Risk of AIDS, Death or Pancreatitis according to Anti-Retroviral Therapy among HIV infected Patients

PH.D. THESIS
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This thesis was carried out in the period 2004-2007 while I was employed at the Copenhagen HIV Programme (CHIP) in Hvidovre University Hospital in Copenhagen.

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Thesis submitted to University of Copenhagen on March 31st, 2008. The defence is planned to take place on June 20th, 2008. Official Opponents: Court Pedersen, Vidar Ormaasen, Jan Gerstoft. Tutors: Nina Friis-Møller, Ole Kirk and Jens D. Lundgren. Statisticians: Colette Smith, Amanda Mocroft and Andrew Phillips. Correspondence: Christian H. Olsen, e-mail: cho@dahlnet.dk
AIDS  Acquired Immune Deficiency Syndrome
ART  AntiRetroviral Therapy
BMI  Body Mass Index
cART  combination AntiRetroviral Therapy
CDC  Centre of Disease Control and Prevention, NIH
CD4  T-helper immune system cell
CHIP  Copenhagen HIV Programme
CI  Confidence Interval
CoDe  Causes of Death
Cps  Copies (of HIV-RNA)
D:A:D  Data Collection on Adverse Events of Anti-HIV Drugs
DC  Drug Conservation
EuroSIDA  European study of ‘Syndrome d'Immuno-Deficience Acquis’
FDA  Food and Drug Administration (US drug regulatory)
FU  Follow Up
HAART  highly active antiretroviral therapy
HBV  Hepatitis B virus
HCV  Hepatitis C virus
HIV  Human Immunodeficiency Virus
HIV-1  Human Immunodeficiency Virus – type 1
HIV-RNA  see HIV and RNA – i.e. HIV viral load / virologic status
IDU  Injecting Drug Use
IQR  Inter Quartile Range
IR  Incidence Rate
IRR  Incidence Rate Ratio
NNRTI  Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI  Nucleoside Reverse Transcriptase Inhibitor
OI  Opportunistic Infection
PI  Protease Inhibitor
PY  Person Years
PYFU  Person Years of Follow Up
RCT  Randomised Controlled Trial
RNA  Ribo Nuclein Acid
SAS  Statistical Analysis System
SMART  Strategies for Management of Anti-Retroviral Therapy
TI  Treatment Interruption
VS  Viral Suppression
PUBLICATIONS

This Ph.D. thesis is based on the following manuscripts:


Background

The purpose of treating HIV-infected persons with antiretroviral treatment (ART) is to prevent death and morbidity from HIV at the lowest possible level of adverse events and thereby ensuring that treatment benefits far outweighs risks at patient and population level.

The introduction of combination antiretroviral therapy (cART) in the mid 1990s in industrialised countries has led to dramatic reductions in morbidity and mortality amongst HIV-infected individuals in the developed world.\textsuperscript{4,5} However, as treatment is required for life and only around a decade of cART experience is available at present, it is unknown whether risks of treatment will remain low and beneficial effects of cART will last, with continued low morbidity and mortality rates.

Prior to the widespread use of cART, clinical trials had to show that a new candidate drug resulted in a reduction in risk of new AIDS events or deaths. Since 1997, drug regulatory authorities indicated that it was sufficient to show that a new drug resulted in sustained suppression of plasma HIV-RNA and to rises in peripheral blood CD4 lymphocyte count.\textsuperscript{6} This was based on evidence from trials, mainly of mono and dual nucleoside therapy regimens, indicating that the effect of a drug regimen on HIV-RNA levels and on CD4 cell counts was strongly correlated with the effect of the regimen on risk of clinical AIDS events or death, even though these markers were known to be far from complete surrogates for the clinical response.\textsuperscript{7-14} Subsequent studies of patients on cART have indicated that the extent of changes in HIV-RNA and CD4 cell count induced by cART correlates with new clinical AIDS or death\textsuperscript{15-21}. Since this change in drug regulatory requirements, several drugs have been licensed for use on the basis of evidence for plasma HIV-RNA and CD4 cell count changes alone.

The assumption underlying the trust in using surrogate markers, and not clinical endpoints, to evaluate new drugs, is that the relationship between the HIV-RNA or CD4 cell count, and risk of clinical disease, continues to hold true also for newer drugs, and that there is no additional effect of such drugs, which leads to a higher or lower AIDS/death risk for given HIV-RNA/CD4 cell count levels when compared with others.\textsuperscript{22,23}

Hence, the aim of (I) was to evaluate the general assumption that the link between the surrogate markers CD4 or HIV-RNA and new clinical AIDS or death holds true across different cART regimens and specific ART drugs.

In clinical practice, it is difficult for many patients to adhere to continuous, long-term therapy.\textsuperscript{24} Up to 50% of patients starting cART discontinue part or all of their initial regimen within a year.\textsuperscript{25} This is often a consequence of the patient’s own choice,\textsuperscript{25-29} but can also be related to the drug toxicities and metabolic side effects associated with cART use.\textsuperscript{30-34} These toxicities and side effects may increase the risk of interruption or stopping of therapy.\textsuperscript{35}

Until recently there were few published data on treatment interruption (TI) and the association with disease progression to
AIDS or death in clinical practice, and results were not entirely consistent\textsuperscript{36-42}.

Thus, the aim of (II) was to assess the incidence of TIs and risk factors for TI, as well as the risk of AIDS and death associated with TIs. Furthermore, the aim was to assess the risk factors for disease progression within the TI group.

Use of antiretroviral drugs, with or without TI, have been reported to be associated with an increase in the incidence of cardiovascular disease\textsuperscript{43-45}, hepatotoxicity\textsuperscript{46, 47} and renal disease\textsuperscript{48, 49}. Cases of pancreatitis in HIV-infected patients were also reported in the cART era\textsuperscript{50-54}. Previous studies have shown incidence rates of pancreatitis amongst HIV-infected people receiving various different antiretrovirals from 0.03\textsuperscript{51} to 1.95\textsuperscript{52} per 100 person-years of follow-up (PYFU).

It has been proposed that the nucleoside reverse transcriptase inhibitor (NRTI) class of antiretroviral in general, and the NRTIs stavudine (d4T) and didanosine (ddI) in particular, may lead to increased mitochondrial toxicity\textsuperscript{55, 56}, which in turn may lead to a higher risk of pancreatitis\textsuperscript{51, 53, 55, 57}. However, other studies have not found such a link, with a recent study by Guo et al of more than 4,000 patients finding no association between different antiretroviral drugs and the incidence of pancreatitis\textsuperscript{52}.

Therefore, the aim of (III) was two-fold, firstly to investigate the incidence of pancreatitis amongst a large, well characterised cohort of HIV-infected individuals and secondly to assess which factors were associated with increased rates of pancreatitis, focusing on ART use in general, and d4T and ddI use in particular.
Objectives

The objectives of this thesis were, based on data from the EuroSIDA study, to

- determine the risk of AIDS or death at latest HIV-RNA and CD4 count levels, for HIV infected people on different cART regimens, thereby investigating if HIV-RNA and CD4 count were good surrogate markers for AIDS or death also for newer drugs.

- determine the incidence of and the risk factors for TI, and the risk of AIDS or death associated with TI. Further to assess the risk factors for AIDS or death within the TI group.

- determine the incidence of pancreatitis and investigate which factors were associated with increased rates of pancreatitis, focusing on ART use in general, and d4T and ddI use in particular.
METHODS
STUDY DESIGN
The EuroSIDA study is a prospective, observational multicohort study of patients with HIV-1 infection across Europe. The study was designed to assess the impact of antiretroviral drugs on clinical disease progression in the general population of HIV-infected patients living in Europe. All manuscripts included in this thesis were performed within the EuroSIDA study.

Study Population
In EuroSIDA, seven cohorts of patients were recruited in the period from 1994 to 2007; figure 1. To include a representative subset of patients followed at the specific centres, patients were enrolled consecutively within specified time intervals, as they were seen in the outpatient clinic, irrespective of disease or treatment status.

Figure 1. Patients included by cohort in the EuroSIDA study

At the time of analysis for all manuscripts, respectively five cohorts with 9,810 patients in 72 centres (I), six cohorts with 11,231 patients in 82 centres (II), and seven cohorts with 14,200 patients in 92 centres (III) were included in the study.

Data Collected
Information from patient notes was provided on a standardized data collection form at baseline and every 6 months thereafter. The latest version of the data form is available at the coordinating centre website (www.cphiv.dk) and can be found under the EuroSIDA study documents.

At enrolment and at every follow-up visit, CD4 counts and HIV-RNA measurements and other laboratory parameters routinely taken since the last follow up were reported, including potential confounding factors described in detail in statistical methods. The dates of starting and stopping every antiretroviral drug together with dates of AIDS-defining illnesses were recorded using the 1993 clinical definition of AIDS from the Centers for Disease Control and Prevention (CDC).

Information on date and cause(s) of death was collected from the start of the EuroSIDA study in 1994, over time with extensions and modifications to specified causes of interest. Since January 2004 information on causes of death for patients under active follow up in EuroSIDA has been reported on a CoDe fatal case report form (CRF); also available on the above mentioned website.

Endpoints
The primary endpoint in manuscript (I) and (II) was either new clinical AIDS events or death; of note, a CD4 cell count below 200 cells per mm$^3$ was not counted as an AIDS event. In manuscript (II) also TIs were used as a separate endpoint, defined as an interruption of all drugs in the cART regimen for 3 months or more. In manuscript (III) the endpoint used was clinical pancreatitis.

**Treatment**

Unless otherwise stated, cART is defined as antiretroviral treatment (ART) regimens containing three or more drugs from one or more of the three widely used drug classes, namely Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs). For the purpose of the analysis in the different manuscripts, cART was more specifically defined. In manuscript I and II, cART was defined as an ART regimen containing either a single (or ritonavir-boosted) PI, a single NNRTI or the NRTI drug abacavir (referred to as 'third' drugs in manuscript I) plus two NRTIs (referred to as the regimen 'backbone' in manuscript I).

In manuscript III, all patients on ART, and not just patients on cART, were included, as the drugs possibly being associated with an increased risk of pancreatitis were often given as mono- or dual drug therapy.

**Quality Assurance**

At enrolment training of study personnel at each site was performed. Standardised instructions for completion of the enrolment and follow up forms were provided to the sites.

Standardised control of data entry was performed at the coordinating centre, with querying for outlying values. The data provided by the centres was further checked at yearly monitoring visits where all clinical events were checked. Further to ensure a high data quality, cross-checking of data against clinical chart notes from ten percent randomly selected patients under active follow up were routinely performed. All cases of AIDS, death and pancreatitis eligible for analyses were validated by source verification 1,15,61.

**Informed Consent and Ethical Approvals**

Informed consent and ethics committee approvals were obtained according to national guidelines.

**STATISTICAL METHODS**

The statistical analyses applied were standard methods for analyses of observational data, summarised in the following. Analyses were performed using Statistical Analysis System (SAS) version 8.2 or 9.1 (SAS Institute Inc, Cary, NC).

**Descriptive Statistics**

Descriptive statistics, using proportions, median and inter quartile range (IQR), were used to describe the sample study populations at baseline in manuscripts I, II and III. Comparisons
between relevant groups were performed using non-parametric tests such as the Wilcoxon test for continuous variables and chi-squared tests for the comparisons of proportions.

**Incidence Rates**

The incidence rate (IR) of an event was calculated as the number of events divided by the total number of PYFU, i.e. for AIDS or death (I and II), TIs (II) and pancreatitis (III). Estimates for IRS were given with 95% confidence intervals assuming that the events followed a Poisson distribution.

**Poisson Regression Analyses**

Poisson regression models were used to investigate which factors were associated with occurrence of the endpoint event(s). Poisson models are fitted to estimate changes in incidence over time with the advantage over other survival analyses in that repeated endpoints can be assessed. In I and II the same patient could contribute with more than one endpoint event, whereas only the first pancreatitis event in the follow-up period per individual was considered (III). The models were used to assess estimates for the IRRs of the dependent variable - i.e. the endpoint events AIDS/death in I and II and pancreatitis in III, for the different groups defined by the independent variables - i.e. specific drugs or drug combinations in I, TI or non-TI group in II, and cumulative ART use according to category in III.

The estimates were assessed in both univariable and multivariable models after adjusting for all known and recorded confounding factors (I, II, III); in II using a stepwise procedure without and with adjustment for latest CD4 count and/or HIV-RNA. The estimates were given with 95% confidence intervals. Statistical significance was denoted at the \( p \leq 0.05 \) level unless otherwise stated.

**Potential confounding factors**

Potential confounding factors were: gender, age, HIV transmission category, ethnicity, geographical region, prior AIDS, HBV/HCV status, calendar year, time from starting therapy, time on last regimen, current/prior ART regimen, cumulative ART use as well as CD4 counts and HIV-RNA. In the main analyses, the following variables were investigated as time-updated values: HBV/HCV status, diagnosis of AIDS, CD4 cell count and HIV-RNA (II), and cumulative use of ART regimens of interest (III). In addition, sensitivity analyses were carried out in (I) and (III) using latest measured value of CD4 and HIV-RNA within three months prior to the endpoint event investigated to ensure that only current values were used in the analyses; i.e., as in (II), if a CD4 was more than 3 months old it was considered to be out of date and no longer relevant to the immediate risk of AIDS or death.

**Follow Up Time**

Time of FU began at baseline of the analyses, i.e. for I and II at the date of starting cART, and for III on June 2001 when prospective data collection on pancreatitis started or the date the patient first visited the HIV clinic, whichever occurred later.
In manuscript I, patients contributed to the analysis during periods of time in which they were on a cART regimen. In manuscript II patients were only included, if they had a CD4 cell count and HIV-RNA measured in the 6 months prior to starting cART, which should be during prospective FU. For the analysis on pancreatitis (III), patients could be either on cART, ART or not on treatment, using a 6-month time lag allocating possible events to the latest used regimen until discontinuation plus 6 months, re-assigning events thereafter to the switch regimen.

For all analysis performed in the manuscripts (I, II, III), patients stopped contributing to observation time on the date that the last FU visit form was completed or on the date of an endpoint event, whichever happened sooner.

**Sensitivity Analyses**

Various sensitivity analyses were performed to test the robustness of the primary analyses in all manuscripts. Sensitivity analyses were conducted using AIDS or death as separate endpoints (II). Analyses with different lag times (I, II, III) were carried out to assess if assumptions between time from specific drug exposure (I), TI (II) or HIV-RNA and CD4 measurement (I, II, III) to endpoint, were influencing the results. Further sensitivity analyses of (sub)groups of patients (I, II, III) with specified strict immunologic (I), virologic (I), treatment (I, III) and endpoint (II, III) criteria were performed to assess if the associations found in the primary model, could be reproduced, when assumptions were changed.
**Results**

**Risk of AIDS or death according to latest CD4 and HIV-RNA**

A total of 6,814 EuroSIDA patients starting cART contributed observation time to the analysis. Of these, 4,773 patients (70%) had used nucleoside mono- or dual therapy prior to starting cART. The median date of starting cART was March 1997 and the median (IQR) age was 36.8 years (32.1-43.8).

The median (IQR) CD4 cell count and HIV-RNA at the start of cART (i.e. within 3 months after starting cART) were 184 cells/mm$^3$ (78–315) and 26,000 cps/mL (3,100–129,000), respectively. The median (IQR) latest measured CD4 cell count and HIV-RNA was 353 cells/mm$^3$ (IQR 206-539), and 199 cps/mL (IQR 49-1,900), respectively.

A total of 900 events of AIDS or death, of which 125 were deaths, were observed in 22,766.6 PYFU, corresponding to 4.0 AIDS or death events per 100 PYFU and 0.55 deaths per 100 PYFU. The IRs of AIDS or death differed markedly according to the latest CD4 cell count or HIV-RNA strata with higher incidences observed in groups of patients having lower CD4 counts or higher HIV-RNA levels; given for HIV-RNA in figure 2. Patients with a latest CD4 count below 50, between 50 and 99, between 100 and 199, between 200 and 349 or above 350 cells/mm$^3$, had IRs (95%CI) of AIDS or death of 33.3 (29.7-36.9), 14.3 (13.2-15.5), 8.3 (7.1-9.5) and 1.0 (0.8-1.2) per 100 PYFU, respectively.

**Figure 2**

IRs of AIDS/death with 95%CIs according to latest HIV-RNA levels (cps/mL) and specific drugs in the cART regimen
Patients on different cART regimens appeared to have similar IRs of AIDS or death for a given strata of CD4 count or HIV-RNA, regardless of the nucleoside pair or specific third drug used; figure 2.

This was tested formally in a multivariable Poisson regression model analysis, where the IRR of AIDS or death for each risk factor (including latest CD4 count and HIV-RNA) was found after adjusting for the effects of all the others; figure 3.

For the different nucleoside pairs and specific third drugs all IRR estimates were close to one. The relatively narrow 95% confidence intervals were all overlapping 1, meaning that the risk of AIDS or death (for a given HIV-RNA and CD4 cell count) was fairly similar to the reference regimen, regardless of which nucleoside pair or specific third drug were used as part of the cART regimen.

Still, even after adjusting for other confounding factors, the IRRs of AIDS or death differed markedly according to the latest CD4 cell count or HIV-RNA strata with the highest risk observed in groups of patients having lower CD4 counts or higher HIV-RNA levels. Further patients with 10 years older age, one year later start or shorter duration of cART or of the current specific third drug had a higher risk of AIDS or death.
The multivariable analysis was adjusted for all factors shown in the figure plus for HIV transmission group and prior AIDS. IDV=indinavir, SQV=saquinavir, * = only counted as third drug, ZDV=zidovudine, 3TC=lamivudine, d4T=stavudine, ddI=didanosine, r = ritonavir, and x/r = drug x boosted with ritonavir.

The reference group for the nucleoside pairs is the group of other nucleoside pairs, and the reference for specific third drugs is indinavir.

The reference group for the latest CD4 cell count is the group of patients having latest CD4 cell counts of 500 cells/mm$^3$ or more, and the reference group for HIV-RNA is the group of patients having a latest HIV-RNA of 500,000 cps/mL or more.
In conclusion, although the IRR of AIDS/death varied across different latest CD4 count and HIV-RNA levels, the IRR of AIDS or death did not appear to differ significantly for various drugs in the cART regimens for patients having the same latest CD4 cell count or HIV-RNA levels. This implies that the markers currently used for gauging patients’ risk of clinical progression can be interpreted similarly, regardless of which regimen the patient is receiving.

Risk of AIDS or death at cART interruption

A total of 3,811 EuroSIDA patients qualified and contributed observation time to the analysis. Of these, 26% had a prior diagnosis of AIDS and 26% were ART naive prior to cART. When patients started cART with the median (IQR) time being July 1997 (February 1997 to July 1999), they had a median (IQR) age of 37.6 (32.9–44.6) years, a CD4 of 226 (114–347) cells/mm³, and a HIV-RNA of 23,000 (2,600–112,000) cps/mL.

A total of 879 patients, corresponding to 23%, experienced one or more episodes of interruption of their cART regimen for three months or more, i.e. TI(s). Further, 1243 TIs in 20,845 PYFU was observed, giving an IR of 6.0 per 100 PYFU (95% CI 5.7–6.3).

In the time-updated multivariable analysis, several factors were associated with TI; figure 4. Being a female, coming from central and northern Europe, starting on a single NNRTI, being HCV positive, having a previous AIDS diagnosis and having a higher latest HIV-RNA or a higher latest CD4 count, were associated with a higher risk of experiencing one or more TIs, while being older were associated with a lower risk.

Figure 4
Adjusted IRRs for risk factors associated with TI
The multivariable analysis with 95% CIs given was adjusted for all factors shown in the figure; * variable included as time-updated.

A total of 406 AIDS/deaths were observed in 13,192 PYFU, i.e. 3.1 events per 100 PYFU (95%CI 2.8-3.4), of which 251 (62%) were deaths, corresponding to a mortality rate of 1.9 events per 100 PYFU.

Patients in the TI group had overall a significantly higher incidence of AIDS or death compared to patients in the non-TI group; with respectively an IR of 4.7 (95%CI of 3.7-5.8) versus an IR of 2.8 (95%CI of 2.5-3.2).

In the univariable analysis, patients in the TI group had an IRR of 1.66 (95%CI 1.30-2.13) of AIDS or death compared with patients in the non-TI group.

After adjustment for confounding factors at baseline in the multivariable analysis, the IRR increased to 2.63 (95%CI 2.01-3.44). The baseline factors were exposure group, HBV and HCV status, prior AIDS, cART regimen started, age, date of cART initiation, and prior antiretroviral therapy.

After further adjusting for latest CD4 cell count, the IRR dropped to 1.45 (95%CI 1.11-1.91), while adjusting for latest HIV-RNA (without adjusting for latest CD4 count) resulted in an IRR of 1.33 (95% CI 1.00-1.77). Adjusting for both latest CD4 cell count and latest HIV-RNA, reduced the IRR to 1.14 (95%CI 0.86-1.51).
Hence, the increased risk of disease progression after interruption of cART seemed to be explained by changes in the latest measured HIV-RNA and CD4 cell count following the interruption of cART.

Among patients in the TI group, patients with a CD4 cell count below 200 cells/mm$^3$ had a 3-fold higher incidence of AIDS or death (i.e. IRR=2.94, 95%CI 1.65–5.26) compared to patients with a CD4 cell count between 201 and 350 cells/mm$^3$, while patients with CD4 cell counts above 350 cells/mm$^3$ had a 4-fold lower incidence (i.e. IRR=0.23 (95%CI 0.13–0.39); figure 5. Similarly, patients with a log higher HIV-RNA had a higher incidence of AIDS or death with an IRR of 1.36 (95% 1.14–1.62). Belonging to the transmission group of injection drug use, being HCV antibody positive, having had prior AIDS or having started cART one year later were not associated with an increased risk of AIDS or death, while being ten years older were associated with an independent increased risk of clinical progression.

Figure 5

Factors associated with AIDS or death in the TI-group

- Exposure Group (IDU)
- HCV* (positive)
- Prior AIDS (yes)
- Age (10 years older)
- Start of cART (1 year later)
- CD4 cell count*
  - ≤200
  - 201–350
  - >350
- HIV-RNA measure* (log higher)

Figure 5 text

The multivariable analysis with 95% CIs given was adjusted for all factors shown in the figure; * variable included as time-updated.

In conclusion, TIs were common in the clinical practice for patients on cART. The risk of progression to new clinical AIDS events or death was more than two-fold higher for people who interrupted all therapy for at least 3 months compared with people who did not. This difference disappeared after adjusting for latest HIV-RNA and CD4 count suggesting that the majority of the increased risk of a TI
can be explained by changes in CD4 cell count and HIV-RNA following a TI.

Risk of pancreatitis in relation to ART

A total of 9,678 patients in EuroSIDA were eligible for inclusion in the analyses and followed for a median (IQR) of 4.3 years (2.2–4.9). The median (IQR) age was 39.8 (34.5–46.8) years at baseline. A total of 43 pancreatitis events were observed in 33,742 person years of FU, corresponding to an IR of 1.27 (95%CI 0.89–1.66) per 1,000 PYFU.

Baseline characteristics were similar for patients experiencing pancreatitis when compared to the entire study population, e.g. age, gender, ethnicity, transmission category, region, HBV/HCV, BMI, haemoglobin, and cumulative exposure to ART, except for a significant lower median (IQR) CD4 of 272 (176 – 427) versus 415 (266 – 593) cells/mm$^3$, a higher median HIV-RNA 1140 (49 – 18900) versus 141 (49 – 3800) cps/mL, and a higher proportion having experienced prior AIDS 41.9% versus 29.1%.

Comparable IRs of pancreatitis was found according to length of exposure to ART and type of antiretroviral combination; figure 6.

Figure 6
IRs of pancreatitis according to length of exposure to specific ART regimens

In a univariable Poisson regression analysis, there was evidence that lower CD4 counts, higher HIV-RNA, being HCV positive, and coming from the central region of Europe were all associated with increased risk of pancreatitis at a p ≤ 0.10 level.
Further, later calendar years or cumulative ART use were not associated with an increased risk.

However, in the multivariable analysis, adjusted for factors significantly (p ≤ 0.10) associated with pancreatitis in the univariable model, only the baseline CD4 count was independently associated with pancreatitis. For every 100 cells/mm³ higher CD4 count, the risk of pancreatitis decreased by 22% (IRR=0.78; 95%CI 0.66-0.93; p=0.002); figure 7. Although not significant at the 5% level (IRR=1.10 per one log HIV-RNA cps/mL higher with a 95%CI 0.99-1.21, p=0.09), our results suggested that higher HIV-RNA was associated with a higher risk of pancreatitis; figure 7. There was no significant difference in IRRs for the different types of ART regimens; figure 7.

Figure 7
Adjusted IRR of pancreatitis (with 95%CIs) according to ART, HIV-RNA and CD4 count

The multivariable analysis with IRRs and 95% CIs given was adjusted for all factors shown in the figure plus HCV status and region.
* = Included as time-updated variable.

Fitting the latest (in stead of baseline) CD4 cell count and HIV-RNA as time-updated variables led to virtually identical results (data not shown) to those presented in the main analysis. This meant that a latest measured 100 cells lower CD4 count and a log higher latest HIV-RNA were associated with a higher incidence of pancreatitis at the 5% significance level.
In conclusion, a low incidence of pancreatitis was found within the EuroSIDA study, the IR being constant over time in the years 2001-2006. During the observation period, there was no association between risk of pancreatitis and specific or combinations of antiretrovirals. The risk of pancreatitis was increased for those with lower CD4 counts, and there was some evidence of an association with higher HIV-RNA, suggesting that people with more advanced disease are at greater risk.
DISCUSSION

This thesis builds on results from the observational study, EuroSIDA, in the first decade of HIV patients on cART. The conduct of longitudinal observational studies allows per design for assessment of the incidence of multiple endpoints over years time, including new clinical AIDS events, death and even relatively rare events, such as pancreatitis.

This discussion focuses on placing our findings in the context of updated evidence, and to draw a line from the risk of AIDS or death for patients being on or off cART (I and II) over the risk of AIDS or death according to latest CD4 and HIV-RNA (I and II). Further to assess the risk of cART interruption (II) and the risk of pancreatitis according to cumulative exposure to ART (III).

Finally, the strength and limitations of the designs of the studies (I-III) performed within the EuroSIDA study will be discussed.

Risk of AIDS or death for patients being on or off cART

The overall beneficial effect of taking ART, and in particular cART, for patients with HIV has been observed in both observational and randomised controlled clinical trials. Observational studies, following patients with access to treatment in industrialised countries have demonstrated dramatically declining IRs of AIDS or death since the introduction of cART, mediated via virological suppression and improvement of immune function. With the increasing use of ART regimens in the HIV population and in particular since the introduction of cART in 1996, the absolute risk of death in HIV infected persons followed in the EuroSIDA study declined from approximately 30 per 100 PYFU before 1996 to less than 3 per 100 PYFU from 2001 and onwards.

In study I, the IR of AIDS or death was 4.0 per 100 PYFU and in II, the IR was 3.1 events per 100 PYFU. These IRs are comparable to IRs found in other cohorts and cohort collaborations reporting on the same period, with recent IRs of AIDS or death found in EuroSIDA and other cohorts tending to be even lower than in the beginning of the cART era.

In study II, patients in the TI group had overall a significantly higher incidence of AIDS or death compared to patients in the non-TI group; with respectively an IR of 4.7 and 2.8 per 100 PYFU. The IRs of AIDS or death, also when looking at the TI and non-TI group separately, were quite similar in the ICoNA study to what was found in our study.

In the SMART RCT study published in late 2006, the IR of opportunistic disease (as described in the supplementary appendix of the SMART study protocol) or death from any cause were 3.3 per 100 PYFU in the drug conservation (DC) group, comparable to the TI-group, and 1.3 per 100 PYFU in the viral suppression (VS) group, comparable to the non-TI group.

When comparing the incidence in the RCT study to the results in our population study, the non-TI group in our study seemed to
have a higher IR of AIDS or death. This probably largely reflects that our population had lower median CD4 counts and higher median HIV-RNAs at baseline. This is also true when comparing the TI groups in SMART and EuroSIDA, to some extent probably also reflecting the generally poorer outcome observed among patients included in an observational as opposed to a RCT setting.

After adjusting for all known and measured confounding factors other than latest CD4 count and HIV-RNA measure, patients experiencing TIs in our study had a 2.63-fold (95%CI 2.01-3.44) increased incidence of AIDS or death compared to patients continuously on cART. These findings are comparable to the multivariable hazard ratio of 2.75 (95%CI 1.14-6.65) found in the ICoNA study 36, and the hazard ratio for clinical progression of 2.6 (95% CI 1.9 - 3.7), comparing the DC with the VS group in the SMART study 71.

In conclusion, the IRs of AIDS or death for both patients on and off cART (I and II) were comparable to what other large observational and RCTs have found. Patients interrupting cART generally had more than two-fold higher risk of AIDS or death compared to patients continuously taking cART regimens adjusting for all other factors than latest CD4 count and HIV-RNA measure. Our study in particular contributed to published evidence in that among patients experiencing TIs, which is common in clinical practice, the risk of AIDS or death were significantly higher among patients with latest CD4 cell counts lower than 350 cells/mm³ or one log higher latest HIV-RNA measures. These findings suggest that patients should stay continuously on cART once started, and that patients having TIs should restart cART, when the CD4 count drops below 350 cells/mm³.

Risk of AIDS or death according to latest CD4 and HIV-RNA

Our results in I showed that the IR of AIDS or death was approximately 33 per 100 PYFU both for patients with a latest CD4 cell count below 50 cells/mm³, and for those with a latest HIV-RNA measure above 500,000 cps/mL. In comparison, the IR of AIDS or death was approximately 1 for patients with a latest HIV-RNA measure below 500 cps/mL and for those with a latest CD4 cell count above 350 cells/mm³. The risk of AIDS or death according to latest CD4 count and HIV-RNA levels found in the EuroSIDA study are in line with data reported from other cohort studies in Europe and North America 66,68.

Among the TI group in study II, patients with a latest CD4 cell count of 200 cells/mm³ or lower had a 3-fold higher incidence of new AIDS/death, with most of this increased incidence being explained by the group of patients with CD4 below 50 cells/mm³, while patients with a latest CD4 cell count above 350 cells/mm³ had a 4-fold lower incidence of AIDS or death, when compared with patients with a latest CD4 cell count between 201 and 350 cells/mm³.

In I, the adjusted IRRs of AIDS or death for a given, latest HIV-RNA or CD4 cell count did not appear to differ for patients on different drugs for which some direct evidence of clinical efficacy exists (zidovudine 72, didanosine 73,74, lamivudine 75,
indinavir \textsuperscript{76}, ritonavir \textsuperscript{77}, saquinavir \textsuperscript{78}), and for those on widely used newer drugs, for which there was no such evidence. Our results seemed quite robust, also when restricting data analysis to person time where a beneficial change in immunologic and virologic levels could be attributed to a specific third drug and when specifically looking at patients having a clear, immediate effect of specific drugs in the cART regimen.

Although the risk of AIDS or death was the same for a given, latest CD4 cell count and HIV-RNA, regardless of the specific antiretroviral drug being used, it is important to note that these results do not suggest that the regimens assessed have equal clinical efficacy. Several published randomized clinical trials have shown that different regimens have different capacities to decrease the HIV-RNA and raise the CD4 cell count, which is likely to translate into differences in clinical efficacy for different drug regimens.

Likewise in study II, the increased risk of AIDS or death among patients experiencing a TI seemed to be explained by the latest CD4 count and HIV-RNA measure, since the IRR decreased to approximately one when adjusting for all confounding factors including CD4 and HIV-RNA; results being in line with those found in the ICoNA study \textsuperscript{36}. These findings suggest that latest CD4 cell count and HIV-RNA seems to be good surrogates of risk of AIDS or death (in I and II) when used in combination as a measure of the treatment effect both in TI and non-TI. Although they are the best surrogate markers available at present time, the CD4 count and HIV-RNA measure are probably not ideal surrogate markers. To be ideal the surrogate marker should be a correlate of the clinical endpoint, being on the only causal pathway of the disease process, and further the intervention’s entire effect on the clinical endpoint should be mediated through its effect on the surrogate \textsuperscript{23, 79}.

The use of HIV-RNA and CD4 cell counts, has been reported not to provide exact estimates of the treatment effect on clinical progression of HIV disease to AIDS and/or death \textsuperscript{9, 80}. CD4 count and HIV-RNA measures can be highly variable \textsuperscript{81-83} and discordant responses have been reported \textsuperscript{18, 84, 85}. Previous analyses from the EuroSIDA study have suggested an effect of ART over and above the effect mediated via CD4 cell count and HIV-RNA \textsuperscript{86, 87}. Equally, the results from the SMART study implied that the full effect on ART interruption was not fully captured by latest CD4 count and latest HIV-RNA measure. Other studies have also suggested, that antiretroviral drugs might have an increasing or decreasing effect on risk of AIDS and/or death, not mediated by the effect of the drugs on HIV-RNA and CD4 cell count \textsuperscript{22, 23, 88}. This could imply that the predictive value of latest CD4/HIV-RNA is not ideal, and that there are benefits of ART, which are mediated via other mechanisms \textsuperscript{88-91}.

When validating a surrogate marker it is however important that the follow up time is longer than the progression time from surrogate marker to the clinical endpoint. In general, this is not fulfilled in trials designed to examine the short-term effects of drugs on risk of AIDS and/or death by HIV-RNA and CD4 cell count measurements.
It is worth noticing that in contrast to our findings the SMART study found an increased risk of death and opportunistic disease in the DC versus the VS group, even after adjustment for latest CD4 count and HIV-RNA, with a hazard ratio of 1.5 (95%CI, 1.0-2.1). The reasons for the remaining excess risk are not known. Recent findings suggest however that apart from the absolute levels of the latest CD4 count and HIV-RNA, the CD4 count change over time calculated as standardized slopes over three months follow up, also seems to have an influence on the incidence on AIDS or death in the cART era.

At present time HIV-RNA and CD4 count in combination however seems to be the best available surrogate markers and have been shown to be independent predictors of progression to new AIDS events and death and therefore clinically useful to assess the efficacy of antiretroviral drugs.

Although AIDS and overall death rates of 2-3 per 100 PYFU have remained relatively stable in the EuroSIDA study within the last 5 years, it is unknown whether the predictive value of CD4 counts and HIV-RNA will continue to hold true in the years to come, with an increasing relative proportion of non-AIDS-related death. The surrogate markers can be expected to be a measure of the risk of AIDS and HIV-immunodeficiency related death, e.g. caused by OIs, but the surrogate markers can not be expected (or rather have not yet been proved) to predict also deaths and diseases from causes generally thought to be non-HIV-immunodeficiency related. Therefore, it is of paramount importance to be able to accurately assess cause of death classification and HIV-induced immunodeficiency relatedness of these deaths, as the CoDe methodology allows.

Further new drugs and even new drug classes, such as entry, fusion inhibitors as well as co receptor antagonists and maybe even immunomodulatory therapeutic drugs e.g. Interleukin-2, will become more widely available. Hence, it will remain necessary to regularly evaluate the clinical treatment effect of newer drugs and regimens and also in the future assess the predictive ability of surrogate markers on relevant endpoints for clinical progression among HIV infected patients.

Risk of cART interruption

In the EuroSIDA study, nearly a quarter of the patients on cART experienced one or more episodes of TI during a median period of follow up of 5.5 years with no evidence of changing the IR of approximately 6 TIs per 100 PYFU over time. Using a similar definition for TIs as in our study, d’Arminio Monforte et al found a comparable IR of TIs among pre-cART naïve patients in the ICoNA cohort study. In both studies, female gender, younger age and injecting drug use were associated with increased rates of TI. Further, patients with higher latest HIV-RNAs and CD4 counts were more likely to interrupt treatment.

Results from the SMART study showed that patients having TIs surprisingly did not have a reduced risk of adverse events, a priori thought to be associated with antiretroviral treatment. On
the contrary, patients having interruptions in the SMART study experienced diseases, such as cardiovascular, kidney or liver disease, at a significantly higher IR, compared to the patients continuously taking antiretroviral treatment. Whether these diseases, not traditionally defined as AIDS-related, are in fact associated with immunodeficiency, further investigation will have to show, but based on the published results a substantial part of the non-AIDS defining diseases observed seemed to be immunodeficiency- rather than treatment-related.

In conclusion, within the first decade of using cART, clinical practice have shown that a large proportion of HIV infected patients, will interrupt the entire cART regimen for three months or more. Further, as based on results from the SMART study, cART interruption seems to be associated with an increased than decreased risk of also non-AIDS related adverse events. Hence, TI should be discouraged and closely monitored.

**Risk of pancreatitis in relation to ART**

Pancreatitis is one of the adverse events seen in the HIV population with a higher rate than in the background population. In the general HIV-negative population, studies in various European countries have demonstrated IRs of pancreatitis between 0.05 to 0.80 cases per 1,000 PYFU. In the observation period from 2001 to 2006 an incidence of 1.27 clinical pancreatitis events per 1,000 PYFU was observed in the EuroSIDA study. There are several possible explanations for the two- to thirty-fold higher incidence observed in our HIV-infected population. Patients in our study were young with a median age of 39.8 years, and may have higher alcohol consumption rates and be more at risk of hyperlipidaemia than the general population, all of which are risk factors for development of pancreatitis. In addition, studies have reported an increasing risk of pancreatitis associated with more advanced HIV disease progression, suggesting that HIV infection itself or the following immunodeficiency may play a role. Studies considering the IR of pancreatitis amongst HIV-infected patients in the calendar years prior to the observational period of our study, have similarly found higher IRs of pancreatitis than those observed in the general population, and even higher than those observed in the present study. Dutta et al considered 321 patients seen in the period 1993-1994 and found that 45 (14%) developed pancreatitis. Reisler et al found an IR of pancreatitis of 6.1 per 1,000 person years in the period 1989-1999 and a rate of 8.5 per 1,000 person years in the period 1996-2001. As these studies were carried out in 'pre- and early-cART' period, where ddI and d4T were more widely used, compared to our 'late-cART' study, possible explanations may be the change in used specific antiretroviral drugs with a higher proportion of patients being on effective cART regimens in more recent calendar years. In addition the exact definition of a pancreatitis event and the verification procedures used differed between studies, which may account for such large differences, even for recently reported IRs of
pancreatitis. For example Fessel and Hurley used a definition based on elevated plasma lipase or amylase, or a pancreatitis diagnosis captured in the electronic medical record, whereas the criteria used in study II were more stringent, in that a consistent, detailed case definition of pancreatitis were applied, and all events were source verified, reviewed, and classified centrally by the study physicians.

Our results did not provide evidence to support the hypothesis that antiretroviral treatment including a NRTI backbone containing ddI and/or d4T was associated with a higher risk of developing pancreatitis compared to patients taking other ART regimens without these two drugs. Moreover, there was no evidence that cumulative exposure to any other antiretroviral regimens was associated with an increased risk of pancreatitis. In contrast, there was a tendency, although not statistically significant, that the risk of pancreatitis decreased with increasing time on treatment. All the relative risk estimates were close to one, suggesting that any effect of different ART combinations on the occurrence of pancreatitis were small.

Some studies found an association between ddI and/or d4T and an increased risk of pancreatitis, and other studies did not. These studies, like ours, are all restricted in precisely estimating the effects of these antiretrovirals due to the low IRs of pancreatitis observed.

Our results showed that the risk of pancreatitis was increased for patients with lower CD4 counts. There was also evidence of an association with higher HIV-RNA, perhaps suggesting that those with more advanced disease were at greater risk. This finding is in concordance with other studies that found an increased risk of pancreatitis amongst those with more advanced disease.

In conclusion, the observed incidence of pancreatitis among HIV patients within the EuroSIDA study were low compared with previously published studies, although still higher than IRs reported in the background population. Furthermore, there was no association between specific antiretrovirals, or combinations of antiretrovirals, associated with development of pancreatitis, and no evidence to suggest an increase in the IR over time in the recent years of cART use. The risk of pancreatitis was higher for patients with lower CD4 counts and higher HIV-RNA, suggesting that those with more advanced disease were at greater risk.

Strengths and limitations in the study methodology

The interpretation of observational data are based on general methodologies applied in the EuroSIDA study, including careful analyses planning, data collection and verification of occurring events in a well-defined, representative study population followed. The hypotheses are tested using multivariable models adjusting for relevant confounding factors known or expected to be associated with the endpoint. The results are presented using IRs and IRRs quantifying the effects of interest with 95% CIs providing a range of values, including the true value for the whole population which the study sample is representing.
Observational studies are per design limited in that a potential relationship between factors of interest and disease or death in a population can be assessed, but causality cannot be proven. Although causal relations can be evaluated in observational studies meeting a number of causation criteria such as those proposed by Hill, biologically plausible findings should if possible be tested in RCTs, providing the most convincing evidence as randomisation controls for even unknown confounding factors.

Further choosing the optimal lag-time between the endpoint and a surrogate marker is important when validating the surrogate marker. Hence, various sensitivity analyses were performed using different lag times in (I) and (II) to test if the results were robust.

A low loss-to-follow-up rate is essential for interpretation of data in a longitudinal study. Patients off (c)ART, at low CD4 cell count and high HIV-RNA were at higher risk of having no recent follow-up, and these patients would be expected to have a worse prognosis with regards to AIDS, pancreatitis and death, compared with those who remained under follow-up. This finding suggests that the assessments of incidences of new AIDS defining events, pancreatitis and the mortality within the EuroSIDA study are minimum estimates. In this connection, it is reassuring that the incidence rate of loss-to-follow-up is fairly stable, being below five per 100 PYFU. This is lower than the level reported from mono-centre, multi-centre or national cohort studies and even lower than reported from some randomised studies. Many, persistent efforts are carried out to collect additional data on patients lost to follow up including repeated education of staff and queries to the sites as well as support, monitoring, feedback and continued FU.

All data within the EuroSIDA study is subject to quality control and source verification. A rigorous quality assurance program including a monitoring of 10% randomly selected patients in the entire EuroSIDA cohort ensures that the risk of missing any clinical events is minimal. The verification of the events during monitoring is conducted within the same year of the event, unless delayed event reporting occurs. For each case, standardised event-checking charts are completed, signed and registered with a unique event ID in the event databases. During the random monitoring of patients, information on ART regimens was also checked. Hence, although TIs are often unplanned, periods off and on cART are carefully registered. However, it is possible that patients interrupt therapy and do not tell the clinician. This potential bias would only tend to give a slight underestimation of the difference between the groups.

Sites participating in the EuroSIDA study are often university associated and identified based on commitment by site investigators in research projects. Thus diagnostics and treatment regimens have been likely to represent the golden standard in the countries involved. This should be taken into consideration when extrapolating results from the EuroSIDA study to patients in other settings, and to patients not attending the clinics regularly. A particular strength of the analyses performed within this multinational study is the size and heterogeneity of the study.
population consisting of patients from sites all over Europe with different treatment politics and patterns of OIs and causes of deaths \(^5,^6,^13^\).

Following clinical observations and concerns about pancreatitis, collection of these events was started prospectively from 2001 onwards in EuroSIDA. If pancreatitis events were more frequent prior to this date, the incidence could be underestimated. However, as concerns about the relationship between pancreatitis and the concomitant use of ddI and d4T have occurred more recently, one might expect any reporting bias to result in an increased incidence with increasing calendar time, as clinicians became more aware of pancreatitis as a potential problem and were more likely to report the diagnosis.

A potential limitation of study III is that it was conducted from 2001 onwards, when ddI use is likely to be less widespread than in earlier years. Therefore, much of the cumulative exposure to ddI without d4T and to ddI with d4T is likely to be previous, rather than current exposure. No information on alcohol consumption and limited lipid data were available, both of which are known to be associated with pancreatitis. Not adjusting for these factors could be a potential limitation of the study. However it seems unlikely that these risk factors are highly unevenly distributed, e.g. only present in patients not taking the regimens under suspicion or only present in patients with low CD4 counts.

Although unable to comment on the IR of pancreatitis in earlier calendar years, the purpose of III was to perform a prospective study, to ensure that information on pancreatic events was accurately collected and verified, and to avoid cases being missed which may have happened in a retrospective study. In addition, sub-clinical disease was not the centre of attention of the study, but rather the focus was to investigate the prospective IR of clinical pancreatitis classified according to strict criteria \(^1^\).
CONCLUSION AND PERSPECTIVES

In conclusion, the widespread use of cART has reduced mortality and morbidity rates in populations of HIV infected individuals. The results from manuscript I in this Ph.D. thesis provide reassurance that the incidence of AIDS or death do not appear to differ significantly for various antiretroviral regimens for patients having the same latest CD4 count or HIV-RNA levels. Likewise, the increased risk of AIDS or death among patients experiencing a TI in our study (II) seemed to be explained by the latest CD4 count and HIV-RNA. Hence, our results indicate that the surrogate markers CD4 and HIV-RNA have the same prognostic meaning even when newer drugs are being used as part of the cART regimen and in situations with interruption of treatment.

With new drugs and drug classes still approved solely on their effect on HIV-RNA and CD4 count, the predictive value of these markers will need to be re-evaluated also in the future. As ART can only be expected to prevent AIDS and HIV-induced immunodeficiency related death, HIV-RNA and CD4 count have not been expected and not yet been demonstrated to accurately predict also deaths and diseases from non-immunodeficiency related causes. Therefore, it is of paramount importance to be able to accurately assess causes of death and their immunodeficiency relatedness as the CoDe methodology allows.

As shown in I and II, the risk of AIDS or death differ markedly according to the latest CD4 cell count or HIV-RNA strata with the highest risk observed in groups of patients having lower CD4 counts or higher HIV-RNA levels. This was true for both patients continuously on cART and those experiencing TIs. The results in manuscript II showed that TIs were associated with an increased risk of AIDS or death. This is in concordance with findings from the RCT study, SMART, where they found that TIs caused patients to have an increased risk of clinical progression to AIDS or death, as well as non-AIDS related disease. Hence, TIs should be avoided and adherence maintained among HIV infected patients having started cART. In our study (II) female gender, younger age and injecting drug use were associated with increased risk of TI, and patients with higher latest HIV-RNAs and CD4 counts were more likely to interrupt treatment.

Although specific or combinations of antiretroviral drugs in certain periods of time could have been associated with an increased risk of pancreatitis, a constant, low incidence of pancreatitis among HIV infected persons was found within the EuroSIDA study in the years 2001 to 2006. No evidence was found in manuscript III to support that development of pancreatitis was associated with specific antiretroviral drugs, or ART regimens. Pancreatitis has generally not been thought to be a disease related to HIV-RNA viral load or CD4 cell immunodeficiency. Surprisingly, a lower CD4 cell count was independently associated with the risk of experiencing pancreatitis, suggesting that immune deficiency could be an independent risk factor for pancreatitis in patients with HIV. To what extent non-AIDS related diseases are immunodeficiency related, and hence preventable by antiretroviral therapy, recent 70, ongoing 102 and future research will hopefully show.
The benefit of cART currently seems to outweigh the risks of taking cART. However, it remains important through long-term observational studies to investigate the risk of clinical endpoints according to antiretroviral drug regimens, as clinical evidence suggest that patients once started should take ART for life. As the EuroSIDA, D:A:D and other studies continue to accumulate follow-up time, the ability to describe the relationship between cART, HIV virologic status, CD4 cell immunodeficiency and risk of disease and death, will increase. Extended follow-up and applying standardised case definitions for endpoints observed, including a widespread use of the CoDe death classification system \(^{103}\) will facilitate comparisons of HIV-related endpoints between studies of HIV-infected persons.
English Summary

This Ph.D. thesis includes three published manuscripts based on work conducted within EuroSIDA, an observational study on European HIV-1 infected patients, in the period 2004-2007 at the Copenhagen HIV Programme. The overall aim in this Ph.D. thesis was to assess the risk of AIDS, death and pancreatitis according to CD4 count, HIV-RNA and use of different antiretroviral treatment with and without interruption.

Since regulatory authorities in 1997 allowed drugs to be approved without data from clinical endpoint trials, it was unknown whether the relationship between the HIV-RNA or CD4 cell count and risk of AIDS or death continued to hold true for newer antiretroviral drugs used also in combination antiretroviral therapy (cART) regimens. To investigate this, 6,814 patients taking different cART regimens were included in the analysis. Person-years at risk, numbers of AIDS and death events and incidence rates (IRs) of these events were calculated for specific categories of the latest CD4 count and HIV-RNA, according to which drugs were currently used in the regimen. Poisson regression models were used to assess the incidence rate ratios (IRRs) comparing specific drugs and combinations of drugs after adjusting for confounding factors, including latest CD4 cell count and HIV-RNA. The results showed that for a given, latest CD4 cell count and HIV-RNA persons were at similar risk for AIDS and death regardless of which cART regimen taken, and that there was no detrimental or additional beneficial effect of the newer cART regimens. This implies that the markers currently used for gauging patients' risk of clinical progression to AIDS or death can be interpreted similarly, regardless of which regimen the patient is receiving.

There were few published data on treatment interruption (TI) and the association with disease progression to AIDS or death in clinical practice, and results were not entirely consistent. Hence, there was a need to investigate this relation and assess the risk factors for developing new AIDS or death among HIV-infected people interrupting their cART regimens for 3 months or more. To look at this, 3,811 patients starting cART with a CD4 cell count and a HIV-RNA available within six months of starting cART were included in the study. The IR and risk factors for TI were determined, and the IRRs for TI and AIDS events or death were assessed using Poisson regression models. In EuroSIDA, one out of five patients followed in a five-year period interrupted the entire cART regimen for three months or more corresponding to IRs published by other cohort studies. Compared to patients staying on cART continuously, these patients have a more than doubled risk of AIDS or death. The increased risk were explained by lower latest CD4 counts and higher HIV-RNAs following cART interruption.

Use of antiretroviral drugs was reported to be associated with long-term toxicities, including cases of pancreatitis. Studies have published widely varying incidence rates of pancreatitis, and although some studies suggested that NRTI drugs in general and ddi/d4T in particular may lead to increased risk of pancreatitis, others did not find evidence for such an association. To study this, 9,678 patients on ART since
prospective collection of pancreatitis events were contributing to follow up time in the analysis until a diagnosis of pancreatitis or the last study visit. Factors associated with pancreatitis were investigated using Poisson regression model. Cumulative lengths of exposure to ddI and/or d4T and other ART were included as time-updated variables. In EuroSIDA, the IR of pancreatitis was higher than in the background population, but lower than published by studies on HIV-infected people in earlier years. There was no evidence that the IR differed according to cumulative ddI/d4T use. Pancreatitis was associated with lower CD4 counts, indicating that immunodeficiency may increase the risk of developing pancreatitis. However, caution must be taken when assessing the association of risk factors with pancreatitis due to a small number of events.

To conclude, also newer cART drugs reduce the risk of AIDS or death, and latest CD4/HIV-RNA can be used as surrogate markers. Interruption of cART (TI) is associated with an increased risk of clinical progression, explained by post-TI changes in CD4/HIV-RNA. The increased risk of pancreatitis in HIV-infected seems to be associated with immunodeficiency and not with specific ART use.
Danish Summary


Anvendelse af anti-retrovirale lægemidler blev rapporteret at være associeret med langtids-toksicitet, inklusive tilfælde af pancreatitis. Studier havde publiceret vidt forskellige incidens rater af pancreatitis, og selv om nogle studier antydede at NRTI

CORRECTIONS TO ORIGINAL MANUSCRIPTS

In manuscript I, the legend in table 3 states in point (iv) ‘at latest given CD4 cell count groups according to’. The correct labelling is ‘at latest given HIV-RNA measure groups according to’, like it is stated in the subheading.

In manuscript I p. 322 in the last sentence of the second paragraph, the text should be: ‘Table 3 shows similar results by HIV-RNA instead of CD4 count; that is, AIDS/death rates are higher in patients with higher HIV-RNAs, but no significant difference in incidence rates (delete: rate ratios) could be shown within the HIV-RNA groups for different cART regimens.’

In manuscript I p. 325, table 3, for HIV-infected persons on Ritonavir with a latest HIV-RNA between 50000-499999 for whom the IR is 20.4, the 95%CI is (12.7-28.2), not (22.5-28.3).

In manuscript II, on page 98, in the first line in the paragraph on ‘disease progression to AIDS or death’ it is stated that: ‘There were 403 clinical events of AIDS/deaths in 13,192 PYFU; (IR 3.7; 95%CI 3.5-4.0);’, whereas the correct number is: ‘There were 406 clinical events of AIDS or death in 13,192 PYFU; (IR 3.1 events per 100 PYFU; 95%CI 2.8-3.4).’

In manuscript II on Fig. 1 the events per PYFU with IR and 95%CI should be switched for the group with the latest HIV-RNA viral load ≥10.000. Hence, the line with ‘155/1489 10.4 (8.8–12.1)’ belong to the TI-group, and the line with ‘55/579 9.5 (7.0–12.0)’ belong to the non-TI group.

In manuscript II on page 100 (5 of 9), paragraph 1, line 2 the text should be:
‘After adjustment for confounding factors known at baseline (exposure group, cART regimen started, HBV and HCV status, prior AIDS diagnosis, age, time since started cART, date of starting cART and prior antiretroviral treatment, CD4 cell count and viral load), the IRR increased slightly (IRR 2.63; 95% CI 2.01-3.44; P<0.0001). After adjustment for current CD4 cell count and HBV and HCV status, the IRR dropped to 1.45 (95% CI 1.11-1.91; P=0.0071), while adjusting for current viral load and HBV and HCV status resulted in an IRR of 1.33 (95% CI 1.00-1.77; P=0.049). Adjustment for both current CD4 cell count and viral load and HBV and HCV status reduced the IRR further to 1.14 (95% CI 0.86-1.51; P=0.37).

In manuscript II, table 3, in the multivariable analysis (MVA), the 95%CIs for Gender – Female is (1.19-1.54), not (0.19-1.54), and the MVA p-value for AIDS diagnosis is 0.0072, not 0.072.
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