

PhD thesis

**Morbidity and mortality in Danish HIV patients after the introduction of
highly active antiretroviral treatment**

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The PhD thesis consists of three published studies performed in The Danish HIV Cohort Study at The Department of Infectious Diseases, Rigshospitalet, Copenhagen University Hospital, in the period 2008 – 2011:

- I. Engsig FN, Hansen AB, Omland LH, Kronborg G, Gerstoft J, Laursen AL, et al. Incidence, clinical presentation, and outcome of progressive multifocal leukoencephalopathy in HIV-infected patients during the highly active antiretroviral therapy era: a nationwide cohort study. *Journal of Infectious Diseases*. 2009 Jan 1;199(1):77-83.
- II. Engsig FN, Hansen AB, Gerstoft J, Kronborg G, Larsen CS, Obel N. Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007. *AIDS*. 2010 Jan 28;24(3):457-61.
- III. Engsig FN, Gerstoft J, Kronborg G, Larsen CS, Pedersen G, Roge B, et al. Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study. *BMC Infectious Diseases*. 2010;10:318.

Preface

This PhD thesis was carried out in the period 2008-2012 at the Department of Infectious Diseases, Rigshospitalet, Copenhagen University Hospital. I thank Rigshospitalet, Copenhagen University Hospital and the Faculty of Health and Medical Science, Copenhagen University whom in part sponsored my PhD. I am grateful for the possibility to write this PhD thesis.

I would like to thank my supervisors, Niels Obel and Jan Gerstoft, whom I think make up a terrific research team. Jan has a remarkable clinical insight and sense for relevant clinical problems and Niels has a unique ability to transform the problems into something that can be measured and estimated. I would especially like to thank Niels for introducing me to clinical epidemiology with a consistent focus on improvement of the care and prognosis of patients. Since I started my research with Niels the Danish HIV Cohort Study research laboratory at the Department of Infectious Diseases has evolved and I am greatly thankful for being a part of it. I would especially like to thank Ann-Brit E Hansen, Frederikke F Rønsholt, Marie Helleberg, Casper Roed and Lars H Omland for great company and inspiration. I would like to thank all co-authors for contributing and commenting on my articles. Finally, I am deeply grateful to my beautiful wife Magaly for her love, support and patience in all aspects of my life.

Bispebjerg, 2012

Frederik Neess Engsig

Abbreviations

AIDS	Acquired immune deficiency syndrome	IR	Immunological responder
CI	Confidence intervals	IQR	Interquartile range
CRS	The Danish Civil Registration System	IRIS	Immune restitution inflammatory syndrome
CSF	Cerebrospinal fluid	JCV	JC virus
CT	Computed tomography	MRI	magnetic resonance imaging
DHCS	The Danish HIV Cohort Study	MRR	Mortality rate ratios
HAART	Highly active antiretroviral treatment	OVR	Outpatient visit rates
HIV	Human immunodeficiency virus	PCR	polymerase chain reaction
IAR	Inpatient admission rates	PhD	Philosophiae doctor
ICD-8	International Classification of Diseases 8 th revision	PML	Progressive multifocal leucoencephalopathy
ICD-10	International Classification of Diseases 10 th revision	PYRS	Person years of observation
IDU	intravenous drug use	RR	Relative risk
INR	Immunological non- responder	VL	Viral load

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1. Introduction

Now, more than 30 years into the epidemic there are approximately 33.3 million HIV infected worldwide with the majority of infected living in Africa (1). Since 1999, the year in which it is thought that the epidemic peaked, the global number of new infections in 2009 has fallen by 19% due to prevention strategies and increasing availability of HAART. The epidemic has now been halted and reversed in many countries. In spite of these advances an estimated 10 million people, who are eligible for treatment under the new World Health Organization guidelines, are still in need of treatment and HIV is in the top five causes of death in more than 30 countries.

In Western and Central Europe approximately 820,000 adults and children are living with HIV (1). The introduction of HAART, which in most countries is free of charge, has decreased HIV related morbidity and mortality in HIV patients due to immunological recovery following viral load suppression (2;3). Initially after the introduction of HAART the main concerns were continued viral suppression and viral resistance but with growing experience in treating HIV patients and an increasingly wider range of drugs, well treated HIV infected patients now have an overall life expectancy close to non-HIV infected individuals (4-6). The main challenges are now drug toxicity and non-HIV related morbidity associated with behavioral risk factors like cigarette smoking (7).

This thesis revolves around the effects of HAART on the Danish HIV infected population. Mainly three aspects which will be investigated; PML in HIV patients, HIV patients use of health care facilities and mortality in successfully treated HIV patients with low CD4 cell count. The backgrounds of the three studies included in this thesis will be presented separately.

2. Objectives

We aimed to test the following hypotheses in this PhD:

1. The incidence and mortality of PML in Danish HIV infected patients have improved after the introduction of HAART.
- 2.1 The use of health care facilities has decreased after the introduction of HAART among Danish HIV patients.
- 2.2 Danish HIV patients still have an increased use of health care facilities compared to that of Danish non-HIV infected individuals.
3. Danish HIV patients with low CD4 cell counts who are successfully treated with HAART for more than three years with an insufficient immunologic response have an increased mortality compared to those with an adequate CD4 cell response.

3. Background

3.1. Background for Study 1

PML is a rare but frequently fatal disease of the central nervous system caused by JC virus, a polyomavirus. It occurs almost exclusively in patients with profound cellular immunodeficiency and in spite of an increasing incidence in patients receiving treatment with monoclonal antibodies HIV infection is still the single most predisposing disorder for PML (8;9). Data from early after the introduction of HAART have described less dramatic improvements for PML than for other opportunistic infections (2;10;11). The clinical presentation of PML varies according to the localization of the disease, and the initial symptoms may be misdiagnosed as other HIV- or non-HIV-related cerebral lesions. To improve the initial diagnostic strategy, it is important to know the major presenting symptoms of PML. There is no known causal effective treatment of the disease. Mainstay of treatment is symptomatic and immune recovery through treatment of the HIV infection (12;13).

3.2. Background for Study 2

The decreased morbidity and mortality in the HIV infected population should transform into a decreased use of health care resources. However, the increased age of the HIV patients and the higher numbers of patients being alive and managed at HIV clinics may have lead to changes in hospitalization patterns and an increased use of health care recourses. Changes in hospitalization have been described in regional settings but to our knowledge no nationwide studies have been reported (14;15). Information regarding changes in the use of health care recourses among HIV

infected patients is important for clinicians and health care planners in order to make budgets and allocate resources.

3.3. Background for Study 3

Shortly after the primary HIV infection a steady decrease in CD4 cell count follows and immunodeficiency ensues over time (years) (16;17). Treatment with HAART suppresses viral replication leading to recovery of CD4 cells and immunologic and clinical improvement (18). Still, the immunological reconstitution after HAART initiation varies depending on the pre-HAART level of immunodeficiency (19;20). Several studies have shown that patients in spite of successful virological response to HAART and incomplete initial CD4 recovery have increased mortality but the outcome is poorly documented for patients with persistent low CD4 count despite several years of HAART with sustained VL suppression (21;22).

4. Methods

4.1. Settings

All studies were carried out in Denmark in the period 1995 - 2008. Denmark had a population of 5.5 million as of 31 December 2008, with an estimated HIV prevalence of approximately 0.07% in the adult population (23;24). Patients with HIV infection are treated in one of the country's nine specialized medical centres, where they are seen on an outpatient basis at intended intervals of 12 weeks. Antiretroviral treatment is provided free of charge to all eligible HIV-infected residents of Denmark. During the follow up period for this thesis (1995 – 2008) national criteria for initiating HAART were HIV-related disease, acute HIV infection, pregnancy, CD4⁺ cell count < 300 cells/μL, and until 2001, plasma HIV-RNA >100.000 copies/ml.

4.2. Study design

All three studies in this thesis are cohort studies.

In epidemiology, a cohort is defined most broadly as “any designated group of individuals who are followed or traced over a period of time” (25). Typically, the persons in a cohort have a common characteristic, in this case HIV, PML or low CD4 cell count, defining the group of persons as “exposed”. The concept of following a cohort is to measure the occurrence of one or more outcomes over time, in the exposed and un-exposed populations, thus the name observational or longitudinal study (26).

The quality of the research done in observational studies depend on the accuracy of the information collected meaning how valid is the information that a patient is emigrated, lost to follow up or still alive. In Denmark we have unique conditions to register this kind of information.

4.3. Data sources

The Danish HIV Cohort Study

The DHCS is described in details elsewhere, is a population-based prospective nationwide cohort study of all HIV-infected individuals 16 years or older at diagnosis and who are treated at Danish HIV centres after 1 January 1995 (27). The number of HIV patients in DHCS as per December 31, 2008 was 5481. Patients are consecutively enrolled, and multiple registrations are avoided through the use of a unique civil registration number. Data are updated yearly and includes demographics, date of HIV infection, AIDS defining events, date and cause of death and antiretroviral treatment. CD4 cell counts and HIV-RNA measurements are extracted electronically from laboratory data files.

The Danish Civil Registration System

The Danish Civil Registration System is a national registry of all Danish residents established in 1968(28). A 10-digit personal identity number (the CPR number) assigned at birth or immigration uniquely identifies each person. The CPR number is used by all public registries.

The Danish National Hospital Registry

The Danish National Hospital Registry was established in 1977 and covers all inpatient admissions and outpatient visits at non-psychiatric hospitals in the country (29). The registry is based on the unique CPR number and contains data on: type of admission, type of speciality, date of admissions and discharges, procedures, primary and secondary diagnoses (coded according to ICD-8 until the end of 1993, and ICD-10 thereafter). From this registry, we extracted data on inpatient admissions and outpatient visits of the HIV patients and the population controls for Study 2.

The Danish Cancer Registry.

The Danish Cancer Register is a population-based register that contains information on incident cancers diagnosed in Danish citizens since 1943. Details about registration can be found elsewhere (30).

4.4. Study populations

In Study 1 we included all HIV infected individuals from the DHCS in the period 1 January 1995 to 31 December 2006.

In Study 2 we identified and included all HIV infected individuals from DHCS with Danish residency in the period 1 January 1995 to 31 December 2007. We also identified and included five population controls matched on gender and date of birth for each HIV patient from The Danish Civil Registration System for every study period.

In Study 3 we identified all HIV-1 positive patients from DHSC who were included in the cohort before 31 December 2008, who 1) started HAART before 1 January 2005, 2) had a CD4 cell count $\leq$ 200 cells/ μ l at start of HAART, 3) had a VL $<$ 50 copies/ml for more than three consecutive years before 1 January 2008, 4) had no intervals of more than seven months between VL tests in the suppressed period, and 5) had a CD4 cell count $\leq$ 200 cells/ μ l at start of the virally suppressed period.

4.5. Study outcomes and statistical analyses

Study 1

In the first part of Study 1 the outcome was the incidence of PML in HIV infected patients during the pre-HAART (1995–1996), early HAART (1997–1999), and late HAART (2000–2006) periods, along with the incidence of PML in patients with CD4 cell counts $< 200 \mu\text{L}$ and $\geq 200 \text{ cells}/\mu\text{L}$. In the second part of the study the main outcome was death. We used Kaplan-Meier analysis to construct survival curves. We also described the presenting clinical and paraclinical characteristics of PML, as well as the neurological outcome of the disease. Risk factors for PML and death were estimated using the Cox proportional Hazard analysis. Selection of potential confounders was performed using the “change in estimate” method with age and gender forced into the model (31).

Study 2

In Study 2 the outcome was IAR (number of inpatient admissions/100 PYRS), and OVR (number of outpatient visits/100 PYRS) for Danish HIV patients and matched population controls. Rates of inpatient admissions and outpatient visits were stratified on admission to departments of infectious diseases vs. all other departments, primary ICD-10 discharge diagnoses and CD4 counts ($\leq 200 \text{ cells}/\mu\text{L}$, $200 \text{ cells}/\mu\text{L}$ to $< 500 \text{ cells}/\mu\text{L}$ and $\geq 500 \text{ cells}/\mu\text{L}$). We calculated crude Poisson confidence intervals (95% CI) and RRs comparing rates of IARs and OVRs in the HIV infected patients to that in the population controls.

Study 3

In the first part of Study 3 we estimated risk factors for immunological non-response (CD4 cell count $\leq 200 \text{ cells}/\mu\text{L}$ after three years of VL suppression) using binary logistic. In the second part of Study 3 we estimated mortality in INRs versus IRs. We used Kaplan Meier analysis to construct survival curves for INRs vs. IRs and further stratified these by time from first CD4 measurement $\leq$

200 cells/ μ L to start of the virologically suppressed period ($\leq$ one year vs. $>$ one year). Cox regression analyses were used to estimate MRRs. Selection of potential confounders was performed using the “change in estimate” method with age and gender forced into the model (31).

General differences in characteristics between groups were estimated using the χ^2 test, Kruskal-Wallis and Fisher’s exact test when appropriate.

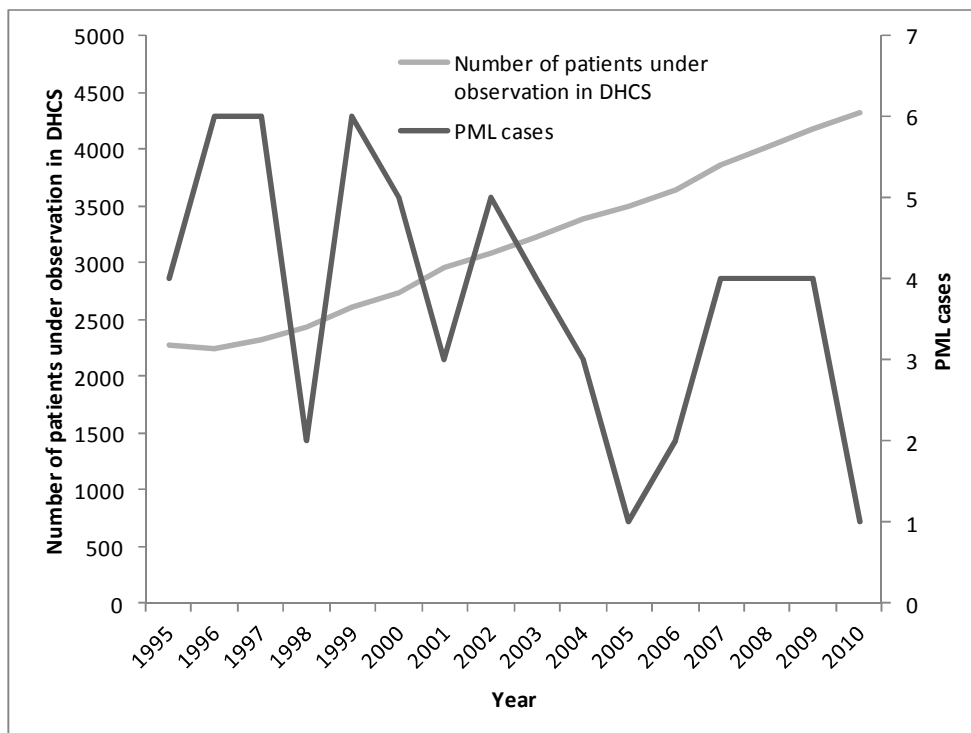
5. Results

5.1. Results from Study 1

Incidence of PML

We identified 47 PML patients in the study period. Incidence rates of diagnosis of PML decreased considerably from the pre-HAART period (3.3 per 1000 PYRS (95% CI; 1.9-5.7)) to the late-HAART periods (1.3 per 1000 PYRS (95% CI; 0.8-1.9)). Incidence rate among patients with CD4+ cell count above or equal to 200 cells/ μ l was 0.2 /1000 PYRS (95%CI; 0.1-0.6) and 9.1/1000 PYRS (95%CI; 6.7-12.3) for patients with less than 200 CD4 cells/ μ l. The annual number of new PML cases in DHCS has been relatively stable after 2006 (figure 5.1.1.)(unpublished data)

Figure 5.1.1. Yearly number of new PML cases and patients under observation in DHCS.



A CD4 cell count > 200 cells/ μ l vs. < 200 cells/ μ l at time of HIV diagnosis was the only significant marker for decreased risk of development of PML (Adjusted incidence rate ratio, 0.20 (95% CI; 0.11-0.47)). Age, sex, race, IDU or being diagnosed with HIV before 1 January 1997 did not confound the beneficial effect of a CD4 cell count $\geq$ 200 cells/ μ l at time of PML diagnosis.

Characteristics and presenting symptoms in patients diagnosed with PML

Most of the patients with PML had advanced HIV disease with low CD4 cell count (median CD4 cell count (IQR); 50 cells/ μ l (27-160)) and high viral load (median HIV-RNA log₁₀ copies/ml (IQR); 4.9 (3.7-5.6)). Almost all patients had a nadir CD4 cell count below 200 cells/ μ L. The predominant neurological symptoms at presentation of PML were coordination disturbances, cognitive affection and limb paresis.

Para clinical characteristics

Very few patients presented with common signs of infection such as fever or leucocytosis along with the onset of neurological symptom. But an elevated sedimentation rate or C-reactive protein were seen in 39% (18/38) of the patients. CSF pleocytosis was uncommon (2%) while protein in the CSF was elevated in 49% of the cases. Abnormal decrease in CSF glucose level was not observed.

PML diagnosis

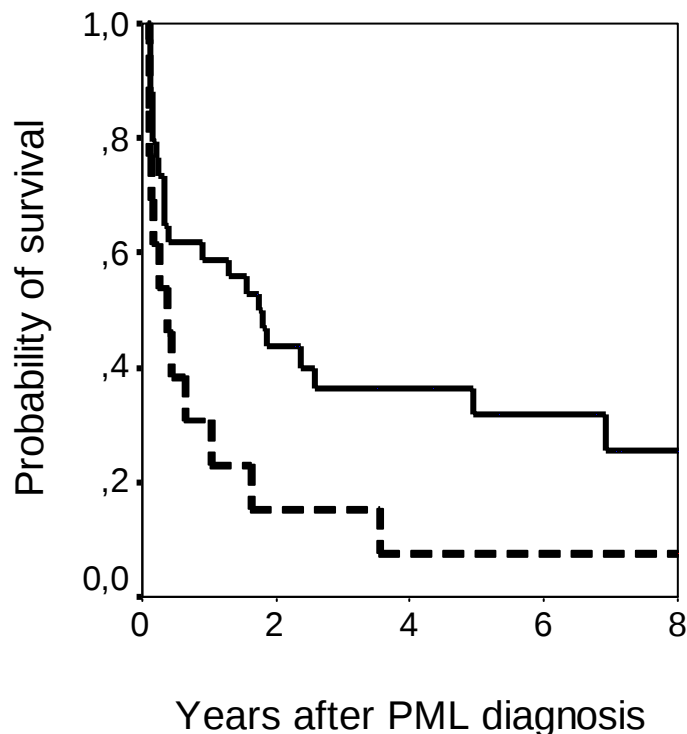
Twenty-five (53%) patients were diagnosed exclusively on clinical symptoms combined with either MRI findings (18 patients) or CT scans (7 patients). In 22 (47%) patients the PML diagnosis was confirmed by brain biopsy (14 patients) and/or positive PCR for JCV in CSF. Thirteen patients out of

31 patients tested had positive test PCR for JCV in the spinal fluid. Interestingly, seven of the 14 patients diagnosed by brain biopsy had negative PCR for JCV in the CSF.

Mortality in PML patients

A total of 35 PML patients died in the study period. Median survival time for all patients diagnosed in the period 1995 to 2006 was 1.02 years (95% CI; 0.0- 2.5). Patients diagnosed with PML after 1997 had a considerably higher median survival time than those diagnosed with PML before 1997 (figure 5.1.2). Median survival time for patients diagnosed with PML who were treated with HAART was 1.8 years (95% CI; 0.8-2.8).

Figure 5.1.2. Kaplan-Meier curves for overall survival by time of PML diagnosis: PML after 1997 (solid line), PML before 1997 (broken line).



In unadjusted analyses, a CD4 cell count above or equal to 50 cell/ μ L at diagnosis of PML (MRR, 0.47 (95% CI, 0.24 – 0.93)) as well as diagnosis of PML after 1997 (MRR, 0.48 (95% CI, 0.24 – 0.97)) were associated with reduced mortality. None of the two were confounded by age, CD4 cell count at index date, sex, race, IDU or AIDS defining diagnose before PML.

Following the publication of the study an interesting question was raised by a reader in regards to the role of contrast enhancement on scans in our study (32). This inspired us to reanalyze our data and in a sub analysis we identified all PML long-term survivors (defined as patients being alive after four years from date of PML diagnose) and PML progressors (defined as patients not alive after four years from date of PML diagnose) and calculated the prevalence of contrast enhancement in these two populations. The prevalence of contrast enhancement (all found at MR scans) in PML progressors was five (14%) of 35 patients and two (18%) of 11 patients in PML long-term survivors. In unadjusted Cox regression analyses, contrast enhancement was not associated with reduced mortality (MMR, 0.88 (95% CI, 0.34 – 2.29)).

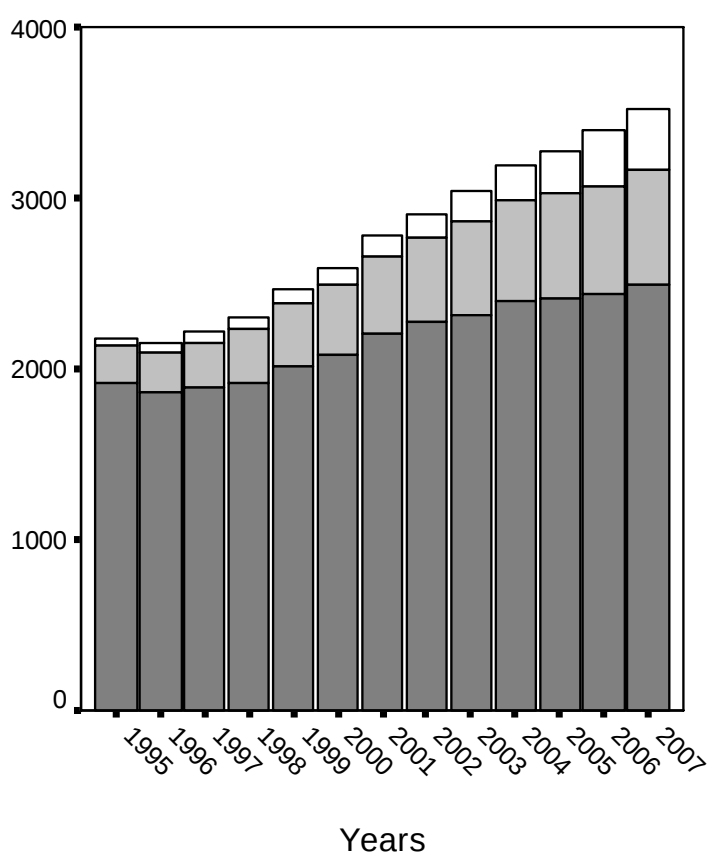
Neurological Outcome

Among those surviving more than three years 73% experienced improvement of their neurological symptoms.

5.2. Results from Study 2

The yearly number of HIV patients seen in the Danish hospital system increased by 61% from 2,184 in 1995 to 3,524 in 2007 (figure 5.2.1) and the number of patients above 50 and 60 years of age increased considerably. The yearly number of PYRS at risk for patients with a CD4 cell count below 200 cells/ μ L decreased from 814 PYRS in 1995 to 216 PYRS in 2007. Correspondingly the yearly number of PYRS at risk for patients with a CD4 cell count between 200 and 500 cells/ μ L increased from 778 PYRS in 1995 to 1347 PYRS in 2007 and from 319 PYRS in 1995 to 1743 PYRS in 2007 for patients with a CD4 cell count > 500 cells/ μ L.

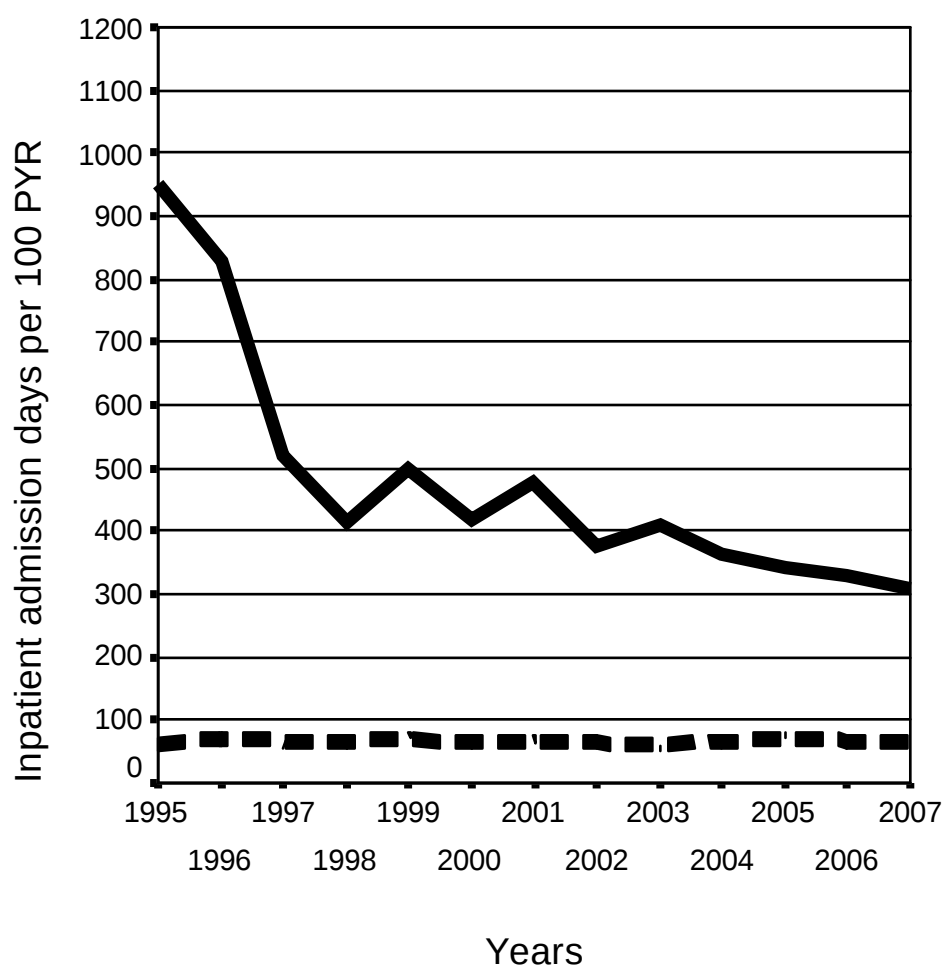
Figure 5.2.1. Number of Danish HIV patients under observation per year divided in persons below 50 years (dark gray), between 50 and 60 years (light gray), above 60 years (white).



Inpatient admission rates

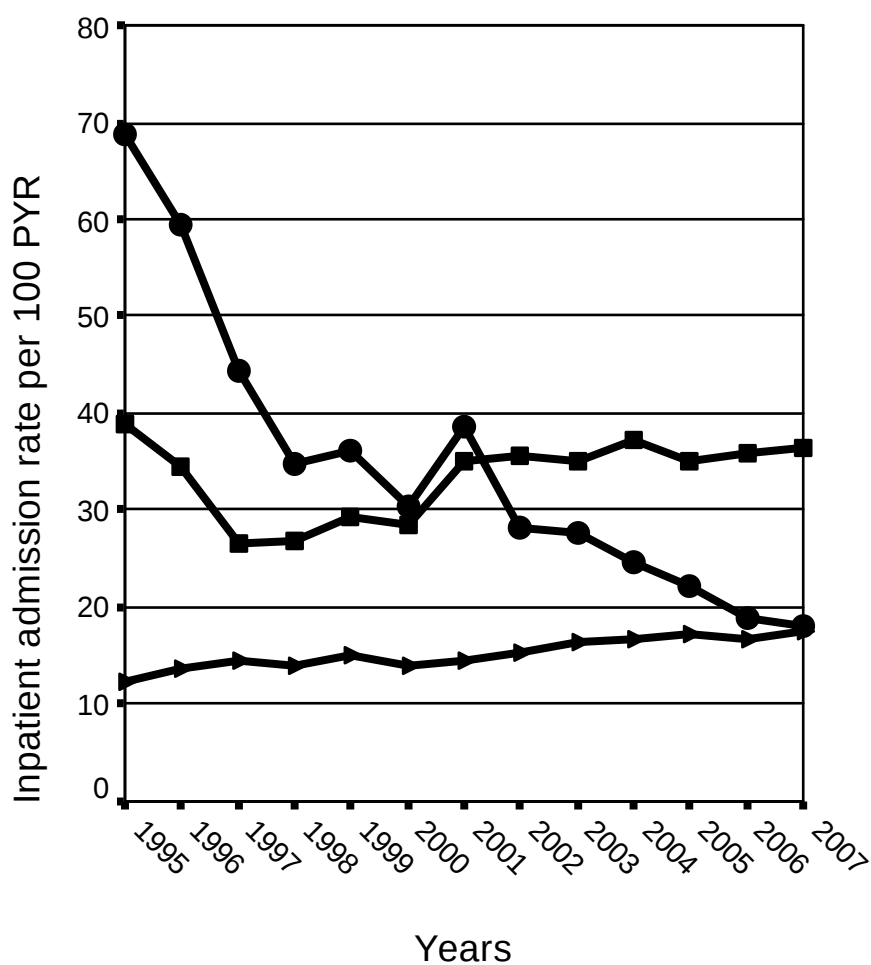
Over the observation period the overall IAR for HIV infected patients decreased (36.8%), while it increased (24.7%) for the population controls and thereby caused the RR to decrease from 6.2 in 1995 - 1997 to 3.1 in 2004 – 2007. This trend can also be seen at the rate of *inpatient admission days per 100 PYRs* at all departments where it even appears that the rate of HIV patients is approaching that of the population controls (figure 5.2.2.)(unpublished data).

Figure 5.2.2. Inpatient admission days pr 100 PYRS at all departments for HIV patients (thick line) and population controls (broken line).



We also observed that the RR between admissions at departments of infectious diseases and departments of non-infectious diseases changed during the observation period where the HIV patients ended up being admitted two times more at departments of non-infectious diseases than at departments of infectious diseases (figure 5.2.3.).

Figure 5.2.3. Inpatient admission rates pr 100 PYRS at departments of infectious diseases for HIV patients (circular), non-infectious diseases (square) for HIV patients and non-infectious diseases for controls (triangular). Inpatient admission rates at departments of infectious diseases for non-HIV patients are not shown, due to the very low rates.

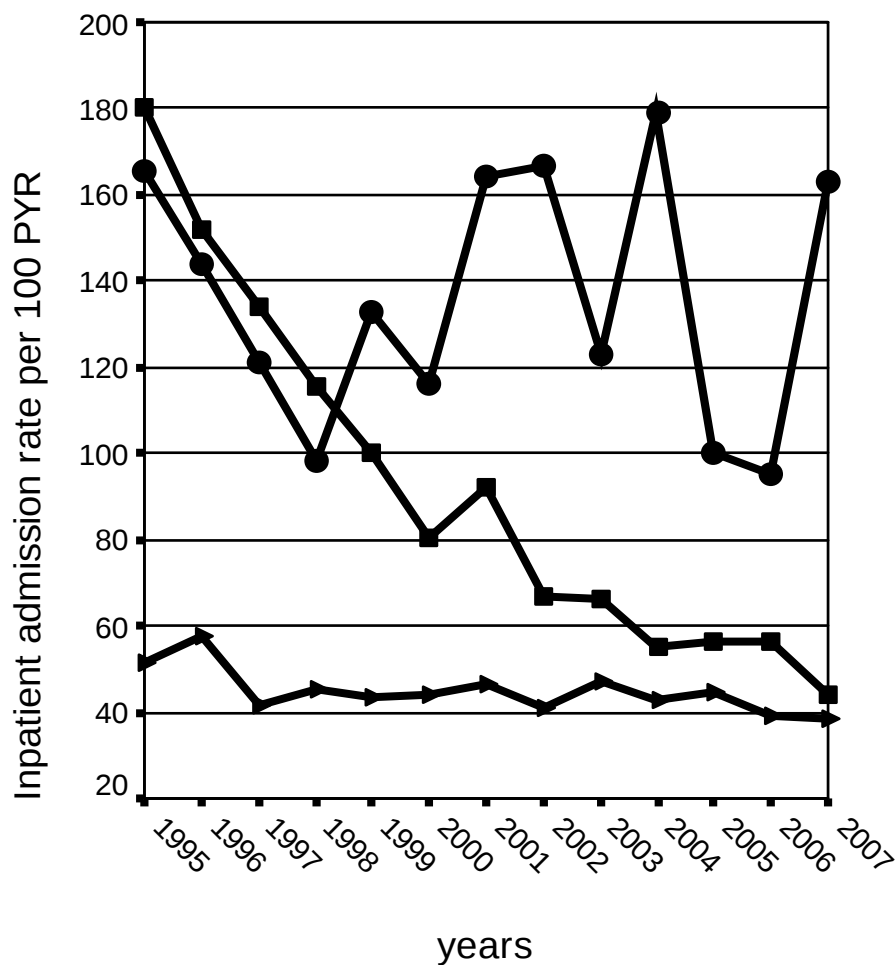


When categorised according to primary discharge diagnosis only, HIV patients had an increased RR due to cancer diagnoses and cardiovascular disease compared to the population controls.

CD4 stratified inpatient admission rates

For HIV infected patients with a CD4 cell count between 200 and 500 cells/ μ L, the overall IARs decreased in the study period to almost the same level as for patients with a CD4 cell count > 500 cells/ μ L (figure 5.2.4.). In contrast IARs for HIV infected persons with a CD4 cell count < 200 cells/ μ L remained high throughout the study period.

Figure 5.2.4. Inpatient admission rates pr 100 PYRS at all departments for Danish HIV patients stratified for; (circular and group 1) CD4 cell count < 200 cells/ μ L; (square and group 2) 200 cells/ μ L $\leq$ CD4 cell count < 500 cells/ μ L; (triangular and group 3) CD4 cell count $\geq$ 500 cells/ μ L.

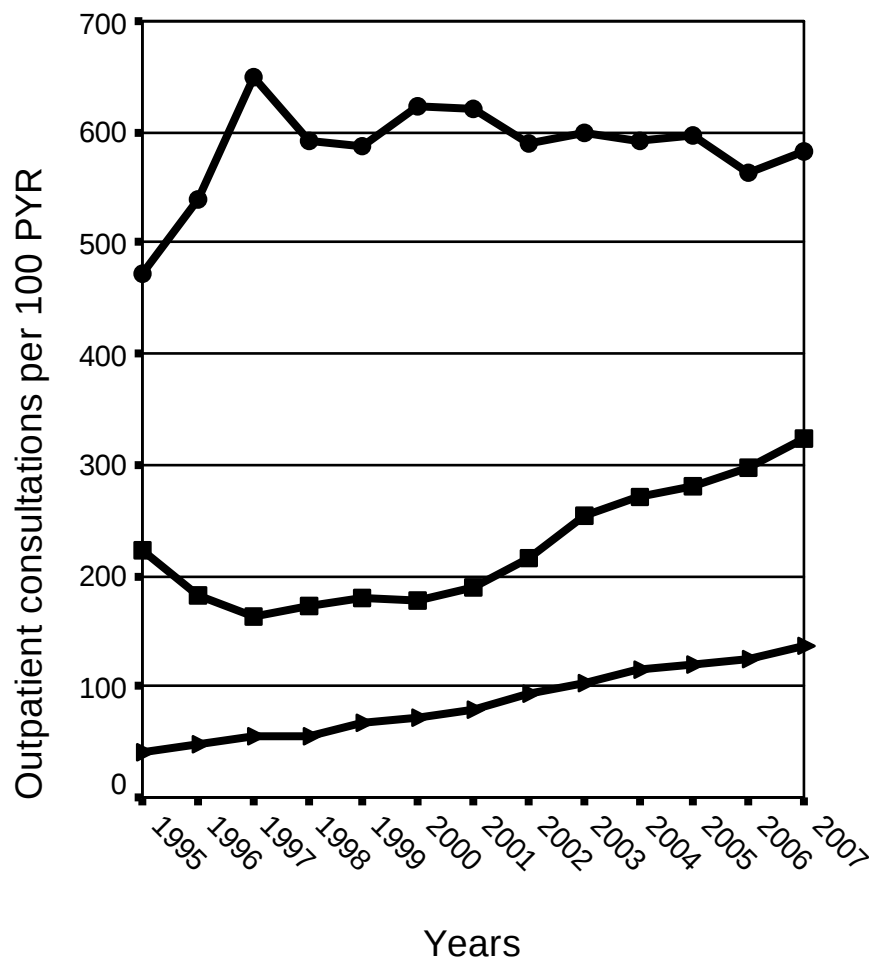


Outpatient visit rates

The overall OVRs for the HIV infected persons increased slightly in the period 1995 – 2007 whereas this rate increases by 175% in the control population, and in consequence, the RR decreased over the period from 16.1 to 7.1. The OVR at departments of infectious diseases was stable around 600 outpatient visits per 100 PYRS whereas it increased considerably at departments of non-infectious diseases (figure 5.2.5.).

Figure 5.2.5. Outpatient visit rates pr 100 PYRS at departments of infectious diseases (circular) and non-infectious diseases (square) for HIV patients and for controls at departments of non-infectious diseases

(triangular). Outpatient visit rates at departments of infectious diseases for non-HIV patients are not shown, due to the very low rate.

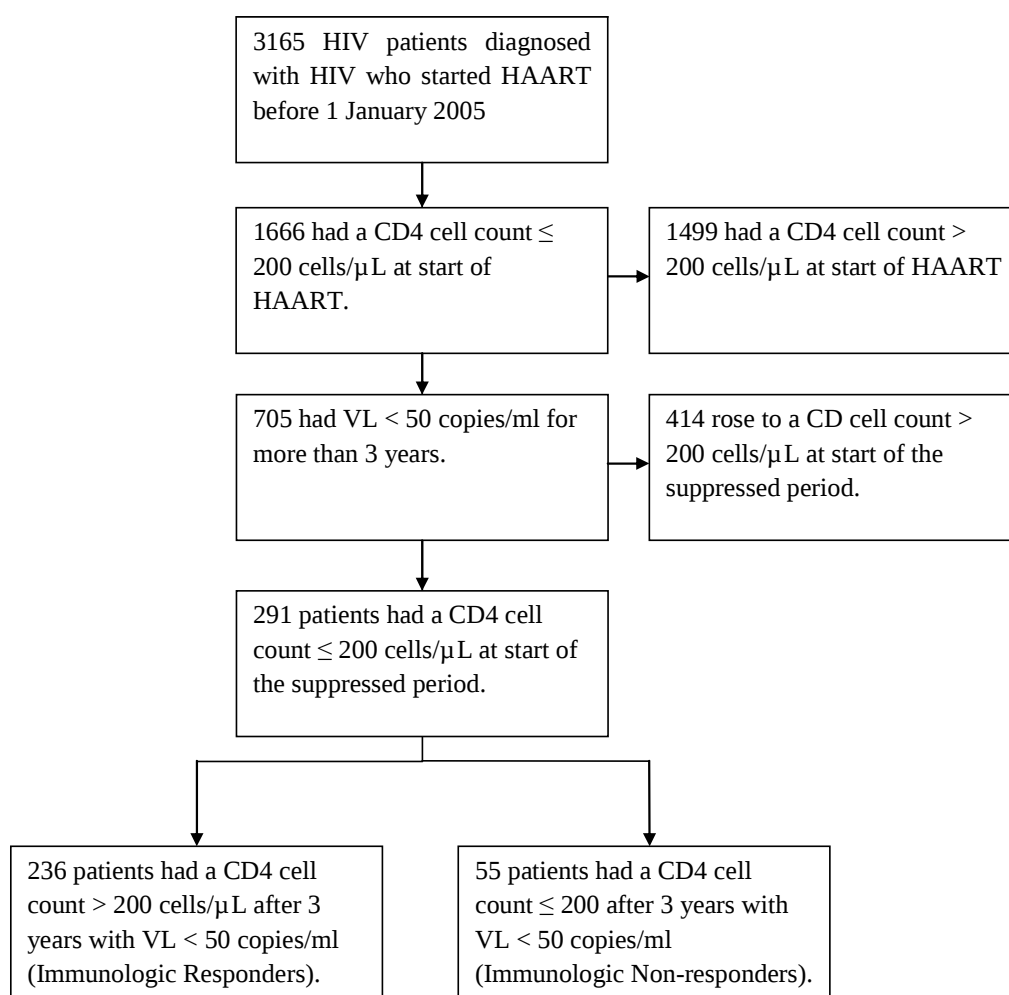


The OVR for cancer diagnoses increased considerably more for the control population (370%) than for the HIV infected population (54%) why the RR decreased from 8.0 to 2.6. Concerning visits under cardiovascular diagnoses an increase was observed in the HIV population (266%) as well in the control population (200%) and the RR increased from 2.0 to 2.5 in the observation period.

5.3. Results from Study 3

In DHCS 3165 patients started HAART before 1 January 2005 (figure 5.3.1). 291 study subjects fulfilled the inclusion criteria. After three years of sustained VL 236 (81.1%) patients had reached a CD4 cell count above 200 cells/ μ L (IRs) and 55 (18.9%) of the HIV infected patients had not (INRs).

Figure 5.3.1. Study flow chart.



In adjusted analysis only age and time from first CD4 cell count ≤ 200 cells/ μ L until start of the virologically suppressed period were associated with increased risk of being INR.

A total of 22 (7.6%) patients died in the observation period, 11 (20.0%) in the INR group and 11 (4.7%) in the IR group. INRs had a substantially higher mortality compared to IRs (adjusted MRR; 3.4 (95%CI; 1.4 – 8.0))(figure 5.3.2).

Figure 5.3.2. Kaplan-Meier curves for overall survival according to immunologic response at index date: 1. CD4 cell count ≥ 200 cells/ μ L (broken line), 2. CD4 cell count < 200 cells/ μ L (full line). Index date was defined at the date of first CD4 measurement after three years of viral suppression (VL <50 HIV-RNA copies/ml).

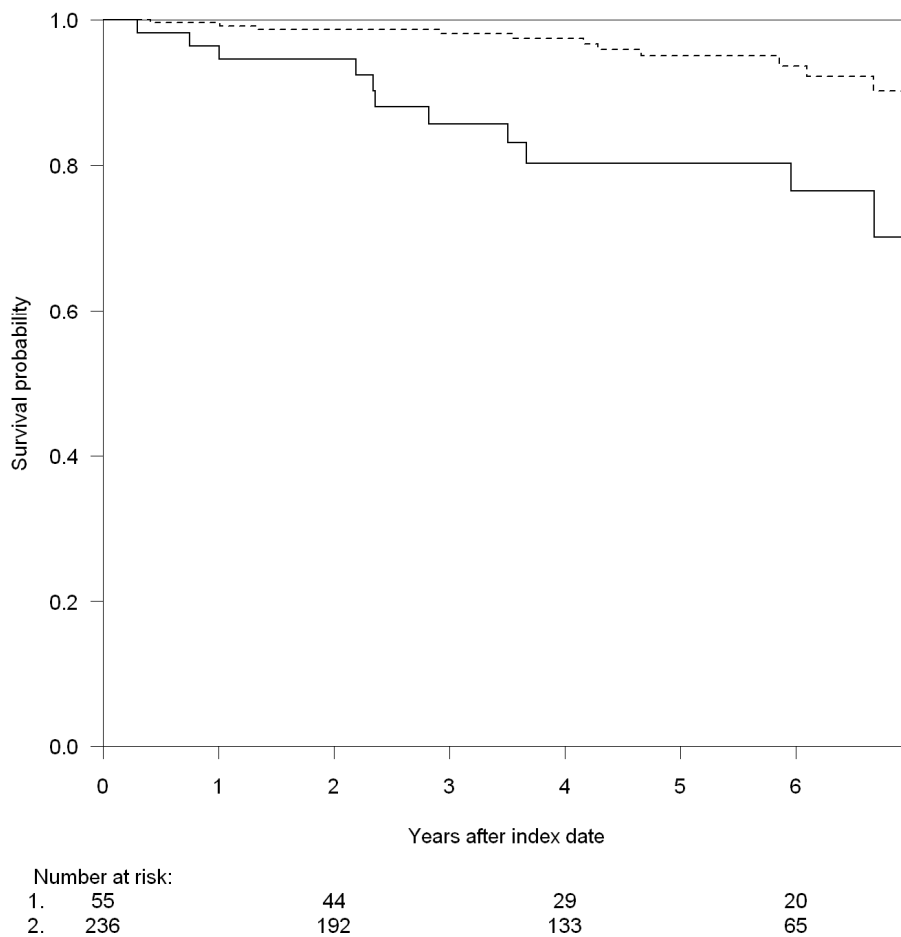
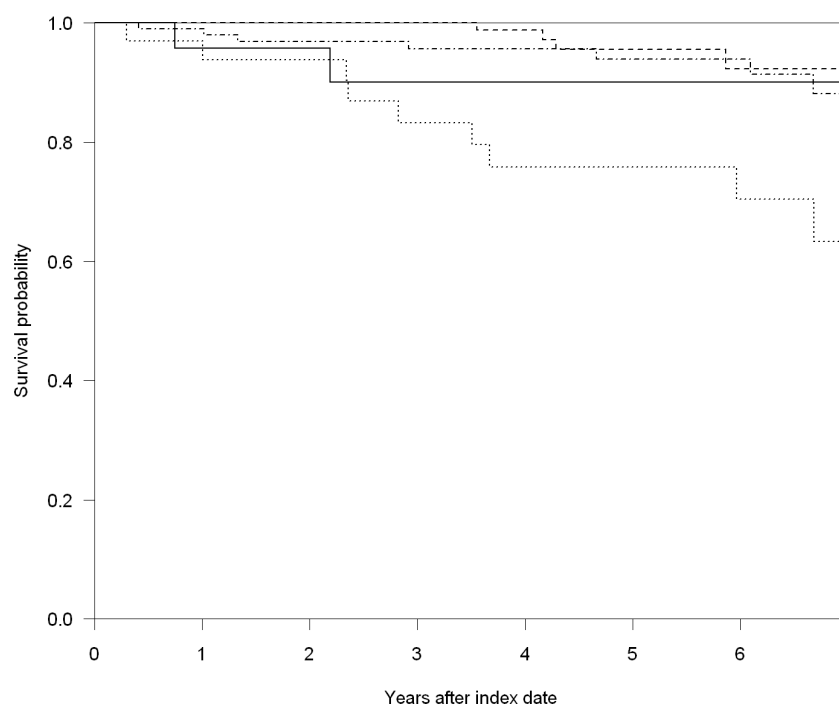


Figure 5.3.3 shows the mortality for IRs vs. INRs stratified by time from first CD4 measurement ≤ 200 cells/ μ L until start of the virologically suppressed period and demonstrates that the main excess mortality was observed in the INRs with more than one year of immunological suppression prior to the virologically suppressed period. Of importance, the excess mortality was observed up to 6.5 years after initiation of virologically successful HAART.

Figure 5.3.3. Kaplan-Meier curves for overall survival according to CD4 count at index date and stratified by time from first CD4 cell count < 200 cells/ μ L to start of the virally suppressed period: 1) CD4 ≥ 200 cells/ μ L and $\leq$ one year (full line), 2) CD4 ≥ 200 cells/ μ L and $>$ one year (bar line), 3) CD4 < 200 cells/ μ L and $\leq$ one year (dot and bar line), 4) CD4 < 200 cells/ μ L and $>$ one year (dot line).



Number at risk:

1.	23	17	9	7
2.	140	107	64	26
3.	32	27	20	13
4.	96	85	69	39

In a stratified analyses excluding the 128 patients (44.0%) who had more than one year from first CD4 measurement ≤ 200 cells/ μ L until start of the virologically suppressed period the mortality was considerably reduced for INRs (adjusted MRR; 1.8 (95%CI; 0.3 – 10.2)). Ninety (70.3%) of these 128 patients were diagnosed before 1995. When excluding IDUs from our analysis, the adjusted MRR was reduced to 1.8 (95%CI; 0.6 – 5.1). The exclusion of patients with previous cancer or AIDS defining events did not change the results substantially.

Only one death in each group (INR vs. IR) appeared to be a classical HIV related cause of death (one PML and one cryptococcal meningitis).

We performed a sensitivity analysis with a cut-off value of 500 copies/ml for viral suppression which did not change the increased mortality among INRs.

6. Discussion

The findings of the three studies in this thesis will be discussed separately followed by a general conclusion.

6.1. Discussion of Study 1

In Study 1 we demonstrated that the incidence of PML decreased after the introduction of HAART and that it continued to decline further over time in the HAART period. In previously unpublished data we have also showed that the number of new cases appears to have reached a level and the situation appears to be the same in other parts of Europe (33). The initial decline in incidence is without doubt associated with the increased effectiveness of HAART due to better and simpler drug regimens as documented by increased survival and decreased occurrence of treatment failure (4;6;34). In concordance with this the main risk factor for development of PML is low CD4 cell count and we clearly demonstrated that the risk of PML is very low when the CD4 cell count is above 200 cells/ μ L. We found that HIV patients with PML are generally characterized by being newly diagnosed patients with low CD4 cell counts. This should be supported by the findings in Study 1 but we erroneously estimated the median time from HIV to PML for all patients diagnosed with PML to be 4.6 years (IQR; 1.2-10.9). When excluding the patients diagnosed before 1995, the median time from HIV to PML is 0.12 (IQR; 0.0-3.5) years which exemplifies the statistical problems with a cohort that is not entirely incident. In regards to the HIV diagnosis, the DHCS is in part prevalent since it includes all HIV patients alive in Denmark per 1 January 1995 and then includes all new HIV cases from 1 January 1995 and forth. Prevalent cohorts have some disadvantages over incident cohorts since they may fail to include patients with short-lived terminal disease and therefore give unreal optimistic estimates of for example survival. On the other hand patients with long-standing morbidity who die shortly after the inclusion date may be

included leading to the opposite effect because of truncation of the length of survival from onset to the event of interest, death (35). Without doubt the patients in DHCS diagnosed with HIV before 1995 differ in some aspects from the incident part of the cohort in that they are more likely to have more viral resistance towards antiretroviral drugs and a slower rate of CD4 cell decay. Still, we think the impact of this phenomenon on our study is small but could lead to both under- and overestimation of the true incidence of PML in the pre-HAART years - it would not affect our estimate of mortality since the inclusion of PML cases was incident.

When is a HIV patient at risk of contracting PML? In our study we included the observation time of all HIV patients under observation which allows for an immortal time bias (36). Immortal time refers to cohort follow-up time in a time-to-event analysis during which, because of the exposure definition (all time after the HIV diagnosis), the outcome under study could not occur. To avoid this bias we could have included only observation time from for example the first 6 months after HIV diagnosis in HIV patients with low CD4 cell counts or used at time-updated analysis. The immortal time bias would tend to underestimate the true risk of PML in those at risk.

Corresponding to our findings concerning PML, Lescure FX et al found a decreasing incidence of severe neurocognitive disorders in DHCS after the introduction of HAART and also here a low CD4 cell count increased the risk of severe neurocognitive disorders (37). Besides immunological restitution, the effect of HAART in our observation period may also be due to increased use of CNS penetrating drugs suppressing viral replication in otherwise “sanctuary” locations (38).

Another main finding in Study 1 was the beneficial effect of HAART on mortality in HIV patients diagnosed with PML. The development of PML after 1997 and high CD4 cell counts at time of PML diagnoses were considerable prognostic factors for survival most likely due to the effect of HAART.

Both Antinori and lately Khanna N et al found similar results (33;39). We did not find that contrast-enhancement on MR scans reduced mortality as proposed by others (40) perhaps due to a low prevalence of IRIS in this study.

In conclusion, we failed to reject hypothesis 1 as the incidence of PML has decreased and that the prognosis of HIV infected patients with PML has improved after the implementation of HAART. But the disease still has a high mortality, and patients who survive are often left with severe neurological sequelae. The main focus in management of PML should be on prophylactic measures by maintaining a high CD4 cell count through early diagnosis of HIV infection and initiation of HAART prior to immunological deterioration.

6.2. Discussion of Study 2

The main result in Study 2 is that in spite of a considerably reduction of the IARs for HIV patients the overall IAR is still three times higher than that of the population controls. Similar changes in the IARs for HIV infected patients have been described previously with regard to hospitalization for pneumonia and in the early HAART era (41;42). Not surprisingly, the reduction of the IARs for HIV patients was mainly seen at departments of infectious diseases among patients with a CD4 cell count 200 - 500 cells/ μ L and their IAR decreased to almost the same level as seen in the population with a CD4 count > 500 cells/ μ L. Without doubt this change in the IARs is largely caused by the decreased mortality and morbidity in HIV patients due to HAART (4;43). The IARs for patients with a CD4 cell count < 200 cells/ μ L and > 500 cells/ μ L changed little. Interestingly, the HIV patients ended up being admitted two times more at departments of non-infectious diseases than at departments of infectious disease, where they were mainly treated in the start of the epidemic. This finding indicates a shift in the morbidity of HIV patients from being primarily HIV

related to something non-HIV related. We believe that this is in part explained by the HAART induced improvement of the incidence and prognosis of HIV related morbidity. Toxicity of HAART could explain part of this finding. This issue have been extensively explored and a study from the Data Collection on Adverse Events of Anti-HIV Drugs study have showed that treatment with protease inhibitors increased the risk of myocardial infarction by 16% with each year of exposure (44). This could not be confirmed in our study since the relative risk for the HIV patients of being admitted or seen on outpatient basis under cardiovascular diagnoses compared to the control population was rather stable during the observation period. Still, the HIV patients were admitted or seen on outpatient basis under cardiovascular diagnoses twice as much as the control population which corresponds well with a twofold increased risk of acute myocardial infection (45). In spite of a decreased risk of lymphoma and Kaposi's sarcoma following HAART the RRs for HIV patients for both outpatient visits and admissions under malignant diagnoses remained high which probably reflects the increased risk of non-AIDS defining cancer found in other studies from DHCS (46-48).

In general, study 2 suggests that the HIV patients have an increased risk of non-HIV comorbidity compared to population controls and this has been confirmed in several other studies from DHCS (45;47-51). Chronic immune activation in well treated HIV patients leading to accelerated ageing has been a major suspect (52). Another possible explanation could be that our results are biased by an unknown confounder. Confounding is defined as a factor related to both the exposed population (the HIV patients) and the outcome (hospitalization). Usually estimates are adjusted for confounding using statistical models but some confounders are not known or difficult to estimate resulting in residual or unmeasured confounding (26). Several of our studies from DHCS have demonstrated an increased risk of both cardiovascular disease and certain cancers in parents of

Danish HIV infected patients compared to parents of population controls (47;48;51). This implies that family associated or behavioral risk factors are involved. Risk-taking behavior is moderately to strongly related to heritability and for example offspring of smokers have a four times higher risk of initiating smoking (53;54). The results in our study may therefore be socially confounded by differences in life style, especially the prevalence in smoking.

Finally, one should keep in mind that inpatient and outpatient visit rates are not a direct measure of morbidity but influenced by changes in health care policies and culture which call for some caution when interpreting the results. Namely the structure of the Danish health care system have changed during the observation period with a decreasing number of hospital beds while the number of outpatient visits has increased substantially (55). Further, many diagnostic and therapeutic procedures now take place in outpatient clinics. This may explain part of the decrease in IARs in our study and emphasises the need to control for calendar effects in observational studies of HIV populations. Even though improved diagnostics may have affected the absolute risk of being admitted to a hospital this is also be the case for the control populations in our study and the relative risk should therefore remain unchanged by this bias.

The OVRs in for HIV patients at departments of infectious diseases increased slightly after the introduction of HAART and thereafter stabilized around six visits per year per patient, which is similar to the findings from other HIV centres in the late-HAART period (15). In Denmark patients on HAART are seen at intended three-month intervals (four times a year), which therefore accounts for almost two thirds of these visits.

In conclusion, we failed to reject hypothesis 2.1 since the use of health care facilities by HIV patients after the introduction of HAART has shifted from inpatient to outpatient treatment. The

decrease in inpatient admissions is mainly observed at departments of infectious diseases and for HIV patients with CD4 cell counts between 200 - 500 cell/ μ L. In contrast, the increased use of outpatient visits mainly takes place in departments of non-infectious diseases and is probably a consequence of the increased age and comorbidity in the HIV population and a general trend towards a shift from inpatient to outpatient treatment in European health care systems. Also hypothesis 2.2 could not be rejected as the HIV patients still have an excess use of health care facilities compared to population controls.

6.3. Discussion of Study 3

In a population of HIV patients on successful HAART for more than three years with initial low CD4 cell count we found that almost one out of five was INR and that older age and more than one year of severe immune deficiency prior to start of sustained VL were risk factors for INR. This finding is in accordance with van Lelyveld SF et al and other studies although there is some variation in the study designs (56-58). The biological mechanism behind poor immunological recovery is not completely understood although decreased thymus size and thymus activity are associated with slow CD4 cell recovery in several studies (6;13;19;59).

Another main finding in Study 3 was the excess mortality seen among the INRs. Recently van Lelyveld et al have also found an increased mortality in HIV patients with poor immunological recovery (56). The study was performed in the Dutch ATHENA cohort and included HAART treated patients virally suppressed for 1.5 and 2 years. They defined poor immunological recovery as a CD4 cell count < 200 cells/ μ L despite two years of virological successful HAART and found that it was associated with a higher risk for overall morbidity and mortality and cardiovascular events in particular. We found that the increased risk of death was mainly explained by prolonged

immunological suppression prior to successful HAART and IDU. Since almost two thirds of the patients with prolonged immunological suppression prior to successful HAART were diagnosed with HIV before 1995 the explanation is in most cases delayed initiation of efficient HAART due to scarce treatment options and viral resistance. Thus it may seem as a historical problem but with a quite constant number of late presenters not just in Denmark the problem is indeed relevant and emphasises the importance of an early diagnosis of HIV (60). The increased mortality in patients with delayed initiation of successful HAART is well documented and the contribution of persistent immunodeficiency to the development of for example cancer has been observed by others (61;62). While IDUs in general do not have an increased risk of HIV-related death in the HAART era, they have a substantially increased risk of non-HIV related death, even when successfully treated with HAART, which corresponds well with our findings (63;64). We encountered few AIDS related deaths among the INRs but we cannot rule out that there is a biological cause of the increased mortality in INRs related to immunodeficiency since our study simply do not have power enough to demonstrate an association. The reason for such a correlation is not clear but chronic immune activation in virally well treated HIV patients has been proposed as a cause of both poor CD4 cell recovery and otherwise non-HIV related endpoints like cardiovascular disease (65;66). As in Study 2 our results may also be confounded by lifestyle factors like smoking and other risk taking behavior.

With the findings from Study 3 we failed to reject hypothesis 3 since HIV patients with poor immunological recovery after more than three years of successful HAART had an increased mortality. Both poor CD4 cell recovery and the increased mortality were related to prolonged immunological suppression. These findings again points toward early diagnosis of HIV as the main

prophylactic measure. Also, the increased mortality in these patients calls for increased concern in terms of treatment and screening for comorbidity.

6.4. Strengths

A major strength of this thesis is the population-based design with long and nearly complete follow-up. This has allowed us to perform nationwide analyses with well validated follow-up without the bias of selected cohorts and study populations.

Another strength is the quality and coverage of The Danish Civil Registration System and the Danish Hospital Registry which enabled us to present the first nationwide study on the use of both inpatient admissions and outpatient visits of HIV infected persons compared with a matched control population (Study 2).

The major strength of Study 3 is the identification of a homogeneous group with stringent viral suppression, essentially eliminating the possibility that incomplete viral suppression explains our findings.

6.5. Limitations

In Study 1 the diagnosis was confirmed by histology or PCR in only half of the patients. This may lead to the concern that some patients have been misclassified. However, the group with confirmed PML did not differ from the group diagnosed by clinical and radiological findings in prognosis. Also, we cannot rule out that some PML cases may have been misclassified as other neurodegenerative diseases such as HIV dementia which may have caused underestimation of the true incidence of PML in our study. Prospective assessment of neurological deficits and performance by standardized scoring systems would have been preferable as patient files are

known to be incomplete in registration. Consequently the retrospective design of this part of the study will probably tend to underestimate the degree of neurological symptoms and sequelae after diagnosis of PML. The neurologic prognosis after diagnosis of PML may therefore be even worse than what we have estimated.

One of the limitations in Study 2 lies in the comparison of HIV patients and non-HIV infected individuals. One concern is that we did not match the two populations on geography, but data from Statistics Denmark indicates that regional IARs differed little in the observation period (23). Further, treatment of HIV-infected patients in Denmark mainly takes place at university hospitals whereas medical treatment among the control population presumably is less centralized why treatment and admission patterns may differ.

A major limitation of Study 3 is the small study population (in part caused by the stringent definitions of INRs and IRs) and the small number of outcomes, which did not allow us to extend the logistic regressions analysis or to calculate cause specific rate ratios of death. The study population encompasses a highly selected group of patients surviving more than three years in spite of low initial CD4 cell count, and a healthy survivor effect may lead to underestimation of the excess mortality in the INRs. Still, by using an extended virally suppressed period we allowed the patients to reach a steadier CD4 plateau without the interference of CD4 cell redistribution as seen after HAART start. At present we are running similar analyses on data from 181,976 HIV infected patients from ART-CC and COHERE, which may answer some of the questions raised by Study 3.

6.6. Conclusion and perspectives

The principle findings of this thesis was 1) both incidence and mortality of PML among Danish HIV patients have decreased after the introduction of HAART, 2) after the introduction of HAART the

use of health care facilities by HIV patients have in general shifted from inpatient to outpatient treatment mainly at departments of non-infectious diseases but Danish HIV patients still have an excess use of health care facilities compared to population controls, 3) Danish HIV patients with poor immunological recovery after more than three years of successful HAART have an increased mortality.

This thesis provides new insight and new information concerning morbidity and mortality in Danish HIV patients. Whereas our result in Study 1 do not lead to changes or improvement of the current treatment of HIV it is relevant for both HIV patients and HIV clinicians in regards to diagnosing PML in HIV patients and the prognosis of HIV patients with PML. Furthermore, Study 2 showed that HIV patients have an increasing use of outpatient health care facilities which from an administrative perspective is valuable in regards to planning, budgeting and distribution of resources in the health care sector. Finally, Study 3 demonstrated that HIV patients, who in spite of successful HAART continued to have a low CD4 cell count, have an excess mortality associated with prolonged immunosuppression which underlines the importance of an early HIV diagnosis. Also, the increased mortality in these patients calls for increased concern in terms of treatment and screening for comorbidity.

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8. Publications

8.1. Study 1

MAJOR ARTICLE

Incidence, Clinical Presentation, and Outcome of Progressive Multifocal Leukoencephalopathy in HIV-Infected Patients during the Highly Active Antiretroviral Therapy Era: A Nationwide Cohort Study

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Background. Human immunodeficiency virus (HIV) infection predisposes to progressive multifocal leukoencephalopathy (PML). Here, we describe the incidence, presentation, and prognosis of PML in HIV-1-infected patients during the period before highly active antiretroviral therapy (HAART) (1995–1996) and during the early HAART (1997–1999) and late HAART (2000–2006) periods.

Methods. Patients from a nationwide population-based cohort of adult HIV-1-infected individuals were included. We calculated incidence rates of PML and median survival times after diagnosis. We also described neurological symptoms at presentation and follow-up.

Results. Among 4649 patients, we identified 47 patients with PML. The incidence rates were 3.3, 1.8, and 1.3 cases per 1000 person-years at risk in 1995–1996, 1997–1999, and 2000–2006, respectively. The risk of PML was significantly associated with low CD4⁺ cell count, and 47% of cases were diagnosed by means of brain biopsy or polymerase chain reaction analysis for JC virus. The predominant neurological symptoms at presentation were coordination disturbance, cognitive defects, and limb paresis. Thirty-five patients died; the median survival time was 0.4 years (95% confidence interval [CI], 0.0–0.7) in 1995–1996 and 1.8 years (95% CI, 0.6–3.0) in both 1997–1999 and 2000–2006. CD4⁺ cell count >50 cells/ μ L at diagnosis of PML was significantly associated with reduced mortality.

Conclusions. The incidence of PML in HIV-infected patients decreased after the introduction of HAART. Survival after PML remains poor. In the management of PML, the main focus should be on prophylactic measures to avoid immunodeficiency.

Progressive multifocal leukoencephalopathy (PML) is a disease of the central nervous system and is caused by JC virus (JCV), a polyomavirus. It occurs almost exclusively in patients with severe defects in the cellular immune system. With the advent of the HIV pandemic,

HIV infection has become the single most predisposing disorder for PML [1, 2].

Only a few studies have estimated the incidence of PML during the highly active antiretroviral therapy (HAART) era. The EuroSIDA study found that the incidence of PML has declined from 10 cases per 1000 person-years of follow-up during the pre-HAART era

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to 1 case per 1000 person-years at risk during the HAART era [3].

The clinical presentation of PML varies according to the localization of the disease, and the initial symptoms may be misdiagnosed as other HIV- or non-HIV-related cerebral lesions. To improve the initial diagnostic strategy, it is important to know the major presenting symptoms of PML.

Although HAART has clearly improved survival after the diagnosis of PML, there is no known causal treatment of the disease [4, 5]. During the pre-HAART area, the median survival time was only a few months after diagnosis [6]. Since the introduction of HAART, survival has improved significantly, with a 1-year probability of survival of ~50% or higher, compared with 5% or lower in patients not receiving HAART [7, 8]. However, PML still frequently induces irreversible neurological sequelae [5, 9–10].

To our knowledge, no nationwide population-based studies have been conducted on the incidence, presenting symptoms, and prognosis of HIV-associated PML during the HAART era. In the present study, we used a nationwide population-based Danish cohort to determine the incidence of PML in HIV-infected patients during the pre-HAART (1995–1996), early HAART (1997–1999), and late HAART (2000–2006) periods, along with the incidence of PML in patients with CD4⁺ cell counts <200 or ≥200 cells/μL. We also describe the presenting clinical and paraclinical characteristics of PML, as well as the mortality and neurological outcome of the disease.

METHODS

In a cohort of HIV-1-infected individuals, we estimated the incidence, clinical presentation, and prognosis of PML. In the first part of the study, the outcome was time to PML in a cohort of HIV-1-infected individuals. In the second part of the study, the population was all patients with PML, and the outcomes were neurological symptoms and mortality.

Setting. Denmark had a population of 5.4 million as of 31 December 2006 [11], with an estimated HIV prevalence of ~0.07% in the adult population [12]. Patients with HIV infection are treated in one of the country's 8 specialized medical centers, where they are seen on an outpatient basis at intended intervals of 12 weeks. Antiretroviral treatment is provided free of charge to all HIV-positive residents of Denmark. During our study's follow-up period (1 January 1995 through 31 December 2006), the national criteria for initiating HAART were HIV-related disease, acute HIV infection, pregnancy, CD4⁺ cell count <300 cells/μL, and, until 2001, plasma HIV RNA level >100,000 copies/mL.

During the whole study period, polymerase chain reaction (PCR) analysis for JCV was performed at 2 laboratories. For details concerning PCR methods, see appendix A, which appears only in the electronic edition of the *Journal*.

Study population and data collection. The Danish HIV Cohort Study is a population-based prospective nationwide cohort study of all HIV-infected individuals ≥16 years old treated at Danish HIV centers after 1 January 1995. The study has been described elsewhere [12]. Patients are consecutively enrolled, and multiple registrations are avoided through use of a unique 10-digit civil registration number assigned to all individuals in Denmark at birth or on immigration. Data are updated yearly and include demographics, date of HIV infection, AIDS-defining events, date and reason of death, antiretroviral treatment, and all CD4⁺ cell counts and HIV RNA measurements. As of 31 December 2006, the cohort included 4660 Danish residents.

Outcomes. In the first part of the study, we estimated PML incidence rates in the entire Danish HIV cohort. PML was defined as either (1) demonstration of JCV by PCR analysis of cerebrospinal fluid (CSF) or by histopathological confirmation at brain biopsy or (2) a combination of characteristic clinical symptoms and neuroimaging findings highly suggestive of PML on magnetic resonance imaging (MRI) or computed tomography (CT) scans (for CT findings, hypodense lesions without contrast enhancement; for MRI findings, hyperintense white matter lesions on T2-weighted images or hypointense white matter lesions on T1-weighted images with no significant contrast enhancement and no mass effect).

In the second part of the study, the study cohort included the patients with a diagnosis of PML, and the outcomes were neurological symptoms and mortality. All patients with PML diagnosed between 1 January 1995 and 31 December 2006 were identified from the Danish HIV Cohort Study. Their patient files were examined by trained physicians, and information was extracted regarding neurological symptoms, laboratory results, and radiological findings at diagnosis of PML. The date of diagnosis of PML was defined as the date of initial clinical symptoms; clinical symptoms were counted as absent if they were not described in the patient files. If the temperature was not recorded in the files, it was assumed to be <37.5°C.

Neurological symptoms were estimated at 4 months and 3 years after the onset of symptoms (using the first available clinical evaluation after 4 months and 3 years, respectively). The neurological condition was registered as better, stable, or deteriorated compared with the initial presentation. In addition, a return to the pre-PML level of activity was noted, along with the need for help on a daily basis. If the patient files concluded that initiation of HAART had led to an immune reconstitution inflammatory syndrome (IRIS) related to PML, this was noted as well. Furthermore, we identified cases in which HAART had led to a rapid increase in CD4⁺ cell counts and reviewed MRI scans for contrast enhancement as a sign of IRIS.

Baseline characteristics. Differences in characteristics between groups were evaluated by the χ^2 test and Fisher's exact test,

as appropriate. Differences were considered significant at $P < .05$.

Incidence rates of PML. To estimate incidence rates, we calculated person-years at risk from the date HIV infection was diagnosed or from 1 January 1995 if the diagnosis preceded this date. Patients were censored at the date of last follow-up, the date of death, or 31 December 2006, whichever came first. PML was the outcome event. Incidence rates for diagnosis of PML and 95% confidence intervals (CIs) were calculated for 1995–1996, 1997–1999, and 2000–2006, corresponding to pre-HAART, early HAART, and late HAART periods. We also calculated the total periods of observation for HIV-infected patients with CD4⁺ cell counts <200 or ≥200 cells/mL. To determine CD4⁺ cell counts at time points falling between measurements, the last measured value was carried forward.

Mortality in patients with PML. We computed person-years at risk from the diagnosis of PML to the date of death, date of last follow-up, or 31 December 2006, whichever came first. We used Kaplan-Meier analysis to construct survival curves and estimate median survival for the calendar periods 1995–1996, 1997–1999, and 2000–2006, corresponding to pre-HAART, early HAART, and late HAART periods. Causes of death were classified into 3 categories: (1) related to PML, (2) not related to PML but related to HIV infection, or (3) not related to HIV infection.

Multivariate models. We used Cox regression analysis to assess the influence of risk factors with regard to the incidence of PML in the HIV-infected population and prognostic factors for mortality in the patients with PML. The following covariates were included in crude and adjusted models: sex, race (white vs. nonwhite), route of infection (injection drug use vs. others), age at diagnosis of HIV infection (≥40 vs. <40 years), and calendar year of diagnosis of HIV infection (before vs. after 1 January 1997). Furthermore, the CD4⁺ cell count at the index date (<200 vs. ≥200 cells/μL) was included in the Cox regression analysis of PML incidence. In the analysis of mortality, we further included AIDS-defining events before PML diagnosis, the CD4⁺ cell count at the onset of PML (<50 vs. ≥50 cells/μL), and antiretroviral treatment status at the diagnosis of PML. Confounding was evaluated by the change-in-estimate method, in which covariates changing the estimate by <10% were excluded from the model [13]. The study was approved by the Danish Data Protection Agency. SPSS statistical software (version 15.0; SPSS) was used for data analysis.

RESULTS

We identified 4660 patients with HIV-1 infection in the Danish HIV Cohort Study, 11 of whom had PML diagnosed before 1 January 1995, leaving 4649 patients in the study with a total of 27,693 person-years of follow-up. Of these, 1971 patients (42%) had HIV infection diagnosed before 1 January 1995, 1050 (23%)

Table 1. Incidence rates (IRs) for progressive multifocal leukoencephalopathy (PML).

Category	PYR	PML events	IR (95% CI), cases/1000 PYR
Observation period			
1995–1996	3903	13	3.3 (1.9–5.7)
1997–1999	6559	12	1.8 (1.0–3.2)
2000–2006	17,231	22	1.3 (0.8–1.9)
Receiving HAART	17,571	13	0.7 (0.4–1.3)
CD4⁺ cell count			
≥200 cells/μL	20,818	5	0.2 (0.1–0.6)
<200 cells/μL	4604	42	9.1 (6.7–12.3)

NOTE. CI, confidence interval; HAART, highly active antiretroviral therapy; PYR, person-years at risk.

died during follow-up, 48 (1%) were unavailable for follow-up, and 182 (4%) emigrated. Of the patients, 3500 (75%) were male, 3669 (79%) were white, 2080 (44.7%) were men who have sex with men, and 534 (11%) were injection drug users. The median age at the index date was 33.9 years (interquartile range, 21.7–56.2 years), and 3327 (72%) of the patients had been exposed to HAART.

Incidence of PML. In the study period, 47 patients met the criteria for a diagnosis of PML. The incidence rates for diagnosis of PML decreased considerably from the pre-HAART period to the early and late HAART periods (table 1). Among all patients receiving HAART and those who had received HAART for >6 months, the PML incidence rates were 0.7 (95% CI, 0.4–1.3) and 0.8 (95% CI, 0.4–1.3) cases per 1000 person-years at risk.

The incidence rates were 0.2 (95% CI, 0.1–0.6) and 9.1 (95% CI, 6.7–12.3) cases per 1000 person-years at risk, respectively, for patients with CD4⁺ cell counts ≥200 versus <200 cells/μL. A CD4⁺ cell count of ≥200 versus <200 cells/μL at diagnosis of HIV infection was the only significant marker for a decreased risk of PML (incidence rate ratio, 0.2 [95% CI, 0.11–0.47]). Evaluation by the change-in-estimate method showed that age, sex, race, injection drug use, or diagnosis of HIV infection before 1 January 1997 did not confound the beneficial effect of a CD4⁺ cell count ≥200 cells/μL.

Twelve patients developed PML despite receiving >6 months of HAART. By review of patient files, 7 of the 12 patients were confirmed to have experienced treatment failure due to viral resistance and/or compliance problems.

Characteristics and presenting symptoms in patients with a diagnosis of PML. Characteristics of the 47 patients with a diagnosis of PML are shown in table 2. At the onset of PML symptoms, most of the patients had advanced HIV disease, with a low CD4⁺ cell count (median, 50 cells/μL) and a high viral load (median, 4.9 log₁₀ HIV RNA copies/mL). In 9 of the 34 patients whose PML was diagnosed after 1997, HIV infection was diagnosed within 3 months before the PML diagnosis. Almost all patients had a nadir CD4⁺ cell count <200 cells/μL.

Table 2. Demographics and HIV-related characteristics of patients with progressive multifocal leukoencephalopathy (PML) diagnosed during the study period.

Characteristic	All (n = 47)	PML diagnosis		P
		1995–1997 (n = 13)	1998–2006 (n = 34)	
HIV RNA level, median (IQR), log ₁₀ copies/mL	4.9 (3.7–5.6)	NA	4.9 (3.7–5.6)	...
CD4 ⁺ cell count at diagnosis, median (IQR), cells/μL	50 (27–160)	40 (18–123)	58 (25–162)	.51
CD4 ⁺ cell count <200 cells/μL at PML diagnosis	6 (13)	2 (15)	4 (12)	>.99
AIDS diagnosis before PML diagnosis	18 (38)	6 (46)	12 (35)	.49
Male	35 (74)	13 (100)	22 (65)	.01*
Age, median (IQR), years	48.7 (43.3–53.0)	50.6 (45.2–54.2)	46.1 (44.2–51.5)	.48
Men who have sex with men	23 (49)	12 (92)	11 (32)	<.001*
Heterosexually infected	18 (38)	1 (8)	17 (50)	.01*
Injection drug user	4 (9)	0 (0)	4 (12)	.56
White	42 (89)	13 (100)	29 (85)	.14
Interval between HIV infection and PML, median (IQR), years	4.6 (1.2–10.9)	4.3 (2.5–10.7)	5.8 (0.1–12.0)	.91
Nadir CD4 ⁺ cell count <200 cells/μL	46 (98)	13 (100)	33 (97)	.53
Died during study period	35 (74)	12 (92)	23 (68)	.08
IRIS reported	2 (4)	0 (0)	2 (6)	>.99

NOTE. Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; NA, not available.

* $P < .05$.

The predominant neurological symptoms at the presentation of PML were coordination disturbances, cognitive defects, and limb paresis (table 3). In accordance with the high frequency of coordination disturbances, 25 patients (53%) had lesions involving the cerebellum and/or brainstem that were visualized radiographically. Very few patients presented with common signs of infection, such as fever and leukocytosis, along with the

neurological symptoms, but 18 (47%) of 38 had an increased sedimentation rate or C-reactive protein level (table 4). CSF pleocytosis was rarely seen (2%), but elevated levels of protein in the CSF were seen in 49% of patients. Abnormal decreases in CSF glucose level were not observed.

In 25 patients (53%), the diagnosis of PML was based exclusively on clinical symptoms combined with either MRI (18 pa-

Table 3. Neurological symptoms at primary presentation of progressive multifocal leukoencephalopathy (PML), at first follow-up after 4 months, and after 3 years.

Category, parameter	Disease presentation (n = 47)	Follow-up	
		4 months (n = 29)	3 years (n = 11)
Neurological symptoms			
Cognitive defects	27 (57)	14 (48)	4 (36)
Coordination disturbance	32 (68)	16 (55)	4 (36)
Speech disturbance	20 (43)	20 (69)	10 (91)
Visual impairment	13 (28)	14 (48)	2 (18)
Facial palsy	3 (6)	3 (10)	0 (0)
Limb paresis	20 (43)	14 (48)	1 (9)
Sensory affection	8 (17)	3 (10)	1 (9)
Seizures	6 (13)	6 (21)	4 (36)
Need help in everyday life	27 (57)	20 (69)	6 (55)
Status at follow-up			
Progression of neurological symptoms	...	11 (38)	2 (18)
Unchanged neurological symptoms	...	4 (14)	1 (9)
Improvement of neurological symptoms	...	14 (48)	8 (73)*

NOTE. Data are no. (%) of patients.

* Five patients returned to their pre-PML level of activity.

Table 4. Clinical and paraclinical data at presentation with progressive multifocal leukoencephalopathy (PML) and results of diagnostic tests.

Category, parameter	Proportion (%)
Clinical and paraclinical signs at presentation	
CSF pleocytosis (>10 cells/ μ L)	1/41 (2)
Elevated CSF protein level (>0.5 g/L)	19/39 (49)
Abnormal CSF glucose level compared with periphery	0/38 (0)
Fever >37.5°C	9/47 (19)
Fever >38.0°C	2/47 (4)
Leukocytosis in peripheral blood (>9 $\times 10^9$ cells/L)	3/46 (7)
Elevated sedimentation rate or CRP level	18/38 (47)
Results of diagnostic tests for PML	
PML diagnosed by brain biopsy ^a	14/47 (30)
JCV detected by PCR in CSF	13/31 (42)
PML diagnosed by brain biopsy with PCR for JCV negative in CSF ^b	6/14 (43)
PML diagnosed by MRI/CT and clinical symptoms	25/47 (53)

NOTE. CRP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; JCV, JC virus; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.

^a PML was diagnosed by means of in situ hybridization for JCV in 7 of the 14 patients and by means of JCV immunohistochemistry in 1 patient; 6 patients had characteristic histopathological features consistent with PML.

^b One of the 14 patients whose PML was diagnosed by means of brain biopsy did not undergo PCR for JCV.

tients) or CT (7 patients) findings. In 22 patients (47%), the PML diagnosis was confirmed by brain biopsy (14 patients) and/or PCR for JCV in the CSF (table 4). Thirteen of 31 patients tested had positive PCR results for JCV in the spinal fluid. Interestingly, 7 of the 14 patients whose PML was diagnosed by means of brain biopsy had negative PCR results for JCV. The negative PCR samples were equally distributed between the 2 laboratories and spread over the whole study period (data not shown).

Mortality. A total of 35 patients with PML died during the study period. According to the records, 28 deaths were related to PML, 5 were due to unknown causes, 1 was HIV related (lymphoma), and 1 was not related to HIV (end-stage liver disease). Eighteen patients died within 4 months after the onset of PML, and 12 were available for evaluation 3 years after the diagnosis of PML. Kaplan-Meier survival curves for the patients with a diagnosis of PML are shown in figure 1. The median survival time for all patients with PML diagnosed during the period 1995–2006 was 1.02 years (95% CI, 0.0–2.5 years). Of 13 patients with PML diagnosed before 1997, 12 died during the study period, with a median survival of 0.4 years (95% CI, 0.0–0.7 years). Of 34 patients whose PML was diagnosed from 1997 onward, 23 died before 31 December 2006; their median survival time was 1.8 years (95% CI, 0.9–2.6 years). We found no differences in survival among patients with PML diagnosed in 1997–1999 versus 2000–2006 (data not shown).

Of the 47 patients with PML, 36 were treated with HAART, and 24 of the 36 died during the study period. The median survival time in this group was 1.8 years (95% CI, 0.8–2.8 years). Of the 12 patients who had PML diagnosed >6 months after the

start of HAART, 11 died; their median survival was 0.4 years (95% CI, 0.0–2.0 years). The median survival times did not differ between patients whose diagnosis was based on brain biopsy

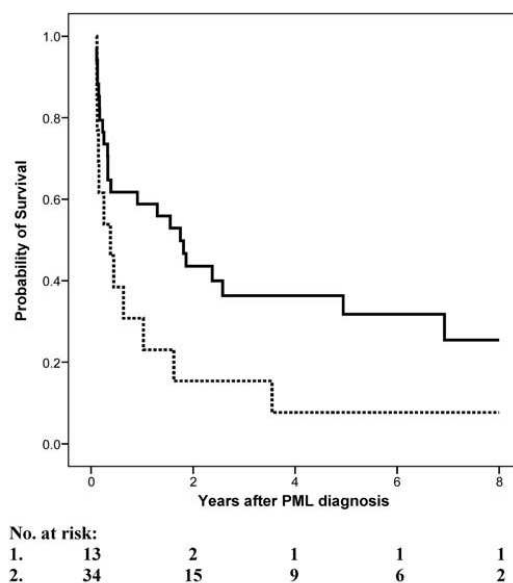


Figure 1. Kaplan-Meier curves for overall survival by time after progressive multifocal leukoencephalopathy (PML) diagnosis, before (dotted line and group 1) and after (solid line and group 2) 1997.

findings before death and those whose diagnosis was based on characteristic clinical and radiological findings (data not shown).

In unadjusted analyses, a CD4⁺ cell count ≥ 50 cells/ μ L at the diagnosis of PML (mortality rate ratio [MRR], 0.47 [95% CI, 0.24–0.93]) and a diagnosis of PML after 1997 (MRR, 0.48 [95% CI, 0.24–0.97]) were associated with reduced mortality. Neither variable was confounded by age, CD4⁺ cell count at index date, sex, race, injection drug use, or AIDS-defining diagnosis before PML. Viral load at the time of the onset of symptoms was not included in the analysis, because of a lack of data (43% missing).

Neurological outcome. The neurological symptoms were largely unchanged after 4 months of follow-up. The level of help needed to perform everyday activities was high during the follow-up period (table 3). Patients surviving >3 years fell into 2 groups: patients with progression of neurological symptoms or unchanged symptoms (27%), and those with a high degree of restitution or a return to their pre-PML level of life activity (73%). Rescanning was not done systematically. Of 15 patients who underwent MRI rescanning after the initiation of HAART, only 3 had a partial regression of lesions, but a wide time interval (varying from weeks to years) precludes systematic conclusions.

IRIS. Only 2 patients had IRIS reported in the patient files as a possible explanation for neurological deterioration after the initiation of HAART. Both patients died, at 40 and 59 days after the diagnosis of PML. Review of their MRI scans revealed no signs of contrast enhancement.

DISCUSSION

In this population-based cohort study, we found that the incidence of PML has steadily decreased after the introduction of HAART. Survival has improved, but the prognosis for patients with PML remains poor, with a high mortality rate and a high degree of neurological sequelae among survivors despite free access to HAART.

To our knowledge, this study is the first to present nationwide analysis of the incidence, presentation, and prognosis of PML in HIV-infected patients. A major strength of the study is the population-based design, with long and nearly complete follow-up. This allowed us to estimate the incidence rates of PML for pre-HAART, early HAART, and late HAART periods and the incidence of PML stratified by CD4⁺ cell count.

Our study has some weaknesses. The diagnosis was confirmed by histology or PCR in only half of the patients. This may lead to the concern that some patients were misclassified. However, the group with confirmed PML did not differ in prognosis from the group whose diagnoses were based on clinical and radiological findings. Assessment of neurological deficits and performance by standardized scoring systems would have been preferable, because patient files are known to be incomplete at registration. Consequently, the retrospective design of this part of the study

will probably tend to underestimate the degree of neurological symptoms and sequelae after the diagnosis of PML. The prognosis after diagnosis may therefore be even worse than what we have observed.

Our results show that the incidence of PML decreased after the introduction of HAART and that it continued to decline over time during the HAART period. This decrease is probably associated with increased effectiveness of HAART due to better and more simple drug regimens, as documented by increased survival [14] and decreased treatment failure [15]. The main risk factor for the development of PML is low CD4⁺ cell count, and we clearly demonstrated that the risk of PML is extremely low when the CD4⁺ cell count is ≥ 200 cells/ μ L. We mainly observed PML in late presenters and in patients with failure of HAART due to compliance problems. Even though we expect the effectiveness of antiretroviral treatment to continue to increase in the near future, the incidence of PML will probably not decrease considerably in coming years unless more measures are taken to diagnose HIV infection earlier. Our data clearly demonstrate that the main focus in the management of PML should be on the prophylactic effect of maintaining a CD4⁺ cell count ≥ 200 cells/ μ L.

The neurological symptoms we observed at the presentation of PML and at follow-up are in accordance with previous findings, in which the main presenting symptoms of PML were cognitive impairment, paresis, and cerebellar affection, with seizures in only a few patients [5, 9, 10, 16]. In accordance with a report from Albrecht et al. [10], half of the patients with PML had elevated protein levels but no pleocytosis in the CSF. These findings indicate that signs of universal or meningeal inflammation are not suggestive of PML.

PCR for JCV in CSF has high specificity (96%–99%), but reported sensitivities have varied from 57% to 90% [17, 18]. We also found a low sensitivity of PCR for JCV. It has been proposed that this low sensitivity is due to periventricular localization of PML lesions, which may result in low shedding of the virus in CSF. Therefore, ante- or postmortem biopsy, which has high sensitivity and specificity, is the reference standard [18]. However, biopsy may be contraindicated, the PML lesions may be difficult to access, and the patient may be unwilling to participate; for these reasons, most agree that the diagnosis is presumptive in patients with progressively deteriorating neurological function and characteristic findings on MRI or CT. This approach to diagnosis was exemplified in the present study; 53% of diagnoses were not based on brain biopsy findings or PCR for JCV. The difficulties in confirming the diagnosis are as likely to lead to the underdiagnosis of PML as to overdiagnosis.

We observed a beneficial effect of HAART on mortality in patients with a diagnosis of PML, with a median survival of 1.8 years. This finding is considerably improved compared with pre-HAART survival [19]; however, compared with other findings in HIV-infected patients with PML, the effect of HAART seemed to

be lower in our study [6, 8, 9]. Previous studies were characterized by shorter observation periods. Furthermore, because they were not population based, they may have suffered from potential selection bias. In selected cohorts, there is a risk that the patients affected most by PML have not been included in the study population. A beneficial effect of cidofovir on PML has been proposed in some studies [7, 20]. Given that only 1 of our patients was treated with cidofovir, it could not have influenced our results.

The development of PML after 1997 was a considerable prognostic factor for survival, most likely because of the effect of HAART. This finding is supported by the identification of CD4⁺ cell counts ≥ 50 cells/ μ L at PML diagnosis as another prognostic factor. Similar observations were made by Antinori et al. [8], who also found that both patients previously exposed to HAART who continued treatment after PML diagnosis and those starting HAART at the time of PML diagnosis had a significantly reduced risk of death compared with patients who did not receive HAART.

Although there is no specific treatment for PML, restoration of the immune defect is essential. Paradoxically, this may lead to PML as a part of IRIS. Of note, only 2 patients had a possible diagnosis of IRIS. The true incidence of IRIS among those with PML is unclear; the reported incidences vary from 6.5% to 18% among HIV-infected patients with PML [21, 22].

In conclusion, the present study shows that the incidence of PML has decreased and that its prognosis has improved in HIV-infected patients after the implementation of HAART, but the disease still has a high mortality rate, and patients who survive are often left with severe neurological sequelae. The main focus in the management of PML should be on prophylactic measures, on maintaining a high CD4⁺ cell count through early diagnosis of HIV infection, and on initiating HAART before immunological deterioration.

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CONCISE COMMUNICATION

Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995–2007

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Gitte Kronborg^b, Carsten Schade Larsen^c and Niels Obel^a

Objective: HAART has changed morbidity and mortality in the HIV-infected population dramatically. We aimed to estimate the use of healthcare facilities in HIV-infected patients after the introduction of HAART.

Design: This is a prospective nationwide cohort study.

Methods: We identified all Danish HIV patients and a cohort of population controls matched on sex and date of birth. The study period was 1995–2007. We calculated inpatient admission rates and outpatient visit rates stratified by medical speciality and International Classification of Diseases-10 diagnose categories. Relative risks were computed.

Results: Four thousand, seven hundred and sixty HIV-infected patients and 23 800 population controls were identified. Overall inpatient admission rates [95% confidence interval (CI)] for HIV-infected patients decreased from 90 (88–93) to 57 (56–58)/100 person-years in the study period. The risk ratio (95% CI) fell from 6.2 (6.0–6.5) to 3.1 (3.1–3.2) predominantly due to reduced inpatient admission rates to departments of infectious diseases. The overall outpatient visit rates (95% CI) for the HIV-infected patients increased from 744 (737–751) to 877 (872–882)/100 person-years, mainly due to visits at departments other than infectious diseases. A marked increase in outpatient visit rates (95% CI) in the background population decreased the risk ratio from 16.5 (16.2–16.8) to 7.1 (7.0–7.2). We observed a decreased relative risk of inpatient admissions and outpatient visits due to cancers and a small increase in relative risk due to cardiovascular disease.

Conclusion: After the introduction of HAART, the inpatient treatment of HIV-infected patients has decreased, especially at departments of infectious disease. In contrast, this population's use of outpatient facilities has increased in noninfectious disease specialities.

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Keywords: control population, HAART, HIV, inpatient admission rate, nationwide cohort study, outpatient visit rate

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Introduction

After the introduction of HAART, HIV has changed from a fatal disease to a chronic condition, and well treated HIV patients now have an overall life expectancy close to non-HIV-infected individuals [1]. The decreased morbidity and mortality may have led to changes in hospitalization patterns and the use of healthcare resources. To elucidate this issue, we describe rates of inpatient admission rates (IARs) and outpatient visit rates (OVRs) in a nationwide population-based cohort study of HIV-infected patients and a cohort of age and sex-matched population controls in the period 1995–2007.

Methods

Setting

Denmark had a population of 5.5 million as of 31 December 2008 [2], with an estimated HIV prevalence of approximately 0.07% in the adult population [3].

Data sources and study population

The Danish HIV Cohort Study (DHCS) is a population-based prospective nationwide cohort study of all HIV-infected patients of age 16 years or older at diagnosis, treated at Danish HIV centres after 1 January 1995. The study has been described previously [4]. From DHCS, we identified and included all HIV-1-infected patients with Danish residency.

We identified five population controls matched on sex and date of birth for each HIV patient from the Danish Civil Registration System (CRS) for every study period [5].

Data on inpatient admissions and outpatient visits for the study participants were obtained from Danish National Hospital Registry (DNHR) [6].

Details on all the above-mentioned registries are described in the Supplementary material (www.aidsonline.com).

Analyses

We calculated the absolute number of HIV-infected patients seen in the healthcare system in each calendar year in the period 1995–2007 and stratified it by age (>50 years, >60 years). To estimate the changes over time in consumption of healthcare resources for the HIV population, we determined the total number of inpatient days and outpatient visits.

For each population, we computed

- (1) IARs (number of inpatient admissions/100 person-years of follow-up) and
- (2) OVRs (number of outpatient visits/100 person-years).

IARs and OVRs were stratified by admission to departments of infectious disease versus all other departments, primary International Classification of Diseases-10 (ICD-10) discharge diagnoses and CD4 cell count. See Supplementary material for further details on calculations of IARs, OVRs and the above-mentioned stratifications.

We calculated crude Poisson 95% confidence intervals (95% CIs) and relative risk (RR) comparing rates in the HIV-infected patients with that in the population controls.

In a subanalysis, we computed RRs for inpatient admissions and outpatient visits exclusively for non-IDUs and patients diagnosed after 1 January 1995.

The study was approved by the Danish Data Protection Agency.

Results

We identified 4760 HIV-1-infected patients with a total observation time of 33 998 person-years. One thousand, nine hundred and thirty-nine patients (40.7%) were diagnosed with HIV before 1 January 1995, and 1157 (24.3%) of the HIV-infected patients died during the study period, 14 (0.3%) were lost to follow-up and 172 (3.6%) emigrated. Three thousand, six hundred and forty (76.5%) of the patients were men and 3800 (79.8%) whites. Two thousand, one hundred and sixty-one patients (45.4%) were MSM and 524 (11.0%) were IDUs. Median age [interquartile range (IQR)] at study inclusion was 34.4 (28.1–42.5) years. Three thousand, four hundred and ninety-one (73.3%) of the patients had been exposed to HAART.

The yearly number of HIV patients seen in the Danish hospital system increased by 61% from 2184 in 1995 to 3524 in 2007. The number of HIV-infected patients under 50 years of age increased by 31%, whereas the number of patients above 50 and 60 years of age increased by 208 and 596%, respectively (Supplementary Fig. 1).

The total number of inpatient admission days for the HIV-infected patients decreased by 43% from 18 460 days in 1995 to 10 464 days in 2007. In contrast, the yearly number of outpatient visits rose by 127% from 13 491 visits in 1995 to 30 607 in 2007.

Inpatient admission rates

Over the observation period, the overall IAR for HIV-infected patients decreased by 37%, whereas it increased by 25% for the population controls and thereby caused the RR to decrease from 6.2 in 1995–1997 to 3.1 in 2004–2007.

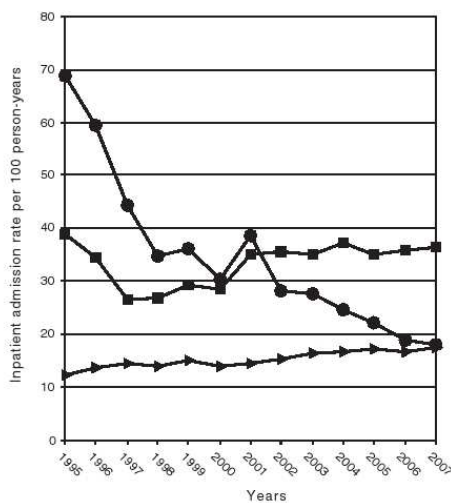


Fig. 1. Inpatient admission rates per 100 person-years at the departments of infectious diseases (circle) and noninfectious diseases (square) for HIV patients and controls (triangle). Inpatient admission rates at departments of infectious diseases for non-HIV patients are not shown due to the very low rates.

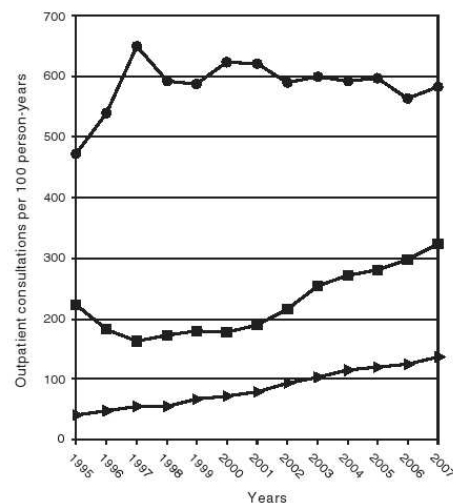


Fig. 2. Outpatient visit rates per 100 person-years at the departments of infectious disease (circle) and noninfectious disease (square) for HIV patients and for controls at departments of noninfectious medicine (triangle). Outpatient visit rates at departments of infectious diseases for non-HIV patients are not shown due to the very low rate.

IARs at departments of infectious diseases fell from 69/100 person-years in 1995 to 18/100 person-years in 2007 (Fig. 1). For the HIV population, the IARs at departments of noninfectious disease specialties initially decreased and then rose to a stable level of around 35/100 person-years, whereas it increased from 12 to 17/100 person-years in the population controls.

When categorized according to primary discharge diagnosis, only the IAR due to cancer diagnoses increased in the observation period for both populations, but less for the HIV population, whereas the RR decreased from 3.1 to 2.5 (Supplementary Table 2). The IAR with primary discharge diagnoses of cardiovascular diseases increased more for the HIV-infected patients (76%) than for the control population (46%) and consequently the RR increased from 1.6 to 2.1.

CD4 cell stratified inpatient admission rates

For HIV-infected patients with a CD4 cell count between 200 and 500 cells/ μ l, the overall IARs decreased in the study period to almost the same level as for patients with a CD4 cell count above 500 cells/ μ l (50/100 person-years, Supplementary Fig. 2). The number of person-years at risk for patients with a CD4 cell count below 200 cells/ μ l decreased by 73%.

Outpatient visit rates

The overall OVRs for the HIV-infected patients increased slightly by 18% in the period 1995–2007. In

the same period, this rate increased by 175% in the control population, causing the RR to decrease from 16.1 to 7.1.

Rates of outpatient visits at departments of noninfectious disease specialties initially decreased for the HIV patients and then increased by 100% from 1997 to 2007. The OVR for the control population increased by 241% in the study period (Fig. 2).

The RR for outpatient visits under the diagnosis for cancer decreased from 8.0 to 2.6. The RR for cardiovascular diagnoses increased from 2.0 to 2.5 in the observation period. OVRs and RRs for all disease categories are presented in Supplementary Table 3.

We observed no changes in RRs for inpatient admissions and outpatient visits in the late HAART period when performing separate calculations restricted to non-IDUs and patients diagnosed after 1 January 1995 (data not shown).

Discussion

In this population-based cohort study, we found that the overall utilization of inpatient services by the Danish HIV-infected population fell dramatically after the introduction of HAART, whereas it continued to increase for outpatient services, especially at noninfectious disease specialties.

To our knowledge, this is the first nationwide study on the use of both inpatient admissions and outpatient visits of HIV-infected patients compared with a matched control population. A major strength of the study is the quality and coverage of the Danish registries and the population-based design with long and nearly complete follow-up.

Our study has some limitations. We did not match the two populations on geography, but data from Statistics Denmark indicate that regional IARs differed little in the observation period [2]. Our population of HIV patients included both IDUs and those diagnosed before 1995, but this did not seem to bias our results. Further, treatment of HIV-infected patients in Denmark mainly takes place at university hospitals, whereas medical treatment among the control population is less centralized, and treatment and admission patterns may differ [2].

Along with the increased survival of HIV patients in the observation period [1], median age has increased and almost one-third of the population is now more than 50 years old. Aging of the HIV population probably explains some of the increase in outpatient contacts to non-infectious disease specialities as it is also seen for the control population.

The overall IAR for HIV patients in 2007 ended up being almost three times as high as in the background population. The decrease was mainly seen at departments of infectious diseases. Similar changes in the IARs for HIV-infected patients have been described previously with regard to hospitalization for pneumonia [7] and in the early HAART era [8]. Part of the decrease in IARs may be explained by changes in the structure of the European healthcare system, which has caused a declining number of beds in Danish hospitals and an increasing number of outpatient visits [9,10]. Our data further emphasize the need to control for calendar effects in observational studies of HIV populations.

We saw an increase in both inpatient admissions and outpatient visits for cardiovascular and endocrinological diseases compared with the population controls. The increase in RR was, however, small and we cannot exclude that later years' focus on the metabolic aspects of HAART has caused an excess referral to endocrinological and cardiological specialities. The RR for outpatient admissions under malignant diagnoses decreased in the study period, probably mainly due to a decreased risk of lymphoma and Kaposi's sarcoma in the HIV population. The IAR for patients with CD4 cell count between 200 and 500 cells/ μ l decreased to almost the same level as seen in the population with a CD4 cell count above 500 cells/ μ l. This is probably caused by the increased use of HAART in the study period [1]. A higher proportion of the patients therefore have suppressed viral replication and increasing CD4 cell count and thereby reduced risk of HIV-related disease and inpatient admission [11]. Our

results correspond to those of Mascolini [12] who found an IAR of around 40/100 person-years among responders to HAART 3 months after initiation of HAART. The OVRs in infectious disease clinics increased slightly after the introduction of HAART and thereafter stabilized at around six visits per year, which is similar to the findings from other HIV centres in the late-HAART period [13]. In Denmark, patients on HAART are seen at intended 3-month intervals (four times a year), which thereby accounts for almost two-thirds of these visits.

The use of outpatient clinic visits at departments of noninfectious diseases increased for both the HIV-infected population and the control population, and the HIV patients were seen almost twice as much as the background population in these clinics. The data suggest that HAART has not decreased the impact of non-HIV-related comorbidity in the HIV population and support the view of Deeks and Phillips [14] that even well treated HIV-infected patients have increased risk of comorbidity.

Conclusion

The introduction of HAART has changed the use of healthcare facilities from inpatient to outpatient treatment. The decrease in inpatient admissions is mainly observed at departments of infectious diseases and for HIV patients with CD4 cell count between 200 and 500 cells/ μ l. In contrast, the increased use of outpatient visits mainly takes place in noninfectious disease specialities and is probably a consequence of increased age of the HIV population and a general trend towards a shift from inpatient to outpatient treatment in European healthcare systems.

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F.N.E. contributed to the study design, data analysis, interpretation of findings and writing of the manuscript. A.B.H. contributed to the interpretation of findings and critical edit of the manuscript. J.G. contributed to the study design, interpretation of findings and critical edit of the manuscript. G.K. contributed to the data collection and critical edit of the manuscript. C.S.L. contributed to the data collection and critical edit of the manuscript. N.O. contributed to the study design, critical review of data analyses, interpretation of findings and critical edit of the manuscript.

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N.O. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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A.B.H., G.K. and C.S.L. have no conflicts of interest.

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Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995–2007.

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Supplementary material

Details on data extraction and registries

The Danish Civil Registration System (CRS)

The Danish Civil Registration System (CRS) is a national registry of all Danish residents established in 1968. [1]. A 10-digit personal identity number (Civil Person Registration (CPR) number) assigned at birth or immigration uniquely identifies each person and thereby avoids double registrations. The CPR number is used by all public registries [2]. For every study period we Identified matched cohorts of population controls from CRS and included five population controls matched on gender and date of birth for each HIV patient. Date of emigration, immigration and death for both the HIV and the control cohorts were identified from this registry.

Danish National Hospital Registry (DNHR)

Supplementary table 2. Inpatient admission rates for HIV patients and population controls with relative risk overall and stratified by ICD-10 diagnoses.

Periods		1995-1997		1998-2000		2001-2003		2004-2007	
		IR* (95%CI) ¹	RR ² (95%CI)	IR (95%CI)	RR (95%CI)	IR (95%CI)	RR (95%CI)	IR (95%CI)	RR (95%CI)
All diagnoses	HIV patients	90.4 (88.1-92.9)	6.2 (6.0-6.5)	61.9 (60.1-63.8)	4.3 (4.1-4.5)	66.6 (34.8-68.4)	4.1 (4.0-4.3)	57.1 (55.8-58.4)	3.1 (3.1-3.2)
	Population Controls	14.6 (14.2-15.0)		14.3 (13.9-14.7)		16.2 (15.9-16.6)		18.2 (17.9-18.5)	
Infectious disease	HIV patients	54.6 (52.8-65.5)	148.0 (97.1-225.5)	29.0 (27.7-30.3)	99.5 (64.0-154.6)	30.2 (29.1-31.4)	89.6 (61.6-130.4)	21.6 (20.8-22.4)	64.5 (47.6-87.5)
	Population Controls	0.4 (0.2-0.6)		0.3 (0.2-0.5)		0.3 (0.2-0.5)		0.3 (0.3-0.5)	
Cancers	HIV patients	3.7 (3.2-4.2)	3.1 (2.3-4.1)	4.1 (3.7-4.6)	3.3 (2.6-4.2)	4.4 (4.0-4.9)	3.1 (2.6-3.9)	4.7 (4.3-5.0)	2.5 (2.2-2.9)
	Population Controls	1.2 (0.9-1.5)		1.3 (1.0-1.5)		1.4 (1.2-1.7)		1.9 (1.6-2.1)	
Endocrinological disease	HIV patients	0.5 (0.3-0.7)	1.2 (0.7-2.1)	0.5 (0.4-0.7)	1.7 (1.0-3.0)	0.6 (0.4-0.8)	1.5 (1.4-1.8)	0.7 (0.6-0.9)	1.6 (1.2-2.3)
	Population Controls	0.4 (0.3-0.5)		0.3 (0.2-0.4)		0.4 (0.3-0.4)		0.4 (0.4-0.5)	
Neurological disease	HIV patients	1.2 (0.9-1.5)	4.2 (2.5-7.2)	0.9 (0.7-1.2)	2.1 (1.4-3.2)	1.0 (0.8-1.2)	2.2 (1.5-3.3)	0.7 (0.6-0.9)	1.5 (1.1-2.0)
	Population Controls	0.3 (0.2-0.3)		0.4 (0.4-0.5)		0.4 (0.4-0.5)		0.5 (0.4-0.6)	
Cardiovascular disease	HIV patients	2.5 (2.1-2.9)	1.6 (1.2-2.1)	3.1 (2.7-3.5)	1.8 (1.5-2.3)	4.0 (3.6-4.4)	2.0 (1.7-2.4)	4.4 (4.1-4.8)	2.1 (1.8-2.4)
	Population Controls	1.5 (1.4-1.7)		1.7 (1.5-1.8)		2.0 (1.8-2.1)		2.2 (2.0-2.3)	
Pulmonary disease	HIV patients	5.5 (4.9-6.1)	7.3 (5.3-10.0)	3.5 (3.1-4.0)	5.1 (3.8-7.0)	4.4 (4.0-4.9)	6.8 (5.1-9.0)	3.9 (3.6-4.3)	4.5 (3.6-5.5)
	Population Controls	0.7 (0.7-0.9)		0.7 (0.6-0.8)		0.7 (0.6-0.7)		0.9 (0.8-1.0)	
GI tract disease	HIV patients	3.8 (3.4-4.4)	2.3 (1.8-3.0)	3.6 (3.2-4.1)	2.5 (2.0-3.2)	3.7 (1.5-1.8)	2.3 (1.8-2.8)	3.4 (3.1-3.7)	2.1 (1.7-2.4)
	Population Controls	1.6 (1.5-1.8)		1.4 (1.3-1.5)		1.6 (1.5-1.8)		1.6 (1.5-1.7)	
Skin disease	HIV patients	3.0 (2.6-3.5)	13.4 (7.7-23.3)	1.7 (1.4-2.1)	7.2 (4.3-12.0)	1.6 (1.3-1.9)	4.5 (3.0-6.7)	1.3 (1.1-1.5)	3.0 (2.2-4.1)
	Population Controls	0.2 (0.2-0.3)		0.2 (0.2-0.3)		0.4 (0.3-0.4)		0.4 (0.4-0.5)	
Urinary tract disease	HIV patients	1.2 (0.9-1.5)	1.1 (0.8-1.5)	1.2 (1.0-1.5)	1.4 (1.0-1.9)	1.0 (0.8-1.2)	0.9 (0.7-1.2)	1.3 (1.1-1.5)	1.1 (0.9-1.3)
	Population Controls	1.1 (1.0-1.2)		0.9 (0.8-1.0)		1.1 (1.0-1.2)		1.2 (1.1-1.3)	

* IR: Incidence rates (number inpatient admissions/100 PYRS).

¹ CI: Confidence interval.

² RR: Relative risk (IR for HIV patients/IR for population control).

DNHR was established in 1977 and covers all inpatient admissions and outpatient visits at non-psychiatric hospitals in the country [3]. The registry is based on the unique CPR number and contains data on: type of admission, type of speciality, date of admissions and discharges, procedures, primary and secondary diagnoses (coded according to the International Classification of Diseases 8th revision [ICD-8] until the end of 1993, and ICD-10 codes thereafter). From this registry, we extracted data on inpatient admissions and outpatient visits of the HIV patients and the population controls.

Calculations of inpatients admission- and outpatient visit rates

Person-years at risk for HIV-infected persons and controls were calculated from date of first positive HIV test or 1 January 1995 if the diagnosis preceded this date, date of immigration if diagnosed with HIV before arrival to Denmark or start of the calendar period, whichever came last. Patients were censored at date of death, lost to follow up, emigration, end of the calendar period of interest or 31 December 2007, whichever came first. If HIV was diagnosed during a hospital admission, observation time was calculated from first day of the admission. In each calendar year, the number of inpatient days was calculated as the sum of days in each admission counting from day of

Supplementary table 3. Outpatient visit rates for HIV patients and population controls with relative risk overall and stratified by ICD-10 diagnoses.

Periods	1995-1997			1998-2000			2001-2003			2004-2007		
	IR* (95%CI)	RR† (95%CI)	IR (95%CI)	RR (95%CI)	IR (95%CI)	RR (95%CI)	IR (95%CI)	RR (95%CI)	IR (95%CI)	RR (95%CI)		
All diagnoses	743.9 (737.0-750.8)	16.5 (16.2-16.8)	778.5 (772.0-785.1)	11.6 (11.5-11.8)	825.0 (818.8-831.2)	9.2 (9.1-9.3)	877.3 (872.2-882.4)	7.1 (7.0-7.2)				
Infectious disease	45.0 (44.3-45.8)	876.4 (1001.9-766.7)	66.9 (66.0-67.7)	882.9 (780.7-998.6)	90.0 (89.0-90.9)	906.2 (808.5-1015.6)	123.6 (122.7-124.5)	504.0 (470.2-540.3)				
Cancers	641.1 (634.7-647.6)	8.0 (7.2-8.8)	656.1 (650.0-662.1)	4.1 (3.8-4.4)	657.2 (651.7-662.7)	3.0 (2.9-3.2)	644.3 (639.9-648.7)	2.6 (2.5-2.7)				
Population Controls	18.0 (16.9-19.1)		23.9 (22.7-25.0)		25.7 (24.6-26.8)		17.7 (26.9-28.7)					
Endocrinological disease	2.3 (2.1-2.4)		5.8 (5.6-6.1)		8.5 (8.2-8.6)		10.8 (10.5-11.1)					
HIV patients	2.5 (2.1-2.9)	0.8 (0.7-1.0)	4.8 (4.3-5.3)	1.1 (1.0-1.2)	7.8 (7.2-8.4)	1.4 (1.3-1.5)	11.9 (11.3-12.5)	1.5 (1.4-1.6)				
Population Controls	3.0 (2.8-3.2)		4.4 (4.3-4.6)		5.7 (5.5-6.0)		8.0 (7.8-8.2)					
Neurological disease	1.3 (1.1-1.7)	1.0 (0.8-1.2)	3.6 (3.2-4.1)	2.2 (1.9-2.6)	3.1 (2.7-3.5)	1.2 (1.1-1.4)	4.1 (3.8-4.5)	1.0 (0.9-1.0)				
HIV patients	1.4 (1.2-1.5)		1.6 (1.5-1.8)		2.5 (2.3-2.6)		4.3 (4.1-4.5)					
Cardiovascular disease	4.7 (4.1-5.2)	2.0 (1.7-2.3)	6.9 (6.3-7.5)	1.5 (1.3-1.6)	11.3 (10.6-12.1)	2.1 (2.0-2.3)	17.2 (16.5-17.9)	2.5 (2.4-2.6)				
Population Controls	2.3 (2.2-2.5)		4.7 (4.4-4.9)		5.3 (5.1-5.5)		6.9 (6.7-7.1)					
Pulmonary disease	5.4 (6.0-4.8)	3.3 (2.8-3.8)	5.5 (4.9-6.2)	2.6 (2.3-3.0)	3.8 (3.4-4.3)	1.8 (1.6-2.0)	3.9 (3.6-4.3)	1.3 (1.2-1.5)				
Population Controls	1.6 (1.5-1.8)		2.1 (1.9-2.2)		2.1 (2.0-2.3)		3.0 (2.8-3.1)					
GI tract disease	4.9 (4.4-5.4)	1.4 (1.2-1.6)	6.1 (5.6-6.7)	1.7 (1.5-1.8)	7.1 (6.6-7.7)	1.5 (1.4-1.7)	11.1 (10.5-11.7)	2.1 (2.0-2.3)				
Population Controls	3.6 (3.4-3.8)		3.7 (3.5-3.9)		4.6 (4.4-4.9)		5.2 (5.1-5.4)					
Skin disease	10.5 (17.4-19.6)	15.2 (13.5-17.1)	10.5 (9.7-11.3)	6.0 (5.4-6.7)	10.0 (9.3-10.7)	3.5 (3.2-3.8)	11.4 (10.8-12.0)	4.0 (3.7-4.2)				
Population Controls	1.2 (0.9-1.5)		2.9 (2.7-3.1)		2.9 (2.8-3.0)		3.0 (2.9-3.1)					
Urinary tract disease	5.1 (4.5-5.7)	1.0 (0.9-1.1)	5.3 (4.8-5.9)	0.7 (0.6-0.8)	6.4 (5.9-7.0)	0.8 (0.7-0.9)	8.5 (8.0-9.1)	0.7 (0.6-0.8)				
Population Controls	5.0 (4.8-5.3)		7.7 (7.4-8.0)		8.3 (8.0-8.5)		11.6 (11.3-11.8)					

*IR: Incidence rates (number outpatient consultations/100 PYRS).

†CI: Confidence interval.

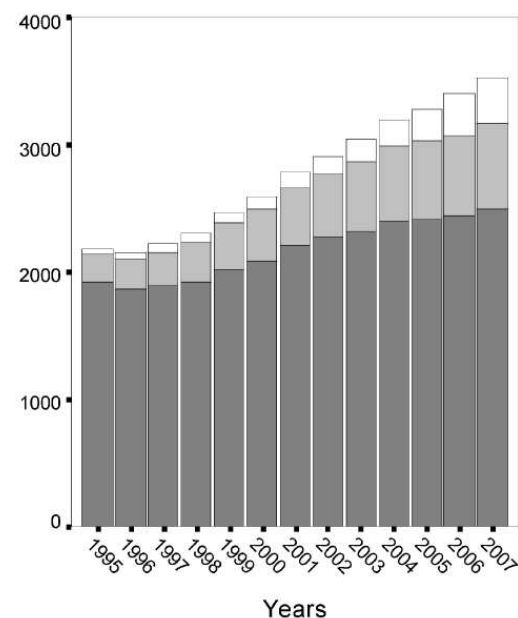
‡RR: Relative risk (IR for HIV patients/IR for population control).

admission to day of discharge and the number of outpatient visits was calculated simply by the sum of visits. In case a person was admitted and discharged on the same day it was counted as one day. If seen in two different outpatient departments on the same date it was counted as two separate visits. Inpatient admission rates (IAR) and outpatient visit rates (OVR) were calculated according to the primary discharge diagnosis in the following *International Classification of Diseases* 10th revision (ICD-10) categories: A & B (infectious disease), C & D (cancer), E (endocrinological disease), G (neurological disease), I (cardiovascular disease), J (pulmonary disease), K (gastro-intestinal disease), L (skin disease) and M (urinary tract disease).

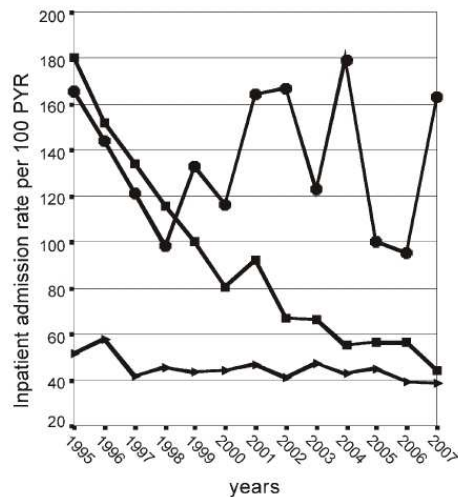
CD4 stratified inpatient admission rate

IARs for the HIV infected patients were stratified for CD4 cell counts in three strata: < 200 cells/ μ L, $\geq$ 200 cells/ μ L and < 500 cells/ μ L, $\geq$ 500 cells/ μ L. Only patients with more than one CD4 cell count measurement were included. In these analyses the last CD4 count was carried forward to the next measurement or to death, emigration, lost to follow up, end of the calendar period of interest or 31 December 2007, whichever came first. In case no CD4 count had been measured in advance of the index date the nearest CD4 count was carried "backwards".

Supplementary figure 1. Number of Danish HIV patients under observation per year divided in persons below 50 years (dark gray), between 50 and 60 years (light gray), above 60 years (white).



Supplementary figure 2. Inpatient admission rates pr 100 PYRS at all departments for Danish HIV patients stratified by; (circular and group 1) CD4 cell count < 200 cells/ μ L; (square and group 2) 200 cells/ μ L $\leq$ CD4 cell count < 500 cells/ μ L; (triangular and group 3) CD4 cell count $\geq$ 500 cells/ μ L.



Person-years at risk:

1.	814	790	644	528	481	426	392	326	378	284	374	364	216
2.	778	817	1014	1121	1196	1246	1278	1299	1306	1318	1335	1362	1347
3.	316	335	389	512	606	765	924	1136	1189	1409	1407	1498	174

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8.3. Study 3

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RESEARCH ARTICLE

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Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: A cohort study

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Abstract

Background: The mortality in patients with persistent low CD4 count despite several years of HAART with sustained viral suppression is poorly documented. We aimed to identify predictors for inadequate CD4 cell recovery and estimate mortality in patients with low CD4 count but otherwise successful HAART.

Method: In a nationwide cohort of HIV patients we identified all individuals who started HAART before 1 January 2005 with CD4 cell count ≤ 200 cells/ μ L and experienced three years with sustained viral suppression. Patients were categorized according to CD4 cell count after the three years suppressed period (≤ 200 cells/ μ L; immunological non-responders (INRs), >200 cells/ μ L; immunological responders (IRs)). We used logistic regression and Kaplan-Meier analysis to estimate risk factors and mortality for INRs compared to IRs.

Results: We identified 55 INRs and 236 IRs. In adjusted analysis age > 40 years and > 1 year from first CD4 cell count ≤ 200 cells/ μ L to start of the virologically suppressed period were associated with increased risk of INR. INRs had substantially higher mortality compared to IRs. The excess mortality was mainly seen in the INR group with > 1 year of immunological suppression prior to viral suppression and injection drug users (IDUs).

Conclusion: Age and prolonged periods of immune deficiency prior to successful HAART are risk factors for incomplete CD4 cell recovery. INRs have substantially increased long-term mortality mainly associated with prolonged immunological suppression prior to viral suppression and IDU.

Background

The introduction of HAART has decreased morbidity and mortality in HIV patients due to viral load (VL) suppression and immunological recovery [1]. Still, the immunological reconstitution after HAART initiation varies depending on the pre-HAART level of immunodeficiency [2-7]. Several studies have shown that patients with successful virological response to HAART and incomplete initial CD4 recovery have increased mortality [5,8,9]. However, the outcome is poorly documented for patients with persistent low CD4 count despite several years of HAART with sustained VL suppression.

Within a nationwide cohort of HIV infected patients we identified all individuals who were on stable HAART, had been virally suppressed for more than three years and had a CD4 cell count ≤ 200 cells/ μ L prior to the virally suppressed period. In this population we identified immunologic non-responders who did not rise to a CD4 cell count > 200 cells/ μ L after the three years of sustained VL suppression. We aimed to identify predictors for inadequate CD4 cell recovery and to determine the mortality in the immunological non-responders compared to those with an adequate CD4 cell response.

Methods

The study was performed as a nationwide cohort study. In the first part of the study we estimated risk factors for immunological non-response (CD4 cell count ≤ 200

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cells/ μ L after three years of VL suppression). In the second part of the study we estimated mortality in immunological non-responders (INRs) versus immunological responders (IRs).

Setting

Denmark had a population of 5.5 million as of 31 December 2008, with an estimated HIV prevalence of approximately 0.07% in the adult population [10,11]. Patients with HIV infection are treated in one of the country's eight specialized medical centres, where they are seen on an outpatient basis at intended intervals of 12 weeks. Antiretroviral treatment is provided free of charge to all HIV-infected residents of Denmark. The national criteria for initiating HAART have been described previously [12].

Data sources

The Danish HIV Cohort study (DHCS), described in details elsewhere, is a population-based prospective nationwide cohort study of all HIV-infected individuals 16 years or older at diagnosis and who are treated at Danish HIV centres after 1 January 1995 [13]. December 31, 2008 the cohort included 5206 Danish residents. Patients are consecutively enrolled, and multiple registrations are avoided through the use of a unique 10-digit civil registration number assigned to all individuals in Denmark at birth or upon immigration. Data are updated yearly and includes demographics, date of HIV infection, AIDS defining events, date and cause of death and antiretroviral treatment. CD4 cell counts and HIV-RNA measurements are extracted electronically from laboratory data files. After 1 January 2000 all viral load (VL) analyses in Denmark were designed to measure VL below 50 copies/ml. Before that period lower limit of detection were in some centres 199 copies/ml and 399 copies/ml and these values are in the present study considered being below 50 copies/ml. Patients are intended tested yearly for hepatitis C antibodies, and if positive further tested for hepatitis C RNA.

Primary causes of death were obtained from The Danish Civil Registration System and The Danish Register of Causes of Death [14]. Data on date of cancer diagnosis were obtained from The Danish Cancer Registry and all patients diagnosed with cancer in the suppressed period or 10 years previous to this were identified [15].

Study population

From DHCS we identified all HIV-1 positive patients, who 1) started HAART before 1 January 2005, 2) had a CD4 cell count ≤ 200 cells/ μ L at start of HAART, 3) had a VL < 50 copies/ml for more than three consecutive years before 1 January 2008, 4) had no intervals of more than seven months between VL tests in the suppressed

period, and 5) had a CD4 cell count ≤ 200 cells/ μ L at start of the virally suppressed period.

Patients were defined as IRs in case the first CD4 cell count measured after 3 years of viral suppression was > 200 cells/ μ L and INRs in case the CD4 count was ≤ 200 cells/ μ L. Mortality was also stratified for more differentiated CD4 cell strata after 3 years of viral suppression (> 200 cells/ μ L and ≤ 350 cells/ μ L, > 350 cells/ μ L and ≤ 500 cells/ μ L and > 500 cells/ μ L) but the prognosis differed little for all strata above 200 cells/ μ L why we chose to pool all CD4 responses above 200 cells/ μ L in one group (data not shown).

Statistics

Differences in characteristics between groups were evaluated by the χ^2 test, Fisher's exact test and Kruskal-Wallis Test when appropriate.

We used binary logistic regression in order to identify predictors for immunological non-response. The following covariates were included in the model: Gender, age at start of the suppressed period (≤ 40 years vs. > 40 years) (The cut point was based on the median age; IRs; 37.6 years (IQR; 32.1 - 45.3) and INRs; 42.6 years (IQR; 36.1 - 51.3)), race (Caucasian vs. non-Caucasian), route of HIV infection (men who have sex with men (MSM) vs. heterosexually vs. injection drug user (IDU) vs. other), chronic HCV infection (positive vs. negative PCR for HCV RNA), cancer prior to start of the suppressed period, one or more AIDS defining event before start of suppressed period, HIV diagnose before 1 January 1995, nadir CD4 cell count < 50 cells/ μ L and > 1 year from first CD4 ≤ 200 cells/ μ L to start of the virologically suppressed period (The cut point of one year was based on the median time from first CD4 cell count ≤ 200 to start of the suppressed period; IRs; 0.7 year (IQR; 0.3 - 2.2) and INRs; 1.5 years (IQR; 0.4 - 3.2)). Selection of potential confounders was performed using the "change in estimate" method with age and gender forced into the model [16].

Index date was defined as date of first CD4 cell count measurement after three years of sustained viral suppression. We calculated person-years at risk from index date until death, 1 January 2008, emigration or lost to follow-up, whichever came first. We used Kaplan Meier analysis to construct survival curves for INRs vs. IRs and further stratified these by time from first CD4 measurement ≤ 200 cells/ μ L to start of the virologically suppressed period (≤ 1 year vs. > 1 year). Cox regression analyses were used to estimate mortality rate ratios (MRR). We further calculated MRRs stratified by cancer (no cancer diagnosed prior to index date vs. cancer diagnosed prior to index date), previous AIDS defining event (none vs. > 1 or more AIDS defining events prior to index date) and time from first CD4 measurement ≤ 200 cells/ μ L to

start of the virologically suppressed period ($\leq$ one year vs. $>$ one year). The estimates were adjusted for gender and age.

In a robustness analysis we used a cut-off value of 500 copies/ml in the virologically suppressed period to test the impact of VL cut-off on our estimates.

VL and CD4 cell response in the observation period

Using a previously described method [13] we grouped all CD4 cell measurements and VL tests in the observation period in 12-weeks intervals and calculated the proportion of CD4 measurements $\leq$ 200 cells/ μ L and VL tests $<$ 50 copies/ml for both IRs and INRs up to six years after index date.

The study was approved by the Danish Data Protection Agency (Denmark has no Institutional review boards). Data in the database is not publicly available. SPSS statistical software, Version 15.0 (Norusis; SPSS Inc., Chicago, Illinois, USA) and R software, version 2.8.1, was used for data analysis.

Results

In The Danish HIV Cohort Study 3165 patients started HAART before 1 January 2005 (figure 1). 291 study subjects fulfilled the inclusion criteria and were followed for 1373 person-years during which 7 patients (2.4%) emigrated. 227 (78.0%) of the patients were males and 218 (74.9%) were Caucasians. Median time between VL tests in the suppressed period was 88 days (IQR; 59 - 98).

After three years of sustained VL 236 (81.1%) patients reached a CD4 cell count above 200 cells/ μ L (IRs) and 55 (18.9%) of the HIV infected patients did not (INRs). Characteristics of IRs and INRs are shown in table 1.

In un-adjusted analysis age, Caucasian race and time from first CD4 cell count $\leq$ 200 cells/ μ L to start of the virologically suppressed period were associated with immunological non-response (table 2). However, after having adjusted for potential confounders only age and time from first CD4 cell count $\leq$ 200 cells/ μ L to start of the virologically suppressed period remained associated with increased risk of being INR (table 2).

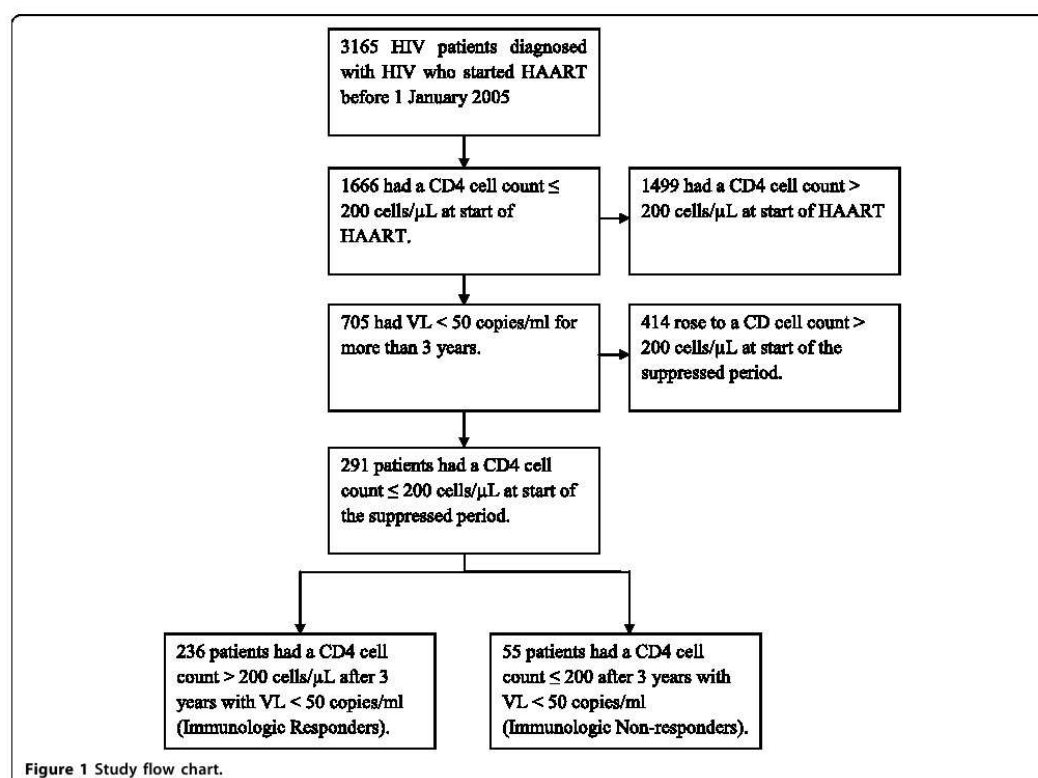


Table 1 Characteristics and demographics of immunological responders and non-responders

Characteristics	Immunologic responders N = 236	Immunologic non-responders N = 55	P
Male gender	179 (75.8)	48 (87.3)	0.065
Age at time of index date, median (IQR), years	37.6 (32.1 - 45.3)	42.6 (36.1 - 51.3)	<0.001
Caucasians	171 (72.5)	47 (85.5)	0.045
Route of HIV infection			0.059
Men who have sex with men	97 (41.1)	29 (52.7)	
Heterosexually infected	98 (41.5)	14 (25.5)	
Injection drug user	14 (5.9)	7 (12.7)	
Other	26 (11.8)	3 (6.1)	
Chronic HCV infection	20 (8.5)	8 (14.5)	0.169
Cancer within 13 years prior to index date	27 (11.4)	10 (18.2)	0.177
One or more AIDS defining events prior to index date	114 (48.3)	24 (43.6)	0.532
One or more AIDS defining events after index date	3 (1.3)	2 (3.6)	0.240
Diagnosed with HIV before 1 January 1995	94 (49.8)	27 (49.1)	0.210
Treated with zidovudine prior to index date	219 (92.8)	54 (98.2)	0.465
Nadir CD4 cell count, median (IQR), cells/ μ L	40 (10 - 82)	40 (15 - 57)	0.289
More than one year from first CD4 cell count $\leq$ 200 cells/ μ L to start of the virologically suppressed period*	96 (40.7)	32 (58.2)	0.019
Time from start of HAART to start of the virologically suppressed period, median (IQR), years	0.4 (0.2 - 1.1)	0.4 (0.3 - 1.3)	0.518

* Median time from first CD4 cell count $\leq$ 200 cells/ μ L to start of the virologically suppressed period was 0.7 year (IQR; 0.3 - 2.2) for IRs and 1.5 years (IQR; 0.4 - 3.2) for INRs.

The distribution of drug classes included in the last HAART regimen prior to index date did not differ between IRs and INRs (Additional file 1).

A total of 22 (7.6%) patients died in the observation period, 11 (20.0%) in the INR group and 11 (4.7%) in the IR group. As shown in figure 2, INRs had substantially higher mortality compared to IRs. Unadjusted

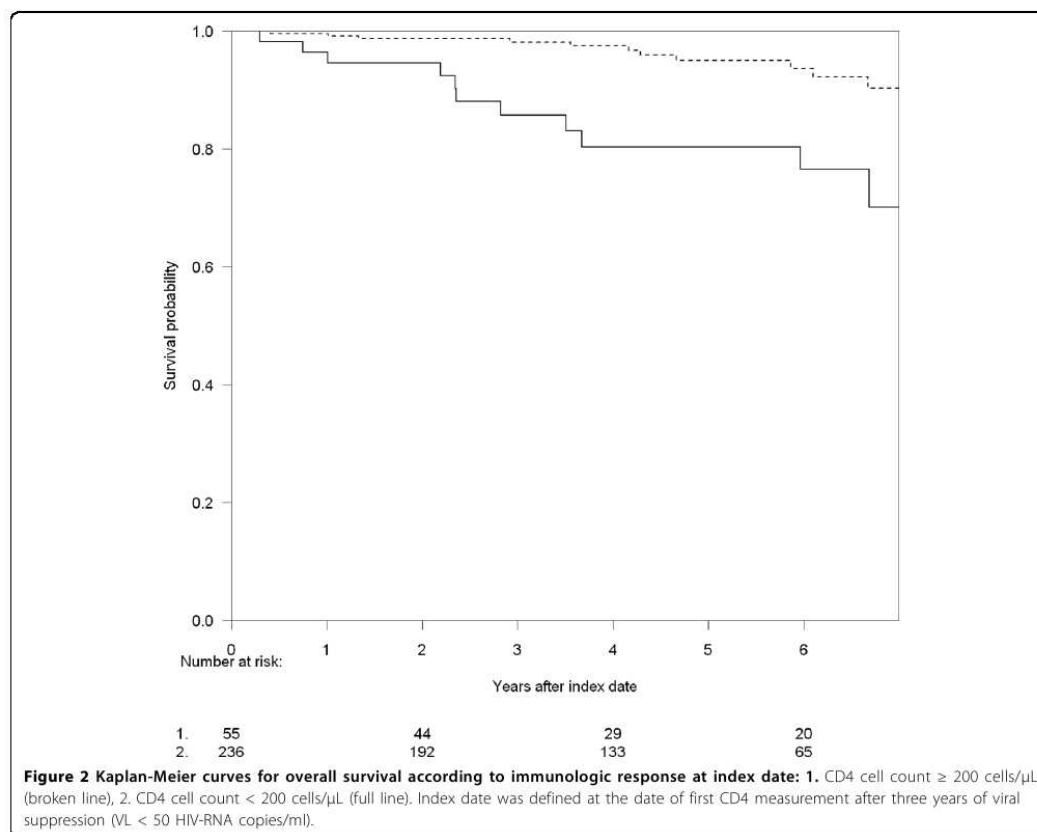
MRR for INRs compared to IRs was 4.2 (95%CI; 1.8 - 9.6) and 3.4 (95%CI; 1.4 - 8.0) after adjustment for age and gender. Figure 3 shows the mortality for IRs vs. INRs stratified by time from first CD4 measurement $\leq$ 200 cells/ μ L to start of the virologically suppressed period and from this analysis it is seen, that the main excess mortality was observed in the INRs with more

Table 2 Odds ratios (OR) for immunological non-response in patients on successful HAART for more than three years

Characteristics	OR Un-adjusted (95% CI*)	OR, Adjusted ** (95% CI)
Male gender	2.18 (0.94 - 5.09)	1.73 (0.73 - 4.14)
More than 40 years old at index date	2.53 (1.390 - 4.63)	2.32 (1.25 - 4.29)
Caucasians	2.23 (1.01 - 4.98)	1.09 (0.36 - 3.36)
Route of HIV infection		
Men who have sex with men	1.0	
Heterosexually infected	0.48 (0.24 - 0.96)	0.73 (0.30 - 1.78)
Injection drug user	1.67 (0.63 - 4.54)	3.06 (0.98 - 9.52)
Other	0.62 (0.22 - 1.75)	0.63 (0.20 - 1.96)
Chronic HCV infection	1.84 (0.76 - 4.43)	2.11 (0.79 - 5.61)
Cancer within 13 years prior to index date	1.72 (0.78 - 3.81)	1.47 (0.63 - 3.44)
One or more AIDS defining events prior to index date	0.83 (0.46 - 1.50)	0.84 (0.46 - 1.53)
Diagnosed with HIV before 1 January 1995	1.46 (0.81 - 2.63)	1.43 (0.72 - 2.86)
Nadir CD4 cell count less than 50 cells/ μ L	1.59 (0.86 - 2.96)	1.06 (0.43 - 1.75)
More than one year from first CD4 $\leq$ 200 cells/ μ L to start of the virologically suppressed period	2.03 (1.12 - 3.68)	2.28 (1.18 - 4.43)

*Confidence interval (95%CI).

**All estimates adjusted for age and gender.

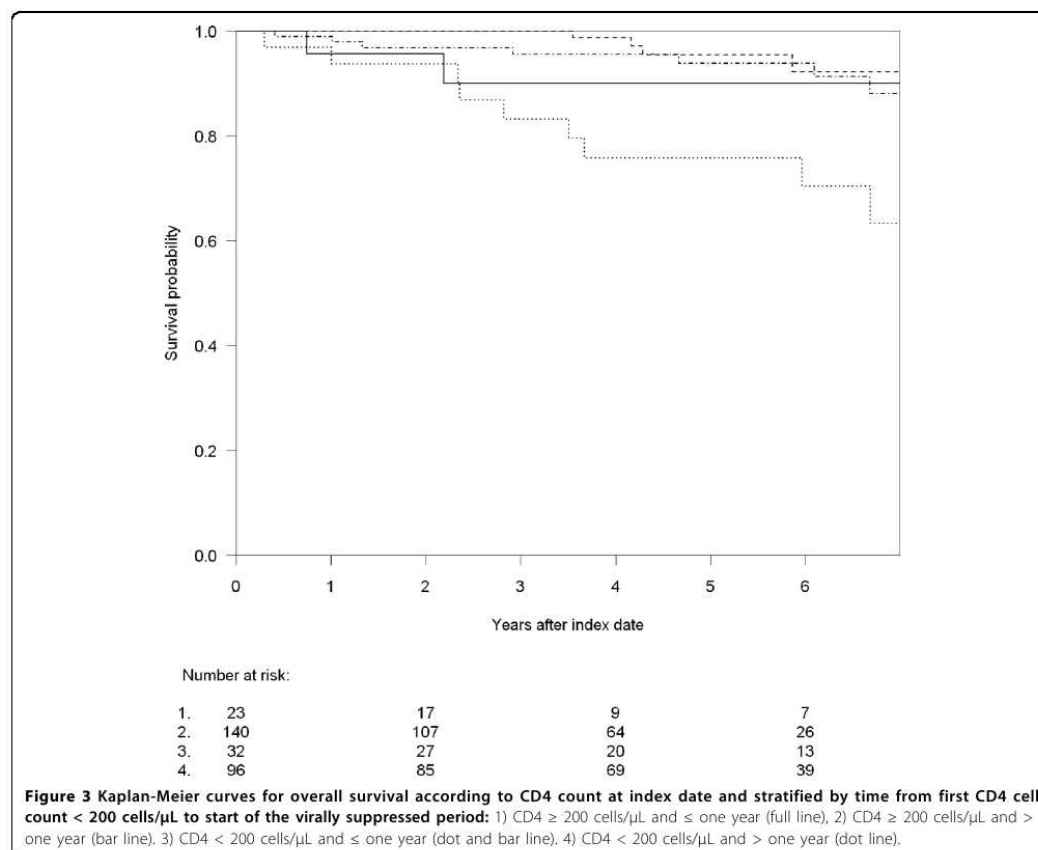


than one year of immunological suppression prior to the virologically suppressed period. Of importance, the excess mortality was observed up to 6.5 years after initiation of virologically successful HAART. The INRs did not experience substantially more AIDS defining events in the observation period (3.6% vs. 1.3%).

128 patients (44.0%) had more than one year from first CD4 measurement ≤ 200 cells/ μ L to start of the virologically suppressed period. The un-adjusted MRR among these 128 patients was 3.9 (95%CI; 1.5 - 10.6) and after adjustment for age and gender it was 3.6 (95%CI; 1.3 - 9.7). When excluding these patients from the analysis the unadjusted MRR was reduced to 3.1 (95%CI; 0.6 - 17.7) and after adjustment for age and gender it was 1.8 (95%CI; 0.3 - 10.2). 90 (70.3%) of the 128 patients who had more than one year of immunological suppression prior to the virologically suppressed period were diagnosed before 1995. When excluding IDUs from our analysis the unadjusted MRR was 2.5 (95%CI; 0.9 - 7.8) and the adjusted MRR was reduced to 1.8

(95%CI; 0.6 - 5.1). The un-adjusted MRR calculated exclusively for IDUs was 12.7 (95%CI; 1.5 - 109.9) and 9.8 (95%CI; 1.1 - 86.1) after adjustment for age and gender. Exclusion of patients with previous cancer or AIDS defining event, respectively, did not change the estimates substantially. Only one death in each group (one PML and one cryptococcal meningitis) appeared to be a classical HIV related cause of death. Other causes of death were: cancer (INR: 3, IR: 2), sudden death (INR: 3, IR: 3), liver related (INR: 2, IR: 1), suicide (INR: 1, IR: 1), opiate overdose (INR: 1, IR: 0), pneumonia (INR: 2, IR: 0) and cachexia (not HIV related) (INR: 0, IR: 1).

80% of the INRs achieved a CD4 cell count > 200 cells/ μ L after six years of observation (i.e. more than nine years after starting HAART). However, only 20 (35.1%) of the INRs were still under observation six years after study inclusion. After six years of observation, 95.5% of the whole study population who were still alive had fully suppressed VL.



When using a cut-off value of 500 copies/ml for viral suppression, 445 study subjects (372 IRs and 73 INRs) were identified, of whom 18 (4.5%) patients in the IR group and 15 in the INR group (20.5%) died during follow-up. In the un-adjusted analysis age, Caucasian race, IDU, time from first CD4 cell count ≤ 200 cells/μL to start of the virologically suppressed period and CD4 cell count at start of the suppressed period were associated with immunological non-response. However, after having adjusted for potential confounders only age, IDU and CD4 cell count at start of the suppressed period remained associated with increased risk of being INR. MRRs were 4.5 (95%CI; 2.3 - 9.0) and 3.69 (95%CI; 1.8 - 7.4) when adjusted for gender and age.

Discussion and Conclusions

In a population of HIV patients with initial low CD4 count we found that almost one out of five was INR after three years of successful HAART. Older age and

more than one year of severe immune deficiency prior to start of sustained VL were associated with INR. The patients with insufficient immunological response had substantially increased long term mortality. The increased mortality was mainly seen in patients with more than one year of immunological deficiency prior to the period of sustained VL suppression and IDUs.

The major strength of this study is the identification of a homogeneous group with stringent viral suppression, essentially eliminating the possibility that incomplete VL suppression explains our findings. The study had a nationwide design with long and almost complete follow-up including data on cancer as well as HCV status.

A limitation of the study is the small study population (in part caused by the stringent definitions of INRs and IRs) and the small number of outcomes, which did not allow us to extend the logistic regressions analysis or to calculate cause specific rate ratios of death. The study population encompasses a highly selected group of

patients surviving more than three years in spite of low initial CD4 cell count, and a healthy survivor effect may lead to underestimation of the excess mortality in the INRs. Still, by using an extended virally suppressed period we allowed the patients to reach a steadier CD4 plateau without the interference of CD4 cell redistribution as seen after HAART start. HCV testing in the cohort is "intended" and may only be performed yearly on known high risk groups in the cohort e.g IDU why chronic HCV may be underestimated in the cohort.

19% of the study patients did not have an adequate immunological response to HAART. Our results corresponds to the findings of other studies who found a negative impact of low initial CD4 cell count on long-term CD4 recovery [2,3,6,17-19].

INRs were significantly older than the IRs and age predicted inadequate CD4 cell recovery which corresponds to the findings of several other studies [3,5,9,19,20]. In contrast to other studies, we did not find a statistically significant association between IDU or chronic HCV infection and INR [3,21]. However, when using a cut-off value of 500 copies/ml, IDU was associated with INR. This is probably due to the inclusion of a larger number of study patients with these characteristics when allowing for viral blips. Nadir CD4 cell count did not differ between the two groups, presumably reflecting that immunological responders without a low nadir were able to escape above the 200 cells/ μ L threshold before full suppression was observed. More INRs had more than one year from first CD4 cell count $\leq$ 200 cells/ μ L to start of the virologically suppressed period, indicating that they had a longer period of immunological suppression than the IRs and this factor was also associated with incomplete CD4 cell recovery. Defect bone marrow function and decreased thymus activity are major suspects in delayed CD4 cell recovery in HIV patients with severe immunodeficiency [4,22-25]. To our knowledge no studies have been made on predictors for bone marrow function in HIV infected patients, but our results are in accordance with the findings of Dion et al, who found that both older age and prolonged immunological suppression were associated with decreased thymic activity in virally suppressed HIV patients resulting in slower CD4 recovery [26].

The excess mortality seen among the INRs was mainly related to prolonged immunological suppression prior to successful HAART and IDU. The median time from start of HAART to start of the virologically suppressed period was less than half a year. A longer period of immunological suppression prior to the three years of sustained VL suppression is therefore explained by delayed initiation of HAART and not poor compliance. This is supported by the fact that most of the patients with more than one year of immunological suppression before start of the virologically suppressed period were diagnosed with HIV

before 1995 when treatment options were scarce. As many of the INRs who died were patients who initiated HAART in the mid-nineties it could be hypothesized that resistance played a role, but the stringent design with full VL suppression eliminates that possibility. We find it unlikely that the late death of these patients were directly related to the toxicity of the antiretroviral agents, but cannot rule out that toxicity should interact with the immune reconstitution. The increased mortality in patients with delayed initiation of HAART is well documented [27] and the contribution of persistent immunodeficiency to the development of e.g. cancer has also been observed by others [28]. It may be speculated that intermittent viraemias in this setting would increase mortality but the estimate remained unchanged in our sensitivity analysis using a cut-off value of 500 copies/ml for viral suppression, suggesting that either the impact of low-level replication on death and immune reconstitution is limited or that the majority of bleeps below 500 remains laboratory artefacts.

We only encountered two AIDS related deaths suggesting that these are rare after prolonged HAART even without adequate CD4 recovery. Thus, it seems that the INRs are not immune-suppressed in the classical sense where a low CD4 count leads to AIDS defining diseases and eventually death. Still, we cannot rule out that there is a biological cause of the increased mortality in immunological non-responders related to immunodeficiency and that our study simply do not have power enough to demonstrate an association.

The risk factors for incomplete CD4 cell recovery and increased mortality points toward earlier diagnosis of HIV as the main prophylactic measure. Also, the increased mortality in these patients calls for increased concern in terms of treatment and screening for co-morbidity.

We conclude that age and prolonged immunological suppression are risk factors for incomplete CD4 cell recovery in patients with otherwise successful HAART. Patients with insufficient immunological response have substantially increased long-term mortality compared to IRs, but the increased mortality is mainly associated with prolonged immunological suppression prior to VL suppression and IDU. We therefore presume that in the modern HAART era where patients are started early on HAART the prevalence of insufficient CD4 recovery will decrease substantially.

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the decision to submit the article for publication. The researchers are independent from the funders.

Additional material

Additional file 1: Table S1. Drugs included in the last HAART regimen prior to index date.

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Authors' contributions

The authors contributions are the following: FNE (MD) contributed with study design, data collection, data analysis, interpretation of findings and writing of the manuscript. JG (MD, Professor, DrMedSc) contributed with study design, data collection, interpretation of findings and critical edit of the manuscript. GK (MD, associate professor, DrMedSc), CSL (MD, DrMedSc), GP (MD), BR (MD, PhD), JJ (MD) and LNN (MD) contributed with data collection, study design, interpretation of findings and critical edit of the manuscript. NO (MD, DrMedSc) contributed with data collection, study design, critical review of data analyses, interpretation of findings and critical edit of the manuscript. All authors read and approved the final manuscript.

Competing interests

Potential competing of interest: N Obel has received research funding from Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, GlaxoSmithKline, Abbott, Boehringer Ingelheim, Janssen-Cilag and Swedish Orphan. F Engsig has received research funding from Merck Sharp & Dohme. J Gerstoft has received research funding from Abbott, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Pharmasia, GlaxoSmithKline, Swedish Orphan and Boehringer Ingelheim.

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Additional table 1.

Drugs included in the last HAART regimen prior to index date	IR	INR
	N (%)	N (%)
NRTI	234 (99.2)	55 (100%)
1 NRTI	8 (3.4)	3 (5.5)
2 NRTI	215 (91.1)	49 (89.1)
3 NRTI	11 (4.7)	3 (5.5)
NNRTI	99 (41.9)	21 (38.2)
PI	158 (66.9)	38 (69.1)
Boosted PI	60 (38.0)	11 (28.9)
Non-boost PI	98 (62.0)	27 (71.1)
Maraviroc, etravirine, darunavir or T20	-	-

Appendix 1

Summary in English

In Western and Central Europe approximately 820,000 adults and children are living with HIV. The introduction of HAART, which in most countries is free of charge, has decreased HIV related morbidity and mortality in HIV patients due to immunological recovery following viral load suppression. Initially after the introduction of HAART the main concerns were continued viral suppression and viral resistance but with growing experience in treating HIV patients and an increasingly wider range of drugs, well treated HIV infected patients now have an overall life expectancy close to non-HIV infected individuals. The main challenges are now drug toxicity and non-HIV related morbidity associated with behavioral risk factors like cigarette smoking.

This thesis included three studies that revolve around the effects of HAART on the Danish HIV infected population. Mainly three aspects was investigated; 1) Incidence and mortality of progressive multifocal leucoencephalopathy (PML) in HIV patients, 2) HIV patients use of health care facilities compared to population controls and 3) the long term mortality in successfully treated HIV patients with low CD4 cell counts.

Study 1 demonstrated that the incidence of PML has decreased and its prognosis improved in HIV infected patients after the implementation of HAART. But the disease still has a high mortality, and patients who survive are often left with severe neurological sequelae. The main focus in management of PML should be on early diagnosis of HIV infection and initiation of HAART prior to immunological deterioration.

Study 2 showed that after the introduction of HAART the use of health care facilities by HIV patients have shifted from inpatient to outpatient treatment. The decrease in inpatient admissions

is mainly observed at departments of infectious diseases and for HIV patients with CD4 cell counts between 200 - 500 cell/ μ L. In contrast, the increased use of outpatient visits mainly takes place in departments of non-infectious diseases and is probably a consequence of the increased age and comorbidity in the HIV population and a general trend towards a shift from inpatient to outpatient treatment in European health care systems. In spite of HAART, Danish HIV patients still have an excess use of hospital health care facilities compared to the background population.

Study 3 showed that poor CD4 cell recovery in HIV patients on otherwise successful HAART was associated with prolonged immunological suppression and that these HIV patients had an increased mortality related to prolonged immunological suppression and intravenous drug use. These findings again point toward early diagnosis of HIV as the main prophylactic measure. Also, the increased mortality in these patients calls for increased concern in terms of treatment and screening for comorbidity.

Altogether, the studies have demonstrated that 1) the incidence and mortality of PML in Danish HIV patients have decreased after the introduction of HAART, 2) The use of health care facilities among HIV patients have decreased but Danish HIV patients still have a excess use of hospital health care facilities compared to the background population, 3) HIV patients who continued to have a low CD4 cell count in spite of successful HAART have an excess mortality. This thesis contributes with new knowledge and information to HIV patients and clinicians regarding diagnosing PML and the prognosis for HIV patients with PML and HIV patients who continued to have a low CD4 cell count in spite of successful HAART. Knowledge about changes and trends in the use of healthcare facilities by HIV patients are valuable in regards to planning, budgeting and distribution of resources in the health care sector.

Appendix 2

Summary in Danish

HIV epidemien startede for 30 år siden og der er i dag cirka 33,3 millioner HIV inficerede på verdensplan hvoraf størstedelen lever i Afrika. I Vest- og Centraleuropa er der i dag cirka 820.000 voksne og børn med HIV. Introduktionen af highly active antiretroviral treatment (HAART) som er gratis i den fleste europæiske lande har sænket morbiditet og mortalitet hos HIV patients via viral supprimering og immunologisk restitution. Således har velbehandlede HIV patienter i dag en næsten normal forventet levealder.

Denne PhD består af tre kohorte studier og omhandler effekten af HAART på danske HIV patienter. Især tre aspekter blev belyst: 1) incidens og mortalitet af progressiv multifokal leukoencefalopati, 2) forbruget af hospitalsydelser hos danske HIV patienter sammenlignet med baggrundsbefolkningen, 3) mortaliteten hos danske HIV patienter succesfuldt behandlet med HAART i mere end tre år med dårlig immunologisk restitution.

Studie 1 demonstrerede at incidensen af PML er faldet og at prognosen er forbedret efter implementeringen af HAART. Mortaliteten er dog fortsat høj og dem der overlever har ofte alvorlige neurologiske sequelae. I behandlingen af PML bør fokus være på profylakse gennem tidlig diagnose af HIV infektion og opstart af behandling før immunforsvaret bliver svækket.

Studie 2 viste at forbruget af hospitalsydelser efter introduktionen af HAART er faldet og at behandlingen af HIV patienter er skiftet fra behandling under indlæggelse til ambulant behandling. Faldet i hospitals indlæggelser blev primært observeret hos patienter med et CD4 celle tal mellem 200 og 500 celler/ μ L på infektionsmedicinske afdelinger. I kontrast hertil observerede vi en

stigning i forbruget af ambulante ydelser på ikke-infektionsmedicinske afdelinger, hvilket sandsynligvis er en konsekvens af stigende alder og komorbiditet i HIV populationen samt generelle ændringer i den danske hospitalssektor. Trods et betydeligt fald i forbruget af hospitalsydelser har danske HIV patienter fortsat et større forbrug af hospitalsydelser end baggrundsbefolkningen.

Studie 3 viste at succesfuldt behandlede HIV patienter med dårlig immunologisk restitution har en øget dødelighed. Dårlig restitution af immunforsvaret var associeret med langvarig suppression af immunforsvaret og den øgede dødelighed i denne patient gruppe var associeret med langvarig suppression af immunforsvaret inden start på effektiv HIV behandling og intravenøst stof misbrug. Disse fund understreger igen vigtigheden af tidlig diagnosticering af HIV. Den øgede dødelighed i denne patient gruppe bør føre til øget opmærksomhed på screening og behandling af komorbiditet.

Overordnet set har studierne vist at 1) PML incidensen og mortaliteten hos danske HIV patienter er faldet efter implementeringen af HAART, 2) danske HIV patienters forbrug af hospitals ydelser er faldet markant, men forbruget er fortsat større end hos ikke-HIV inficerede kontrolpersoner og 3) succesfuldt behandlede HIV patient med dårlig restitution af immunforsvaret har en øget dødelighed. Denne afhandling bidrager med klinisk anvendelig viden om diagnosticering af PML hos HIV patienter, prognosen for HIV patienter med PML samt prognosen for succesfuldt behandlede HIV patienter med dårlig immunologisk restitution. Yderligere er viden om ændringer og trends i HIV patienters forbrug af hospitals ydelser nødvendige fra et administrativt synspunkt til planlægning, budgettering og allokering af resurser i hospitalssektoren.

3.1. Coauthor declarations for Study 1

Declaration of co-authorship

This declaration concerns the article: "Incidence, clinical presentation, and outcome of progressive multifocal leukoencephalopathy in HIV-infected patients during the highly active antiretroviral therapy era: a nationwide cohort study", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

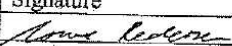
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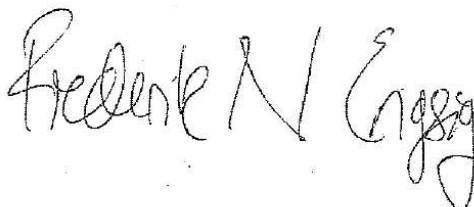
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Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments.	B
2. Planning of the experiments and methodology design, including selection of methods and method development.	B
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

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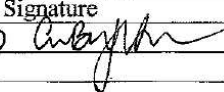
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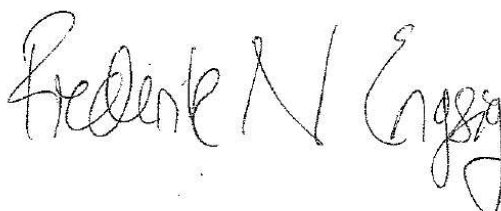
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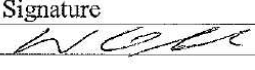
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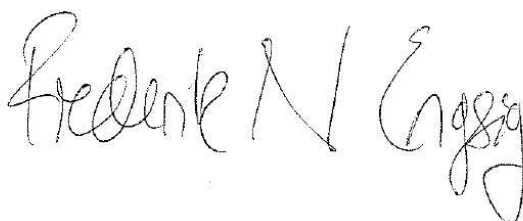
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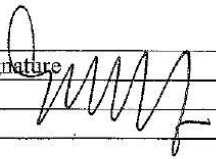
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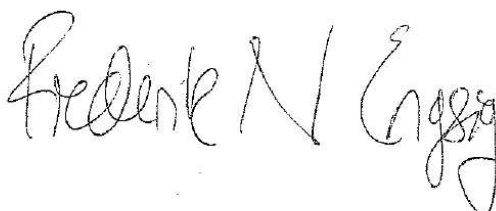
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Declaration of co-authorship

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4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
29.8.11	Christ. Bak Dy	MD	CHRISTIAN BACKER DY

Signature of the PhD student:

Frederik N Engsig

3.2. Coauthor declarations for Study 2

ejby Sygehus

003

Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

- A. Has contributed to the co-operation (0-33%)
- B. Has contributed considerably to the co-operation (34-66%)
- C. Has predominantly executed the work independently (67-100%)

Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments.	B
2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
19-11	CHRISTEN SCHULDE LARSEN	DC	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

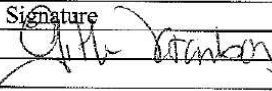
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

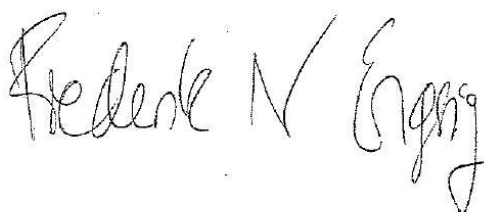
- A. Has contributed to the co-operation (0-33%)
- B. Has contributed considerably to the co-operation (34-66%)
- C. Has predominantly executed the work independently (67-100%)

Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
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2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
18.11	Gert Krarup	MD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

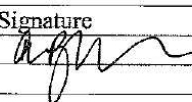
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

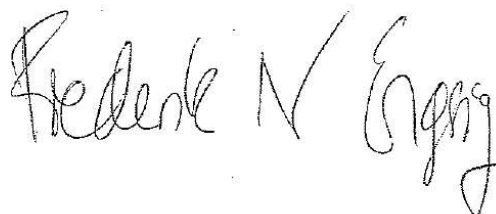
- A. Has contributed to the co-operation (0-33%)
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- C. Has predominantly executed the work independently (67-100%)

Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
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2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
14/8-2011	Ann-Brit Egholm Jensen	lektor, ph.d.	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995–2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

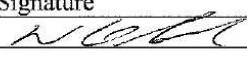
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

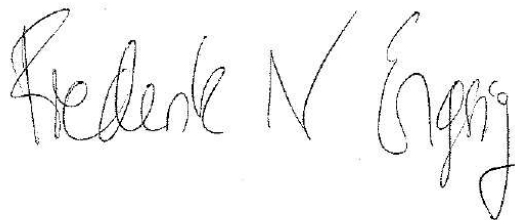
- A. Has contributed to the co-operation (0-33%)
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- C. Has predominantly executed the work independently (67-100%)

Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments.	B
2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
18-11	N. OBEK		

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

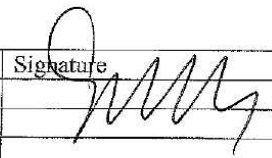
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

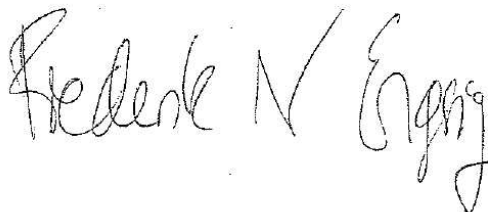
- A. Has contributed to the co-operation (0-33%)
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- C. Has predominantly executed the work independently (67-100%)

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2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
17/11	JAN CENSTADT	MD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Inpatient admissions and outpatient visits in persons with and without HIV infection in Denmark, 1995-2007", a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

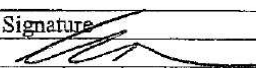
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

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2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
19/8-11	CARSTEN SCHMIDT LARSEN	DC	

Signature of the PhD student:



3.3. Coauthor declarations for Study 3

Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study."

, a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

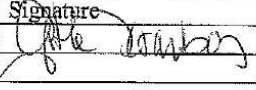
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

- A. Has contributed to the co-operation (0-33%)
- B. Has contributed considerably to the co-operation (34-66%)
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Declaration regarding specific elements (1.-4.)	Extent (A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments.	C
2. Planning of the experiments and methodology design, including selection of methods and method development.	C
3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
08.11	Lotte Jørgensen	MD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study," a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

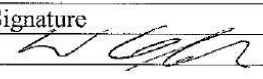
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

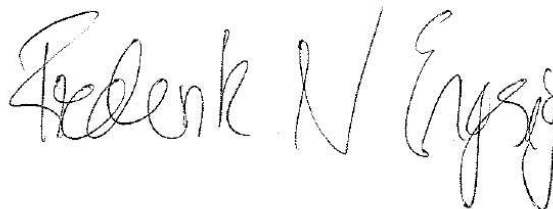
- A. Has contributed to the co-operation (0-33%)
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4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
18/11	N. OBECK		

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study."
 , a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

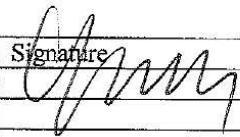
Name of the PhD student: Frederik Neess Engsig

The proportion of the PhD students contribution to the article in question is evaluated from the following scale:

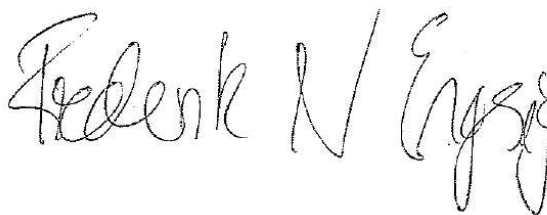
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3. Involvement in the experimental work.	C
4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
18/11	JAN CENSTRI	MD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study." , a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

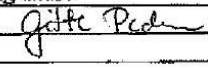
Name of the PhD student: Frederik Neess Engsig

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Signature of the co-authors:

Date	Name	Title	Signature
18/8	GITTE PEDERSEN	MD, PhD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study."
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3. Involvement in the experimental work.	C
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Signature of the co-authors:

Date	Name	Title	Signature
12/8	Sci Biegi - Thorup Reg E	MD, PhD	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study," a part of the thesis "Morbidity and mortality in Danish HIV patients after the introduction of highly active antiretroviral treatment" submitted for the defense/obtainment of the PhD degree at the University of Copenhagen.

Name of the PhD student: Frederik Neess Engsig

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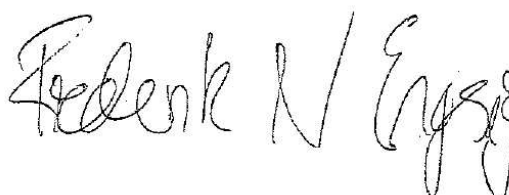
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4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
19-11	CORSTEN SCHADE LARSEN	DR	

Signature of the PhD student:



Declaration of co-authorship

This declaration concerns the article: "Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: a cohort study."
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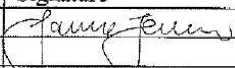
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4. Presentation, interpretation and discussion in a journal article format of the obtained data.	C

Signature of the co-authors:

Date	Name	Title	Signature
17/8 2011	JANNE JENSEN	MD	

Signature of the PhD student:

