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Prevalence and Incidence of Renal Dysfunction in Patients initiating Antiretroviral Therapy at a Primary Health Care Centre in Gugulethu, Cape Town: a Cohort Study

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PREAMBLE

Declaration

I, Monika Kamkuemah (KMKMON001), hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Background

Tenofovir disoproxil fumarate (TDF) is used worldwide for the treatment of HIV-1 infection. Tenofovir has been found to be associated with declines in renal function and chronic kidney disease in HIV-infected patients. There are limited data on how soon after antiretroviral therapy (ART) initiation any loss of renal function can be detected. We studied a cohort of HIV-infected adults initiating TDF-containing ART regimens at the Hannan Crusaid Antiretroviral Treatment Centre in Gugulethu. The centre provides ART to the residents of the Gugulethu and Nyanga districts situated on the outskirts of Cape Town. We described the prevalence and incidence of renal dysfunction in this cohort, the patterns of change in their renal function in the first 12 months on therapy and factors associated with renal dysfunction. We also examined the diagnostic value of early serum creatinine tests in identifying incident renal dysfunction after 12 months.

Methods

All consecutive HIV-infected, adult patients who initiated ART regimens with TDF at Hannan Crusaid between February 2010 and April 2012 were eligible for the study. Renal function was assessed for the first 12 months on ART by estimating glomerular filtration rate (eGFR) calculated using the Cockroft-Gault equation. Creatinine clearance was categorised into normal, mild, moderate and severe renal dysfunction according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Criteria based on values of eGFR >90, 60-89, 30-59 or <30 ml/min/1.73m^2, respectively. We examined predictors of renal dysfunction using multivariable logistic regression. Linear mixed effect regression models were used to describe the mean eGFR trajectory over 12 months on TDF, using stratified models for sub-group analysis and maximum likelihood estimation. We used imputed data to assess the usefulness of early serum creatinine tests in predicting the change in eGFR at 12 months.

PART I presents the study protocol with a brief motivation for the relevance of the study and the methodology used in the analysis.

PART II presents a structured literature review on HIV and the kidney in the pre- and post-antiretroviral therapy eras. It provides an overview of empirical evidence on tenofovir and its effects on kidney function from both the developed and developing world.

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PART III summarizes the methodology, results and interpretation of the analysis conducted in a journal-ready manuscript according to BioMed Central's *AIDS Research and Therapy* journal requirements.

Findings

The cohort consisted of 62% women and 38% men, median age at baseline was 34 years (IQR 29; 41 years). Most of the patients had normal baseline renal function; eGFR >90 μmol/L (62%), 34% had mildly impaired eGFR and 4% had moderate-severe renal function impairment. Age greater than 41 years, female gender, higher WHO stage (III and IV) and anaemia were all independently associated with increased probability of moderate or severe renal dysfunction at baseline. The estimated glomerular function improved in most sub-groups of patients over the first 12 months on TDF (0.960 ml/min/1.73m^2 mean increase over 12 months (95% CI: 0.67; 1.26) and this increase was not significantly confounded by baseline covariates. Female gender, higher baseline serum creatinine and age greater than 29 years were associated with faster growth in mean eGFR over 12 months. Overall incidence of eGFR decline over 12 months was low (4.4% developed eGFR <50 ml/min/1.73m^2) and the crude incidence rate for a decline >10 ml/min/1.73m^2 in 12 months was 3.60 per 100 person years. Earlier creatinine tests that were done before 4 months on ART had limited diagnostic value in predicting overall renal function change after a year on ART.

Conclusions

Renal dysfunction was uncommon in HIV-infected adults initiating ART in this primary health care setting. Renal function generally improved during the first year on ART even in those with lowest creatinine clearance at initiation. Creatinine tests done earlier than four months after baseline screening may be unnecessary. This is similar to the results found in previous studies and has important public health implications in settings were creatinine tests might be less accessible.

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University of Cape Town

Contents

University of Cape Town

II LITERATURE REVIEW

University of Cape Town

III JOURNAL MANUSCRIPT

University of Cape Town

IV APPENDIX

University of Cape Town

List of Tables

RESEARCH PROTOCOL

LITERATURE REVIEW

JOURNAL MANUSCRIPT

APPENDIX

University of Cape Town

List of Figures

JOURNAL MANUSCRIPT

APPENDIX

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List of Abbreviations

3TC lamivudine.

AACTG Adult AIDS Clinical Trials Group.

ABC abacavir.

AIDS acquired immunodeficiency syndrome.

AKI acute kidney injury.

ANOVA analysis of variance.

ARF acute renal failure.

ART antiretroviral therapy.

ATV atazanavir.

ATV/r ritonavir-boosted atazanavir.

AZT zidovudine.

BMI body mass index.

CD4 T-lymphocite cell bearing CD4 receptor.

CDC United States Centers for Disease Control and Prevention.

CG Cockroft-Gault equation.

CKD chronic kidney disease.

CKD-EPI Chronic Kidney Disease Epidemiology Collaboration equation.

CrCl creatinine clearance.

CVD cardiovascular disease.

d4T stavudine.

DART Development of Antiretroviral Therapy in Africa trial.

EFV efavirenz.

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eGFR estimated glomerular filtration rate.

FTC emtricitabine.

GEE generalized estimating equations.

GFR glomerular filtration rate.

HAART highly active antiretroviral therapy.

HBAC Home Based AIDS Care trial.

HBV hepatitis B virus.

HCTC Hannan Crusaid Treatment Centre.

HCV hepatitis C virus.

HIVAN HIV-associated nephropathy.

HPT hypertension.

IDU intravenous drug user.

IDV indinavir.

K/KDOQI National Kidney Foundation Kidney Disease Outcomes Quality Initiative.

LPV/r ritonavir-boosted lopinavir.

LTFU lost to follow-up.

MDRD Modification of Diet in Renal Disease Study Equation.

mice Multiple imputation by chained equations.

NHLS National Health Laboratory Service.

NRTI nucleoside reverse-transcriptase inhibitor.

NtRTI nucleotide analogue reverse-transcriptase inhibitor.

NVP nevirapine.

PI protease inhibitor.

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PI/r ritonavir-boosted protease inhibitor.

SCr serum creatinine.

TDF tenofovir disoproxil fumarate.

UK United Kingdom.

US United States.

US FDA United States food and drug administration.

WHO World Health Organization.

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Part I

RESEARCH PROTOCOL

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1. Prevalence and Incidence of Renal Dysfunction in Patients initiating Antiretroviral Therapy at a Primary Health Care Centre in Gugulethu, Cape Town: a Cohort Study

1 Protocol Summary

The South African government included tenofovir disoproxil fumarate (TDF) as a first-line antiretroviral regimen drug in April 2010. Tenofovir is a known nephrotoxin and observational studies have found tenofovir to be associated with significant, though modest declines in glomerular function. There are few data on patterns of change in renal function after tenofovir initiation. The World Health Organization (WHO) recommends that renal monitoring be conducted before initiation of tenofovir. Patients with compromised renal function and high-risk patients for developing kidney disease should not be started on tenofovir. In South Africa, the antiretroviral therapy (ART) guidelines recommend that serum creatinine and creatinine clearance be measured at ART initiation, at months 3 and 6 and yearly thereafter for patients on tenofovir in order to identify toxicity. In a primary health care setting, periodic monitoring might be difficult to maintain. In addition, research has shown that the prevalence of pre-existing kidney disease in HIV-infected patients is higher than in the general population which poses a significant burden on the health care system if compounded by the use of tenofovir. We intend to address key questions regarding the prevalence and incidence of renal dysfunction and patterns of change in renal function in the first year after initiating tenofovir in a primary health care setting in this analysis.

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2 Introduction

2.1 Background

The South African government included tenofovir as a first-line antiretroviral regimen drug in April 2010 [1]. Tenofovir is a nucleoside reverse transcriptase inhibitor which is widely used in combination antiretroviral therapy including as a fixed dose combination pill [2]. The first efficacy and safety trials conducted in HIV-infected patients found tenofovir to be an effective drug with a good safety profile and minimal risk [3–6]. However, several case reports and case series reported

afterwards found tenofovir to be associated with a host of renal complications including acute kidney injury (AKI), renal failure, chronic kidney disease (CKD) and proximal tubular injury [7–12] [1].

Subsequent observational cohort studies have found tenofovir to be associated with significant but modest declines in glomerular function [2]. A pooled measure from a meta-analysis of 17 studies in 2010 found tenofovir to be associated with a mean decrease in creatinine clearance of 3.9 ml/min/1.73 m^2 over the time of treatment exposure [13]. Although it has been established that tenofovir is related to significant but small loss of renal function, the general consensus is that risk of nephrotoxicity should not be a reason to withhold treatment since the benefits of highly active antiretroviral therapy (HAART) may outweigh potential risks. Moreover, renal toxicity resulting from tenofovir can be reversed after cessation of the drug in acute cases [2, 14]. Early detection of any nephrotoxic effects and cessation of TDF treatment are therefore important to avoid kidney damage [15].

The WHO recommends that serum creatinine screening must be conducted before initiation of TDF and that patients with compromised renal function should not be started on TDF [16]. However, laboratory monitoring is not a requirement for the initiation of ART and lack of access to laboratory testing facilities to measure serum creatinine should not be a barrier to TDF use in remote and resource-limited settings [16]. Nevertheless, the current guidelines emphasize that more data are needed on whether routine or periodic laboratory monitoring of renal function among TDF users is required for all individuals or only high-risk patients [17]

High-risk groups identified include those with pre-existing renal disease (also higher serum creatinine levels prior to starting TDF), advanced HIV disease (acquired immunodeficiency syndrome (AIDS) and low CD4 counts), age >40 years, low body weight (<50 kg or body mass index (BMI) <18.5 kg/m^2), having other risk factors for renal disease like untreated hypertension and diabetes, and using a protease inhibitor (PI) or other nephrotoxic drugs in addition to TDF [17]. Patients that fall into these subsets should have their creatinine clearance monitored to detect and limit further progression of renal impairment [6, 16, 18].

It is still not known how early after treatment initiation the loss of renal function can be detected. Several studies have suggested that changes in the renal function after ART initiation (with or without TDF) mostly occur during the first year [19]. However, knowing when to screen for elevated serum creatinine levels within this year would enable optimal monitoring of incident renal dysfunction in order to detect it early and prevent severe disease. Some studies have suggested that renal dysfunction can occur as early as during the first two to three months after initiating TDF in those with normal baseline estimated glomerular filtration rate (eGFR) [18, 20, 21] but there are

[1] Other complications reported in the literature: Fanconi syndrome, hypophosphatemia, normoglycemic glycosuria, proteinuria, and decreased creatinine clearance [7–12]

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few data from resource limited settings in sub-Saharan Africa.

The South African National ART guidelines recommend that serum creatinine and creatinine clearance be measured at ART initiation, after 3 and 6 months and annually thereafter for patients on tenofovir to identify TDF toxicity. A creatinine test and urinalysis should be done and if found to be abnormal, the patient ought to be referred for specialist opinion [22].

In a primary health care, periodic monitoring might be difficult to maintain, particularly where there is limited access to laboratory infrastructure [23]. In attempting to scale up ART in resource-limited settings, simplification of treatment is important for successful implementation of ART [24]. Evidence from resource-limited settings has shown that TDF implementation without prior screening of serum creatinine and routine renal monitoring could still be a viable option, especially in high HIV-prevalence settings where the cost of laboratory monitoring exceeds the benefits [23].

Coupled with this complication is the high prevalence of pre-existing chronic kidney disease in HIV-infected patients ranging from 5 - 15% of patients presenting with a creatinine clearance eGFR <60ml/min/1.73 m^2 [25, 26]. Overall, both acute kidney injury (AKI) and CKD are more prevalent in HIV-infected persons than in the general population [27]. The prevalence of HIV-related kidney diseases varies in sub-Saharan Africa from as high as 34% in Zambia and 20 - 49% in Uganda, to 6% in South Africa [28]. Fabian et al. report that it is hard to quantify the exact prevalence due to varying criteria used for diagnosis of kidney disease in patients with HIV across sub-Saharan Africa [28]. All in all, HIV-infected patients of African descent are at higher risk of renal disease both within Africa and in the diaspora [28, 29].

Our key interests in this analysis are to describe the prevalence of different degrees of renal dysfunction in patients initiating ART in primary health care in South Africa. We are interested in exploring the risk factors associated with impaired renal function and whether it is possible to identify high-risk groups at baseline using disease characteristics, instead of laboratory testing. Our second objective is to describe the incidence of renal dysfunction and patterns of change in renal function in patients starting tenofovir in the first 12 months on therapy. Thirdly, we want to determine the diagnostic role of early serum creatinine measures in identifying incident renal dysfunction and whether we can optimize screening to detect onset of renal impairment.

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2.2 Rationale and Study Objectives

The following objectives are outlined for the study:

1. To describe the prevalence of different degrees of renal dysfunction in patients initiating TDF-containing ART regimens both overall and by patient characteristics: demographic characteristics (age, gender) and HIV disease characteristics (T-lymphocite cell bearing CD4 receptor (CD4) count, WHO staging)
   Key question: How common is prevalent renal dysfunction in patients starting ART, and what factors are associated with this?

2. To describe the patterns of change in renal function in patients initiating TDF-containing ART regimens, with measures at baseline and after 1, 2, 4 and 12 months on ART by patient characteristics: demographic characteristics (age, gender) and HIV disease characteristics (CD4 count, WHO staging)
   Key questions: What is the overall pattern of change in renal function among patients starting TDF? How common is incident renal dysfunction in patients starting TDF (during the first 12 months on therapy) and what factors are associated with this?

3. To examine the diagnostic role of early serum creatinine measures on ART in identifying incident renal dysfunction
   Key questions: What proportion of all incident renal dysfunction detected during the first 12 months on therapy is detected at month 1, 2, 4 and 12? What is the prognostic role of early changes in serum creatinine in the subsequent detection of renal dysfunction?

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3 Methods

3.1 Study Design

This is a retrospective cohort study based on routinely collected clinical data. We will review clinical data from HIV-infected patients enrolled in a community-based ART program between February 2010 and April 2012 before ART initiation and for the first year after starting a TDF-containing regimen.

3.2 Population and Sampling

Population

The Gugulethu/Nyanga district has a population of about 420 000, and has the highest prevalence of HIV in the Western Cape, after Khayelitsha, reported at 29% in 2008 [30]. The majority of the population is of low socio-economic status, being either unemployed or earning less than R 1000 per month [31]. The Hannan Crusaid Treatment Centre (HCTC) is based at the Gugulethu Community Health Centre and provides ART to the residents of this district [31, 32].

Patients and Data Collection

Patients become eligible for ART according to the South African Antiretroviral Treatment Guidelines for 2010 (CD4 count below 200 cells/mm^3 irrespective of clinical stage or CD4 count below 350 cells/mm^3 in pregnant women and TB co-infected patients, or WHO stage IV irrespective of CD4 count [1]). If eligible, they are seen twice before initiating ART, at least 4 weeks and at 2 weeks prior to initiation. Thereafter they are seen at scheduled visits at 4 weeks, 8 weeks, 16 weeks and then at 16-week intervals [33]. Some patients do not initiate treatment at Hannan Crusaid either due to death, transferring out and other reasons [32]. During the study period, first-line ART at the HCTC consists of TDF, lamivudine (3TC) and either efavirenz (EFV) or nevirapine (NVP) [34]. Patients that had started regimens containing zidovudine (AZT) or stavudine (d4T) prior to 2010 were kept on these regimens unless they had developed any side-effects [34].

Sampling Strategy and sample size

The sample will consist of all consecutive adult patients (>18 years) enrolled in the ART programme at the HCTC between February 2010 and April 2012. We will use existing information of approximately 1800 patients started on TDF at HCTC since 2010 for whom pre-ART and follow up serum creatinine tests were done.

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3.3 Measurement

Outcome of interest and Independent Variables

The main outcome of interest is renal function assessed by glomerular filtration rate which we will estimate via creatinine clearance. Using the level of serum creatinine (SCr) in the blood, an individual's age, weight and sex, a measure of the creatinine clearance can be calculated [35]. The eGFR will be calculated using the Cockroft-Gault equation (CG) equation [36].

$$\text{Cockcroft-Gault formula: eGFR} = \frac{140 - Age(years) \times Weight(kg)}{(72 \times SCr) \times (0.85 \text{ if female})} \tag{1.1}$$

The degree of renal dysfunction will be determined using the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/KDOQI) criteria [37]. Severe renal dysfunction is defined as eGFR <30 ml/min/1.73 m^2, moderate renal dysfunction as eGFR of 30-59 ml/min/1.73 m^2 and mild renal dysfunction as an eGFR of 60-89 ml/min/1.73 m^2. Other variables of interest are displayed in Table 1.

Table 1.1: List and definition of variables that will be collected in the study

Variables	Scale	Categorical
Demographics		
Age (years)	Numerical- continuous	(quartile category proportions)
Sex	Categorical - binary	Male, Female
Weight (kg)	Numerical - continuous	(quartile category proportions)
Clinical Characteristics		
WHO Stage	Categorical- ordinal	Stages I- IV
CD4 count (cells/mm^3)	Numerical- continuous	<100 cells/mm^3 100-200 cells/mm^3 ≥200 cells/mm^3
viral load (copies/ml)	Numerical- continuous log(10)	<5log; ≥5 log
Haemoglobin (g/dl)	Numerical- continuous	Anaemia classification: Hb[a] <11 pregnant women Hb <12 women Hb <13 men
Serum creatinine (μmol/L)	Numerical- continuous	quartile category proportions
CrCl[b] (eGFR in ml/min/1.73m^2)	Numerical- continuous	Severe: <30 ml/min/1.73m^2 Moderate: 30-59 ml/min/1.73m^2 Mild: 60-89 ml/min/1.73m^2 Normal: >90 ml/min/1.73m^2

[a]Hb: Haemoglobin
[b]CrCl: Creatinine Clearance

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3.4 Potential Bias and Confounding

Loss to Follow Up

Patients are regarded as lost to follow up if they are not seen at the clinic for longer than three months at any time after starting ART [31]. There might be some differential loss to follow up if more patients with particular characteristics are lost. We will assess this by comparing the characteristics of patients lost during the study period to those retained.

Survivor Bias

Similarly, the effect of differential attrition might result in a specific cohort of patients being retained and for whom measurements are available after 12 months. If these patients are different from the initial baseline sample, our results might not be representative of the average patient's progression and change in renal function after the first year on ART. We will assess this by comparing the

characteristics of those known to have died during the study period to those who are alive after 12 months.

Confounding

We will adjust for possible confounding in the analysis for the variables that are available. Due to the constraints of secondary data, we are unable to collect additional information to assess renal function apart from serum creatinine measures. We do not know if the patients had other comorbidities like diabetes or hypertension. We are also unable to assess BMI, which would provide additional information for a more standardized estimate of creatinine clearance.

4 Data Management and Analysis Plan

A database will be set up for capturing the data to be collected from patient folders. Data from the central National Health Laboratory Service (NHLS) database will then be matched on patient id keys with the additionally collected data. Demographic and disease characteristics are mostly available for all patients at baseline, but a large number of follow-up weights may be missing. We suspect that the weight measures may be missing at random. Multiple imputation by chained equations (mice) will be used to impute missing weights in order to include more eGFR measures for patients with missing data [38]. All statistical analyses will be performed using `STATA/SE` version 12 (StataCorp).

4.1 Objective 1

We will explore descriptive statistics at baseline and conduct a baseline analysis of renal dysfunction in the adult patients screened for ART. We will use Wilcoxon rank-sum tests for comparing medians, t-tests for means and Pearson chi-square statistics for proportions. Logistic regression will be used to examine factors associated with renal dysfunction.

4.2 Objective 2

We will describe observed eGFR profiles both overall and within subgroups of patients. In addition, we will model the mean estimated GFR profile at baseline, month 1, month 2, month 4 and month 12 using linear mixed effect regression models and maximum likelihood parameter estimation (MLE). Mixed effect regression models take the correlation between repeated measurements for individual subjects into account [39].

In order to investigate whether the profiles had significantly different rates of change in different subgroups of patients over time, we will use stratified models and explore the effect of group×time interaction. Baseline demographic and disease characteristics will be adjusted for in the models to

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account for possible confounding. Model diagnostics will be performed after building each model to check whether the linearity assumption is valid and whether the appropriate covariance structure was fitted.

4.3 Objective 3

In order to examine the prognostic role of early serum creatinine measures, a second outcome variable: the change in eGFR from baseline to month 12, will be derived. Using a prognostic modelling framework, we will determine which early creatinine measurements best predict change in eGFR. Separate models for change in eGFR will be built using creatinine measurements at month 1, month 2 and month 4 as predictors. The covariates (age, sex, CD4 cell count, WHO Stage and baseline creatinine) that improve the model fit will be included in these models. All models will be checked for goodness of fit and residual plots will be examined to determine whether the regression assumptions are valid.

5 Ethical Considerations

5.1 Consent

This is a secondary data analysis. Although no direct participation is required, we will need to access the records and patient folders of participants. Data collection at the site had been initiated prior to this study by approval of the Human Research Ethics Committee at the University of Cape Town (*See Appendix A*). Permission to undertake the study will be sought from the Desmond Tutu HIV Centre and staff at the Hannan Crusaid Treatment Centre to access the patient folders and the NHLS database.

5.2 HIV-positive, vulnerable population

The participants of this study are vulnerable due to their HIV status and may be subject to stigmatization and discrimination. The information provided will be regarded as sensitive and privacy and confidentiality will be maintained at all times during the data collection and analysis. No patient names will be collected and an anonymous patient code, unrelated to the patient's personal identity, will be used for data entry. Computer-based records will only be available to the researchers and data capturers involved in the study through the use of passwords. All paper-based records will be destroyed on completion of the study.

Although there are no direct benefits to the patients whose data will be used, there is an indirect societal benefit. It will be beneficial to optimize and simplify screening algorithms and to allocate

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resources where they are most needed. The future societal benefits of this research by way of scaling-up and simplifying of ART services outweigh any potential harm that might arise.

6 Stakeholders and Dissemination Strategy

Given the objectives of the research project, the findings of the study are valuable to the existing service provision of monitoring patients on ART. We believe that the research question is relevant and will add to the current knowledge and guidelines on screening for toxicities in patients in similar resource-limited settings. Relevant stakeholders who are likely to be interested in the findings include the department of health, community-based health care centres running similar ART programmes, clinicians in every-day practice and research and academic institutions. The results will be made available by publication in a peer-reviewed journal.

7 Logistics

An outline of the proposed study time is given below:

April 2012	Data collection, data capturing and cleaning
May 2012	Data validation and merging with existent database
June- July 2012	Baseline data exploration, modelling
August- September 2012	Longitudinal data merging, exploration and summary tables
October- November 2012	Abstract and poster preparation of baseline analysis
February- March 2013	Imputation and mixed-effect modelling
April- May 2013	Prognostic modelling
June- August 2013	Final analysis, preparation of manuscript
September 2013	Submission

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Part II

LITERATURE REVIEW

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2. Literature Review

1 Introduction

The advent of highly active antiretroviral therapy (HAART) signified a new era in the treatment of HIV with significant decreases in HIV-related morbidity and mortality. With the roll-out of new drugs came a host of new complications associated with antiretroviral therapy (ART). Among these drugs is tenofovir disoproxil fumarate (TDF) which is currently used in first-line ART worldwide and across sub-Saharan Africa. TDF is an effective nucleotide analogue reverse-transcriptase inhibitor (NtRTI) that inhibits HIV replication but is also a known nephrotoxin. It has been found to be associated with declines in glomerular function and chronic kidney disease in HIV-infected patients.

The aim of this review is to determine the prevalence and the incidence of renal dysfunction in the general population of HIV-infected patients and in those initiating ART, and particularly TDF-containing, regimens. We will investigate risk factors for renal dysfunction in relation to HIV. This requires an exploration of different definitions of renal dysfunction used for classification in the literature. A review of longitudinal studies that observed HIV-infected patients over time will be done to explore patterns of change in renal function among patients starting TDF, and to examine the diagnostic role of periodic serum creatinine tests for patients on ART. A comparison of various definitions and methods used as well as analytical approaches applied will be done. We will evaluate the available evidence from both developed and developing settings and identify gaps requiring further research.

1.1 Literature Search Strategy

We queried the following search engines for any available articles on tenofovir-related renal dysfunction: PubMed, Google Scholar and Science Direct. The following search terms were used: '(renal OR kidney OR nephron*) AND (toxicity OR failure OR dysfunction OR impairment OR reduction OR reduced) AND (antiretroviral OR ARV OR ART OR haart OR tenofovir OR TDF OR viread OR HIV OR aids) NOT children* NOT adolescent NOT babies NOT pregnant NOT knee NOT liver'

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2 Concepts and Definitions

2.1 Glomerular Filtration Rate

The central function of the kidneys is to filter metabolic waste products, excess sodium and water from the blood and to eliminate them from the body [1]. Kidney function can be gauged by analysing blood samples or urine samples. The glomerular filtration rate (GFR) is the most widely used and accepted measure of kidney function [2]. It is the rate at which blood passes through the kidney and is filtered as filtrate (clear filtered fluid) into the renal tubule.GFR varies with age and sex, but is approximately 120 - 130 ml/min/ 1.73 m^2 surface area in adults [3]. The GFR generally remains constant in a healthy person, but will fall due to disease and other complications [3]. This is because the ability to excrete waste material and to regulate the volume and composition of body fluid declines leading to a rise in plasma urea or creatinine which then results in a reduction in the measured GFR [3].

2.2 Estimating the Glomerular Filtration Rate

Although GFR cannot be measured directly, it can be estimated using the concentration of certain substances in the blood [2]. One such substance is the fructose inulin. Inulin clearance: the volume of blood that is cleared of inulin per unit time, is regarded as the gold standard for estimating GFR [2].

GFR can also be estimated by measuring serum creatinine. In fact, the most widely used measure of GFR is creatinine clearance (CrCl) [3]. Using the level of serum creatinine in the blood, an individual's age, weight and sex, a measure of the creatinine clearance can be estimated. In the ideal scenario, a 24-hour urine sample would be needed to determine creatinine clearance exactly [1]. This makes the estimation of GFR time-consuming and inaccurate if 24-hour urine samples are not obtained. Instead a host of prediction equations have been developed to estimate GFR or creatinine clearance using serum creatinine and patient characteristics [3].

The Cockroft-Gault equation for creatinine clearance

The Cockroft-Gault equation (CG) was derived in 1976 from a cohort of 290 patients [4]. Using their age, gender, weight and serum creatinine, a measure of creatinine clearance was derived to gauge their GFR.

$$eGFR = \frac{140 - Age(years) \times Weight(kg)}{(72 \times SCr) \times (0.85 \text{ if female})} \tag{2.1}$$

The Cockroft-Gault equation was validated in 200 hospital patients with a broad range of creatinine

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clearance values [4].

The Modification of Diet in Renal Disease Equation

The Modification of Diet in Renal Disease Study Equation (MDRD) equation was derived in 1999 from a sample of 1628 patients with chronic kidney disease (CKD) who were part of the Modification of Diet in Renal Disease study [5]. The equation uses a patient's age, race and gender to derive an estimate of GFR

$$eGFR = 186 \times SCr^{-1.154} \times Age^{-0.203} \times (0.742 \text{ if female})(\times 1.21 \text{ if black}) \tag{2.2}$$

The MDRD formula was validated in a sample of patients in the MDRD study who also had CKD and GFR values between 20-60 ml/min/1.73 m^2 [5]

A slight difference in the formulae is that the Cockcroft-Gault equation estimates creatinine clearance, while the MDRD estimates glomerular filtration rate directly [4, 5].

The Chronic Kidney Disease Epidemiology Collaboration equation

The Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) equation was derived in 2009 from a collaboration of clinical studies and data from the National Health and Nutrition Examination Survey in the United States (US) between 1999 and 2006. A pooled dataset consisting of 8254 participants from 10 studies was used to develop the equation and an additional sample of 3896 participants from 16 studies was used as the validation sample [6].

The equation depends on categories of race, sex and the range of serum creatinine:

$$GFR = 141 \times min(Scr/\kappa, 1)^{\alpha} \times max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018\ [if female] \times 1.159\ [if black] \tag{2.3}$$

where SCr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

The CKD-EPI equation was found to be more accurate than the MDRD study equation especially at high glomerular filtration rates in the validation sample [6]. Ibrahim and Hamzah assessed use of the CKD-EPI equation in routine clinical care for HIV-infected patients and found similar results [7].

The prediction equations for GFR give different results depending on the context in which they are applied. Reid et al. [8] caution that estimated GFR changes have to be interpreted carefully, bearing in mind specific populations and the formulae used. The Cockroft-Gault equation and

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MDRD formulae yield different results and associated risk factors particularly in the context of HIV-infected adults in Africa who might be underweight and be sensitive to significant weight changes after introduction of ART [8].

3 Renal Impairment and HIV

Kidney damage as defined by the US National Kidney Foundation is any structural or functional abnormality of the kidney when the kidneys fail to adequately excrete toxic substances from the body [1, 2]. In the beginning this may be without decreased GFR, but over time it can lead to decreased GFR [2]. Indications of kidney damage include abnormalities in blood composition or urine (proteinuria) or abnormalities seen in kidney biopsy or imaging tests [2]. Renal failure can be attributed to a range of underlying causes, with some leading to a rapid decline in kidney function (acute renal failure), while others lead to a more gradual decline in kidney function over time (chronic renal failure) [1].

A wide spectrum of HIV-associated kidney diseases has been described in case reports and observational cohorts in the literature since as far back as 1987 [9]. Renal impairment related to HIV can be acute or chronic, depending on the pathway by which it occurred. HIV-associated nephropathy (HIVAN) is the most common cause of chronic kidney disease in HIV-infected patients, particularly among African-Americans and others of African descent [10–12]. Other kidney diseases that occur in HIV-infected patients include mesangial glomerulonephritis, lupus-like glomerulonephritis, allergic interstitial nephritis, crystal-induced nephropathy, acute tubular necrosis and Fanconi syndrome [13].

There is lack of consensus on the exact mechanism that drives the HIV-renal disease association, but some hypothesize that it can be caused directly by HIV or indirectly from other pathways like diabetes, hypertension and drug-related effects [14]. Acute renal failure commonly results from the toxic effects of antiretroviral therapy, while chronic renal failure can be caused by multiple other physiological pathways [14].

In a prospective cohort study conducted in ambulatory HIV-infected patients receiving primary HIV care in the US between 2000 and 2002, Franceschini et al. found an incidence of acute renal failure (ARF) of 5.9 per 100 person years (95% CI: 4.9, 7.1). Factors associated with ARF included immunosuppression (defined as CD4 levels below 200 cells/mm^3 and viral loads greater than 10,000 copies/ml), advanced stage of disease (AIDS) and liver-disease associated hepatitis C virus (HCV) [15]. Patients who experienced ARF were also more likely to have received HAART previously [15].

A similar prospective cohort study conducted in 2010 found a lower incidence of ARF in HIV-

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infected patients of 2.8 per 100 person-years (95% CI: 2.41, 3.24). Unlike the first study, the investigators did not find HCV coinfection, exposure to HAART (with or without tenofovir) and HIV viraemia to be associated with ARF. Only levels of immunodeficiency and renal function were independent predictors of HIV-associated ARF [16]. This study and others seem to suggest that HIV-replication may be a mediator of CKD in patients at advanced disease stage [17].

The prevalence of CKD in HIV-infected patients has been found to be quite high, ranging from 5 - 15% of patients presenting with a GFR less than 60 ml/min/1.73 m^2 [18, 19]. Overall, both ARF) and CKD are more prevalent in HIV-infected persons than in the general population [20]. The prevalence of HIV-related kidney diseases varies in sub-Saharan Africa from as high as 34% in Zambia and 20 - 49% in Uganda, to 6% in South Africa [21]. Fabian et al. report that it is hard to quantify the exact prevalence due to varying criteria used for diagnosis of kidney disease in patients with HIV across sub-Saharan Africa as shown in Table 2.1.

Table 2.1: Prevalence of HIV-related kidney diseases in sub-Saharan Africa

Country	Prevalence [a]
South Africa	6 %
Nigeria	38 %
Ivory Coast	26 %
Tanzania	28 %
Kenya	25 %
Uganda	20- 49 %
Zambia	34 %

[a] Adapted from results by Fabian and Naicker, 2009

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4 HAART and the Kidney

4.1 Nephrotoxicity

Drugs are mainly excreted through the kidney, particularly through the proximal tubule [17]. The proximal tubule is thus exposed to substantial levels of toxins and may be susceptible to damage [17]. The increased roll-out of antiretroviral drugs in the HAART era has achieved unprecedented survival rates in HIV-infected patients, but has also brought a host of new complications [17]. Several antiretroviral drugs before tenofovir including ritonavir-boosted atazanavir (ATV/r), unboosted ATV, indinavir (IDV), ritonavir-boosted lopinavir (LPV/r), and other ritonavir-boosted protease inhibitor (PI/r) drugs, have been associated with chronic renal impairment in HIV-infected populations with different degrees of pre-existing renal impairment [22, 23]. Tenofovir, the first NtRTI to be approved by the United States food and drug administration (US FDA), has been

found to lead to damage of the proximal tubule and mitochondrial toxicity resulting in a spectrum of moderate-to-severe kidney injuries [17,24]. In addition, increased life expectancy of HIV-infected patients as a result of HAART means that patients are more likely to experience chronic diseases like hypertension and diabetes which are risk factors for decreased renal function [17].

4.2 Benefits of HAART

HAART may offset and repair the kidney damage caused by HIV infection in patients with severe immune suppression and renal dysfunction, resulting in a much milder form of renal dysfunction. Dolin et al. explain that ART suspends the disease process by stopping new rounds of renal cell infection [12]. Before the advent of HAART, patients would progress to renal failure requiring renal replacement therapy within weeks of onset [12].

In a longitudinal study conducted between 1988 and 2004, Lucas et al. observed a cohort of African- American patients before HAART and after HAART and found that the incidence of CKD decreased in the HAART era while the prevalence increased as the survival rate of HIV-infected CKD sufferers improved [25]. The ARV regimens analysed in their study included TDF after 2001. Kalayjian et al. reported similar findings in a US-based cohort study, showing that HIV-1 viral suppression as a result of HAART helped to improve the glomerular filtration rate [26]. Tenofovir was included in the regimens of many patients in this study but the authors did not break down HAART by regimen type.

In the African setting, the Development of Antiretroviral Therapy in Africa trial (DART) study investigators found that there were renal benefits of HAART for patients with mild to moderate impairment at baseline. These patients experienced the greatest increase in estimated glomerular filtration rate (eGFR) over 96 weeks [8]. Peters et al. reported a 53% increase in the median creatinine clearance of HIV-infected patients with renal dysfunction prior to HAART initiation in the Home Based AIDS Care trial (HBAC) in Uganda after 2 years of HAART [1].

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5 Tenofovir and the Kidney

5.1 History, Development and Efficacy Trials

TDF was developed in 2001 by Gilead Sciences and is currently used worldwide as a first-line antiretroviral therapy drug [24]. TDF is structurally similar to two antiviral nucleotide analogues cidofovir and adefovir, which are established nephrotoxins [24]. The recommended dosage in

[1] 74% of the cohort in the DART trial initiated ART with tenofovir as part of first-line therapy although tenofovir was not part of routine care in Uganda and Zimbabwe at the time (2003- 2008). HBAC trial participants were initiated on stavudine plus lamivudine with either nevirapine (496 participants, 98%) or efavirenz (12 participants, 2%).

adults is 300 mg/day [27]. Kearney et al. explain how tenofovir is excreted renally via glomerular filtration and active tubular secretion which necessitates dose-interval adjustments in patients with pre-existing renal impairment before starting a TDF-containing regimen [27].

The first efficacy and safety trials conducted in HIV-infected patients found tenofovir to have a good safety profile with minimal risk [28–31] (*See Appendix C for an outline of the trials*). However, several case reports and case series reported afterwards found tenofovir to be associated with decreased creatinine clearance, renal failure, chronic kidney disease and proximal tubular injury [17,32–36] [2]. Subsequent observational cohort studies have found tenofovir to be associated with significant but modest declines in eGFR [24]. A pooled measure from a meta-analysis of 17 studies in 2010, found tenofovir to be associated with a mean decrease in creatinine clearance of 3.9 ml/min/1.73 m^2 over the time of treatment exposure [37].

Tenofovir toxicity appears to target the proximal tubule leading to mitochondrial toxicity of the cells in the proximal tubule. Toxicity may either present as proximal tubular dysfunction without altering renal function, or proximal tubular dysfunction with decreased renal function [24, 38]. However, measures of tubular function have not been reported as extensively as glomerular function in the literature [24].

Three large cohort studies from the developed world have found tenofovir to be associated with increased risk of developing CKD defined as an eGFR of less than 60 ml/min/1.73m^2 (cohort of patients from seven large HIV reference centres in France [39]; HIV-infected patients from the Veterans Health Administration database in the US [40] and a multicenter cohort of ART-naïve patients from the Center for AIDS Research Network of Integrated Clinical Systems (CNICS) Cohort in the US [41]). Tenofovir is also associated with an increased risk of AKI that was observed within a few months of starting tenofovir in patients with predisposing factors [37]. Renal function was found to recover after discontinuation of TDF, except in cases of CKD [38].

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5.2 Guidelines and Recommendations for Use

Frequent renal monitoring and dose adjustment where necessary are the best strategies for effective tenofovir administration [24]. International guidelines, like those of the HIV Medicine Association of the Infectious Diseases Society of America recommend that patients on TDF have their serum creatinine and eGFR assessed at baseline and at routine check-ups every 3 - 4 months thereafter [42]. In addition, serum phosphate, proteinuria and glycosuria should be measured at least twice a year in order to test for possible proximal tubular dysfunction [42].

Following evidence on increased toxicities associated with stavudine (d4T), the WHO recommended

[2] Other kidney complications associated with TDF: Fanconi syndrome, hypophosphatemia, normoglycemic glycosuria, proteinuria, acute kidney injury (AKI) and decreased bone mineral density [17,32–36]

that countries take progressive steps to reduce the use of d4T in first-line regimens. In 2010, the WHO advised countries to transition towards complete AZT- or TDF-based first-line regimens in settings where d4T regimens were still the main option for starting ART [43]. In the current WHO guidelines, TDF is recommended for first-line therapy in combination with lamivudine (3TC) (or emtricitabine (FTC) + efavirenz (EFV)) or as a fixed-dose combination for treatment-naïve patients. Prior to the updated World Health Organization (WHO) guidelines for 2013, serum creatinine screening was recommended before ART initiation and every 6 months when possible [43]. However, laboratory monitoring was and is currently not a requirement for the initiation of ART. Therefore lack of access to laboratory testing facilities to measure serum creatinine should not be a barrier to TDF use in remote and resource-limited settings [43]. The new guidelines rather advise on symptom-directed laboratory monitoring for safety and toxicity as opposed to strict bi-annual monitoring [44].

TDF is contraindicated in long-term diabetes sufferers, those with uncontrolled hypertension or renal failure and when the estimated glomerular filtration rate is <50 ml/min/1.73 m^2 [44]. If any altered creatinine clearance is detected after initiation, the TDF dosage can be adjusted or an alternative regimen started [43, 44]. However, for those that do start treatment with TDF, more data are needed on whether routine or periodic laboratory monitoring for toxicities (renal and bone toxicities) is required for all individuals or only high-risk people [3].

Although it has been established that tenofovir is related to significant albeit small loss of renal function, the general consensus is that fear of nephrotoxicity should not be a reason to withhold treatment since the benefits of HAART far outweigh the risks. The renal toxicity resulting from tenofovir can be reversed after cessation of the drug in acute cases, although not in all patients [24]. Herlitz et al. studied 13 confirmed cases of acute tubular necrosis due to TDF nephrotoxicity and found that 20 months after discontinuation of tenofovir, almost 50% (6/13) had completely recovered their baseline renal function [46]. Early detection of any nephrotoxic effects and cessation of TDF treatment are therefore very important to avoid tubular damage [38].

Several studies have suggested that changes in renal function after ART initiation (with or without TDF) mostly occur during the first year [17, 47]. Knowing when to screen for elevated serum creatinine within this year would enable optimal monitoring of incident renal dysfunction in order to detect it early and treat or prevent severe disease. Some studies have suggested that this can occur as early as during the first two to three months after initiating TDF in those with normal baseline eGFR [45, 48]. These include studies in sub-Saharan Africa like the Johannesburg cohort

[3] High-risk groups identified: those with pre- existing renal disease (also higher serum creatinine levels prior to starting TDF), advanced HIV disease (acquired immunodeficiency syndrome (AIDS) and low CD4 levels), age >40 years, low body weight (<50 kg or body mass index (BMI) <18.5 kg/m^2), have other risk factors for renal disease like untreated hypertension and diabetes, and are using a PI/r or other nephrotoxic drugs in addition to TDF should have their creatinine clearance monitored to detect and limit further progression of renal impairment [31, 43, 45].

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study [49]. In this cohort of patients with contraindications that prevented them from starting d4T or AZT-based regimens, a median time to nephrotoxicity after tenofovir initiation of 3.6 months was found, confirming the importance of the 3-month creatinine clearance screening [49]. In a Senegalese cohort, during 12 months of treatment, patients on TDF experienced a higher rate of change in impairment from mild (eGFR 60-90 ml/min/1.73 m^2) to moderate (eGFR 30-60 ml/min/1.73 m^2) compared to patients not receiving TDF, which persisted after the first year [50].

5.3 Use and Introduction in South Africa

The South African government added TDF as a first line regimen drug in April 2010 for all new patients needing treatment, including pregnant women [51]. The South African National ART guidelines recommend that serum creatinine and creatinine clearance be measured at ART initiation, at months 3 and 6 and yearly thereafter for patients on tenofovir in order to identify toxicity. A creatinine screening test and urinalysis should be done and if found to be abnormal, the patient ought to be referred for specialist opinion [52].

The Southern African HIV Clinician's Society ART guidelines for adults are similar, but advise that creatinine should be measured at baseline, at months 3 and 6 and then *every 6 months*. Particular attention should be given to high-risk patients with concurrent co-morbidities (hypertension or diabetes), for whom creatinine should also be checked at 1 and 2 months after initiation [53]. The guidelines suggest that renal function can be estimated either by the modified Cockroft-Gault equation or the modification of diet in renal disease (MDRD) method [4].

In primary health care, periodic monitoring might be difficult to maintain, particularly where there is limited access to laboratory infrastructure [54]. Bygrave et al. described low levels of toxicity outcomes observed in a routine programme in rural Lesotho where the cohort of those who developed a creatinine clearance below 50 ml/min/1.73m^2 dropped by less than 10 ml/min/1.73m^2 and subsequently returned above the threshold after HAART. They conclude that a small proportion of those patients who dropped to levels between 40 and 50 ml/min/1.73 m^2 may have been unnecessarily switched off TDF if renal function had been rechecked in the interim. The option of TDF implementation without prior screening of serum creatinine and routine renal monitoring could still be a viable option in high HIV-prevalence settings where the cost of laboratory monitoring exceeds the benefits [54].

[4] The modified Cockcroft-Gault equation:

$$creatinine\ clearance = \frac{(140 - age(years)) \times ideal\ weight(kg)}{serum\ creatinine}$$

For women, multiply the total by 0.85.
The guidelines stipulate that the results of these formulae differ slightly but either can be used for clinical management [53]. We chose to use the Cockroft-Gault formula in section 2.2 because we had weight measurements available and it is the formula that is applied internationally.

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6 Observational Studies on Tenofovir Use

Important differences exist in the studies that have explored the effects of tenofovir, both in study settings, study designs, definitions used and length of follow-up. This makes it difficult to draw definitive conclusions about the effects of tenofovir use.

Table 2.2 displays different observational studies from established cohorts in developed settings (Europe, North America and Australia). These studies mostly consisted of patients in long-term HIV care, with some who were ART-experienced and others completely treatment-naïve (to ART and TDF), observed over long periods of follow-up. The D:A:D study [23] and the Veterans Health Administration study [40] each had up to 10 years of follow-up. The least period of observation was 6 months in the study by Scott et al. in which the short-term effects of TDF use on renal function were studied [55]. Some important differences in participants were the large proportion of white male, intravenous drug users [56], and male patients infected through male-to-male sex [23].

In contrast, Table 2.3 displays different observational studies from resource- limited settings (Thailand, Uganda, Zimbabwe, Zambia, South Africa, Senegal, Lesotho and Malawi). The study participants were mostly obtained from undergoing trials (Staccato Trial in Thailand [57], DART in Uganda/Zimbabwe [8], HBAC in Uganda [58], a Senegalese cohort from a clinical trial [50] and a pooled trial analysis from Malawi [59]). As a result, the study participants were subject to certain screening and monitoring criteria that may have been different from the underlying HIV-infected populations in these countries. The HBAC trial excluded participants with severe renal dysfunction (CrCl <25 ml/min) from the study [58]. In certain settings like Malawi, the participants had serum creatinine tests done by virtue of being enrolled in the trial despite it being non-routine in standard care. However, Johnson et al. explain that the participants in their study were drawn from the general HIV-infected population in Malawi and would not have been excluded based on comorbidities associated with kidney disease [59].

Most patients were ART-naïve at baseline and were newly initiating ART. As TDF was only introduced as a first-line drug much later in these settings compared to well-resourced settings [5], the studies had much shorter lengths of follow up and outcomes were assessed after HAART initiation in general and not just TDF-containing regimens in particular. The longest length of follow-up was for four years in the Johannesburg cohort which included ART- experienced patients switched on to TDF (79%) [49]. The majority of participants were female [49, 54, 59].

[5] TDF was introduced in 2007 in Zambia and in 2010 in South Africa [60]

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6.1 Methodological Approaches

Definition of Outcome

Different outcomes relating to varying degrees of renal dysfunction were assessed as shown in Table 2.2. Some studies looked at serum creatinine elevations [15, 61, 62] while others looked at changes/losses in creatinine clearance or decline in kidney function [48,55,56,63,64]. Scherzer et al. included proteinuria among the outcomes they assessed [40]. Creatinine clearance and glomerular filtration rate were assessed by either the Cockroft-Gault or MDRD equations in most studies, except for the study by Laprise et al. which made use of the CKD-EPI formula [56]. They advise that the CKD-EPI equation is better suited for routine clinical use in HIV-infected patients as the MDRD equation may overestimate the severity of renal impairment in HIV-infected patients as found by Ibrahim et al. [7].

In the studies from the developing world shown in Table 2.3, the assessed outcomes range from changes in creatinine clearance and glomerular filtration rate, severely decreased glomerular filtration rate (<30 ml/min/1.73 m^2), significant and persistent renal impairment, nephrotoxicity and death. Renal function was assessed using either the CG equation or the MDRD equation. Most studies defined grades of eGFR impairment by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/KDOQI) grading scale for normal, mild, moderate and severe impairment [2]. Peters et al. [58], Bygrave et al. [54] and Johnson et al. [59] also binarized renal function at CrCl <50 ml/min/1.73 m^2 and >50 ml/min/1.73 m^2 which is the recommended dosage modification criteria stipulated in the WHO ART guidelines [43, 44]. Franey et al. performed additional urinalysis in order to detect significant proteinuria and haematuria [65].

Scott et al. [55] and Peters et al. [58] made use of toxicity grading scales to define levels of creatinine beyond the normal range. These include the Adult AIDS Clinical Trials Group (AACTG) toxicity grades for Creatinine and the National Institutes of Health Division of AIDS (DAIDS) Table for grading the severity of adult and pediatric adverse events [66].

Creatinine measures were assessed at varying times either according to standard practice in the studies conducted under routine care settings or according to study protocol. A baseline creatinine measure was available in all the studies, either prior to HAART or at initiation. In the studies were follow-up measures were collected, the time frames used were either weekly or monthly measurements.

Confounding

Common variables adjusted for in the analyses were demographic characteristics (age, sex and ethnicity), clinical characteristics (HIV disease stage, CD4 level, viral load, mode of HIV transmission and haemoglobin) other co-morbidities (diabetes, hypertension, hepatitis B or C virus coinfection,

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baseline renal function and pre-existing renal risk factors) as well as concurrent ARV use and other nephrotoxic drugs. Body mass index and information on weight change after initiating ART was not always available. Although weight and BMI are important variables in assessing renal function, only a few studies took these variables into account [8, 50, 57–59, 67]. These were mostly studies from the African setting for which changes in patient weights after ART initiation have a significant effect on renal function.

6.2 Analytical Approaches

The modelling approaches applied in the studies ranged from survival analysis modelling by Cox proportional hazards and Kaplan-Meier curves [40,56,61,63,67], generalized linear models (logistic [61,65], linear [56,58], and Poisson regression models [15,23]), marginal structural models [40] and generalized estimating equations (GEE) [56].

Apart from survival analysis models in which time to an event was modelled explicitly, only a few studies utilized a repeated measurement analysis approach despite the longitudinal structure of the data. Laird et al. [68] in their derivation of mixed models for longitudinal data, stress that in order to analyse longitudinal data the models must adjust for the relationship between serial observations on the same measurement unit. The correlation between repeated observations has to be accounted for in order to derive correct unbiased estimates of effect [69].

Gallant et al. modelled the change in creatinine clearance using a single value computed from the difference between maximum CrCl within one year after initiation and baseline CrCl [48]. Multivariate least squares linear regression was then used to assess the effect of other covariates on this change. A similar approach was used by Peters et al. who modelled the change from baseline to 24 months using general linear regression [58]. Preferably, all available creatinine measurements should have been incorporated in the analysis. Averaging out measurements and modelling one single measurement led to a considerable loss of statistical power. Similarly, other studies compared means and medians at different time points to assess changes in CrCl [54]. Scott et al. compared the mean differences in serum creatinine and CrCl values at baseline and at subsequent time points (12 and 24 weeks). It is difficult to profile the renal function trajectory over time by comparing averages, especially in attempting to identify the critical time point when renal dysfunction sets in.

Gayet-Ageron et al. studied CrCl longitudinally by comparing the average CrCl at different time periods using Spearman correlations and one-way ANOVA [57]. Others used mixed-effect models to model the mean profile of eGFR over a given time period. Reid et al. [8] used a random effects model to model changes in eGFR over 96 weeks on ART. De Beaudrap et al. used latent growth analysis and mixed-effect models to take the correlation between repeated measurements

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into account [50], while Laprise et al. [56] used GEE logistic regression with an exchangeable correlation pattern for repeated measures. These modelling approaches enabled them to trace the complete trajectory of patients with multiple repeated measurements in order to study any effect there might be of TDF on kidney function over time.

Step-wise model-building procedures were often applied in the studies. Step-wise regression has been criticized because it leads to biased estimation of regression coefficients. Using step-wise procedures increases the probability of inappropriately selecting non-confounders in analysis models and omitting confounders [70, 71]. Important variables may have been omitted due to stringent, arbitrary p-value cut-off criteria.

To illustrate, Peters et al. [58] and Bygrave et al. [54] relied on step-wise regression to identify risk factors and variables that were independently associated with their renal outcome. Peters et al. explain that they sequentially dropped each variable with a p.value >10% in order to build a parsimonious model. However, they only included variables that they found to be associated with baseline renal dysfunction in univariate analysis. It is unclear whether they were adjusting for potential confounders, or blindly trying to build the best parsimonious model, increasing the risk of inappropriately selecting non-confounders in their model.

Bygrave et al. built multivariate logistic regression models by backwards elimination, despite having a limited number of baseline variables. They calculated adjusted odds ratios for CrCl ≥50 ml/min compared to CrCl <50 ml/min and identified risk factors for reduced CrCl. They then used these associations to develop algorithms for screening baseline creatinine based only on age and CD4 level. They acknowledge that the algorithm development would have benefited from including a broader range of risk factors [54].

Reid et al. [8] built a predictive model to predict baseline eGFR using backward elimination and deleting the covariate with the least contribution to the coefficient of determination (R^2). However, even for predictive modelling, the use of step-wise regression procedures leads to inflated measures of R^2 and biased F-statistics [72,73]. Application of step-wise regression is erroneous in its attempt to find a best-model and has been criticized for the above-mentioned reasons.

Another critical point is how the studies dealt with loss to follow-up and missing values in analysis. Very few of the studies address this directly. Studies that used the survival analysis approach, like Cox proportional hazards, automatically censored participants with missing outcomes by nature of the modelling framework used. Standard regression methods used in statistical packages use complete-case analysis and simply exclude missing data [74]. This may lead to selection bias if the cases included in the analysis are different from those excluded due to incomplete data. The study by Ryom et al. assessed possible selection bias by comparing patients included to those excluded

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from analysis, which they found to be similar [23]. Brennan et al. found that excluding over 400 patients with missing creatinine clearance led to selection bias since CD4 levels were different for those included compared to those excluded in the analysis. They proceeded by using marginal structural models with inverse probability treatment weights to account for loss to follow-up and confounding [49].

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Table 2.2: Observational Studies from the Developed World

Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Jones, R. & Stebbing, J. (2004)	UK/ Chelsea and Westminster cohorts, excluded participants from matching who had a creatinine level >120 μmol/L before receiving TDF	To investigate the overall incidence and risk of renal dysfunction in individuals receiving TDF compared to other antiretrovirals	Cohort and matched case-control (n= 4183)	TDF & ART-naïve patients vs patients exposed to TDF and non-TDF-containing regimens; Creatinine elevation vs none in matched case-control patients all on TDF	Up to 4 years	creatinine value >120 μmol/L at any time	Renal dysfunction = sCr >120 μmol/L at any time, classified patients according to ARV exposure before this level	Conditional logistic regression with the Proportional Hazards Regression Model (PHREG) procedure, using the discrete logistic model stratified by the matching criteria	The overall occurrence of renal failure was rare in this context. TDF is not associated with renal dysfunction more frequently than other ARVs. The occurrence of renal dysfunction in this context is attributable to other causes.
Franceschini, N. & Napravnik, S. (2005)	UNC CFAR Cohort Study: US, North Carolina. HIV patients (18 years) visiting a university-based Infectious Disease Clinic between 2000 and 2002.	To assess the incidence and causes of mild severe ARF and its impact on clinical outcomes in ambulatory HIV-infected patients receiving primary care in the post-HAART era	Prospective cohort (n= 754)	none directly (Some patients on HAART, others not)	3 years	Incidence of Acute Renal Failure and its clinical out-comes/possible causes	ARF sustained increase in serum creatinine (over 2 days) [6]. The number of episodes of ARF per patient and underlying clinical conditions at the time of the ARF event reviewed to determine potential causes.	ARF incidence rates (IR) calculated assuming an underlying Poisson Distribution. T-test or chi-square tests used to compare patients with and without ARF.	IDV, TDF, and NVP were the only ARVs clearly associated with ARF in this cohort, but were associated with only a few events of ARF. Tenofovir was associated with ATN in two patients. Investigators conclude that ARF is common in ambulatory HIV patients.
Gallant, J. E. & Parish, M. A. (2005)	Johns Hopkins HIV Clinical Cohort. All patients who started therapy between 1 January 2001 and 31 December 2003 with either TDF or an alternative NRTI as part of a HAART regimen	To assess change in CrCl in TDF-treated patients compared to NRTI-treated patients in clinical practice and associations with covariates (age, ethnicity, sex, HIV transmission risk factor, diabetes, hypertension, CrCl at baseline, CD4 cell count and HIV-1 RNA load, and other ARVs).	Retrospective cohort (n= 658)	TDF (n=344) compared to other NrTI 5 (n=314)	Up to 1 year of follow-up (median duration= 322 days)	Changes in calculated CrCl between the start of treatment and the end of the observation period	CrCl calculated using CG equation	Compared the change in CrCl from baseline for TDF-treated patients to other NRTI-treated patients. Multivariate linear regression to assess the associations of multiple factors (age, ethnicity, sex, HIV transmission risk factor, diabetes, hypertension, CrCl at baseline, CD4 cell count and HIV-1 RNA load, and concurrent ARVs.)	4% relative decline in CrCl with use of TDF, compared to other NRTIs. Small and statistically significant decline but not clinically significant to prompt discontinuation. Advanced immunosuppression (CD4 <50 cells/mm^3), diabetes, decreased baseline renal function (CrCl of <50 mL/min) also strongly associated with CrCl decline.

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[6]Criteria by Nash et al: increase of 0.5 mg/dL for patients with baseline serum creatinine level <2.0 mg/dL, 1.0 mg/dL for patients with baseline level of 2.0 - 4.9 mg/dL, and 1.5 mg/dL for patients with a baseline level 5.0 mg/dL

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Padilla, S. & Gutierrez, F. (2005)	HIV-infected patients in a Spain Hospital from July 2001 to October 2003.	To describe the frequency and characteristics of TDF-related renal function impairment in a real-world scenario.	Retrospective case-control (n= 316)	TDF-naïve patients who started treatment with TDF-containing regimens since the drug was marketed (n=122) compared to patients receiving ART with other (non-TDF) ARVs	Retrospec-tive case-control looking back at a 48 week period.	Serum creatinine level elevations measured throughout a 48-week period or until TDF withdrawal.	Grade 1 or higher serum creatinine elevations according to AACTG grading scale [7]. Toxicity defined and graded according to the WHO toxicity scale.	Univariate associations determined by Chi-square test, or Fisher's exact test where appropriate, to compare proportions of qualitative variables and the Wilcoxon rank sum test to compare continuous variables (5% Significance level).	Authors conclude that in clinical practice during a 12-month period renal failure is infrequent and TDF is associated with low risk of mild renal failure. However, they caution that only half of their patients had a follow-up period >1 year and the long-term incidence of renal failure might be higher.
Scott, J. D. & Wolfe, P. R. (2006)	HIV-infected, ART experienced patients who received TDF at two clinical practice settings Los Angeles, California, USA, >=18 years of age who received TDF as part of HAART for $\geq$ 3 months with at least one viral load and CD4 count obtained before and after starting treatment.	To evaluate the short-term effects (up to 6 months) of TDF use on renal function in patients being treated for HIV-1 infection	Retrospective cohort (n= 447)	At least three months of TDF treatment versus none	Up to 6 months of follow-up	The development of renal impairment, as measured by graded changes in SCr and CrCl, through 24 weeks in patients taking TDF.	CrCl calculated and compared to baseline. Serum creatinine elevations were classified by AACTG grading scale[2].	Mean and standard deviations for SCr and CrCl values determined before (baseline) and after starting TDF (at 12 and 24 weeks)	All three patients with grade 2 increases in SCr had other medical reasons for an increased SCr. No patients experienced any complications from these increases in SCr. Increases in SCr and CrCl within the first 6 months of TDF therapy were rare (1.3%) in patients taking TDF.
Fux, C. A.& Simcock, M. (2007)	Swiss HIV Cohort Study (SHCS), which prospectively enrols HIV-infected adults throughout Switzerland.	To assess TDF-associated changes in calculated GFR in a large observational HIV cohort.	Prospective cohort (n= 1078)	TDF-based ART (n = 363) versus TDF-sparing regime (n =715)	24 months	Time to a 10 ml/min loss of calculated GFR maintained over 1 month during follow up. Patients without an event were censored at their last cGFR value.	Calculated GFR based on CG equation, confirmed by a follow-up measurement at least 1 month later. Included calculations based on the MDRD formula in sensitivity analyses.	Endpoints modelled using pre-specified covariates in a multiple Cox proportional hazards model. Conducted sensitivity analyses with secondary endpoints including calculations based on the MDRD formula.	TDF use (HR = 1.84 [95% CI 1.35-2.51]) and boosted protease inhibitor use (HR = 1.71 [95% CI 1.30-2.24]) significantly increased the risk for reaching a loss of 10ml/min in eGFR in the multiple Cox model. Found a significant reduction in eGFR associated with TDF use in HIV-infected patients

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[7] Adult AIDS Clinical Trials Group toxicity grades for creatinine

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Guaraldi, G. & Roverato, A. (2009)	HIV-infected ART-experienced adults, naïve to TDF, with a GFR 60-90 ml/min (by MDRD equation) who attended a metabolic clinic in Italy, between January 2004 and December 2005. Excluded patients with chronic renal failure (GFR <60 ml/min) or normal renal function (GFR = 90 ml/ min).	To assess the impact of plasma HIV-1 viral load (VL) level variation and TDF exposure on kidney function by analysing changes in eGFR assessed by mean MDRD changes over a 48 week period in patients with mild renal impairment (60-90 ml/min).	Prospective cohort (n= 99)	TDF exposed patients initiated TDF at baseline (n= 57) & TDF-unexposed patients whose ARV therapy did not include TDF (n= 42).	48 weeks	Change in GFR by MDRD equation	Evaluating SCr, urine protein: creatinine ratios. GFR estimated by MDRD equation. Stratified study population into three sub-groups according to HIV-1 VL changes during follow-up [8]	Evaluated differences between means using the Student's t-test and ANOVA for between group mean differences. Used Fisher's exact test for differences between proportions (5% Significance level).	At week 48, the mean GFR was 79.7+13.2 ml/min in the TDF-exposed group and 80.2+13.6mL/ min in the TDF-unexposed group. None of these measures was significantly different in the unexposed non-TDF group. The three VL response sub-groups had significantly different mean GFR changes at week 48. Improvements in GFR subsequent to HAART-induced viral suppression seemed to counteract any potential negative effects of TDF on renal function
Scherzer, R. & Estrella, M.(2012)	California, USA. HIV-infected patients from the Veterans Health Administration who initiated ART from 1997 to 2007	To explore whether TDF use is associated with higher risk of kidney disease.	Retrospective cohort (n= 10841)	All patients on TDF	10 years	Proteinuria (by urine dipstick measurements ≥30 mg/dl), rapid decline in kidney function (≥3 ml/min/1.73 m^2 annual decline), and CKD (eGFR <60 ml/min/1.73 m^2).	Used MDRD formula for eGFR	Cox proportional hazards and marginal structural models used to evaluate associations between TDF and time to first occurrence of proteinuria.	Pre-existing renal risk factors did not aggravate the effects of TDF. Among those who discontinued TDF use, risk of kidney disease events did not appear to decrease during follow-up. TDF exposure was independently associated with increased irreversible risk for three types of kidney disease events.

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[8]Sub-group 1, viral load remained below limit of detection (50 copies/ml); sub-group 2, viral load increased ≥0.5 log10; and sub-group 3, viral load decreased ≥0.5 log10.

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Laprise, C. & Baril, J. (2013)	HIV-infected patients in Canada who are part of an open cohort. Mostly white homosexual men (only 3.8% of the cohort were women) and a large proportion of intravenous drug users (IDUs)	To evaluate the association between long-term TDF exposure and kidney dysfunction in a cohort of HIV-positive patients followed up for 10 years and to quantify the loss in eGFR in patients exposed to TDF in comparison with those exposed to other ART.	Ambidirectional cohort (n= 1043)	Patients who had taken TDF alone or in combination were considered to be exposed to TDF compared to patients exposed to any other ARV	Up to 10 years of ARV exposure (median of 7.9 years)	Decreased kidney function, defined as eGFR < 90 ml/min/1.73 m^2. Failure outcome= 2 consecutive measurements of eGFR < 90 ml/min/1.73 m^2 > 3 months apart	GFR estimated using the CKD-EPI formula for every visit for which a sCr test was available.	Kaplan-Meier analysis for cumulative incidence of reduced kidney function by exposure status. Log-rank test for differences in incidence by exposure status. Cox proportional hazards and GEE models to find adjusted hazard ratios (HR) and odds ratios (OR) for the association between TDF and kidney dysfunction. Multivariate linear regression for cumulative mean loss in eGFR per year attributable to TDF exposure, controlling for empirical confounders.	TDF exposure was associated with reduced kidney function; exposure increased the risk of kidney dysfunction by 63% compared with other ARV exposure (HR, 1.63; 95% CI, 1.26-2.10). Loss in eGFR attributable to TDF is relatively mild in the long-term, occurs in the first year of exposure and stabilizes thereafter.

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
G.Ryom, L. & Mocroft,A. (2013)	HIV-positive persons undergoing active follow-up in the D:A:D study from established cohorts in Europe, the US, and Australia, with $\geq$ 3 SCr measurements after 1 January 2004 and a normal eGFR of 90 ml/min. Mostly white, male treatment-naïve patients, infected through male-male sex.	To establish the extent to which several ARVs are associated with chronic renal impairment among HIV- positive persons with initially normal renal function.	Prospective cohort (n= 49734)	TDF, ATV/r, and LPV/r	<5 years. ART exposure assessed categorically (ie, never exposed and exposed for <1, 1-2, 2-3, and >3 years)	Development of a confirmed eGFR of 70 ml/min or 60 ml/min (moderately severe CKD) or until the last eGFR measurement during follow-up.	GFR calculated by CG equation, standardized for BSA. Baseline: all had eGFR $\geq$90 ml/min (normal). Followed until confirmed eGFR $\geq$70 ml/min or $\geq$60 ml/min or until the last eGFR measurement during follow-up. [9] Calculated a 28-day mean for those with frequent eGFR measurements.	Incidence rates of progression from an eGFR of $\geq$90 ml/min to eGFRs of <70 ml/min and CKD per 1000 person-years of follow-up. Poisson regression models to find predictors for confirmed eGFR < 70ml/min. Multivariate analysis with TDF and other ARV exposures adjusting for age, sex, ethnicity, CD4 count, HIV load, and prior AIDS-defining illness; HBV, HCV + established risk factors for renal impairment (HPT, diabetes, and CVD). Included interactions between ARVs and risk factors significant in multivariate analysis for a confirmed eGFR of <70 ml/min	TDF, ATV/r, and LPV/r each independently associated with an adverse chronic effect on renal function in persons without pre-existing renal impairment. Other significant predictors of progression to a confirmed eGFR of $\leq$70ml/min and CKD = age, female sex, diabetes, IDU (vs male-male sex) transmission, prior AIDS-defining illness, and current CD4 count. Relationship between cumulative ART exposure and a confirmed eGFR of $\leq$ 70ml/min not modified by age, HCV, AIDS-defining illness or immunosuppression. HPT and HBV not associated with either end point.

[9] eGFR was considered confirmed if detected at 2 consecutive measurements >3 months apart

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Table 2.3: Observational Studies from the Developing World

Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Gayet-Ageron, A. & Ananworanich, J. et al. (2007)	Thailand, Staccato trial patients with CD4 >350 cells/mm^3 and viral load <50 copies/ml.	To evaluate the impact of standard tenofovir disoproxil fumarate therapy (300 mg once daily) on renal function among Thai patients treated within the Staccato trial who are of a lower average body weight than patients from Western Europe or the USA.	Prospective cohort (n= 264)	300 mg of TDF daily, continuous HAART (tenofovir/lamivudine combined with ritonavir-boosted saquinavir) but no control group	up to 108 weeks	CrCl used as proxy for GFR, calculated using CG formula and the MDRD formula. Creatinine values (in mg/dl), weight (in kg) and age were available before TDF and then measured every 12 weeks after.	Creatinine values were measured before the start of TDF and at every 12 weeks. Renal function assessed using CG formula and the MDRD formula.	Used one-way ANOVA and Spearman's correlation coefficients to study CrCl longitudinally. Compared the mean CrCl between different time periods (12-36, 36-60, 60-84 and 84-108 weeks) using one-way ANOVA and tested the effect of gender on CrCl over time using two-way ANOVA.	CrCl remained stable after a median of 21 weeks on TDF (difference of +1.06 ml/min; 95% CI -2.7-4.8, P =0.58), even among patients with underlying diseases. TDF could be prescribed safely at a standard dosage of 300 mg once daily in the Thai population.
Reid, A. & Stohr, W. et al. (2008)	DART 9 trial participants in Uganda and Zimbabwe from January 2003-October 2006, symptomatic (WHO stage II), HIV-infected, ART-naïve adults aged 18 years, CD4 <200 cells/mm^3	To describe the effect of ART on eGFR in a resource-limited setting (sub-Saharan Africa) in which 74% of participants received TDF as first-line treatment.	Observational cohort within an RCT (n= 3316)	Zidovudine-lamivudine plus a third drug, nevirapine (NVP) or abacavir (ABC) or TDF based on availability. Clinical monitoring only versus laboratory + clinical monitoring	96 weeks	Severely decreased eGFR (<30 ml/min/1.73 m^2) and changes in eGFR to 96 weeks	SCr levels measured before ART initiation, at weeks 4 and 12 of therapy, and every 12 weeks thereafter. Calculated eGFR by CG formula and defined according to grading of eGFR impairment by K/KDOQI criteria.	Estimated prevalence and incidence of impaired renal function. Random effects models for incidence of severely decreased eGFR (<30 ml/min/1.73 m^2) and changes in eGFR to 96 weeks, including demographic data, type of ART, WHO stage, and baseline biochemical and haematological characteristics as predictors. Used backward selection in a linear model to select a final model to predict baseline eGFR.	Lower baseline eGFR or haemoglobin level, lower BMI, younger age, higher baseline CD4 cell count, and female sex associated with greater increases in eGFR over baseline, with small but statistically significant differences between regimens (P <0.001 for all). Mild-to-moderate baseline renal impairment was common, severe infrequent. Compromised participants showed greatest increases in eGFR after starting ART.

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Mulenga, L. B. & Kruse, G. et al. (2008)	HIV-infected, ART-naïve adult patients (>15 years), had a baseline creatinine result, and initiated ART between 1 May 2004 and 30 September 2007 across 18 primary care facilities in Lusaka, Zambia	To examine the association between baseline renal insufficiency and mortality among adults initiating ART in an urban African setting	Cohort study (n= 25779)	All on TDF	<4 years	Primary measure of renal function: Estimates of CrCl by CG formula. Secondary analyses using SCr levels and GFR calculated by the MDRD equation. Primary outcome was death	Mortality examined according to baseline renal function among adults initiating ART. Renal function assessed by CG and MDRD equations, and absolute SCr levels. Used K/KDOQI guidelines to categorize renal insufficiency. Used commonly used cut-points from own clinical practice as thresholds for SCr. [10].	Compared crude mortality rates by the z-test. Modified multivariate Poisson regression to derive adjusted relative risk (ARR) and robust error variances, adjusted for baseline CD4 count, WHO stage, and haemoglobin. Kaplan-Meier curves stratified by disease severity for the association of renal insufficiency with mortality over time. Cox proportional hazards regression to estimate the hazard ratios for mortality over two periods: before and after 90 days.	About 1/3 of participants had renal insufficiency at time of ART initiation; 73.5% of them were mild, 23.4% were moderate, and 3.1% were severe. Covariates associated with renal disease =female sex , increasing age, haemoglobin <8 g/dl, BMI <16 kg/m^2, and WHO stage III or IV. Risk for renal insufficiency increased as CD4 cell counts decreased. Baseline renal function found to be an important independent predictor of survival

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[10]SCr ≤120 mmol/l= normal; SCr 121- 150 mmol/l= mild renal insufficiency; SCr 151-200 mmol/l= moderate; SCr >200 mmol/l= severe

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Peters, P. J. & Moore, D. M. et al. (2008)	HBAC 10 cohort: Participants aged 18 years in rural Uganda, had either a CD4 250 cells/ mm^3 or symptomatic HIV disease (WHO stage III or IV disease), baseline CrCl <25 ml/min (severe renal dysfunction) were excluded from the study.	To evaluate the impact of HAART on renal function among HIV-infected Ugandans in the Home- Based AIDS Care clinical trial.	Cohort nested in an RCT (n= 508)	HAART (stavudine plus 3TC with either NVP (98%) or EFV (2%)).	At least 24 months after HAART initiation	Renal dysfunction defined as CrCl <50ml/min evaluated at baseline and at 12-month and 24-month follow-up visits for participants followed for at least 24 months after HAART initiation.	CrCl using CG equation and simplified MDRD equation. Renal dysfunction defined as a CrCl of 25-50 ml/min. Binarized renal function at 50 ml/min according to HIV treatment guidelines dosage modification cut-off. Analysis repeated with renal function dichotomized at 60 ml/min. Used DAIDS table to grade the severity of adverse events [11].	Tested differences in serum creatinine, CrCl, and change in CrCl over 24 months on HAART between groups. Multivariable general linear regression model (GLM) to identify variables independently associated with changes in CrCl from baseline to 24 months. Backward elimination used to create a parsimonious model. Logistic regression to identify variables independently associated with baseline renal dysfunction.	Renal dysfunction prevalent in the rural Ugandan population with advanced HIV disease (20% baseline), but generally improved during 2 years on HAART. Baseline creatinine ≥133 mmol/l (1.5 mg per 100 ml), a weight gain ≥5kg on HAART, female gender, and WHO stage IV disease all associated with a greater improvement in CrCl. Participants with renal dysfunction at baseline had the biggest improvements in CrCl (about 53% increase over 24 months).
Franey, C. & Knott, D. et al. (2009)	South Africa, patients initiating ART at a public sector service in rural KZN (Hlabisa) between 2004 and 2007.	To assess prevalence and risk factors for impaired renal function in a large rural South African population initiating ART.	Retrospective review (cross-sectional prevalence, n= 2189)	d4T, 3TC and either EFV or NVP	not given	Outcome for logistic regression: Significant renal impairment defined as mild+ moderate renal impairment (eGFR ≤60 ml/min/1.73 m^2).	GFR was estimated (eGFR) with the MDRD method. Used K/KDOQI criteria to define impaired renal function.	Logistic regression was used to determine odds ratio (OR) of significantly impaired renal function (combining severe and moderate impairment). Co-variates for analysis were age, sex and CD4 count at initiation.	There was a low prevalence of severe renal impairment (29/2189, 1.3% 95% C.I. 0.8- 1.8), moderate renal impairment was more frequent, with many patients having advanced immunosuppression at initiation. Age >40, male gender and CD4 <100 cells/μl all associated with risk of significant renal impairment.

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[11] National Institutes of Health Division of AIDS DAIDS tables for grading the severity of adult and paediatric adverse events

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
De Beaudrap, P. & Diallo, M. B. et al. (2010)	Senegal; The source population consisted of 444 adults infected with HIV-1 enrolled in an observational cohort between August 1998 and December 2004	To describe and compare the changes in renal function between HIV-1 infected adult patients receiving ART with and without TDF and associated risk factors.	Observational prospective cohort and data from a clinical trial (n= 428)	TDF-regimen (n=40) and non-TDF regimen (n=388)	42 months	Primary: Change in eGFR over time. Secondary outcome: persistence of renal impairment from 12-42 months	Estimated GFR by CG and MDRD equations. Graded renal function (by CG and MDRD) into three states according to K/KDOQI criteria; normal (eGFR >90 ml/min), mild (60-90 ml/min), and moderate impairment (<60 ml/min).	Used latent class growth analysis (LCGA)for change in eGFR the first year of follow-up (0-12 months). For the remaining period (12-42 months), used Cox models stratified by states to assess transition rates between states and the effect of baseline covariates on them (CD4, BMI, United States Centers for Disease Control and Prevention (CDC) stage, viral load, age, sex, the first-line regimen (TDF-containing regimen versus other)), and the duration in the previous state. Used mixed effect models to take into account the correlation between repeated measurements of log-eGFR in an individual. [12]	After a year, patients receiving TDF experienced a higher rate of transition from mild to moderate renal impairment when compared with patients not receiving TDF (rate ratio of 2.74, 95% CI = [1.40; 5.37]). Other rates of transition not significantly different between patients receiving TDF or not. A significant though moderate decline in the renal function observed in 1/3 of the patients on TDF compared to patients not on TDF, this impairment was persistent after the first year of treatment.

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[12]Used the log transformation of eGFR (log-eGFR) in the regression models to achieve normality

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Brennan, A. & Evans, D. et al. (2011)	HIV-infected adults who received TDF, had a CrCl done at initiation at the Themba Lethu Clinic, Johannesburg, South Africa, 18 years, either ART-naïve at initiation (21%), or switched onto an ART regimen containing 300 mg of TDF daily between April 2004 and September 2009. Mostly women (73.5 %)	To analyse the relationship between renal dysfunction at tenofovir initiation, nephrotoxicity and mortality	Retrospective Cohort study (n= 890)	All given TDF	48 months	ART outcomes (nephrotoxicity and mortality) by 48 months of follow-up stratified by renal function at TDF initiation	Nephrotoxicity any decline in kidney function from baseline (acute or chronic) that is secondary to a toxin (including drugs) and documented by a clinician, within 48 months after initiation onto TDF. Mortality ascertained via South African National Vital Registration Initiative for patients who were LTFU [13] Creatinine clearance by the CG equation, categorized according to the K/KDOQI criteria.	Modeled the relationship between renal dysfunction, nephrotoxicity and mortality for patients initiated onto TDF-containing regimens using marginal structural models and inverse probability of treatment weights to adjust for lost to follow-up (LTFU) and confounding. Fitted a weighted pooled logistic model controlling for baseline covariates and current viral load (<400 or 400 copies/ml) to estimate predictors of death and nephrotoxicity.	A total of 2.4% experienced nephrotoxicity, 7.8% died and 9.7% were lost during 48 months of follow-up. Patients with mild [HR 4.8; 95% confidence interval (CI) 1.5-15.2] or moderate (HR 15.0; 95% CI 3.4-66.5) renal dysfunction were at greatest risk of nephrotoxicity. Those with mild (HR 1.2; 95% CI 0.7-2.3) or moderate (HR 3.2; 95% CI 1.3-7.8) renal dysfunction vs. normal renal function were at highest risk of death by 48 months. It was likely that most of the incident renal dysfunction in TDF patients was related to pre-existing renal disorder, which may be compounded by TDF.

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[13]Lost to follow-up (LTFU) was defined as not having attended the clinic in 4 months.

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Bygrave, H. & Kranzer, K. et al. (2011)	Lesotho; All adults initiating ART from 1 January 2008 to 31 December 2008 who had a baseline serum creatinine were included in the analysis. Majority (62.5%) were women	To investigate the prevalence of abnormal renal function at baseline and factors associated with abnormal renal function from a community cohort in Lesotho	Retrospective Cohort study (n= 933)	All on TDF	up to 18 months	Creatinine Clearance as a measure of renal function	CrCl by CG equation using weight taken on the same day as creatinine but without adjusting for body surface area. Baseline renal function categorized according to K/KDOQI criteria. Baseline renal function also categorized into CrCl <50 ml/min and >50 ml/min. Calculated changes in CrCl from baseline for patients on TDF at 6 and 12 months and the proportion of patients initiated on TDF who developed renal impairment. Defined adequate renal function as a CrCl >50 ml/min calculated using the CG equation.	Compared baseline characteristics for patients starting TDF correctly and those started in error. Derived crude and adjusted odds ratios for CrCl $\geq$50 ml/min versus CrCl<50 ml/min by univariate and multivariate logistic regression models (by backwards elimination) and identified risk factors for reduced CrCl. Developed screening algorithms using the risk factors identified. Determined the median change in CrCl in the TDF group from baseline at 6 and 12 months. (5% Significance level)	Renal function improved during follow-up. Patients who developed renal toxicity during follow up remained on TDF (n= 19); renal function improved (CrCl $\geq$50 ml/min) in all but 3 of these patients. TDF-associated renal toxicity was rare and mainly a transitory state in this setting.

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Study	Countries, participants & year of analysis	Objective(s) of study	Type of Study (Sample size)	Exposure & Comparison	Duration of exposure/ follow-up time	Health outcomes measured	Definitions and Methods	Analytical methods	Findings and Conclusions
Johnson, D. C. & Chasela, C. et al. (2012)	Malawi, Lilongwe; HIV-infected, ART-naïve adults screened for enrolment into 5 HIV clinical trials in Lilongwe, Malawi. Screened individuals drawn from the general HIV-infected population. No exclusion criteria excluded patients based on co-morbid diseases associated with kidney disease prior to the study. Most subjects were female (90.6%).	To determine prevalence and predictors of CrCl<50 ml/min among HIV-infected, ART-naïve individuals in Lilongwe, Malawi.	cross-sectional study of pooled data from 5 clinical trials (n= 3508)	First-line ART (d4T, 3TC and NVP) and PMTCT [14] regimen (TDF/ 3TC/ EFV) as a fixed dose combination tablet	cross-section (no follow-up)	Outcome= CrCl <50 ml/min based on categorization of CrCl from CG equation	Used the CG equation to estimate CrCl using data collected at screening before an individual was selected for recruitment into a clinical trial. All trials included in this analysis collected information at screening on serum creatinine, CD4 cell count, and haemoglobin on participants drawn from the general HIV-infected population.	Bivariate analyses to assess the crude association for each covariate with CrCl <50 ml/min according to both CrCl equations. Multivariable logistic regression for association between age, BMI, haemoglobin, CD4 <350 cells/mm^3, gender, and pregnancy with CrCl <50 ml/min.	Reduced CrCl <50 ml/min was rare (only 1.1 %) Baseline creatinine clearance assessment may not be necessary for implementation and can be offered to high-risk patients (individuals with low BMI, low haemoglobin, non pregnant women who were at greatest risk for a CrCl <50 ml/min).

[14]Prevention of mother to child transmission

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7 Summary of Findings and Interpretation of Literature

7.1 Prevalence and risk factors

In the study conducted by Jones et al. in a European cohort of 4183 HIV-infected patients, renal dysfunction was rare and mostly attributable to a non-TDF cause [61]. Franceschini et al. also found that in a US-based cohort of HIV-infected patients in primary care, most cases of acute renal failure were associated more with opportunistic infections resulting from immunosuppression than with HAART (including tenofovir) [15]. In a similar US cohort consisting of patients who had received TDF as part of HAART for at least three months in two clinical practice settings, all patients with serum creatinine increases within 6 months of using TDF had other medical reasons for this increase [55].

Advanced immunosuppression (low CD4 count, high viral load, prior AIDS-defining illness) [23, 48, 65] and decreased renal function at baseline prior to ART initiation [48, 56, 63] frequently emerge as strongly predictive variables for impaired baseline renal function and decline over time both in resource-rich and resource-limited settings [48, 65]. Other associated factors include HCV coinfection [65], diabetes [48, 63], age >40 years [56, 62]), lower weight/ BMI <18 kg/m^2 [58, 59], low haemoglobin and anaemia [49, 59]. Female gender was sometimes also found to be associated with higher risk at baseline [54, 58, 67].

In the African setting, mild-to-moderate renal impairment was more common with a prevalence of 20% in rural Ugandans prior to ART [58]), 13% in rural Kwazulu Natal (KZN) [65], 74% mild and 23% moderate renal insufficiency in Zambia [67], 30% mild and 5% moderate in the Johannesburg cohort [49]. Severe renal impairment was less common, ranging from over 1% in rural KZN [65] to 3% in Zambia [67]. Many of these patients had advanced immunosuppression before starting treatment [50, 54, 65].

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7.2 Incidence, risk and patterns of change over time

Jones and others have found that TDF is not associated with renal dysfunction more often than other ARVs [61]. In contrast, Gallant and others found that TDF use was associated with significantly greater decrease in creatinine clearance compared to other nucleoside reverse-transcriptase inhibitors (NRTIs) [48]. Both these studies were conducted in resource-rich settings, the UK and US respectively.

In a study in Senegal more patients on TDF moved from mild (60-90 ml/min/1.73 m^2) to moderate renal impairment (30 - 60 ml/min/1.73 m^2) after a year, compared to patients not on TDF with a rate ratio of transition from mild to moderate renal impairment of 2.74 in patients receiving TDF

(95% CI: 1.40, 5.37). In the DART [15] trial the incidence of grade 3 or 4 decreased eGFR was 1.7% for participants on TDF [8]. These changes occurred in a median of 14 weeks after starting ART (IQR, 4-52 weeks; range, 2-96 weeks).

In Western studies, incidence ranged from 1.3% changes in creatinine clearance and serum creatinine elevations in ART-experienced patients after starting TDF [55], to a cumulative incidence rate of a confirmed GFR <70 ml/min of 1.18 per year of TDF use (95% CI: 1.12; 1.25) in patients in the D:A:D study with normal baseline renal function (eGFR ≥90 ml/min) [23]. It was more difficult to quantify incidence in terms of movements between grades in developed country studies due to the occurrence of fewer renal events .

The risk of significant renal impairment after initiating ART depends both on baseline characteristics and on clinical characteristics over time and other comorbidities for kidney disease like diabetes [48, 63]. Some patients with advanced immunosuppression at baseline improved over time, while others experienced declines in eGFR [48]. Scherzer et al. conclude that pre-existing renal risk factors did not appear to aggravate the effects of TDF, with TDF exposure itself independently related to irreversible risk of proteinuria, CKD and rapid declines in GFR [40]. On the other hand, De Beaudrap found that those with persistent decrease in eGFR did not have different baseline characteristics from those that did not, apart from impaired immune reconstitution [50]. TDF had an independent effect on adverse renal function even in person's without pre-existing baseline renal impairment and this was not significantly modified by age, HCV, prior AIDS-defining illness or current CD4 cell count [23].

7.3 Time and detecting changes in renal function

The change in CrCl occurred mostly in the first year of TDF use [56]. In the Johns Hopkins HIV cohort, change in CrCl was evident after 2 months of treatment and was found to persist for the rest of the year [48], while there were no significant changes in creatinine clearance in the first 6 months of TDF use in a similar cohort of patients taking TDF in the US [55]. After observing patients cared for in routine clinical practice in Spain, Padilla et al. found that renal failure was infrequent during a 12 month period and TDF was associated with a low risk of mild renal failure [62]. In another study in Italy, renal function was found to improve after 48 weeks due to HAART-induced viral load suppression [64].

Similar findings were found in the African setting where renal function improved during follow-up [54], particularly in severely immunosuppressed patients at baseline, who had the greatest increases in eGFR over time (after 96 weeks [8], and after 24 months [58]). Results from the

[15] Development of Antiretroviral Therapy DART trial in Uganda and Zimbabwe

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HBAC cohort [16] in Uganda found that a higher baseline serum creatinine level (>133 mmol/l), a weight gain of ≥5kg on HAART, WHO stage IV disease and female gender were associated with greater improvement in creatinine clearance after 24 months on HAART [58].

Although TDF was found to be associated with a statistically significant decline in CrCl, this was not found to be clinically significant, at least not enough to discontinue its use [48]. Bygrave et al. hypothesize that by screening patients in a rural Lesotho clinic, some of the patients whose creatinine clearance dropped to 40-50 ml/min may have been unnecessarily switched off TDF if renal function had been rechecked as more than 80% of them actually experienced an improvement in renal function over time [54].

7.4 Identification of gaps and needs for further research

Prevalence and incidence of renal dysfunction varies according to definitions and methods used [75]. Severe renal dysfunction was rare in most settings but mild-to-moderate dysfunction was more common in the African setting.

It is evident that we cannot extrapolate the findings from well-resourced settings to the developing world. Even within the developed world itself, clear differences in prevalence of kidney disease are evident. Severe renal dysfunction was rare in both settings, but mild-to-moderate dysfunction was more common in the African setting. Coupled with the additional burden of diabetes and hypertension in the African setting and patients initiating ART with more advanced disease, the incidence of renal impairment associated with tenofovir needs a country-specific lense.

Some studies found TDF to be an independent risk factor for renal dysfunction [40]. Others suggest that pre-existing renal risk factors are in fact aggravated by TDF to progress to renal failure, while others argue that the beneficial effects of HAART outweigh these risk factors.

While guidelines stress the importance of monitoring patients receiving TDF for early detection of renal impairment, there has been some debate about when this should be done if at all. There is no clear evidence as to when would be the optimal time after the first pre-ART screening. The WHO consolidated guidelines for 2013 are still not clear on how to best monitor renal function in people using TDF- containing regimens. Unanswered questions persist on whether toxicity monitoring should be routine or targeted to high-risk patients [44].

[16] Home- Based AIDS Care Trial Uganda

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8 Conclusion

The studies reviewed report diverse populations with various risk factors and renal outcomes. However, there are limited data on the prevalence of renal dysfunction in patients starting ART in primary care, and the factors associated with impaired creatinine clearance. There is conflicting evidence about the change in renal function among patients on tenofovir and little is known about the incidence of renal dysfunction in patients starting tenofovir during the first year on therapy.

If renal dysfunction can be detected at a particular time point after ART initiation, this can optimize the use of laboratory monitoring and resources. In settings where resources for laboratory testing are limited, the associations between decreased renal function and markers of advanced HIV disease may help to focus monitoring of renal function in those high-risk individuals.

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Part III

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Prevalence and Incidence of Renal Dysfunction in Patients initiating Antiretroviral Therapy at a Primary Health Care Centre in Gugulethu, Cape Town: a Cohort Study

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Abstract

Background: Tenofovir disoproxil fumarate (TDF) is used worldwide for the treatment of HIV-1 infection. Tenofovir has been found to be associated with declines in glomerular function and chronic kidney disease in HIV-infected patients. There are limited data on the prevalence and incidence of HIV-related kidney disease in sub-Saharan Africa. We described the prevalence and incidence of renal dysfunction in primary health care in a cohort of 1092 HIV-infected individuals initiating TDF. We analysed the patterns of change in their renal function in the first 12 months on therapy, factors associated with renal dysfunction and examined the diagnostic role of early serum creatinine measures in identifying incident renal dysfunction after 12 months on ART.

Results: The cohort consisted of 62% women and 38% men, median age at baseline was 34 years (IQR 29; 41 years). Most of the patients had normal baseline renal function; eGFR >90 μmol/L (62%), 34% had mildly impaired eGFR and 4% had moderate-severe renal function impairment calculated using the Cockroft-Gault equation. Age greater than 41 years, female gender, higher WHO stage (III and IV) and anaemia were all independently associated with increased probability of moderate or severe renal dysfunction at baseline. The estimated glomerular function improved in most sub-groups of patients over the first 12 months on TDF (0.960 ml/min/1.73m^2 mean increase over 12 months (95% CI: 0.67; 1.26) and this increase was not significantly confounded by baseline covariates. Female gender, higher baseline serum creatinine and age greater than 29 years were associated with faster growth in mean eGFR over 12 months. Overall incidence of eGFR decline over 12 months was low (4.4% developed eGFR <50 ml/min/1.73m^2) and the crude incidence rate for a decline >10 ml/min/1.73m^2 in 12 months was 9.76 per 100 person years. Earlier creatinine tests that were done before 4 months on ART had limited diagnostic value in predicting overall renal function change after a year on ART.

Conclusion: Renal dysfunction was uncommon in HIV-infected adults initiating ART in this primary health care setting. Renal function generally improved during the first year on ART even in those with lowest creatinine clearance at baseline. Creatinine tests done earlier than four months after baseline screening may be unnecessary.

The article meets the requirements set out in the Instructions for Authors for the AIDS Research and Therapy Journal, an extract of these instructions is included in Appendix E of the dissertation. For readability purposes, figures and tables are inserted in the text of the manuscript rather than appended as required by the Journal. Supplementary materials referred to in the article are included in Appendix D. No co-authors are listed here. The contribution of collaborators and supervisors is listed in the acknowledgements section of the dissertation.

3. Journal Manuscript

1 Background

Tenofovir disoproxil fumarate (TDF)is prescribed world wide as part of antiretroviral therapy (ART) for the treatment of HIV-1 infection. The South African government included TDF as a first line regimen drug in April 2010 [1]. The first efficacy and safety trials conducted in HIV-infected patients found that tenofovir had a good safety profile with minimal risk [2–4]. However, subsequent observational studies have found tenofovir to be associated with modest but significant declines in glomerular function in HIV-infected patients [5]. The severity of this decline has implications for the development of chronic kidney disease and the long-term health of HIV-infected patients.

Coupled with this is the increased prevalence of pre-existing kidney disease in HIV- infected patients compared to the general population. Approximately 5-15% of patients present with a reduced creatinine clearance (estimated glomerular filtration rate (eGFR)) of <60 ml/min/1.73m^2 pre-ART) [6]. But the exact prevalence of HIV related kidney diseases is hard to quantify due to the use of different criteria for diagnosis and different patient populations [7]. In South Africa, the prevalence of HIV-related kidney disease is estimated at approximately 6% in ART-naïve out-patients who present with persistent proteinuria [8] and 27% prevalence of HIV-associated nephropathy in hospitalized patients [9]. But the prevalence of abnormal renal function may be different in a primary health care setting.

There are limited data on how soon after ART initiation any loss of renal function can be detected. While frequent monitoring is recommended for patients on TDF, no clear evidence exists on optimal creatinine screening times. The South African National ART guidelines recommend that serum creatinine and creatinine clearance be measured at ART initiation to rule out pre-existing disease and again at 3 and 6 months and annually thereafter in order to identify possible TDF toxicity [1, 10]. The recommendations draw on evidence from both the developed world and from studies conducted in limited resource settings that found that renal function decline after ART initiation

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occurs sometime in the first year [11–13]. The World Health Organization (WHO) recommends screening for renal impairment prior to treatment with TDF, but this is not mandatory to start treatment [14, 15].

However, the updated WHO guidelines for 2013 are still unclear on whether laboratory screening and toxicity monitoring after ART initiation should be routine or targeted to high risk groups to best monitor renal function in TDF patients [15]. High risk patients identified include those who are older, those with pre-existing renal disease, diabetes, untreated hypertension and those using a boosted protease inhibitor or other known nephrotoxic drugs [15]. The guidelines emphasize that more research is required in order to devise a suitable monitoring strategy [14, 15].

The objectives of this study were to describe the prevalence and incidence of renal dysfunction in HIV-infected patients initiating ART in primary health care in South Africa. We explored and described patterns of change in renal function in patients starting tenofovir in the first 12 months on therapy and the factors associated with renal dysfunction in these patients. In addition we examined the diagnostic role of early serum creatinine measures done in the first 12 months on therapy (at months 1, 2, 4 and 12) in identifying incident renal dysfunction.

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2 Methods

2.1 Study Setting

The Hannan Crusaid Treatment Centre (HCTC) is based at the Gugulethu Community Health Centre situated in the Gugulethu district of Cape Town and provides ART to the residents of this district. The service has been described in detail before [16–18]. Patients become eligible for ART according to the South African antiretroviral treatment guidelines (either WHO Stage IV disease or a CD4 cell count below 200 cells/mm^3) [14]. If eligible, they are seen twice before initiating ART, at least 4 weeks and at 2 weeks prior to initiation. Thereafter they are seen at scheduled visits at 4 weeks, 8 weeks, 16 weeks and then at 16-week intervals [16].

2.2 Cohort Description

Patient records were reviewed for the first year after starting a TDF-containing ART regimen. Participants were consecutive HIV-infected, adult patients (>18 years old) who initiated ART

regimens at HCTC containing 300 mg of TDF daily between February 2010 and April 2012. A total of 1861 patients were screened for ART during the period; we excluded women who were pregnant at ART initiation, patients who were not treatment-naïve and those who died before starting ART.

2.3 Measures

Patient information was extracted from patient folders at HCTC and an ongoing electronic database updated with National Health Laboratory Service (NHLS) diagnostic data. The main outcome of interest was renal function defined as creatinine clearance after 12 months on ART. This was assessed by estimating the glomerular filtration rate calculated using the Cockroft-Gault (CG) equation [19].

Creatinine tests were done before initiating ART, after 1 month, 2 months, 4 months, and again after 12 months on ART. We extended the window around the 12 month interval to capture creatinine measurements done up to 18 months after initiation in cases where no measurement was available by 12 months. Patients were regarded as lost to follow up if they were not seen at the clinic for longer than three months after starting ART.

Severe renal dysfunction was defined as eGFR <30 ml/min/1.73m^2, moderate renal dysfunction as eGFR of 30-59 ml/min/1.73m^2 and mild renal dysfunction as an eGFR of 60- 89 ml/min/1.73m^2, according to the US National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/KDOQI) criteria [20]. Incident renal function decline was defined as any reduction in baseline eGFR of more than 10 ml/min/1.73m^2 or any movement below 50 ml/min/1.73m^2, the threshold indicated by the WHO as a possible indication of TDF nephrotoxicity [15].

2.4 Study Variables

Demographic characteristics (age, sex) and disease characteristics (CD4 cell count, WHO staging, and viral load) were collected for 1092 patients included in the analysis. Viral load measurements were done at pre-ART screening which was done (4-2 weeks) prior to ART initiation. Laboratory diagnostics (CD4 count, viral load, and serum creatinine) were performed by the NHLS diagnostic pathology services, while WHO staging and weight measurements were done by health care personnel at HCTC. Patient weights were extracted by retrospective review of patient folders at the

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clinic. Anaemia was classified as haemoglobin <12 g/dL for women, and haemoglobin <13 g/dL for men.

2.5 Statistical Analysis

Prevalence and associated risk factors

Baseline was defined as the date of ART initiation. The prevalence of moderate or severe renal dysfunction was estimated at baseline using a prevalence ratio of all those with baseline eGFR <59 ml/min/1.73m^2 as a fraction of the total sample. Risk factors for severe or moderate renal dysfunction at baseline were identified using logistic regression analysis. Prevalence odds ratios (POR) were estimated for individual baseline covariates and in the final model controlling for age, gender, WHO stage and anaemia status.

Patterns of change in eGFR

The mean observed GFR profiles over time were plotted by patient and disease characteristics. Linear mixed effect models using maximum likelihood estimation were used to model the association between eGFR profiles over time and relevant covariates. The mean eGFR profiles were modelled from month 1 in order to avoid circularity between baseline covariates used as independent variables in the models and in the calculation of the eGFR outcome (baseline weight and serum creatinine). Stratified models were used to investigate whether mean eGFR profiles had significantly different rates of change in different subgroups of patients over time.

We used model-based imputation by maximum likelihood estimation in the mixed effect models to account for missing data. For objective 3, Multiple imputation by chained equations (mice) was used to impute missing weights in order to include measures for patients with missing data at month 12 [21]. A detailed outline of the imputation process is included in *Appendix 1.2*.

Incidence of GFR impairment

Incident renal function decline was estimated using incidence proportions calculated using the number of cases who experienced a reduction in eGFR below 50 ml/min/1.73m^2 over the 12 month period with available weight and serum creatinine measures. A second estimate of incidence was calculated using incidence rates per 100 person years for any reduction in eGFR of more than 10 ml/min/1.73m^2 using the accrued person-time at each interval.

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Diagnostic Role of early Creatinine Tests

In order to examine the prognostic role of early creatinine tests, we defined a second outcome variable: the absolute change in eGFR from baseline to month 12 in ml/min/1.73m^2.

Separate models for change in eGFR were built using serum creatinine measurements at month 1, month 2 and month 4 as predictors including baseline covariates. The predictive power of these models was compared using the coefficient of determination *R-squared* statistic. Nested models of serum creatinine measurements at months 1, 2 and 4 were compared to determine which measurements were relatively more useful in predicting the outcome. The nested models were compared using the likelihood ratio χ^2-squared statistic. All statistical analyses were performed using STATA/SE version 12 (StataCorp). The study was approved by the Human Research Ethics Committee at the University of Cape Town.

3 Results

3.1 Baseline Characteristics of Patients initiating TDF

The baseline characteristics of the 1092 patients included in the analysis are shown in Table 3.1. The cohort consisted of more women than men (62% vs. 38%) and the median age at time of ART initiation was 34 years (IQR 29; 41 years). Most of the patients had renal function within the normal range of >90 μmol/L at ART initiation (62%), 34% had mildly impaired eGFR and 4% had moderate-severe renal function impairment. More than half the patients presented with advanced HIV disease at baseline (43% WHO Stage III and 11% Stage IV) with a median CD4 cell count of 154 cells/mm^3 (IQR 84; 218 cells/mm^3) and a high prevalence of anaemia (63%).

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Table 3.1: Baseline demographic, clinical and laboratory characteristics of 1092 participants stratified by pre- ART renal function

Variable Median (IQR)	Normal [a] 676 (62%)	Mild [b] 368 (34%)	Moderate- severe [c] 48 (4%)	Total 1092 (100%)
Age (years) Median (IQR)	32 (28; 38)	38 (32; 46)	42 (33; 54)	34 (29; 41)
Age category N (%)				
<29 years	227 (33%)	69 (19%)	6 (13%)	302 (28%)
29-34 years	181 (27%)	66 (18%)	9 (19%)	256 (23%)
34-41 years	173 (26%)	97 (26%)	6 (13%)	276 (25%)
>41 years	95 (14%)	136 (37%)	27 (57%)	258 (24%)
Sex N (%)				
Male	224 (33%)	172 (47%)	16 (33%)	412 (38%)
Female	452 (67%)	196 (53%)	32 (67%)	680 (62%)
WHO disease stage N (%)				
I	176 (27.5%)	65 (19%)	6 (13%)	247 (24%)
II	144 (22.5%)	77 (22%)	6 (13%)	227 (22%)
III	252 (39%)	164 (47%)	26 (57%)	442 (43%)
IV	69 (11%)	41 (12%)	8 (17%)	118 (11%)
CD4 cells/mm^3 Median (IQR)	161 (93; 226)	150 (71; 209)	114 (60; 184)	154 (84; 218)
CD4 category N (%)				
<100 cells/mm^3	156 (27%)	105 (33%)	14 (40%)	275 (30%)
100-200 cells/mm^3	215 (37%)	117 (37%)	14 (40%)	346 (37%)
≥200 cells/mm^3	204 (36%)	95 (30%)	7 (20%)	306 (33%)
Viral load ($\log_{10}$) copies/ml Median (IQR)	4.68 (4.17; 5.18)	4.82 (4.29; 5.33)	4.74 (4.20; 5.60)	4.74 (4.21; 5.22)
Viral load category N (%)				
copies/ml <5 $\log_{10}$	351 (65%)	185 (60%)	21 (60%)	557 (63%)
copies/ml ≥5 $\log_{10}$	189 (35%)	121 (40%)	14 (40%)	324 (37%)
Haemoglobin g/dL Median (IQR)	11.7 (10.3- 13)	11.8 (10.1; 13.1)	10.25 (8.8; 11.55)	11.7 (10.1; 13)
Haemoglobin g/dL - Males	12.7 (11.3; 14.0)	12.5 (10.8; 13.8)	11.4 (10.1; 11.7)	12.5 (11; 13.9)
Haemoglobin g/dL - Females	11.4 (10.0; 12.5)	11.1 (9.6; 12.2)	9.7 (8.1; 11.3)	11.2 (9.7; 12.3)
Anaemia [d] N (%)	384 (61%)	218 (62%)	40 (91%)	642 (63%)
Serum Creatinine μmol/L Median (IQR)	59 (52; 68)	72 (64; 81)	87 (77; 105)	64 (56; 74)
Weight kg Median (IQR)	67 (59.4; 76.2)	56.8 (50.7; 64.1)	50.5 (46.7; 56.6)	62.4 (54.4; 71.3)
eGFR ml/min/1.73m^2[CG] Median (IQR) [e]	110.6 (99.5; 130.5)	78.5 (72.4; 84.7)	53.5 (48.2; 56.7)	97.1 (81.8; 116.6)

[a]Normal: eGFR >90 ml/min/1.73m^2
[b]Mild: eGFR 60-89 ml/min/1.73m^2
[c]Moderate & Severe: eGFR <60 ml/min/1.73m^2
[d]Anemia status: haemoglobin <12 g/dL for women, and haemoglobin <13 g/dL for men
[e]eGFR: estimated glomerular filtration rate, determined using the Cockroft-Gault equation

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3.2 Risk factors associated with prevalent renal dysfunction at baseline

In univariate analysis, older age (age >41 years), Stage III and IV disease and anaemia were significantly associated with increased probability of severe-moderate renal dysfunction ($p < 0.05$). In a multivariable logistic regression model, age greater than 41 years, female gender, higher WHO stage (III and IV) and anaemia were all independently associated with increased probability of moderate or severe renal dysfunction at baseline ($p < 0.05$) *(See Appendix Table D.1 for crude and adjusted model output).*

3.3 Follow up measurements and loss to follow up

The patient flow diagram in Figure 3.1 displays the available serum creatinine measures at screening visits during the first year of treatment. Baseline GFR was estimated for 1092 patients who initiated a TDF-containing regimen and had a serum creatinine measurement at baseline. By month 1, 44 patients had been lost to follow up for reasons other than death and 9 had died (5%). Serum creatinine measures for 641 patients were available at month 1 (59%), 697 at month 2 (64%) and 614 measurements were recorded at month 12 (56%).

By the end of the first year, a total of 237 patients had been lost to follow up corresponding to a retention rate of 78%. Reasons for loss to follow up were either known (patient transfers to other clinics and reported deaths (10%)) or unknown (90%). The patients who were lost during the first year on ART had similar age, WHO stage, creatinine and eGFR levels as the retained patients at baseline *(See Appendix Table D.2 for full table).* There was a greater proportion of men who were lost compared to women, and some differences in proportions with respect to CD4 cell count, viral load category and anaemia. Relatively more of those lost had CD4 cell counts <100 cells/mm^3, detectable viral loads (≥ 5 $\log_{10}$ copies/ml) and had anaemia. These proportions were inflated by the clinical characteristics of patients who died, who were sicker than those who remained in care.

Although, the median baseline weight for those lost to follow up was slightly lower (60.1 kg compared to 63.2 kg), there was no significant difference in median eGFR or renal function categories. More importantly, there were no significant differences in baseline characteristics for those patients with missing creatinine versus measured creatinine for any reason at month 12, except for gender which showed a relatively smaller proportion of men who had creatinine tests done at month 12.

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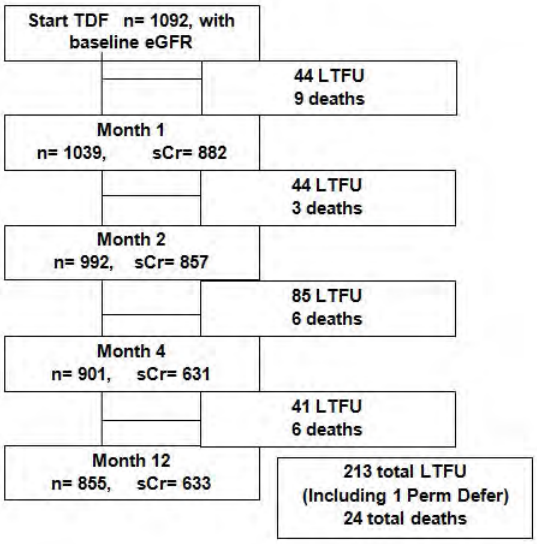

Figure 3.1: Patient flow diagram for the first year after tenofovir initiation. The boxes between the stages represent the numbers lost from care and those that died during each stage.
N = number of patients, LTFU = loss to follow up, Perm defer = Permanent deferrals defined as patients who did not receive ART for a reason other than pre-treatment death and who were subsequently excluded from the program. SCr = number of serum creatinine measurements

(See Appendix Table D.5 for full table).

3.4 Observed changes in eGFR over time

We explored eGFR profiles by patient demographic and disease subgroups as displayed in Figure 3.2. Panel 2.A displays the overall eGFR profile over the 12 month period with point-wise confidence intervals for the monthly estimates. The plot displays an increasing linear trajectory which shows that overall, renal function improved on average in the 12 months after ART initiation.

The additional panels (B-F) display the observed eGFR profiles by age groups, gender and baseline disease characteristics. Some notable eGFR profile differences exist between age groups, gender, WHO disease stage and anaemia status at baseline. On average, the age groups had similar slopes except the oldest age group (age >41 years) which had lower mean eGFR compared to the other three groups. Women had higher mean eGFR than men, and their eGFR increased at a faster rate over time. Mean eGFR increased steadily for WHO stage III and IV disease patients. Patients with stage I or II disease started off at a higher mean level of eGFR, but the two profiles had similar

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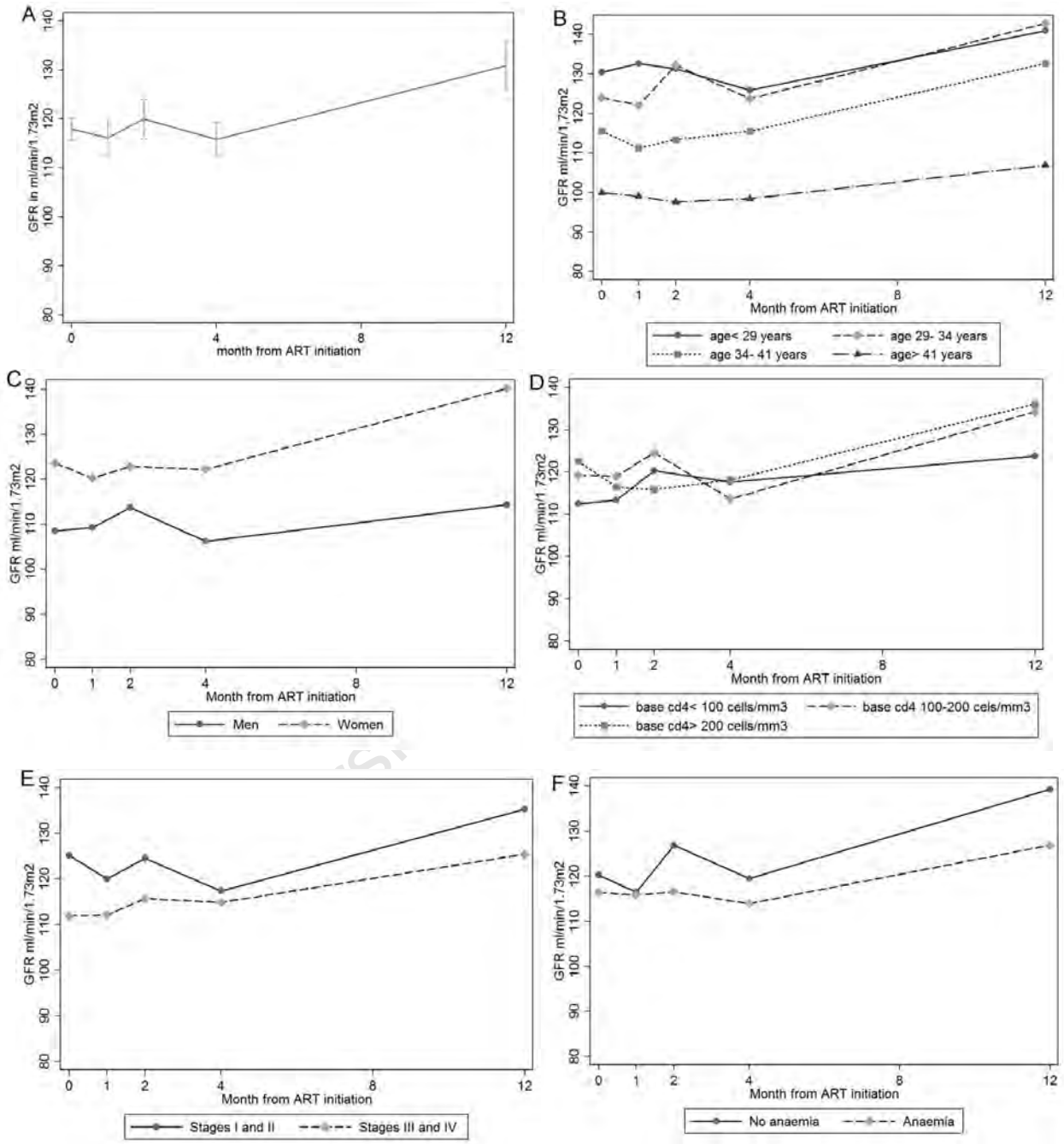

Figure 3.2: Profile plots of mean estimated creatinine clearance values (y- axis) and time on TDF in months (x-axis) by patient demographic and clinical characteristics: A- overall average profile, B- by age group, C- by gender, D- by baseline CD4 cell count, E- by WHO disease stage, F- by anaemia status at baseline

slopes after month 4. Patients with anaemia had lower mean eGFR levels compared to patients with no anaemia but the profiles were parallel indicating no significant group effect over time. The mean profiles for baseline CD4 cell count levels overlap, indicating no significant group differences between CD4 cell count categories.

3.5 Estimated glomerular function changes over 12 month period

The estimated glomerular function displayed a positive trend over time; eGFR increased by 0.960 ml/min/1.73m^2 for every one month increase as shown in Table 3.2 (95% CI: 0.67; 1.26). There was a negative association with baseline serum creatinine and age. On average, for every μmol/L increase in baseline serum creatinine, mean eGFR decreased by 0.277 ml/min/1.73m^2 (95% CI: -0.381; -0.172). For every 10 year increase in age, mean eGFR decreased by 12.50 ml/min/1.73m^2 (95% CI: -14.68; -10.33). There was a positive association with baseline weight and CD4 cell count. For every 10 kg increase in baseline weight, mean eGFR increased by 12.2 ml/min/1.73m^2 (95% CI: 10.9;13.6).

The average eGFR estimates for the reference categories at month 1 are displayed in Table 3.2 for those categories. The estimates for the other categories are relative changes compared to the reference category. Women had higher mean eGFR profiles than men (12.72 ml/min/1.73m^2 higher at month 1 (95% CI: 7.97; 17.47 unadjusted). Patients with anaemia had lower mean eGFR compared to patients with normal haemoglobin levels, while WHO stage III and IV disease patients had lower mean eGFR profiles compared to stage I and II patients. The mean eGFR profiles for patients with CD4 cell counts greater than 100 cells/mm^3 were higher than those for patients with CD4 cell counts below 100 cells/mm^3.

After adjusting for baseline serum creatinine, baseline weight, age, sex and baseline CD4 cell count, the adjusted slope estimate showed that the mean effect over time was not significantly confounded by baseline covariates and eGFR improved in most subgroups of patients over the first 12 months on TDF (*See Table 3.2*).

3.6 Stratified Models

The stratified models displayed in Table 3.3 show estimated rates of growth in eGFR in different subgroups of patients. Decreased statistical power as a result of stratification made it difficult to

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Table 3.2: Linear mixed model results for estimated glomerular filtration rate over time

Variable [a]	Model coefficient [b] & group intercept	95 % CI [c]	Model coefficient [d] & group intercept	95 % CI[c]
Month (continuous)	0.960	(0.665; 1.255)	0.926	(0.630; 1.221)
Baseline creatinine μmol/L (cont)	-0.277	(-0.381; -0.172)	-0.272	(-0.352; -0.193)
Creatinine category				
≤56 μmol/L reference	112.836	(108.400; 117.272)		
56- 64 μmol/L	-12.479	(-18.806; -6.151)		
64- 74 μmol/L	-14.732	(-20.926; -8.537)		
>74 μmol/L	-23.520	(-29.733; -17.307)		
Baseline weight (kg)(cont)	1.224	(1.093; 1.355)	1.256	(1.137; 1.375)
Weight category				
≤54.5 kg reference	82.121	(78.025; 86.217)		
54.5- 62.9 kg	13.268	(7.632; 18.904)		
62.9- 72.3 kg	19.760	(14.258; 25.262)		
>72.3 kg	43.751	(37.985; 49.518)		
Age (years) (cont)	-1.265	(-1.500; -1.030)	-1.238	(-1.426; -1.050)
Age category				
≤29 years reference	110.910	(106.546; 115.274)		
29- 34 years	-2.673	(-8.692; 3.346)		
34- 41 years	-12.409	(-18.502; -6.316)		
≥41 years	-28.098	(-34.195; -22.000)		
Sex (female)	12.723	(7.975; 17.472)	1.988	(-1.717; 5.693)
Anaemia	-5.514	(-10.460; -0.567)		
Baseline CD4 cells/mm^3 (cont)	0.027	(0.003; 0.051)	-0.004	(-0.021; 0.012)
CD4 category				
<100 cells/mm^3 reference	96.374	(91.790; 100.958)		
100-200 cells/mm^3	6.128	(0.179; 12.077)		
≥200 cells/mm^3	5.272	(-0.977; 11.520)		
WHO Stage (III & IV combined)	-3.791	(-6.180; -1.401)		

[a] **NOTE**: All models are linear mixed effect models with time as a fixed effect and subjects as random effects starting at month 1

[b] Univariate Analysis; slope coefficient for model with month and intercept relative to the mean for indicated reference group

[c] 95 % Confidence Interval

[d] Multivariate Analysis adjusting for baseline covariates shown; coefficients for model with month and baseline creatinine, baseline weight, age, gender and baseline CD4 cell count

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detect differences in the rates of growth between the groups.

The slopes suggest possible differences in the rates of growth in eGFR for different levels of baseline creatinine, gender, age and baseline CD4 cell count where the degree of overlap of the groups' confidence intervals showed some differences. Patients with age greater than 29 years experienced a faster increase in eGFR compared to younger age groups. Women experienced a faster increase in eGFR over 12 months compared to men. Patients with CD4 cell counts $\geq$200 cells/mm^3 at baseline experienced faster growth in mean eGFR compared to lower CD4 cell counts. There was insufficient power to detect differences in the slopes for baseline weight category, anaemia status and WHO disease stage.

Table 3.3: Linear stratified mixed models for estimated glomerular filtration rate over time

Variable [a]	Month Slope Coefficient	95 % CI
Month (cont)	0.960	(0.665; 1.255)
Baseline creatinine category		
$\leq$56 μmol/L	1.205	(0.454; 1.956)
56- 64 μmol/L	1.272	(0.751; 1.793)
64- 74 μmol/L	0.603	(0.110; 1.096)
>74 μmol/L	0.808	(0.315; 1.301)
Baseline weight category		
$\leq$54.5 kg	0.655	(0.0968; 1.213)
54.5- 62.9 kg	1.107	(0.568; 1.647)
62.9- 72.3 kg	0.834	(0.288; 1.380)
>72.3 kg	1.072	(0.432; 1.712)
Age category		
$\leq$29 years	0.710	(0.0315; 1.388)
29- 34 years	1.011	(0.367; 1.655)
34-41 years	1.121	(0.623; 1.620)
$\geq$41 years	0.937	(0.510; 1.365)
Men	0.247	(-0.104; 0.598)
Women	1.329	(0.922; 1.735)
No anaemia	1.081	(0.579; 1.582)
Anaemia	0.948	(0.570; 1.327)
CD4 category		
<100 cells/mm^3	0.946	(0.382; 1.510)
100-200 cells/mm^3	0.883	(0.326; 1.440)
$\geq$200 cells/mm^3	1.265	(0.772; 1.758)
WHO Stage (I & II combined)	0.946	(0.496; 1.396)
WHO Stage (III & IV combined)	0.871	(0.474; 1.268)

[a]Individual models for effect of time on eGFR restricted to the categories shown

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3.7 Incidence of GFR impairment

The proportion of patients who experienced deteriorated renal function below 50 ml/min/1.73m^2 over 12 months was approximately 4.4% (seven people by month 1, four people by month 2 and two people by month 4 of a subset of 293 patients). Using a more sensitive criterion of any reduction in eGFR of more than 10 ml/min/1.73m^2 from baseline eGFR, the incidence rate decreased from 79.31 per 100 person years in month 1 to 9.76 per 100 person years at month 12. Table 3.4 displays the results.

Table 3.4: Incidence of reduced renal function

	Baseline	Month 1	Month 2	Month 4	Month 12
Reduced eGFR [a]		69	76	79	33
LTFU [b]		5	47	91	46
Persons at risk (N)	1044	1039	992	901	855
Person time (in months)		1044	1046.5	2125	4059
Person time (in years)		87	87.21	177.08	338.25
Incidence rate (per 100 person years))[c]		79.31	87.15	44.61	9.76

[a]Patients with eGFR at least 10 ml/min/1.73m^2 less than baseline eGFR
[b] Patients lost to follow up
[c]Incidence calculated between time intervals using interval censoring method

3.8 Early Serum Creatinine Measures as Predictors of eGFR at Month 12

Table 3.5 displays the results of the linear regression models for predicting change in eGFR at month 12 compared to baseline using the imputed data. For the total follow-up visits over 12 months, 55.8% of the weight measures were missing. The univariate model R^2 value showed that and 3% of the variation in change in eGFR at month 12 was explained by the change in creatinine from baseline to month 1, 17% by change in creatinine from baseline to month 2 and 31% by change in creatinine from baseline to month 4. Adding more baseline covariates increased the predictive power of the models by almost 25% as shown in the increase in the multivariate R^2. The likelihood ratio tests comparing the nested models showed that serum creatinine measurements at months 1 and 2 are not as useful in predicting eGFR change after 12 months compared to month 4. Month 4 may be more useful.

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Table 3.5: Linear models for continuous change in eGFR at month 12

Variable	Univariate [a] Estimate	95% CI [b]	R^2	Multivariate [c] Estimate	95% CI	R^2	χ^2 *P.value*[d]
SCr Month 1 [e]	-0.384	(-0.574; -0.193)	0.0276	-0.131	(-0.429; 0.167)	0.221	0.1785
SCr Month 2[e]	-0.317	(-0.496; -0.138)	0.174	-0.198	(-0.484; 0.089)	0.292	0.0844
SCr Month 4[e]	-0.534	(-0.773; -0.295)	0.308	-0.656	(-1.188; -0.123)	0.387	

[a]Univariate estimated coefficient for serum creatinine measure at indicated month

[b]95% confidence interval (CI)

[c]Multiple regression estimated coefficient for model including age, sex, baseline weight, baseline serum creatinine, CD4 category, baseline haemoglobin and WHO disease stage

[d]Chi-squared likelihood ratio test p-value for comparison of models with SCr 1 or SCr 2 additional to SCr 4

[e]Serum creatinine (SCr) at the indicated month

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4 Discussion

The prevalence of moderate-severe renal dysfunction in patients initiating ART in this primary health care centre was low (4%). Decreased renal function was associated with increased age, female gender, more advanced HIV disease stage and lower haemoglobin. Incident reduction in renal function of more than 10 ml/min/1.73m^2 was rare (16.3 per 100 person years), with very few patients reaching the 50 ml/min/1.73m^2 threshold at which switching is advised by WHO guidelines [15]. The creatinine test at month 4 was more predictive of renal function after 12 months compared to earlier tests conducted at months 1 and 2.

Renal function generally improved over the first year on ART with no significant confounding by baseline variables (age, sex, baseline weight and serum creatinine, anemia, WHO stage or CD4 cell count). There were significant differences in the eGFR growth trajectories of patient subgroups with a faster rate of increase in mean eGFR in those with higher baseline serum creatinine, age greater than 29 years, CD4 cell counts $\geq$200 cells/ mm^3 at baseline and in women. There might be modification in eGFR growth by baseline characteristics over time, but our models were not suitably powered to test this and the clinical significance of the small variations observed here is uncertain.

A previous study in rural Uganda found similar results in a cohort of patients in the Home-Based AIDS Care clinical trial Home Based AIDS Care trial (HBAC) [22]. Before ART initiation, 20% of the patients had compromised renal function but after 2 years on highly active antiretroviral therapy

(HAART) they experienced the greatest improvements in renal function with a 53% increase in their creatinine clearance. Similar factors were found to be associated with improvements in creatinine clearance on HAART; higher baseline serum creatinine, female gender and a WHO stage IV disease status [22].

The prognostic model for the change in eGFR using early creatinine measurements had low predictive power, but the results were in line with those from a similar primary care setting. Brennan et al. found that in a Johannesburg cohort of patients on TDF, the 3 month creatinine clearance was crucial for detecting nephrotoxicity [13]. This suggests that early creatinine measures done at 3-4 months after initiation are important for picking up patients who are at risk of experiencing deteriorating renal function. Earlier creatinine clearance measures done at 1 or 2 months after initiation are less predictive of change in renal function after a year on ART.

The same risk factors for renal dysfunction at baseline emerged as found in previous studies from sub-Saharan Africa: low haemoglobin /anaemia [23, 24], older age and lower CD4 levels [25, 26]. However, we found that female gender had a higher odds of renal dysfunction at baseline, but also showed greater improvement in renal function over time. Some studies have found the converse, showing that male gender is associated with greater risk of significant renal impairment [24, 26].

Our study had several limitations. Firstly, tenofovir is mainly excreted through the proximal tubule, which potentially leads to tubular dysfunction [5, 27]. However, we were unable to asses this in our study as neither proteinuria nor glycosuria were assessed by urinalysis. Another major factor we were unable to assess was the prevalence of other non-communicable diseases correlated with kidney failure, including diabetes mellitus, hypertension and cardiovascular disease [28]. Considering the high prevalence of hypertension and cardiovascular diseases in South Africa, it is possible that the change in kidney function was confounded by these associated conditions [29]. Confounding by these conditions could lead to a mixing up of the true effect of TDF, attenuating the true increase in mean eGFR. Moreover, patients that experienced renal impairment might have had other co-morbidities that put them at increased risk of renal impairment unrelated to TDF exposure.

Our data may have been prone to selection bias, particularly since participants who were lost to follow up had different risk factors at baseline that may have predicted their probability of being lost. They had lower CD4 levels, higher viral load, lower weight and were more likely to be male

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and have anaemia at baseline. Closer inspection revealed that some of these patients were those who died during the 12 month period as a result of disease complications. However, they generally had the same renal function at baseline compared to those who were retained and did not differ significantly in serum creatinine levels.

The setting is a well-researched clinic with patient records that are maintained regularly. This allowed access to a large sample of ART-naïve patients with available weight and creatinine measures recorded after a year on ART. Moreover, the serum creatinine screening policy in this clinic is different from the National Department of Health standards, with more frequent screening tests during the first year of ART initiation. This allowed for a closer examination of early changes in renal function after months 1 and 2 on ART; times that these measures are not usually available in routine settings in South Africa.

Another strength of our study lies in the fact that we did not exclude those with missing weights. We used multiple imputation to derive an estimate of weight for all participants for whom serum creatinine tests were done at subsequent visits. We assumed that the weights were missing at random as there was no indication that there was informative censoring. By using the Cockroft-Gault equation we took the influence of weight changes after ART initiation into consideration giving a more robust measure for monitoring kidney function.

Our results highlight that tenofovir can be administered safely in a primary health care setting after the initial pre-ART screening of creatinine clearance. Renal function generally improves as general health improves due to HAART [30]. This contributes to the growing body of evidence in support of less restriction on tenofovir use in settings where frequent monitoring of renal function might be difficult or impractical [31].

Given the low prevalence of renal dysfunction before ART initiation, the identified risk factors are important for monitoring purposes and planning ART services. It is still not clear whether frequent toxicity monitoring is necessary for all patients on tenofovir. Developing a suitable prognostic tool can help to limit the pool by identifying high risk patients without requiring costly laboratory tests. A more cohesive collection and analysis of associated variables on related comorbidities and urinalysis should be done in order to identify them.

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5 Conclusions

In conclusion, this analysis indicates that renal dysfunction appears uncommon in HIV-infected adults initiating ART in a primary health care setting. In this group of patients without pre-existing glomerular disease, renal function generally improved during the first year on ART even in those with lowest creatinine clearance before initiation. Furthermore, creatinine tests done earlier than four months after baseline screening may be unnecessary. The benefits of tenofovir initiation potentially outweigh the nephrotoxic effects it might have at least during the first 12 months of use.

6 Authors' contributions

MK carried out data collection, performed the statistical analysis and drafted the manuscript.

7 Authors' information

MK is a masters student in the School of Public Health and Family Medicine at the University of Cape Town.

8 Acknowledgements

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Potential conflicts of interest The authors declare that they have no competing interests.

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Part IV

APPENDIX

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Appendix A

Ethics Approval Letter

Letter of approval from the UCT Human Research Ethics Committee

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Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
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Website: www.health.uct.ac.za/research/humanethics/forms

18 September 2013

HREC REF: 569/2013

Ms M Kamkuemah
c/o A/Prof L Myer
Public Health and Family Medicine

Dear Ms Kamkuemah

PROJECT TITLE: THE PREVALENCE AND INCIDENCE OF RENAL DYSFUNCTION IN PATIENTS INITIATING ANTERETROVIRAL THERAPY AT A PRIMARY HEALTH CARE CENTRE IN GUGULETHU CAPE TOWN

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th September 2014

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

L Samuels

Appendix B

Data Extraction

Data Extraction Form Tenofovir Renal Study
Gugulethu

Patient ID #:_ _ _ _ _ _ _/_ _/_ _ _ _ _ _ Date completed: __/___/____
DD / MMM / YYYY

Folder number :_ _ _ _ _ _ _

	Date recorded	Week recorded	Weight recorded in kg	Tick if missing
1	D D / M M M / Y Y Y Y			
2	D D / M M M / Y Y Y Y			
3	D D / M M M / Y Y Y Y			
4	D D / M M M / Y Y Y Y			
5	D D / M M M / Y Y Y Y			
6	D D / M M M / Y Y Y Y			
7	D D / M M M / Y Y Y Y			
8	D D / M M M / Y Y Y Y			

Appendix C

Efficacy and Safety trials for the Development of TDF

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Table C.1: Safety and Efficacy trials

Trial+ Authors	GS-97-901: Barditch-Crovo, P. & Deeks, S. G. 2001	GS-98-902: Schooley, R. T. & Ruane, P. 2002	GS-00-907: Gallant, J. E. & Staszewski, S.2004	Nelson, M. R. & Katlama, C.2007	Gallant, J. E. & Winston, J. A.:2008
Study	Phase I/II trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults	Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study	Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral- naive patients: a 3-year randomized trial (GS 903). The primary registrational trial for TDF.	The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years	The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue- containing regimen in antiretroviral-naive patients
Journal	Antimicrob Agents Chemother 45(10): 2733-2739	AIDS 16(9): 1257-1263	JAMA 292(2): 191-201	AIDS 21(10): 1273-1281	AIDS 22(16): 2155-2163
Countries, participants & year of analysis	Eligible subjects: men and women with HIV-1 infection, HIV-1 RNA> 10,000 copies/ml, CD4≥ 200 cells/mm^3, sCr level ≤1.5 mg/dl, calculated CrCl rate ≥60 ml/min (by CG formula), ART-naive and experienced	Treatment-experienced HIV-1-infected patients with incomplete virological suppression; plasma HIV-1 RNA 400-100,000 copies/ml & stable ART (≥ 8 weeks)	81 centers in the United States, South America, and Europe from 9 June 2000, to 30 January 2004 in ART-naive HIV-infected patients.	Patients enrolled in the TDF EAP [1] from March 2001 to March 2004, HIV-positive, with advanced disease and limited treatment options. Participating countries: Australia (654), Belgium (348), Canada (1761), France (1857), Germany (411), Ireland (12), Italy (553), the Netherlands (256), Portugal (58), Spain (576), United Kingdom (819), and the United States (3038). Plus safety data from the manufacturer's database; reports of all postmarketing adverse drug reactions received up to 30 April 2005.	ART-naive HIV-infected patients (from Studies 903 and 934); baseline sCr <1.5 mg/dl, serum phosphorus ≥2.2 mg/dl and calculated CrCl ≥60 ml/min (for Study 903) and ≥50 ml/min (for Study 934)using CG equation.
Objective(s) of study	To evaluate the anti-HIV-1 activity, safety, tolerance, and pharmacokinetics of oral TDF when administered as a single daily dose for 28 consecutive days.	To evaluate the safety and efficacy of once daily doses of TDF administered in combination with other antiretroviral therapy (ART)	To evaluate the efficacy and safety of TDF compared with stavudine in ART-naive patients.	To characterize the safety profile of TDF for the treatment of HIV infection in adults over the first 4 years of use using data from SAE reports to detect signals of new safety events not observed in clinical trials and to explore changes in patterns of known safety events.	To assess the renal safety of TDF using data from over 1000 patients, thereby providing greater power to detect renal events and small changes in renal function.
Type of Study	Randomized, double-blind, placebo-controlled, escalating-dose study	Randomized double-blind, placebo-controlled	Randomized, double-blind, placebo-controlled trial (Phase III)	Analysis of TDF expanded access program (EAP) and Postmarketing SADR [2] data	2 clinical trials (RCTs from Studies 903 and 934)
Number of subjects	49	189	602	10 343	1111
Intervention/ exposure	TDF (4 doses): 75 mg, 150 mg, 300 mg, 600 mg	TDF 75 mg, 150 mg, or 300 mg or placebo to existing ART	TDF (n = 299) in combination with lamivudine and efavirenz	TDF in expanded access and SAE [3] reports from manufacturer's database	TDF (n= 556)
Comparison	Placebo	Placebo	Stavudine (n = 303), with placebo, in combination with lamivudine and efavirenz.	all patients were treated with TDF	Thymidine analogue-containing (control) regimens (n=555) either stavudine or zidovudine in combination with efavirenz and either lamivudine or emtricitabine

continued . . .

[1]Expanded Access Program
[2]Serious Adverse Drug Reactions
[3]Serious Adverse Event

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. . . continued

Trial+ Authors	GS-97-901: Barditch-Crovo, P. & Deeks, S. G. 2001	GS-98-902: Schooley, R. T. & Ruane, P. 2002	GS-00-907: Gallant, J. E. & Staszewski, S.2004	Nelson, M. R. & Katlama, C.2007	Gallant, J. E. & Winston, J. A.:2008
Duration	4 weeks	48 weeks	144 weeks	4 years since start for SAE reports, but different mean duration of treatment in the EAP, United States (13 weeks), European Union/Australia (24 weeks), Canada (29 weeks).	144 weeks, 3 years
Health outcomes measured	Signs, symptoms, and laboratory abnormalities recorded by using a modified graded toxicity scale [4]. Toxicities or abnormalities classified as: mild, grade I; moderate, grade II; severe, grade III; possibly life threatening, grade IV.	Efficacy analyzed by the mean changes in HIV-1 RNA levels (log10 copies/ml plasma) from week 0 to weeks 4, 24, and 48. Safety analyzed by incidence of grade 3 or 4 clinical and laboratory adverse events.	Proportion of patients with HIV RNA levels of less than 400 copies/mL at week 48	any post drug SAE	Abnormalities in serum creatinine (sCr) (>1.5 mg/dl); serum phosphorus (<2.0 mg/dl); urine proteinuria $\geq$ 100 mg/dl; median change from baseline to week 144 in glomerular filtration rate by CG and by MDRD. Changes from baseline through week 144 in sCr, serum phosphorus and urine protein. The proportions of patients developing a maximum graded toxicity in sCr, serum phosphorus and proteinuria through 144 weeks
Findings and Explanations (and conclusions)	No renal abnormalities at 28 days. Grade III or IV adverse events were limited to laboratory abnormalities, including elevated creatine phosphokinase and liver function tests, which resolved with or without drug discontinuation and without sequelae. In summary, oral tenofovir DF exhibits a pharmacokinetic profile that supports once-daily dosing. With good antiviral potency and tolerability.	The incidence of adverse events was similar among the TDF groups and placebo through week 24. Throughout the 48-week study, no significant changes in renal function were observed. In treatment-experienced patients with baseline nucleoside resistance mutations, TDF provided dose-related, durable reductions in HIV-1 RNA. Through 24 weeks, the safety profile of TDF was similar to that of placebo.	The number of bone fractures and the renal safety profile were similar between the 2 groups. Through 144 weeks, the combination of TDF, lamivudine, and efavirenz was highly effective and comparable with stavudine, lamivudine, and efavirenz in antiretroviral-naive patients. TDF appeared to be associated with better lipid profiles and less lipodystrophy.	Serious adverse events (SAEs) were reported in 631 (6%)of patients enrolled in the EAP. A renal SAE of any type was observed in 0.5% of patients, and graded elevations in sCr occurred in 2.2% of the patients studied.Significant baseline risk factors for development of nephrotoxicity identified in multivariate analysis were elevated sCr, simultaneous use of nephrotoxic medications, increased age, lower weight, and lower CD4 cell count of which preexisting kidney disease, nephrotoxic medications, and older age are known risk factors for renal disease. For postmarketing safety data the most commonly reported SADRs [5] were renal events. In summary, The most common SADRs reported for TDF were renal, including renal failure, FanconiâĂŹs syndrome, and sCr increase, but an overall favorable safety profile for TDF demonstrated in the treatment of adults with HIV infection in expanded access and postmarketing safety surveillance.	Small but statistically significant decreases in estimated GFR were observed in the TDF group through 144 weeks. A significant increase in GFR was seen in the control group using both formulae. But no clinically relevant renal disease, adverse events or changes in sCr and phosphorus were seen with both the TDF and control groups in ART-naive patients in 144 weeks. The incidence of sCr elevation or hypophosphatemia was less than 1% in both treatment groups. Likewise, a similar incidence of proteinuria (5âĂŞ6%) was seen in both treatment groups.

[4]based on the AIDS Clinical Trials Group common toxicity grading scale
[5]serious adverse drug reactions

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Appendix D

Journal Manuscript Appendices

1 Appendix 1

1.1 Fraction of missing data

```
Patterns of missingness in the data:  319

Fraction Missing for original variables:
----------------------------------------

            Fraction Missing
pidno             0.00000000
viralload         0.54589643
cd4               0.54140961
hb                0.49242849
creatinine_       0.28510002
age               0.00000000
sex               0.00000000
stage             0.05010282
base_cd4          0.23761451
base_vL           0.27444382
base_hb           0.19424191
weight_           0.55804823
basecreat         0.02879043
week4             0.00000000
baseweight        0.06655450
```

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1.2 Imputation Model

Not all patients had weight measurements at all five time points. We assumed a missing at random structure, i.e. missing weights were dependent on the observed data but not on missing characteristics as defined by Rubin [1] and used mice to impute missing weights.

Framework

`MICE` is a practical approach used to create imputed datasets using a set of imputation models, with one model for each variable with missing values.

Let complete data Y be a partially observed random sample from a p-variate multivariate distribution $P(Y|\theta)$ where θ is a vector of unknown parameters. The aim is derive the multivariate

[1] Rubin DB: **Inference and missing data**. *Biometrika*1976, 63(3): 581-592

distribution of θ, either explicitly or implicitly. The chained equations algorithm is designed to obtain the posterior distribution of θ by sampling iteratively from conditional distributions of the form $P(Y_1|Y_{-1}, \theta_1) \ldots P(Y_p|Y_{-p}, \theta_p)$ [2].

The process starts with a simple draw from observed marginal distributions and the *t-th* iteration of chained equations is a Gibbs Sampler that successively draws from:

$$\begin{aligned}
\theta_1^{*(t)} &\sim P(\theta_1|Y_1^{obs}, Y_2^{(t-1)}, \ldots, Y_p^{(t-1)}) \\
Y_1^{*(t)} &\sim P(Y_1|Y_1^{obs}, Y_2^{(t-1)}, \ldots, Y_p^{(t-1)}, \theta_1^{*(t)}) \\
&\vdots \\
\theta_p^{*(t)} &\sim P(\theta_p|Y_p^{obs}, Y_1^{(t)}, \ldots, Y_{p-1}^{(t)}) \\
Y_p^{*(t)} &\sim P(Y_p|Y_p^{obs}, Y_1^{(t)}, \ldots, Y_p^{(t)}, \theta_p^{*(t)})
\end{aligned}$$

where $Y_j^{(t)} = (Y_j^{obs}, Y_j^{*(t)})$ is the jth imputation of the missing variable at iteration t.

Model

For our third objective, mice was used to impute missing weights in `STATA` (version 12) as described by White and Royston [3]. The imputed data allowed us to include more eGFR measures for patients with missing data at month 12. We performed the imputation of missing weights using weight measurements taken at different time points and assuming normality of weights. A detailed outline of the imputation modeling process is shown below.

a) Imputation: First we used the `nscore` command to correct for out of range values. This is a transformation command.

```
nscore  weight_0 weight_1  weight_2  weight_4  weight_12, gen(nscore)
```

b) Then a linear regression command was used to specify the imputation model. The `dryrun` was done to check how ice would impute the missing variables. The procedure was then repeated 10 times, giving 10 imputed datasets.

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[2]Buuren S, and Groothuis-Oudshoorn K: **MICE: Multivariate imputation by chained equations in R**. *Journal of statistical software*2011, 45(3)

[3]White IR, Royston P, Wood AM: **Multiple imputation using chained equations: Issues and guidance for practice**. *Stat Med* 2011, 30(4):377âĂŞ399.

```
. ice  nscore1-nscore5, dryrun
   #missing |
     values |      Freq.     Percent        Cum.
------------+-----------------------------------
          6 |          1        0.08        0.08
          7 |         23        1.91        2.00
          8 |         56        4.66        6.66
          9 |        138       11.48       18.14
         10 |        180       14.98       33.11
         11 |        234       19.47       52.58
         12 |        379       31.53       84.11
          . |        191       15.89      100.00
------------+-----------------------------------
      Total |      1,202      100.00

   Variable | Command | Prediction equation
------------+---------+-------------------------------------------------------
    nscore1 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore2 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore3 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore4 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore5 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
------------------------------------------------------------------------------
End of dry run. No imputations were done, no files were created.

. ice  nscore1-nscore5,saving( "C:\Imputations_data\Imputedweights.dta",replace) m(10) seed(999)

   #missing |
     values |      Freq.     Percent        Cum.
------------+-----------------------------------
          6 |          1        0.08        0.08
          7 |         23        1.91        2.00
          8 |         56        4.66        6.66
          9 |        138       11.48       18.14
         10 |        180       14.98       33.11
         11 |        234       19.47       52.58
         12 |        379       31.53       84.11
          . |        191       15.89      100.00
------------+-----------------------------------
      Total |      1,202      100.00

  Variable | Command | Prediction equation
------------+---------+-------------------------------------------------------
    nscore1 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore2 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore3 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore4 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
    nscore5 | regress |  nscore1 nscore2 nscore3 nscore4 nscore5
------------------------------------------------------------------------------
```

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```
Imputing ..........
1.........2.........3.........4.........5.........6.........7........8.........9.........10
file C:\Imputations_data\Imputedweights.dta saved

use  "C:\Imputations_data\Imputedweights.dta",clear
```

c) Back-transformation of nscore variables to untransformed range of original data

```
invnscore  weight_0 weight_1  weight_2  weight_4  weight_12
```

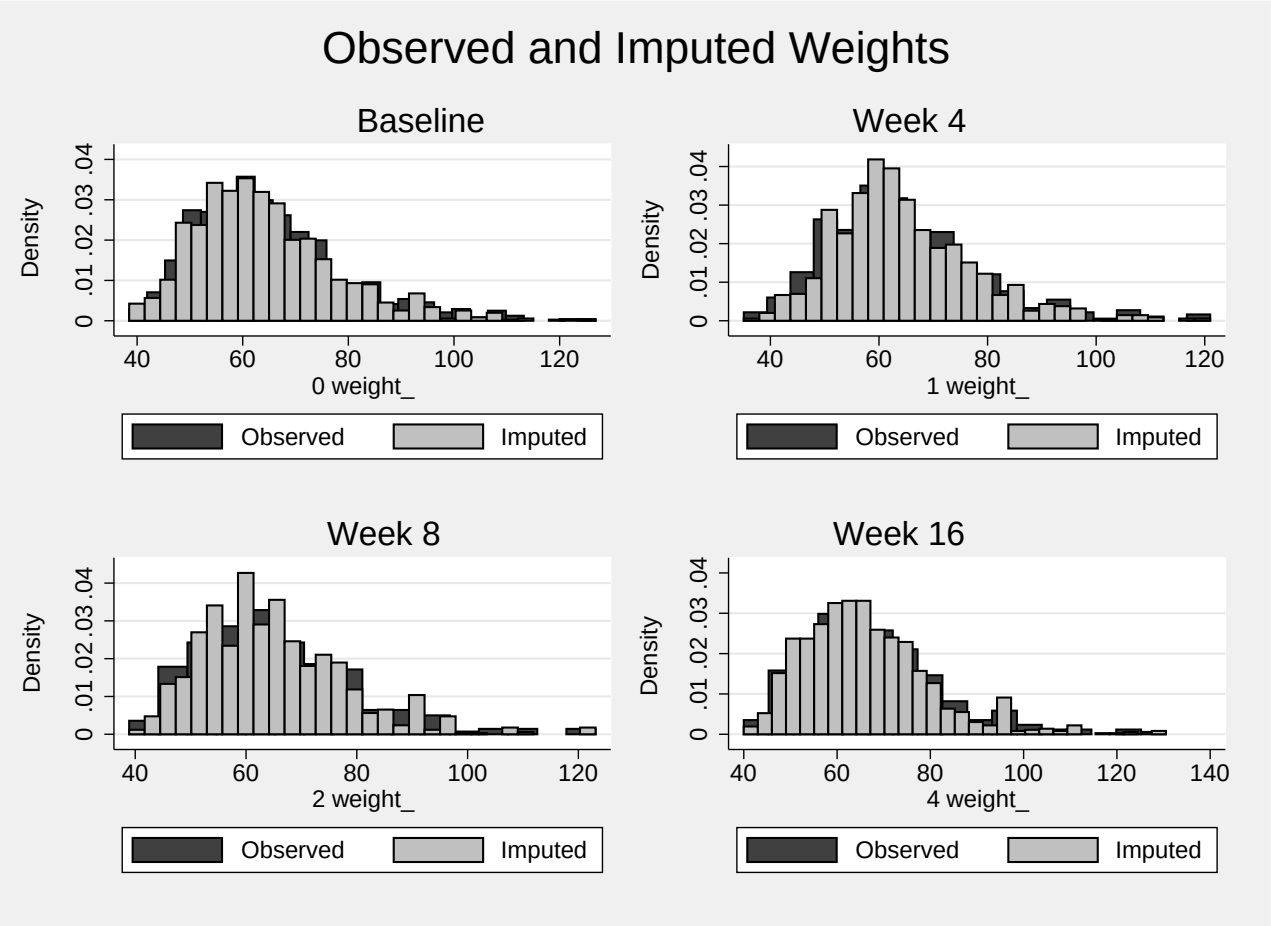

Figure D.1: Distribution of observed and imputed weights at baseline, months 1, 2 and 4

The distribution of the imputed data is similar to that of the observed data as shown in Figure D.1.

2 Appendix 2

2.1 Model for Baseline Renal Dysfunction

Logistic regression models specifying the prevalence odds ratio option, were estimated using STATA. The results for univariate and multivariable analysis models are displayed in Table D.1.

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Table D.1: Crude and Adjusted Associations between covariates and severe-moderate renal dysfunction for 1092 participants

Variable	crude estimate (POR)[a]	95% CI	*P.value*	Adjusted estimate[b] (POR)	95% CI	*P.value*
Age years (continuous)	1.10	(1.07; 1.13)	<0.001			
Age category						
<29 years	1.00					
29-34 years	1.72	(0.54; 5.49)	0.363	2.03	(0.58; 7.75)	0.252
34-41 years	1.29	(0.39; 4.29)	0.675	1.72	(0.45; 6.60)	0.43
>41 years	6.04	(2.27; 16.05)	<0.001	8.67	(2.85; 26.38)	<0.001
Sex						
male	1.00					
female	1.30	(0.68; 2.49)	0.433	2.43	(1.11; 5.29)	0.026
Stage						
I and II	1.00					
III and IV	3.00	(1.42; 6.37)	0.004	2.35	(1.03; 5.37)	0.043
CD4 cells/mm^3 (cont)	0.998	(0.99; 1.00)	0.237			
CD4 category						
<100 cells/mm^3	1.00					
100-200 cells/mm^3	0.93	(0.42; 2.08)	0.865			
>200 cells/mm^3	0.48	(0.18; 1.29)	0.146			
Log_{10} Viral Load copies/ml (cont)	1.36	(0.83; 2.21)	0.225			
Viral Load category						
<5 log_{10} copies/ml	1.00					
≥5 log_{10} copies/ml	1.27	(0.61; 2.64)	0.519			
Anaemia status						
No anaemia	1.00					
Anaemia [c]	5.40	(1.90; 15.34)	0.002	5.62	(1.67; 18.90)	0.005

[a]POR: Prevalence odds ratio of moderate/severe renal dysfunction
[b]Adjusted for age category, sex, WHO disease stage, CD4 count and anaemia
[c]Anaemia classification: Hb <12g/dL women, Hb <13g/dL men

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3 Appendix 3

3.1 Comparison of retained patients and those lost to follow up during the first year on ART

Total LTFU (including death) compared to retained at month 12

Table D.2: Total patients lost to care compared to retained patients

	Retained 855 (78%)	Lost 237 (22%)
Median age (IQR) (years)	35 (29; 41)	33 (28; 40)
Age category		
<29 years	228 (27%)	74 (31%)
29-34 years	195 (23%)	61 (26%)
34-41 years	224 (26%)	52 (22%)
>41 years	208 (24%)	50 (21%)
Sex		
Male	300 (35%)	112 (47%)
Female	555 (65%)	125 (53%)
WHO stage n (%)		
I	193 (24%)	54 (24%)
II	187 (23%)	40 (17%)
III	342 (43%)	100 (44%)
IV	84 (10%)	34 (15%)
Median CD4 cells/mm^3 (IQR)	157 (92; 218)	142 (64; 219)
CD4 category n (%)		
<100 cells/mm^3	201 (28%)	74 (36%)
100-200 cells/mm^3	284 (39%)	62 (31%)
>200 cells/mm^3	239 (33%)	67 (33%)
Median Viral load (log_{10}) copies/ml (IQR)	4.70 (4.20; 5.21)	4.83 (4.33; 5.29)
Viral load category n (%)		
<5 log_{10} copies/ml	449 (65%)	108 (56%)
≥5 log_{10} copies/ml	240 (35%)	84 (44%)
Median Hemoglobin g/dL (IQR)	11.7 (10.2; 13.0)	11.5 (9.8; 12.8)
non-anemic n (%)	308 (39%)	71 (31%)
anemic n (%)	487 (61%)	155 (69%)
Median creatinine μmol/L (IQR)	64 (56; 75)	64 (56; 73)
Median weight kg (IQR)	63.2 (55.0; 71.7)	60.1 (53.7; 68.7)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	97.2 (81.3; 116.8)	96.9 (83.1; 115.5)
renal function category		
moderate/severe	36 (4%)	12 (5%)
mild	291 (34%)	77 (33%)
normal	528 (62%)	148 (62%)

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LTFU for reasons other than death compared to retained at month 12

Table D.3: Loss to follow up (not known death) compared to retained

	Retained 855 (80%)	Lost 213 (20%)
Median age (IQR) (years)	35 (29; 41)	33 (27; 39)
Age category		
<29 years	228 (27%)	70 (33%)
29-34 years	195 (23%)	53 (25%)
34-41 years	224 (26%)	46 (21%)
>41 years	208 (24%)	44 (21%)
Sex		
Male	300 (35%)	101 (47%)
Female	555 (65%)	112 (53%)
WHO stage n (%)		
I	193 (24%)	51 (25%)
II	187 (23%)	35 (17%)
III	342 (43%)	94 (46%)
IV	84 (10%)	24 (12%)
Median CD4 cells/mm^3 (IQR)	157 (92; 218)	156 (78; 227)
CD4 category n (%)		
<100 cells/mm^3	201 (28%)	58 (32%)
100-200 cells/mm^3	284 (39%)	57 (32%)
>200 cells/mm^3	239 (33%)	64 (36%)
Median Viral load ($\log_{10}$) copies/ml (IQR)	4.70 (4.20; 5.21)	4.89 (4.40; 5.28)
Viral load category n (%)		
<5 $\log_{10}$ copies/ml	449 (65%)	93 (54%)
≥5 $\log_{10}$ copies/ml	240 (35%)	78 (46%)
Median Hemoglobin g/dL (IQR)	11.7 (10.2; 13.0)	11.6 (10.0; 12.8)
Non-anaemic n (%)	308 (39%)	68 (34%)
Anaemic n (%)	487 (61%)	134 (66%)
Median creatinine μmol/L (IQR)	64 (56; 75)	64 (56; 73)
Median weight kg (IQR)	63.2 (55.0; 71.7)	60.12 (54.0; 68.7)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	97.2 (81.3; 116.8)	98.7 (83.4; 116.3)
Renal function category		
moderate/severe	36 (4%)	8 (4%)
mild	291 (34%)	71 (33%)
normal	528 (62%)	134 (63%)

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Confirmed deaths on ART compared to those alive

Table D.4: Patients who died in 12 month period compared to those alive

	Alive 1068 (98%)	Died 24 (2%)
Median age (IQR) (years)	34 (29; 41)	34.5 (30; 42.5)
Age category		
<29 years	298 (27%)	4 (17%)
29-34 years	248 (23%)	8 (33%)
34-41 years	270 (25%)	6 (25%)
>41 years	252 (24%)	6 (25%)
Sex		
Male	401 (38%)	11 (46%)
Female	667 (62%)	13 (54%)
WHO stage n (%)		
I	244 (24%)	3 (12%)
II	222 (22%)	5 (21%)
III	436 (43%)	6 (25%)
IV	108 (11%)	10 (42%)
Median CD4 cells/mm^3 (IQR)	156 (89; 219)	41.5 (7.5; 172)
CD4 category n (%)		
<100 cells/mm^3	259 (29%)	16 (67%)
100-200 cells/mm^3	341 (38%)	5 (21%)
>200 cells/mm^3	303 (33%)	3 (12%)
Median Viral load ($\log_{10}$) copies/ml (IQR)	4.74 (4.22; 5.22)	4.68 (4.16; 5.58)
Viral load category n (%)		
<5 $\log_{10}$ copies/ml	542(63%)	15(71%)
≥5 $\log_{10}$ copies/ml	318 (37%)	6 (29%)
Median Hemoglobin g/dL (IQR)	11.7 (10.2; 13.0)	9.9 (8.95; 12.2)
non-anemic n (%)	376 (38%)	3 (13%)
anemic n (%)	621 (62%)	21 (87%)
Median creatinine μmol/L (IQR)	64 (56; 74)	65.5 (55.5; 76.5)
Median weight kg (IQR)	62.5 (54.8; 71.3)	54.2 (50.0; 67.0)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	97.2 (81.9; 116.7)	91.5 (71.6; 107.3)
Renal function category		
moderate/severe	44 (4%)	4 (17%)
mild	362 (34%)	6 (25%)
normal	662 (62%)	14 (58%)

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3.2 Comparison of patients with missing and measured creatinine at follow-up months

Month 12

Table D.5: Baseline characteristics: missing creatinine vs measured creatinine at month 12

	Missing 478 (44%)	Measured 614 (56%)
Median age (IQR) (years)	34 (29- 41)	34 (29- 41)
Age category		
<29 years	131 (27%)	171 (28%)
29-34 years	119 (25%)	137 (22%)
34-41 years	116 (24%)	160 (26%)
>41 years	112 (24%)	146 (24%)
Sex		
Male	196 (41%)	216 (35%)
Female	282 (59%)	398 (65%)
WHO stage n (%)		
I	116 (25%)	131 (23%)
II	90 (20%)	137 (24%)
III	191 (42%)	251 (43%)
IV	58 (13%)	60 (10%)
Median CD4 cells/mm^3 (IQR)	155.5 (78; 218)	152 (92; 218)
CD4 category n (%)		
<100 cells/mm^3	131 (32%)	144 (28%)
100-200 cells/mm^3	140 (34%)	206 (40%)
>200 cells/mm^3	139 (34%)	167 (32%)
Median Viral load ($\log_{10}$) copies/ml (IQR)	4.75 (4.18; 5.22)	4.73 (4.25; 5.24)
Viral load category n (%)		
<5 $\log_{10}$ copies/ml	251 (63%)	306 (63%)
≥5 $\log_{10}$ copies/ml	146 (37%)	178 (37%)
Median Hemoglobin g/dL (IQR)	11.8 (10.1; 13.0)	11.6 (10.2; 12.9)
Non-anaemic n (%)	172 (38%)	207 (37%)
Anaemic n (%)	285 (62%)	357 (63%)
Median creatinine μmol/L (IQR)	65 (56; 75)	63 (56; 74)
Median weight kg (IQR)	61.7 (55; 71.5)	63.0 (54.4; 71.0)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	95.9 (80.1; 116.3)	97.7 (82.3; 116.7)
Renal function category		
moderate/severe	26 (6%)	22 (4%)
mild	168 (35%)	200 (32%)
normal	284 (59%)	392 (64%)

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Month 4

Table D.6: Baseline characteristics: missing creatinine vs measured creatinine at month 4

	Missing 722 (66%)	Measured 370 (34%)
Median age (IQR) (years)	34 (29; 41)	34 (29; 42)
Age category		
<29 years	204 (28%)	98 (26%)
29-34 years	166 (23%)	90 (24%)
34-41 years	192 (27%)	84 (23%)
>41 years	160 (22%)	98 (27%)
Sex		
Male	269 (37%)	143 (39%)
Female	453 (63%)	227 (61%)
WHO stage n (%)		
I	165 (24%)	82 (24%)
II	144 (21%)	83 (24%)
III	293 (43%)	149 (43%)
IV	85 (12%)	33 (9%)
Median CD4 cells/mm^3 (IQR)	163 (92; 227)	141.5 (78; 204)
CD4 category n (%)		
<100 cells/mm^3	164 (28%)	111 (33%)
100-200 cells/mm^3	215 (36%)	131 (40%)
>200 cells/mm^3	216 (36%)	90 (27%)
Median Viral load (log_{10}) copies/ml (IQR)	4.70 (4.21; 5.23)	4.78 (4.22; 5.22)
Viral load category n (%)		
<5 log_{10} copies/ml	354 (63%)	203 (63%)
≥5 log_{10} copies/ml	207 (37%)	117 (37%)
Median Hemoglobin g/dL (IQR)	11.6 (10; 12.9)	11.7 (10.4; 13.1)
Non-anaemic n (%)	244 (36%)	135 (39%)
Anaemic n (%)	428 (64%)	214 (61%)
Median creatinine μmol/L (IQR)	64 (55; 74)	65 (57; 76)
Median weight kg (IQR)	61.8 (54.1; 70.9)	63.7 (55.1; 71.7)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	97.2 (81.9; 118.3)	96.4 (80.8; 115.4)
Renal function category		
moderate/severe	31 (4%)	17 (5%)
mild	239 (33%)	129 (35%)
normal	452 (63%)	224 (60%)

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Month 2

Table D.7: Baseline characteristics: missing creatinine vs measured creatinine at month 2

	Missing 395 (36%)	Measured 697 (64%)
Median age (IQR) (years)	35 (29- 41)	34 (29- 41)
Age category		
<29 years	105 (27%)	197 (28%)
29-34 years	90 (23%)	166 (24%)
34-41 years	111 (28%)	165 (24%)
>41 years	89 (22%)	169 (24%)
Sex		
Male	163 (41%)	249 (36%)
Female	232 (59%)	448 (64%)
WHO stage n (%)		
I	98 (26%)	149 (23%)
II	74 (19%)	153 (23%)
III	154 (41%)	288 (44%)
IV	52 (14%)	66 (10%)
Median CD4 cells/mm^3 (IQR)	161 (92; 224)	150.5 (82; 216)
CD4 category n (%)		
<100 cells/mm^3	86 (27%)	189 (31%)
100-200 cells/mm^3	119 (37%)	227 (38%
>200 cells/mm^3	116 (36%)	190 (31%)
Median Viral load ($\log_{10}$) copies/ml (IQR)	4.66 (4.12; 5.26)	4.78 (4.27; 5.22)
Viral load category n (%)		
<5 $\log_{10}$ copies/ml	194 (65%)	363 (62%)
≥5 $\log_{10}$ copies/ml	104 (35%)	220 (38%)
Median Hemoglobin g/dL (IQR)	11.8 (10.2; 13.1)	11.6 (10.1; 12.9)
Non-anaemic n (%)	147 (40%)	232 (36%)
Anaemic n (%)	222 (60%)	420 (64%)
Median creatinine μmol/L (IQR)	64 (56; 74)	64 (56; 74)
Median weight kg (IQR)	62.2 (54.5; 70.6)	62.7 (54.4; 71.6)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	97.1 (82.6; 114.9)	97.2 (81.0; 118.2)
Renal function category		
moderate/severe	19 (5%)	29 (4%)
mild	130 (33%)	238 (34%)
normal	246 (62%)	430 (62%)

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Month 1

Table D.8: Baseline characteristics: missing creatinine vs measured creatinine at month 1

	Missing 451 (41%)	Measured 641 (59%)
Median age (IQR) (years)	35 (29; 41)	34 (29; 41)
Age category		
<29 years	132 (29%)	170 (26%)
29-34 years	91 (20%)	165 (26%)
34-41 years	117 (26%)	159 (25%)
>41 years	111 (25%)	147 (23%)
Sex		
Male	172 (38%)	240 (37%)
Female	279 (62%)	401 (63%)
WHO stage n (%)		
I	110 (26%)	137 (22%)
II	89 (21%)	138 (23%)
III	171 (40%)	271 (45%)
IV	55 (13%)	63 (10%)
Median CD4 cells/mm^3 (IQR)	155.5 (84; 221)	154 (85; 217)
CD4 category n (%)		
<100 cells/mm^3	112 (30%)	163 (30%)
100-200 cells/mm^3	139 (37%)	207 (37%)
>200 cells/mm^3	123 (33%)	183 (33%)
Median Viral load (log_{10}) copies/ml (IQR)	4.79 (4.21; 5.29)	4.70 (4.22; 5.21)
Viral load category n (%)		
<5 log_{10} copies/ml	207 (61%)	350 (65%)
≥5 log_{10} copies/ml	132 (39%)	192 (35%)
Median Hemoglobin g/dL (IQR)	11.7 (10.1; 12.8)	11.7 (10.2; 13.1)
Non-anaemic n (%)	149 (35%)	230 (39%)
Anaemic n (%)	276 (65%)	366 (61%)
Median creatinine μmol/L (IQR)	64 (56; 76)	64 (56; 73)
Median weight kg (IQR)	62.3 (54.4; 72.3)	62.6 (54.5; 70.9)
Median eGFR ml/min/1.73 m^2 [CG] (IQR)	96.5 (81.8; 116.5)	97.9 (81.3; 116.7)
Renal function category		
moderate/severe	21 (4%)	27 (4%)
mild	152 (34%)	216 (34%)
normal	278 (62%)	398 (62%)

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Appendix E

Journal Submission Guidelines
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AIDS RESEARCH AND THERAPY

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- Methods
- List of abbreviations used (if any)
- Competing interests

- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
- Tables and captions
- Preparing additional files

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In press article
Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract
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Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal
Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings
Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book
Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series
Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.* Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology,* vol 1.]

Book with institutional author
Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis
Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL
The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)
Corpas M: **The Crowdfunding Genome Project: a personal genomics community with open source values** [http://blogs.biomedcentral.com/bmcblog/2012/07/16/the-crowdfunding-genome-project-a-personal-genomics-community-with-open-source-values/]

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