



PhD Thesis

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Estimation of renal function and renal complications in Danish HIV-infected individuals

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1. PUBLICATIONS

This Ph.D. thesis is based on the following four publications:

1. Rasch MG, Helleberg M, Feldt-Rasmussen B, Kronborg G, Larsen CS, Pedersen C, *et al.* **Increased risk of dialysis and end-stage renal disease among HIV patients in Denmark compared with the background population.** *Nephrol Dial Transplant* 2014; 29:1232–8.
2. Ahlström MG, Feldt-rasmussen B, Legarth R, Kronborg G, Pedersen C, Larsen CS, *et al.* **Smoking and renal function in people living with human immunodeficiency virus : a Danish nationwide cohort study.** *Clin Epidemiol* 2015; 7:391–399.
3. Glindvad Ahlstrom M, Feldt-Rasmussen B, Gerstoft J, Obel N. **Routine urine protein/creatinine ratio testing in an outpatient setting of Danish HIV-infected individuals.** *Infect Dis* 2016; 48:560–562.
4. Ahlström MG, Kjær A, Gerstoft J, Obel N. **Agreement between Estimated and Measured Renal Function in an Everyday Clinical Outpatient Setting of Human Immunodeficiency Virus-Infected Individuals.** *Nephron* Published Online First: 2017.

2. PREFACE

This PhD thesis was carried out in the period 2010-2017 at the Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet.

First of all I would like to thank the research board at Copenhagen University Hospital, Rigshospitalet for sponsoring my studies. I have been very grateful for the opportunity to spend two full years to immerse into this deep and interesting subject, and have the freedom to learn the skills I have thought necessary in order to grasp the subject in a satisfactory manner.

I would like to thank my primary supervisor, Niels Obel, with whom I have had a terrific cooperation the past years, he introduced me to clinical epidemiology in my time as a research year student and has since then constantly been focusing on developing my research skills from the first idea to the final draft of the manuscript. Also he has been a great support in the more difficult times of my studies. I would like to thank my primary co-supervisor, Bo Feldt-Rasmussen for a good collaboration; his great insight in his own field of expertise has been an invaluable source to draw inspiration and knowledge from throughout my entire study period. Further, I would like to thank Jan Gerstoft for his clever comments on my project.

I would like to thank the centers in the Danish HIV Cohort Study, including all individuals involved in the collection of data, for making this thesis possible: Departments of Infectious Diseases at Copenhagen University Hospitals, Rigshospitalet and Hvidovre, Odense University Hospital, Aarhus University Hospitals, Skejby and Aalborg Hospital, Herning Hospital, Helsingør Hospital and Kolding Hospital.

When I started off as a research year student I met Lars Omland, Casper Rod and Frederik Engsiig. This was a very enjoyable time that I look back on with joy. Thanks to the three of you for making my first research experience nice and enjoyable.

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I would like to thank all co-authors for contributing and commenting on my articles. I would like to thank my mother Henriette and Father Jens for their interest and support in my project. Finally, I must express my deep and inner gratitude to my amazing wife Malin, and our two fantastic daughters Alma and Laura for their love and support. Without you to hold my hand on the way, this would not have been possible.

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3. ABBREVIATIONS

⁵¹Cr-EDTA: 51-chromium-ethylenediaminetetraacetic acid

AIDS: Acquired immunodeficiency syndrome

aRRT: Any renal replacement therapy

ATV: Atazanavir

cART: Combination antiretroviral therapy

CCC: Concordance correlation coefficient

CI: Confidence interval

CKD: Chronic kidney disease

CKD-EPI: the chronic kidney disease epidemiology collaboration

CP: Coverage probability

CVD: Cardiovascular disease

cRRT: Chronic renal replacement therapy

DCRS: Danish civil registration system

DHCS: Danish HIV cohort study

DNPR: Danish national patient registry

DNR: Danish nephrology registry

EACS: European AIDS clinical society

eGFR: Estimated glomerular filtration rate

GFR: Glomerular filtration rate

HIV: Human immunodeficiency virus

HIVAN: HIV-associated nephropathy

ICD: International classification of diseases

IDU: Injection drug use

IR: Incidence rate

IRR: Incidence rate ratio

KDIGO: Kidney disease - improving global outcome

MDRD: Modification of diet in renal

mGFR: Measured glomerular filtration rate

MR: mortality rate

MRR: Mortality rate ratio

MSM: Men who have sex with men

PH: Proportional hazard

PI: Protease inhibitor

PIN: Personal identification number

PY: Person years of follow up

TAF: Tenofovir alafenamide

TDF: Tenofovir disoproxil fumarate

TDI: Total deviation index

UA/Cr: Urine albumin/creatinine ratio

UP/Cr: Urine protein/creatinine ratio

4. INTRODUCTION

In this section I will discuss the background for this thesis. I will start by giving a short presentation of the human immune deficiency virus (HIV) pandemic, further I will introduce renal function and its relation to the pandemic. Second I will discuss some of the challenges when working with renal function and discuss this in relation to the existing literature, and finally a presentation of the hypotheses and objectives of this thesis.

4.1 RENAL FAILURE IN THE CONTEXT OF THE HIV PANDEMIC

In the developing part of the world, there are still substantial challenges with HIV and AIDS, which unfortunately is not mirrored by the academic output in these regions[1]. The discussion of this subject is out of the scope of this thesis, but should be mentioned.

In the developed part of the world, HIV-infection has transformed from a rapidly decaying disease, with an almost certain fatal outcome, to a chronic disease with a favourable prognosis. This transformation has happened mainly on the basis of combination antiretroviral therapy (cART)[2]. Since this dramatic change in the epidemiology of HIV, focus has shifted from treating opportunistic infections and AIDS to the treatment of co-morbidities and side-effects of cART[3–5]. This is mirrored by a shift in the academic output with an increasing number of papers published on co-morbidity related topics (such as renal disease) and a decreasing amount of papers published on opportunistic infections and AIDS (Figure 1).

Several renal diseases have been found to be associated with HIV-infection, i.e. HIV associated nephropathy (HIVAN), Thrombotic microangiopathy and Immune complex-mediated glomerulonephritis[6], these diseases are primarily confined to the pre-cART era. In the post-cART era, data are scarce on the causes of acute and chronic renal failure in HIV-infected individuals. Franceschini et al, reported that acute renal failure was associated with male gender, HCV co-infection, advanced HIV disease, including patients with low CD4 cell count, higher HIV RNA levels, and prior AIDS-defining diseases. In addition, patients with acute renal failure were more likely to have received cART[7]. Some antiretrovirals have been found to be associated with renal failure, e.g. tenofovir disoproxil fumarate (TDF) which is associated with proximal tubular damage and Fanconi syndrome[8–11], protease

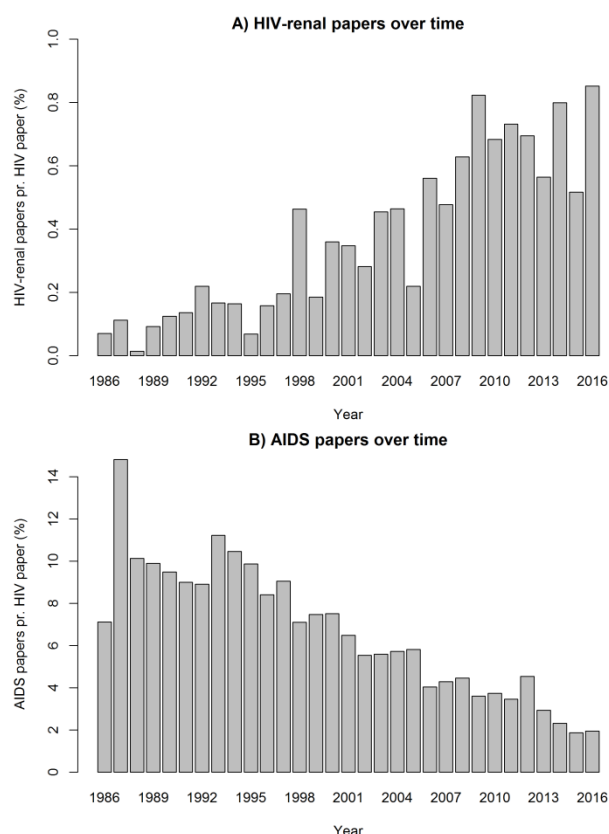


Figure 1: Illustrates the number of papers published relative to the total number of HIV papers published on **(A)** Renal function and **(B)** AIDS (Data acquired from PubMed).

inhibitors (PIs) have also been suspected to cause damage to the kidneys[12–16]. Furthermore several other antiretrovirals and antibiotics to treat superinfections have been associated with renal diseases in HIV-infected individuals[17]. The mortality rate of renal diseases have not changed substantially in the pre-/early cART era compared with the post-cART era, however when compared to the background population the mortality rate (MR) of renal diseases is substantially increased in HIV-infected individuals even when intravenous drug users (IDUs) are excluded from analyses[18].

4.2 ESTIMATING RENAL FUNCTION

The kidneys have a variety of important functions in human physiology, which includes: control of acid-base homeostasis, regulation of hydration and electrolyte status, removal of toxins, control of blood pressure, control of erythrocyte production and vitamin D metabolism. Renal function is commonly evaluated by estimates of the glomerular filtration rate (GFR). Measured GFR (mGFR) is a method where the clearance of different exogenous substances, e.g. inulin, iohexol, technetium 99m -diethylenetriaminepentaacetic acid and chromium-ethylenediaminetetraacetic acid (^{51}Cr -EDTA) is used to measure renal function. This has been found to correspond well with the true GFR [19–21]. However these methods are time consuming, expensive and laborious. Estimated GFR (eGFR) are formula based estimations of GFR which includes the level of one or more endogenous substances and different other parameters such as age, sex, race and weight. The first of these formulas was developed by Effersø et al. in 1957[22]. Since then more than 20 different formulas to estimate GFR from endogenous substances has been developed[21,23–29]. Several of

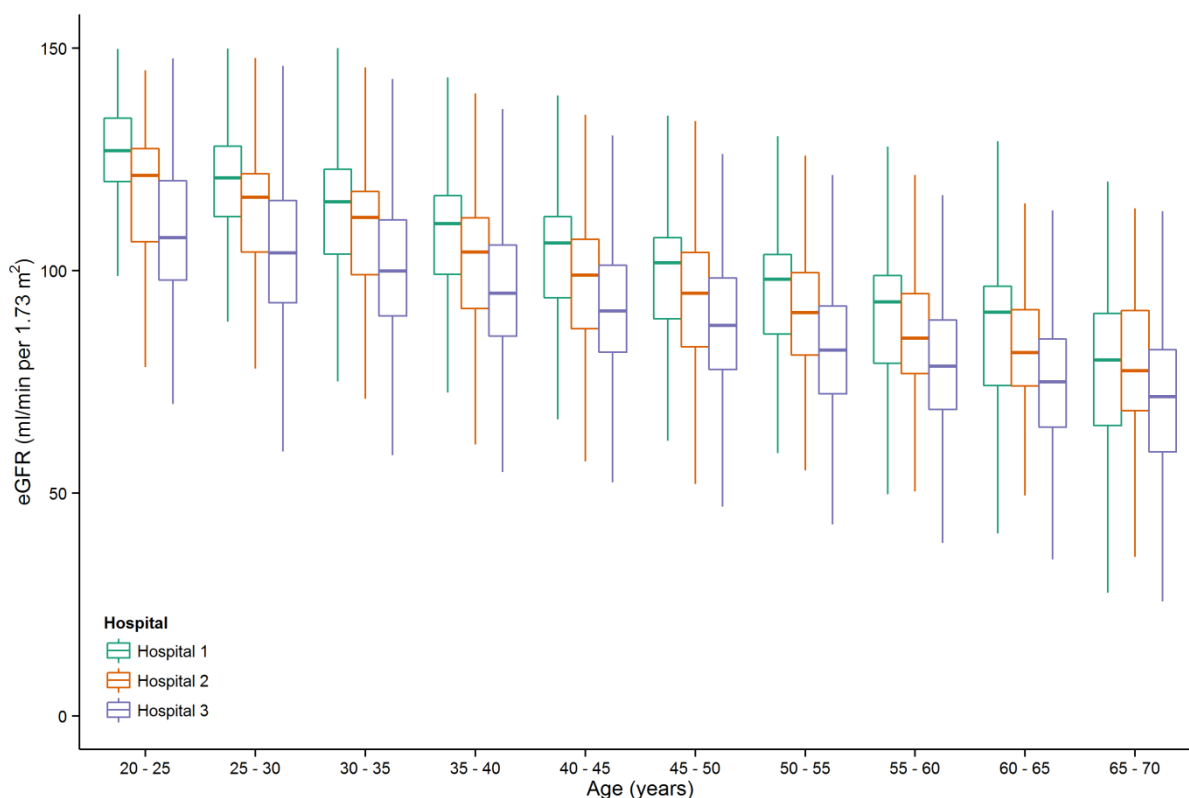


Figure 2: Creatinine based estimated glomerular filtration rate (calculated with the CKD-EPI equation) with increasing age at three hospitals in Denmark.

these equations have been evaluated in controlled settings of HIV-infected individuals[30–36], the conclusion of these in general was that the formulas perform well enough to make the base for clinical decisions. In a study we performed within the context of this thesis we found a large inter-hospital difference between eGFRs measured on comparable HIV-infected individuals (Figure 2, unpublished data). This problem has also been highlighted in other settings. A study that evaluated the state of the art in measuring serum-creatinine in the US, found that 30 of 50 labs had substantial bias in their measurement of serum-creatinine[37]. This bias obviously will affect the result from a serum-creatinine based eGFR. Comparisons of eGFR between hospitals could therefore prove to be problematic.

4.3 ESTIMATING RENAL COMPLICATIONS

Damage to the kidneys results in one or more of the above mentioned functions of the kidneys to be affected. This is used when physicians need to evaluate renal function. The most commonly used ways to evaluate renal function is by GFR, assessed with either eGFR or mGFR, and urine albumin/creatinine ratio (UA/Cr) or urine protein/creatinine ratio (UP/Cr). The Kidney Disease Improving Global Outcome (KDIGO) group recommends that chronic kidney disease (CKD) is classified based on cause, GFR category and albuminuria category (Figure 3)[38]. Based on this individuals are placed in a group of low-, moderate, high or very high risk of complications to CKD[38]. As discussed previously, one major concern regarding renal function in HIV-infected individuals is TDF associated nephropathy which is predominated by non-albuminuric proteinuria[39], why the European AIDS Clinical Society (EACS) guidelines recommend screening with UP/Cr rather than Albuminuria[40]. However, as discussed previously creatinine-based estimation of renal function is associated with uncertainty. This leads to potential misclassification of CKD. Misclassification will bias estimates of the absolute risk of a condition. If the misclassification is non-differential, i.e. there is the same degree of misclassification between the groups of interest, then the impact on the relative risk is negligible. In the case of differences in eGFR measurements on hospitals, there is a risk of differential misclassification since the composition of groups of individuals could differ between the hospitals, and in such cases the relative risk is also affected which is problematic. In our studies we used hard undisputable end-points from one data source for all groups of individuals in combination with the vaguer CKD event in order to reduce differential misclassification.

4.4 HYPOTHESES AND OBJECTIVES

Before this thesis was commenced several questions were unanswered. The current thesis sought to address some of these questions.

The hypotheses of the current thesis were:

- The risk of any renal replacement therapy (aRRT) and chronic renal replacement therapy (cRRT) (synonymous to end stage renal disease (ESRD)) is increased in HIV infected individuals compared to the background population.

Prognosis of CKD by GFR and albuminuria category

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased <30 mg/g <3 mg/mmol	Moderately increased 30-300 mg/g 3-30 mg/mmol	Severely increased >300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73 m ²) Description and range	G1	Normal or high	≥90	Green	Yellow	Orange
	G2	Mildly decreased	60-89	Green	Yellow	Orange
	G3a	Mildly to moderately decreased	45-59	Yellow	Orange	Red
	G3b	Moderately to severely decreased	30-44	Orange	Red	Red
	G4	Severely decreased	15-29	Red	Red	Red
	G5	Kidney failure	<15	Red	Red	Red

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

Figure 3: KDIGO Guidelines regarding classification of chronic kidney disease[38].

- Exposure to TDF and/or PIs is associated with increased risk of aRRT and cRRT
- Smoking is associated with: 1) decreased renal function, 2) increased risk of CKD, 3) increased risk of aRRT and 4) increased mortality following aRRT in HIV-infected individuals.
- The usefulness of routine UP/Cr testing is limited in settings of well treated HIV-infected individuals.
- The agreement between estimated glomerular filtration rate (eGFR) and measured glomerular filtration rate (mGFR) based on real-life data from outpatient settings is poor in HIV-infected individuals.
- The use of a new antiretrovirals that elicit a benign increase in serum-creatinine is associated with poorer agreement between mGFR and eGFR.

The objectives of the current thesis were to:

- Estimate the risk of aRRT and cRRT in HIV-infected individuals and compare this to that of the general population and to evaluate whether exposure to TDF or PIs were associated with increased risk of either aRRT or cRRT (Study 1; Rasch et al., 2014).
- Evaluate the impact of smoking on overall renal function, the risk of CKD, the risk of aRRT and mortality following aRRT in HIV-infected individuals (Study 2, Ahlström et al., 2015).
- Evaluate the usefulness of routine UP/Cr testing as a screening tool for renal disease in HIV-infected individuals (Study 3, Ahlström et al., 2016).
- Evaluate the agreement between mGFR and eGFR in an everyday clinical setting, and evaluate whether exposure to antiretroviral drugs that elicit a benign increase in serum-creatinine were associated with poorer agreement between mGFR and eGFR (Study 4, Ahlström et al., 2017).

5. METHODS

5.1 SETTING

All studies were based on data acquired in Denmark. The prevalence of known HIV-infection in the adult population in Denmark has remained almost constant throughout the study period, with a slight increase (0.10 % in 2012 and 0.11 % in 2016). HIV-infected individuals are treated in one of eight specialized medical centers, where they are seen on an outpatient basis at intended intervals of 24 weeks. Antiretroviral treatment is provided free of charge to all HIV-infected individuals.

5.2 DATA SOURCES

All studies in the current PhD thesis were register based. Our primary source of data was the Danish HIV cohort study (DHCS). In this database we identified all HIV-infected individuals with a Danish 10-digit unique person identifier (PIN) to avoid multiple registrations and to be able to merge data and track individuals from the following databases.

5.2.1 THE DANISH HIV COHORT STUDY (DHCS)

DHCS is a prospective study of all HIV-infected individuals 16 years or older at HIV diagnosis, that are treated at Danish HIV-centres after 1 January 1995. Individuals are consecutively enrolled. Data is collected annually and includes: Date of first positive HIV-test, route of infection, sex, race, AIDS-defining events, date and cause of death and data on antiretroviral treatment. CD4 cell counts and HIV viral load are extracted electronically from laboratory data files. The study is described in detail elsewhere[41,42], validity of data is continually being evaluated. From this database we extracted demographic and clinical data (Studies 1, 2, 3 and 4).

5.2.3 DANISH CIVIL REGISTRATION SYSTEM (DCRS)

All individuals in Denmark are given a PIN at birth or immigration. DCRS was established in 1968 and stores information on all Danish citizens. From DCRS we identified background population controls for study 1; furthermore we extracted data on vital status, residency and migration (studies 1 and 2).

5.2.2 THE DANISH NATIONAL HOSPITAL REGISTRY (DNHR)

DNHR was established in 1977 and stores information on all admission to non-psychiatric hospitals in Denmark. Diagnoses are classified according to the international classification of diseases 8th revision (ICD8) until 31 December 1993 and 10th revision (ICD10) thereafter. DNHR is described in detail elsewhere[43]. We used this registry to identify individuals who were treated with dialyses (ICD-10: BJFD.xx, ZZ43.40- ZZ43.50 and ICD-8 as 923.90, 943.99, 943.40, and 943.50), any renal replacement therapy (aRRT) was defined as the first date of treatment with dialysis or the first date of registration in the Danish Nephrology registry (described below), whichever came first. Furthermore we extracted data on comorbidities diabetes (ICD-10: DE10.0-DE14.9 and ICD-8: 249.00-250.09) and hypertension (ICD1: DI10-DI5.9 and ICD-8: 400.09-414.99) (Studies 1 and 2).

5.2.3 DANISH NEPHROLOGY REGISTRY (DNR)

DNR was established in 1990, but contains data dating back to 1964. DNR contains data on all Danish citizens who have received chronic dialysis or renal transplantation. Underlying cause is categorized according to ICD-10 after 1 January 1990. Data from this registry was used to identify individuals on chronic renal replacement therapy (cRRT) (Study 1).

5.2.4 LABORATORY DATA

From electronic clinical laboratory databases we extracted data on serum-creatinine measurements, eGFR, UP/Cr, provided by the Department of Clinical Biochemistry, at Copenhagen University Hospital, Rigshospitalet, Copenhagen University Hospital, Hvidovre Hospital and Southern Denmark University Hospital, Odense University Hospital (all studies). ^{51}Cr -EDTA clearance measurements were provided by the Department of Nuclear Physiology at University Hospital, Rigshospitalet (Study 4).

Serum-creatinine was converted to eGFR via the following formulas: 1) the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation ($\text{eGFR}_{\text{CKD-EPI}}$)[28] (study 2 and 4), 2) the 4-variable modification of diet in renal disease (MDRD) equation ($\text{eGFR}_{\text{MDRD}}$)[29] (study 1, 2, and 4), 3) the Cockcroft-Gault equation (eGFR_{CG})[27] (study 2 and 4), 4) the Bjørnson equation ($\text{eGFR}_{\text{Bjørnson}}$)[23], 5) the Mawer equation ($\text{eGFR}_{\text{Mawer}}$)[44], 6) the Hull equation ($\text{eGFR}_{\text{Hull}}$)[25], 7) the Gates equation ($\text{eGFR}_{\text{Gates}}$)[26], 8) the Jelliffe-1973 equation ($\text{eGFR}_{\text{Jelliffe}}$)[24], and 9) the Effersø equation ($\text{eGFR}_{\text{Effersø}}$) (Study 4)[22].

5.3 STUDY DESIGNS

All 4 studies included in the current thesis evaluated some aspect of either estimating renal function or estimating renal complications in HIV-infected individuals.

5.3.1 STUDY 1

Study 1 was a population based matched cohort study, designed to estimate the relative risk of aRRT and cRRT in HIV-infected individuals compared to the background population and to evaluate if exposure to TDF, atazanavir (ATV) or the combination TDF/ATV were associated with increased risk of aRRT (we did not have power to evaluate differences in the risk of cRRT). We included all HIV-infected individuals who had a Danish PIN and were aged ≥ 16 years at HIV diagnosis. Study inclusion was defined as 1 January 1995, the date of HIV diagnosis or date of immigration, whichever came last. Time was calculated from study inclusion to death, emigration, loss to follow up or date of aRRT/cRRT whichever came first. Individuals with a diagnosis of aRRT or cRRT before study inclusion were excluded from analyses. For each HIV-infected individual we identified 10 individuals matched on age and gender, from the background population to comprise the comparison cohort. Study outcome was 1) time to first date of aRRT and 2) time to first date of cRRT. Furthermore, we also evaluated risk factors for aRRT in HIV-infected individuals. cRRT was defined as the first date an individual was registered in DNR and aRRT was defined as the first date an individual was registered with any dialysis procedure code as registered in DNHR or the first cRRT.

5.3.2 STUDY 2

Study 2 was a population based cohort study, designed to estimate the effect of smoking on renal function in HIV-infected individuals. The study was divided into three parts. We included all HIV-infected individuals who 1) had data available on smoking status, 2) had a Danish PIN 3) were aged ≥ 16 years at HIV diagnosis 4) were alive and living in Denmark at time of inclusion and 5) did not have aRRT at or prior to study inclusion. This comprised the study population for part 2. For part 1 the following criteria for inclusion was added: 1) at least 1 eGFR available 2) data on weight available and 3) no CKD at or prior to study inclusion. In part 3, all patients from part 2 with aRRT were included. CKD was defined as two consecutive eGFRs of ≤ 60 mL/min, ≥ 3 months apart and aRRT was defined as the first registration of a procedure code for dialysis in DNHR.

In part 1, we evaluated the effect of smoking on 1) overall renal function estimated with eGFR calculated with the Cockcroft-Gault equation (eGFR_{CG}) and 2) the risk of CKD. When evaluating risk of CKD (as defined below) time was calculated from January 1, 1995, date of HIV diagnosis, date of first available data on smoking, first available eGFR or date of immigration whichever occurred last to date of CKD, last available eGFR, loss to follow-up, emigration, death, or 10 years after study inclusion, whichever occurred first. Outcome was time to CKD.

In Part 2, we evaluated risk of aRRT. Time was calculated from January 1, 1995, date of HIV diagnosis, date of first available data on smoking or date of immigration whichever occurred last to date of aRRT, loss to follow-up, emigration, death, or 10 years after study inclusion, whichever occurred first. Outcome was time to aRRT, as defined earlier.

In part 3 Time was calculated from aRRT to death, emigration, loss to follow-up, December 31, 2013, or 56 days after study inclusion whichever came first. Outcome was time from aRRT to death.

5.3.3 STUDY 3

Study 3 was a single center study designed to evaluate the implementation and usefulness of routine UP/Cr testing at HIV-outpatient clinic at the University Hospital of Copenhagen, Rigshospitalet. We included all HIV-infected individuals who: 1) had a Danish PIN and 2) were seen at the clinic in the period 1 July 2013–30 June 2015. The study period was divided into two sub-periods. For the statistical analyses we included only the first UP/Cr and the corresponding eGFR in each sub-period. To evaluate UP/Cr as a marker of early renal damage, we excluded individuals with already known renal disease (eGFR < 60 mL/min pr. 1.73 m^2 (abnormal eGFR) or a UP/Cr ≥ 0.5 mg/mmol (abnormal UP/Cr)), within 1 year prior to the date of blood/urine testing. The degree of implementation was estimated as the numbers who did not have a UP/Cr performed divided by the number with no UP/Cr performed. The usefulness was evaluated via the number of abnormal tests actually commented in the patient files as a percentage of the total number of abnormal tests performed, and the consequences on an individual level of the abnormal tests.

5.3.4 STUDY 4

Study 4 was a single center study designed to evaluate 1) the agreement between eGFR and mGFR in an everyday clinical setting of HIV-infected individuals, 2) whether the use of antiretrovirals that elicit a benign increase in creatinine or potentially nephrotoxic drugs influences the agreement

and 3) to evaluate if intra-individual differences were smaller than inter-individual differences. We used the eGFR reported by the department of clinical biochemistry ($eGFR_{LAB}$) and eGFR calculated with 9 different equations from serum-creatinine: (1) the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation ($eGFR_{CKD-EPI}$) [28], (2) the 4-variable modification of diet in renal disease (MDRD) equation ($eGFR_{MDRD}$) [29], (3) the Cockcroft-Gault equation ($eGFR_{CG}$) [27], (4) the Björnson equation ($eGFR_{Björnson}$) [23], (5) the Mawer equation ($eGFR_{Mawer}$) [44], (6) the Hull equation ($eGFR_{Hull}$) [25], (7) the Gates equation ($eGFR_{Gates}$) [26], (8) the Jelliffe-1973 equation ($eGFR_{Jelliffe}$) [24], and (9) the Effersø equation ($eGFR_{Effersø}$) [22]. We used agreement statistics as proposed by L. Lin to analyse agreement between mGFR and eGFR [45].

5.4 STATISTICAL ANALYSES

The studies in the current PhD are quite different in regards to overall design, and therefore the statistical methods to analyse the data are also quite different from study to study. In this section I will describe and discuss the statistical methods used in the 4 studies.

5.4.1 ANALYSIS OF SURVIVAL DATA

Study 1 and study 2 are primarily based on survival data. Survival statistics deals with time to event analyses; e.g. time to cRRT or time to aRRT in study 1 and time to CKD, time to aRRT and time to death in study 2. In survival statistics we need to understand two important concepts, survival and hazard. The survival function is defined as:

$$S(t) = \Pr(T > t),$$

where T is a variable describing the time of the event of interest and t is time. The survival function has a very nice interpretation which is simply the probability for an individual in the sample population to be alive at time t . It is however difficult to calculate on the survival scale, why calculations are often done on the hazard scale. The hazard function is defined as:

$$\lambda(t) = \lim_{dt \rightarrow 0} \frac{\Pr(t \leq T < t + dt)}{dt \cdot S(t)}$$

The interpretation of hazard in words is: “the instantaneous risk of experiencing the event of interest at time t , given that you are at risk at time t ”. This concept is difficult to comprehend. However it is easier to calculate on the hazard scale and we know the hazard function we can calculate the cumulative hazard (denoted $\Delta(t)$). This has a nice relationship with the survival function which is written as:

$$\begin{aligned} \Delta(t) &= -\log(S(t)) \Leftrightarrow \\ S(t) &= \exp(-\Delta(t)) \end{aligned}$$

From this follows that if we know the hazard function we can calculate the cumulative hazard and then the survival function.

To analyse survival data we require specific statistics in order to deal with censoring of individuals. Censoring is when the value of interest is only partially known e.g. when an individual’s follow-up ends before he experiences the event of interest. This could be caused by the individual emigrating, or simply because the individual has not experienced the event at the end of the follow-up

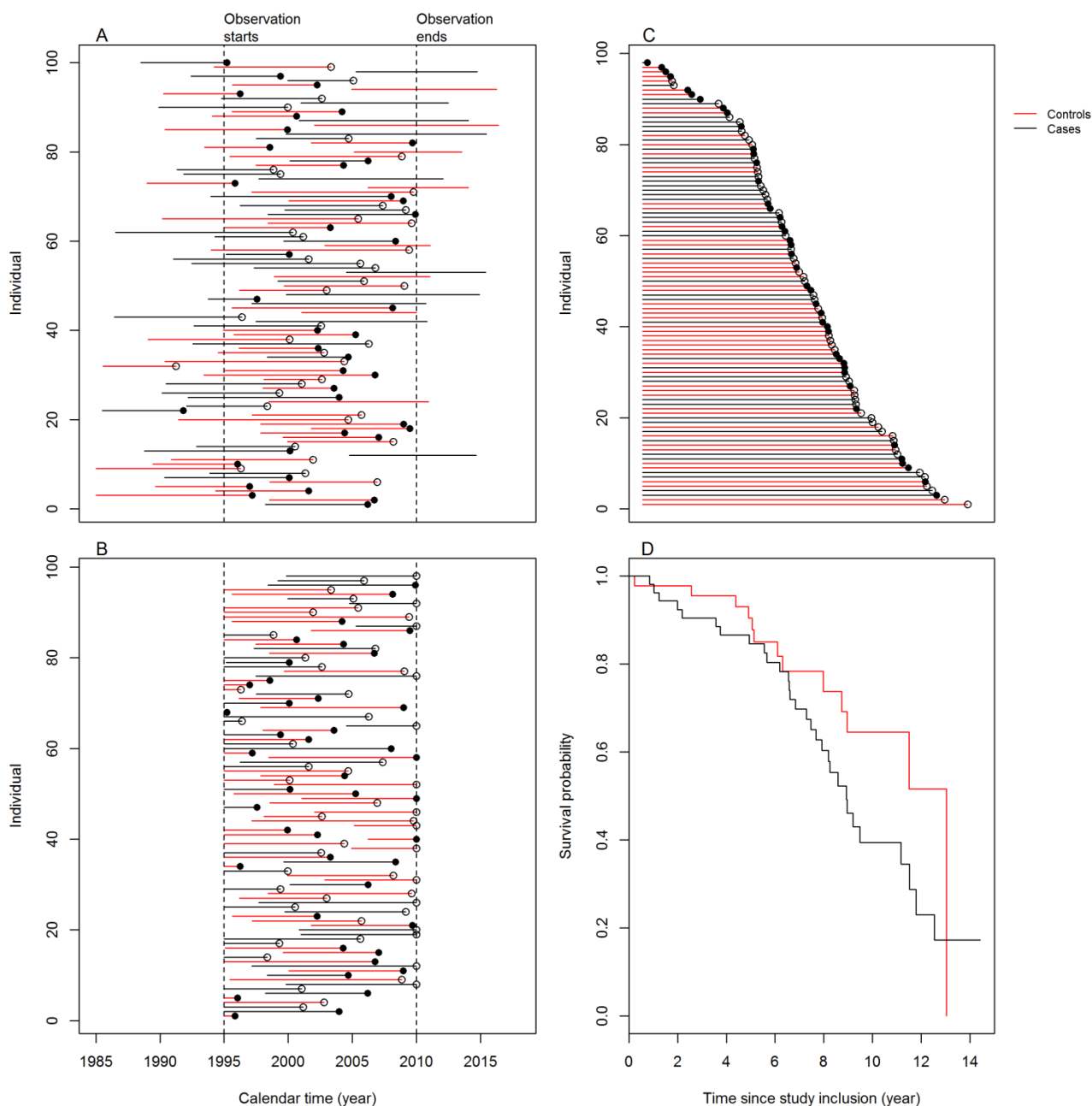


Figure 4: An illustration of how data is processed when doing a survival analysis on data in a prospective cohort with consecutive enrolment. The x axis is calendar time for A and B and time since study inclusion for C and D. The y-axis is all the individuals in the simulated data. The points in the end illustrate how the observation time is ended for each individual with either censoring (open dot) or an event (filled dot), the colour illustrates if the line represents case e.g. HIV-infected individuals or a control e.g. an individual from the comparison cohort. **(A)** Illustrates the raw data before we have selected the study inclusion criteria and end of follow up, **(B)** Illustrates the data and follow up of each individual after the exact follow-up period has been determined, and the appropriate censoring/event label has been added, **(C)** Illustrates the data in an ordered fashion and **(D)** Is the Kaplan-Meier estimate of the survival in this sampled dataset.

period (Figure 4A-C for illustration). In this case we know that the subject did not experience the event up until the time follow-time ended, but we do not know when or if he will experience it

later. This particular type of censoring is called right-censoring. In this thesis we will only deal with right-censoring. In this thesis we have used several different methods to analyse survival data.

An important consideration in survival statistics is the assumption that the probability of experiencing an event is the same for censored individuals and those who remain under follow-up (the independent censoring assumption)[46]. This assumption cannot be checked from the data, so we need to consider this before conducting analyses. In the analyses where the event of interest is CKD, aRRT or cRRT this assumption could be violated if individuals were censored at death and death was somehow related to the risk of cRRT or aRRT, e.g. death from cardiovascular disease or death from chronic kidney disease[47]. Competing risks (described later) addresses the issue of independent censoring[48].

5.4.1.1 KAPLAN-MEIER ESTIMATOR

The Kaplan-Meier estimator (also known as the product limit estimator) is a non-parametric statistical model used to estimate the survival function from survival data. The Kaplan-Meier estimator has the following formula[49]:

$$\begin{aligned}\hat{S}(t_j) &= \widehat{Pr}(T > t_1 | T \geq t_1) \cdot \widehat{Pr}(T > t_2 | T \geq t_2) \cdot \dots \cdot \widehat{Pr}(T > t_j | T \geq t_j) \\ &= \hat{S}(t_{j-1}) \cdot \widehat{Pr}(T > t_j | T \geq t_j)\end{aligned}$$

The above described in words reads that the estimated survival probability at time j can be calculated as the estimated survival probability at time j minus 1 multiplied by the probability of being alive at time j given that the individual is at risk. If an individual is censored the probability of survival does not change so we only need to consider the survival probability every time an event occurs. The graphical presentation of the estimator will be a series of horizontal declining steps, where the steps downward is calculated each time an event occurs and the new level is given by the estimated survival probability multiplied by the number of events divided by the number at risk (Figure 4D).

One important limitation of the Kaplan-Meier estimator is that it is not possible to calculate adjusted estimates of survival. For this you need other models.

5.4.1.2 POISSON REGRESSION

Poisson regression is a type of regression analyses that belongs to the family of generalized linear regression models; it is used to model counts or rates and has the following formula:

$$\log(\lambda_i) = \beta_0 + \beta_1 X_{i1} + \dots + \beta_p X_{ip}$$

Here λ_i is the expected hazard rate of subject i , the X_i 's indicate the specific predictors of subject i , e.g. gender or age and the β_{1-p} are the coefficients associated with the predictors. The β_0 is interpreted as the baseline rate, i.e. the rate if all other co-variables are 0.

From the formula we see that the baseline rate is estimated directly from the model but also that it is assumed to be constant, i.e. a Poisson regression model assumes constant risk. This assumption however, holds for piecewise constant rates. In practice this means that if the time for each individual is divided into reasonably small intervals the coefficients are interpretable in the normal way.

As the name suggests the model assumes that the response variable, e.g. risk of dialysis, follows a Poisson distribution. A Poisson distribution has the property that the expected value of the distri-

bution $E(Y)$ is equal to the variance of the distribution $\text{Var}(Y)$. If this assumption does not hold, (the usual problem is that $\text{Var}(Y) > E(Y)$), the model is said to be over dispersed.

The link function, i.e. the function that provides the relationship between the linear predictors and the mean of the response variable, is the natural logarithm. When modelling survival data, we need to take into account both the number of events and the time elapsed, as described above. This is handled in Poisson regression by including the logarithm of time as an offset predictor variable, i.e. a predictor where the regression coefficient is fixed at 1, as demonstrated in the following.

$$\begin{aligned}\log\left(\frac{\text{Events}}{\text{Time}}\right) &= \beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p \Leftrightarrow \\ \log(\text{Events}) - \log(\text{Time}) &= \beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p \Leftrightarrow \\ \log(\text{Events}) &= \log(\text{Time}) + \beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p\end{aligned}$$

5.4.1.3 COX PROPORTIONAL HAZARDS MODEL

The cox proportional hazards model was proposed by David Cox in 1972[50], and is still one of the most popular statistical methods to analyse survival data.

If we let $X_i = \{x_{1i}, x_{2i}, \dots, x_{pi}\}$, be the predictors of the subject i , then the hazard of subject i can be estimated by the following formula:

$$\lambda(t, X_i) = \lambda_0(t) \cdot e^{\beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_p x_{pi}}$$

$\lambda_0(t)$ is the baseline hazard, i.e. the hazard if all covariates are 0, and $\beta_1 - \beta_p$ are the regression coefficients. Consider a model where we estimate the hazard of a particular event of HIV-infected individuals compared with population controls, so that HIV-infected individuals have the X_1 -value of 1, and population controls have the X_1 -value of 0.

$$\begin{aligned}\lambda(t, X_{HIV-status=0}) &= \lambda_0(t) \cdot e^{\beta_1 \cdot 0} \quad \wedge \quad \lambda(t, X_{HIV-status=1}) = \lambda_0(t) \cdot e^{\beta_1 \cdot 1} \Rightarrow \\ \frac{\lambda(t, X_{HIV-status=1})}{\lambda(t, X_{HIV-status=0})} &= \frac{\lambda_0(t) \cdot e^{\beta_1 \cdot 1}}{\lambda_0(t) \cdot e^{\beta_1 \cdot 0}} = e^{\beta_1}\end{aligned}$$

So e^{β} is interpreted as the ratio between the hazard of the HIV-infected individuals and the HIV-negative individuals – the hazard ratio (HR). One nice property of cox regression is that we do not assume anything about the baseline hazard, the baseline hazard is a function of time, and can take any form, as long as it fulfils the requirements for being a hazard [51]. What we do assume, however is that the hazard between groups is proportional i.e. there is no interaction with time from the particular co-variate. There is a risk that this assumption is violated with HIV-infected individuals when the event of interest is a result of acute disease. Approximately 48 % of Danish HIV infected individuals present with a CD4 cell count < 350 cells pr. μL (late presenters) [52]. These are potentially at increased risk of acquiring opportunistic infections (OIs) and sepsis and as a result renal impairment requiring dialysis. After they commence treatment CD4 cell count rise, and the increased risk of OIs and sepsis diminishes, this is not true for the background population, and as such we could get two hazards that are not proportional in the beginning of the study period, which could threaten to violate the proportional hazards assumption.

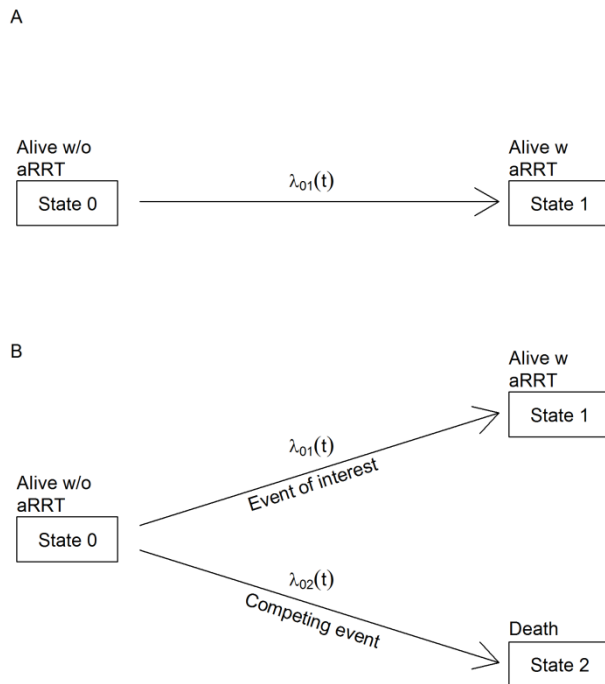


Figure 5: An Illustration of the difference between (A) a two-state model used in study 2, part 3 and (B) a multistate model with competing risks as we used in study 1 and study 2, part 1 and part 2.

We can check this assumption graphically by:

- Plotting the survival e.g. by the means of the Kaplan-Meier estimator.
- Plotting the cumulative hazard against time and checking for constant slope.
- Plotting the log of cumulative hazard (also described as the log-log survival) against time, and looking for parallelism[53].

If any of the co-variables violates the PH assumption there are two ways to fix this. 1) Fit a stratified cox-model, where we allow the different strata to have different baseline hazards. This solution is only appropriate for co-variables that can be considered as nuisance co-variables, which is not the case in the above example, since we are interested in estimating the impact of being HIV-infected. 2) Another solution is to include time-dependent covariates and

let the effect of that co-variate vary with time, which is the solution we used in study 2.

5.4.1.4 COMPETING RISKS AND CUMULATIVE INCIDENCE FUNCTION

In the Kaplan-Meier estimator, Poisson regression, and Cox regression we have a model that assumes two states. In the case of my thesis this could be Alive without aRRT (state 1) and alive with aRRT (state 2) (Figure 5A). In this case we would treat loss to follow up, emigration and death as censorings. However this will, in many cases, be too simple. If individuals are at risk of one or more other events than the event of interest and the other events exclude the event of interest from happening or modify the probability of that event, the event is said to be a competing risk to the event of interest, in that case we deal with a multi-state model (figure 5B). If we consider aRRT as the event of interest, dying from any cause excludes aRRT from happening and as such should be considered a competing risk, whether emigration and loss to follow modifies the probability of aRRT is debatable. If we believe that loss to follow-up or emigration modifies the probability of aRRT they should be considered competing risks, if not they should be considered censorings, in our studies we have considered loss to follow up and emigration as competing risks. In cases where we are working with acute diseases as is the case in study 2, part 3 where we consider death following aRRT, it should be sufficient to use a normal two-state model, since we have a predominant single event of interest (Death)[54]. In the other settings if we model time to different kinds of events, in individuals who are followed over long time, and are not generally considered to be acutely ill, it would be more appropriate to consider using a multistate model as described in stud-

ies 1 and 2. The cumulative incidence function is a non-parametric method to model competing risks data.

5.4.2 ANALYSIS OF AGREEMENT

If you wish to evaluate whether two different methods for measuring some quantity yields the same result, one common approach is to evaluate this via simple correlation, e.g. Pearson's r or the non-parametric counterpart to Pearson's r , i.e. Spearman's ρ . Pearson's r is a statistic that measures how far each observation is from the line that best fits the observed data. However

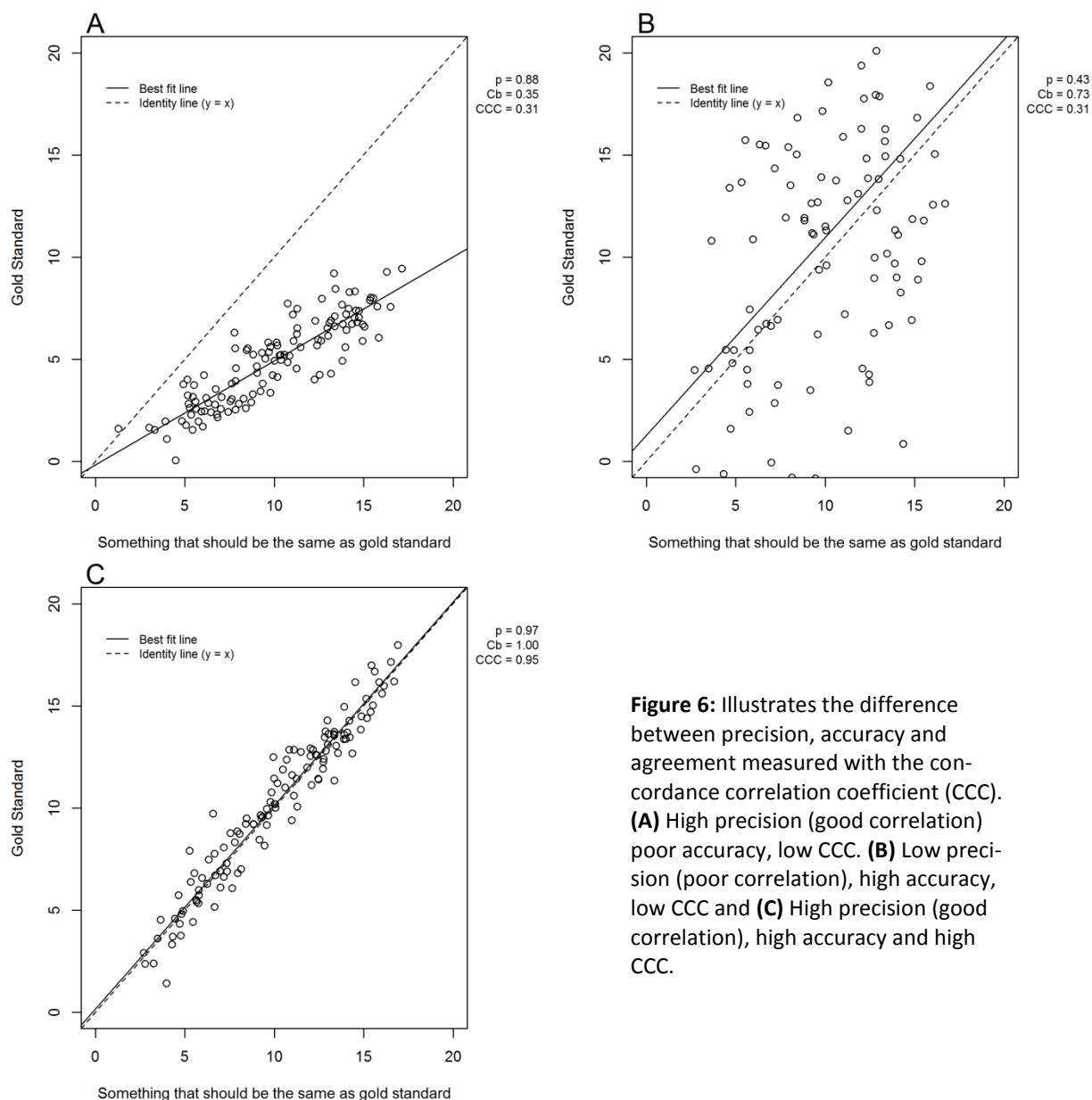


Figure 6: Illustrates the difference between precision, accuracy and agreement measured with the concordance correlation coefficient (CCC). **(A)** High precision (good correlation) poor accuracy, low CCC. **(B)** Low precision (poor correlation), high accuracy, low CCC and **(C)** High precision (good correlation), high accuracy and high CCC.

since this coefficient only tells how well the two variables fit to the line that best fits the data, the two variables could give very differing results even though the correlation coefficient indicates a very good correlation (Figure 6). In order to address this problem directly one needs to use statistical methods that are specifically developed to deal with these kinds of problems. Lawrence Lin has developed such methods [45,55]. In the next section I will briefly describe the methods he

suggested to use when one wishes to evaluate agreement. These are the methods we used in study 4.

5.4.2.1 CONCORDANCE CORRELATION COEFFICIENT (CCC)

The concordance correlation coefficient has the formula

$$\rho_c = p \cdot C_b$$

Where p , is the Pearson correlation coefficient, as described above which is a measure of precision and C_b is a factor that describes how far the best fit line is from the identity line ($x = y$)[55].

5.4.2.2 TOTAL DEVIATION INDEX (TDI) AND COVERAGE PROBABILITY (CP)

Like the Pearson correlation coefficient, CCC is difficult to interpret. TDI and CP are easier to interpret and gives clinicians the opportunity to define what they believe are relevant margins for the difference of two measurements.

Consider that we have two sets of values X (the non-Gold standard) and Y (the Gold-standard) and D is the difference between these two. Intuitively a good agreement is when X captures a large amount of Y , i.e. when the probability of the absolute value of D less than some predefined boundary κ is high. We can fix κ at some value, and then calculate the coverage probability (CP), or we can fix the CP, and calculate the κ . This can be described mathematically as.

$$CP = \Pr(|D| < \kappa)$$

And TDI is the κ that solves the equation:

$$\Pr(D^2 < \kappa^2) = CP$$

In our agreement study we chose a TDI value of 10% to calculate the CP values, in other words if we allow a difference of 10% between X (eGFR) and Y (mGFR) what will the value of CP be [45]. For the calculation of TDI we chose a CP of 90%, in other words, if we want to have 90% coverage of the data, how big a difference will we have to accept. These values were chosen because most other studies concerning agreement between eGFR and mGFR has used these values [56,57].

5.4.3 OTHER ANALYSES AND TESTS

For study 2, we divided time into three intervals and calculated median and IQR of eGFR for each subgroup of HIV-infected individuals. This was then plotted against time since study inclusion.

In study 3 we tested for independence between groups in regards to a variable. Here we used the Pearson's Chi-squared test (We did not use Fishers exact test, since no expected values were less than 5). We also tested for differences in the mean of continuous variables between two groups in study 3. Here we used the unpaired two sample t-test.

In study 4 we also used the following statistics to evaluate agreement. Bias: eGFR - mGFR; Relative accuracy: the cumulative percentage of eGFRs falling within 10% (P10) and 30% (P30) of mGFR; finally agreement was evaluated graphically with the Bland-Altman method [58].

6. RESULTS AND DISCUSSION

6.1 STUDY 1

Hypotheses (as a reminder):

- The risk of any renal replacement therapy (aRRT) and cRRT is increased in HIV infected individuals compared to the background population.
- Exposure to TDF and/or PIs is associated with increased risk of aRRT and cRRT.

6.1.1 RESULTS

For study 1 we included 5300 HIV-infected individuals and 53.000 individuals from the background population. In HIV-infected individuals we identified 68 cases of aRRT during 42.833 person years of follow up (PYs); among individuals from the background population we identified 182 cases of aRRT during 534.282 PYs. This corresponded to Incidence rates (IR) of 159 (95% CI: 125-201) and 34 (29-39) pr. 100.000 PYs among HIV-infected individuals and individuals from the background population. Poisson regression analysis yielded an Incidence Rate Ratio (IRR) of 4.7 (95% CI: 3.5 – 6.2). We did sensitivity analyses where we 1) only included individuals diagnosed after 1 Jan 1995 (no substantial change in estimates of IRR) and 2) non-IDUs and non-Africans. Here found an IRR of 3.5 (95% CI: 2.5-4.), so a substantial decrease in IRR, but still highly significant. The risk of aRRT was highest the first year following HIV-diagnosis Adjusted IRR 3.4 (95% CI: 1.5-8.1) (Figure 6).

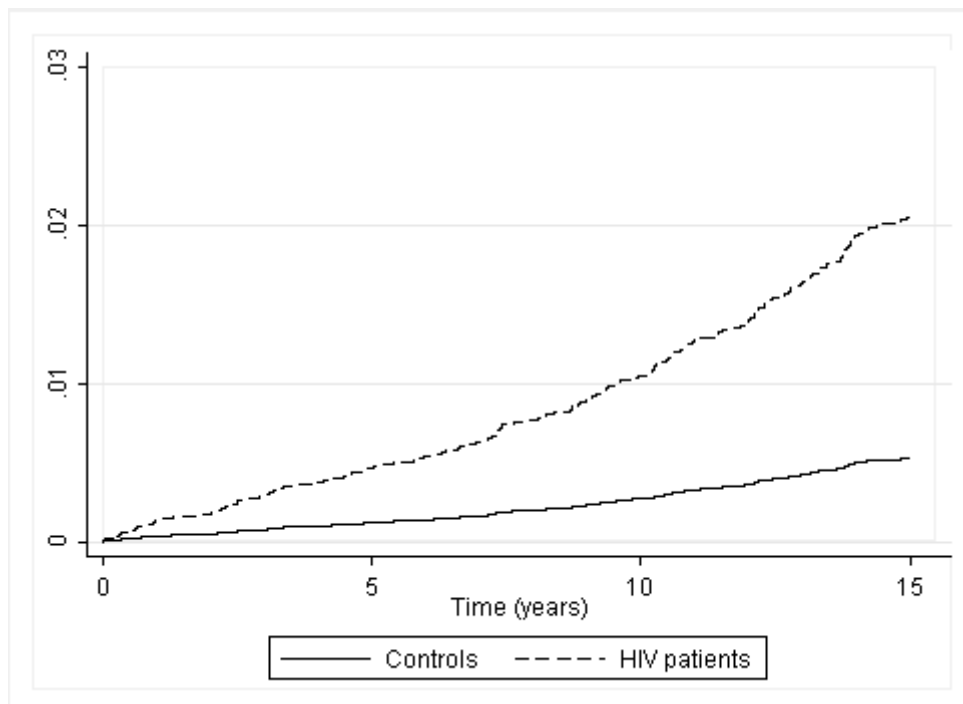


Figure 6: Cumulative incidence of any renal replacement therapy (aRRT) For HIV-infected individuals vs. background population controls.

Of the 68 cases of aRRT among HIV-infected individuals 19 cases progressed to cRRT during 42.879 PYs (IR 44 (95% CI: 28-69) pr. 100.000 PYs). 66 of the 182 individuals from the background population that had aRRT progressed to cRRT during 534.476 PYs (IR 12 (95% CI: 10-16) pr. 100.000 PYs.). This corresponded to an IRR of 3.6 (95% CI: 2.2-6.6) in Poisson regression analyses (Figure 7). Among HIV-infected individuals the only risk factors that were associated with increased risk of cRRT with statistical significance were hypertension and baseline eGFR < 60 mL/min pr. 1.73 m². Specifically we did not find that black ethnicity was associated with increased risk of cRRT. In two sensitivity analyses we included 1) only HIV-infected individuals that were diagnosed after 1 January 1995 (Incident cohort) and 2) non-IDU and non-Africans, which yielded results that did not change the overall conclusion substantially.

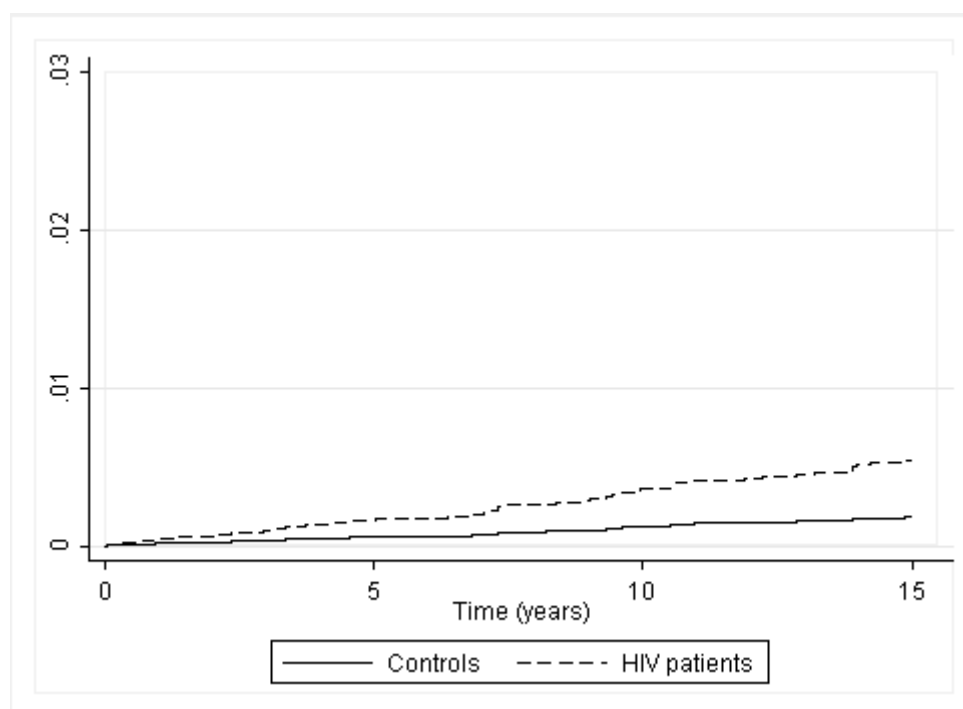


Figure 7: Cumulative incidence of any chronic replacement therapy (cRRT) For HIV-infected individuals vs. background population controls.

We did not find evidence to suggest that exposure to TDF, ATV or TDF/ATV was associated with increased risk of aRRT.

6.1.2 DISCUSSION

The main findings in this study was that: 1) HIV-infected individuals are at substantially increased risk of both aRRT and cRRT compared to the background population and 2) exposure to TDF, ATV or TDF/ATV was not associated with risk of aRRT in HIV-infected individuals.

The first finding was not surprising. Several studies have stated that there was an ongoing epidemic of HIV-associated ESRD/HIVAN resulting in an increased number of HIV-infected individuals receiving cRRT[59–61]. Our estimates of IR corresponded well with previous studies which found overall IRs ranging from 29.8 – 51.5 pr. 100.000 PYs[60,62] in non-Africans. Individuals of black ethnicity were found to at substantially higher risk than individuals of Caucasian ethnicity. However these studies had mainly focused on the fact that HIV-associated ESRD/HIVAN was mainly ob-

served in individuals of black ethnicity. We were not able to find an association between black ethnicity and cRRT. One possible explanation could be that the majority of Africans that we observed were from the eastern part of Africa (69%). Studies have shown that HIVAN is associated with risk variants of the APOL1 gene[63], which was predominant in the western and central parts of Africa[64], this could in part explain this discrepancy.

It was somewhat surprising that we were not able to find an association between exposure to TDF, ATV or the combination TDF/ATV and the risk of aRRT or cRRT since many case reports and previous studies have suggested an association[9,11–13,65–67]. However this finding most likely indicates that clinicians had been aware of the nephrotoxic potential of these drugs and switched individuals from TDF/ATV-containing regimens to TDF/ATV-free regimens if they were considered at risk of cRRT/aRRT or had progressive loss of renal function. This is supported by studies that have found increases probability of TDF/ATV being discontinued with decreasing renal function[68,69].

The results from this study did not give a clear answer as to why HIV-infected individuals are at increased risk of aRRT or cRRT, so we decided to evaluate the impact of smoking on the risk of CKD and aRRT.

6.2 STUDY 2

Hypotheses:

- Smoking is associated with: 1) decreased renal function, 2) increased risk of CKD, 3) increased risk of aRRT and 4) increased mortality following aRRT in HIV-infected individuals.

6.2.1 RESULTS

This study was divided into three parts. Overall 4,515 HIV-infected individuals fulfilled the inclusion criteria with a total of 29,196 PYs. One-thousand-four-hundred-and-five (32.7%) HIV-infected individuals were categorized as never smokers, 768 (17.0%) as previous smokers, and 2,272 (50.3%) as current smokers.

6.2.1.1 PART 1 - SMOKING, RENAL FUNCTION, AND CKD

For part 1 one-thousand-three-hundred-seventy-three individuals were excluded because they did not have data available on eGFR. We did not find differences in median eGFR based on smoking status (Figure 8A). When we modelled risk of CKD, 128 individuals were excluded because they had a diagnosis of CKD prior to study inclusion leaving 3,014 HIV-infected individuals with a total of 18,131 PYs. We identified 180 cases of CKD, 47 among never smokers, 43, among previous smokers and 90 among smokers, corresponding to overall IRs (pr. 100,000 PYs) of 832 (95% CI: 625 - 1108) for never smokers, 1327 (95% CI: 984 - 1789) for previous smokers and 974 (95% CI: 792 - 1197) for current smokers. In unadjusted Poisson regression analyses we calculated IRRs of 1.6 (95% CI: 1.1 - 2.4) for previous smokers vs. never smokers and 1.2 (95% CI: 0.8–1.6) for current smokers vs. never smokers. In adjusted models we found IRR of 1.1 (95% CI: 0.7–1.7) for previous vs. never smokers and 1.3 (95% CI: 0.9–1.8) for current vs. never smokers with age accounting for most of decrease in IRR (Figure 6b). As observed in figure 8B, we found that there was not proportional hazard, why we included a time-updated variable after 5 years. In this analysis we found no

differences before 5 years after study inclusion but after 5 years the IRR was 3.4 (95% CI: 1.3–8.7) for previous smokers vs. never smokers.

6.2.1.2 PART 2 - SMOKING AND aRRT

In part 2 the full cohort of 4,515 HIV-infected individuals with data on smoking status was included. We identified 62 cases of aRRT (20, 11, and 31 among never, previous, and current smokers) corresponding to IRs (per 100,000 PYs) of aRRT of 206 (95% CI: 133–319) for never smokers, 220 (95% CI: 122–397) for previous smokers, and 214 (95% CI: 150–304) for current smokers. We did not find evidence to suggest that the risk of aRRT was different in current and previous smokers compared to never smokers. Only 17 patients (nine never smokers, two previous smokers, and six current smokers) progressed to cRRT in the study period which did not allow us to make robust conclusions about the association of smoking and risk of cRRT. In sensitivity analyses where IDUs and individuals of non-Danish origin were excluded we found results that did not differ substantially from the ones reported (Figure 9A).

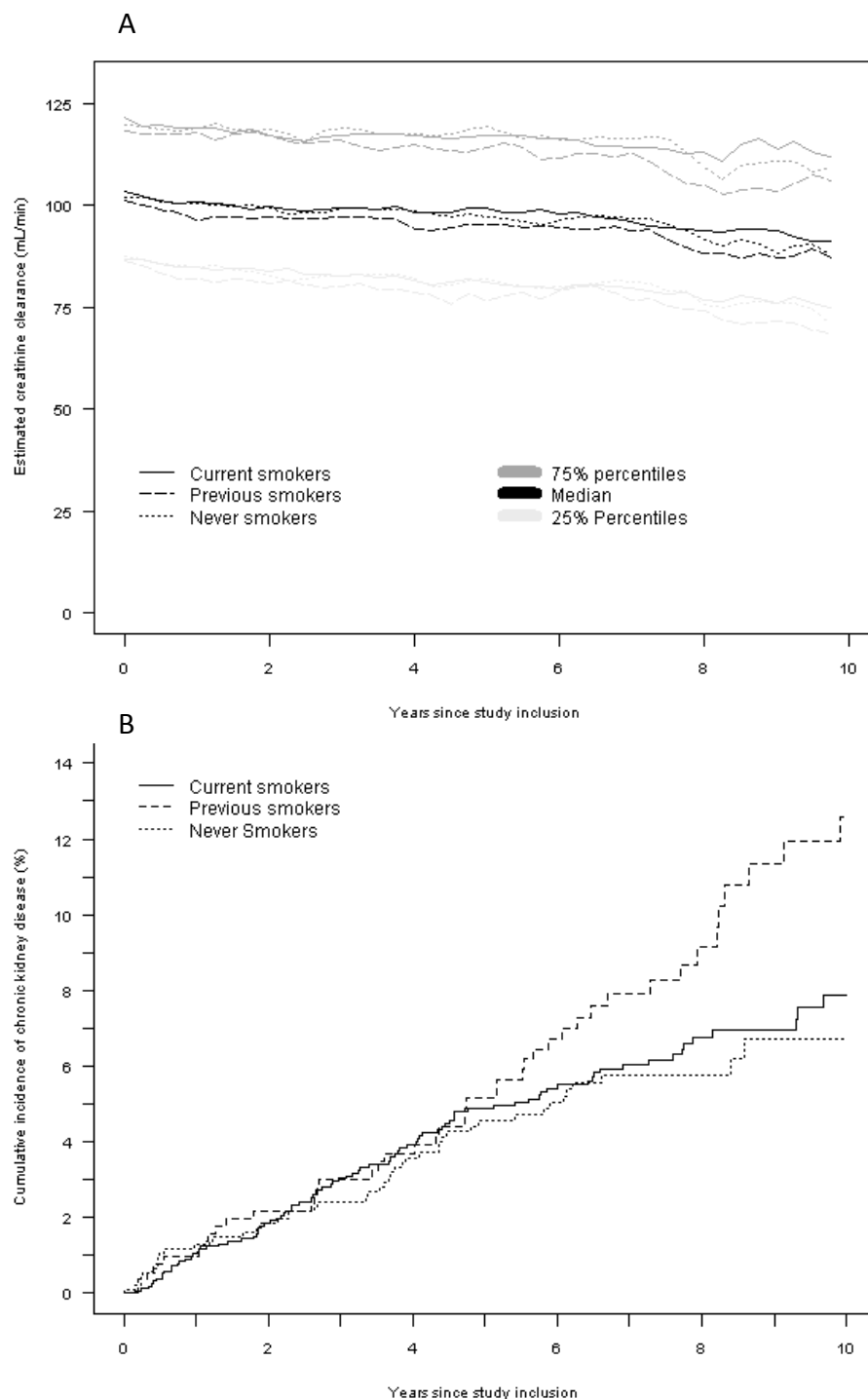


Figure 8: Estimated glomerular filtration rate and risk of chronic kidney disease stratified on smoking status. **(A)** 25%, median, and 75% percentiles of estimated glomerular filtration rate stratified on smoking status. We used the Cockcroft–Gault equation to calculate estimated glomerular filtration rate ($eGFR_{CG}$) (displayed as mL/min). We divided time from study inclusion until end of follow-up in 3-month intervals. In each interval, all participants contributed with one $eGFR_{CG}$. The $eGFR_{CG}$ in a time interval was either the median of all $eGFR_{CG}$ in that time interval. If no $eGFR_{CG}$ was available in a 3-month time interval, the $eGFR_{CG}$ was calculated as the weighted mean of the $eGFR_{CG}$ measurements determined before and after the actual 3-month period. At study inclusion, there were 984 never smokers, 530 previous smokers, and 1,628 current smokers. At 10 years of follow-up, 110 never smokers, 98 previous smokers, and 170 current smokers were still under follow-up. **(B)** Risk of chronic kidney disease stratified on smoking status. Definition of chronic kidney disease: two consecutive $eGFR_{CG}$ of ≤ 60 mL/min > 3 months apart. **Abbreviation:** $eGFR_{CG}$, estimated glomerular filtration rate calculated with the Cockcroft–Gault equation.

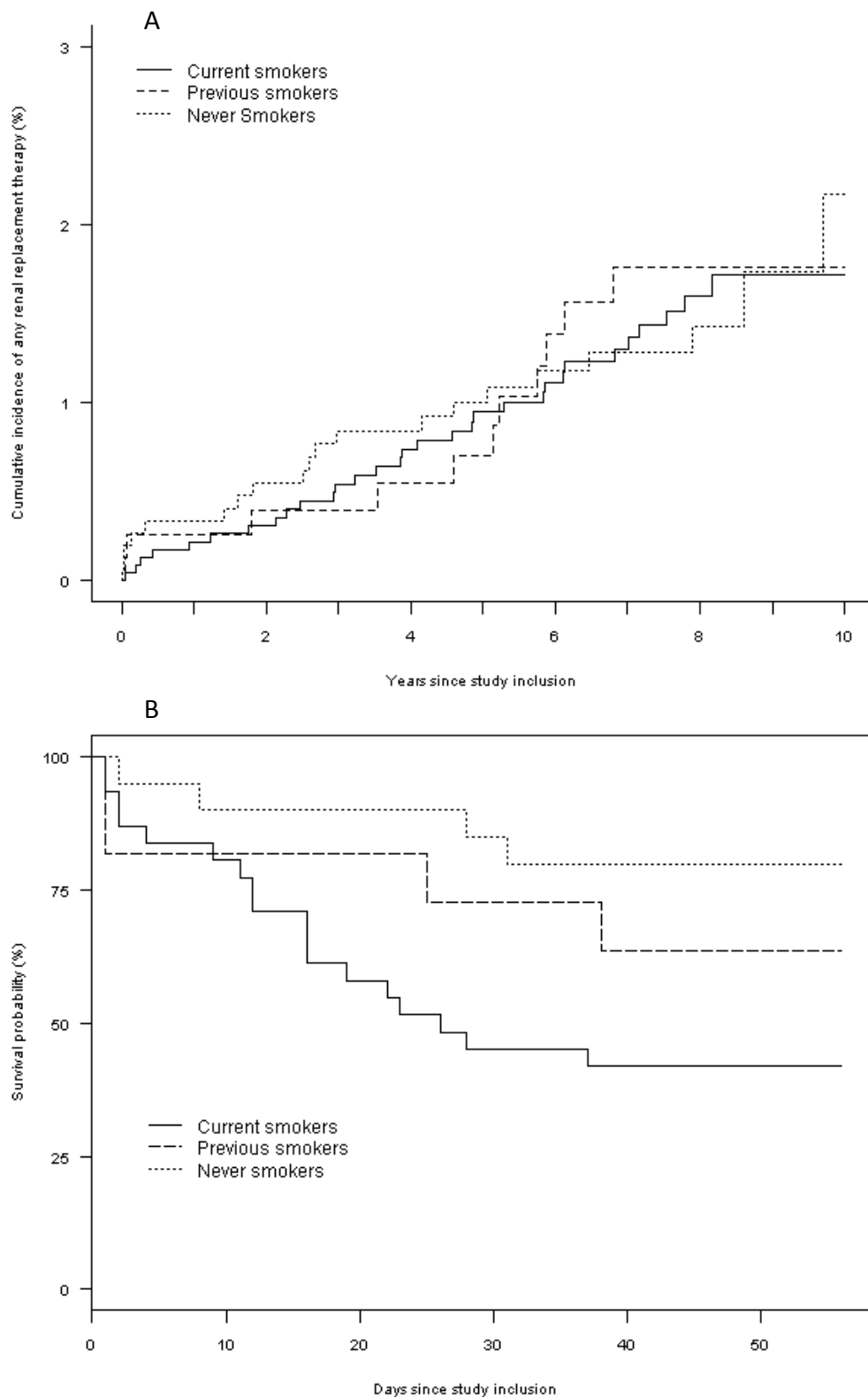


Figure 9: (A) Risk of any renal replacement therapy stratified on smoking status, **(B)** Mortality following any renal replacement therapy stratified on smoking status.

6.2.1.3 PART 3 - SMOKING AND MORTALITY FOLLOWING ARTT

In part 3 we included 62 HIV-infected individuals (The ones who commenced aRRT in part 2 of the study). They had a total of 344 person days of follow-up (PDs), which corresponded to MRs (per 1,000 PDs) of 29.0 (95% CI: 10.9–77.3), 65.3 (95% CI: 23.0–163.0), and 128.0 (95% CI: 80.6–203.0) for never smokers, previous smokers, and current smokers. The unadjusted hazard ratios (HR) were 2.0 (95% CI: 0.5–8.2) for previous smokers vs. never smokers and 3.9 (95% CI: 1.30–11.5) for current smokers vs. never smokers (Figure 3). When we adjusted for gender and age, the HRs were 1.9 (95% CI: 0.5–7.8) and 3.8 (95% CI: 1.3–11.2). In sensitivity analysis where we excluded IDUs and individuals of non-Danish origin we found results that differed very little from the overall results (Figure 9B).

6.2.2 DISCUSSION

The results from this study were surprising. Smoking has been shown to be a major risk factor in the HIV-infected population, increasing the risk of cardiovascular disease (CVD), smoking related cancers and overall mortality[70–72]. In fact it was estimated that HIV-infected individuals as a population lose more life-years than from the HIV-infection itself[72]. Also several studies from the background population have suggested an association between smoking and the risk of renal diseases[73–76]. However this association is not unambiguous[77]. Yamagata et al. conducted a large prospective cohort study in Japan of all residents that had their annual health examination at a community center and included 123,764 individuals. They defined CKD stage III as a single eGFR < 60 mL/min pr. 1.73 m². HR of CKD for smokers were 1.26 (95% CI: 1.14–1.41) among men and 1.40 (95% CI: 1.16–1.69) among women[75]. Worth noticing is the relatively small HR and their definition of CKD stage III, which is not the strict definition of CKD, which requires the reduced eGFR to be present > 3 months[38]. Briganti et al. found Odds Ratios of impaired renal function (eGFR < 60 mL/min pr. 1.73 m²) of the same magnitude as Yamagata et al. (1.38 [95% CI: 0.75–2.56]), however their population was only big enough to show an association for men where smoking was associated with an odds ratio of 3.59 (95% CI, 1.27 to 10.09). They also found a statistically significant negative association between the number of pack-years smoked and eGFR (3.6 mL/min pr. 1.73 m² (95% CI, 2.3 to 4.9) lower eGFR for every 10 pack-years smoked for men and 2.6 mL/min pr. 1.73m² (95% CI, 1.2 to 4.0) lower for every 10 pack-years for women). However these results should be interpreted with caution since they are not based on a regression analysis, and as such age is not adjusted for which could change the estimates substantially[74]. Halimi et al. did not find an association between smoking and low eGFR but found an association with proteinuria[78]. The simultaneous occurrence of normal/high eGFR and proteinuria in smokers have led to the hypothesis that smokers are subject to hyperfiltration[79,80], which is a phenomenon also described in diabetes and probably is one of the mediators of renal dysfunction in diabetics[81]. Haroun et al. used a fairly strict definition of CKD and found that smoking was associated with the risk of CKD, but only after 60 years of age[82]. Klag et al. conducted a large cohort study with more than 300,000 participants with the purpose of evaluating the impact of hypertension on risk of cRRT. They reported that smoking was associated with increased the risk of cRRT but not the magnitude[83].

Only a few studies have evaluated smoking as a risk factor of renal disease in HIV-infected individuals. Ryom et al. found that in adjusted analyses, smoking was associated with a statistically significant increase in IRR of advanced CKD/ESRD of approximately 1.9. Their study was not designed to

evaluate the effect of smoking specifically, as such the precise manor of how smoking was included, whether or not there were missing values, and how these were handled was not reported. Furthermore the population was slightly older than in our study, which could explain the difference[69].

Smoking as a risk factor of mortality among individuals on renal replacement therapy is well established and corresponds well with the results from the current study[84].

6.3 STUDY 3

Hypothesis:

- The usefulness of routine UP/Cr testing is limited in settings of well treated HIV-infected individuals.

6.3.1 RESULTS

For study 3 we identified 1,510 individuals who attended the HIV outpatient clinic at Rigshospitalet in period one and 1,441 individuals in period two (1,369 individuals were seen in both periods). Individuals were generally well treated with CD4 cell counts of 660 cells/ μ L (IQR = 490 - 858) and 650 cells/ μ L (IQR = 490 - 850) and the fractions of virally suppressed (viral load < 50 copies/ μ L) were 86% and 85% for periods one and two. In period one, 643 (42.6%) individuals had at least one UP/Cr performed, compared to 415 (28.8%) in period two ($p < 0.001$). In period one, 13 (2.0% of the tested population) and in period two, four individuals (1.0% of the tested population) had an abnormal UP/Cr. For period one, the abnormal test was commented in the medical files for three individuals (0.5% of the tested population), one individual (0.2% of the tested population) had further analyses made (Chrome-EDTA clearance) and two individuals (0.4% of the tested population) started a treatment regime for CKD. For period two, none of the abnormal tests were commented in the patient files or had consequences for the patient.

6.3.2 DISCUSSION

Clearly evidence exists that HIV-infected individuals are at increased risk of adverse renal events[85]. This is especially evident in high risk populations such as IDUs and individuals of black ethnicity [69,85]. As a consequence guidelines on the management of renal function in HIV-infected individuals have widely implemented the use of annual UP/Cr screening, however the evidence to support this is poor (C-III or based on consensus decisions)[86]. Before this study no studies, to our knowledge, had evaluated the effectiveness of routine UP/Cr screening in HIV-infected individuals in a modern cART era.

Fink et al. conducted a systematic review on screening versus no screening for CKD in the general population. They found that no randomized clinical trials had been performed on the effect of UP/Cr screening, and they concluded that the benefits of screening was uncertain[87]. Based on this, screening is not recommended by the US preventive task force in low risk populations[88].

6.4 STUDY 4

Hypotheses:

- The agreement between estimated glomerular filtration rate (eGFR) and measured glomerular filtration rate (mGFR) based on real-life data from outpatient settings is poor in HIV-infected individuals.
- The use of new antiretrovirals that elicit a benign increase in serum-creatinine is associated with poorer agreement between mGFR and eGFR.

6.4.1 RESULTS

6.4.1.1 CHARACTERISTICS

For this study we identified 98 HIV-infected individuals who had at least one mGFR performed during the study period. Eight individuals (8.2%) were exposed to either RLP/COB/DTG and 62 individuals (63.3%) were exposed to TDF. Seven (7.1%) were not receiving any ARVs at time of eGFR. 15 individuals did not have data on weight available and so eGFR calculation with equations relying on weight was not possible for these individuals.

Table 1 – Performance characteristics of the different equations to estimate renal function

Estimation equation	Number of Individuals	CCC	TDI ₉₀	CP ₁₀	P10	P30	RMSE
Department of Clinical Biochemistry*	98	0.42 (0.33)	33.59 (36.59)	0.37 (0.34)	36 (37%)	72 (73%)	16.9
CKD-EPI equation	98	0.41 (0.27)	35.16 (39.28)	0.36 (0.32)	29 (30%)	69 (70%)	18.6
MDRD equation	98	0.49 (0.38)	30.24 (33.28)	0.42 (0.38)	36 (37%)	78 (80%)	16.8
Cockcroft-Gault equation	83	0.67 (0.58)	26.94 (30.65)	0.45 (0.40)	28 (34%)	66 (80%)	13.7
Björnsson equation	83	0.64 (0.54)	28.68 (32.61)	0.43 (0.38)	29 (35%)	69 (83%)	14
Mawer equation	83	0.42 (0.33)	56.09 (63.79)	0.23 (0.20)	23 (28%)	49 (59%)	15.3
Hull equation	83	0.43 (0.29)	32.51 (36.79)	0.38 (0.34)	30 (36%)	62 (75%)	16.7
Gates equation	98	0.18 (0.14)	59.23 (61.95)	0.09 (0.07)	7 (7%)	25 (26%)	16.2
Jelliffe-1973 equation	98	0.38 (0.27)	36.59 (40.16)	0.34 (0.31)	18 (18%)	66 (67%)	17.9
Effersø equation	98	0.35 (0.25)	34.08 (36.86)	0.37 (0.35)	31 (32%)	73 (74%)	17.9

* During the study period The Department of Clinical Biochemistry calculated eGFR with the MDRD equation but without adjustment for race.

Abbreviations: CCC; concordance correlation coefficient, TDI₉₀; Total Deviation Index (CP fixed at 90%), CP₁₀; Coverage Probability (TDI fixed at 10%), P10; Relative accuracy 10%, P30; Relative accuracy 30%, RMSE; root mean squared error, MDRD; modification of diet in renal disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration.

6.4.1.2 PERFORMANCE CHARACTERISTICS OF EGFR EQUATIONS

Overall, the equations showed poor performance characteristics. CCC ranged from 0.18 to 0.67, CP₁₀ ranged from 0.09 to 0.45 and TDI₉₀ ranged from 26.94 to 59.23%. P₃₀ ranged from 26 to 83%. eGFR_{LAB}, eGFR_{CKD-EPI} and eGFR_{MDRD} all had poor performance characteristics that did not differ sub-

stantially from each other. CCC ranged from 0.41 to 0.49, CP_{10} ranged from 0.36 to 0.42, TDI_{90} ranged from 30.24 to 35.16, and P_{30} ranged from 70 to 80%. The equation that performed worst of the 3 was $eGFR_{CKD-EPI}$. The equations that rely on weight to calculate eGFR ($eGFR_{CG}$, $eGFR_{Björnsen}$, $eGFR_{Mawer}$ and $eGFR_{Hull}$) were the equations that overall performed best (all performance characteristics are summarized in table 1). Bland-Altman plots for $eGFR_{CKD-EPI}$, $eGFR_{MDRD}$, $eGFR_{CG}$ and $eGFR_{Effersø}$ all showed wide limits of agreement ranging from -39.01 to 45.45 with many points lying far from the mean line, which also indicated poor agreement between mGFR and eGFR (Figure 10A). In sensitivity analyses where we only included individuals that had an mGFR performed with the indication falling/lowered eGFR and non-IDUs we found results that did not differ substantially from the overall results (Figure 10B).

6.4.1.3 AGREEMENT BETWEEN MGFR AND EGFR ACCORDING TO DRUG EXPOSURE

Eight of 98 individuals were exposed to RLP/COB/ DTG at the time of serum-creatinine measurement. We were not able to find any statistically significant difference, between the exposed and unexposed individuals, of the agreement between mGFR and eGFR for any of the 10 eGFRs included; however, average mGFR was higher for RLP/COB/DTG exposed individuals (median 79.0 (IQR: 70.2–96.5) ml/min pr. 1.73 m^2) compared to the overall level of mGFR (median 66.0 (IQR: 54.8–82.5) ml/min pr. 1.73 m^2). Sixty-two of 98 individuals were exposed to TDF. TDF-exposed individuals on average had higher mGFR (median 73.5 (60.2–86.2) ml/min pr. 1.73 m^2) compared with the overall level of mGFR. Exposure to TDF was associated with a statistically significant difference in agreement for 2 of the equations that rely on weight ($eGFR_{Björnsen}$ and $eGFR_{Hull}$) and $eGFR_{Gates}$, evaluated with P_{30} . For $eGFR_{CG}$ that also relies on weight, the difference was only of borderline significance. For $eGFR_{Mawer}$, the final equation that relies on weight, we were not able to detect any differences. For these formulas, the agreement between mGFR and eGFR was better for individuals exposed to TDF than for individuals unexposed to TDF. Forty-five of 98 individuals were exposed to any PI. We were not able to find any differences in either P_{10} or P_{30} for individuals exposed to any PI compared to unexposed individuals (Tables 2).

6.4.1.4 INTER- AND INTRA-INDIVIDUAL REPRODUCIBILITY

We identified 14 individuals who had 2 or more mGFRs performed. The inter-individual difference was large with SDs ranging from 14.6 to 19, the intra-individual difference was far smaller than the inter-individual difference with SDs ranging from 7.49 to 9.9 (Figure 11), however even the intra-individual differences was substantial. For 7 of the individuals, the bias was almost the same for each measurement. For 6 of the individuals, the bias was substantially different from measurement to measurement; for the final individual, the bias was almost the same for most measurements except for 2 outliers.

6.4.2 DISCUSSION

The agreement between mGFR and eGFR was poor for all the evaluated equations, even measurements within the same individual showed substantial differences. The equation that showed the least discrepancy between mGFR and eGFR was $eGFR_{CG}$.

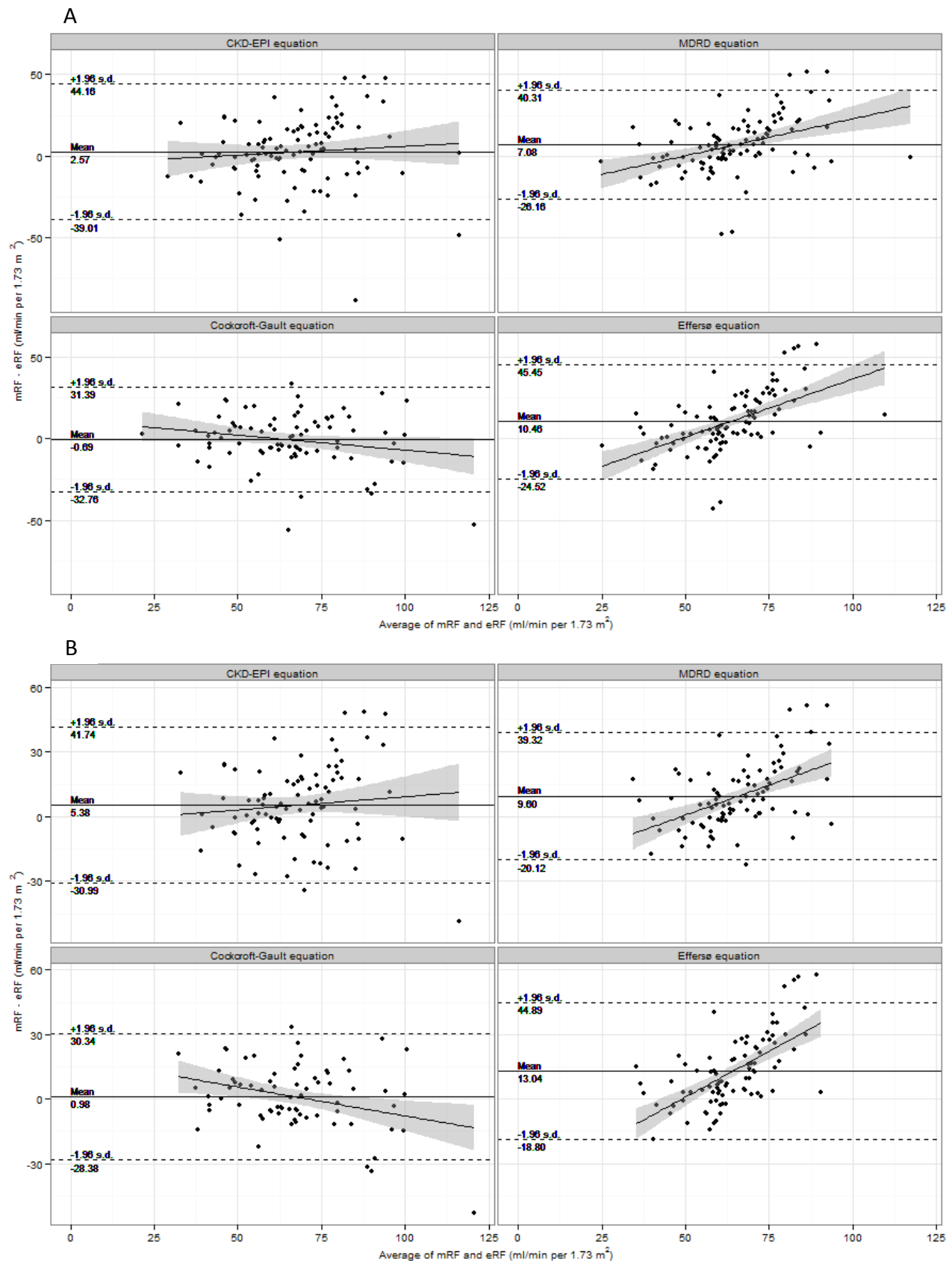


Figure 10: Bland Altman plots: difference between measured and estimated GFR vs. average of measured and estimated GFR for 4 renal function estimation equations. **(A)** All individuals included, **(B)** non-IDUs and individuals for which the indication was lowered/falling eGFR included.

Table 2 – Agreement between mGFR and eGFR for individuals exposed and individuals unexposed to 3 drug categories

	RLP/COB/DTG		TDF		PI	
	P10	P30	P10	P30	P10	P30
Department of Clinical Biochemistry*						
Exposed	2 (25.0)	7 (87.5)	22 (35.5)	49 (79.0)	17 (37.8)	32 (71.1)
Unexposed	34 (37.8)	65 (72.2)	14 (38.9)	23 (63.9)	19 (35.8)	40 (75.5)
P-value for difference between exposed and unexposed	0.706	0.677	0.829	0.154	1.000	0.653
CKD-EPI equation						
Exposed	1 (12.5)	6 (75.0)	20 (32.3)	48 (77.4)	15 (33.3)	33 (73.3)
Unexposed	28 (31.1)	63 (70.0)	9 (25.0)	21 (58.3)	14 (26.4)	36 (67.9)
p-value for difference between exposed and unexposed	0.430	1.000	0.498	0.066	0.510	0.659
MDRD equation						
Exposed	2 (25.0)	7 (87.5)	25 (40.3)	52 (83.9)	18 (40.0)	37 (82.2)
Unexposed	34 (37.8)	71 (78.9)	11 (30.6)	26 (72.2)	18 (34.0)	41 (77.4)
p-value for difference between exposed and unexposed	0.706	1.000	0.389	0.198	0.674	0.621
Cockcroft-Gault equation						
Exposed	2 (40.0)	4 (80.0)	20 (38.5)	45 (86.5)	14 (34.1)	32 (78.0)
Unexposed	26 (33.3)	62 (79.5)	8 (25.8)	21 (67.7)	14 (33.3)	34 (81.0)
p-value for difference between exposed and unexposed	1.000	1.000	0.337	0.052	1.000	0.791
Björnsson equation						
Exposed	3 (60.0)	4 (80.0)	22 (42.3)	47 (90.4)	10 (24.4)	34 (82.9)
Unexposed	26 (33.3)	65 (83.3)	7 (22.6)	22 (71.0)	19 (45.2)	35 (83.3)
p-value for difference between exposed and unexposed	0.337	1.000	0.096	0.033	0.065	1.000
Mawer equation						
Exposed	1 (20.0)	3 (60.0)	16 (30.8)	33 (63.5)	12 (29.3)	23 (56.1)
Unexposed	22 (28.2)	46 (59.0)	7 (22.6)	16 (51.6)	11 (26.2)	26 (61.9)
p-value for difference between exposed and unexposed	1.000	1.000	0.459	0.358	0.810	0.658
Hull equation						
Exposed	2 (40.0)	4 (80.0)	23 (44.2)	45 (86.5)	11 (26.8)	32 (78.0)
Unexposed	28 (35.9)	58 (74.4)	7 (22.6)	17 (54.8)	19 (45.2)	30 (71.4)
p-value for difference between exposed and unexposed	1.000	1.000	0.060	0.002	0.110	0.615
Gates equation						
Exposed	1 (12.5)	3 (37.5)	5 (8.1)	21 (33.9)	2 (4.4)	11 (24.4)
Unexposed	6 (6.7)	22 (24.4)	2 (5.6)	4 (11.1)	5 (9.4)	14 (26.4)
p-value for difference between exposed and unexposed	0.460	0.417	1.000	0.016	0.447	1.000
Jelliffe-1973 equation						
Exposed	2 (25.0)	7 (87.5)	10 (16.1)	45 (72.6)	10 (22.2)	30 (66.7)
Unexposed	16 (17.8)	59 (65.6)	8 (22.2)	21 (58.3)	8 (15.1)	36 (67.9)
p-value for difference between exposed and unexposed	0.637	0.267	0.589	0.182	0.437	1.000
Effersø equation						
Exposed	1 (12.5)	6 (75.0)	19 (30.6)	47 (75.8)	17 (37.8)	33 (73.3)
Unexposed	30 (33.3)	67 (74.4)	12 (33.3)	26 (72.2)	14 (26.4)	40 (75.5)
p-value for difference between exposed and unexposed	0.429	1.000	0.824	0.811	0.278	0.821

* During the study period The Department of Clinical Biochemistry calculated eGFR with the MDRD equation but without adjustment for race.

Abbreviations: RLP; rilpivirine, COB; cobicistat, DTG; dolutegravir, TDF, tenofovir disoproxil fumarate, P₁₀; Relative accuracy 10%, P₃₀; Relative accuracy 30%, MDRD; modification of diet in renal disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration.

Several studies have evaluated the agreement between mGFR and eGFR in HIV-infected individuals[32–36,89–91]. The studies overall find very differing degrees of agreement between mGFR and eGFR and generally there were large discrepancies between mGFR and eGFR, that is, the P_{30} of the best performing equations were around 80%. Recently Gagneux-Brunon et al. conducted a large European study with 203 included HIV-infected individuals[36]. They found values of P_{30} that ranged from 75-82 for the evaluated equations, which corresponded well with the results from the current study. However they also found that the CKD-EPI equation performed better than the MDRD equation, which we did not find. Tebogo et al. also found that the CKD-EPI equation performed better than the MDRD formula, and if the ethnicity-correction factor was not included performance increased, however they found that performance characteristics were generally worse than previous studies with P_{30} ranging from 41 - 78[91].

The small number of individuals who received treatment with RLP/COB/DTG did not allow us to establish whether exposure to these drugs was associated with poorer agreement between mGFR and eGFR. In contrast, we found a trend that TDF exposure was associated with a better agreement between mGFR and eGFR for exposed vs. unexposed individuals for the equations that rely on weight to calculate eGFR (Table 2). Sax et al. [92] studied the safety of tenofovir alafenamide (TAF), and found that $eGFR_{CG}$ decreased a median of 11 mL/min immediately after initiation of TDF; TAF was associated with a smaller decrease in $eGFR_{CG}$ of approximately 6 mL/min. The results from our study suggested that the effect on $eGFR_{CG}$ is a benign increase in creatinine and does not reflect a true decrease in renal function.

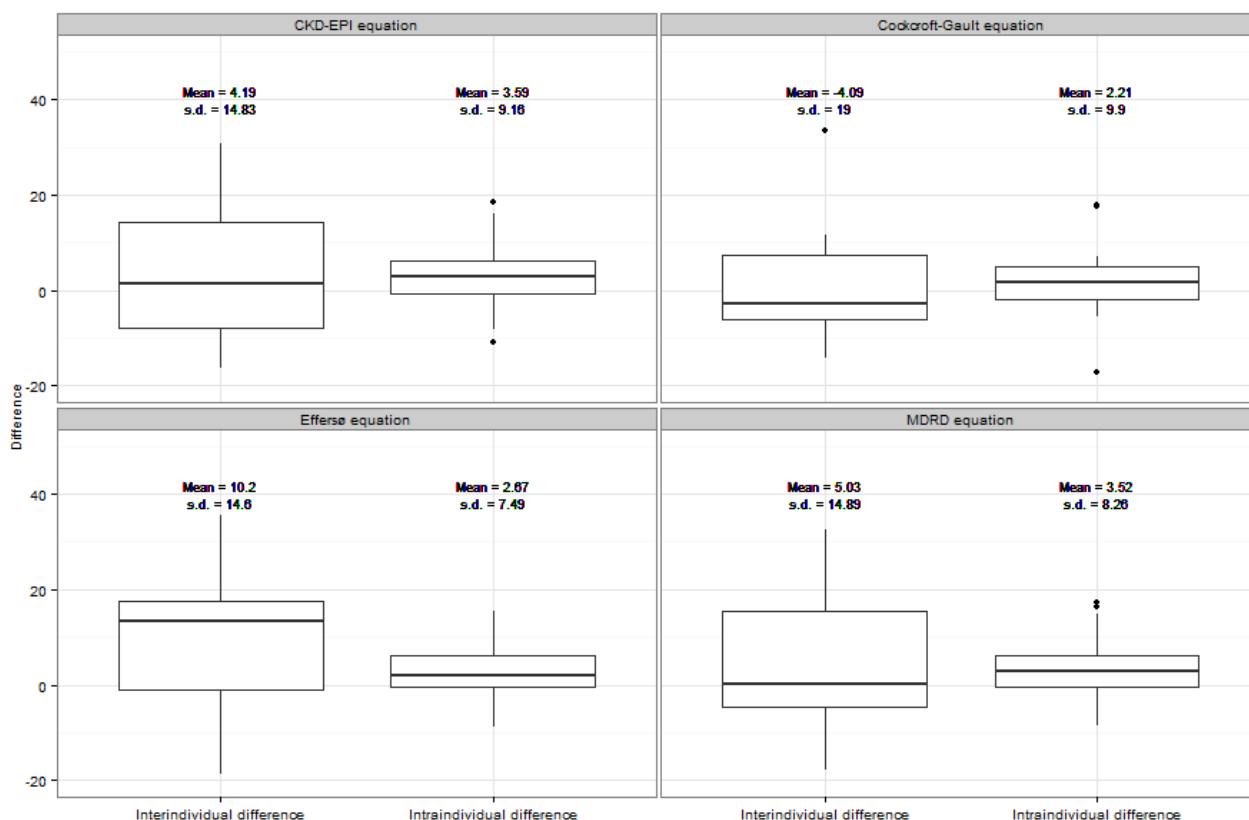


Figure 11: Inter- and intra-individual differences. The inter-individual difference is the bias (mGFR – eGFR) for the first measurement for each individual; the intra-individual difference is the difference between the bias for the first and the second measurement for each individual.

7. STRENGTHS AND LIMITATIONS

7.1 STRENGTHS

A general strength in all studies was the architecture of the Danish health care system with coupling of individuals to the PIN. This made it possible to link individuals in databases with almost 100% accuracy. In study 1 we used the DCRS which is based on the PIN to generate a well-matched population-based control cohort with long and almost complete follow-up. We made a great effort to extract data from well validated Danish databases, i.e. DNHR (studies 1, 2 and 3) and DNR (study 2). If this was not possible we used clinical databases which made it possible to make estimates from everyday clinical settings. Data was extracted from the same sources of all groups of individuals, which minimized the risk of differential misclassification and thereby a negligible impact on relative risk. Finally we used hard and undisputable endpoints, i.e. aRRT and cRRT to estimate renal complications, in addition to the vaguer endpoint CKD. We had a large number of individuals included with data on eGFR available and in study 4 we also had a relatively large number of individuals included with data on mGFR and also the ability to estimate the agreement of mGFR and eGFR over time in 14 individuals.

7.2 LIMITATIONS

There are some limitations. The number of individuals who developed cRRT was small, so we were not able to make robust analyses in subgroups regarding this endpoint in any of our studies. In study 2 we only had data on smoking available for about three quarters of Danish HIV-infected individuals. Serum-creatinine was measured with different testing methods throughout the study period. Furthermore they were measured at different sites, which is known to increase the inter-measurement variability[37]. Implementation of standardization of serum-creatinine measurements was introduced at varying time points at Danish hospitals. In order to minimize the risk of bias because of differences in standardization we chose to only include data after 1 January 2004 (date of implementation of standardization at Rigshospitalet) in Study 4. In study 4 the number of individuals who had received RLP/COB/DTG did not make it possible to determine whether exposure to any of these drugs impacted the agreement between mGFR and eGFR. In study 4 we did not have weight available for 15 of the individuals. In these cases we were not able to calculate eGFR with equations that rely on weight; this could pose a bias in regards to the analyses of these equations. However we did sensitivity analyses where we excluded individuals with no weight available from all analyses which did not change our conclusions substantially.

8. CONCLUSION AND PERSPECTIVES

8.1 CONCLUSION

In the current thesis we have focused on estimation of renal function and renal complications in HIV-infected individuals. We were able to make several conclusions:

- HIV-infected individuals are at increased risk of aRRT and cRRT.
- The increased risk is caused in part by 1) high risk groups within the HIV infected population, i.e. IDUs, 2) severe illness during the initial phase of HIV-infection.
- Smoking is not a major risk factor of CKD and aRRT in HIV-infected individuals. However smoking is associated with increased mortality in individuals that commence aRRT.
- The usefulness of routine UP/Cr is of limited use in well-treated HIV-infected individuals.
- Renal function cannot be accurately assessed with creatinine based estimation of renal function.

8.2 PERSPECTIVES

- The reason for the increased risk of CKD, aRRT and cRRT in HIV-infected individuals is not fully understood. Future studies should try to address this subject. A recent large cohort study by Sumida et al. performed in the context of the United States Veterans Cohort found a statistically significant, but weak (HR 1.09 (95% CI: 1.01-1.18)), association between constipation and CKD/cRRT[93]. In the article they argue that the association could be caused by chronic inflammation generated by microbial translocation in the gut. The association was weak and therefore difficult to find HIV-infected individuals, which tend to far smaller populations than populations of the general population; however microbial translocation has been shown to be a major driver of chronic inflammation and morbidity in HIV-infected individuals, and as such could prove to be of importance[94].
- To our knowledge no studies have investigated the influence of co-morbidities on the risk of CKD/aRRT/cRRT in HIV-infected individuals compared with comparable individuals from the background population, which could also be of interest.
- Screening of individuals should only be done if it benefits the individuals that are screened. In the case of UP/Cr screening of well-treated HIV-infected individuals this was not the case. Future studies should elucidate the beneficial effects of UP/Cr screening in larger cohorts.
- Equations to better estimate renal function in HIV-infected individuals with biomarkers are warranted. Future studies should address this issue in both the background population and in populations of HIV-infected individuals.

9. Summary

Since 1984, HIV has been the subject of major research activity. Early research focused on treating the infections that were developed due to failure of the immune system. In the middle of the 1990's, combination antiretroviral therapy (cART) was introduced which significantly improved prognosis for HIV-infected individuals. However, HIV-infected individuals still has increased mortality compared to the background population. This mortality is probably the result of an interaction between social/ genetic factors and HIV-related factors.

In recent years there has been a lot of focus on renal function in HIV-infected individuals. Partly because renal diseases that specifically develop in HIV-infected individuals long has been known, but also because some of the antiretroviral drugs used in cART regimens have been shown to cause renal complications. The purpose of this PhD study was therefore:

- To estimate the risk of dialysis and end stage renal disease (ESRD) in HIV-infected individuals and compare this to the general population. As well as assess whether exposure to specific antiretroviral drugs was associated with increased risk of dialysis or ESRD (Study 1; Rasch et al., 2014).
- To evaluate the effect of smoking on general renal function, risk of chronic renal failure, risk of dialysis and mortality after dialysis in HIV-infected individuals (Study 2, Ahlström et al., 2015).
- To evaluate the usefulness of routine screening for chronic renal impairment in HIV-infected subjects with urinary protein/creatinine ratio (UP/Cr) (Study 3, Ahlström et al., 2016).
- To evaluate the agreement between measured renal function (mGFR) and estimated renal function (eGFR) in an everyday clinical setting. To assess whether exposure to antiretroviral drugs that elicits a benign increase in serum-creatinine was associated with a poorer agreement between mGFR and eGFR and to assess whether the intra-individual agreement between mGFR and eGFR is better than inter-individual agreement between mGFR and eGFR (Study 4, Ahlström et al., 2017).

Study 1 showed that HIV-infected individuals are at increased risk of both dialysis and ESRD compared to the background population. It was not possible to find an association between exposure to specific antiretroviral drugs and the risk of dialysis or ESRD.

Study 2 showed that smoking in HIV-infected individuals is not associated with increased risk of CKD or dialysis, but smokers were had increased mortality Increased mortality after initiation of dialyses.

Study 3 showed that the usefulness of routine screening for chronic renal impairment in well-treated HIV-infected individuals with UP/Cr is poor.

Study 4 showed that the agreement between mGFR and eGFR is poor in HIV-infected individuals in an everyday clinical setting. This cannot be explained by changes in the use of antiretroviral drugs. The agreement between measurements was significantly better when evaluated as intra-individuals agreement compared to inter-individuals agreement; however there was still a substantial discrepancy.

The studies in this thesis have helped to first of all determine the extent of the problem regarding renal diseases in HIV-infected individuals. In addition, some important questions regarding the management of HIV-infected individuals have been answered. A broad screening of HIV-infected individuals should not be a focus area, but physicians should focus more on specific risk groups, e.g. IDUs and individuals who are given potentially nephrotoxic drugs. In addition, we have shown that new methods to estimate renal function are needed, as the methods available so far are insufficient.

10. Dansk resume

HIV har siden det blev opdaget i 1984 været genstand for stor forskningsmæssig aktivitet. Den tidlige forskning fokuserede overvejende på at behandle de infektioner, som udvikledes på baggrund af det svigtende immunforsvar. I midten af 1990'erne indførtes 'combination antiretroviral therapy' (cART) som markant bedre prognosen for HIV-inficerede individer. HIV-inficerede individer har dog stadig en overdødelighed sammenlignet med baggrundsbefolkningen. Denne overdødelighed er formentlig resultatet af et samspil mellem sociale-/genetiske faktorer og HIV relaterede faktorer.

Der har de senere år været meget fokus på nyrefunktionen hos HIV-inficerede individer. Dels fordi man længe har kendt til specifikke nyresygdomme som udvikles hos HIV-inficerede individer, men også fordi nogle af de stoffer man bruger som led i cART behandlingen har vist sig at kunne give nyrekomplikationer. Formålet med dette PhD studium var:

- At estimere risikoen for dialyse og terminalt nyresvigt hos HIV-inficerede individer og sammenligne dette med den generelle befolkning. Samt at vurdere om eksponering for specifikke antiretrovirale stoffer var forbundet med øget risiko for dialyse eller terminalt nyresvigt (Studie 1; Rasch et al. 2014).
- At estimere effekten af rygning på den generelle nyrefunktion, risikoen for kronisk nyrepåvirkning, risikoen for dialyse og dødelighed efter dialyse hos HIV-inficerede individer (Studie 2; Ahlström et al., 2015).
- At evaluere anvendeligheden af rutinemæssig screening for kronisk nyrepåvirkning hos HIV-inficerede individer med urin protein/kreatinin ratio (Studie 3; Ahlström et al., 2016).
- At vurdere overensstemmelse mellem målt nyrefunktion og estimeret nyrefunktion i en klinisk hverdag. At vurdere om eksponering for antiretrovirale stoffer, der fremkalder en godartet stigning i serum-kreatinin, var forbundet med en dårligere overensstemmelse mellem målt nyrefunktion og estimeret nyrefunktion og vurdere om overensstemmelsen mellem målt og estimeret nyrefunktion er bedre hos det enkelte individ i forhold til hele gruppen af HIV-inficerede individer (Studie 4; Ahlström et al., 2017).

Studie 1 viste at HIV-inficerede individer er i øget risiko for både dialyse og terminalt nyresvigt sammenlignet med baggrundsbefolkningen. Det var ikke muligt at finde en association mellem eksponering for specifikke antiretrovirale stoffer og risikoen for dialyse eller terminalt nyresvigt.

Studie 2 viste at rygning hos HIV-inficerede individer ikke er associeret med overrisiko for kronisk nyrepåvirkning eller dialyse, men rygere er i højere risiko for at dø hvis de bliver så syge at de får brug for dialyse sammenlignet med ikke rygere.

Studie 3 viste at anvendeligheden af rutinemæssig screening for kronisk nyrepåvirkning hos velbehandlede HIV-inficerede individer med urin protein/kreatinin ratio er ringe.

Studie 4 viste at overensstemmelsen mellem målt nyrefunktion og estimeret nyrefunktion er dårlig hos HIV-inficerede individer i en almindelig klinisk hverdag. Dette kan ikke umiddelbart forklares ved ændringer i brugen af antiretrovirale stoffer. Overensstemmelsen mellem målinger var markant bedre hos de individer der havde fået målt nyrefunktionen flere gange, men selv målinger på det samme individ var behæftet med betydelig uoverensstemmelse.

Studierne i denne afhandling har været med til først og fremmest at fastslå omfanget af problemet vedrørende nyresygdomme hos HIV-inficerede individer. Derudover er nogle vigtige spørgsmål vedrørende håndteringen af HIV-inficerede individer blevet besvaret. En bred screening af HIV-inficerede individer bør ikke være et fokusområde, men der bør nok i højere grad fokuseres på risikogrupper, f.eks. stofmisbrugere og individer som får potentielt nyretoksiske stoffer. Derudover har vi vist at der er behov for nye måder at vurdere nyrefunktionen på, da de hidtil tilgængelige metoder ikke er adækvate.

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12. PAPER 1

Original Article

Increased risk of dialysis and end-stage renal disease among HIV patients in Denmark compared with the background population

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ABSTRACT

Background. HIV patients have increased risk of impaired renal function. We aimed to estimate the incidence of any renal replacement therapy (aRRT) and start of chronic renal replacement therapy (cRRT) among HIV patients compared with population controls.

Methods. In a nationwide, population-based cohort study we analysed incidence rates (IR), incidence rate ratios (IRR) and risk factors for aRRT and cRRT among HIV patients compared with an age- and gender-matched population control cohort using Poisson regression.

Results. We identified 5300 HIV patients and 53 000 population controls. The IRs per 10 000 person-years of aRRT and cRRT among HIV patients were 15.9 (95% CI: 12.5–20.1) and 4.4 (95% CI: 2.8–6.9), respectively. The IRR was 4.7 (95% CI: 3.5–6.2) for aRRT and 3.6 (95% CI: 2.2–6.0) for cRRT compared with population controls. Risk of aRRT was increased during the first year after HIV diagnosis [IRR 3.5 (95% CI: 1.5–8.1)], after a diagnosis of AIDS [IRR 2.3 (95% CI: 1.3–3.9)], in intravenous drug users [IRR 6.0 (95% CI: 2.9–12.2)] and in patients with hypertension [IRR 7.0 (95% CI: 3.7–13.2)]. Factors associated with increased risk of cRRT were hypertension [IRR 20 (95% CI: 6.8–61)] and baseline eGFR < 60 mL/min pr. 1.73 m² [IRR 7.8 (95% CI: 1.2–50)]. Exposure to tenofovir and/or atazanavir was not associated with risk of aRRT or cRRT.

Conclusions. The risk of aRRT is increased more than 4-fold and the risk of cRRT is increased more than 3-fold in HIV patients in Denmark compared with the background population. We found no association between exposure to tenofovir, atazanavir or the combination of the two and risk of aRRT or cRRT.

Keywords: Dialysis, ESRD, eGFR, renal replacement therapy, HIV, CKD

INTRODUCTION

After the introduction of highly active antiretroviral therapy (HAART), the mortality and morbidity of HIV patients have decreased dramatically and with aging of this patient population the incidence of comorbidity has increased [1, 2]. HIV patients lose kidney function faster than the background population [3, 4], but the mechanism of this decline is not fully understood. Exposure to antiretrovirals (ARVs) (indinavir, atazanavir and tenofovir) has been associated with deterioration of renal function [5–7]. Furthermore, HIV replicates in other cells than CD4 cells, e.g. renal epithelial cells [8]. This seems to be directly associated with HIV-associated nephropathy (HIVAN) [9].

Although the increased incidence of mild-to-moderate renal impairment in HIV patients is well documented, few studies have addressed the prevalence and incidence of renal replacement therapy (dialysis or renal transplantation)

[10, 11]. Only a few studies have evaluated ARVs as risk factors of renal replacement therapy [12].

In the present study, we aimed to assess the risk and risk factors for renal replacement therapy in the HIV population in Denmark compared with an HIV-negative control population. We evaluated risk of any renal replacement therapy (aRRT) including dialysis because of acute kidney failure and as a marker of end-stage renal disease we estimated risk of chronic renal replacement therapy (cRRT).

PATIENTS AND METHODS

In a nationwide, population-based cohort we evaluated risk factors for aRRT and cRRT among HIV patients compared with an age- and gender-matched cohort of population controls.

Setting

Denmark has a population of 5.5 million, with an estimated HIV prevalence of 0.09% among adults in January 2010. HIV patients are treated in eight specialized HIV care centres and are seen as outpatients at intended intervals of 12 weeks. A letter specifying a date for a new appointment reminds patients who miss a planned visit. Antiretroviral treatment is provided at the centres free of charge. The national criteria for initiating HAART have been described previously [13].

Data sources

The unique 10-digit personal identification number assigned to all Danish citizens at birth or immigration was used to avoid multiple registrations and to track individuals in the following registries.

The Danish HIV Cohort Study (DHCS) is a prospective study of all HIV patients 16 years or older at diagnosis, who were treated at Danish HIV centres after 1 January 1995. Patients are consecutively enrolled. Data are collected annually and include demographics, date of HIV diagnosis, AIDS-defining events, date and cause of death and ARV treatment. CD4 cell counts and viral loads (VL) are extracted electronically from laboratory data files. The study is described in detail elsewhere [13].

Date of cRRT was defined as the first date of chronic dialysis or renal transplantation as registered in the Danish Nephrology Registry (DNR). DNR contains data on all Danish patients who have received chronic dialysis or renal transplantation, and the underlying causes [categorized according to International Classification of Diseases 10th revision (ICD-10)], after 1 January 1990. Chronic dialysis was defined as dialysis during a minimum of 3 months for a minimum of 12 times. More than 99% of patients in Denmark with cRRT are included in DNR [14, 15].

For both HIV patients and controls diagnoses of dialysis (ICD-10: BJFD00-BJFD27), diabetes [categorized according to ICD-10 as Type 1, Type 2 and other diabetes (ICD10: DE10.0-DE14.9)] and hypertension (ICD10: DI10-DI5.9) were extracted from the Danish National Hospital Registry (DNHR) [16].

Data on vital status, residency and migration were extracted from the Danish Civil Registration System (DCRS) which were established in 1968 and stores information on all Danish residents.

Serum creatinine measurements were extracted from electronic laboratory databases. All serum creatinines were standardized according to the Jaffe method as described by the local laboratories (data not published). We calculated eGFR using the modification of diet in renal disease (MDRD) formula [17]. Baseline eGFR was defined as the eGFR closest to—and within 6 months of—index date (defined below). In analyses only including time after start of HAART, baseline eGFR was calculated as the eGFR closest to initiation of HAART.

Study population

We included all HIV patients who had a Danish person identification number and were aged ≥ 16 years at HIV diagnosis. The index date was defined as 1 January 1995, the date of HIV diagnosis or date of immigration whichever came last. Patients with a diagnosis of aRRT or cRRT before index date were excluded from the analyses.

Population control cohort. From DCRS, we identified 10 population controls for each HIV patient matched on date of birth and gender. The population controls were assigned the same index date as the HIV patient, to whom they were matched.

Study outcomes

Outcomes were (i) time to first date of aRRT and (ii) time to cRRT. cRRT was defined as described above and aRRT was defined as the first date an individual was registered with dialysis in DNHR (with ICD codes as stated above) or cRRT, and thereby includes patients who receive RRT because of acute kidney failure. As a consequence, all patients with cRRT will by definition have an outcome in the analyses of time to aRRT.

Statistics

Time was calculated from the index date to 1 January 2010, emigration, death, loss to follow-up or first date of aRRT/cRRT whichever occurred first.

Because death and emigration can be considered events competing with the events of interest (aRRT and cRRT), we used Cumulative Incidence Functions to construct survival curves for aRRT and cRRT (taking into account these competing risks) and Poisson regression analyses to estimate incidence rate ratios (IRR) of aRRT and cRRT among HIV patients compared with controls. Separate analyses were conducted for aRRT and cRRT. Further, we analysed risk factors for aRRT and cRRT among HIV patients using Poisson regression. The following variables were included in the model: route of infection [men who have sex with men (MSM), intravenous drug use (IDU), heterosexually infected or other], date of HIV diagnosis (before versus after 1 January 1997, reflecting the pre- and post HAART era), hepatitis C co-infection in non-IDUs (defined as positive hepatitis C antibodies or positive PCR for hepatitis C RNA in non-IDU

patients), CD4 cell count at baseline (<200 versus ≥ 200 cells/ μL), viral load at baseline (copies/mL) (<5000, 5000–100 000 and >100 000), baseline eGFR (≥ 90 , 60–90 and <60 mL/min pr. 1.73 m^2), race (Caucasian, African or other) and gender. The following were included as time-updated variables: HAART exposure, diabetes (yes/no), hypertension (yes/no) and AIDS (yes/no), after the date 1 year after diagnosis, age (5-year intervals) and year after index date (5-year intervals). The African population was categorized as West/Central African, East African or unspecified.

We analysed the association between treatment with tenofovir, atazanavir and the combination tenofovir/atazanavir and risk of first dialyses by including exposure of the ARVs as time-updated variables in the Poisson regression model (in three separate analyses). Exposure to the specific drugs was calculated from date of initiation until 1 year after discontinuation. In this analysis, time on tenofovir or atazanavir was stopped once the combination tenofovir/atazanavir was started.

We performed a sensitivity analysis in which only time after 1 January 2000 was included as no tenofovir was used in Denmark before that date. Statistical analyses were performed using SPSS for Windows (Norusis; SPSS Inc., Chicago, IL, version 13.0) and STATA (Stata Corporation, College Station, TX, version 11.0).

RESULTS

We identified 5300 HIV patients and 53 000 population controls. The African population accounted for 13% of the HIV population and the majority of the African HIV population were from East Africa (69%), 24% were from Western and Central regions and 7% were from unknown or other regions. Characteristics of the study population are summarized in Table 1.

Among HIV patients, there were 68 cases of aRRT during 42 833 person years of follow-up (PY) [IR 15.9/10 000 PY (95% CI: 12.5–20.1)]. Among population controls, there were 182 cases of aRRT during 534 282 PY [IR 3.4/10 000 PY (95% CI: 2.9–3.9)]. The IRR of aRRT was 4.7 (95% CI: 3.5–6.2) among HIV patients compared with the population control cohort (Figure 1a). In two sensitivity analyses, we included only (i) individuals diagnosed after 1 January 1995 [IRR 5.0 (95% CI: 3.3–7.4)] and (ii) non-IDUs and non-Africans [IRR 3.5 (95% CI: 2.5–4.8)]. The incidence of aRRT was highest during the first year after HIV diagnosis [IR 24.8/10 000 PY (95% CI: 12.4–49.5)] compared with the period >1 year after HIV diagnosis [IR 15.2/10 000 PY (95% CI: 11.8–19.5)].

Of the 68 cases of aRRT among HIV patients, 19 (28%) progressed to cRRT during 42 879 PY [IR 4.4/10 000 PY (95%

Table 1. Characteristics of the study population

	HIV patients (aRRT/cRRT) ^a	Controls (aRRT/cRRT) ^a
Number	5300	53 000
Number of events (%)	68 (1.3)/19 (0.4)	182 (0.3)/66 (0.1)
Follow-up, median (IQR), years	8.0 (3.1–13.7)/8.0 (3.1–13.8)	11.2 (5.8–15.0)/11.2 (5.8–15.0)
Lost to follow-up (%)	22 (0.4)	98 (0.2)
Emigrated during follow-up (%)	219 (4.1)	2037 (3.8)
Died during study period (%)	1338 (25.2)	2454 (4.7)
Age at study inclusion, median (IQR), years	37 (30–44)	37 (30–44)
Male (%)	4036 (76.2)	40 360 (76.2)
Race		
Caucasian	4167 (78.6)	–
African	683 (12.9)	–
Other/unknown	450 (6.7)	–
Route of infection		
MSM (%)	2407 (45.5)	–
IDU (%)	563 (10.6)	–
Heterosexually (%)	1928 (36.4)	–
Others (%)	402 (5.6)	–
HIV diagnosis after 1 January 1997	2486 (46.9)	–
Diabetes during study period (%)	131 (2.5)/132 (2.5)	1303 (2.5)/1309 (2.4)
Type 1 (%)	36 (0.7)	230 (0.4)/232 (0.5)
Type 2 (%)	80 (1.5)	975 (1.8)/979 (1.8)
Other (%)	15 (0.3)/16 (0.3)	117 (0.2)/117 (0.2)
Hypertension during study period (%)	219 (4.1)/220 (4.1)	2557 (4.8)/2574 (5.0)
Hepatitis C co-infection (non-IDU)	354 (6.7)	–
AIDS diagnosis during study period (%)	788 (14.8)/790 (14.9)	–
CD4 cell count at baseline (IQR)	290 (120–480)	–
eGFR baseline (mL/min pr. 1.73 m^2)		
>90 (%)	1776 (33.5)	–
60–90 (%)	1177 (22.2)	–
≤60 (%)	69 (1.3)	–
Missing (%)	2278 (43)	–

aRRT, any renal replacement therapy; cRRT, start of chronic renal replacement therapy; IQR, interquartile range; MSM, men who have sex with men; IDU, intravenous drug users; eGFR, estimated glomerular filtration rate.

^aPresented if there is a difference between aRRT and cRRT.

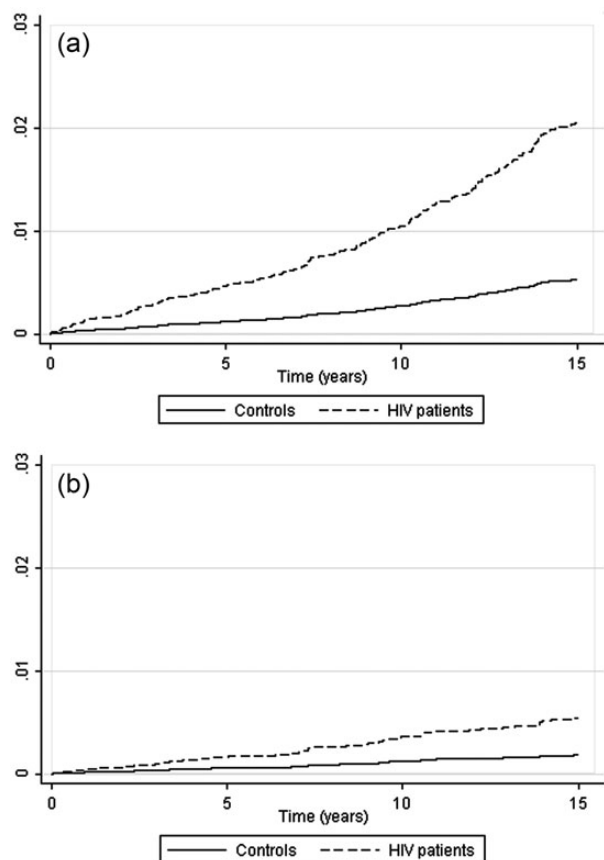


FIGURE 1: (a) Time to any renal replacement therapy (aRRT). (b) Time to chronic renal replacement therapy (cRRT).

CI: 2.8–6.9)], none of these patients had received renal transplantation (for underlying causes of cRRT, see Supplementary data, Table S1). Of the 182 cases of aRRT among controls, 66 (36%) progressed to cRRT during 534 476 PY [IR 1.2/10 000 PY (95% CI: 1.0–1.6) pr. 10 000 PY], IRR 3.6 (95% CI: 2.2–6.0) (Figure 1b).

Factors associated with increased risk of aRRT were, age, IDU, hypertension and AIDS. HAART exposure, hepatitis C co-infection in non-IDU patients and baseline eGFR <60 mL/min pr. 1.73 m² were also associated with the increased risk of aRRT, although associations did not reach the statistical significance (Table 2).

Factors associated with the increased risk of cRRT were hypertension and baseline eGFR <60 mL/min pr. 1.73 m² (Table 2).

Neither tenofovir, atazanavir nor their combination was associated with increased risk of aRRT (Table 3). A sensitivity analysis including only time after 1 January 2000 did not change the estimates substantially.

DISCUSSION

In this Danish, nationwide, population-based cohort study we found that the risk of aRRT was increased more than 4-fold and the risk of cRRT was increased more than 3-fold in HIV patients compared with the background population. The risk of aRRT was highest the first year after HIV diagnosis. Factors

associated with increased risk of aRRT were IDU, hypertension and AIDS-defining illness. Risk factors for cRRT were hypertension and baseline eGFR. We found no association between tenofovir, atazanavir or the combination atazanavir/tenofovir and risk of aRRT or cRRT.

We found an overall incidence of cRRT of 4.4 pr. 10 000 PY, which is comparable with the findings in a large German cohort study who found an incidence of 5.2 pr. 10 000 PY [10]. A study performed in a US veteran cohort by Choi *et al.* [11] found an incidence of 39.1 pr. 10 000 PY, which is almost 10 times higher than in our study. However, the latter study included a much larger proportion of both diabetics and Afro-Americans than our study (2.4 versus 14.4% and 12.9 versus 53.6%, respectively), and the study subjects were older (median age 49 versus 37 years) which may explain some of the difference. No patients in the study population received renal transplantation; however, during the study period two Danish HIV patients were registered with transplantation, these were excluded from the analysis because they had a diagnosis of aRRT prior to HIV diagnosis.

HIV-infected individuals have a faster decline in renal function than the background population, but estimates of the magnitude of this difference varies [3, 4, 18]. The increased risk of impaired renal function may both be related to a direct effect of the HIV infection and to toxic effects of the ARVs. HIV itself can cause impaired renal function as HIVAN, HIV-associated thrombotic microangiopathy and HIV-associated immunomediated glomerulonephritis [19, 20] and in these cases HAART can improve renal function. Tenofovir and atazanavir have been associated with increased risk of impaired renal function [3, 21, 22]. This effect seems to be reversible when the drugs are discontinued [23]. We found that AIDS-defining illness, but not baseline CD4<200 cells/μL was associated with risk of aRRT. It is possible that the increased risk of aRRT is caused by hypotension, dehydration or intensive care treatment during the acute phase of some AIDS-defining diseases.

Tenofovir was introduced in 2001 and the first case report of acute renal failure associated with exposure to tenofovir was published in December 2002 [24]. Several studies have shown an association between tenofovir exposure and decline in eGFR and risk of chronic kidney disease [25–27]. It is difficult to assess the risk of adverse events associated with specific ARVs in observational studies due to the risk of confounding by indication. In the present study, we found no indication of association between tenofovir and risk of aRRT or cRRT. In our analyses, time of exposure to tenofovir, atazanavir and the combination tenofovir/atazanavir was calculated from date of first exposure of the drug to the date 1 year after discontinuation. The ‘tail’ of 1 year was included since a previous study showed that the effect of drug exposure wanes and is no longer significant 1 year after discontinuation, suggesting that the nephrotoxic effect is reversible [23]. The lack of association between tenofovir and aRRT/cRRT in the present study may be due to the fact that clinicians, knowing the nephrotoxic effects of tenofovir, discontinued the drug in patients who developed mild-to-moderate impairment in renal function and thereby prevented further decrease in renal function and descent to renal failure.

Table 2. Risk factors for aRRT–Poisson regression analyses and cRRT–Poisson regression analyses

	Events	IR pr. 10 000 PY (95% CI)	Unadjusted IRR (95% CI)	Adjusted IRR ^a (95% CI)
aRRT				
HIV patients compared to controls				
Controls	182	3.4 (2.9–3.9)	Ref.	–
HIV patients	68	16 (13–20)	4.7 (3.5–6.2)	–
Only including the HIV patients				
Female gender	14	13 (7.6–22)	0.8 (0.4–1.4)	0.8 (0.4–1.5)
Age (pr. 5 year interval)	–	–	1.07 (1.04–1.09)	1.06 (1.03–1.08)
Race				
Caucasian	57	17 (13–22)	Ref.	Ref.
African	6	11 (4.9–24)	0.7 (0.3–1.5)	1.2 (0.4–3.0)
Other	5	16 (6.8–39)	1.0 (0.4–2.5)	1.4 (0.6–3.7)
HAART exposed—time updated	59	20 (16–26)	3.0 (1.5–6.0)	2.0 (0.9–4.5)
Route of infection				
MSM	23	12 (7.7–17)	Ref.	Ref.
IDU	15	34 (20–56)	2.9 (1.5–5.6)	5.3 (2.6–11.0)
Heterosexual	23	15 (9.6–22)	1.3 (0.7–2.2)	1.4 (0.7–2.8)
Other	7	27 (13–56)	2.3 (1.0–5.3)	1.6 (0.6–4.0)
Time of diagnosis				
After 1 January 1997	27	16 (11–23)	Ref.	Ref.
Before 1 January 1997	41	16 (12–22)	1.0 (0.6–1.6)	0.8 (0.4–1.9)
Comorbidity				
Diabetes—time updated	7	73 (35–153)	5.0 (2.3–10.9)	1.2 (0.5–3.0)
Hypertension—time updated	16	151 (83–247)	12.2 (7.0–21.3)	6.7 (3.5–13.0)
Hepatitis C co-infection (non-IDU)	7	22 (11–46)	1.4 (0.7–3.1)	2.1 (0.9–4.8)
AIDS—time updated	23	34 (22–51)	2.7 (1.6–4.5)	2.2 (1.3–3.8)
CD4 cell count baseline (cells/ μ L)				
>200	15	20 (11–33)	Ref.	Ref.
<200	33	15 (11–22)	1.4 (0.7–2.7)	0.6 (0.3–1.4)
Viral load at baseline (copies/mL)				
<5000	1	4 (0.6–31)	Ref.	Ref.
5000–100 000	9	15 (8–28)	3.3 (0.4–26.2)	2.5 (0.3–19.8)
>100 000	12	22 (13–39)	5.0 (0.6–38.4)	2.9 (0.4–23.4)
Baseline eGFR (mL/min pr. 1.73 m ²)				
>90	25	18 (12–26)	Ref.	Ref.
60–90	18	18 (11–28)	1.0 (0.6–1.9)	0.8 (0.4–1.6)
<60	3	94 (30–293)	5.4 (1.6–17.8)	2.0 (0.5–7.5)
First year after HIV diagnosis	8	25 (12–50)	1.6 (0.8–3.4)	3.4 (1.5–8.1)
cRRT				
HIV patients compared to controls				
Controls	66	1.2 (1.0–1.6)	Ref.	–
HIV patients	19	4.4 (2.8–6.9)	3.59 (2.15–5.98)	–
Only including the HIV patients				
Female gender	3	2.8 (0.9–8.6)	0.6 (0.2–1.9)	0.3 (0.1–2.4)
Age (pr. 5 year interval)	–	–	1.06 (1.02–1.10)	1.03 (0.97–1.08)
Race				
Caucasian	14	4.1 (2.4–6.9)	Ref.	Ref.
African	3	5.4 (1.8–17)	1.3 (0.4–4.6)	2.8 (0.6–13)
Other	2	6.5 (1.6–26)	1.6 (0.4–7.1)	3.4 (0.7–17)
HAART exposed—time updated	15	5.1 (3.1–8.4)	1.7 (0.6–5.1)	1.1 (0.3–3.9)
Route of infection				
MSM	8	4.0 (2.0–8.0)	Ref.	Ref.
IDU	2	4.5 (1.1–18)	1.1 (0.2–5.3)	2.0 (0.4–11)
Heterosexual	8	5.0 (2.5–10)	1.3 (0.5–3.3)	1.3 (0.4–4.4)
Other	1	3.8 (0.5–27)	0.9 (0.1–7.5)	0.7 (0.1–6.4)
Time of diagnosis				
After 1 January 1997	7	4.1 (2.0–8.7)	Ref.	Ref.
Before 1 January 1997	12	4.6 (2.6–8.1)	1.1 (0.4–2.8)	1.5 (0.3–7.4)
Comorbidity				
Diabetes—time updated	3	31 (10–96)	8.1 (2.4–28)	1.6 (0.4–6.7)
Hypertension—time updated	8	75.0 (38–150)	29 (12–71)	19 (6.2–56)

Continued

Table 2. Continued

	Events	IR pr. 10 000 PY (95% CI)	Unadjusted IRR (95% CI)	Adjusted IRR ^a (95% CI)
Hepatitis C co-infection (non-IDU)	2	6.3 (1.6–25)	1.5 (0.3–6.3)	2.2 (0.5–11)
AIDS—time updated	5	7.3 (3.0–18)	1.9 (0.7–5.2)	1.6 (0.5–5.0)
CD4 cell count baseline (cells/ μ L)				
>200	5	3.6 (1.5–8.7)	Ref.	Ref.
<200	4	5.3 (2.0–14)	1.5 (0.4–5.4)	0.7 (0.2–3.3)
Viral load at baseline (copies/mL)				
<5000	0	–	Ref.	Ref.
5000–100 000	3	4.9 (1.6–15)	–	–
>100 000	3	5.5 (1.8–17)	–	–
Baseline eGFR (mL/min pr. 1.73 m ²)				
>90	6	4.2 (1.9–9.4)	Ref.	Ref.
60–90	7	7.0 (3.3–14)	1.7 (0.6–4.9)	1.3 (0.4–4.3)
<60	2	61 (15–24)	14 (2.9–72)	7.8 (1.2–50)
First year after HIV diagnosis	1	3.1 (0.5–22)	0.7 (0.1–5.1)	0.8 (0.1–6.5)

aRRT, any renal replacement therapy; cRRT, chronic renal replacement therapy; IR, incidence rate; PY, person years at risk; IRR, incidence risk ratio; MSM, men who have sex with men; IDU, intravenous drug users; eGFR, estimated glomerular filtration rate.

^aAdjusted for: HAART, route of infection, time of diagnosis, first year after diagnosis, diabetes, hypertension, hepatitis C co-infection, AIDS, CD4 count at baseline, baseline eGFR, age, year after index date, race and gender.

Table 3. Associations between tenofovir and atazanavir and risk of aRRT

	Events	PY	IR pr. 10 000 PY (95% CI)	Unadjusted IRR ^a (95% CI)	Adjusted IRR ^{a,b} (95% CI)
Including only time after initiation of HAART					
HAART exposed but not tenofovir/atazanavir exposed	46	23 674	19.4 (14.6–25.9)	Ref.	Ref.
Tenofovir exposed	5	3181	15.7 (6.54–37.8)	0.77 (0.31–1.91)	0.63 (0.25–1.59)
Atazanavir exposed	5	1520	32.9 (13.7–79.0)	1.70 (0.68–4.26)	1.67 (0.65–4.29)
Atazanavir/tenofovir exposed	3	1111	27.0 (8.71–83.8)	1.37 (0.43–4.37)	1.10 (0.34–3.60)
Including only time after 1 January 2000					
HAART exposed but not tenofovir/atazanavir exposed	42	19 613	21.4 (15.8–29.0)	Ref.	Ref.
Tenofovir exposed	5	3181	15.7 (6.54–37.8)	0.70 (0.27–1.75)	0.68 (0.27–1.73) ^c
Atazanavir exposed	5	1520	32.9 (13.7–79.0)	1.57 (0.63–3.94)	1.50 (0.58–3.78) ^c
Atazanavir/tenofovir exposed	3	1111	27.0 (8.71–83.8)	1.26 (0.39–4.04)	1.07 (0.33–3.50) ^c

PY, person-years at risk; IR, incidence rate; CI, confidence interval; IRR, incidence rate ratio; eGFR, estimated glomerular filtration rate.

^aThe Poisson regression model.

^bAdjusted for route of infection, year of diagnosis, diabetes, hypertension, hepatitis C co-infection, AIDS, CD4 count at baseline, eGFR at initiation of HAART, age, 1 year after diagnosis, year after index date, race and gender.

^cAdjusted for eGFR at initiation of tenofovir rather than at start of HAART.

Though previous studies have shown increased risk of HIVAN in African HIV-infected individuals, we found no association between African race and the risk of aRRT or cRRT. The increased risk of end-stage renal disease in African populations has been shown to be associated with the Apol1 gene [28], which is more frequent in Western and Central regions of Africa [29]. The majority of the African population in the present study were from East Africa (~69%) and only ~24% were from Western and Central regions. This may to some extent explain why we did not find increased risk of either aRRT or cRRT among Africans. It is, however, also possible that the lack of association between race and risk of aRRT/cRRT in our analyses was explained by unmeasured confounders.

Major strengths of the study are the population-based, nationwide design. The use of DCRS, which assign a personal identification number to all Danish citizens and the well-organized structure of Danish national registers allowed us to generate a well-matched population-based control cohort with

long and almost complete follow-up. Also this system enabled us to extract data on study outcomes from two well-validated Danish databases, the DNHR [16, 30] and the DNR [14]. Data on study outcomes were extracted from the same data sources for both HIV patients and the control cohort, which decrease the risk of differential misclassification and thereby leads to a negligible impact on estimates of relative risks. Also our study did not use surrogate markers for impaired renal function but hard and indisputable end points as dialyses. There are some limitations in the study. The number of cRRT events was relatively small, which limits statistical power of analyses. We did not have information of race in the population control group, but analyses restricted to HIV patients of Danish origin yielded similar results. We were unable to assess socioeconomic and lifestyle-related factors, which may influence the risk of renal disease.

Our study has several clinical implications. It demonstrates that HIV patients have substantially increased risk of aRRT and cRRT, compared with an age- and gender-matched control

population, and are especially vulnerable to these conditions in the first year after HIV diagnosis. Important risk factors are, an AIDS-defining illness, being IDU and having hypertensive disease. Furthermore, it illustrates that in a setting with regularly monitoring of renal function, treatment with tenofovir or atazanavir or their combination does not seem to increase the risk of severe and irreversible renal disease.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://ndt.oxfordjournals.org>.

CONFLICT OF INTEREST STATEMENT

None declared.

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13. PAPER 2

Smoking and renal function in people living with human immunodeficiency virus: a Danish nationwide cohort study

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Introduction: Smoking is a main risk factor for morbidity and mortality in people living with human immunodeficiency virus (PLHIV), but its potential association with renal impairment remains to be established.

Methods: We did a nationwide population-based cohort study in Danish PLHIV to evaluate the association between smoking status and 1) overall renal function and risk of chronic kidney disease (CKD), 2) risk of any renal replacement therapy (aRRT), and 3) mortality following aRRT. We calculated estimated creatinine clearance using the Cockcroft–Gault equation (CG-CrCl), and evaluated renal function graphically. We calculated cumulative incidence of CKD (defined as two consecutive CG-CrCls of ≤ 60 mL/min, ≥ 3 months apart) and aRRT and used Cox regression models to calculate incidence rate ratios (IRRs) for risk of CKD, aRRT, and mortality rate ratios (MRRs) following aRRT.

Results: From the Danish HIV Cohort Study, we identified 1,475 never smokers, 768 previous smokers, and 2,272 current smokers. During study period, we observed no association of smoking status with overall renal function. Previous and current smoking was not associated with increased risk of CKD (adjusted IRR: 1.1, 95% confidence interval [CI]: 0.7–1.7; adjusted IRR: 1.3, 95% CI: 0.9–1.8) or aRRT (adjusted IRR: 0.8, 95% CI: 0.4–1.7; adjusted IRR: 0.9, 95% CI: 0.5–1.7). Mortality following aRRT was high in PLHIV and increased in smokers vs never smokers (adjusted MRR: 3.8, 95% CI: 1.3–11.2).

Conclusion: In Danish PLHIV, we observed no strong association between smoking status and renal function, risk of CKD, or risk of aRRT, but mortality was increased in smokers following aRRT.

Keywords: chronic kidney disease, renal replacement therapy, mortality, creatinine clearance, incidence rate ratio, mortality rate ratio

Introduction

Since the introduction of highly active antiretroviral therapy (HAART), the natural course of human immunodeficiency virus (HIV) infection has changed dramatically from being invariably fatal to a chronic disease with an overall favorable prognosis. However, people living with HIV (PLHIV) still suffer from a significantly increased all-cause mortality compared to the background population.¹

PLHIV have a faster decline in renal function and a higher risk of any renal replacement therapy (aRRT) and end-stage renal disease (ESRD) compared to the background population, but the mechanisms of the deterioration in renal function are not fully understood.^{2–4} Exposure to antiretrovirals (eg, tenofovir and atazanavir) has been associated with deterioration of renal function.^{5–7} Furthermore, HIV has been found to replicate in other cells than CD4 cells, for example, renal epithelial cells.⁸ This seems

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to be directly associated with HIV-associated nephropathy seen mainly in PLHIV of black-African origin.⁹

Recent studies indicate that lifestyle-related factors (eg, smoking and other substance abuse) are more important than HIV-related factors as predictors of long-term survival among PLHIV.^{10–13}

We hypothesized that smoking is a risk factor for decline in renal function, chronic kidney disease (CKD), aRRT, and death following aRRT in PLHIV. We therefore assessed the association between smoking status and 1) renal function and CKD, 2) risk of aRRT, and 3) mortality following aRRT.

Methods

Study design

We performed a population-based cohort study divided into three parts in which we estimated the association of smoking with the following: 1) overall renal function, evaluated by estimated creatinine clearance calculated with the Cockcroft–Gault equation (CG–CrCl), and the risk of CKD (as defined in the section Categorization of smoking, renal function, CKD and CD4 cell count), 2) risk of aRRT, and 3) mortality following aRRT.

Setting

Denmark had a population of approximately 5.5 million people, and an estimated HIV prevalence of 0.1% among adults in December 2013.¹⁴ PLHIV are treated in eight specialized HIV care centers and are seen on an outpatient basis at intended intervals of 12–24 weeks. Antiretroviral treatment is provided free of charge. Management of PLHIV is well organized, and rates of treatment failure and loss to follow-up are low.^{15,16}

Data sources – registries

The unique ten-digit personal identification number assigned to all Danish residents at birth or immigration was used to track individuals in the following registries.

The Danish HIV Cohort Study (DHCS) is a prospective study of all PLHIV, 16 years or older at diagnosis, treated at Danish HIV centers after January 1, 1995. Individuals are consecutively enrolled, and data are annually updated and include demographics, date of HIV diagnosis, acquired immunodeficiency syndrome (AIDS)-defining events, and antiretroviral treatment. CD4 cell counts and viral loads are extracted electronically from laboratory data files. The study is described in detail elsewhere.^{17,18}

Data on vital status, residency, and migration were extracted from the Danish Civil Registration System (DCRS) which was established in 1968 and stores information on all Danish residents.¹⁹

From the Danish National Hospital Registry (DNHR), we identified dialysis according to the International Classification of Diseases tenth revision (ICD-10) as BJFD.xx, ZZ43.40–ZZ43.50 or International Classification of Diseases eighth revision (ICD-8) as 92390, 94399, 94340, and 94350. aRRT was defined as the first date of dialysis. With this definition of aRRT, we include both patients with chronic renal replacement therapy (cRRT) and patients who develop acute renal failure and require acute dialysis. We defined cRRT as dialysis for ≥ 3 months. Furthermore, we identified individuals with diagnoses of hypertension (ICD-10: D110–D115.9 and ICD-8: 40009–41499) and diagnoses of diabetes (ICD-10: DE10.0–DE14.9 and ICD-8: 24900–25009). DNHR is described in detail elsewhere.²⁰

Categorization of smoking, renal function, CKD, and CD4 cell count

Data on smoking were obtained by interview. Individuals were categorized as smokers if they smoked any type of tobacco at least once a week or if they categorized themselves as smokers. Individuals were categorized as never, previous, and current smokers according to their status at study inclusion and did not change category during study period.

Serum creatinines were extracted from electronic laboratory databases and included all serum creatinines measured in the period 1995–2013 during inpatient admittances and outpatient planned controls. All serum creatinines were standardized according to the Jaffe method as described by the local laboratories. As an estimate of glomerular filtration rate (GFR), we calculated CG–CrCl without correction of body surface area.^{21,22}

$$\text{CG – CrCl} = (140 - \text{age}) \times \text{weight (in kg)} \times \frac{1.04}{\text{serum – creatinine} \left(\text{in } \frac{\mu\text{mol}}{\text{L}} \right)} \text{ (if female)} \quad (1)$$

$$\text{CG – CrCl} = (140 - \text{age}) \times \text{weight (in kg)} \times \frac{1.23}{\text{serum – creatinine} \left(\text{in } \frac{\mu\text{mol}}{\text{L}} \right)} \text{ (if male)}$$

To calculate CG–CrCl, the age at the date of the actual serum creatinine measurement and the weight closest to that date were used. Thus, an individual with only one weight available was assumed to have the same weight throughout the entire study period.

In sensitivity analyses, we used the CKD epidemiological collaboration (CKD-EPI) equation to estimate GFR.²³

$$\begin{aligned} \text{GFR}_{\text{CKD-EPI}} = & 141 \times \min\left(\frac{\text{serum} - \text{creatinine}}{\kappa}, 1\right)^{\alpha} \\ & \times \max\left(\frac{\text{serum} - \text{creatinine}}{\kappa}, 1\right)^{-1.209} \times 0.993^{\text{age}} \quad (2) \\ & \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]} \end{aligned}$$

where serum creatinine is measured in $\mu\text{mol/L}$, κ is 61.9 for females and 79.6 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of serum creatinine/ κ or 1, and max indicates the maximum of serum creatinine/ κ or 1. CKD was defined as two consecutive CG-CrCl of $\leq 60 \text{ mL/min}$, ≥ 3 months apart. The date of the second CG-CrCl measurement was defined as date of CKD. CD4 cell count and CG-CrCl at study inclusion were defined as the CD4 cell count and the CG-CrCl closest to and within 2 years before and 6 months after study inclusion.

Study population

We included all PLHIV who 1) had available data on smoking status, 2) had a unique Danish person identification number, 3) were 16 years or older at study inclusion, 4) were alive and living in Denmark at study inclusion, and 5) did not have aRRT at or prior to study inclusion. This population was included in part 2.

In part 1, the following inclusion criteria were added. Individuals who 1) had at least three CG-CrCl available during the study period, 2) had data on weight available, and 3) did not have CKD prior to study inclusion, for analyses evaluating risk of CKD.

In part 3, all patients from part 2 with aRRT were included.

Study design

Part 1 – smoking, renal function, and CKD

For the assessment of overall renal function over time, time was calculated from January 1, 1995, date of HIV diagnosis, date of first available data on smoking, first available CG-CrCl or date of immigration whichever occurred last to date of aRRT, last available CG-CrCl, loss to follow-up, emigration, death, or 10 years after study inclusion, whichever occurred first. Observation time was divided into intervals of 3 months starting from study inclusion. If an individual had more than one CG-CrCl within a 3-month interval, median CG-CrCl was used. If no CG-CrCl was recorded within a 3-month interval, we calculated the CG-CrCl as a weighted mean of the two nearest CG-CrCl measurements, thereby assuming linearity between the two measurements. In consequence, all individuals contributed with a CG-CrCl in

every 3-month time interval. We then graphically evaluated median CG-CrCl according to smoking status for every 3-month interval. When we evaluated risk of CKD, time was calculated from January 1, 1995, date of HIV diagnosis, date of first available data on smoking, first available CG-CrCl or date of immigration whichever occurred last to date of CKD, last available CG-CrCl, loss to follow-up, emigration, death, or 10 years after study inclusion, whichever occurred first. Outcome was time to CKD. We calculated cumulative incidence with death, emigration, and loss to follow-up as competing risks to estimate cumulative risk of CKD. We calculated incidence rates (IRs) and used Cox regression to calculate incidence rate ratios (IRRs) of CKD. The following covariates were included in the final model: gender (male vs female), race (Caucasian, African, or other), route of infection (men who have sex with men, intravenous drug use [IDU], heterosexually infected, or other), CD4 cell count ($<200 \text{ cells}/\mu\text{L}$ vs $\geq 200 \text{ cells}/\mu\text{L}$) at study inclusion, and CG-CrCl ($<90 \text{ mL/min}$ vs $\geq 90 \text{ mL/min}$) at study inclusion. The following covariates were included as time-updated variables: age (5-year intervals), HAART exposure (yes/no), tenofovir exposure (yes/no), diabetes (yes/no), hypertension (yes/no), and AIDS (yes/no). In sensitivity analyses, we estimated GFR with the CKD-EPI equation.

Part 2 – smoking and aRRT

Time was calculated from January 1, 1995, date of HIV diagnosis, date of first available data on smoking or date of immigration whichever occurred last to date of aRRT, loss to follow-up, emigration, death, or 10 years after study inclusion, whichever occurred first. Outcome was time to aRRT, as defined earlier.

We calculated cumulative incidence with death, emigration, and loss to follow-up as competing risks to estimate cumulative risk of aRRT. We calculated IRs and used Cox regression to calculate IRR of aRRT. The following covariates were included in the final model: gender (male vs female), race (Caucasian, African, or other), route of infection (men who have sex with men, IDU, heterosexually infected, or other), and CD4 cell count ($<200 \text{ cells}/\mu\text{L}$ vs $\geq 200 \text{ cells}/\mu\text{L}$) at study inclusion. The following covariates were included as time-updated variables: age (5-year intervals), HAART exposure (yes/no), tenofovir exposure (yes/no), diabetes (yes/no), hypertension (yes/no), and AIDS (yes/no). To test for effect modification, we stratified the analyses on gender, route of infection, race, and CD4 cell count at study inclusion. We did sensitivity analyses excluding IDUs from the analyses and analyses excluding

individuals of non-Danish origin. Furthermore, we analyzed the risk of cRRT according to smoking status.

Part 3 – smoking and mortality following aRRT

Time was calculated from aRRT to death, emigration, loss to follow-up, December 31, 2013, or 56 days after study inclusion whichever came first. Outcome was time from aRRT to death, and Cox regression analyses were used to estimate mortality rate ratios (MRRs). We included age at inclusion and gender as covariates in the model. We did not include comorbidities in the adjusted analyses as these covariates most likely are facilitators and not confounders of the increased mortality in smokers. Kaplan–Meier survival tables were used to construct survival curves following aRRT. In secondary analyses, IDUs were excluded from the analyses. Furthermore, we did a sensitivity analyses only including individuals of Danish origin.

For part 1 of the study, we did sensitivity analyses using the CKD-EPI equation to estimate GFR.²³ We used the tests of the nonzero slope to test for proportional hazard assumptions. The study was approved by the Danish Data Protection Agency (journal no 2008-41-1781). Stata software, Version 11.0 (StataCorp, College Station, TX, USA) and R version 3.1.3 were used to perform statistical analyses.

Results

Overall

On December 31, 2013, a total of 6,239 PLHIV were registered in DHCS. Approximately 1,707 had missing data on smoking, and 17 had aRRT before inclusion (five patients [0.3%] among never smokers, three patients [0.4%] among previous smokers, and nine patients [0.4%] among current smokers) leaving 4,515 PLHIV in the study with a total of 29,196 person years of follow-up (PYFU).

Approximately 1,475 (32.7%) PLHIV were categorized as never smokers, 768 (17.0%) as previous smokers, and 2,272 (50.3%) as current smokers. Median age among previous smokers (39.7 years, interquartile range [IQR]: 32.3–48.3) was higher than among never and current smokers (35.2 years, IQR: 29.5–43.9; 36.4 years, IQR: 30.4–43.7). Number of deaths during study period was greater among current smokers than among never and previous smokers. Characteristics of the study population are summarized in Table 1.

Part 1 – smoking, renal function, and CKD

In part 1, 3,142 PLHIV with at least three CG-CrCl_s available were included. No association between smoking

Table 1 Characteristics of study population

	Smoking status		
	Never	Previous	Current
Individuals	1,475 (32.7)	768 (17.0)	2,272 (50.3)
Follow-up (years)	9,694	5,000	14,502
Loss to follow-up	2 (0.1)	3 (0.4)	5 (0.2)
Emigrated	81 (4.0)	30 (3.9)	75 (3.3)
Deaths	75 (5.1)	81 (10.5)	411 (18.1)
Age at study inclusion (years) ^a	35.2 (29.5–43.9)	39.7 (32.3–48.3)	36.5 (30.4–43.7)
Male	912 (61.8)	617 (80.6)	1,840 (80.9)
Race ^b			
Caucasian	919 (62.2)	628 (81.9)	1,983 (87.3)
African	406 (27.5)	82 (10.7)	129 (5.7)
Others/unknown	150 (10.2)	58 (7.4)	160 (7.0)
Route of infection ^b			
MSM	596 (40.4)	404 (52.6)	1,114 (49.0)
IDU	12 (0.8)	37 (4.7)	316 (14.0)
Heterosexually	769 (52.0)	277 (36.1)	736 (32.3)
Others	98 (6.7)	52 (6.6)	106 (4.7)
Comorbidities			
Diabetes	35 (2.4)	19 (2.5)	56 (2.5)
Hypertension	98 (6.6)	46 (6.1)	99 (4.4)
AIDS	38 (2.6)	40 (5.2)	107 (4.7)
CD4 cell count (cells/ μ L) ^{a,b}	435 (291–600)	410 (256–600)	445 (273–650)
Weight (kg) ^{a,b}	74.5 (65.0–84.0)	75.0 (67.0–84.2)	71.2 (63.4–80.0)
Missing	491 (33.2)	238 (31.0)	644 (28.3)
CG-CrCl (mL/min) ^{a,b}	102 (88–120)	101 (87–119)	103 (87–121)
Missing CG-CrCl at study inclusion	491 (33.2)	238 (31.0)	644 (28.3)

Notes: Data are numbers (%) unless otherwise specified; ^amedian (interquartile range); ^bat study inclusion.

Abbreviations: MSM, men who have sex with men; IDU, intravenous drug user; AIDS, acquired immunodeficiency syndrome; CG-CrCl, estimated creatinine clearance calculated with the Cockcroft–Gault equation.

status and median CG-CrCl was observed (Figure 1A). When modeling risk of CKD, we excluded 128 individuals with CKD prior to study inclusion leaving 3,014 PLHIV with a total of 18,131 PYFU. We identified 180 cases of CKD, 47 among never smokers, 43 among previous smokers, and 90 among current smokers. IRs (per 10,000 PYFU) of CKD were 83.2 (95% confidence interval [CI]: 62.5–110.8) for never smokers, 132.7 (95% CI: 98.4–178.9) for previous smokers, and 97.4 (95% CI: 79.2–119.7) for current smokers. In the unadjusted model, previous smoking was associated with increased risk of CKD compared to never smoking (IRR: 1.6, 95% CI: 1.1–2.4), whereas current smoking was not associated with risk of CKD (IRR: 1.2, 95% CI: 0.8–1.6). In the adjusted models, this association was not present (IRR: 1.1, 95% CI: 0.7–1.7 and IRR: 1.3, 95% CI: 0.9–1.8) for previous smokers and current smokers (Figure 1B and Table 2), with differences in age

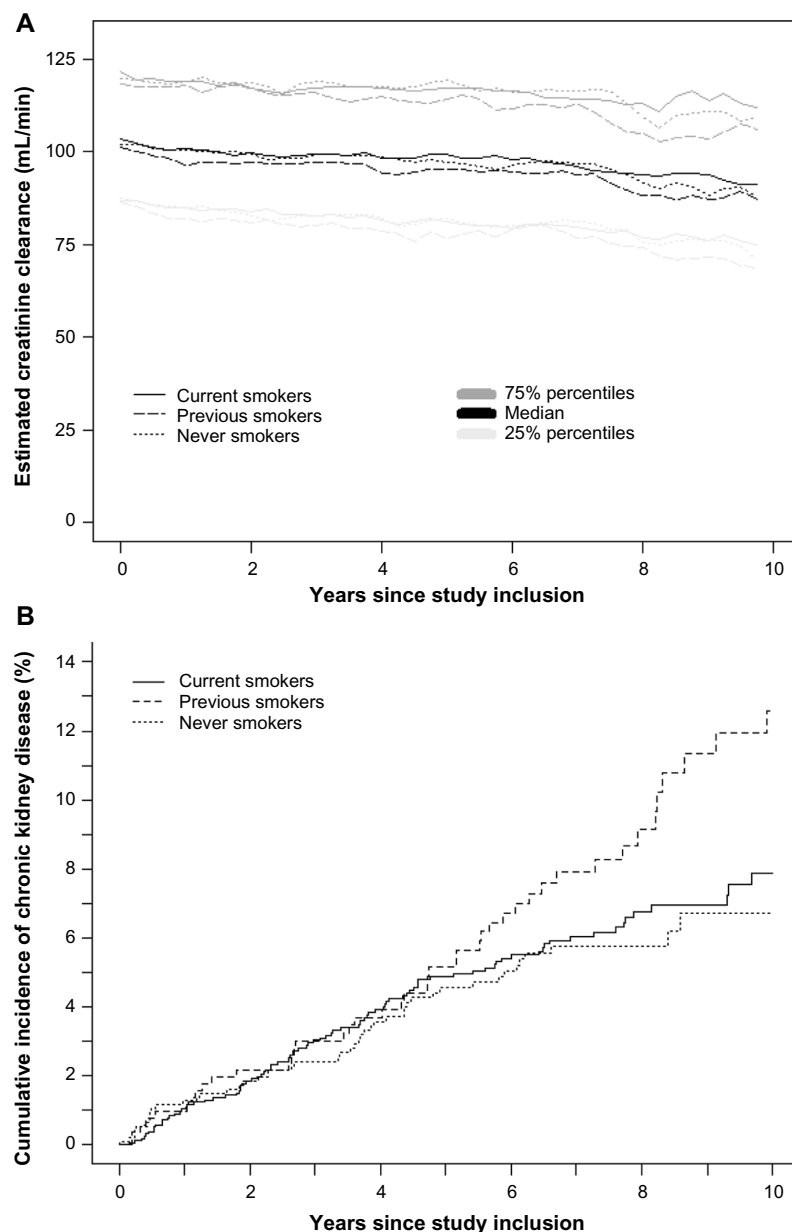


Figure 1 Estimated creatinine clearance and risk of chronic kidney disease stratified on smoking status.

Notes: (A) 25%, median, and 75% percentiles of estimated creatinine clearance stratified on smoking status. We used the Cockcroft–Gault equation to calculate estimated creatinine clearance (displayed as mL/min) as estimate of glomerular filtration rate. We divided time from study inclusion until end of follow-up in 3-month intervals. In each interval, all participants contributed with one CG-CrCl. The CG-CrCl in a time interval was either the median of all CG-CrCls in that time interval. If no CG-CrCl was available in a 3-month time interval, the CG-CrCl was calculated as the weighted mean of the CG-CrCl measurements determined before and after the actual 3-month period. At study inclusion, there were 984 never smokers, 530 previous smokers, and 1,628 current smokers. At 10 years of follow-up, 110 never smokers, 98 previous smokers, and 170 current smokers were still under follow-up. (B) Risk of chronic kidney disease stratified on smoking status. Definition of chronic kidney disease: two consecutive CG-CrCls of ≤ 60 mL/min ≥ 3 months apart.

Abbreviation: CG-CrCl, estimated creatinine clearance calculated with the Cockcroft–Gault equation.

between never smokers and previous smokers explaining most of the risk difference observed between these groups (adjusting for only age – IRR: 1.2, 95% CI: 0.8–1.8 and IRR: 1.3, 95% CI: 0.9–1.9 for previous and current smokers, respectively). As seen in Figure 1B, previous smokers seemed to have increased risk of CKD from 5 years after study inclusion. We therefore performed a Cox regression

analysis in which time was split at 5 years after study inclusion. In this analysis, adjusted IRR for previous smokers vs never smokers was 3.4 (95% CI: 1.3–8.7). When we used the CKD-EPI equation to estimate GFR, it did not change the results substantially (IRR: 1.1, 95% CI: 0.7–1.6 and IRR: 0.8, 95% CI: 0.5–1.2 for previous smokers and current smokers, respectively).

Table 2 Risk of CKD and aRRT

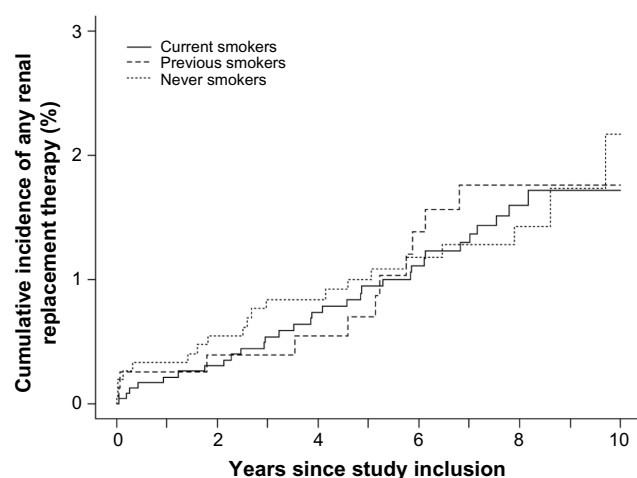
Smoking status ^a	Individuals with CKD during follow-up	IR per 10,000 PYFU (95% CI)	IRR (95% CI)	
			Unadjusted	Adjusted
Risk of CKD ^b				
Never	47	83.2 (62.5–110.8)	Ref	Ref
Previous	43	132.7 (98.4–178.9)	1.6 (1.1–2.4)	1.1 (0.7–1.7)
Current	90	97.4 (79.2–119.7)	1.2 (0.8–1.6)	1.3 (0.9–1.8)
Risk of aRRT				
Never	20	20.6 (13.3–31.9)	Ref	Ref
Previous	11	22.0 (12.2–39.7)	1.1 (0.5–2.2)	0.8 (0.4–1.7)
Current	31	21.4 (15.0–30.4)	1.0 (0.6–1.8)	0.9 (0.5–1.7)

Notes: ^aAt study inclusion; ^bdefinition of CKD: two consecutive CG-CrCl of ≤ 60 mL/min ≥ 3 months apart. For CKD: adjusted for age, gender, HAART exposure, tenofovir exposure, race, route of infection, comorbidities (diabetes, hypertension, and AIDS), included as time-updated variables, CD4 cell count, and CG-CrCl at study inclusion. For aRRT: adjusted for age, gender, HAART exposure, tenofovir exposure, race, route of infection, comorbidities (diabetes, hypertension, and AIDS), included as time-updated variables and CD4 cell count.

Abbreviations: CKD, chronic kidney disease; aRRT, any renal replacement therapy; IR, incidence rate; PYFU, person years of follow-up; CI, confidence interval; IRR, incidence rate ratio; CG-CrCl, estimated creatinine clearance calculated with the Cockcroft–Gault equation; HAART, highly active antiretroviral treatment; AIDS, acquired immunodeficiency syndrome; Ref, reference.

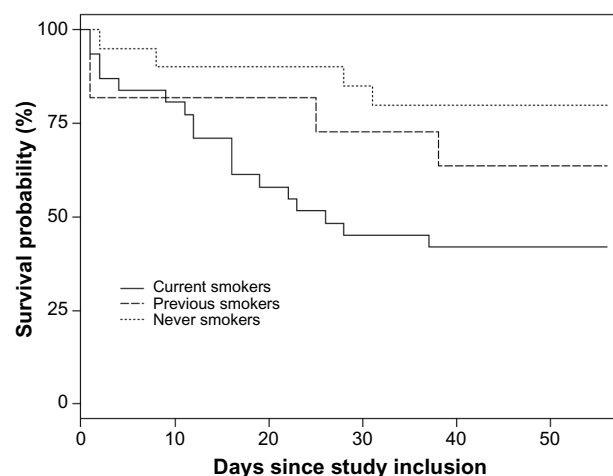
Part 2 – smoking and aRRT

In this part, we included the complete cohort of 4,515 PLHIV with known smoking status. We identified a total of 62 cases of aRRT (20, 11, and 31 among never, previous, and current smokers). IRs (per 10,000 PYFU) of aRRT were 20.6 (95% CI: 13.3–31.9) for never smokers, 22.0 (95% CI: 12.2–39.7) for previous smokers, and 21.4 (95% CI: 15.0–30.4) for current smokers. The risk of aRRT in previous and current smokers did not differ substantially from that of never smokers (Figure 2 and Table 2). Excluding IDUs or individuals of non-Danish origin only changed the estimates marginally (results not shown). Only 17 patients (nine never smokers, two previous smokers, and six current smokers) were categorized with cRRT in the study period, and did not allow us to make robust conclusions about association of smoking and risk of cRRT.

**Figure 2** Risk of any renal replacement therapy stratified on smoking status.

Part 3 – smoking and mortality following aRRT

In this part of the study, we included the 62 individuals from part 2 who initiated aRRT. They had a total of 344 person days of follow-up (PDFU). During the observation time, 26 deaths were recorded, and mortality rates (MRs per 1,000 PDFU) were 29.0 (95% CI: 10.9–77.3), 65.3 (95% CI: 23.0–163.0), and 128.0 (95% CI: 80.6–203.0) for never smokers, previous smokers, and current smokers, respectively. The unadjusted MRRs were 2.0 (95% CI: 0.5–8.2) and 3.9 (95% CI: 1.30–11.5) for previous smokers and current smokers, respectively, compared to never smokers (Figure 3). When adjusting for gender and age at study inclusion, the MRRs were 1.9 (95% CI: 0.5–7.8) and 3.8 (95% CI: 1.3–11.2) for previous and current smokers, respectively, compared to never smokers. Excluding IDUs or individuals

**Figure 3** Mortality following any renal replacement therapy stratified on smoking status.

of non-Danish origin only changed the estimates marginally (results not shown).

Discussion

In this Danish nationwide population-based cohort study, we found no evidence that smoking status was associated with decline in renal function, risk of CKD, or risk of aRRT. Mortality was high following aRRT, and substantially increased in smokers.

GFR in smokers has not convincingly been shown to be decreased compared to nonsmokers. A large cross-sectional study from France which included 28,409 individuals from the background population found that smoking was associated with a slightly increased CG-CrCl and albuminuria.²⁴ Increased GFR and albuminuria in smokers simultaneously have led to the hypothesis that smoking is associated with glomerular hyperfiltration, a phenomenon also described in other disease entities such as diabetes.^{25,26} Thus, glomerular hyperfiltration could be part of the pathogenesis leading to proteinuria in smokers. It could also hide a potential harmful effect of smoking on renal function. Another explanation for an elevated CG-CrCl in smokers could be that smokers tend to have more sedentary activities compared to nonsmokers,²⁷ which results in lower muscle mass and thereby lower serum creatinine and higher CG-CrCl. To compensate for differences in muscle mass, we chose to use the CG-CrCl as an estimate of GFR. However, a change in body weight is not solely explained by a change in total muscle mass, and hence, this equation only partly adjusts for differences in muscle mass.

It is controversial whether smoking increases the risk of renal insufficiency in the general population.²⁸ Some studies have evaluated the association between smoking and aRRT or ESRD in the general population. Haroun et al found that smoking was associated with ESRD or CKD as cause of death in both men and women with adjusted IRR of 2.4 (95% CI: 1.4–4.0) and 2.9 (95% CI: 1.7–5.0) for men and women, respectively. However, this effect was primarily present after the age of 60.²⁹ In a study including more than 300,000 individuals from the background population, Klag et al concluded that smoking was significantly associated with ESRD, but the magnitude of the effect of smoking was not reported.³⁰ The populations in the above-mentioned studies were older than the population in our study, which may explain some of the discrepancies as only 4% of the population in our study were older than 60 years of age at study inclusion. We observed a trend toward increased risk of CKD in previous smokers from 5 years after study inclusion. We presume that this is a

chance finding but cannot exclude that the morbidity, which prompted smoking cessation, later leads to deteriorating renal function.

Only a few studies have evaluated smoking as a risk factor for renal disease in PLHIV. Ryom et al included smoking as a covariate when evaluating HAART exposure as a risk factor for advanced CKD or ESRD in the D:A:D study. They found that smoking was associated with a statistically significant increased risk of advanced CKD/ESRD (IRR approximately 1.9).³¹ In contrast to our study, the D:A:D study included patients with unknown smoking status at enrollment and was not designed to show effects of smoking on renal function. Furthermore, their population was slightly older than ours. As discussed earlier, one study has indicated that the effect of smoking on the risk of ESRD primarily affects people aged 60 or older, and hence, a potential harmful effect of smoking on renal function may be observed in our population as it grows older.²⁹ We have shown that the mortality is higher among smokers than never smokers and previous smokers following aRRT. One could speculate that the reporting of aRRT may be more likely to take place if a patient dies. This could bias the results toward aRRT being associated with smoking. The registration of dialysis in DNHR is coupled to the reimbursement system, and the hospital is only economically compensated if the correct diagnosis is registered in DNHR. We presume that this leads to a high and accurate registration of dialysis, and represents a significant difference from other studies evaluating dialysis as an outcome.^{32,33}

Following aRRT, the mortality is increased in smokers compared to never smokers and previous smokers. Increased mortality in smokers while receiving renal replacement therapy is well established. In a large meta-analysis of ESRD patients performed in 2011, Lieberman et al found a hazard ratio of 1.65 of death among smokers compared to nonsmokers.³⁴ This is comparable to the estimates in our study. However, MRs in the present study are much higher than in previous studies. Our definition of aRRT results in an overrepresentation of patients on acute dialysis, compared to patients on cRRT. Acute dialysis most often occurs with severe acute illnesses such as septic shock with a high mortality, which is the probable explanation for high MRs in our study.

Major strengths of the study are the following: 1) a population-based, nationwide design; 2) the use of DCRS, which assigns a personal identification number to all Danish citizens; 3) the well-organized structure of Danish national registers which allowed us to use well-validated data of high quality on vital status; and 4) the use of another well-validated

Danish database the DNHR which enabled us to extract robust data on study outcomes.²⁰

There are some limitations of the study: 1) We did not have access to data on CG-CrCl or aRRT in Danish individuals from the background population with known smoking status. 2) Smoking status was only available for about three quarters of Danish PLHIV. 3) Serum creatinine was measured by different testing methods over the 18-year study period, and we therefore cannot exclude some intra- and interlaboratory variation.³⁵ In contrast to previous studies, we corrected for the varying testing methods as suggested by the local laboratories. 4) Our study period started from January 1, 1995, and standardization of serum creatinine measurements was implemented in Denmark around 2004, and hence, a substantial part of the serum creatinine measurements has not been subject to standardization. 5) We chose to use CG-CrCl as an estimate of GFR, since a large number of PLHIV gain weight while recovering from their infection upon commencing HAART. This may introduce a bias as some patients did not have a specific weight available for each serum creatinine measurement. However, a sensitivity analysis using the CKD-EPI equation instead of the Cockcroft–Gault equation gave comparable results. 6) We did not have access to data on proteinuria or albuminuria, and hence, we were not able to test for association between smoking and proteinuria/albuminuria. 7) Our definition of aRRT included both patients with ESRD on cRRT and patients on acute dialysis. However, IRR of CKD in the present study showed results that only differed marginally from the IRR of aRRT. We therefore believe that our results are rather robust.

We conclude that in Danish PLHIV, the overall renal function, the risk of CKD, or the risk of aRRT is not substantially associated with smoking status, but smoking increases mortality following aRRT.

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14. PAPER 3



Routine urine protein/creatinine ratio testing in an outpatient setting of Danish HIV-infected individuals

Magnus Glindvad Ahlstrom, Bo Feldt-Rasmussen, Jan Gerstoft & Niels Obel

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LETTER TO THE EDITOR

Routine urine protein/creatinine ratio testing in an outpatient setting of Danish HIV-infected individuals

Due to the availability of antiretroviral treatment (cART), infection with human immunodeficiency virus (HIV) is now considered a chronic manageable disease. Focus has shifted from treatment of opportunistic infections to management of comorbidities and side-effects of cART, including cardiovascular disease and renal dysfunction. To meet these problems, patients are subjected to extensive screening procedures. Based on increasing cost and inconvenience, however, it is concomitantly important to establish what is gained from the implementation of new procedures.

We read with interest a recent report in the present journal,[1] contradicting a clinical utility of a well-established screening tool for peripheral arterial disease among HIV-infected patients. According to the study, the prevalence of the condition was very low. Along a similar line of rationality, we here report a lack of clinical utility of a screening procedure for kidney damage with urine protein/creatinine ratio (UP/Cr), introduced in the EACS guidelines 5.0 in 2009. The evidence in terms of prevalence of abnormal values and the confidence with the test among clinicians were found to be poor.

In accordance with the EACS guidelines, we implemented UP/Cr testing in our clinic from summer 2013. Before the implementation we planned an analysis of the outcome of annual UP/Cr screening 2 years after the implementation. In the present study we report (1) the degree of implementation, (2) the prevalence of UP/Cr > 0.5 (abnormal UP/Cr) in HIV-infected individuals not diagnosed with renal disease and (3) the clinical consequences of an abnormal UP/Cr.

As of 31 June 2015, Denmark had a prevalence of known HIV-infected individuals of 0.1% in the adult population, corresponding to ~4600 individuals. The current study was conducted at the HIV-outpatient clinic at the University Hospital of Copenhagen, Rigshospitalet (further-on referred to as the clinic) caring for approximately one third of Danish HIV-infected individuals. In the study period, HIV-infected individuals were intended to be seen in the clinic twice a year. Antiretroviral treatment is provided free of charge at all Danish HIV-centres.

The unique 10-digit personal identification number assigned to all Danish residents at birth or immigration was used to avoid multiple registrations and to track individuals in the following registries.

The Danish National Health Registry (DNHR), established in 1977, stores information on all inpatient and outpatient admissions to non-psychiatric hospitals in Denmark.[2] From this register we extracted information on outpatient visits of the HIV-infected individuals at the clinic.

Demographic and clinical data were extracted from the Danish HIV Cohort Study (DHCS), which is a prospective study

of all HIV-infected individuals 16 years or older at HIV diagnosis and treated at Danish HIV-centres after 1 January 1995. Individuals are consecutively enrolled and more than 98% of the HIV-infected individuals seen at the clinic have a Danish 10-digit personal identification number. The study is described in detail elsewhere.[3]

Before implementation of annual UP/Cr testing, the staff were instructed to inform all HIV-infected individuals seen at the clinic to bring a urine sample at their next visit, at the last visit before the annual control visit. The urine sample was brought to the laboratory by the HIV-infected individual in conjunction with blood testing. The results of the serum-creatinine and UP/Cr measurements were extracted from electronic laboratory databases. We calculated eGFR with the modification of diet in renal disease (MDRD) formula.[4] If an eGFR was not available at the particular date of a UP/Cr, a weighted average of the two adjacent eGFRs was used; if only one adjacent eGFR was available, it was used as an estimate of eGFR on the date of the UP/Cr.

We included all HIV-infected individuals who: (1) had a Danish personal identification number and (2) were seen at the clinic in the period 1 July 2013–30 June 2015. The study period was divided into two sub-periods; 1 July 2013–30 June 2014 (period one) and 1 July 2014–30 June 2015 (period two). For the statistical analyses we included only the first UP/Cr and the corresponding eGFR in each sub-period. To evaluate UP/Cr as a marker of early renal damage, we excluded individuals with already known renal disease (eGFR < 60 mL/min pr. 1.73 m^2 (abnormal eGFR) or a UP/Cr ≥ 0.5 mg/mmol (abnormal UP/Cr)), within 1 year prior to the date of blood/urine testing.

To evaluate the clinical consequences of an abnormal UP/Cr, the primary investigator (MGA) examined the patient files of HIV-infected individuals with an abnormal UP/Cr to clarify whether the abnormal test: (1) was described as abnormal in the patient file by the treating physician, (2) led to further examinations/tests, (3) led to initiation of a treatment regime for CKD, (4) led to referral to a specialist in nephrology or (5) led to a diagnosis of a specific renal disease. A treatment regime for CKD was defined as interventions to improve CKD outcome (e.g. changes in medication, initiation of an ACE-inhibitor or watchful waiting including extra control visits). Further it was examined whether the individual according to the patient file had known pre-existing renal disease. For each sub-period we calculated (1) the total number of individuals seen in the clinic, (2) the fraction of individuals with at least one UP/Cr, (3) the fraction of individuals with an abnormal UP/Cr of those tested and (4) the fractions of patients with a UP/Cr, for whom the test had any of the above-

mentioned clinical consequences. Differences in characteristics between groups were evaluated with student's *t*-test or the χ^2 -test as appropriate. $p < 0.05$ was considered statistically significant. The study was approved by the Danish Data Protection Agency (journal no 2008-41-1781). Statistical analyses were performed using R version 3.2.

We identified 1510 individuals who attended the clinic in period one and 1441 individuals in period two (1369 individuals were seen in both periods). Median age was 47.0 years (IQR = 39.6–54.4) and 48.4 years (IQR = 40.9–56.5) for periods one and two, respectively. For each sub-period the study population was primarily Caucasians, men who have sex with men (MSM) and males (Table 1). For periods one and two, median CD4 cell counts were 660 cells/ μ L (IQR = 490–858) and 650 cells/ μ L (IQR = 490–850) and the fractions of virally suppressed (viral load < 50 copies/ μ L) were 86% and 85%. We found that characteristics of the individuals that were tested with UP/Cr only differed slightly from those who were not tested. In period one, 643 (42.6%) individuals had at least one UP/Cr performed, compared to 415 (28.8%) in period two ($p < 0.001$). In period one, 13 (2.0% of the tested population) and in period two, four individuals (1.0% of the tested population) had an abnormal UP/Cr.

For period one, the abnormal test was commented in the medical files for three individuals (0.5% of the tested population), one individual (0.2% of the tested population) had further analyses made (Chrome-EDTA clearance) and two individuals (0.4% of the tested population) started a treatment regime for CKD. For period two, none of the abnormal tests were commented in the patient files or had consequences for the patient (Table 2).

In this single centre study we found that, although the staff was well informed and the test was available as routine, UP/Cr screening was performed in less than 45% of the individuals seen in the clinic; 0.8% of the total population and

2% of the tested individuals were identified with an abnormal UP/Cr and the abnormal test results had almost no clinical consequences. Less than half of the individuals seen in the clinic had a UP/Cr measured. Whether or not this is due to forgetfulness of the patients or the staff, that the measurement is perceived as unpleasant or the lack of specific measures to enhance implementation by the staff, e.g. SMS reminders, cannot be established from the current study.

When a new procedure is introduced to clinical practice, it is important to evaluate the effectiveness of this procedure in an everyday clinical setting. To our knowledge this is the first study to present data on the effectiveness of UP/Cr screening in HIV-infected individuals in an everyday, clinical setting.

Gardner et al. [5] defined proteinuria as a urine dipstick protein 2+ or more and observed a proteinuria prevalence of 5%. Their definition corresponds to UP/Cr ≥ 0.5 , which is comparable to our definition. Of importance, Gardner et al.'s study was conducted in the pre-cART era and included mainly black patients which could lead to a higher rate of CKD compared to our study. In a study from 2015, Mocroft et al. [6] found a prevalence of proteinuria of 4.2%, which is twice as

Table 2. Clinical consequences of UP/Cr.

	Period one	Period two
Individuals with ≥ 1 UP/Cr	643 (100.0%)	415 (100.0%)
Individuals with an abnormal UP/Cr	13 (2.0%)	4 (1.0%)
Commented as abnormal in patient files	3 (0.5%)	0 (0.0%)
Known pre-existing renal disease	0 (0.0%)	1 (0.2%)
Further examinations	1 (0.2%)	0 (0.0%)
Nephrological treatment ^a	2 (0.4%)	0 (0.0%)
Referral to nephrologist	0 (0.0%)	0 (0.0%)
Diagnosed with specific renal disease	0 (0.0%)	0 (0.0%)

Period one is from 1 July 2013–30 June 2014, period two is from 1 July 2014–30 June 2015.

UP/Cr; Urine Protein/Creatinine ratio.

Data are *n* (%) unless specified otherwise.

^aDefined as interventions to improve CKD outcome (e.g. change in medication, initiation of ACE-inhibitors or watchful waiting including extra control visits).

Table 1. Characteristics.

	Period one		Period two	
	Individuals with no UP/Cr	Individuals with ≥ 1 UP/Cr	Individuals with no UP/Cr	Individuals with ≥ 1 UP/Cr
Individuals	(<i>n</i> = 867)	(<i>n</i> = 643)	(<i>n</i> = 1026)	(<i>n</i> = 415)*
Individuals included in both periods	—	—	966 (94.2%)	403 (97.1%)
Individuals with UP/Cr in both periods ^b	—	—	—	224 (54.0%)
Individuals with an abnormal UP/Cr	—	13 (2.0%)	—	4 (1.0%)
Age ^a	46.2 (38.4–52.7)	48.2 (40.6–56.6) ^{&}	47.3 (39.7–54.3)	48.6 (41.0–57.1) [#]
Males	717 (82.7%)	563 (87.6%)	848 (82.7%)	383 (92.3%)
Route of infection				
MSM	543 (62.6%)	476 (74.0%) ^{&}	654 (63.7%)	331 (79.8%) [#]
IDU	35 (4.0%)	7 (1.1%) ^{&}	34 (3.3%)	0 (0.0%) [#]
Heterosexually	239 (27.6%)	124 (19.3%) ^{&}	276 (26.9%)	64 (15.4%) [#]
Others/unknown	50 (5.8%)	36 (5.6%)	62 (6.0%)	20 (4.8%)
Race				
White	705 (81.3%)	550 (85.5%)	845 (82.4%)	363 (87.5%)
African	78 (9.0%)	56 (8.7%)	90 (8.8%)	28 (6.7%)
Others/unknown	84 (9.7%)	37 (5.8%) ^{&}	91 (8.9%)	24 (5.8%)
Virally suppressed ^c	711 (82%)	586 (91.1%) ^{&}	859 (83.7%)	361 (87.0%)
CD4 ^a	670 (490–855)	660 (490–870)	670 (490–850)	650 (490–870)

Period one is from 1 July 2013–30 June 2014, period two is from 1 July 2014–30 June 2015.

Data are *n* (%), unless specified otherwise.

UP/Cr, Urine Protein/Creatinine ratio; MSM, men who have sex with men; IDU, intravenous drug users.

*Statistically significant difference between period one and period two.

[&]Statistically significant difference in tested and untested individuals in period one.

[#]Statistically significant difference in tested and untested individuals in period two.

^aMedian (IQR).

^bPercentage of the total number tested with UP/Cr.

^cDefined as viral load < 50 copies/mL.

high as our estimate for period one. Their definition of proteinuria was two consecutive dipstick urine proteins of 2+ or more, which is comparable to our definition. Their study population did not differ markedly from ours.

Clearly evidence exists that HIV-infected individuals are at increased risk of adverse renal events.[5,7,8] In consequence, guidelines for the management of renal disease in HIV-infected individuals have agreed to implement general UP/Cr screening, but the evidence is poor (C-III or based on consensus decisions). Unambiguous evidence to support general UP/Cr screening is lacking and the effectiveness of the implementation on hard clinical end points such as end stage renal disease and death has not yet been described to our knowledge.

Fink et al. [9] conducted a systematic review of the evidence of CKD screening in the general population and found that no randomised clinical trials compared screening with no screening and concluded that the benefits of these interventions are uncertain. Based on this study, the US Preventive Task Force made guidelines not recommending screening for CKD in low risk populations.[10]

WHO guidelines state that, to implement a screening procedure, the disease must be an important health problem. In the current study we found that the prevalence of an abnormal UP/Cr was low (2.0%) and the clinical consequences of an abnormal test was minor. The WHO guidelines also state that the cost of the screening procedure must be economically balanced with overall gain in health.

With the growing knowledge and increasing costs of novel treatments and tests it is important to establish what is gained from implementation of new procedures. The proof of concept of implementation of UP/Cr in the HIV population would be a randomised trial with long-term follow-up.

A major strength of this study is the general architecture of the Danish healthcare system, which is based on the unique person identifier, which means that we had complete follow-up and were able to link all patients with laboratory test results.

Our study is limited by the short follow-up period that prevented us from analysing impact and risk for clinical hard end-points like end stage renal disease and death.

Based on: (1) the low implementation rate of the UP/Cr, (2) the small prevalence of abnormal UP/Cr, (3) the low rate of clinical consequences of an abnormal test results and (4) the lack of evidence to support UP/Cr screening procedures in HIV-infected individuals, we decided to withdraw the UP/Cr screening procedure in our outpatient clinic.

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15. PAPER 4

Agreement between Estimated and Measured Renal Function in an Everyday Clinical Outpatient Setting of Human Immunodeficiency Virus-Infected Individuals

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Keywords

Glomerular filtration rate · Human immunodeficiency virus · Nephrotoxicity · Predicted creatinine clearance

Abstract

Introduction: Estimated renal function (eRF) has been widely implemented as a screening tool in handling human immunodeficiency virus (HIV)-infected individuals. Our primary objective was to investigate the agreement between measured renal function (mRF) and eRF in HIV-infected individuals in an everyday clinical setting. **Methods:** A single-center study at the HIV-outpatient clinic at Copenhagen University Hospital, Rigshospitalet. Study period from January 1, 2004–June 1, 2015. We included all HIV-infected individuals who had an mRF performed and compared this with eRF assessed with 9 different serum-creatinine-based equations and the eRF reported by the Department of Clinical Biochemistry. We evaluated performance characteristics of the different eRFs, with concordance correlation coefficient, total deviation index, coverage probability, relative accuracy, and Bland Altman plots. We also evaluated whether exposure to (1) rilpivirine, cobicistat, or dolutegravir (RLP/COB/DTG), (2) protease inhibitors (PIs), or (3) tenofovir disoproxil fumarate (TDF) had an impact on agreement. Furthermore, we compared inter- and intra-individual differences between mRF and eRF. **Results:** Ninety-eight individuals had an mRF performed during the study period. We found that the

agreement between mRF and eRF was poor irrespective of the eRF equation. Exposure to RLP/COB/DTG and PIs was not associated with different agreement. Exposure to TDF was associated with statistically significant better agreement for 3 of the evaluated equations. **Conclusion:** Irrespective of calculation methods, the agreement between mRF and eRF is poor. Surprisingly TDF exposure was associated with a better agreement compared with TDF-unexposed individuals.

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Introduction

Due to combination antiretroviral therapy (cART), human immunodeficiency virus (HIV) is now considered a chronic disease with a benign prognosis [1, 2]. Focus has shifted from treatment of opportunistic infections to the management of comorbidities and side effects of cART, including renal dysfunction. Since the first equation to estimate renal function was developed by Effersø [3] in 1957, more than 20 biomarker-based equations have been developed to estimate renal function (eRF) [4–6] benchmarked against measured renal function (mRF) using different exogenous substances such as inulin, iothexol, technetium ^{99m} diethylenetriaminepentaacetic acid, and chromium-ethylenediaminetetraacetic acid (⁵¹Cr-EDTA) [7–9]. The performance of these equations in estimating renal function in HIV-infected individuals has

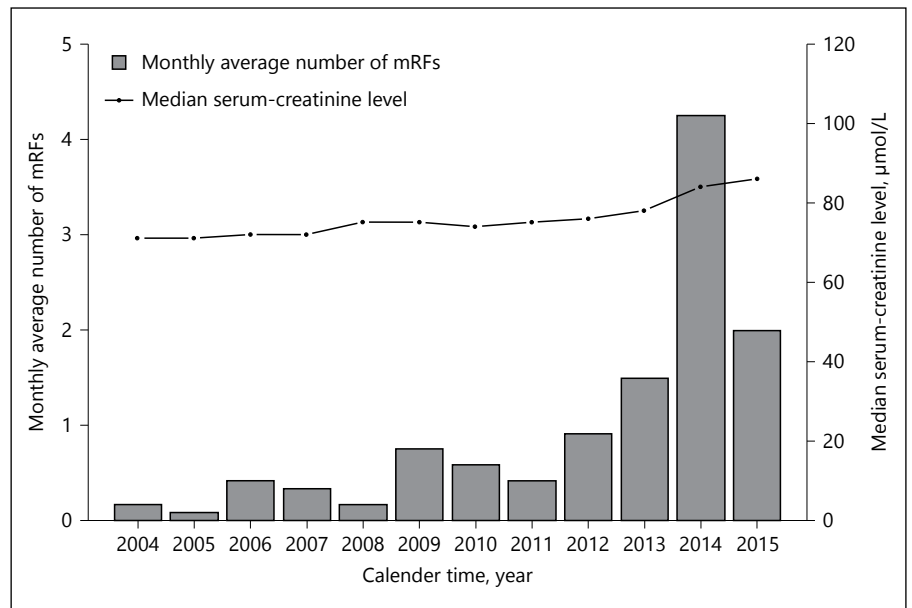


Fig. 1. Median serum creatinine level and monthly average number of measured renal functions January 2004–May 2015.

been evaluated in several controlled trials [10–16]. To our knowledge, the performance of these equations has not been evaluated with real-life data from an everyday clinical setting. Furthermore, no studies have evaluated the effect of antiretroviral drugs (ARVs) that elicit a benign increase in serum-creatinine on the performance of these equations.

During the past 3 years, we have observed an increase in median serum-creatinine in the HIV-infected individuals at our clinic, which has been accompanied by a dramatic increase in the use of mRFs, assessed by ^{51}Cr -EDTA clearance (Fig. 1). We hypothesised that treatment with ARVs that elicit a benign elevation in serum-creatinine (rilpivirine [RLP], cobicistat [COB], and dolutegravir [DTG]) has induced this trend.

The objectives of the current study was (1) to evaluate the agreement between mRF and estimated renal function (eRF) in HIV-infected individuals at the HIV-outpatient clinic at the University Hospital of Copenhagen, Rigshospitalet, (2) to evaluate whether treatment with drugs that elicit a benign elevation in serum-creatinine (RLP/COB/DTG) or potentially nephrotoxic drugs (protease inhibitors [PIs] and tenofovir disoproxil fumarate [TDF]) are associated with a poorer agreement between mRF and eRF and evaluate whether this could explain the increased number of mRFs at our clinic, and (3) to evaluate whether potential differences between mRF and eRF were smaller for intra-individual measurements compared with inter-individual measurements.

Methods

Setting

As of June 1, 2015, Denmark had a prevalence of known HIV-infected individuals of 0.1% in the adult population, corresponding to approximately 4,600 individuals. The current study was conducted at the HIV-outpatient clinic at the University Hospital of Copenhagen, Rigshospitalet (further-on referred to as the clinic), caring for approximately one third of Danish HIV-infected individuals. In the study period, HIV-infected individuals were intended to be seen at the clinic twice a year. Antiretroviral treatment is provided free of charge at all Danish HIV centers.

Study Population

The Danish HIV Cohort Study

Demographic and clinical data were extracted from the Danish HIV Cohort Study (DHCS), which is a prospective study of all HIV-infected individuals 16 years or older at HIV diagnosis and treated at Danish HIV centers after January 1, 1995. Individuals were consecutively enrolled. More than 98% of the HIV-infected individuals seen at the clinic have been assigned a unique Danish 10-digit personal identification number. The study is described in detail elsewhere [17, 18]. From DHCS we identified all HIV-infected individuals who (1) had a Danish personal identification number, (2) attended the clinic during January 1, 2004 to June 1, 2015 and (3) had an mRF performed at Rigshospitalet in this period, and (4) were aged 18 or more at the time of mRF. This constituted our study population.

Other Data Sources

Estimated Renal Function

The serum-creatinine immediately prior to the first mRF performed on each individual was identified and eRF was calculated from the following equations: (1) the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation ($\text{eRF}_{\text{CKD-EPI}}$) [6], (2) the 4-variable modification of diet in renal disease (MDRD)

equation (eRF_{MDRD}) [5], (3) the Cockcroft-Gault equation (eRF_{CG}) [4], (4) the Bjørnson equation ($eRF_{Bjornson}$) [19], (5) the Mawer equation (eRF_{Mawer}) [20], (6) the Hull equation (eRF_{Hull}) [21], (7) the Gates equation (eRF_{Gates}) [22], (8) the Jelliffe-1973 equation ($eRF_{Jelliffe}$) [23], and (9) the Effersø equation ($eRF_{Effersø}$) [3].

Moreover, we extracted the eRF immediately prior to the mRF as calculated by the Department of Clinical Biochemistry and reported in the patient file (eRF_{lab}). The Department of Clinical Biochemistry calculated eRF with the MDRD-equation. They did not have access to data on race and so correction for race was not performed.

Measured Renal Function

Data on mRFs were provided by the Department of Nuclear Physiology at University Hospital, Rigshospitalet. mRF is measured by ^{51}Cr -EDTA clearance. For this measurement, 2–4 MBq ^{51}Cr -EDTA is injected as a single shot in a peripheral vein catheter. Three hours and 20 min after injection, a blood sample is drawn. If the renal function is reduced, a new blood sample is drawn every 20 min up to a maximum of 5 h after the initial injection. The patient is instructed (1) not to smoke, (2) not to attend strenuous physical activity, and (3) not to have any examinations performed that require X-ray contrast. They reported both a BSA-adjusted and a BSA-unadjusted ^{51}Cr -EDTA clearance; BSA was calculated by the Dubois equation. For statistical analyses, we used BSA-adjusted mRF rather than the BSA-unadjusted mRF. For each mRF, the indication for the test was extracted from the patient files and categorized into the following (1) decreased/falling eRF, (2) monitoring of existing renal disease, (3) screening before treatment with nephrotoxic drugs, (4) screening before nephrotoxic procedures, and (5) other/unspecified.

Statistics

For the statistical analyses, we only used the first mRF for each individual. Standardization of serum-creatinine measurements was implemented at Rigshospitalet January 1, 2004, and that is why we only included mRFs performed after January 1, 2004. Inspired by Gaspari et al. [24] performance characteristics for the different renal function estimation equations were evaluated by the following parameters:

1. Concordance correlation coefficient (CCC) defined as: $\rho_c = p * C_b$, where p is the Pearson correlation coefficient, a measure of how far each observation deviates from the best-fit line (a measure of precision) and C_b is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy [25].
2. Total deviation index (TDI): this statistic measures how large the difference between eRF and mRF can be in order to obtain a predefined coverage of the data. In the statistical analyses, we defined the coverage of data to be 90%, meaning that only 10% of measures can deviate more than TDI. Further on we will refer to TDI as TDI_{90} . Low values indicate good agreement between eRF and mRF.
3. Coverage probability (CP): this statistic measures the probability that the difference between mRF and eRF is within a predefined boundary. In the statistical analyses, we defined the boundary to be 10%. Further on, we will refer to CP as CP_{10} . High values indicate a good agreement between eRF and mRF.
4. Relative accuracy: the cumulative percentage of eRFs falling within 10% (P_{10}) and 30% (P_{30}) of mRF.

5. Root mean squared error: this serves as a measure of how far the difference between eRF and mRF is from zero. Difference between mRF and eRF vs. average of mRF and eRF was depicted with Bland–Altman plots for $eRF_{CKD-EPI}$, eRF_{MDRD} , eRF_{CG} .

In order to evaluate, whether treatment with drugs that elicit benign increase in serum-creatinine and potentially nephrotoxic drugs are associated with a poorer agreement between mRF and eRF, we calculated P_{10} and P_{30} for individuals exposed and unexposed to (RLP, COB, or DLT), any PI or TDF at the time of eRF measurement. Additionally, we calculated p values for difference in P_{10} and P_{30} for the exposed vs. the unexposed individuals.

To evaluate inter- vs. intra-individual differences of mRF vs. eRF, we identified all individuals who had 2 or more mRFs performed. We then calculated the bias ($mRF - eRF$) for the first measurement as a measure of inter-individual difference; second, we calculated the difference between the bias for the first and the second measurement as a measure of the intra-individual difference. The inter- and intra-individual differences were calculated for $eRF_{CKD-EPI}$, eRF_{MDRD} , eRF_{CG} , and $eRF_{Effersø}$ equations; we then plotted the differences and reported this together with the mean and SD for the differences.

Statistical analyses were performed using R version 3.2.2.

Results

Characteristics

We identified 98 HIV-infected individuals during the study period, which had at least one mRF performed. The median time elapsed between mRF and eRF was 21 days (interquartile range 14–30). Most individuals were male (81.6%), men who have sex with men (55.1%), and Caucasians (86.7%). Eighty-three individuals (84.7%) had viral load below 50 copies/ μL . Eight individuals (8.2%) were exposed to either RLP/COB/DTG and 62 individuals (63.3%) were exposed to TDF. Seven (7.1%) were not receiving any ARVs at time of eRF. eRF_{CG} , $eRF_{Bjornson}$, eRF_{Mawer} , and eRF_{Hull} could not be calculated for 15 individuals due to lack of information on weight. Overall, eRF underestimated renal function assessed by mRF. This trend was more pronounced for individuals exposed to RLP/COB/DTG or TDF. The characteristics of the study population are summarized in Table 1.

Performance Characteristics of eRF Equations

Overall, the equations showed poor performance characteristics with CCC ranging from 0.18 to 0.67 and CP_{10} ranging from 0.09 to 0.45. This means that if we accept a difference of 10% between mRF and eRF we would at the most capture 45% of the data. TDI_{90} ranged from 26.94 to 59.23. This means that if we want to capture 90% of data we would have to accept a difference between 26.94 and

Table 1. Patient characteristics

Individuals	All individuals (<i>n</i> = 98)	RLP/COB/DTG exposed (<i>n</i> = 8)	TDF exposed (<i>n</i> = 62)	PI exposed (<i>n</i> = 45)
Age, years ^a	54.0 (45.5–63.5)	42.8 (34.0–54.6)	52.7 (46.7–61.9)	54.1 (47.9–60.6)
Males	80 (81.6)	6 (75.0)	51 (82.3)	38 (84.4)
Serum-creatinine level, $\mu\text{mol/L}$ ^a	114.5 (107.0–124.8)	107.5 (99.5–116.2)	110.5 (106.0–116.0)	120.0 (109.0–136.0)
Measured GFR, mL/min/1.73 m ^{2a}	66.0 (54.8–82.5)	79.0 (70.2–96.5)	73.5 (60.2–86.2)	62.0 (50.0–79.0)
eRF according to different equations				
Department of Clinical Biochemistry*	58.0 (51.8–62.8)	61.5 (56.5–71.0)	59.0 (55.2–63.0)	54.0 (49.0–61.0)
CKD-EPI equation	63.7 (55.1–72.9)	74.0 (62.1–84.5)	68.0 (59.9–72.9)	60.4 (52.7–70.4)
MDRD equation	61.6 (55.0–67.5)	65.1 (59.7–75.2)	63.1 (59.2–67.7)	59.1 (51.3–66.0)
Cockcroft-Gault equation	65.8 (50.6–75.8)	98.3 (72.7–98.9)	69.0 (59.6–81.4)	65.8 (49.4–76.7)
Björnsson equation	68.5 (53.8–78.6)	98.8 (78.2–99.0)	71.0 (61.8–83.9)	68.2 (51.3–79.1)
Mawer equation	70.4 (46.4–91.1)	102.3 (84.5–116.7)	74.5 (56.9–92.3)	74.1 (47.5–90.9)
Hull equation	66.0 (55.5–76.3)	81.1 (63.6–87.6)	70.5 (61.4–78.3)	61.9 (53.4–73.1)
Gates equation	100.6 (94.8–106.7)	105.8 (99.6–115.6)	102.4 (98.0–107.8)	98.0 (94.1–105.9)
Jelliffe-1973 equation	56.3 (46.2–64.3)	67.4 (53.8–73.6)	57.7 (51.2–64.7)	51.7 (44.4–59.8)
Effersø equation	59.1 (53.1–63.9)	61.9 (59.0–66.2)	60.4 (57.2–64.3)	56.9 (48.1–62.6)
Time from eRF to mRF, days ^a	21 (14–30)	24 (12–30)	21 (15–35)	21 (15–32)
Route of infection				
MSM	54 (55.1)	5 (62.5)	37 (59.7)	24 (53.3)
IDU	3 (3.1)	0 (0.0)	0 (0.0)	2 (4.4)
Heterosexually	35 (35.7)	3 (37.5)	21 (33.9)	16 (35.6)
Others/unknown	6 (6.1)	0 (0.0)	4 (6.5)	3 (6.7)
Race				
White	85 (86.7)	8 (100.0)	53 (85.5)	39 (86.7)
African	12 (12.2)	0 (0.0)	8 (12.9)	6 (13.3)
Others/unknown	1 (1.0)	0 (0.0)	1 (1.6)	0 (0.0)
Weight, kg ^{a, b}	73.0 (64.0–81.0)	77.0 (71.0–81.5)	73.5 (64.8–81.1)	74.0 (62.0–82.0)
Viral load <50 copies/ μL	83 (84.7)	8 (100.0)	58 (93.5)	36 (80.0)
Exposed to RLP/COB/DTG	8 (8.2)	8 (100.0)	7 (11.3)	2 (4.4)
Exposed to any PI	45 (45.9)	2 (25.0)	24 (38.7)	45 (100.0)
Exposed to TDF	62 (63.3)	7 (87.5)	62 (100.0)	24 (53.3)

Data are *n* (%) unless specified otherwise.

* During the study period, the Department of Clinical Biochemistry calculated eRF with the MDRD equation but without adjustment for race.

GFR, glomerular filtration rate; eRF, estimated renal function; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; MDRD, Modification of Diet in Renal Disease; mRF, measured renal function; MSM, men who have sex with men; IDU, intravenous drug users; TDF, tenofovir disoproxil fumarate; RLP, rilpivirine; COB, cobicistat; DTG, dolutegravir; PI, protease inhibitor.

^a Median (interquartile range).

^b 15 individuals did not have a weight available.

59.23%. P_{30} ranged from 26 to 83% meaning that 26–83% of eRF fell within 30% of mRF. eRF_{LAB}, eRF_{CKD-EPI}, and eRF_{MDRD} all had poor performance characteristics that did not differ substantially from each other. CCC ranged from 0.41 to 0.49, CP₁₀ ranged from 0.36 to 0.42, TDI₉₀ ranged from 30.24 to 35.16, and P_{30} ranged from 70 to 80%. The equation that performed the worst of the 3 was eRF_{CKD-EPI}. The equations that rely on weight to calculate eRF (eRF_{CG}, eRF_{Björnsson}, eRF_{Mawer}, and eRF_{Hull}) were the equations that overall performed the best. The oldest equation eRF_{Effersø},

which only rely on serum-creatinine and gender, had performance characteristics that did not differ substantially from eRF_{CKD-EPI} (Performance characteristics are summarized in Table 2). Bland-Altman plots for eRF_{CKD-EPI}, eRF_{MDRD}, eRF_{CG}, and eRF_{Effersø} all showed wide limits of agreement ranging from –39.01 to 45.45 with many points lying far from the mean line, which also indicated poor agreement between mRF and eRF (Fig. 2). In sensitivity analyses where we only included individuals that had an mRF performed with the indication falling/lowered eRF

Table 2. Performance characteristics of the different equations to estimate renal function

Estimation equation	Number of individuals	CCC	TDI ₉₀	CP ₁₀	P ₁₀ , n (%)	P ₃₀ , n (%)	RMSE
Department of Clinical Biochemistry*	98	0.42 (0.33)	33.59 (36.59)	0.37 (0.34)	36 (37)	72 (73)	16.9
CKD-EPI equation	98	0.41 (0.27)	35.16 (39.28)	0.36 (0.32)	29 (30)	69 (70)	18.6
MDRD equation	98	0.49 (0.38)	30.24 (33.28)	0.42 (0.38)	36 (37)	78 (80)	16.8
Cockcroft-Gault equation	83	0.67 (0.58)	26.94 (30.65)	0.45 (0.40)	28 (34)	66 (80)	13.7
Björnsson equation	83	0.64 (0.54)	28.68 (32.61)	0.43 (0.38)	29 (35)	69 (83)	14
Mawer equation	83	0.42 (0.33)	56.09 (63.79)	0.23 (0.20)	23 (28)	49 (59)	15.3
Hull equation	83	0.43 (0.29)	32.51 (36.79)	0.38 (0.34)	30 (36)	62 (75)	16.7
Gates equation	98	0.18 (0.14)	59.23 (61.95)	0.09 (0.07)	7 (7)	25 (26)	16.2
Jelliffe-1973 equation	98	0.38 (0.27)	36.59 (40.16)	0.34 (0.31)	18 (18)	66 (67)	17.9
Effersø equation	98	0.35 (0.25)	34.08 (36.86)	0.37 (0.35)	31 (32)	73 (74)	17.9

* During the study period, the Department of Clinical Biochemistry calculated eRF with the MDRD equation but without adjustment for race.

CCC, concordance correlation coefficient; TDI, total deviation index; CP, coverage probability; P₁₀, relative accuracy 10%; P₃₀, relative accuracy 30%; RMSE, root mean squared error; MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

and only individuals that did not report IDU as route of infection, we found results that did not differ substantially from results with all individuals included.

Change in Agreement between mRF and eRF According to Drug Exposure

Eight of 98 individuals were exposed to RLP/COB/DTG at the time of serum-creatinine measurement. We were not able to find any significant difference between the exposed and unexposed individuals for the agreement between mRF and eRF for any of the 10 eRFs included, evaluated with either P₁₀ or P₃₀; however, average mRF was higher for RLP/COB/DTG exposed individuals compared to the unexposed. Sixty-two of 98 individuals were exposed to TDF. TDF-exposed individuals on average had higher mRF compared with TDF-unexposed individuals. Exposure to TDF was associated with a significantly different agreement for 2 of the equations that rely on weight (eRF_{Björnsson} and eRF_{Hull}) and eRF_{Gates}, evaluated with P₃₀. For eRF_{CG} that also relies on weight, the difference was only of borderline significance. For eRF_{Mawer}, the final equation that relies on weight, there was no significant difference. For these formulas, the agreement between mRF and eRF was better for individuals exposed to TDF than for individuals unexposed to TDF. Forty-five of 98 individuals were exposed to any PI. We were not able to find any differences in either P₁₀ or P₃₀ for individuals exposed to any PI compared to unexposed individuals (Tables 1, 3).

Inter- and Intra-Individual Reproducibility

We identified 14 individuals who had 2 or more mRFs performed. The inter-individual difference was large with SDs ranging from 14.6 to 19, the intra-individual difference was far smaller than the inter-individual difference with SDs ranging from 7.49 to 9.9 (Fig. 3). For 7 of the individuals, the bias was almost the same for each measurement. For 6 of the individuals, the bias was substantially different from measurement to measurement; for the final individual, the bias was almost the same for most measurements except for 2 outliers (data not shown).

Discussion

In this single-center study, we found that the agreement between mRF and eRF for all evaluated equations was poor. For the eRF_{Lab}, this difference was substantial. We found that the equation with the least disagreement between mRF and eRF in the current setting was eRF_{CG}, eRF_{CKD-EPI}, which is currently recommended by the EACS guidelines to calculate eRF in HIV-infected individuals, performed worse than both eRF_{MDRD}, eRF_{CG}, and eRF_{Lab}. Exposure to ARVs, which elicit a benign increase in serum-creatinine, that is, RLP/COB/DTG, was not associated with a significantly different agreement between mRF and eRF; however, for some of the equations, TDF exposure was associated with a better agreement between mRF and eRF. To our knowledge, this is the first study to

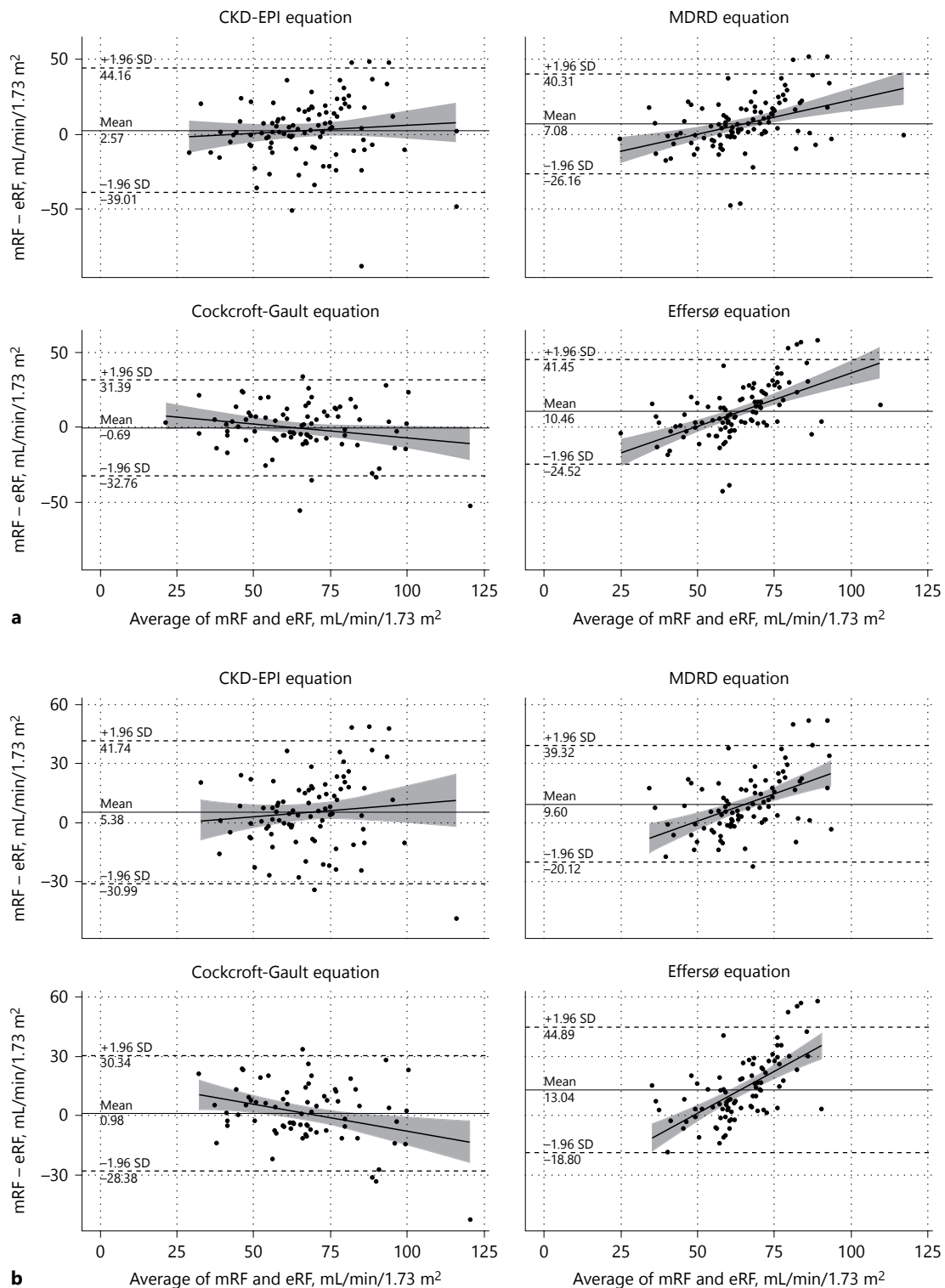


Fig. 2. Bland Altman plots: difference between measured and estimated renal function vs. average of measured and estimated renal function for 4 renal function estimation equations. **(a)** All individuals included, **(b)** non-IDUs and individuals for which the indication was lowered/falling eRF included.

Table 3. Agreement between mRF and eRF for individuals exposed and individuals unexposed to 3 drug categories

	RLP/COB/DTG		TDF		PI	
	P ₁₀	P ₃₀	P ₁₀	P ₃₀	P ₁₀	P ₃₀
Department of Clinical Biochemistry*						
Exposed	2 (25.0)	7 (87.5)	22 (35.5)	49 (79.0)	17 (37.8)	32 (71.1)
Unexposed	34 (37.8)	65 (72.2)	14 (38.9)	23 (63.9)	19 (35.8)	40 (75.5)
<i>p</i> value for difference between exposed and unexposed	0.706	0.677	0.829	0.154	1.000	0.653
CKD-EPI equation						
Exposed	1 (12.5)	6 (75.0)	20 (32.3)	48 (77.4)	15 (33.3)	33 (73.3)
Unexposed	28 (31.1)	63 (70.0)	9 (25.0)	21 (58.3)	14 (26.4)	36 (67.9)
<i>p</i> value for difference between exposed and unexposed	0.430	1.000	0.498	0.066	0.510	0.659
MDRD equation						
Exposed	2 (25.0)	7 (87.5)	25 (40.3)	52 (83.9)	18 (40.0)	37 (82.2)
Unexposed	34 (37.8)	71 (78.9)	11 (30.6)	26 (72.2)	18 (34.0)	41 (77.4)
<i>p</i> value for difference between exposed and unexposed	0.706	1.000	0.389	0.198	0.674	0.621
Cockcroft-Gault equation						
Exposed	2 (40.0)	4 (80.0)	20 (38.5)	45 (86.5)	14 (34.1)	32 (78.0)
Unexposed	26 (33.3)	62 (79.5)	8 (25.8)	21 (67.7)	14 (33.3)	34 (81.0)
<i>p</i> value for difference between exposed and unexposed	1.000	1.000	0.337	0.052	1.000	0.791
Björnsson equation						
Exposed	3 (60.0)	4 (80.0)	22 (42.3)	47 (90.4)	10 (24.4)	34 (82.9)
Unexposed	26 (33.3)	65 (83.3)	7 (22.6)	22 (71.0)	19 (45.2)	35 (83.3)
<i>p</i> value for difference between exposed and unexposed	0.337	1.000	0.096	0.033	0.065	1.000
Mawer equation						
Exposed	1 (20.0)	3 (60.0)	16 (30.8)	33 (63.5)	12 (29.3)	23 (56.1)
Unexposed	22 (28.2)	46 (59.0)	7 (22.6)	16 (51.6)	11 (26.2)	26 (61.9)
<i>p</i> value for difference between exposed and unexposed	1.000	1.000	0.459	0.358	0.810	0.658
Hull equation						
Exposed	2 (40.0)	4 (80.0)	23 (44.2)	45 (86.5)	11 (26.8)	32 (78.0)
Unexposed	28 (35.9)	58 (74.4)	7 (22.6)	17 (54.8)	19 (45.2)	30 (71.4)
<i>p</i> value for difference between exposed and unexposed	1.000	1.000	0.060	0.002	0.110	0.615
Gates equation						
Exposed	1 (12.5)	3 (37.5)	5 (8.1)	21 (33.9)	2 (4.4)	11 (24.4)
Unexposed	6 (6.7)	22 (24.4)	2 (5.6)	4 (11.1)	5 (9.4)	14 (26.4)
<i>p</i> value for difference between exposed and unexposed	0.460	0.417	1.000	0.016	0.447	1.000
Jelliffe-1973 equation						
Exposed	2 (25.0)	7 (87.5)	10 (16.1)	45 (72.6)	10 (22.2)	30 (66.7)
Unexposed	16 (17.8)	59 (65.6)	8 (22.2)	21 (58.3)	8 (15.1)	36 (67.9)
<i>p</i> value for difference between exposed and unexposed	0.637	0.267	0.589	0.182	0.437	1.000
Effersø equation						
Exposed	1 (12.5)	6 (75.0)	19 (30.6)	47 (75.8)	17 (37.8)	33 (73.3)
Unexposed	30 (33.3)	67 (74.4)	12 (33.3)	26 (72.2)	14 (26.4)	40 (75.5)
<i>p</i> value for difference between exposed and unexposed	0.429	1.000	0.824	0.811	0.278	0.821

Data are are *n* (%).

* During the study period, the Department of Clinical Biochemistry calculated eRF with the MDRD equation but without adjustment for race.

RLP, rilpivirine; COB, cobicistat; DTG, dolutegravir; TDF, tenofovir disoproxil fumarate; P₁₀, relative accuracy P10; P₃₀, relative accuracy P30; MDRD, modification of diet in renal disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

present real-life data from an everyday clinical setting on the performance characteristics of creatinine-based renal function estimation equations in HIV-infected individuals, and to evaluate the effect of different ARVs on agreement between mRF and eRF.

Several studies have evaluated the agreement between mRF and eRF in HIV-infected populations with very differing results [10–16]. A recent large European study with 203 individuals by Gagneux-Brunon et al. [16] found that P₃₀ ranged between 75 and 82%, which corresponds well

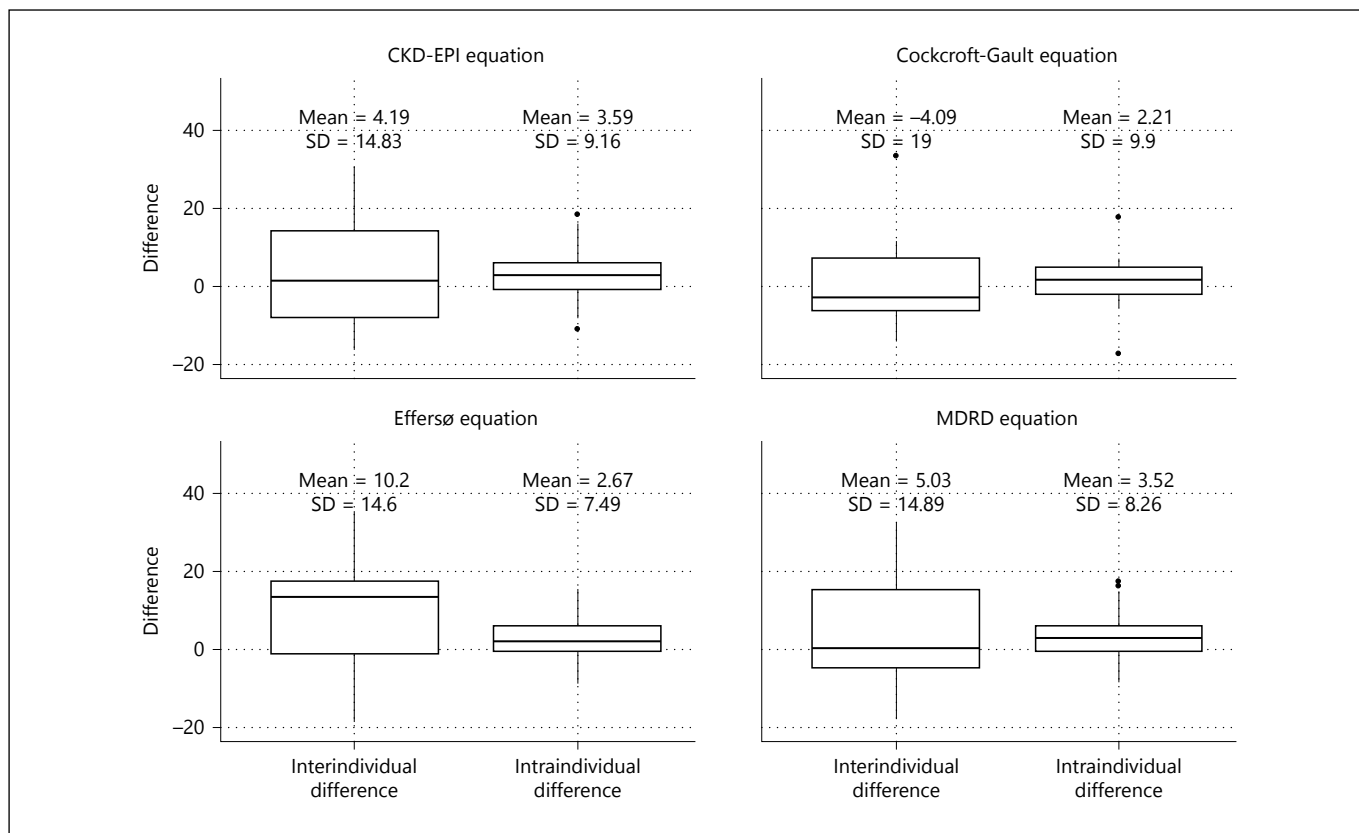


Fig. 3. Inter- and intra-individual differences. The inter-individual difference is the bias ($mRF - eRF$) for the first measurement for each individual, the intra-individual difference is the difference between the bias for the first and the second measurement for each individual.

with the findings of this study. However, Gagneux-Brunon et al. [16] also found that $eRF_{CKD-EPI}$ outperforms the eRF_{MDRD} , which is not in agreement with our findings, as we found that $eRF_{CKD-EPI}$ performed worse than eRF_{MDRD} . Gagneux-Brunon [16] did not evaluate the performance of eRF_{CG} , which has been found to perform better than $eRF_{CKD-EPI}$ and eRF_{MDRD} in other studies including the current study [12]. In general, all studies have found large discrepancies between mRF and eRF , that is, the P_{30} of the best-performing equations was around 80%, meaning that approximately 20 percent of eRF s deviates more than or equal to 30% from mRF . Also, the large degree of discrepancy between the different studies regarding which equation performs best is of concern.

The CKD-EPI study group found a P_{30} of 80.4% in their original work [6]; this is in agreement with subsequent studies that have observed P_{30} ranging from 72 to 89%.

The small number of individuals who received treatment with ARVs known to induce a benign increase in

creatinine levels (RLP/COB/DTG) did not allow us to establish whether exposure to these drugs was associated with larger discrepancies between mRF and eRF . In contrast, we found a trend that TDF exposure was associated with a significantly different agreement between exposed and unexposed individuals for the equations that rely on weight to calculate eRF (Table 3). In these analyses, the trend was that exposure to TDF was associated with better agreement between mRF and eRF . In a study evaluating the safety of tenofovir alafenamide (TAF), Sax et al. [26] found that eRF_{CG} decreased a median of 11 mL/min immediately after initiating TDF; TAF is associated with a smaller decrease in eRF_{CG} of approximately 6 mL/min. The results from the current study suggest that this effect on eRF_{CG} is a benign increase in creatinine and does not reflect a true decrease in renal function. Also, the observed bias associated with TDF exposure may be due to the awareness created by the treating physicians of the nephrotoxic effects of TDF, thereby prompting the need for an mRF . This study is performed

in a real-life setting and that is why eRF and mRF were not necessarily tested the same day and we cannot exclude the fact that our findings are influenced by regression toward the mean.

A major strength of the study is the Danish unique person identification number, which enabled us to track all individuals who had an mRF performed in the study period and allowed us to link our data with all laboratory test results. Furthermore, the design of DHCH made it possible to identify all HIV-infected individuals treated at our clinic and track all clinical data. Also, the large number of individuals who had an mRF performed during the study period makes our estimates rather robust. We did not have weight available for 15 of the included individuals, which may pose a bias with regards to our results for eRF_{CG}. However, in sensitivity analyses excluding individuals with no weight available, we found estimates that did not differ substantially from the primary findings of our study. Cystatin C has not been implemented as a routine measurement at our clinic, and that is why we were not able to evaluate the performance of eRF based on cystatin C.

Conclusions

We found that the agreement between mRF and eRF was poor, irrespective of the equation used to calculate eRF. Due to a few study individuals exposed to RLP/COB/DTG, we were not able to demonstrate a statistically significant association between exposure to these drugs and differences in agreement between mRF and eRF. How-

ever, the small number of study individuals exposed to RLP/COB/DTG makes it unlikely that the use of these drugs increased the frequency of mRF tests at our clinic. HIV clinicians should use serum-creatinine-based eRF with caution and important clinical decisions should not be based on eRF.

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Statement of Ethics

According to Danish legislations individual consent is not required for register based research. The study was approved by the Danish Data Protection Agency (journal no 2008-41-1781).

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16. DECLARATION OF CO-AUTHORSHIPS



DECLARATION OF CO-AUTHORSHIP

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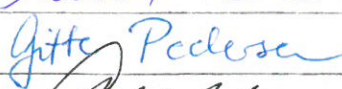
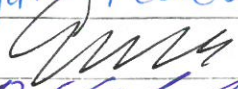

Title of PhD thesis:
Estimation of Renal function and renal complications in Danish HIV-infected individuals.

This declaration concerns the following article:
Increased risk of dialysis and end-stage renal disease among HIV patients in Denmark compared with the background population

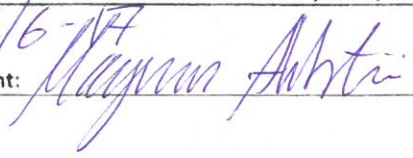

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	A
2. Planning of the experiments and methodology design, including selection of methods and method development	B
3. Involvement in the experimental work	B
4. Presentation, interpretation and discussion in a journal article format of obtained data	B

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
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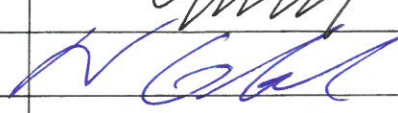
Title of PhD thesis:
Estimation of Renal function and renal complications in Danish HIV-infected individuals.

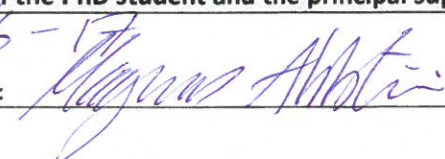
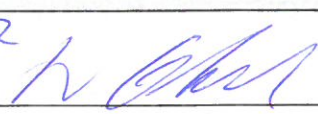
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The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
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2. Planning of the experiments and methodology design, including selection of methods and method development	B
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Estimation of Renal function and renal complications in Danish HIV-infected individuals.

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Routine urine protein/creatinine ratio testing in an outpatient setting of Danish HIV-infected individuals

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

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Agreement between Estimated and Measured Renal Function in an Everyday Clinical Outpatient Setting of Human Immunodeficiency Virus-Infected Individuals

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