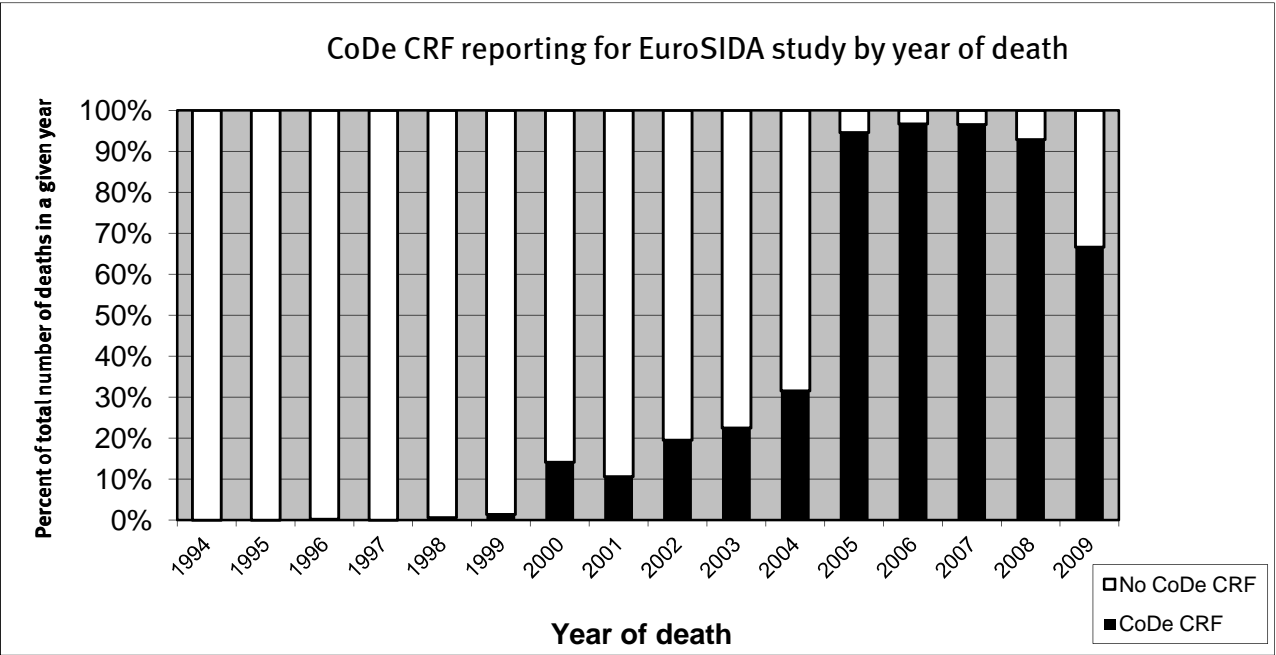


Figure 2. The proportion of deaths reported on CoDe CRF in the EuroSIDA study in 1994 – 2009



EuroSIDA study methods

Design and end-points

EuroSIDA was initiated in 1994 and is a prospective, observational study of 16,597 HIV-1-positive patients at 103 centres across Europe, Israel, and Argentina. To date, eight cohorts of patients have been recruited. Data is collected prospectively at clinical sites, extracted and sent to the coordinating centre at 6-month intervals. For cohorts I–III, eligible patients were those who had had a CD4 count below 500 cells/mm³ at recruitment or during the previous 4 months. The CD4 count restriction was removed for cohorts IV–VIII. In addition to demographic and clinical information, a complete antiretroviral treatment history is obtained, including dates of starting and stopping each antiretroviral drug, all CD4 counts, plasma HIV RNA and other laboratory measurements since the last EuroSIDA follow-up. Data have been routinely collected on AIDS events since the beginning of the study, on CVD since 1999 and on NADM, pancreatitis, end-stage liver disease (ESLD) and end stage renal disease (ESRD) since 2001. AIDS events were diagnosed using the clinical definition from the CDC. Full details of the study and sample follow-up forms can be found at www.cphiv.dk.

Evaluation of causes of death in the study

For patients who died, date and cause of death (19 predefined causes) are reported by the site investigator in Section H of the EuroSIDA follow-up form. Since 2004, a 4-page CoDe CRF has been required to be completed for each fatal case (see Methods page 11)¹⁰². Cause of death is determined by conducting a central review based on the data collected on the form. The overall coverage for CoDe CRFs in the recent years is 95% (**Figure 2**).

Data collection on causes of death in the EuroSIDA study has expanded since 1994 to a more detailed categorization for non-AIDS-related causes³¹ and a large part of the current PhD thesis is dedicated to ascertain that all

causes of death collected before CoDe introduction reflect the now established uniform classification (CoDe project classification) and that only one underlying cause per death is finally assigned.

The cause of death was unknown for approximately 17% of all deaths reported in the study. This proportion has steadily decreased over time in the study, from 19.8% before 1999 to 11.9% after 2004. In order to include these events into statistical analyses, a special algorithm was developed and tested as part of this PhD study classifying cause of death allowing for the assigning of unknown deaths to either an AIDS or non-AIDS related category (see paper III, page 22).

Data ascertainment and quality assurance

EuroSIDA has an extensive quality assurance process. All participating sites are monitored at least once annually. All clinical events, including deaths, are verified by the monitoring team, which also monitors a random selection of all patients without clinical events.

Summary of papers

Paper I

Implementing the number needed to harm in clinical practice: Risk of myocardial infarction in HIV-1 infected patients treated with abacavir.

In this paper we combine estimates of the underlying risk of an event with drug-attributed risk of this event and present it in terms of ARI and NNH. As an example of an adverse event, we use the recently reported findings from the D:A:D study group, which show an association between current or recent exposure to abacavir and increased rate of MI ^{56,87}. Abacavir is a common antiretroviral used in the treatment of HIV-1 infection and is recommended as one of the possible components of initial cART ^{71, 103-106}. It is therefore of great importance to ensure optimal drug application through the individual patient's risk interpretation.

Results

In the first step we investigated the relationship between NNH, ARI and the underlying risk of MI (Fig 3). The NNH decreases quickly from 185 to 5 as the underlying risk of MI increases from 0.6% to above 20%. As the first relationship is reciprocal, the same absolute change in the underlying risk of MI results in a small change in NNH for patients with a high MI risk and a large change for patients with a small underlying risk of MI. Relating ARI to the underlying risk of MI is not capturing this relationship.

Next we have investigated how particular risk components contribute to the underlying risk of MI and translate into different NNH. This has been presented by choosing a sample patient profile and re-calculating NNH for different risk component modifications. For example, NNH changes from 1111 to 555 when diabetes is considered diagnosed, but to 277 if the patient is considered to be a smoker (Table 1).

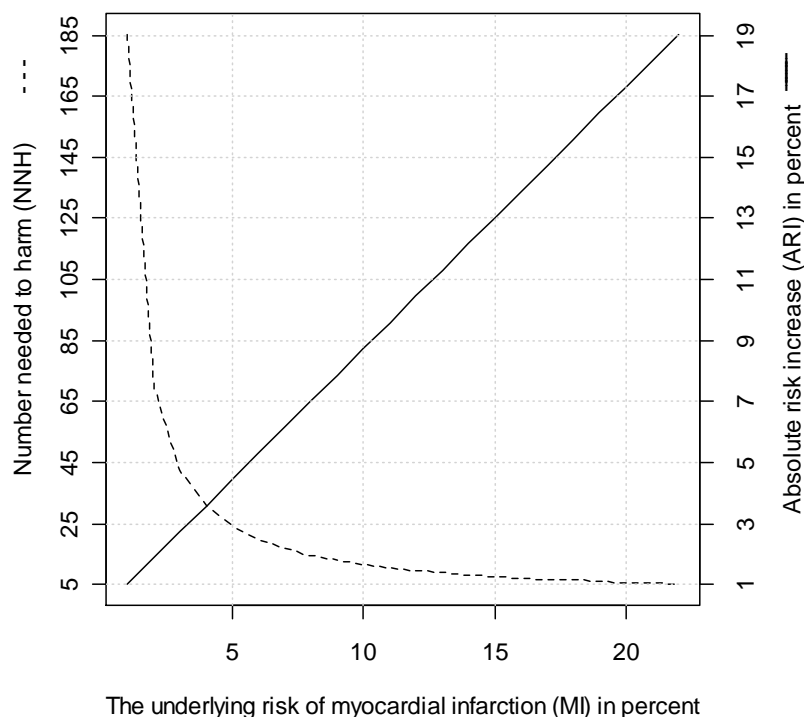


Figure 3. Relationships among NNH (dashed line), ARI (continuous line) and underlying risk of MI estimated for a 5-year period for a drug associated with increased risk of MI

Table 1. NNH for a drug associated with an increased risk of MI for different risk components

Change in factors contributing to underlying risk	Underlying risk of MI (%)	NNH (95%CI)	
Example low risk profile ^a	0.1	1111 (689-2127)	
If total cholesterol unfavourable ^b	0.2	555 (344-1063)	
If diabetic	0.2	555 (344-1063)	
If ECG-LVH	0.2	555 (344-1063)	
If sBP 160 mmHg	0.3	370 (229-709)	
If HDL unfavourable ^c	0.3	370 (229-709)	
If smoker	0.4	277 (172-531)	
If lipids unfavourable ^{b,c}	0.8	138 (86-265)	
If smoker and diabetes	1.1	101 (62-193)	
If smoker and sBP 160 mmHg	1.3	85 (53-163)	
If smoker and HDL unfavourable ^c	1.6	69 (43-132)	
If smoker and lipids unfavourable ^{b,c}	3.1	35 (22-68)	
If all unfavourable combined	15.0	7 (4-14)	

^a Forty-year-old man, non-smoking, no diabetes, left ventricular hypertrophy not present on ECG, systolic blood pressure (120 mmHg), favourable total cholesterol (170mg/dL; 4.4 mmol/L), and favourable HDL (60 mg/dL; 1.6 mmol/L).

^b unfavourable total cholesterol: 240 mg/dL, 6.2 mmol/L

^c unfavourable HDL: 35 mg/dL, 0.9 mmol/L

The NNH was calculated using the underlying risk of myocardial infarction (MI) estimated with rounding to one decimal. CVD, cardiovascular disease.

Figure 4 reflects the same approach in risk assessment as described above yet presented in a series of coloured three-dimensional illustrations relating NNH to age and systolic blood pressure (sBP) and categorizes it according to smoking status and two chosen lipid profiles. These graphs illustrate how different risk factors, the examples here being smoking and an unfavourable lipid profile, add to abacavir's attributable risk of MI expressed in the number of patients that can be treated to observe one additional experiencing an MI. For example, a comparison of graphs A and B demonstrates that smoking produces a marked decrease in NNH, which means that one would need to treat considerably fewer smokers to observe one additional MI; and a comparison of graphs C and D demonstrates that a further decrease in NNH is seen with an additional risk of an unfavourable lipid profile.

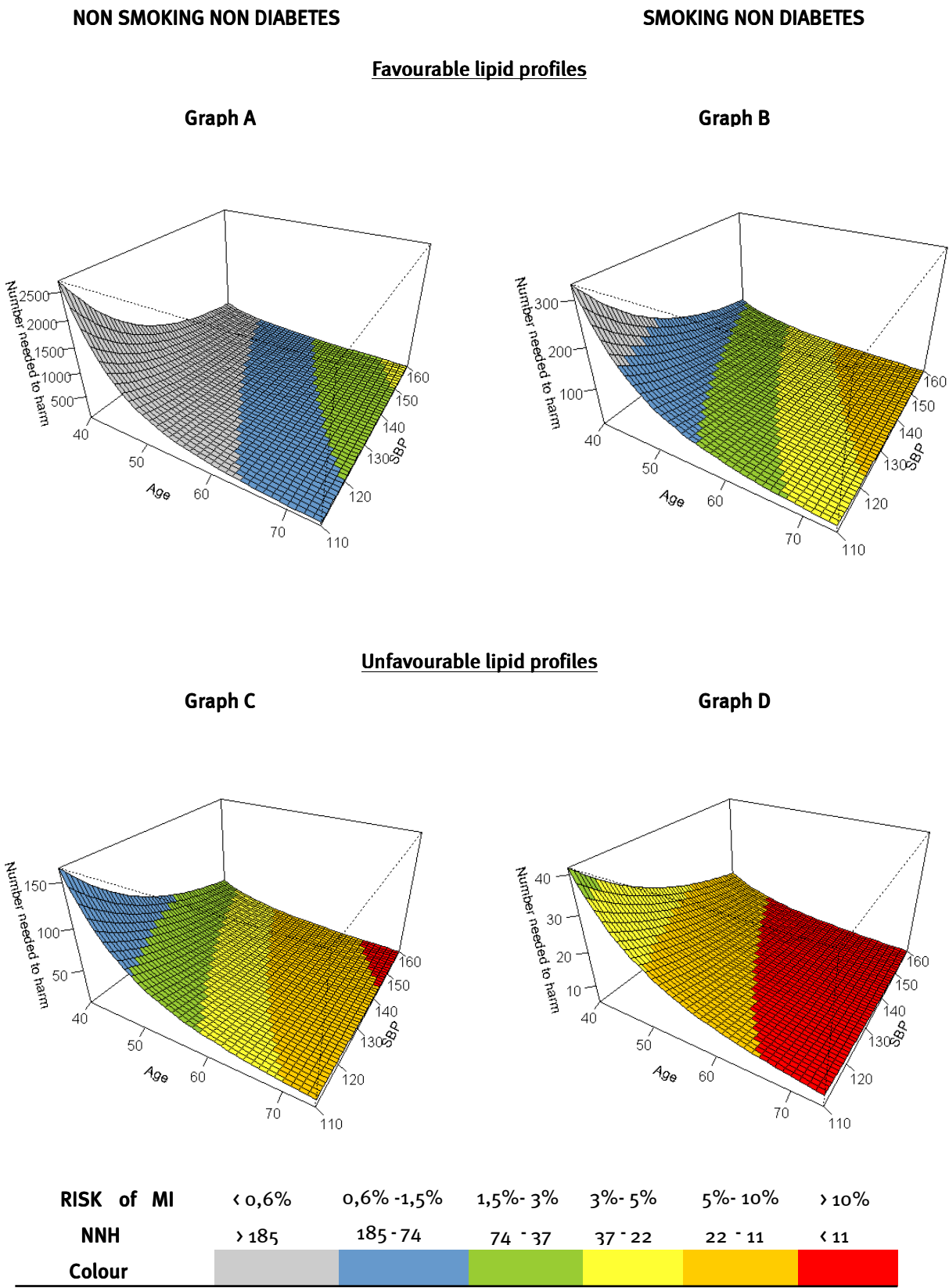
The graphs enable easy identification of high or low NNH and help one to understand the dynamics of NNH change when particular risk components are modified in a way which reflects possible clinical interventions. For example, it is readily apparent that red, reflecting the lowest NNH (graph D), shifts to

orange and yellow if the risk factor of smoking is removed (graph C). Therefore, introducing smoking cessation in this group of patients will eventually increase the NNH from <11 to >22.

Discussion

We show in this paper that if the risk of a drug-attributed adverse effect, here an MI, is investigated and properly assessed it can result in choosing the best fit clinical strategy, increasing the number of patients that can be safely treated with abacavir. Although at the population level the benefit of cART (including abacavir) is unquestionable, we present here the extent to which it can be limited in certain patient sub-groups. From a clinical perspective it is essential that this risk is put in to context and appropriate consideration given as to whether patients should continue taking abacavir or whether the drug should be discontinued.

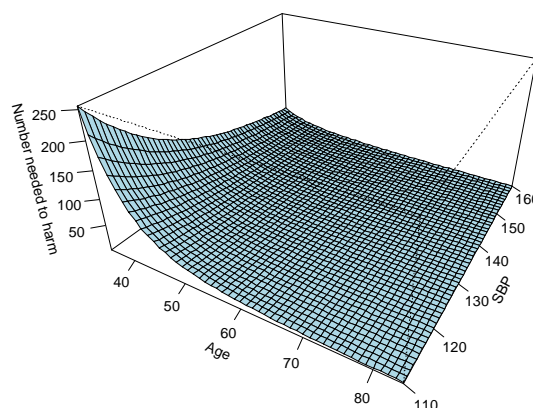
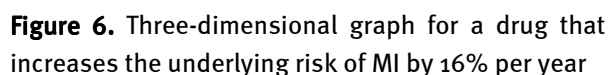
Figure 4. Three-dimensional graphs relating NNH for a drug that increases the underlying risk of MI by 90% to age and systolic blood pressure (sBP).



The y-, x- and z-axes present NNH, age and sBP values, while colours on the plane reflect NNH ranges as described in the key. **Graphs A and B** present NNH for non-smoking and smoking patients with favourable lipid profiles. **Graphs C and D** present NNH for non-smoking and smoking patients with unfavourable lipid profiles.

Clinical application

Figure 5. NNH and underlying risk calculator



This approach can also be used for a drug that has a cumulative risk, for example the RR of MI of 1.16 per additional year of exposure to protease inhibitors, also reported earlier by the D:A:D group ⁹⁰. Applying this risk over a 5-year exposure period in a patient with a 5% underlying risk of MI results in an increase in the underlying risk of 2.1 for protease inhibitors ($RR=1.16^5$) and NNH equal 18. **Figure 6** presents a three-dimensional graph relating the NNH for a drug that increases the underlying risk of MI by 16% annually to age and sBP as changing risk components.

Paper II

The Coding Causes of Death in HIV (CoDe) Project - Initial Results and Evaluation of Methodology.

In this paper we present results from the CoDe Project application in the D:A:D study during the period of 2004–2008. Results of the review process were stratified according to initial agreement or disagreement on the underlying cause of death and logistic regression models were used to identify factors associated with achieving initial agreement by reviewers.

Results

A total of 491 reported deaths went through the review process. The median time from death to the receipt of the CoDe CRF at the coordinating centre was 7.4 months (IQR 4.7–11.0). Deaths occurred from February 2000 to January 2007 and 135 (27%) were defined by investigators as “sudden”. An autopsy was performed in 62 (13%) cases, and a summary of the autopsy findings was available for 51 (10%). For the majority of sections in the CoDe CRF the completeness of answers provided was > 90%. Sections collecting information on treatment related to death and whether the death was sudden were often completed erroneously. Of the 22 cases where investigators had listed associations between the death and drugs/treatment, the coordinating centre could confirm only 17 cases. Of all deaths determined by investigators to be sudden, 61 (45%) were identified with a chronic, ongoing terminal condition that was subsequently assigned as the underlying cause of death by reviewers.

The underlying cause of death was completed by site investigator in 135 cases and in 79 (58%) of these cases agreed with the underlying cause of death determined through the adjudication process. In all cases where the underlying cause of death was not provided by investigator reviewers were able to assign cause of death based on information from the CoDe CRF.

The review process assigned a code for the underlying cause of death to all 491 cases. For 457 (93%) cases, a specific or general classification code was assigned for the underlying cause of death. Eight (2%) cases were coded as ‘other’, reflecting the lack of an applicable code, and 26 (5%) as unknown, reflecting insufficient information to code the case (Table 2).

Table 2. The underlying cause of death provided by 2 independent reviewers in the peer-review process

CoDe codes	Illness/condition	All cases N	Initial agreement N (%)
Total number of events		491	339 (69)
Specific code classification		424	320 (65)
1	AIDS	157	130 (83)
2	Infection (other than 1)	29	16 (55)
3	Chronic viral hepatitis	86	71 (83)
4	Malignancies (other than 1 and 3)	63	43 (68)
5	Diabetes	4	2 (50)
6	Pancreatitis	2	1 (50)
7	Lactic acidosis	1	0 (0)
8	MI and other ischemic	20	17 (85)
9	Stroke	2	1 (50)
10	GI haemorrhage	1	0 (0)
11	Primary pulmonary	2	2 (100)
12	Lung embolus	2	1 (50)
13	Chronic obstructive	2	1 (50)
14	Liver failure (other than 3)	1	1 (100)
15	Renal failure	1	0 (0)
16	Accident or violence	14	6 (43)
17	Suicide	16	14 (87)
19	Substance abuse	21	14 (67)
General classification		33	12 (36)
20	Haematological disease	2	1 (50)
22	Psychiatric disease	14	6 (43)
23	CNS disease	4	0 (0)
24	Heart or vascular	9	5 (56)
25	Respiratory disease	1	0 (0)
26	Digestive system	3	0 (0)
Unclassifiable causes		34	7 (21)
90	Other cause	8	0 (0)
92	Unknown	26	7 (27)

In 339 (69%) cases the 2 reviewers initially agreed and in 152 (31%) there was initial disagreement. In all cases reviewers achieved a final consensus without the need for intervention from a third reviewer.

Factors associated with initial agreement after adjustment were: ongoing chronic hypertension, a history of depression, the number of diagnoses at the time of death, and the underlying cause of death as determined by the review process (Table 3).

For each additional diagnosis listed at the time of death, the odds of agreement were increased by approximately 20% (odds ratio OR 1.19 95% CI: 1.05–1.35). As compared with forms where deaths were ultimately deemed to be due to AIDS-related causes, the odds of agreement were more than 80% lower when deaths were ultimately not deemed to be due to CVD, non-AIDS malignancy, hepatitis or violent cause (other non-AIDS-related) (0.17 [0.08–0.37]) or undetermined causes (0.11 [0.04–0.36]).

There was no difference in odds for achieving initial agreement between deaths ultimately deemed to be due to AIDS-related causes and those due to chronic viral hepatitis, malignancy, CVD, or violence. The odds of agreement between reviewers on cause of death were lower for patients with ongoing chronic hypertension and a history of depression.

Discussion

The initial results from the CoDe Project document that the extent and format of the data collection are sufficient for an informed review, and that the coding scheme proposed with CoDe includes an adequate range of possible causes of death in HIV-positive patients.

We identified sections where data were often omitted (underlying cause of death) or entered erroneously (sudden death, death related to medication). This illustrates a discrepancy between clinicians' and reviewers' perception of terms such as "sudden death" and "underlying cause of death", supporting the

necessity of an external adjudication process.

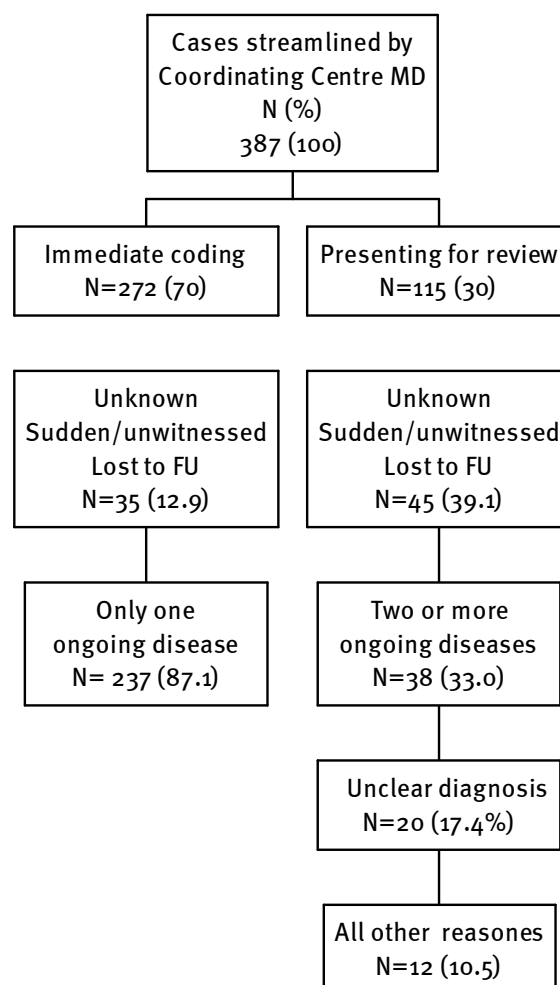
Table 3 . Univariate and multivariate odds ratios for factors associated with agreement by reviews on cause of death

	Univariate OR (95% CI)	Multivariate OR (95% CI)
Demographics		
Age (per 10 year increase)	0.79 (0.66-0.94)	0.90 (0.71-1.14)
Data sources available for completion of form		
Hospital	1.00	1.00
Other	0.60 (0.41-0.88)	1.06 (0.57-1.98)
Co-morbidities		
Ongoing chronic hypertension		
No	1.00	1.00
Yes	0.54 (0.33-0.89)	0.43 (0.22-0.85)
Unknown	0.57 (0.36-0.91)	0.65 (0.20-2.15)
Ongoing chronic diabetes mellitus		
No	1.00	1.00
Yes	0.88 (0.48-1.64)	1.34 (0.61-2.97)
Unknown	0.51 (0.32-0.80)	0.74 (0.23-2.46)
History of depression		
No	1.00	1.00
Yes	0.59 (0.35-0.99)	0.43 (0.23-0.80)
Unknown	1.15 (0.74-1.79)	2.06 (1.05-4.05)
Chronic HCV infection		
No	1.00	1.00
Yes	1.31 (0.84-2.03)	1.35 (0.76-2.40)
Unknown	0.44 (0.24-0.80)	0.56 (0.26-1.18)
Cause of death		
Was the death sudden?		
No	1.00	1.00
Yes	0.48 (0.31-0.76)	0.92 (0.48-1.78)
Unknown	0.39 (0.24-0.66)	0.58 (0.28-1.19)
Number of diagnoses at time of death (per 1 increase)		
	1.16 (1.07-1.26)	1.19 (1.05-1.35)
Cause of death (adjudicated)		
AIDS-related	1.00	1.00
Chronic viral hepatitis	0.98 (0.49-1.97)	1.28 (0.56-2.92)
Malignancy (non-AIDS)	0.45 (0.23-0.88)	0.75 (0.34-1.64)
Cardiovascular disease	0.65 (0.25-1.68)	1.13 (0.35-3.63)
Violent death	0.33 (0.17-0.64)	0.45 (0.19-1.07)
Other non-AIDS-related	0.14 (0.07-0.26)	0.17 (0.08-0.37)
Undetermined	0.08 (0.03-0.20)	0.11 (0.04-0.36)
Laboratory values most recent prior to death		
CD4 count (cells/mm ³)		
<50	1.00	1.00
50-199	0.45 (0.25-0.83)	0.58 (0.28-1.20)
200-349	0.44 (0.23-0.87)	0.58 (0.25-1.36)
≥350	0.33 (0.18-0.61)	0.79 (0.34-1.83)
Unknown	0.61 (0.26-1.41)	0.87 (0.28-2.73)
HIV RNA viral load (copies/mL)		
<50	1.00	1.00
50-9999	1.09 (0.65-1.84)	1.15 (0.61-2.17)
≥10000	1.93 (1.16-3.22)	1.71 (0.87-3.33)
Unknown	1.48 (0.74-2.98)	0.95 (0.35-2.56)

The importance of adjudication is also underlined by the fact that in over two-thirds of cases, site investigators did not report an underlying condition that caused death, yet reviewers were able to do so. These events would not have been coded without central adjudication.

Analyses reviewing the application of the CoDe methodology in practice identified a number of modifications that could help to further strengthen the data collection and adjudication process. The observations suggest a better use of existing resources by expanding the role of the coordinating centre physician to include that of reviewer and by allowing the review by this physician to be sufficient in “obvious” cases. The principles for the process of streamlining events into one or another group were developed and evaluated prior to implementation in a batch of 387 events. A 10% random sample of 25 cases was sent to an external reviewer as a quality control with a disagreement found in 2 (8%) of the cases. Results from this evaluation are presented in **Figure 7**.

Figure 7. Results from evaluation of revised procedures introduced to CoDe project



Paper III

A standardized algorithm for determining the underlying cause of death in HIV infection as AIDS or non-AIDS related: Results from the EuroSIDA Study.

In this paper we develop and test a method which aims for the unification of data on causes of death that has been collected throughout a long-term follow up. Additionally, missing data posed significant analytical limitations for planned analyses of changes in causes of death over time. Therefore we have explored this issue within the EuroSIDA study and propose a standardized protocol allowing for the classification of all deaths collected in the study as AIDS or non-AIDS related.

All patients who died before August 2008 were included in this analyses and three methods of identifying the underlying cause of death were compared: central classification (reference group) based on an externally standardized method (the CoDe procedures)¹⁰², local cohort classification as reported by the site investigator, and four algorithms created based on length of survival time after specific AIDS events¹⁰⁹ (**Figure 8a**). Kappa agreements (κ) were used to compare central classification with local cohort classification and all algorithms¹¹⁰.

Results

In total, 2,783 deaths occurred and 488 events had a definite central classification. The best agreement was between central and local cohort classification ($\kappa = 0.70$). For all 4 computerized algorithms, the agreement with central classification was moderate ($\kappa < 0.60$); the highest for algorithm 1, using survival time upper quartile for the specific disease and 17 months where it was unknown ($\kappa = 0.59$), which also had highest sensitivity for AIDS-related

death. Algorithms allowing longer survival after AIDS event to still classify death as AIDS related, showed higher sensitivity; and algorithms, allowing shorter time, showed higher specificity.

Based on these results, a step-wise algorithm (**Figure 8b**) was identified for classifying cause of death, which prioritized central classification over local cohort classification and used algorithm 1 for patients with no information from these two sources. This step-wise algorithm was applied to all deaths with 1,332 (47.9%) being finally classified as AIDS-related and 1,451 (52.1%) as non-AIDS-related.

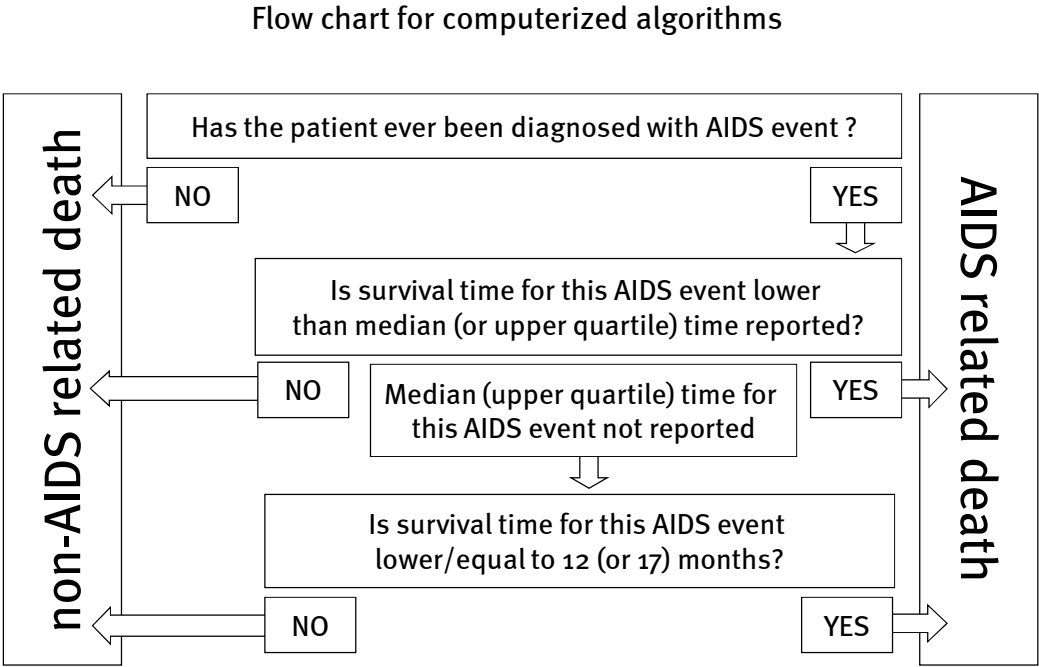
Discussion

After 2004 when the CoDe project was introduced, information on death and its contributory factors has been collected in the EuroSIDA study simultaneously on standard follow-up forms and the CoDe CRF. This practice creates a unique opportunity for evaluating data already collected and for developing algorithms estimating the cause of death when it is unknown.

Although information collected according to the CoDe principles and protocol is considered to be of the highest quality, and will be prioritized in the future, we were able to confirm that causes of death available from local cohort classifications are a reliable source when a CoDe CRF is unavailable. Similarly, estimates received from predefined algorithms showed modest yet satisfactory agreements allowing for the classification of all deaths as AIDS or non-AIDS-related. At the same time, detailed causes of death are not lost in the process but rather available for a smaller subset of patients. The step-wise algorithm is now included in the EuroSIDA study methods, enabling a range of analyses of cause-specific mortality.

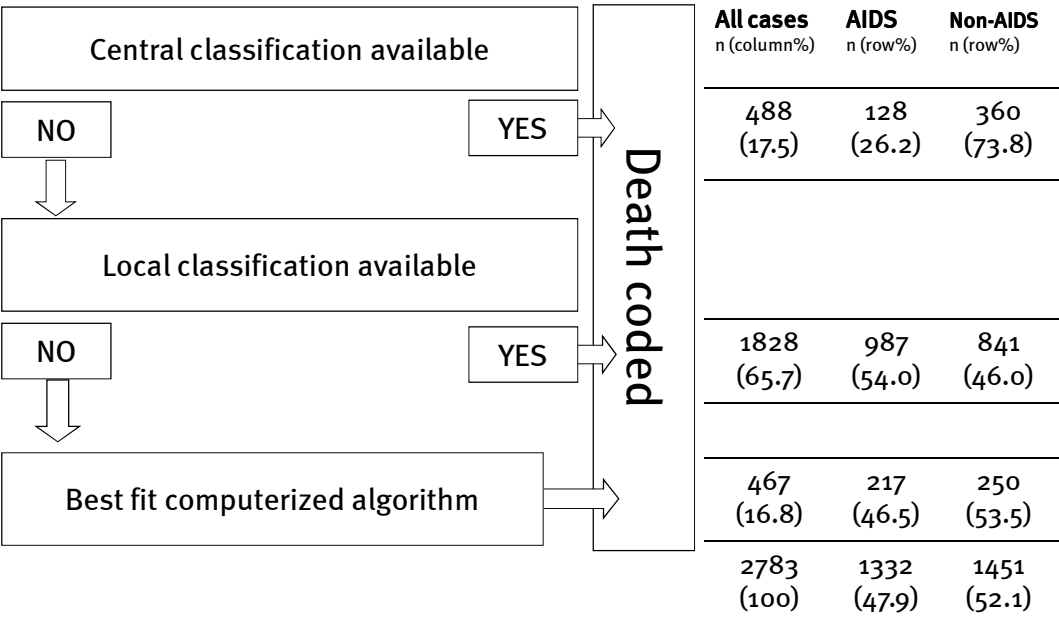
Figure 8. Flow charts of predefined algorithms (a) and step-wise algorithm (b) for assigning death as AIDS or non-AIDS-related

a.



b.

A step-wise algorithm for assigning deaths as AIDS or non-AIDS related



Paper IV

Long-term exposure to combination antiretroviral therapy and risk of death from specific causes: no evidence for any previously unidentified increased risk due to antiretroviral therapy.

In this paper we investigated changes in the rate of cause-specific death with cumulative exposure to cART. All patients recruited to the EuroSIDA cohort who were on cART at some point during follow-up were included into the analyses. Non-AIDS-related deaths were classified in to the following categories: non-AIDS infections (NARI-death), liver related (LR-death), non-AIDS defining malignancies (NADM-death), cardiovascular disease (CVD-death), violent (accidental or violent death, suicide, euthanasia, substance abuse or overdose), other (if < 20 deaths) and unknown death. Incidence rates (IR) of death were calculated per 1000 PYFU and stratified by time of exposure to cART (≥ 3 antiretrovirals): <2, 2-3.99 (reference), 4-5.99, ≥ 6 years. Any time when the patient was off cART was not counted as exposure time. Poisson regression models were fitted for each cause of death separately. As a post-hoc analysis duration of cART exposure was fitted as a continuous variable per year longer on cART and from 2 years of exposure onwards.

Results

During 70613 PYFU, 1297 patients died. AIDS accounted for 32% of all deaths, NARI-death 9%, LR-death 14%, NADM-death 10%, CVD-death 9%, violent 7%, other 7% and 12% of the cases remained unknown (but were classified as non-AIDS-related based on the previously established algorithm). The overall crude IR of all cause death, AIDS related death and non-AIDS-related death were 18.3 (95%CI: 17.4-19.4), 5.85 (5.28-6.41) and 12.5 (11.7-13.3) per 1000 PYFU, respectively. The crude IR of all-cause death decreased with longer exposure to cART, which was largely attributed to a

decrease in AIDS-related mortality, but the rates of non-AIDS related death remained fairly constant. In the multivariate analyses of cumulative exposure to cART adjusted for CD4 cell count, HIV RNA viral load and other factors there was a significant decrease in the rate of all-cause and AIDS-related deaths between 2-3.99 years and any longer exposure time. Additionally the rate of unknown and violent deaths decreased significantly with over 6 years of exposure to treatment (adjusted IRR 0.61, 95%CI 0.40-0.93, $p=0.020$ and 0.54, 95%CI: 0.31-0.96, $p=0.037$, respectively).

No significant difference in the rate of any other non-AIDS cause-specific deaths between 2-3.99 years and longer exposure to cART was observed (**Figure 9**).

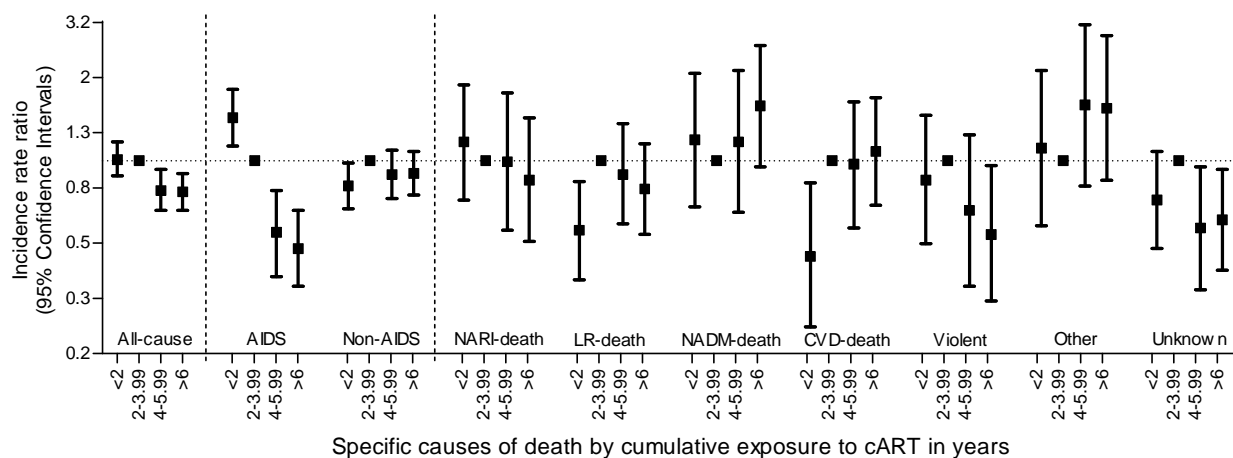
When time on cART was fitted as continuous variable from 2 years of exposure onwards there was a 5% decrease in the risk of all-cause death (IRR 0.95, 95% CI: 0.92-0.97, $p<0.001$) and 14% decrease in the risk of AIDS-related death (IRR 0.86, 95%CI: 0.81-0.91, $p<0.001$) per one additional year on cART, and a borderline significant decrease in the risk of non-AIDS death (IRR 0.97, 95%CI 0.95-1.00, $p=0.060$).

Discussion

The main finding of our study is the lack of increase in the risk of non-AIDS-related death with prolonged exposure to cART. This is the first investigation looking into trends of detailed non-AIDS-related mortality over the actual time spent on cART and with long-term exposure to treatment^{42,73}.

Despite that EuroSIDA is an observational cohort we were able to adjust the models for a large variety of parameters as well as introduce an external validation of cause of death evaluation, namely the Coding Causes of Death in HIV (CoDe)¹⁰² which is a clear advantage for these analyses. In addition we used a step-wise algorithm, unifying data collected before CoDe implementation and classifying all deaths without known causes as either AIDS or non-AIDS-related, which allowed for inclusion of all

Figure 9. The incidence rate ratio of death due to specific cause by cumulative exposure to cART (reference group is 2-3.99 years on cART).



Models adjusted for HIV exposure group (all except All-cause, other), smoking status, age (all except AIDS, violent), region of Europe (all except NADM, CVD, violent), HCV status (all except AIDS, NADM), HBV status (all except NARI, violent, other, unknown), gender (all except NARI, LR-death), race (all except AIDS, NARI, violent, unknown), diabetes, hypertension (all except non-AIDS, NARI, NADM, other), HIV RNA (all except non-AIDS) and CD4 count. All variables as at baseline.

deaths from the follow-up period into the analyses⁹⁸.

Our analyses confirm prolonged benefit of cART over more than two years spent on treatment, with an annual decrease of approximately 5% in overall mortality, yet mainly driven by decrease in the risk of AIDS-related death. We were not able to investigate further the effect of particular antiretroviral drugs or drug classes on cause-specific mortality due to the low number of events received after such stratification. For the same reason we have merged together rare or emerging causes of death (occurring for less than 20 events) in the 'other' category. Therefore although we did not find the risk of non-AIDS-related death to increase with prolonged exposure to cART, we cannot at present exclude that such risk may exist for rare events, specific sub-groups of patients or individual antiretroviral drugs.

As follow-up data accumulate, such analyses will be possible and of relevance for the future clinical management of HIV-positive patients, enabling clinicians to compose cART regimens with the optimal risk-benefit ratio for the individual patients.

Conclusions and perspectives

Combination antiretroviral therapy is administered in order to prevent morbidity and mortality that otherwise would develop as a consequence of HIV-1 infection. In achieving this, treatment guidelines have significantly changed over last 10 years, although it is still recommended to initiate treatment with selected preferred regimens for majority of patients^{103,105,106,108}. While first line drugs are chosen to present optimal safety profile they are still linked to considerable adverse events, which are more likely to occur when the underlying risk of the event is increased^{67,111,112}. When treatment is not individualized according to the patient's pre-treatment risks an important heterogeneity in the expected net treatment benefit, which depends on certain measurable characteristics of a patient, is omitted. In lack of detailed recommendations in this area it is up to clinicians to make a final decision, but at the same time there are no tools that would guide individualized therapy according to a given patient's risk profile. We have presented how easy to understand

measures, namely NNH and absolute risk, can be incorporated into tools that serve to identify patients who are not best candidates for a given antiretroviral drug due to a high pre-treatment risk. This approach can be also used to identify the most effective risk-lowering methods in a specific treatment scenario. We have confirmed further application of these methods in different settings. An extension of this idea and the next step for EuroSIDA analyses presented in this thesis is to estimate the probabilities of developing different AIDS and non-AIDS events for different cART components and patient's pre-treatment risks. Results would be presented as 'matrix' of absolute risks for a given individual patient's characteristic. Such a risk matrix must additionally present estimates for AIDS and non-AIDS events in a cART-free scenario representing maximum benefit received from applying treatment. **Figure 10** presents hypothetical results of this tool showing risk estimates for two different patients' characteristics.

Figure 10. A hypothetical example of risk matrix for different cART components and two chosen patients' characteristics

Patient 1:

CD4 250 cells/mm³; HIV RNA 70.000 copies/mL
5 year CVD risk > 15%
GFR > 60 ml/min

Events	No ART	cART component				
		ABC	TDF	ATV	LPV	EFV
AIDS						
Infection						
Malignancy						
Non-AIDS						
NARI						
CVD						
CKD						
ESLD						
NADM						

Patient 2:

CD4 250 cells/mm³; HIV RNA 70.000 copies/mL
5 year CVD risk < 0.1%
GFR < 60 ml/min

Events	No ART	cART component				
		ABC	TDF	ATV	LPV	EFV
AIDS						
Infection						
Malignancy						
Non-AIDS						
NARI						
CVD						
CKD						
ESLD						
NADM						

Risk <1.0% <2.5% <5% 5-10% 10-20% >20%

The first column of each table presents the risk of developing a particular event if there is no cART (benefit received from cART) and the following columns present risk estimates for the same choice of events yet attributable to particular cART components (treatment harm). Such presentation enables quick orientation to the optimal balance between risk and benefit for particular ARVs and in respect to individual patient characteristics. For example, while it is obvious that both of the patients would benefit from starting cART, the net benefit might be largely impaired by developing CVD for Patient 1 and CKD for Patient 2. This net benefit could be assured if certain cART components were avoided in these patients. Such a tool would also allow for the continuous evaluation of the risk and benefit balance in patients who are already on treatment allowing for guided treatment switches.

In order to increase the power of such analyses in the EuroSIDA study, fatal and non-fatal events will be combined together⁶⁷. However this approach might not be enough when rare events i.e. CKD or NADM are stratified according to single antiretroviral drug exposure or analyses restricted to groups of patients with high underlying risk (e.g. patients with liver disease or of older age). Therefore opportunities for multi-cohort collaboration are under investigation including possibilities for applying a uniform and standardized approach for end-point classification, especially for the underlying cause of death.

The increasing burden of non-AIDS-related comorbidities highlights the need to collect information with a longitudinal perspective and continue to focus on the underlying causes of death. Analyses from the EuroSIDA study presented in this thesis indicate that the proportion of unknown causes of death in the study is decreasing, while studies not using external validation of cause of death reported an increased proportion and incidence of unknown causes^{32,33}, also much higher than in the general population⁴⁰. With a decreasing number of deaths, an increasing proportion of

unknown causes may largely impair results. In this perspective, standardization of the process of determining the cause of death is indispensable¹¹³⁻¹¹⁵. The CoDe Project was launched in response to these needs, tested and modified based on practical experience. Further wide-spread of this method is planned and will significantly improve the utilization and quality of data collected. A crucial step in future analysis of cause-specific mortality will be to investigate its association with individual drugs and drug combination. Large collaborations would enable such analyses even for extremely rare end-points such as death related to antiretroviral treatment. Another vital step is to break down the 'other' category, which usually merges rarely observed causes of death that are of various pathomechanism and associated with different prognosis, like chronic pulmonary disease and diabetes. Such surveillance may also serve as an important element of pharmacovigilance and pharmacoepidemiology.

A remaining area of concern in cohort collaborations is the lack of a standardized approach for unifying data that has already been collected through longitudinal observation and before CoDe procedures were available. Using a range of routinely collected data, we have developed an algorithm that allows the underlying cause of death to be determined for all patients in the EuroSIDA study and we would like to validate this approach in other cohort settings. As the algorithm we propose uses just one element from the patient's history, namely an AIDS event, could be made considerably more comprehensive by incorporating information on non-AIDS morbidity. Non-AIDS events are a heterogeneous group of diseases for which survival in the HIV-infected population and causal relation with prior AIDS events has not yet been sufficiently studied, however the knowledge in this field is continuously increasing and could form a basis for the development of additional algorithms.

In the EuroSIDA study the algorithm unified data collected through the entire follow-up period and enabled the review of the incidence of cause-specific deaths over a long-term exposure to cART. Although we did not find that the risk of any non-AIDS-related death increases with prolonged exposure to treatment, due to limited power we were not able to exclude that for some specific antiretroviral drugs and in sub-groups of patients such risk exists. Recent estimates show that early cART initiation i.e. above 500 CD4 cell count would only have an impact on AIDS-free survival, but not on general survival¹¹⁶. Clearly more needs to be understood about cART risk and benefit balance, both in terms of when and with what to start treatment, and future research should take an individualized approach to antiretroviral therapy by adding a harm perspective to treatment choices.

English summary

Combination antiretroviral therapy (cART) has dramatically improved prognosis of HIV-positive persons. The effect of cART is achieved by suppressing the HIV replication, thereby allowing the person's immune system to regenerate, as depicted by an increasing CD4 lymphocyte count. This response leads to a marked decreased risk of developing an AIDS event and of all cause mortality. Among the various causes of death, the decline in non-AIDS death has been less pronounced than the decline in AIDS-related death. Consequently, the proportion of non-AIDS-related deaths - such as death due to cardiovascular diseases, accelerated liver cirrhosis or chronic kidney disease - has substantially increased in the last decade after cART was widely used. Besides the traditional risk factors for non-AIDS death, untreated HIV-infection, as well as adverse drug reactions from cART, may contribute to the risk of such deaths. The latter contribution is not likely to affect overall net cART benefit on a population level, but may translate into net harm for certain subgroups of persons. Identifying these subgroups and ensuring that research circumvents this problem is of clinical relevancy.

Although rational use of any treatment needs to be weighed against its potential risks, scientific methods for such assessment are limited. Hence, it is challenging to differentiate potential negative effects from the use of cART from those that HIV infection by itself may contribute with and that cART is potentially correcting. Despite these challenges, further investigation of whether - and if so in whom - long-term cART toxicities may limit the overall treatment benefit is necessary in order to further guide the most appropriate use of cART. This challenge was a major objective for this PhD thesis. In the first step we investigated whether it is possible to identify patients where the risk of serious adverse events is high and may compromise the beneficial effect of the drug (**Paper I**). We discuss how to balance risk and benefit in practical terms, using an example of the recently reported increased risk of myocardial infarction associated with abacavir. By translating relative risk to an absolute risk increase and number needed to harm we illustrated how to identify patients who could remain on abacavir-containing regimens and those for whom such an approach would result in an unacceptably high risk of myocardial infarction. We

created an on-line tool designed to help clinicians assess patient risk (www.cphiv.dk).

It is still unknown whether and how the adverse effects of cART translate into cause-specific mortality. Analysing changes in cause of death with cumulative exposure to treatment is necessary to further understand this issue. The EuroSIDA study, following large numbers of patients across Europe since 1994, is in a unique position to investigate it. Further investigation requires accurate assessments of the causal link between the disease or condition and death followed by establishing the underlying cause of death. Such procedures have been developed at the Copenhagen HIV Programme, namely the Coding Causes of Death in HIV (CoDe) project (**Paper II**) and in 2004 were included into the EuroSIDA study methods. For all events collected before 2004, an algorithm was developed and tested to unify accessible information and classify all deaths as either AIDS or non-AIDS-related (**Paper III**). Finally, we analysed the incidence of cause-specific death in relation to cumulative exposure to cART. We did not find any association between prolonged exposure to treatment and dying from any cause, after accounting for the latest CD4 count, viral load and other factors (**Paper IV**). This suggests that adverse effects of cART do not compromise the overall benefit received from treatment at a population level.

With increasing patient survival, new antiretrovirals approved for routine care and earlier initiation of antiretroviral treatment, surveillance of the rate of cause-specific deaths remains a vital tool in monitoring long-term treatment benefit and drug safety. Future research should continue to provide insight into groups of patients with pre-existing unfavourable risk profiles and focus on adding a harm perspective to treatment choice.

Danish summary (Resume på dansk)

Antiretroviral kombinationsbehandling (cART) har i væsentlig grad forbedret prognosen for HIV positive personer. Effekten af cART opnås ved at undertrykke replikationen af HIV og dermed muliggøre, at personens immunsystem regenererer, hvilket ses ved en stigning i CD4 celletallet. Dette respons fører til en markant nedsat risiko for udvikling af AIDS hændelse samt mortalitet af alle årsager. Blandt de forskellige årsager til død, har faldet i non-AIDS død været mindre udtalt end faldet i AIDS relateret død. Derfor er andelen af non-AIDS relateret død - såsom død pga. kardiovaskulære sygdomme, fremskreden levercirrose eller kronisk nyresygdom - steget væsentligt i de ti år efter udbredelsen af cART. Udover de traditionelle risikofaktorer for non-AIDS død, bidrager ubehandlet HIV infektion samt utilsigtede virkninger relaterede til cART, måske også til risikoen for non-AIDS død. Det er ikke sandsynligt, at den sidstnævnte faktor påvirker den samlede nettofordel af cART på befolkningsniveau, men den vil eventuelt kunne vise at have en, i sig selv, skadelig påvirkning hos bestemte delgrupper i befolkningen. At få identificeret disse delgrupper samt sikre, at forskningen kommer rundt om dette problem, er af klinisk relevans.

Selvom de potentielle fordele og ulemper skal afvejes ved rationel anvendelse af alle former for behandling, er de videnskabelige metoder til vurdering af dette begrænsede.

Det er derfor en udfordring at differentiere mellem den potentielle negative effekt ved brugen af cART og den negative effekt der kunne være afledt af HIV infektionen selv og som cART potentielt vil kunne korrigere. Til trods for disse udfordringer, er det nødvendigt at gennemføre flere undersøgelser af om, og i bekræftende fald, hos hvem, de langsigtede cART bivirkninger vil kunne begrænse den samlede behandlingsfordel, for således at kunne vejlede om den bedst mulige måde at administrere cART. Denne udfordring var hovedformålet for denne PhD afhandling.

Først undersøgte vi, om det er muligt at identificere patienter hos hvem risikoen for en alvorlig utilsigtet hændelse er høj og eventuelt vil kunne kompromittere den gavnlige effekt af lægemidlet (**Artikel I**). Vi gør rede for, hvordan fordele og ulemper kan afbalanceres rent praktisk og anvender det for nyligt rapporterede eksempel omkring den

øgede risiko for myokardieinfarkt associeret med abacavir.

Ved at omsætte relativ risiko til en absolut risikoøgning og number needed to harm, viste vi hvordan man identificerer de patienter, der kunne fortsætte behandlingen med regimer indeholdende abacavir og de patienter for hvem det ville betyde en uacceptabel høj risiko for myokardieinfarkt. Vi udviklede et on-line værktøj til at hjælpe klinikere med at vurdere patientrisikoen (www.cphiv.dk)

Det vides stadig ikke om, og hvordan, de utilsigtede hændelser relaterede til cART udmønter sig til årsagsspecifik mortalitet. Det vil være nødvendigt at analysere ændringerne i årsag til død ved kumulativ behandling for nærmere at kunne forstå dette spørgsmål.

EuroSIDA studiet, der siden 1994 har fulgt og stadig følger en stor gruppe patienter over hele Europa, står i en unik position til at undersøge dette. Yderligere undersøgelser kræver præcise vurderinger af kausal sammenhæng mellem sygdommen eller lidelsen og død efterfulgt af en fastsættelse af den underliggende dødsårsag. Sådanne procedurer er blevet udviklet af Copenhagen HIV Programme igennem projektet Coding Causes of Death in HIV (CoDe) (**Artikel II**) og i 2004 blev disse procedurer inkluderet i EuroSIDAs forsøgs-metoder. Der blev, for alle hændelser indsamlet før 2004, udviklet og testet en algoritme til at indsamle tilgængelig information og klassificere alle dødsfald som enten AIDS eller non-AIDS relaterede (**Artikel III**). Til slut analyserede vi incidensen af årsagsspecifik død i relation til kumulativ behandling med cART. Vi fandt ingen association mellem længerevarende behandling og død af alle årsager, efter at korrigeret rede for det seneste CD4 tal, HIV-virusmængde samt andre faktorer (**Artikel IV**). Dette indikerer, at utilsigtede hændelser ved cART ikke kompromitterer den samlede fordel ved behandlingen på befolkningsniveau. Med øget patientoverlevelse, nye antiretrovirale lægemidler godkendt til standardbehandling og en tidligere påbegyndelse af antiretroviral behandling, forbliver overvågning af antallet af årsagsspecifikke dødsfald et vigtigt redskab i monitoreringen af langsigtede behandlings-virkninger og lægemiddel-sikkerhed. Fremtidig forskning bør fortsætte med at give indsigt i patientgrupper med præsisterende ufordelagtig risikoprofil og fokusere på at tilføje et bivirkningsperspektiv til valget af behandling.

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