Renal Impairment in HIV infected patients receiving tenofovir-based Antiretroviral Therapy in a South African Hospital

Faheem Seedat

A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine

Johannesburg, 2017

i. Declaration

I, Faheem Seedat, do hereby declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine. This research report is submitted in the publishable format as recognized by the Faculty of Health Sciences. I further declare that this work has not been submitted for any other examination or degree at this or any other University.

……

The ……. day of …… 2017

ii. Dedication

To my amazing wife, Nureen, for her love, understanding, motivation and unwavering support.

To my parents, Mohammed Ameen and Yasmin for the many years of encouragement and patience.

For my beloved son, Rayyan.

iii. Publications and presentations originating from this research

1. Publication - Seedat F, Martinson N, Motlhaoleng K, Abraham P, Mancama D, Naicker S, *et al*. Acute Kidney Injury, Risk Factors, and Prognosis in Hospitalized HIV-Infected Adults in South Africa, Compared by Tenofovir Exposure. *AIDS Res Hum Retroviruses* 2017,**33**:33-40.
2. Oral presentation at the SoMCHAT Young Researchers Conference, Johannesburg, 24 July 2015
3. Oral presentation at the School of Clinical Medicine Research Day, University of Witwatersrand, Johannesburg, 30 September 2015
4. Selected for Poster presentation at 2nd International Conference on Retroviruses and Novel Drugs, Cape Town, 30 June – 1 July 2016

iv. Ethical considerations

Permission for this prospective study was obtained from Prof. E. Variava (Head of Department, Department of Internal Medicine, Klerksdorp Tshepong Hospital Complex), Dr N.D. Leburu (Clinical Manager, Klerksdorp Tshepong Hospital Complex), and the Human Research Ethics Committee of the University of Witwatersrand (clearance number – M140742)

v. Abstract

Objective: There is limited data describing acute kidney injury (AKI) in HIV-infected adult patients in resource-limited settings where increasingly, tenofovir (TDF), which is potentially nephrotoxic, is prescribed. We describe risk factors for, and prognosis of AKI in HIV-infected individuals receiving and naïve to TDF.

Methods: This was a prospective case cohort study of hospitalized HIV-infected adults with AKI (as defined by the 2012 KDIGO Clinical Practice Guideline for AKI) stratified by TDF exposure. Adults (≥18 years) were recruited: clinical and biochemical data was collected at admission; their renal recovery, discharge or mortality was ascertained as an in-patient and, subsequently, to a scheduled 3-month follow-up.

Results: Amongst this predominantly female (61%), almost exclusively black African cohort of 175 patients with AKI, 93 (53%) were TDF exposed; median age was 41 years (IQR 35-50). Median CD4 count and VL and creatinine at baseline was 116 cells/mm^3 and 110159 copies/ml, respectively. A greater proportion of the TDF group had severe AKI on admission (61% v 43% p=0.014); however, both groups had similar rates of newly diagnosed tuberculosis (TB) (52%) and NSAID (32%) use. Intravenous fluid was the therapeutic mainstay; only 7 were dialyzed. Discharge median serum creatinine (SCr) was higher in the TDF group (p=0.032) and fewer in the TDF group recovered renal function after 3-months (p=0.043). 3-month mortality was 27% in both groups but 55% of deaths occurred in hospital. Those that died had a higher SCr and more severe AKI than survivors; TB was diagnosed in 33 (70%) of those who died.

Conclusions: AKI was more severe and renal recovery slower in the TDF group; co-morbidities, risk factors and prognosis were similar regardless of TDF exposure.

Because TB is linked to higher mortality, TB co-infection in HIV-infected patients with AKI warrants more intensive monitoring. In all those with poor renal recovery, our data suggests that a lower threshold for dialysis is needed.

iv. Acknowledgements

The support and assistance of the following persons in preparation of this research report is gratefully acknowledged:

- Professor Ebrahim Variava and Dr Neil Martinson – for their guidance as supervisors of this study, review of the prepared manuscript and constant encouragement
- Mr Pattamukkil Abraham – for his willingness and assistance with statistics
- Miss Masindi Nthabiseng and Miss Motshidisi Khumalo – for their hard work and invaluable assistance with record gathering

vii. Contents

viii. List of tables

ix. List of figures

x. Nomenclature

AIN	Acute interstitial nephritis
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
ATN	Acute tubular necrosis
ART	Antiretroviral therapy
ARVs	Antiretrovirals
CKD	Chronic kidney disease
CKD – EPI	Chronic Kidney Disease Epidemiological Collaboration
CG	Cockroft - Gault
eGFR	Estimated glomerular filtration rate
FDC	Fixed dose combination
FDA	Food and Drug Administration
HIVAN	HIV associated nephropathy
HIVICK	HIV immune complex kidney disease
IRIS	Immune reconstitution inflammatory syndrome
IQR	Interquartile range
KDIGO	Kidney Disease Improving Clinical Outcomes
mtDNA	Mitochondrial DNA
MDRD	Modification of Diet in Renal Disease
MRP	Multidrug resistance protein
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NSAIDs	Non-steroidal anti-inflammatory drugs
NRTI	Nucleotide reverse-transcriptase inhibitor
OAT	Organic anion transporters

PI	Protease Inhibitor
RRT	Renal replacement therapy
SCr	Serum creatinine
TDF	Tenofovir
TB	Tuberculosis
USD	United States Dollars
WHO	World Health Organization

Chapter 1: Protocol and Extended review of the literature

Background

1.1 Introduction

Acute kidney injury (AKI) is a major cause of mortality and morbidity in HIV infected individuals and has a multifactorial aetiology.[1-3] HIV is directly nephrotoxic and viral infection of renal glomerular and tubular cells can cause an HIV associated nephropathy, but the role of HIV in renal disease remains complex and includes: the nephrotoxic effects of HIV; the direct effects or sequelae of opportunistic infections on renal function; and the nephrotoxic effects of antiretroviral drugs.[4-6] Tenofovir (TDF), with emtricitibine and efavirenz is dispensed as part of the fixed dose combination (FDC) antiretroviral therapy (ART) tablet and, since April 2010, has been the mainstay of the first line ART regimen in South Africa.[7-9] TDF has been cited as a cause for AKI and it is postulated host genetic polymorphisms play a role in increasing the risk of developing TDF-related nephrotoxicity in certain individuals.[10, 11] There is a paucity of data describing AKI in HIV-infected individuals both in those receiving TDF-based ART and in ART-naïve individuals. With greater TDF use in the health sector this prospective case-cohort study, comparing HIV positive patients with AKI who are TDF exposed to those TDF un-exposed, reports the presentation, severity and risk factors for AKI in TDF exposed HIV positive patients, and assesses short – term survival and response to treatment.

1.2 HIV

1.2.1 HIV – a South African Health Issue

HIV is a major in health issue in South Africa with significant cost implications – particularly with regard to in-patient hospital care. 18% of adults in South Africa are HIV seropositive and the 6.2 million people infected in South Africa accounts for 17% of all HIV positive people worldwide.[12, 13] Only since 2004 has ART been available and in mid – 2015 3.1 million South Africans were on ART. The number of AIDS – related deaths in South Africa for 2015 was 162445, comprising 30.5% of the total deaths for 2015.[14, 15] A multi – country analysis of treatment costs for ART provision estimated the cost of ART per patient year to be $682 (United States Dollars [USD]), whilst a South African study from a Gauteng hospital of the cost of in-patient care for HIV positive patients estimated an average cost of $1783 (USD) per hospital admission.[16, 17]

1.2.2 HIV Lifecycle

Following acute infection with HIV - an RNA virus – viral entry into the CD4 cell occurs via gp120 and gp41 viral membrane proteins binding to CD4 cell membrane receptors and an adjacent chemokine co-receptor (either CC Chemokine receptor CCR5 or CXR4). Fusion between the virus envelope and CD4 cell membrane facilitates viral entry and once within the cell the enzyme reverse transcriptase un-coats the viral core. Viral double stranded DNA is produced and is integrated into host DNA by the enzyme integrase. Transcription of mRNA, translation and subsequent production of viral proteins and viral RNA follows. These products are assembled and the virus matures before being released from the cell as a fully matured new HIV copy.[18, 19] HIV has a high rate of viral replication and

inaccuracies during reverse transcription may result in mutations in newly produced viral copies. Consequently, genetic diversity exists both within and between individuals.[20]

1.3. Renal Disease

Renal disease is a heterogeneous group of disorders and newer classifications consider disease duration an important differentiating factor.[21]

1.3.1 Definitions of Renal Disease

1.3.1.1 Acute kidney injury

The traditional definition of acute renal failure is: "a rapid fall in the rate of glomerular filtration, which manifests clinically as an abrupt and sustained increase in the serum levels of urea and creatinine with an associated disruption of salt and water homeostasis."[22] This definition has limitations in clinical practice, as the terms "rapid", "abrupt" and "sustained" are unclear. Several alternative consensus definitions of acute renal failure have been considered over the last decade.[23] In 2000, the RIFLE definition was proposed by the Acute Dialysis Quality Initiative and refers to 5 stages of acute renal failure – **r**isk of renal dysfunction, **i**njury to the kidney, **f**ailure of kidney function, **l**oss of kidney function and **e**nd stage renal disease. In 2005 the Acute Kidney Injury Network (AKIN) proposed the newer term "acute kidney injury".[22] Thereafter, in 2012 the Kidney Disease Improving Clinical Outcomes (KDIGO) re-defined AKI as any one of three entities: an increase in serum creatinine by more than 26.4umol/l in 48 hours; an increase in serum creatinine by more than 1.5 times the baseline which is known or presumed to have occurred within the last seven days or urine output less than 0.5ml/kg/hr for the last 6 hours.[24] Furthermore, a staging system of severity dividing acute kidney injury into 3 stages,

depending on degree of creatinine elevation from baseline or decline in urine output, was added.[24]

1.3.1.2 Chronic kidney disease

KDIGO define chronic kidney disease (CKD) by abnormalities of kidney structure or function, which is present for more than 3 months, with implications for health. Abnormalities of kidney structure and function are defined by: an estimated glomerular filtration rate (eGFR) of less than 60 or by one of the following markers of kidney damage: albuminuria > 30mg/24 hours or albumin creatinine ratio > 30mg/g, urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by imaging or renal biopsy or a history of kidney transplantation.[25] The glomerular filtration rate is then used to classify the stage of CKD into one of five stages.[25]

1.3.2 Acute kidney injury in HIV

The risk of AKI in all HIV positive individuals, both ART naive and those on ART, is increased. Although HIV positive individuals share similar risk factors and causes for AKI to those who are uninfected, certain factors are unique to HIV-infected individuals that predispose to and cause AKI.[26, 27]

1.3.2.1 Epidemiology of AKI in HIV

Many studies have highlighted the increased incidence of AKI in HIV infected individuals. In a cohort study of 754 ambulatory HIV infected patients from North Carolina the incidence of AKI was 5.9 per 100 person years.[28] Whilst, in a retrospective cohort of 2274 HIV infected outpatients from London 2.7 episodes of AKI occurred per 100 person – years.[29] A New York study that compared adult hospitalized patients with AKI during the year 1995 (pre-ART era) and the year 2003

(post –ART era), reported significantly more AKI in HIV infected patients compared to HIV negative patients in both the pre- and post ART era: 1995 (2.9 verses 1%) and 2003 (6 verses 2.7%). Furthermore, the odds ratios for the occurrence of AKI in HIV – infected patients were 4.62 (pre-ART) and 2.82 (post ART).[1] Two retrospective cohorts reported similarly high rates of AKI amongst hospitalized HIV positive patients. One in six of 17325 patients from a U.S. registry of hospitalized HIV-infected patients developed AKI and 18% of 489 HIV – infected hospitalized patients in Lisbon were diagnosed with AKI.[30, 31] A 2012 systematic review and meta-analysis calculated the relative risk of kidney disease in people living with HIV of 3.87 compared to HIV – uninfected people.[32] South African data detailing the incidence of AKI in HIV positive patients is limited.[27, 33] In a Johannesburg retrospective cohort of 684 hospitalized adult patients with AKI 14.8% were HIV positive.[34] In this cohort HIV positive patients presented with more severe AKI: 72% of HIV positive patients were classified in the Failure category of the RIFLE classification compared to 44% of HIV negative patients.[34] In a study from rural Kwa-Zulu Natal of 2189 patients initiating ART, 14.4% of patients had moderate to severe renal impairment (an eGFR of less than 60).[35]

1.3.2.2 HIV associated risk factors and AKI

Traditional risk factors for AKI in seronegative patients, also apply to HIV seropositive individuals, include: increasing age (age greater than 65), black race, hypertension, diabetes, chronic kidney disease, exposure to radio-contrast, injection drug use, viral hepatitis co – infection and cirrhosis. [1, 28-32, 36, 37] In patients with HIV, hepatitis C co-infection is associated with an increase risk of AKI but no association with hepatitis B co-infection is noted.[29, 36, 38, 39] Risk factors for AKI specific to HIV seropositive individuals comprise: male sex, immunosuppression -

evidenced by CD4 counts less than 200, presence of an AIDS defining condition, advanced HIV disease - HIV RNA level greater than 10000copies/mL, long term ART use, use of prophylactic cotrimoxazole, admission to an intensive care unit during hospital stay and pre-existing renal or liver disease.[28-32, 36-41] In South Africa: male sex, age greater than 40 and a CD4 count of less than 100 are associated with increased risk for AKI in ambulatory patients initiating ART, while in hospitalized HIV positive patients with AKI, active tuberculosis (TB) was reported as a risk factor for AKI and 98% of patients were black - although this finding may be as a result of the epidemiological characteristics and spread of HIV unique to South Africa.[34, 35] Moreover, exposures to herbal or traditional medications are important risk factors for AKI in South African HIV seropositive patients.[26, 27]

1.3.2.3 Aetiology of AKI in HIV

The aetiology of AKI in the HIV positive patient is diverse. Mechanisms of AKI can be divided into 3 categories: pre – renal AKI, intrinsic renal injury or post renal obstruction.[2, 3, 6, 28]

1. Pre-renal AKI from renal hypo perfusion due to intravascular fluid depletion occurs commonly in this setting.[6] Pre-renal AKI was present in 43% of ambulatory patients with AKI, in a North Carolina study, whilst the in a Lisbon hospital prevalence was 21.6%.[28, 30] Pre-renal AKI from renal hypo-perfusion may be multifactorial and aetiology includes: gastroenteritis, vomiting, septicemia (often from opportunistic infections), decompensated liver cirrhosis or cardiomyopathy.[2, 3, 6, 28, 29] In South Africa, gastroenteritis and dehydration are often cited as causes pre-renal AKI whilst at a Johannesburg hospital 20% of hospitalized HIV positive patients with AKI result from volume depletion and haemodynamic instability.[27, 34, 42]

2. Intrinsic renal injury may lead to AKI in HIV-infected individuals and can be categorized as: acute tubular necrosis (ATN), acute interstitial nephritis (AIN), glomerular disease and crystal nephropathies.[2, 6]

a) AKI from ATN occurred in 41% of ambulatory HIV-infected patients in North Carolina, whilst, in a Cape Town review 58% of HIV positive patients requiring renal replacement therapy were clinically diagnosed with ATN. ATN may result from numerous scenarios.[26, 28]

i) Sepsis causes ATN due to direct tubular toxicity or via hypotension leading to renal ischemia and is the commonest cause of AKI in the HIV positive population – occurring in 59% of HIV positive patients in a Lisbon hospital.[2, 6, 30, 43] A similar experience is noted in the South African literature at a Johannesburg hospital where 62% of HIV seropositive patients with AKI were sepsis related.[26, 27, 34, 42]

ii) ATN occurs from co – administration of nephrotoxic drugs and in the Lisbon cohort was implicated in 37.5% HIV positive patients with AKI.[30, 38] Nephrotoxic drugs include: aminoglycosides, amphotericin b, pentamidine, foscarnet, cidofovir - agents frequently used to treat opportunistic infections.[2, 6, 28, 38] Furthermore, non-steroidal anti-inflammatory drugs (NSAID) and lithium have been linked to the development of ATN.[6, 28] The antiretrovirals (ARVs) TDF (a nucleoside reverse transcriptase inhibitor or NRTI) and ritonavir (a protease inhibitor or PI) cause ATN.[5, 44] In South Africa this is further complicated by traditional or herbal medication use and may be derived from nephrotoxic plants such as Impila (*Callilepis laureola*) which may used by traditional healers, to treat AIDS related illness.[26, 27, 42, 45]

iii) Other causes of ATN include: contrast nephropathy from radiological contrast media and rhabdomyolysis due to ARVs (zidovudine or didanosine), statin use or HIV-related myopathy.[2, 3, 6, 28, 30, 44]

b) Acute Interstitial nephritis (AIN) - inflammation of the interstitium surrounding the tubules - causes AKI in HIV infected individuals.[6] AIN induced AKI, in North Carolina, occurred in 5% of ambulatory HIV positive patients with AKI and may be due to an allergic idiosyncratic drug reaction from opportunistic infections or the drugs used to treat them.[2, 6, 28] Beta – lactam antibiotics, rifampicin, quinolones, sulfamethaxazole/trimethoprim can all cause AIN whilst other drugs implicated include: histamine – 2 –receptor blockers and NSAIDs.[2, 3, 6, 44] Opportunistic infections causing AIN include: TB, cytomegalovirus and candidiasis.[2] Histologically, renal TB appears as diffuse interstitial disease but may be difficult to distinguish from AIN caused by anti – TB drugs.[26] Furthermore, AIN has been described as part of the immune reconstitution inflammatory syndrome (IRIS), whereby, restoration of the immune system following ART may result in interstitial inflammation to both non – infectious and infectious agents in the kidney, such as TB, and can be confirmed by granulomatous nephritis on renal biopsy.[3]

c) Glomerular disease contributes to AKI in HIV infected individuals.[2, 3, 40, 44] In South Africa, thrombotic microangiopathies - thrombotic thrombocytopenic purpura and hemolytic uremic syndrome - cause AKI in the HIV positive population.[2, 3, 27, 40, 44] A Paris retrospective cohort of 92 HIV positive patients with AKI – 60 of whom had renal biopsies – described 32 cases of hemolytic uremic syndrome with 26 of these biopsy-proven.[40] Direct infection of renal cells by the HIV virus may result in an HIV associated nephropathy (HIVAN) with rapid progression to end stage renal disease – particularly in black patients.[44] In a Johannesburg study HIVAN was

present in 27% of renal biopsies performed over a 2-year period whilst the immune mediated glomerulonephritide, HIV immune complex kidney (HIVICK), was found in 21% of the biopsies.[4] Other glomerulonephritides in the HIV population that may present with AKI include: immune mediated glomerulonephritides - membranous glomerulopathy (which can be caused by hepatitis B or C virus) or post-infectious glomerulonephritides; IgA nephritis; mixed sclerotic/inflammatory disease and lupus-like disease.[2, 4, 44]

d) A crystal nephropathy or post-renal injury can be caused by nephrolithiasis as a result of tumour-lysis syndrome from lymphoma or drugs used to treat opportunistic infections such as: acyclovir, the protease inhibitor atazanavir as well as the rarely utilized ARV indinavir.[2, 3, 6, 28, 44]

3. Obstructive uropathy may result from bladder dysfunction syndromes – neuropathic bladder - and external obstruction from cervical carcinoma or retroperitoneal lymph nodes in patients with lymphoma. Moreover, therapeutic radiotherapy may contribute, due to resulting fibrosis and bilateral ureteral encasement.[2, 3, 6, 28]

Despite the diverse range of disease processes causing AKI the leading cause in the HIV-infected adults remains pre-renal AKI or ATN with sepsis the predominant underlying cause of both entities.[26-28, 30, 34, 40, 42]

1.3.2.4 Mortality of AKI in HIV

HIV-infected patients admitted to hospital with AKI have a greater mortality rate than those without AKI. Mortality, in a cohort of HIV positive patients in Lisbon, admitted with AKI has been reported as 27.3% compared to 8% in those without AKI.[30] Even in the era of ART use, a New York study reported a six-fold increase in mortality in HIV positive patients with AKI compared to those without AKI.[1] HIV

seropositive hospitalized patients with AKI who subsequently recovered to normal renal function still had a 20% higher risk of mortality compared to those who never developed AKI, furthermore, a more severe stage of AKI (measured by the AKIN criteria) was associated with a greater risk of mortality.[31] South African data is limited but a mortality rate of 42% was reported in a Johannesburg cohort in HIV positive patients with AKI and factors predicting mortality included: hyponatremia, acidosis, hyperphosphatemia and the presence of anaemia.[34]

1.3.2.5 Measures of Severity: Glomerular Filtration Rate

Current estimates of glomerular filtration rate (eGFR) include: the Cockroft - Gault equation (CG), Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiological Collaboration (CKD – EPI). No formula has been validated for use in HIV positive patients.[46, 47] Each of these formulas have limitations and concerns exist over their use in HIV positive patients who may have reduced muscle mass and an altered surface area to volume ratio that may influence their reliability and result in underestimation of eGFR.[47-49] Current recommendations support the use of CKD – EPI for the calculation of eGFR in HIV positive individuals.[47, 49]

1.4. Tenofovir

1.4.1 TDF use in South Africa

In October 2001, TDF was approved by the Food and Drug Administration (FDA) as the first NRTI for the treatment of HIV.[50] Following recommendations from the World Health Organization (WHO), in 2010 the South African Department of Health recommended the use of TDF as a first line ARV – ahead of stavudine and zidovudine due to its favorable side effect profile.[7, 51] The latest South African Department of Health guideline recommends the once daily FDC antiretroviral regime – consisting

of: TDF, emtricibine and efavirenz - as first line therapy for HIV positive patients to be used in both those initiated onto ART and to those already on 1st line ART.[8, 52] The CD4 count threshold for the initiation of ART was raised from 200 cells/mm^3 to 350 cells/mm^3 in 2013 and again in 2015 it was increased to 500 cells/mm^3.[8, 9] Consequently, the number of patients being exposed to TDF has increased since 2010.

1.4.2 Pharmacology

TDF is a nucleotide analogue HIV-1 and HIV-2 reverse transcriptase inhibitor and is the only nucleotide reverse transcriptase inhibitor available.[50]

It is initially administered as tenofovir disoproxil fumarate – its ester – derived prodrug. Following diester hydrolysis in the intestinal lumen and plasma and subsequent phosphorylation by cellular enzymes it is converted to the active form - tenofovir diphosphate.[50, 53] Tenofovir diphosphate, the nucleotide base analogue, is incorporated into HIV DNA by the reverse transcriptase enzyme during viral DNA formation. These nucleotides cannot form the necessary chemical bonds to other DNA base pairs and prevent addition of functional nucleotides to the replicating DNA. DNA elongation is halted resulting in the inability to produce new HIV virus.[18, 50, 53] The oral bioavailability of TDF is 25% and despite initial manufacturer recommendations, currently, it is recommended TDF be taken once daily irrespective of related food consumption.[50, 53] Elimination of TDF occurs via glomerular filtration and tubular secretion – over 72 hours 70 – 80% of unchanged drug is found in the urine. TDF has an intracellular half-life of 10 – 50 hours and levels decline in a biphasic manner with a terminal elimination half-life of 17 hours.[50, 53]

1.4.3 Tenofovir and Nephrotoxicity

1.4.3.1 History of the association between Tenofovir and nephrotoxicity

TDF is structurally similar to other acylic nucleotide analogues that cause renal dysfunction and hypophosphatemia - adefovir and cidofovir.[54, 55] Despite this similarity, two initial U.S. based phase I/II randomized double blind placebo-controlled clinical trials did not demonstrate nephrotoxicity with TDF use after 28 days or 48 weeks.[56-58] However, as TDF was introduced into clinical practice, case reports and series emerged from the USA and Europe reporting declining renal function with TDF use.[59-68] Subsequently, TDF associated nephrotoxicity was described in various observational cohort studies conducted mostly in the developing world - USA, Europe and Australasia.[41, 54, 69-75] The most significant cohort, *Nelson et al,* included 10343 patients enrolled in the TDF expanded access program over a 4 year period from the USA, Europe and Australia - all of whom had failed previous ART and were now switched to TDF. Post-marketing safety data, reporting serious adverse events associated with TDF use submitted to Gilead- the pharmaceutical company developing TDF- over a 6-month period was also examined.[72] A decline in creatinine clearance of more than 20% was found in 16% of the TDF exposed group verses 11% of the TDF unexposed group in an American prospective cohort comparing renal function in 593 HIV positive TDF exposed outpatients to 521 TDF – unexposed patients.[76] In 2010, the association of TDF with declining renal function was described in a prospective Senegalese cohort of 40 patients starting a TDF based ART regimen compared to 388 initiating Non-TDF based ART.[71] South African data supporting the association of TDF and nephrotoxicity is described in two cohort studies. The first, *Brennan et al,* comprised 890 HIV positive ambulatory patients from Johannesburg, the majority of whom were

black and female, that were either newly initiated or switched to TDF from a pre-existing ART regimen and the second, *Kamkuemah et al,* included 1092 ambulatory HIV positive ART naïve patients initiated on TDF – based ART in a primary healthcare Clinic in Cape Town.[74, 77] In 2010 a systemic review and meta-analysis by *Cooper et al:* 11 studies confirmed significant decline in renal function associated with TDF use with a mean difference in CG eGFR of 3.92mL/min; [95% confidence interval, 2.13 to 5.70mL/min]. Six more studies that used the MDRD formula noted a difference in kidney function of 2.56mL/min; [95% confidence interval, -0.57 to 5.69].[78] It is postulated that exclusion of patients with underlying risk factors for renal disease from early clinical trials led to publication bias and may explain the discrepancy noted in later findings in clinical practice.[78, 79]

1.4.3.2 Incidence and Prevalence

TDF related nephrotoxicity is considered uncommon and its incidence and prevalence is described in numerous studies.[41, 69, 70, 72, 74-78, 80] In *Nelson et al,* 2.2% of the cohort had a grade 1 rise in creatinine of 44umol/L from baseline whilst 0.6% of patients had a rise of serum creatinine to greater than 177umol/L from baseline.[72] A U.S. retrospective cohort studied ART naïve patients commenced on ARVs: 964 were initiated onto TDF containing regimens compared to 683 initiated onto TDF sparing regimens. In the TDF exposed group 4.8% of patients had a 50% or more decline in eGFR from baseline but the serum creatinine did not rise to greater than 177umol/L.[80] A recent Madrid based prospective cohort assessed both ART experienced and naïve patients and observed AKI in 10% of patients exposed to TDF with a calculated incidence of 5.9 cases per 100 patient years.[41] Other cohorts from the Western world calculated a prevalence of 0.3% of AKI in the TDF group whilst the incidence of AKI in TDF was reported as: 1.6 per 100 person years and 0.71 per

100 person years with an incidence ratio of 1.98 - but greater grades of creatinine elevation were associated with a lesser incidence ratio of 1.27.[70, 75, 76] In South Africa, *Brennan et al* report 2.4% of TDF exposed patients experienced nephrotoxicity (any decline in kidney function from baseline) at 48 month follow up and from *Kamkuemah et al* the incidence of decline of renal function of more than 10ml/min/1.73m^2 at 12 months after TDF initiation was 96 per 100 person – years.[74, 77] *Cooper et al,* calculated the risk of developing AKI with TDF use as 0.7%.[78]

1.4.3.3 Duration of Tenofovir exposure to onset of declining renal function

The onset of declining renal function associated with TDF occurs several months following TDF initiation. A number of cohorts have described nephrotoxicity at a mean and median time of 6 – 9 months following TDF initiation.[41, 57, 69, 70, 72] In contrast, cohorts from South Africa report a shorter time to nephrotoxicity: *Brennan et el* report a median time of 3.6 months to the development of nephrotoxicity and *Kamkuemah et al* had the greatest incidence of a decline in renal function during the first 2 months on TDF based ART.[74, 77]

1.4.3.4 Pathophysiology

During renal elimination, TDF crosses cells of the proximal tubule to be excreted via the urine. Initially, TDF enters the proximal tubule cell via the basolateral membrane through organic anion transporters (OAT), OAT 1 and OAT 3, and crosses the cell to exit the cell via the apical membrane through the transmembrane multidrug resistance protein (MRP) transporter 4. (The apical surface also has a second transporter, MRP 2 – for which implications on TDF transport across the apical membrane is incompletely understood.)[5, 11, 79, 81, 82] The imbalance between the influx and

efflux of TDF into cells results in intracellular TDF accumulation which inhibits mitochondrial DNA (mtDNA) replication through inhibition of the mtDNA-producing enzyme DNA polymerase γ.[5, 83] This effect is toxic to the mitochondria of these cells and mitochondrial dysfunction ensues resulting in proximal tubule dysfunction.[5, 11, 67, 79, 81, 84]

1.4.3.5 Clinical manifestations

The clinical presentation of proximal tubulopathy is characterized by the following: Fanconi syndrome, ATN with AKI and TDF related bone disease. [5, 11, 67, 79, 81, 84] The proximal tubule has two notable functions: the reabsorption of fluid and nutrients filtered by the glomerulus and production of 1.25-dihydroxy-vitamin D. In the proximal tubule, 60 – 70% of the water and sodium chloride from glomerular ultrafiltrate and important nutrients such as: glucose, potassium, amino acids, bicarbonate and phosphate are reabsorbed and 25–hydroxy–vitamin D is converted to its active form 1.25-dihydroxy-vitamin D.[85] Proximal tubular toxicity from TDF results in loss of these functions. Reabsorption fails and increased quantities of important nutrients and anions are lost in the urine. A complete or partial Fanconi syndrome may occur, characterized by: tubular proteinuria, aminoaciduria, glycosuria in the presence of normoglycemia, phosphaturia leading to hypophosphatemia, hypokalemia due to potassium losses and bicarbonaturia, which leads to a renal tubular acidosis.[11, 79, 80, 86] This can be further categorised as proximal tubular dysfunction with preserved or reduced renal function.[79] As proximal tubular dysfunction progresses ATN with AKI may occur – evident by an increasing serum creatinine.[11, 79, 81, 87] Bone disease, reduction in bone density and subsequent osteomalacia, associated with TDF is due to excess urinary phosphate losses and

decreased active vitamin D production resulting in reduced calcium absorption.[11, 67, 79]

1.4.3.6 Biochemical markers

Biochemical markers of proximal tubulopathy include a rising serum creatinine, glycosuria on urine dipsticks and an elevated spot urine protein: creatinine ratio. A urine dipsticks test cannot be used to detect urine protein losses as mainly albuminuria is detected and not tubular proteins.[67] Other tests include serum hypophosphatemia, serum hypokalemia and serum ph.[79, 84] Subclinical renal tubular toxicity may be diagnosed through the detection of smaller sized tubular proteins lost in the urine during the early stages of proximal tubular dysfunction: β 2 – microglobulin, retinol binding protein or N- Acetyl – β – D – glucosaminidase.[67, 84, 88]

1.4.3.7 Histological features

Studies have described histological features of patients with TDF related nephrotoxicity that support TDF is toxic to mitochondria of the proximal tubule.[44, 67, 83, 87, 89] Large eosinophilic intracytoplasmic inclusions representing giant mitochondria are noted within the proximal tubule epithelium and electron microscopy of the proximal tubule epithelium reveals dysmorphic mitochondria with variable size and shape and reduced in number.[67, 83, 87] Features in keeping with toxic ATN are noted on light microscopy.[87, 89]

1.4.3.8 Risk Factors

A number of studies have considered risk factors, with differing results, that predispose patients to TDF nephrotoxicity. Older patients and males are shown to be at a higher risk of nephrotoxicity with TDF use, as is white race. [70, 76, 78, 80, 82, 90] Co – morbid diabetes, hypertension, cardiovascular disease and cirrhosis is

associated with an increase risk of TDF related nephrotoxicity.[54, 72, 80, 84, 90, 91] Patients who are underweight, with hepatitis B or C co-infection, or are admitted with sepsis or severe infections are predisposed to TDF associated nephrotoxicity.[61, 72, 76, 90] Regarding HIV, patients with advanced HIV disease (baseline CD4 counts – less than 50 cells/mm^3 – and higher baseline HIV viral load – more than 20000copies/ml) have a greater risk of TDF associated AKI.[41, 54, 61, 70, 72, 74, 76, 78, 84] Furthermore, patients who are ART naïve and initiated on TDF based regimens have less risk of AKI compared to those who are ART experienced and subsequently switched to a TDF containing regimen.[54, 74, 76, 78] The duration of TDF exposure is described by one study as a risk factor for AKI.[76] In patients with pre-existing renal disease - elevated baseline serum creatinine or reduced baseline eGFR – the risk of TDF associated nephrotoxicity is higher.[54, 69, 72, 74, 76, 78] Concomitant nephrotoxic drug use such as the non – steroidal anti-inflammatory diclofenac and concomitant use of the antibiotic sulfamethoxazole-trimethoprim is associated with a greater risk of AKI due to TDF.[41, 72, 84, 92] Concomitant PI use, as part of a TDF containing ART regimen – such as ritonavir - is noted to increase the risk of TDF related nephrotoxicity due to the inhibition of the MRP 2 transporter in the apical surface of the proximal tubule cell resulting in excess TDF accumulation within the cell.[41, 54, 61, 70, 80, 84, 90, 91]

1.4.3.9 Genetic Predisposition

Genetic polymorphisms in genes that code for the drug transporters of the apical membrane of the proximal tubule result in transporter malfunction and intracellular TDF accumulation.[81] The two transporters on the apical surface, MRP 2 and 4, are coded for by the ABCC2 and ABCC4 genes.[82, 93] Genetic polymorphisms in the ABCC2 – encoding MRP 2 - are associated with the development of proximal tubular

dysfunction but there is no association between proximal tubulopathy and genetic polymorphisms in the ABCC4 gene.[82, 93] Interestingly, MRP 4 is described as the transporter that extrudes TDF from the proximal tubules rather than MRP 2. Although the mechanism by which defective MRP 2 causes TDF accumulation is poorly understood, it is postulated that MRP 2 may have a complimentary role to MRP 4 in TDF transport or alternatively excretes an unknown co-factor that drives TDF induced mitochondrial toxicity.[82]

1.5 Summary

As more patients are being exposed to TDF questions exist regarding its association with AKI in the South African clinical setting. Little is known of this relationship with only two studies examining South African patients initiated on TDF in the ambulatory setting.[74, 77] Evidence suggests that AKI associated with TDF use is multifactorial, and occurs in patients with several predisposing factors but it is possible that a genetic predisposition, due to polymorphisms, play a role in TDF related nephrotoxicity.[11] As limited data describing TDF and AKI in South African HIV positive patients exists, this study will characterize this association, in particular: to describe presentation, severity and risk factors and assess short-term survival and response to treatment.

Objectives

To describe the aetiology and spectrum of renal disease observed in adult hospitalized HIV-infected patients.

Specific Aims:

1. To describe the patient population and disease patterns in patients presenting with acute renal failure whilst on a TDF based ART Regimen with respect to the following variables:
    - Demographics
    - Co-morbidities
    - Stage of HIV infection
2. To assess the proportion of those with acute renal failure and receiving ART as:
    i. Immediate (less than 6 weeks after ART initiation)
    ii. Intermediate (6 weeks - 6months after ART initiation)
    iii. Delayed (>6 months after ART initiation)
3. To compare the patient population and disease patterns in patients with acute renal failure exposed to TDF to all other HIV positive patients not exposed to TDF with renal failure.

    The TDF - exposed group is as follows:

    - Those currently on TDF based ART
    - Those who have defaulted TDF for less than 1 week

    The Non - TDF exposed group is as follows:

    - Those presenting with renal failure on non-TDF based ART
    - Those who have defaulted non-TDF based ART for any period of time
    - Those who have defaulted TDF based ART for more than 1 week
    - Those not on ART

4. To assess short-term survival and response to treatment in HIV positive patients exposed to TDF with acute renal failure

Methodology

1.Study design

A prospective case cohort study with descriptive and comparative elements

2.Study population and sample

2.1 LOCATION & DURATION

The study is a single-centre study and will take place at Klerksdorp/Tshepong Hospital.

2.2 POPULATION

The study will recruit patients admitted to the Klerksdorp/Tshepong hospital who are HIV positive and present with acute renal failure.

INCLUSION CRITERIA

1. Age Older than 18
2. Confirmed HIV positive diagnosis (hard copy dated documentation of one of the following: ART treatment, positive HIV ELISA, positive double rapid HIV antibody tests, detectable HIV viral load)
3. Acute Renal failure as defined by one or more of the following:
    a. Creatinine increase by > 26.4umol/l above:
        i. The upper limit of normal (as per the local laboratory standards)
        ii. A recent normal Creatinine level prior to admission
    b. Creatinine increase by more than 1.5 times the baseline level (baseline taken prior to ARV initiation)
    c. Urine output < 0.5ml/kg/hr for 6 hours

d. eGFR decreased by greater than 25% compared to any prior eGFR calculation using the Cockroft-Gault Equation[46]

EXCLUSION CRITERIA

1. Unable to provide written informed consent
2. Reluctant to comply with study procedures
3. Incomplete or insufficient clinical records for collection of parameters to be measured, as enlisted in the data sheet.
4. Known or Newly Diagnosed with Chronic Kidney Disease defined as: abnormalities of kidney structure or function, which is present for more than 3 months, with implications for health evidenced by one or more of the following:
    a. eGFR < 60ml/min/1.73m^2 for a period of more than 3 months
    b. Proteinuria, urine sediment abnormalities or abnormalities detected by imaging or renal biopsy for a period of more than 3 months

2.3 SAMPLE SIZE AND DURATION

A sample size of approximately 144 patients prospectively recruited over a 6 month period was chosen, based the number of patients admitted to the adult medicine wards at the Klerksdorp/Tshepong Hospital Complex per month. We anticipate recruitment will take place over a period of 24 weeks. However, we will continue to recruit patients until the target of 144 has been reached.

Table showing: Projected patient numbers recruited for study

ADMISSIONS WITH RENAL FAILURE PER WEEK	ADMISSIONS WITH RENAL FAILURE ON TDF ART PER WEEK	STUDY TIME FRAME (WEEKS)	TOTAL PATIENTS RECRUITED
6	3	24	144

3. Description of methods and techniques

1. INTERVIEW: Patients will be counseled about the study and written informed consent obtained. All patients will then undergo a structured interview. Questions will be asked about patient's demographics, HIV history – including current and previous ART use, history of tuberculosis, co-morbid diseases, medication use and traditional medicine and possible heavy metal exposure.

2. LABORATORY INVESTIGATIONS: HIV relevant parameters: the CD4 count and HIV viral load will be measured. Biochemical parameters: urea and electrolytes will be measured and using the Cockroft-Gault equation the eGFR will be calculated. The creatinine and eGFR will be the parameters used to define patients as having renal failure. Other investigations will include: calcium, magnesium and phosphate; serum ferritin; full blood count and hepatitis B status. Urine parameters will include a urine dipsticks, urine protein creatinine ratio, urine for amino acids and phosphate. These investigations will be performed prior to any renal specific treatment being administered and preferably in the acute phase of presentation.

3. RADIOLOGICAL INVESTIGATIONS: Renal sonar to determine kidney sizes and a Chest X-ray to assess the presence of possible pulmonary tuberculosis.

4. PATIENT RECORDS: Patient admission medical records will be assessed to evaluate if patients are diagnosed or treated for current tuberculosis infection or cryptococcal meningitis. Furthermore, the reason for the admission will be abstracted from clinical records.

5. FOLLOW UP: Patients records will be reviewed on discharge and at routine follow up at one month, two months and three months following discharge. Repeat urea and electrolytes, all treatment received for: HIV, acute renal failure and other intercurrent illnesses will be documented.

Statistical analysis

This research project is intended as a pilot study. Aim 1: participant characteristics will be analysed descriptively using frequencies and medians and compared between renal failures exposed to TDF and renal failures not exposed to TDF using chi-square and Kruskall-Wallis tests as appropriate. Aim 2: proportions will be compared using the chi-square test of proportions. Aim 3: comparisons will be made using the chi-square test of proportions and for continuous variables the Kruskall-Wallis non-parametric test. Aim 4: Kaplan-Meier and Cox regression analysis will be used to address short-term survival. A Kaplan Meier graph will be drawn showing time to renal failure using: as the start of follow up time - the initiation of ART and the date of censoring the date - when symptoms first developed or when the first diagnosis of renal failure was made.

Ethics

Ethics approval will be obtained from the Human Resource Ethics Committee. Consent for the use of patient's records and will be gained from the Academic Head of Internal Medicine and CEO/Superintendent of Klerksdorp/Tshepong Hospital Complex. Individual patient consent will be obtained from the patients examined in the study. Each data sheet will be assigned a study number. No patient names or hospital numbers will be used so patient identity will remain confidential. Data will only be made available too the supervisors, primary researcher and statistician.

Timing

Gant Chart showing the timeline of the study:

2014	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Literature Review										

Preparing Protocol										
Protocol Assessment										
Ethics Application										
Data Collection										

2015	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Data Collection										
Data Analysis										
Writing Up - Thesis										

Funding

The budget is expected to involve photocopying of data sheets and travelling costs. Other costs will include study nurse and counselor and costs of non-routine investigations - urine phosphate and amino acids.

Limitations

Due to the prospective nature of the study problems may be encountered in finding an adequate number of patients to meet the study aims. Furthermore, logistical difficulties of travelling from Johannesburg too Klerksdorp may be faced. The sample size may not be adequate to identify small differences in the prevalence of risk factors for renal failure between groups.

Note of review recommendations:

- *Eligibility criteria of Non - TDF exposed group: Patients who defaulted TDF for a period of more than 1 week be increased to 8 weeks and those who defaulted for 1 – 8 weeks to be excluded.*
- *Replace the term acute renal failure with acute kidney injury*

References – (Extended Literature Review)

1. Wyatt CM, Arons RR, Klotman PE, Klotman ME. Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. *AIDS* 2006,**20**:561-565.
2. Cohen SD, Chawla LS, Kimmel PL. Acute kidney injury in patients with human immunodeficiency virus infection. *Curr Opin Crit Care* 2008,**14**:647-653.
3. Kalim S, Szczech LA, Wyatt CM. Acute kidney injury in HIV-infected patients. *Semin Nephrol* 2008,**28**:556-562.
4. Gerntholtz TE, Goetsch SJ, Katz I. HIV-related nephropathy: a South African perspective. *Kidney Int* 2006,**69**:1885-1891.
5. Roling J, Schmid H, Fischereder M, Draenert R, Goebel FD. HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. *Clin Infect Dis* 2006,**42**:1488-1495.
6. Perazella MA. Acute renal failure in HIV-infected patients: a brief review of common causes. *Am J Med Sci* 2000,**319**:385-391.
7. The South African antiretroviral treatment guidelines. In: Department of Health; 2010. pp. 1-7.
8. The South African antiretroviral treatment guidelines. In: Department of Health; 2013. pp. 1-10.
9. National consolidated guidelines for the prevention of mother-to-child transimission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. In: Department of Health; 2015. pp. 1 - 136.
10. Gitman MD, Hirschwerk D, Baskin CH, Singhal PC. Tenofovir-induced kidney injury. *Expert Opin Drug Saf* 2007,**6**:155-164.

11. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. *Am J Kidney Dis* 2011,**57**:773-780.

12. Mayosi BM, Benatar SR. Health and health care in South Africa--20 years after Mandela. *N Engl J Med* 2014,**371**:1344-1353.

13. Campion EW. Treating millions for HIV--the adherence clubs of Khayelitsha. *N Engl J Med* 2015,**372**:301-303.

14. Mid - year population estimates. In: Statistics South Africa; 2015. pp. 1-20.

15. HIV & AIDS 2014/2015 data fact sheet. In: *7th South African AIDS Conference*: Department of Health 2015. pp. 1-2.

16. Long LC, Fox MP, Sauls C, Evans D, Sanne I, Rosen SB. The High Cost of HIV-Positive Inpatient Care at an Urban Hospital in Johannesburg, South Africa. *PLoS One* 2016,**11**:e0148546.

17. Tagar E, Sundaram M, Condliffe K, Matatiyo B, Chimbwandira F, Chilima B, *et al.* Multi-country analysis of treatment costs for HIV/AIDS (MATCH): facility-level ART unit cost analysis in Ethiopia, Malawi, Rwanda, South Africa and Zambia. *PLoS One* 2014,**9**:e108304.

18. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med* 2012,**2**:a007161.

19. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. *N Engl J Med* 2011,**364**:1943-1954.

20. Abram ME, Ferris AL, Shao W, Alvord WG, Hughes SH. Nature, position, and frequency of mutations made in a single cycle of HIV-1 replication. *J Virol* 2010,**84**:9864-9878.

21. Levey AS, Levin A, Kellum JA. Definition and classification of kidney diseases. *Am J Kidney Dis* 2013,**61**:686-688.

22. Hilton R. Defining acute renal failure. *CMAJ* 2011,**183**:1167-1169.

23. Palevsky PM, Liu KD, Brophy PD, Chawla LS, Parikh CR, Thakar CV*, et al.* KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am J Kidney Dis* 2013,**61**:649-672.

24. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter., Suppl.* 2012,**2**:1-138.

25. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Inter., Suppl.* 2013:1-150.

26. Arendse CG, Wearne N, Okpechi IG, Swanepoel CR. The acute, the chronic and the news of HIV-related renal disease in Africa. *Kidney Int* 2010,**78**:239-245.

27. Naicker S, Aboud O, Gharbi MB. Epidemiology of acute kidney injury in Africa. *Semin Nephrol* 2008,**28**:348-353.

28. Franceschini N, Napravnik S, Eron JJ, Jr., Szczech LA, Finn WF. Incidence and etiology of acute renal failure among ambulatory HIV-infected patients. *Kidney Int* 2005,**67**:1526-1531.

29. Roe J, Campbell LJ, Ibrahim F, Hendry BM, Post FA. HIV care and the incidence of acute renal failure. *Clin Infect Dis* 2008,**47**:242-249.

30. Lopes JA, Melo MJ, Viegas A, Raimundo M, Camara I, Antunes F*, et al.* Acute kidney injury in hospitalized HIV-infected patients: a cohort analysis. *Nephrol Dial Transplant* 2011,**26**:3888-3894.

31. Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG. Long-term clinical consequences of acute kidney injury in the HIV-infected. *Kidney Int* 2010,**78**:478-485.

32. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. *BMC Public Health* 2012,**12**:234.

33. Han TM, Naicker S, Ramdial PK, Assounga AG. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. *Kidney Int* 2006,**69**:2243-2250.

34. Vachiat AI, Musenge E, Wadee S, Naicker S. Renal failure in HIV-positive patients—a South African experience. *Clin Kidney J* 2013,**0**:1-6.

35. Franey C, Knott D, Barnighausen T, Dedicoat M, Adam A, Lessells RJ*, et al.* Renal impairment in a rural African antiretroviral programme. *BMC Infect Dis* 2009,**9**:143.

36. Lopes JA, Fernandes J, Jorge S, Neves J, Antunes F, Prata MM. Acute renal failure in critically ill HIV-infected patients. *Crit Care* 2007,**11**:404.

37. Lopes JA, Melo MJ, Raimundo M, Fragoso A, Antunes F. Long-term risk of mortality for acute kidney injury in HIV-infected patients: a cohort analysis. *BMC Nephrol* 2013,**14**:32.

38. Miro JM, Cofan F, Trullas JC, Manzardo C, Cervera C, Tuset M*, et al.* Renal dysfunction in the setting of HIV/AIDS. *Curr HIV/AIDS Rep* 2012,**9**:187-199.

39. Franceschini N, Napravnik S, Finn WF, Szczech LA, Eron JJ, Jr. Immunosuppression, hepatitis C infection, and acute renal failure in HIV-infected patients. *J Acquir Immune Defic Syndr* 2006,**42**:368-372.

40. Peraldi MN, Maslo C, Akposso K, Mougenot B, Rondeau E, Sraer JD. Acute renal failure in the course of HIV infection: a single-institution retrospective study of ninety-two patients anad sixty renal biopsies. *Nephrol Dial Transplant* 1999,**14**:1578-1585.

41. Wikman P, Safont P, Del Palacio M, Moreno A, Moreno S, Casado JL. The significance of antiretroviral-associated acute kidney injury in a cohort of ambulatory human immunodeficiency virus-infected patients. *Nephrol Dial Transplant* 2013,**28**:2073-2081.

42. Fabian J, Naicker S. HIV and kidney disease in sub-Saharan Africa. *Nat Rev Nephrol* 2009,**5**:591-598.

43. Rao TK, Friedman EA, Nicastri AD. The types of renal disease in the acquired immunodeficiency syndrome. *N Engl J Med* 1987,**316**:1062-1068.

44. Fine DM, Perazella MA, Lucas GM, Atta MG. Renal disease in patients with HIV infection: epidemiology, pathogenesis and management. *Drugs* 2008,**68**:963-980.

45. Luyckx VA, Steenkamp V, Stewart MJ. Acute renal failure associated with the use of traditional folk remedies in South Africa. *Ren Fail* 2005,**27**:35-43.

46. Stohr W, Walker AS, Munderi P, Tugume S, Gilks CF, Darbyshire JH*, et al.* Estimating glomerular filtration rate in HIV-infected adults in Africa: comparison of Cockcroft-Gault and Modification of Diet in Renal Disease formulae. *Antivir Ther* 2008,**13**:761-770.

47. Ibrahim F, Hamzah L, Jones R, Nitsch D, Sabin C, Post FA. Comparison of CKD-EPI and MDRD to estimate baseline renal function in HIV-positive patients. *Nephrol Dial Transplant* 2012,**27**:2291-2297.

48. Barraclough K, Er L, Ng F, Harris M, Montaner J, Levin A. A comparison of the predictive performance of different methods of kidney function estimation in a well-characterized HIV-infected population. *Nephron Clin Pract* 2009,**111**:c39-48.

49. Okparavero AA, Tighiouart H, Krishnasami Z, Wyatt CM, Graham H, Hellinger J*, et al.* Use of glomerular filtration rate estimating equations for drug dosing in HIV-positive patients. *Antivir Ther* 2013,**18**:793-802.

50. DeChristoforo R, Penzak SR. Tenofovir: a nucleotide analogue reverse-transcriptase inhibitor for treatment of HIV infection. *Am J Health Syst Pharm* 2004,**61**:86-98; quiz 99-100.

51. Antiretroviral therapy for HIV infection in adults and adolescents. In: World Health Organisation; 2010. pp. 1-145.

52. Clinicians Society SH. Fixed-dose combination for adults accessing antiretroviral therapy. *Southern African Journal of HIV Medicine* 2013,**14**.

53. Antoniou T, Park-Wyllie LY, Tseng AL. Tenofovir: a nucleotide analog for the management of human immunodeficiency virus infection. *Pharmacotherapy* 2003,**23**:29-43.

54. Gallant JE, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clin Infect Dis* 2005,**40**:1194-1198.

55. Cihlar T, Ho ES, Lin DC, Mulato AS. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. *Nucleosides Nucleotides Nucleic Acids* 2001,**20**:641-648.

56. Schooley RT, Ruane P, Myers RA, Beall G, Lampiris H, Berger D*, et al.* Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *Aids* 2002,**16**:1257-1263.

57. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V*, et al.* Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. *Nephrol Dial Transplant* 2005,**20**:743-746.

58. Barditch-Crovo P, Deeks SG, Collier A, Safrin S, Coakley DF, Miller M*, et al.* Phase i/ii trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother* 2001,**45**:2733-2739.

59. Karras A, Lafaurie M, Furco A, Bourgarit A, Droz D, Sereni D*, et al.* Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clin Infect Dis* 2003,**36**:1070-1073.

60. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K. Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. *Clin Infect Dis* 2003,**37**:e41-43.

61. Barrios A, Garcia-Benayas T, Gonzalez-Lahoz J, Soriano V. Tenofovir-related nephrotoxicity in HIV-infected patients. *AIDS* 2004,**18**:960-963.

62. Malik A, Abraham P, Malik N. Acute renal failure and Fanconi syndrome in an AIDS patient on tenofovir treatment--case report and review of literature. *J Infect* 2005,**51**:E61-65.

63. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM*, et al.* Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis* 2002,**40**:1331-1333.

64. Coca S, Perazella MA. Rapid communication: acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. *Am J Med Sci* 2002,**324**:342-344.

65. Rifkin BS, Perazella MA. Tenofovir-associated nephrotoxicity: Fanconi syndrome and renal failure. *Am J Med* 2004,**117**:282-284.

66. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, Mauboussin JM*, et al.* Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 2004,**35**:269-273.

67. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D*, et al.* Tenofovir-associated renal and bone toxicity. *HIV Med* 2009,**10**:482-487.

68. Creput C, Gonzalez-Canali G, Hill G, Piketty C, Kazatchkine M, Nochy D. Renal lesions in HIV-1-positive patient treated with tenofovir. *Aids* 2003,**17**:935-937.

69. Antoniou T, Raboud J, Chirhin S, Yoong D, Govan V, Gough K*, et al.* Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. *HIV Med* 2005,**6**:284-290.

70. Madeddu G, Bonfanti P, De Socio GV, Carradori S, Grosso C, Marconi P*, et al.* Tenofovir renal safety in HIV-infected patients: results from the SCOLTA Project. *Biomed Pharmacother* 2008,**62**:6-11.

71. De Beaudrap P, Diallo MB, Landman R, Gueye NF, Ndiaye I, Diouf A*, et al.* Changes in the renal function after tenofovir-containing antiretroviral therapy initiation in a Senegalese cohort (ANRS 1215). *AIDS Res Hum Retroviruses* 2010,**26**:1221-1227.

72. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B*, et al.* The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *Aids* 2007,**21**:1273-1281.

73. Kinai E, Hanabusa H. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses* 2009,**25**:387-394.

74. Brennan A, Evans D, Maskew M, Naicker S, Ive P, Sanne I*, et al.* Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. *AIDS* 2011,**25**:1603-1609.

75. Winston A, Amin J, Mallon P, Marriott D, Carr A, Cooper DA*, et al.* Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy. *HIV Med* 2006,**7**:105-111.

76. Young B, Buchacz K, Baker RK, Moorman AC, Wood KC, Chmiel J*, et al.* Renal function in Tenofovir-exposed and Tenofovir-unexposed patients receiving highly active antiretroviral therapy in the HIV Outpatient Study. *J Int Assoc Physicians AIDS Care (Chic)* 2007,**6**:178-187.

77. Kamkuemah M, Kaplan R, Bekker LG, Little F, Myer L. Renal impairment in HIV-infected patients initiating tenofovir-containing antiretroviral therapy regimens in a Primary Healthcare Setting in South Africa. *Trop Med Int Health* 2015,**20**:518-526.

78. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010,**51**:496-505.

79. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J*, et al.* Tenofovir nephrotoxicity: 2011 update. *AIDS Res Treat* 2011,**2011**:354908.

80. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L*, et al.* Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. *J Acquir Immune Defic Syndr* 2010,**53**:62-69.

81. Perazella MA. Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy. *Kidney Int* 2010,**78**:1060-1063.

82. Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J*, et al.* Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 2009,**48**:e108-116.

83. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R*, et al.* Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest* 2009,**89**:513-519.

84. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J*, et al.* Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *Aids* 2009,**23**:689-696.

85. Curthoys NP, Moe OW. Proximal tubule function and response to acidosis. *Clin J Am Soc Nephrol* 2014,**9**:1627-1638.

86. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. *Am J Kidney Dis* 2003,**41**:292-309.

87. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, Markowitz GS. Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. *Kidney Int* 2010,**78**:1171-1177.

88. Hall AM, Edwards SG, Lapsley M, Connolly JO, Chetty K, O'Farrell S*, et al.* Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. *Am J Kidney Dis* 2009,**54**:1034-1042.

89. Zimmermann AE, Pizzoferrato T, Bedford J, Morris A, Hoffman R, Braden G. Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. *Clin Infect Dis* 2006,**42**:283-290.

90. Milinkovic A, Pavlou M, Furco A, Copas A, Arenas-Pinto A, O Connolly J*, et al.* Factors associated with increased risk of Tenofovir-related renal toxicity: Case-Control Study.

91. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, Opravil M*, et al.* Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. *Antivir Ther* 2007,**12**:1165-1173.

92. Bickel M, Khaykin P, Stephan C, Schmidt K, Buettner M, Amann K*, et al.* Acute kidney injury caused by tenofovir disoproxil fumarate and diclofenac co-administration. *HIV Med* 2013,**14**:633-638.

93. Izzedine H, Hulot JS, Villard E, Goyenvalle C, Dominguez S, Ghosn J*, et al.* Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis* 2006,**194**:1481-1491.

Chapter 2. Manuscript

Background

Acute kidney injury (AKI) is a major cause of morbidity and mortality in HIV infected individuals.[1-3] Mortality in hospitalized HIV-infected adults is higher in those with AKI, whilst in adults living with HIV the risk of kidney disease is almost four-fold that of HIV-uninfected adults.[4, 5] Antiretroviral therapy (ART) mitigates this risk.[1]

Numerous studies from developed settings have reported AKI in HIV-infected individuals; suggesting that aetiology of AKI is multi-factorial. Moreover both HIV and ART are directly nephrotoxic.[1, 4-9] Pre-renal AKI from renal hypoperfusion (commonly from gastroenteritis), sepsis induced acute tubular necrosis (ATN) and concomitant nephrotoxic drug use are common causes of AKI in HIV positive patients.[2, 3, 6, 7, 10-17] Well-described risk factors for AKI apply to both HIV-seronegative and seropositive individuals.[1, 4-7, 11, 18-21] However, specific risk factors in HIV seropositive individuals include: male sex; advanced immunosuppression; long term ART use and pre-existing renal disease.[4-7, 11, 18-23]

In October 2001, the Food and Drug Administration approved tenofovir (TDF) as the first nucleotide reverse-transcriptase inhibitor (NRTI) for the treatment of HIV.[24] Since April 2010, TDF has been used as the mainstay of first line ART in South Africa, massively increasing the number of South Africans receiving TDF.[25, 26] Although not initially reported in the early clinical trials, subsequent case reports, case series and observational studies reported TDF associated nephrotoxicity and, in 2010, a systematic review and meta-analysis reported a significant decline in renal function associated with TDF use.[27-42] The mechanism of action appears to be the

accumulation of TDF in proximal tubular cells (due primarily to genetic polymorphisms encoding defective transmembrane drug transporters), which results in mitochondrial toxicity and subsequent renal injury, clinically presenting as Fanconi syndrome or ATN with AKI.[9, 38, 43-52] The reported prevalence of TDF-related nephrotoxicity is 2.4% occurring at 6 – 9 months following TDF initiation, although South African studies suggest earlier onset.[23, 28, 40-42, 47, 53-57] We report the presentation, severity and risk factors for AKI in TDF-exposed South African patients, and assess response to treatment and short-term outcomes.

Methods

This prospective case cohort study was a primary data collection, overseen and conducted by the first author (FS), and recruited patients from the Klerksdorp/Tshepong Hospital Complex, a secondary level public hospital in South Africa's North West province, from October 2014 to June 2015. We enrolled HIV-infected patients admitted to the adult medicine wards with AKI, and compared those exposed to TDF to those who had not received TDF and followed them up after discharge. Eligible patients were: hospitalized, HIV-infected adults, ≥18 years of age with a diagnosis of AKI. We excluded patients with prior or pre-existing kidney disease (either self-reported or as indicated by medical records or laboratory results). Those who appeared unwilling or incapable of attending follow up, and those whose clinical records were inadequate to assess renal function or HIV treatments prior to the study were also excluded. The University of Witwatersrand Human Research Ethics Committee approved this study.

Study Definitions and Measures

Patients were categorized as TDF-exposed if they were on TDF-based ART at the time of admission, or had defaulted TDF for less than 1 week prior to admission. The

TDF unexposed group included individuals who: were ART naïve, were taking non-TDF ART, had defaulted non-TDF based ART for any duration, and those who had defaulted TDF based ART for at least 8 weeks prior to the onset of AKI.

In all study patients, AKI was diagnosed and staged according to the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice AKI Guideline.[58] Baseline serum creatinine (SCr) prior to AKI was determined either by a SCr taken prior to, but within the preceding year, of admission, or the lowest normal SCr at discharge or on follow up.[59, 60] If no baseline SCr was available, an estimated baseline SCr was calculated based on a Modification of Diet in Renal Disease Equation (MDRD) eGFR of 75ml/min per $1.73m^2$.[58, 61, 62] Renal recovery was defined as a decreasing SCr over time and no need for continuing renal replacement therapy (RRT); and was categorized as complete (SCr recovery below 1.5 times the baseline) or partial (SCr recovery above 1.5 times the baseline).[63, 64] New admissions to the acute care adult medical ward were screened for eligibility on weekdays. If eligible, written informed consent was obtained and a structured interview administered assessing: ART use, previous tuberculosis (TB) history, co-morbidities, concomitant gastrointestinal symptoms, drug history and other important exposures; necessary blood and urine samples were taken, results of radiological investigations recorded and relevant information from clinical records noted. On discharge, a summary of the clinical course of the patient was abstracted from the medical records. Patients were then actively followed up at a visit scheduled 3 months after discharge.

Statistical Analysis

Patient demographic and clinical characteristics are reported as medians and interquartile ranges (IQR) for continuous variables and proportions with 95%

confidence intervals for categorical variables. These were compared between groups using the Mann – Whitney, Kruskal-Wallis and Chi – square tests as appropriate. All statistical analyses were performed using Statistica version 12 (StatSoft).

Results

From 1 October 2014 to 30 June 2015, 199 HIV-infected patients with presumed AKI were enrolled in this study, 24 were subsequently excluded as they either had prior kidney disease (16) or had defaulted TDF-based ART for less than 8 weeks (8). Of the remaining 175, their median age was 41years (IQR 35-50); 61% were women; virtually all (99%) were black Africans; their median BMI was 21kg/m^2 (IQR 19-24) on admission *(Table 1)*. Ninety-three (53%) were TDF-exposed for a median treatment duration of 30 weeks (IQR 8-61) of whom 27 (29%) had prior ART exposure before switching to a TDF containing regimen. The majority of the TDF group (94%) received a concomitant non-nucleoside reverse transcriptase inhibitor (NNRTI) and 6% a protease inhibitor (PI). Thirty-one patients in the non-TDF group were receiving ART for a median duration of 306 weeks (IQR 88-729); 62% of the non-TDF group was ART-naïve on admission. .

Table 1: Baseline demographic characteristics, co-morbidities, risk factors and laboratory characteristics in acute kidney injury (AKI) patients at admission stratified by TDF exposure (n=175)			
	TDF	**NON TDF**	**p -value**
	93 (53)	82 (47)	
Demographics			
Age (yrs) [median (IQR)]	42 (35 - 53)	40 (35 - 49)	0.260
Female [n (%)]	62 (67)	45 (55)	0.110
Black [n (%)]	91 (98)	83 (100)	0.182
Serum Electrolytes			
Potassium (mmol/L) [median(IQR)]	4.1 (3.3 - 5.1)	4.3 (3.5-5.2)	0.348
Bicarbonate (umol/L) [median(IQR)]	14 (10 - 18)	16 (13 - 19)	0.047
Phosphate (mmol/L) [median(IQR)]	1.2 (0.8 - 1.7)	1.3 (0.9 - 1.6)	0.496
Renal Parameters			
Admission Creatinine (umol/L) [median(IQR)]	282 (172 - 542)	189 (146 - 343)	0.007
Admission eGFR - CKD - EPI (mL/min per 1.73m2) [median(IQR)]	14.2 (4.3 - 28)	25.6 (10.4 - 39.5)	0.002
Baseline Creatinine (umol/L) [median(IQR)]	77 (65 - 88)	77 (62 - 92)	0.920
Urine Protein:Creatinine Ratio (g/mmol creat) [median(IQR)]	0.212 (0.111 - 0.316)	0.128 (0.087 - 0.212)	0.003
Normoglycemic glycosuria [n (%)]	7 (11)	6 (10)	0.863
Hyperechoiec [n(%)]	71 (91)	48 (84)	0.226
Right kidney size (cm) [median(IQR)]	11,25 (10,3 - 12,2)	11 (10,2 - 11,8)	0.347
Left kidney size (cm) [median(IQR)]	11,4 (10,7 - 12,3)	11 (10,4 - 11,8)	0.135
Co-morbidities			
Hypertension [n(%)]	22 (24)	16 (20)	0.507
Diabetes [n(%)]	8 (9)	3 (4)	0.179
Hepatitis B Positive [n(%)]	11 (12)	9 (11)	0.86
Risk factors			
Tuberculosis (TB) [n(%)]			
Completed TB treatment before admission	35 (38)	34 (41)	0.605
Newly diagnosed TB	46 (50)	45 (55)	0.474
Sputum or culture proven tuberculosis	22 (48)	22 (49)	0.919
Pneumonia [n(%)]	9 (10)	12 (17)	0.148
Sepsis [n(%)]	4 (4)	7 (9)	0.249
Vomiting [n(%)]	32 (34)	14 (17)	0.009
Diarrhoea [n(%)]	36 (39)	17 (21)	0.008
Non-steroidal anti-inflammatory drugs use [n(%)]	26 (28)	30 (37)	0.784
HIV-related and infective parameters			
CD4 Count (cells/mm3) [median(IQR)]	147 (57 - 304)	87 (41 - 210)	0.067
HIV Viral Load (copies/ml)			
Virally suppressed (LDL or < 1000 [n(%)])	56 (60)	14 (17)	<0.001
Median HIV Viral load in those > 1000 [median(IQR)]	39456 (4537 - 326300)	414365 (110159 - 1521015)	<0.001
Haemoglobin (g/dl) [median(IQR)]	9.6 (7.8 - 11.3)	9.8 (7.4 - 12)	0.622
C-reactive protein (mg/L) [medain(IQR)]	127 (39 - 189)	89 (37 - 180)	0.386
Albumin (g/L) [median(IQR)]	20 (16 - 24)	18 (15 - 23)	0.122
Ferritin (ug/L) [median(IQR)]	490 (205 - 1171)	750 (366 - 1415)	0.047

AKI severity and measures of kidney disease

Half (53%) of all participants had severe AKI, most were in the TDF group (62%; n=57); whereas mild AKI was predominant in the non-TDF group (62%; n=23) *(Figure 1).* The TDF group had: a higher median admission SCr (282umol/L; IQR 172-542 vs 189umol/L IQR 146-343; p=0.007); higher median urine protein creatinine ratio (0.212g/mmol creat; IQR 0.111-0.316 vs 0.128g/mmol creat; IQR 0.087-0.212; p=0.003); a lower median eGFR (CKD-EPI) (14.2mL/min; IQR 4.3-28 vs 25.6mL/min; IQR 10.4-39.5; p=0.002) and was more acidotic (serum bicarbonate 14umol/L; IQR 10-18 vs 16umol/L; IQR 13-19; p=0.047). Markers of proximal tubular dysfunction (urine phosphaturia and glycosuria, serum hypophosphatemia and hypokalemia) were not markedly deranged in either group. Laboratory results suggestive of interstitial nephritis were present in many, with high rates of leucocyturia (n=47; 32%) and hematuria (n= 81; 55%) in sterile urine specimens, but there was no significant difference in rates in the two groups - only 29 (17%) of all patients had culture positive urinary tract infections.

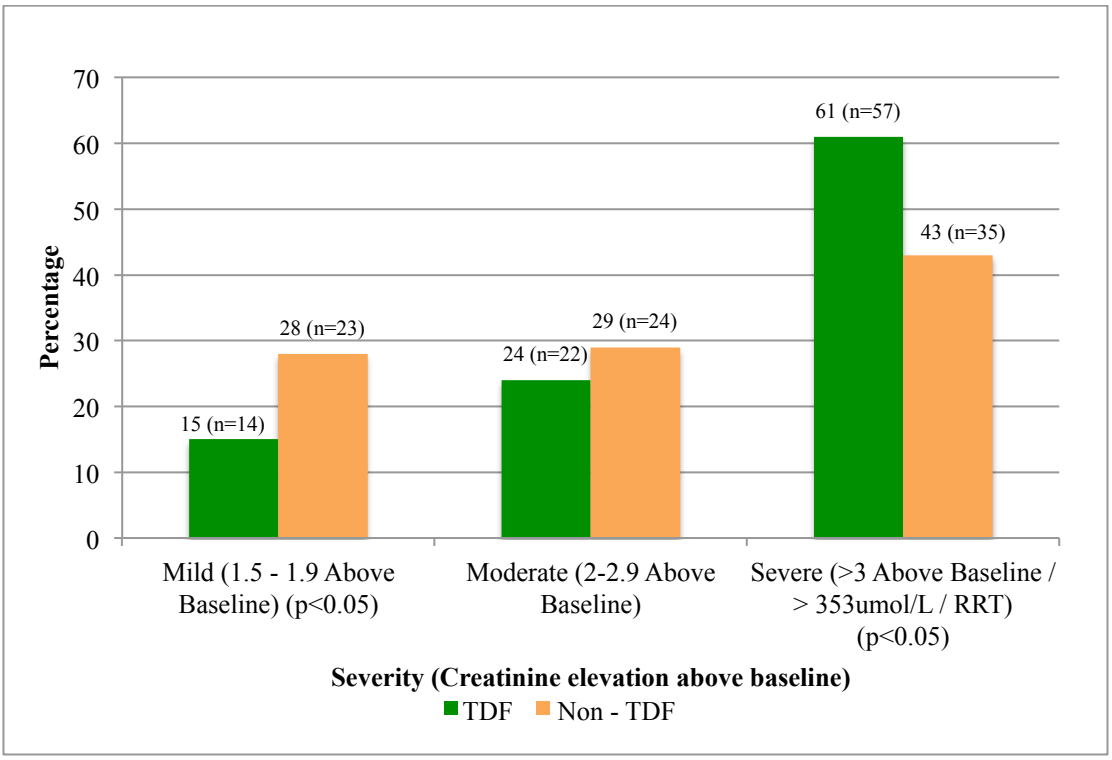

Figure 1: Severity of acute kidney injury on admission stratified by TDF exposure

Co-morbidities and risk factors for AKI

Half of both groups (50% TDF vs 55% non-TDF; p=0.474) were newly diagnosed with TB, and overall a prior history of TB treatment was reported by 39%. Gastrointestinal symptoms, vomiting and diarrhea were reported more frequently in the TDF group (39% v 21%; p=0.008) and, twice as many patients in the TDF group reported chronic diarrhea (p=0.522). Regarding hemodynamic status, using clinical and biochemical parameters, there was no statistically significant difference between the two groups. Non-infectious co-morbidities such as hypertension (22%) and diabetes (6%) were reported at similar rates in both groups. Receipt of traditional medication was infrequently self-reported but high rates of non-steroidal anti-inflammatory drug (NSAID) use, within the month prior to admission, was reported by both groups. Rates of exposure to other nephrotoxins, such as amphotericin B, were similar in both groups. A higher proportion of TDF-exposed patients were

virally suppressed than ART-treated patients from the non-TDF group (p=0. 017); and in those not suppressed, the median viral load was lower in the TDF group (p=0.002).

Treatment and outcomes

The overwhelming majority of patients received intravenous fluid therapy (96%) and only seven received RRT; 85% of all patients recovered sufficiently to be discharged from hospital. Even though the median length of hospital stay was the same in both groups (9 days), the TDF group had delayed renal recovery with higher SCr on discharge: median SCr 120umol/L; IQR 87-240 vs 97umol/L; IQR 75-137; p=0.032 *(Figure 2)*. Moreover, a smaller proportion of TDF exposed patients had complete renal recovery at the 3-month post-discharge follow-up visit (61% vs 78%; p=0.043) *(Table 2).* Although TDF was stopped on admission in all patients, at 3-month follow up 55 patients (31%) had been re-started on full dose TDF-based ART without apparent adverse effects, 38 (22%) of whom had been TDF-exposed on admission. Only 1 patient who was re-initiated on TDF was subsequently changed to non-TDF based ART. No significant difference in renal recovery (p=0.114) or mortality (p=0.105) at 3-month follow-up was found in those re-initiated on TDF compared to those where non-TDF based ART was used following discharge.

In those who died during their hospital stay, no difference was noted in either SCr values measured prior to death or on renal recovery when comparing the TDF and non-TDF groups. *(Table 2)* Fifty-five percent of deaths occurred in-hospital and they had a higher median serum SCr prior to death compared to the median SCr at discharge in survivors (p<0.001). Furthermore, most patients who died in hospital had severe AKI immediately prior to death (p<0.001).

Sixteen (9.1%) patients were lost to follow-up after discharge. In those who died following discharge, similar mortality rates were found in both groups (p=0.383).

When assessing overall outcome, from admission to 3-month follow-up, the mortality rate in both groups was 27% but the duration from admission to death and discharge to death was significantly shorter in the TDF group *(Table 2)*. Seventy percent (n=33) of those who died were newly diagnosed with TB compared to 42% of survivors (p=0.001). Similarly, elevated CRP and anaemia were more frequently found in patients who died in this cohort.

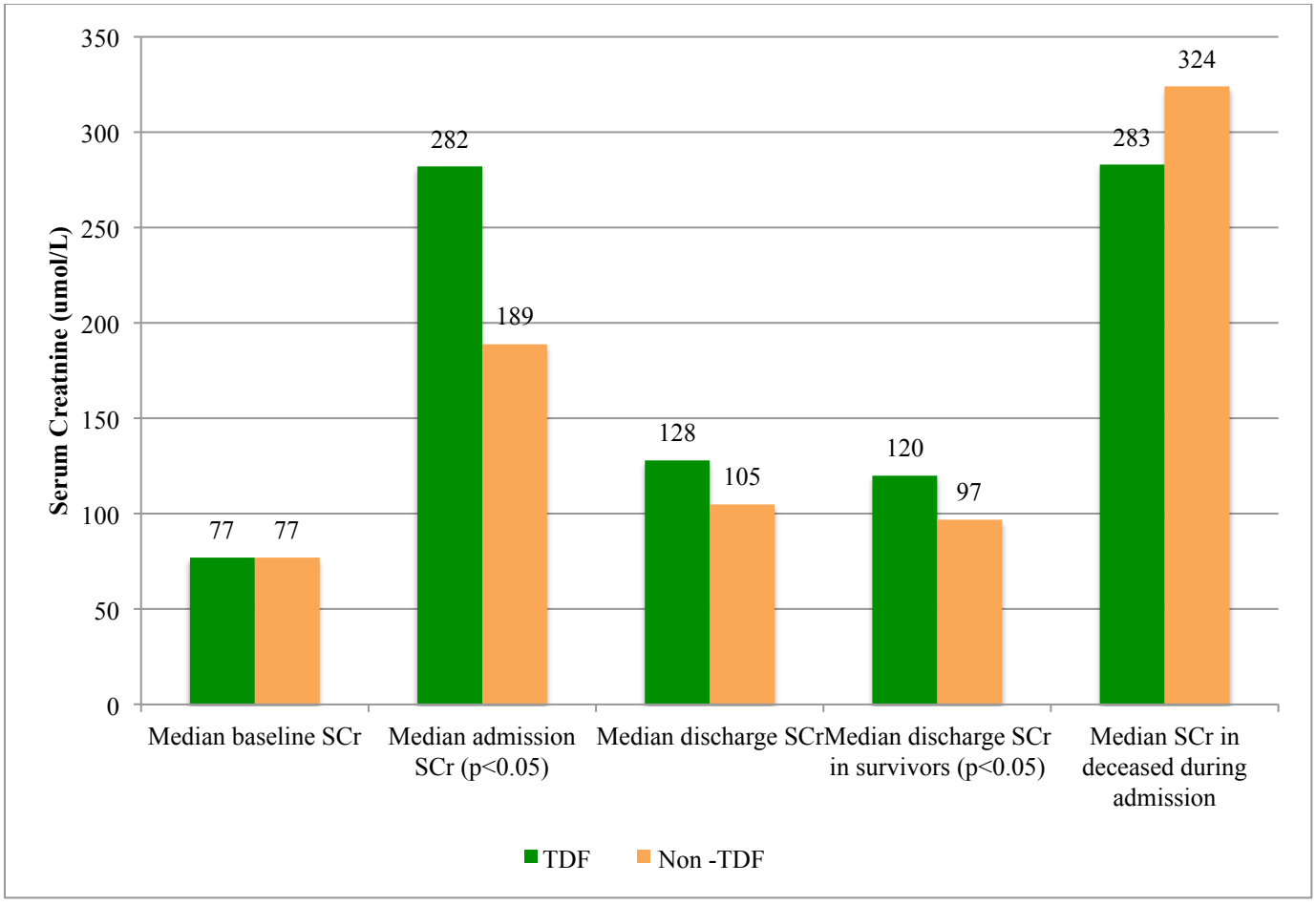

Figure 2: Comparison of median serum creatinine (SCr) values stratified by TDF exposure at various time intervals: Baseline prior to admission; On admission; On discharge; In survivors to discharge and In deaths during hospital stay.

Table 2: Outcomes of mortality, renal recovery and co-morbidities measured at discharge and 3-month follow up startified by TDF exposure (n=175)			
	TDF	**NON TDF**	**p -value**
	93 (53)	82 (47)	
Mortality at discharge			
Deceased [n (%)]	16 (17)	10 (12)	0.353
Fluid therapy alone [n (%)]	88 (95)	80 (98)	0.322
Deceased [n (%)]	13 (15)	9 (11)	0.499
Fluid and Renal replacement therapy [n(%)]	5 (5)	2 (2)	0.322
Deceased [n (%)]	3 (60)	1 (50)	0.809
Duration of stay (days) [median[IQR])			
If Survived	9 (7-15)	9 (7-14)	0.722
If Deceased	10 (6-16)	13 (9-16)	0.286
Mortality after 3-month follow up			
Deceased [n (%)]	25 (27)	22 (27)	0.921
Duration to death (days) (median[IQR])			
Admission to death	30 (13-40)	64 (57-129)	0.049
Discharge to death	24 (18-30)	52 (47-122)	0.016
Renal parameters on discharge			
Renal recovery amongst deceased [n (%)]			
Complete creatinine recovery	3 (20)	1 (10)	0.504
Partial creatinine recovery	7 (47)	5 (50)	0.870
Worsening Creatinine	5 (33)	4 (40)	0.734
Renal recovery amongst survivors [n (%)]			
Complete creatinine recovery	37 (48)	39 (54)	0.456
Partial creatinine recovery	32 (42)	19 (26)	0.051
Creatinine at death by renal recovery (umol/L) [median(IQR)]			
Deceased and complete recovery	79 (78 - 83)	92 (92 - 92)	0.5
Deceased and partial recovery	283 (131 - 368)	271 (139 - 513)	1
Deceased and worsening creatinine	572 (527 - 627)	388 (366 - 577)	0.413
Renal parameters after 3-month follow up			
Renal Recovery amongst survivors [n (%)]			
Complete creatinine recovery	37 (61)	40 (78)	0.043
Partial creatinine recovery	9 (15)	2 (4)	0.055

Discussion

This prospective cohort suggests that HIV-infected patients with AKI who also received TDF ART had more severe AKI and a higher presenting median SCr compared to those on non-TDF ART. Moreover, the renal recovery was slower in those taking TDF. In-hospital outcomes, duration of admission, and overall mortality, however, were similar in both TDF-exposed and unexposed groups. AKI in HIV-infected individuals has a poor prognosis, as more than a quarter of patients in this cohort died.

In our study TDF-exposed HIV-infected patients who develop AKI have similar aetiology and rate and range of nephrotoxic risk factors that we measured, as those not receiving TDF. However, our data suggests that TDF has an added nephrotoxic effect in patients with AKI, causing more rapid worsening of renal function and higher proportions with proteinuria and acidosis, and appears to delay recovery.

The data on TDF use and AKI in Southern Africa is limited; previous studies in ambulatory patients report a frequency of TDF-associated nephrotoxicity of just under 3% with pre-existing renal dysfunction, anaemia and immunosuppression risk factors for nephrotoxicity with TDF use.[55, 56] This study is the first we are aware of that reports AKI, stratified by TDF exposure, in hospitalized HIV positive patients in South Africa, and is particularly relevant due to the massive increase in prescriptions of TDF as part of first line ART in the country.[26]

Renal replacement therapy use was limited in this cohort, despite increasing SCr in over a third of those who died during admission, and despite no formal restrictions or eligibility criteria for the initiation of RRT in this setting. We postulate that RRT may have prevented some deaths. Moreover, the substantial mortality following discharge from hospital suggests that more intensive monitoring and follow-up after discharge

from hospital is necessary in all patients with AKI. Further studies are required to assess the likely causes of, and contributors to death, and to better identify potential interventions to prevent mortality. Consistent with our findings, international studies have reported the duration of TDF exposure prior to onset of decline in renal function to be 6 - 9 months [41, 54], although studies of ambulatory patients in South Africa report a shorter median time to nephrotoxicity (3.6 months).[55, 56] Current recommendations suggest that TDF dose adjustment is necessary in those with renal injury whilst on TDF.[65] Although published literature reports male gender and lower body weight as risk factors associated with AKI [17, 41, 42, 54, 56], the high proportion of women with AKI that we report is likely due to higher seroprevelance of HIV amongst South African women and national policy to initiate ART in all HIV-positive pregnant women irrespective of CD4 count.[26, 66] The apparent role of TB, which was frequently diagnosed in this cohort, in contributing to both morbidity and mortality following discharge, is substantial. The association between AKI and TB is described in a retrospective cohort from Johannesburg, in whom 26% of HIV positive patients with AKI were diagnosed with TB, almost half what we report.[14] However, over half of the cases of TB we report were not laboratory confirmed.[67] 53% of our cohort had laboratory findings suggestive of interstitial nephritis, which could be at least partially attributed to TB and/or NSAID use, although this finding is limited by the absence of renal biopsies in this cohort.[68, 69] Whilst the immune re-constitution syndrome (IRIS) is a known cause of an interstitial nephritis, the duration of ART exposure in the majority of our patients was for a period longer than that associated with the onset of IRIS; moreover, some patients were ART naive.[69, 70]

Limitations

This study has several limitations. We restricted our sample to patients admitted to a single regional hospital in South Africa and we are unable to report the overall rate of AKI in TDF and other ART recipients. Furthermore, the incidence or prevalence of AKI relative to all HIV positive admissions was not measured, nor is there reliable data on which to base an estimate of incidence or prevalence. Tubular dysfunction using urine beta-2 microglobulin and fractional excretion of phosphate was not measured and, in some patients, not every study parameter was measured but all had sufficient measured data to be eligible for inclusion. Baseline SCr values were not available in all patients and missing baseline values were inferred using the MDRD formula (calculated using an eGFR of 75ml/min/ 1.73m^2); this may have biased the classification of AKI severity in both the TDF, but particularly in the non – TDF group where baseline SCr levels were less readily available as ART-naïve patients infrequently had a measured SCr prior to admission or on follow-up.[60, 71] Furthermore, no renal biopsies were performed in this cohort, particularly in those with partial renal recovery, however in most instances the aetiology of AKI was clinically evident.

Conclusion

Comparison of the two groups suggests that risks factors, aetiology and mortality of AKI in HIV infected in-patients, irrespective of TDF use, is similar but that co - existing TDF use appears to result in more severe and longer lasting AKI. Our findings suggest that a lower threshold for RRT may be needed in those with limited or no SCr recovery and intensive follow after discharge from hospital may reduce mortality in all HIV-infected AKI patients, and especially in those with concomitant TB.

References – (Manuscript)

1. Wyatt CM, Arons RR, Klotman PE, Klotman ME. Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. *AIDS* 2006,**20**:561-565.
2. Cohen SD, Chawla LS, Kimmel PL. Acute kidney injury in patients with human immunodeficiency virus infection. *Curr Opin Crit Care* 2008,**14**:647-653.
3. Kalim S, Szczech LA, Wyatt CM. Acute kidney injury in HIV-infected patients. *Semin Nephrol* 2008,**28**:556-562.
4. Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG. Long-term clinical consequences of acute kidney injury in the HIV-infected. *Kidney Int* 2010,**78**:478-485.
5. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. *BMC Public Health* 2012,**12**:234.
6. Franceschini N, Napravnik S, Eron JJ, Jr., Szczech LA, Finn WF. Incidence and etiology of acute renal failure among ambulatory HIV-infected patients. *Kidney Int* 2005,**67**:1526-1531.
7. Roe J, Campbell LJ, Ibrahim F, Hendry BM, Post FA. HIV care and the incidence of acute renal failure. *Clin Infect Dis* 2008,**47**:242-249.
8. Gerntholtz TE, Goetsch SJ, Katz I. HIV-related nephropathy: a South African perspective. *Kidney Int* 2006,**69**:1885-1891.
9. Roling J, Schmid H, Fischereder M, Draenert R, Goebel FD. HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. *Clin Infect Dis* 2006,**42**:1488-1495.

10. Perazella MA. Acute renal failure in HIV-infected patients: a brief review of common causes. *Am J Med Sci* 2000,**319**:385-391.

11. Lopes JA, Melo MJ, Viegas A, Raimundo M, Camara I, Antunes F*, et al.* Acute kidney injury in hospitalized HIV-infected patients: a cohort analysis. *Nephrol Dial Transplant* 2011,**26**:3888-3894.

12. Naicker S, Aboud O, Gharbi MB. Epidemiology of acute kidney injury in Africa. *Semin Nephrol* 2008,**28**:348-353.

13. Fabian J, Naicker S. HIV and kidney disease in sub-Saharan Africa. *Nat Rev Nephrol* 2009,**5**:591-598.

14. Vachiat AI, Musenge E, Wadee S, Naicker S. Renal failure in HIV-positive patients—a South African experience. *Clin Kidney J* 2013,**0**:1-6.

15. Arendse CG, Wearne N, Okpechi IG, Swanepoel CR. The acute, the chronic and the news of HIV-related renal disease in Africa. *Kidney Int* 2010,**78**:239-245.

16. Fine DM, Perazella MA, Lucas GM, Atta MG. Renal disease in patients with HIV infection: epidemiology, pathogenesis and management. *Drugs* 2008,**68**:963-980.

17. Franey C, Knott D, Barnighausen T, Dedicoat M, Adam A, Lessells RJ*, et al.* Renal impairment in a rural African antiretroviral programme. *BMC Infect Dis* 2009,**9**:143.

18. Lopes JA, Fernandes J, Jorge S, Neves J, Antunes F, Prata MM. Acute renal failure in critically ill HIV-infected patients. *Crit Care* 2007,**11**:404.

19. Lopes JA, Melo MJ, Raimundo M, Fragoso A, Antunes F. Long-term risk of mortality for acute kidney injury in HIV-infected patients: a cohort analysis. *BMC Nephrol* 2013,**14**:32.

20. Miro JM, Cofan F, Trullas JC, Manzardo C, Cervera C, Tuset M*, et al.* Renal dysfunction in the setting of HIV/AIDS. *Curr HIV/AIDS Rep* 2012,**9**:187-199.

21. Franceschini N, Napravnik S, Finn WF, Szczech LA, Eron JJ, Jr. Immunosuppression, hepatitis C infection, and acute renal failure in HIV-infected patients. *J Acquir Immune Defic Syndr* 2006,**42**:368-372.

22. Peraldi MN, Maslo C, Akposso K, Mougenot B, Rondeau E, Sraer JD. Acute renal failure in the course of HIV infection: a single-institution retrospective study of ninety-two patients anad sixty renal biopsies. *Nephrol Dial Transplant* 1999,**14**:1578-1585.

23. Wikman P, Safont P, Del Palacio M, Moreno A, Moreno S, Casado JL. The significance of antiretroviral-associated acute kidney injury in a cohort of ambulatory human immunodeficiency virus-infected patients. *Nephrol Dial Transplant* 2013,**28**:2073-2081.

24. DeChristoforo R, Penzak SR. Tenofovir: a nucleotide analogue reverse-transcriptase inhibitor for treatment of HIV infection. *Am J Health Syst Pharm* 2004,**61**:86-98; quiz 99-100.

25. The South African antiretroviral treatment guidelines. In: Department of Health; 2010. pp. 1-7.

26. The South African antiretroviral treatment guidelines. In: Department of Health; 2013. pp. 1-10.

27. Schooley RT, Ruane P, Myers RA, Beall G, Lampiris H, Berger D*, et al.* Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *Aids* 2002,**16**:1257-1263.

28. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V, *et al.* Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. *Nephrol Dial Transplant* 2005,**20**:743-746.

29. Barditch-Crovo P, Deeks SG, Collier A, Safrin S, Coakley DF, Miller M, *et al.* Phase i/ii trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother* 2001,**45**:2733-2739.

30. Karras A, Lafaurie M, Furco A, Bourgarit A, Droz D, Sereni D, *et al.* Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clin Infect Dis* 2003,**36**:1070-1073.

31. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K. Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. *Clin Infect Dis* 2003,**37**:e41-43.

32. Barrios A, Garcia-Benayas T, Gonzalez-Lahoz J, Soriano V. Tenofovir-related nephrotoxicity in HIV-infected patients. *AIDS* 2004,**18**:960-963.

33. Malik A, Abraham P, Malik N. Acute renal failure and Fanconi syndrome in an AIDS patient on tenofovir treatment--case report and review of literature. *J Infect* 2005,**51**:E61-65.

34. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM, *et al.* Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis* 2002,**40**:1331-1333.

35. Coca S, Perazella MA. Rapid communication: acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. *Am J Med Sci* 2002,**324**:342-344.

36. Rifkin BS, Perazella MA. Tenofovir-associated nephrotoxicity: Fanconi syndrome and renal failure. *Am J Med* 2004,**117**:282-284.

37. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, Mauboussin JM*, et al.* Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 2004,**35**:269-273.

38. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D*, et al.* Tenofovir-associated renal and bone toxicity. *HIV Med* 2009,**10**:482-487.

39. Creput C, Gonzalez-Canali G, Hill G, Piketty C, Kazatchkine M, Nochy D. Renal lesions in HIV-1-positive patient treated with tenofovir. *Aids* 2003,**17**:935-937.

40. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010,**51**:496-505.

41. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B*, et al.* The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *Aids* 2007,**21**:1273-1281.

42. Young B, Buchacz K, Baker RK, Moorman AC, Wood KC, Chmiel J*, et al.* Renal function in Tenofovir-exposed and Tenofovir-unexposed patients receiving highly active antiretroviral therapy in the HIV Outpatient Study. *J Int Assoc Physicians AIDS Care (Chic)* 2007,**6**:178-187.

43. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J*, et al.* Tenofovir nephrotoxicity: 2011 update. *AIDS Res Treat* 2011,**2011**:354908.

44. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. *Am J Kidney Dis* 2011,**57**:773-780.

45. Perazella MA. Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy. *Kidney Int* 2010,**78**:1060-1063.

46. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J*, et al.* Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *Aids* 2009,**23**:689-696.

47. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L*, et al.* Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. *J Acquir Immune Defic Syndr* 2010,**53**:62-69.

48. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. *Am J Kidney Dis* 2003,**41**:292-309.

49. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R*, et al.* Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest* 2009,**89**:513-519.

50. Izzedine H, Hulot JS, Villard E, Goyenvalle C, Dominguez S, Ghosn J*, et al.* Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis* 2006,**194**:1481-1491.

51. Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J*, et al.* Predictors of kidney tubular dysfunction in HIV-infected patients

treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 2009,**48**:e108-116.

52. Bickel M, Khaykin P, Stephan C, Schmidt K, Buettner M, Amann K*, et al.* Acute kidney injury caused by tenofovir disoproxil fumarate and diclofenac co-administration. *HIV Med* 2013,**14**:633-638.

53. Antoniou T, Raboud J, Chirhin S, Yoong D, Govan V, Gough K*, et al.* Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. *HIV Med* 2005,**6**:284-290.

54. Madeddu G, Bonfanti P, De Socio GV, Carradori S, Grosso C, Marconi P*, et al.* Tenofovir renal safety in HIV-infected patients: results from the SCOLTA Project. *Biomed Pharmacother* 2008,**62**:6-11.

55. Brennan A, Evans D, Maskew M, Naicker S, Ive P, Sanne I*, et al.* Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. *AIDS* 2011,**25**:1603-1609.

56. Kamkuemah M, Kaplan R, Bekker LG, Little F, Myer L. Renal impairment in HIV-infected patients initiating tenofovir-containing antiretroviral therapy regimens in a Primary Healthcare Setting in South Africa. *Trop Med Int Health* 2015,**20**:518-526.

57. Winston A, Amin J, Mallon P, Marriott D, Carr A, Cooper DA*, et al.* Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy. *HIV Med* 2006,**7**:105-111.

58. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter., Suppl.* 2012,**2**:1-138.

59. Siew ED, Ikizler TA, Matheny ME, Shi Y, Schildcrout JS, Danciu I*, et al.* Estimating baseline kidney function in hospitalized patients with impaired kidney function. *Clin J Am Soc Nephrol* 2012,**7**:712-719.

60. Wald R. Predicting baseline creatinine in hospitalized patients. *Clin J Am Soc Nephrol* 2012,**7**:697-699.

61. Okusa MD, Davenport A. Reading between the (guide)lines--the KDIGO practice guideline on acute kidney injury in the individual patient. *Kidney Int* 2014,**85**:39-48.

62. Gaiao S, Cruz DN. Baseline creatinine to define acute kidney injury: is there any consensus? *Nephrol Dial Transplant* 2010,**25**:3812-3814.

63. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004,**8**:R204-212.

64. Kashani K, Kellum JA. Novel biomarkers indicating repair or progression after acute kidney injury. *Curr Opin Nephrol Hypertens* 2015,**24**:21-27.

65. Jafari A, Khalili H, Dashti-Khavidaki S. Tenofovir-induced nephrotoxicity: incidence, mechanism, risk factors, prognosis and proposed agents for prevention. *Eur J Clin Pharmacol* 2014,**70**:1029-1040.

66. Shisana O, Rehle T, Simbayi LC, K. Z, Jooste S, Zungu N*, et al.* South African National HIV Prevalence, Incidence and Behaviour Survery, 2012. *Cape Town: HSRC Press* 2014:1 - 198.

67. Hoffmann CJ, Hoffmann JD, Kensler C, van der Watt M, Omar T, Chaisson RE*, et al.* Tuberculosis and hepatic steatosis are prevalent liver pathology

findings among HIV-infected patients in South Africa. *PLoS One* 2015,**10**:e0117813.

68. Parkhie SM, Fine DM, Lucas GM, Atta MG. Characteristics of patients with HIV and biopsy-proven acute interstitial nephritis. *Clin J Am Soc Nephrol* 2010,**5**:798-804.

69. Zaidan M, Lescure FX, Brocheriou I, Dettwiler S, Guiard-Schmid JB, Pacanowski J*, et al.* Tubulointerstitial nephropathies in HIV-infected patients over the past 15 years: a clinico-pathological study. *Clin J Am Soc Nephrol* 2013,**8**:930-938.

70. Leone S, Nicastri E, Giglio S, Narciso P, Ippolito G, Acone N. Immune reconstitution inflammatory syndrome associated with Mycobacterium tuberculosis infection: a systematic review. *Int J Infect Dis* 2010,**14**:e283-291.

71. Siew ED, Peterson JF, Eden SK, Moons KG, Ikizler TA, Matheny ME. Use of multiple imputation method to improve estimation of missing baseline serum creatinine in acute kidney injury research. *Clin J Am Soc Nephrol* 2013,**8**:10-18.

Chapter 3: Appendix

i. Data collection sheet

BASELINE DATA

PID

DATE OF RECRUITMENT

DATE OF BIRTH

AGE

GENDER – Male/Female

ETHNICITY

Black

Coloured

White

Indian

Other

Date of Admission

Date of Discharge

FOR CURRENT AND PREVIOUS ARV STATUS

Is the patient on ARVs now or has the patient ever taken ARVs before this admission?

IF YES -> PLEASE GIVE INFORMATION OF ALL ARVS PATIENT HAS

TAKEN BOTH NOW AND PREVIOUSLY

CODING

FDC – 1, TDF – 2, D4T – 3, AZT – 4, ABC – 5 , DDI – 6, 3TC – 7, EFV – 8,

NVP – 9, ALLUVIA – 10

CODE	DATE STARTED	DATE STOPPED	REASON FOR STOPPING		
	DD/MM/YYYY	DD/MM/YYYY	SIDE EFFECT	DRUG RESISTANCE	DEFAULT

TB AND OTHER OPPORTUNISTIC INFECTIONS

1. Has the patient been treated for TB before this admission? - Y/N

IF NO -> STOP

IF YES - > DATE OF TREATMENT STARTED

- DATE 1 – MM/YYYY
- DATE 2 – MM/YYYY

2. Is the patient on TB treatment that was started before this admission? – Y/N

IF NO -> STOP

IF YES – a) *What was the date of treatment started?*

- Date

b) *Were was the TB Diagnosed?*

CHOOSE ONE:

- Local Clinic
- Hospital

c) How was the TB Diagnosed?

- Empiric
- Sonar
- Sputum
- Chest X – Ray
- Other

3. Is the patient being tested for TB on this admission? – Y/N

IF NO -> STOP

IF YES Were the following tests done?

– a) Sputum GXP Done? – Y/N

Barcode

GXP Result – Pos/Neg

If Positive – Rif Sens / Rif Resis

- b) LPA Done? Y/N
- Barcode
- LPA Result – Pos/Neg
- If Positive – Rif Sens/Rif Resis
- If Positive – INH Sens/INH Resis
- c) TB Culture Done?
- Barcode
- TB Culture Result – MTB Pos / MTB Neg

4. Does the patient currently have cryptococcal meningitis? – Y/N

- IF YES – Date of diagnosis – DD/MM/YYYY

5. Has the Patient previously had cryptococcal meningitis? – Y/N

- IF YES – Date of diagnosis – MM/YYYY

CO – MORBIDITIES

Does the patient have any co-morbidities? – YES/NO

YES – *Select from List*

- Hypertension
- Diabetes
- Asthma
- Epilepsy
- Chronic Kidney Disease
- Cardiac Disease
- Other

For Each Co – Morbidity Selected as Yes

- What is the duration of the co – morbidity?
- Wks / mths/ yrs

LIST ANY KNOWN MEDICATIONS TAKEN WITHIN ONE MONTH PRIOR TO ADMISSION

Medication 1 – Name

ETC

GIT HISTORY

1. Is the patient vomiting? – Y/N

YES – *For How long is the patient vomiting - DURATION IN DAYS*

2. Does the patient have diarrhea? – Y/N

YES – *For How Long does the patient have diarrhea – DURATION IN DAYS*

- *How many Stools per day?* - patient to give number
- *Is it watery? Is there Blood?*

- SELECT
    - Watery
    - Blood
    - Both
- *Has a stool MC/S been sent*
- YES – Barcode

IMPORTANT EXPOSURES

1. Has the patient taken traditional medications in the last two months? – Y/N

IF YES – What is the name of the traditional medication?

2. Has the patient worked in a mine? – YES/NO

YES – *What type of mining (gold, coal, uranium)?*

For how long did the patient work in the mine?

3. Does the patient use Non –Steroidal Inflammatories? – Y/N

Eg: Aspirin, Disprin, Grandpa, Brufen, Feldene, Myprodol

IF YES – HAS THE PATIENT USED NSAIDS IN THE LAST MONTH BEFORE ADMISSION – Y/N

QUANTIFY – More Than 5 Doses per Week OR Less Than 5 Doses per Week

OUTCOMES

1. How was the Patient treated?

SELECT

- Ward – Fluids
- Dialysis

2. What was the patient outcome?

SELECT

- Recovered = UE Normal/Improving on Discharge

- Dialysis
- Death

3. Has the patient been started on TB treatment on this admission? – Y/N

IF YES – *What is the reason for TB Treatment?*

SELECT

1) Empiric
2) Sonar
3) Sputum – Barcode
4) Chest X – Ray
5) Other

4. Discharge diagnosis

List Discharge diagnosis from Discharge Summary

Particular Attention to Cause of Renal Failure to be stated

5. Discharge ARVs

CODING

FDC – 1, TDF – 2, D4T – 3, AZT – 4, ABC – 5 , DDI – 6, 3TC – 7, EFV – 8,

NVP – 9, ALLUVIA – 10

CODE	**DATE STARTED**
	DD/MM/YYYY

LABORATORY AND RADIOLOGICAL INVESTIGATIONS

	ADMISSION	**DATE**	**BARCODE**	**DISCHARGE**	**DATE**	**BARCODE**
HIV BLOODS						
CD4 – cells/mm3						
VIRAL LOAD (WITHIN THE LAST 3 MONTHS) – copies/ml						
RENAL IX						
WEIGHT (KG)						
HEIGHT (M)						
NA						
K						
HCO3						
CL						
UREA						
CREATININE						
eGFR						
WCC						
HB						
PLATLETS						
CRP						
CALCIUM						
MAGNESIUM						
PHOSPHATE						
ALBUMIN						
FERRITIN						
HEPATITIS B	SAg Pos / Neg					
URINE						
DIPSTICKS	GLUCOSE					
BEDSIDE HGT						
UPCR						
U PHOSPHATE	U AMINO ACIDS					
SONAR						
KIDNEYS						
RIGHT KIDNEY SIZE						
LEFT KIDNEY SIZE						
HYDRONEPHROSIS	YES	NO				
HYPERECHOIEC	YES	NO				
ABDOMEN						
ASCITES	YES	NO				
PARAAROTIC LYMPHNODES	YES	NO				
MICROSPLENIC ABSCESSES	YES	NO				

XRAY

Is a Chest Xray Done?

YES

SELECT ONE OF THE FOLLOWING

- Cavities
- Infiltrates
- Pleural Effusion
- Pulmonary Oedema
- Normal

X – RAY IMAGE UPLOADED

YES - > Image

2 WEEK / 1 MONTH FOLLOW UP – DATA SHEET NUMBER:

		DATE	**BARCODE**
WEIGHT (KG)			
NA			
K			
HCO3			
CL			
UREA			
CREATININE			
eGFR			

TREATMENT	
ARVS	TDF / AZT / ABC / D4T 3TC EFV / NVP / ALLUVIA
TB TREATMENT	
OTHER INTERCURRENT ILLNESS **TREATMENT** EG: CRYPTO HT/DM/EPILEPSY ETC	

2-MONTH FOLLOW UP – DATA SHEET NUMBER:

		DATE	BARCODE
WEIGHT (KG)			
NA			
K			
HCO3			
CL			
UREA			
CREATININE			
eGFR			

TREATMENT	
ARVS	TDF / AZT / ABC / D4T 3TC EFV / NVP / ALLUVIA
TB TREATMENT	
OTHER INTERCURRENT ILLNESS **TREATMENT** EG: TB, CRYPTO HT/DM/EPILEPSY ETC	

3-MONTH FOLLOW UP – DATA SHEET NUMBER:

		DATE	BARCODE
WEIGHT (KG)			
NA			
K			
HCO3			
CL			
UREA			
CREATININE			
eGFR			
CALCIUM			
MAGNESIUM			
PHOSPHATE			
ALBUMIN			
UPCR			
U PHOSPHATE			

TREATMENT	
ARVS	TDF / AZT / ABC / D4T 3TC EFV / NVP / ALLUVIA
TB TREATMENT	
OTHER INTERCURRENT ILLNESS TREATMENT EG: TB, CRYPTO HT/DM/EPILEPSY ETC	

ii. Ethics Clearance Certificate

M140742

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140742

NAME: (Principal Investigator)	Dr Faheem Seedat
DEPARTMENT:	Department of Internal Medicine CHBAH/Tshepong Hospital
PROJECT TITLE:	Renal Impairment in HIV Infected Patients Receiving Tenofovir-Based Antiretroviral Therapy in a South African Hospital
DATE CONSIDERED:	25/07/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Neil Martinson
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/07/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

M140742Date 20/8/14

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

iii. Plagiarism Report

The plagiarism software Turn it in was used to review this dissertation. A similarity index of 9% was reported. This relates to the use of standardised definitions; furthermore, all other similarities have been appropriately referenced.

Department of Internal Medicine

The Chair

Postgraduate Studiess Committee

Faculty of Health Sciences

Universtity of Witwatersrand

Re: Turn – it – in report: Dr Faheem Seedat MMed: "Renal Impairment in HIV infected patients receiving tenofovir-based Antiretroviral Therapy in a South African Hospital"

I have reviewed the Turn – it – in report of DrF. Seedat'sMMed dissertation. The report identifies a similarity index of 9%. Much of this relates to the use of standardised definitions; furthermore, the other information that bears a similarityhas been appropriately referenced

Yours sincerely,

Professor E VariavaDr N Martinson
Head of department
Internal Medicine
Klerksdorp/Tshepong Hospital Complex
Supervisor

Executive Director
Perinatal HIV Research Unit

Supervisor

iv. Published Manuscript

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 33, Number 1, 2017
Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2016.0098

Acute Kidney Injury, Risk Factors, and Prognosis in Hospitalized HIV-Infected Adults in South Africa, Compared by Tenofovir Exposure

Faheem Seedat,[1] Neil Martinson,[2,3] Katlego Motlhaoleng,[2] Pattamukkil Abraham,[2] Dalu Mancama,[4] Saraladevi Naicker,[1] and Ebrahim Variava[1]

Abstract

There are limited data describing acute kidney injury (AKI) in HIV-infected adult patients in resource-limited settings where tenofovir disoproxil fumarate (TDF), which is potentially nephrotoxic, is increasingly prescribed. We describe risk factors for and prognosis of AKI in HIV-infected individuals, stratified by those receiving and those naive to TDF. A prospective case cohort study of hospitalized HIV-infected adults with AKI stratified by TDF exposure. Adults (≥18 years) were recruited: clinical and biochemical data were collected at admission; their renal recovery, discharge, or mortality was ascertained as an in-patient and, subsequently, to a scheduled 3-month follow-up. Among this predominantly female (61%), almost exclusively black African cohort of 175 patients with AKI, 93 (53%) were TDF exposed; median age was 41 years (interquartile range 35–50). Median CD4 count and viral load and creatinine at baseline were 116 cells/mm^3 and 110,159 copies/ml, respectively. A greater proportion of the TDF group had severe AKI on admission (61% vs. 43%, $p = .014$); however, both groups had similar rates of newly diagnosed tuberculosis (TB; 52%) and nonsteroidal anti-inflammatory drug (NSAID; 32%) use. Intravenous fluid was the therapeutic mainstay; only seven were dialyzed. Discharge median serum creatinine (SCr) was higher in the TDF group ($p = .032$) and fewer in the TDF group recovered renal function after 3 months ($p = .043$). Three-month mortality was 27% in both groups, but 55% of deaths occurred in hospital. Those that died had a higher SCr and more severe AKI than survivors; TB was diagnosed in 33 (70%) of those who died. AKI was more severe and renal recovery slower in the TDF group; comorbidities, risk factors, and prognosis were similar regardless of TDF exposure. Because TB is linked to higher mortality, TB coinfection in HIV-infected patients with AKI warrants more intensive monitoring. In all those with poor renal recovery, our data suggest that a lower threshold for dialysis is needed.

Keywords: acute kidney injury, renal insufficiency, HIV, tenofovir, South Africa, mortality

Background

Acute kidney injury (AKI) is a major cause of morbidity and mortality in HIV-infected individuals.[1,2] Mortality in hospitalized HIV-infected adults is higher in those with AKI, while in adults living with HIV the risk of kidney disease is almost fourfold that of HIV-uninfected adults.[3,4] Antiretroviral therapy (ART) mitigates this risk.[1]

Numerous studies from developed settings have reported AKI in HIV-infected individuals, suggesting that the etiology of AKI is multifactorial. Moreover, both HIV and ART are directly nephrotoxic.[1,3–6] Prerenal AKI from renal hypoperfusion (commonly from gastroenteritis), sepsis-induced acute tubular necrosis (ATN), and concomitant nephrotoxic drug use are common causes of AKI in HIV-infected adults.[2,5,7,8] Well-described risk factors for AKI apply to both HIV-seronegative and seropositive individuals.[1,3–7,9,10]

[1]Department of Internal Medicine, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.
[2]Perinatal HIV Research Unit (PHRU), MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, University of the Witwatersrand, Johannesburg, South Africa.
[3]Johns Hopkins University Centre for TB Research, Baltimore, Maryland.
[4]Council for Scientific and Industrial Research, Pretoria, South Africa.

However, specific risk factors in HIV-seropositive individuals include: male sex; advanced immunosuppression; long-term ART use; and preexisting renal disease.[3–7,9–11]

In October 2001, the Food and Drug Administration approved tenofovir disoproxil fumarate (TDF) as the first nucleotide reverse-transcriptase inhibitor (NRTI) for the treatment of HIV.[12] Since April 2010, TDF has been the mainstay of first line ART in South Africa: massively increasing the number of South Africans receiving TDF.[13] Although not initially reported in the early clinical trials, subsequent case reports, case series, and observational studies reported TDF-associated nephrotoxicity and, in 2010, a systematic review and meta-analysis reported a significant decline in renal function associated with TDF use.[14–19]

The mechanism of action appears to be the accumulation of TDF in proximal tubular cells (due primarily to genetic polymorphisms encoding defective transmembrane drug transporters), with resultant mitochondrial toxicity and subsequent renal injury, clinically presenting as the Fanconi syndrome or ATN with AKI.[16,20–22] The reported prevalence of TDF-related nephrotoxicity is 2.4% occurring at 6–9 months following TDF initiation, although South African studies suggest an earlier onset.[11,17–19,22–25]

We report the presentation, severity, and risk factors for AKI in HIV-positive South African patients and assess the response to treatment and short-term outcomes. Furthermore, to assess the role of TDF in HIV-positive patients with AKI, analysis was stratified by TDF exposure.

Materials and Methods

This prospective case cohort study recruited patients from the Klerksdorp/Tshepong Hospital Complex, a secondary level public hospital in South Africa's North West province, from October 2014 to June 2015. We enrolled HIV-infected patients admitted to the adult medicine wards with AKI and compared those exposed to TDF to those who had not received TDF and followed them up after discharge. Eligible patients were: hospitalized, HIV-infected adults, and ≥18 years of age with a diagnosis of AKI. We excluded patients with prior or preexisting kidney disease (either self-reported or as indicated by medical records or laboratory results). Those who appeared unwilling or incapable of attending follow-up and those whose clinical records were inadequate to assess renal function or HIV treatments before the study were also excluded. The University of Witwatersrand Human Research Ethics Committee approved this study.

Study definitions and measures

Patients were categorized as TDF exposed if they were on TDF-based ART at the time of admission or had defaulted TDF for <1 week before admission. The TDF unexposed group included individuals who: were ART naive, were taking non-TDF-based ART, had defaulted non-TDF based ART for any duration, and those who had defaulted TDF based ART for at least 8 weeks before the onset of AKI.

In all study patients, AKI was diagnosed and staged according to the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice AKI Guideline.[26] Baseline serum creatinine (SCr) before AKI was determined either by a SCr taken before, but within the preceding year of admission, or the lowest normal SCr at discharge or on follow-up.[27,28] If no baseline SCr was available, an estimated baseline SCr was calculated based on a Modification of Diet in Renal Disease (MDRD) equation estimated glomerular filtration rate (eGFR) of 75 ml/min per 1.73 m^2.[26,29,30]

Renal recovery was defined as a decreasing SCr over time and no need for continuing renal replacement therapy (RRT) and was categorized as complete (SCr recovery below 1.5 times the baseline) or partial (SCr recovery above 1.5 times the baseline).[31,32] New admissions to the acute care adult medical ward were screened for eligibility on weekdays. If eligible, written informed consent was obtained and a structured interview administered assessing: ART use, previous tuberculosis (TB) history, comorbidities, concomitant gastrointestinal symptoms, drug history, and other important exposures; necessary blood and urine samples were taken, results of radiological investigations recorded, and relevant information from clinical records noted.

On discharge, a summary of the clinical course of the patient was abstracted from the medical records. Patients were then actively followed up at a visit scheduled 3 months after discharge.

Statistical analyses

Patient demographic and clinical characteristics are reported as medians and interquartile ranges (IQRs) for continuous variables and proportions with 95% confidence intervals (95% CIs) for categorical variables. These were compared between groups using the Mann–Whitney, Kruskal–Wallis, and Chi-square tests as appropriate. Univariable logistical regression was used to control for TDF use and the presence of TB coinfection. Multivariable regression was not performed due to the small sample size and some missing data. All statistical analyses were performed using STATISTICA version 12 (StatSoft).

Results

From 1 October 2014 to 30 June 2015, 199 HIV-infected patients with presumed AKI were enrolled in this study; 24 were subsequently excluded as they either had prior kidney disease (16) or had defaulted TDF based ART for <8 weeks (8). Of the remaining 175, their median age was 41 years (IQR 35–50); 61% were women; virtually all (99%) were black Africans; and their median body mass index was 21 kg/m^2 (IQR 19–24) on admission (Table 1). Ninety-three (53%) were TDF exposed for a median treatment duration of 30 weeks (IQR 8–61) of whom 27 (29%) had prior ART exposure before switching to a TDF containing regimen. The majority of the TDF group (94%) received a concomitant non-NRTI (NNRTI) and 6% a protease inhibitor. Thirty-one patients in the non-TDF group were receiving ART for a median duration of 306 weeks (IQR 88–729); 62% of the non-TDF group was ART-naive on admission.

AKI severity and measures of kidney disease

Half (53%) of all participants had severe AKI, most were in the TDF group (62%; $n=57$), whereas mild AKI was predominant in the non-TDF group (62%; $n=23$) (Fig. 1). The TDF group had: a higher median admission SCr (282 μmol/l; IQR 172–542 vs. 189 μmol/l; IQR 146–343; $p=.007$); higher median urine protein creatinine ratio (0.212 g/mmol creat; IQR 0.111–0.316 vs. 0.128 g/mmol creat; IQR 0.087–0.212; $p=.003$); and a lower median eGFR (CKD-EPI) (14.2 ml/min;

Table 1. Baseline Demographic Characteristics, Comorbidities, Risk Factors, and Laboratory Characteristics in Acute Kidney Injury Patients at Admission Stratified by Tenofovir Disoproxil Fumarate Exposure ($N=175$)

	TDF	*Non-TDF*	p
	93 (53)	82 (47)	
Demographics			
Age (years), median (IQR)	42 (35–53)	40 (35–49)	.260
Female, *n* (%)	62 (67)	45 (55)	.110
Black, *n* (%)	91 (98)	83 (100)	.182
Serum electrolytes			
Potassium (mmol/l), median (IQR)	4.1 (3.3–5.1)	4.3 (3.5–5.2)	.348
Bicarbonate (μmol/l), median (IQR)	14 (10–18)	16 (13–19)	.047
Phosphate (mmol/l), median (IQR)	1.2 (0.8–1.7)	1.3 (0.9–1.6)	.496
Renal parameters			
Admission creatinine (μmol/l), median (IQR)	282 (172–542)	189 (146–343)	.007
Admission eGFR-CKD-EPI (ml/min per 1.73 m^2), median (IQR)	14.2 (4.3–28)	25.6 (10.4–39.5)	.002
Baseline creatinine (μmol/l), median (IQR)	77 (65–88)	77 (62–92)	.920
Urine protein:creatinine ratio (g/mmol creat), median (IQR)	0.212 (0.111–0.316)	0.128 (0.087–0.212)	.003
Normoglycemic glycosuria, *n* (%)	7 (11)	6 (10)	.863
Hyperechoic, *n* (%)	71 (91)	48 (84)	.226
Right kidney size (cm), median (IQR)	11.25 (10.3–12.2)	11 (10.2–11.8)	.347
Left kidney size (cm), median (IQR)	11.4 (10.7–12.3)	11 (10.4–11.8)	.135
Comorbidities			
Hypertension, *n* (%)	22 (24)	16 (20)	.507
Diabetes, *n* (%)	8 (9)	3 (4)	.179
Hepatitis B positive, *n* (%)	11 (12)	9 (11)	.86
Risk factors			
TB, *n* (%)			
Completed TB treatment before admission	35 (38)	34 (41)	.605
Newly diagnosed TB	46 (50)	45 (55)	.474
Sputum or culture proven TB	22 (48)	22 (49)	.919
Pneumonia, *n* (%)	9 (10)	12 (17)	.148
Sepsis, *n* (%)	4 (4)	7 (9)	.249
Vomiting, *n* (%)	32 (34)	14 (17)	.009
Diarrhea, *n* (%)	36 (39)	17 (21)	.008
NSAID use, *n* (%)	26 (28)	30 (37)	.784
HIV-related and infective parameters			
CD4 count (cells/mm^3), median (IQR)	147 (57–304)	87 (41–210)	.067
HIV viral load (copies/ml)			
Virally suppressed (LDL or <1,000), *n* (%)	56 (60)	14 (17)	<.001
Median HIV viral load in those >1,000, median (IQR)	39,456 (4,537–326,300)	414,365 (110,159–1,521,015)	<.001
Hemoglobin (g/dl), median (IQR)	9.6 (7.8–11.3)	9.8 (7.4–12)	.622
C-reactive protein (mg/l), median (IQR)	127 (39–189)	89 (37–180)	.386
Albumin (g/l), median (IQR)	20 (16–24)	18 (15–23)	.122
Ferritin (μg/l), median (IQR)	490 (205–1,171)	750 (366–1,415)	.047

eGFR, estimated glomerular filtration rate; IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug; TB, tuberculosis; TDF, tenofovir disoproxil fumarate.

IQR 4.3–28 vs. 25.6 ml/min; IQR 10.4–39.5; $p=.002$) and was more acidotic (serum bicarbonate 14 μmol/l; IQR 10–18 vs. 16 μmol/l; IQR 13–19; $p=.047$). The odds ratio (OR) for severe AKI in the TDF group was 1.2 (1.02–1.40; 95% CI; $p=.013$) compared to those who were not exposed to TDF (Table 2).

Markers of proximal tubular dysfunction (urine phosphaturia and glycosuria, serum hypophosphatemia and hypokalemia) were not markedly deranged in either group. Laboratory results suggestive of interstitial nephritis were present in many, with high rates of leukocyturia ($n=47$; 32%) and hematuria ($n=81$; 55%) in sterile urine specimens, but there was no significant difference in the two groups—only 29 (17%) of all patients had a culture positive urinary tract infection.

Comorbidities and risk factors for AKI

Half of both groups (50% TDF vs. 55% non-TDF; $p=.474$) were newly diagnosed with TB, and overall a prior history of TB treatment was reported by 39%. Gastrointestinal symptoms, vomiting, and diarrhea were reported more frequently in the TDF group (39% vs. 21%; $p=.008$) and twice as many patients in the TDF group reported chronic diarrhea

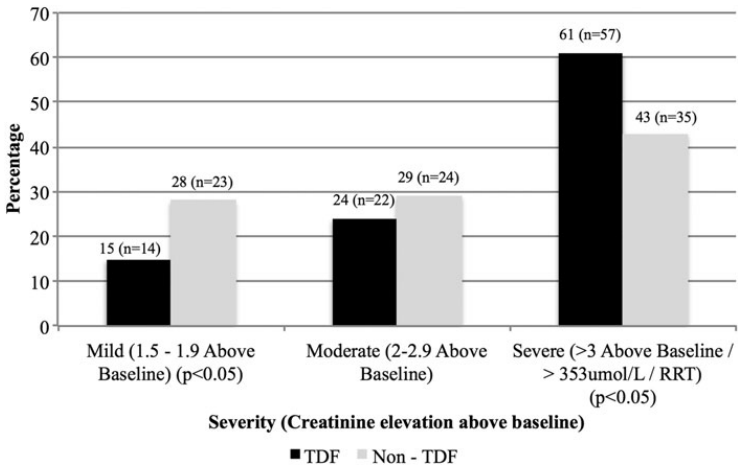

FIG. 1. Severity of acute kidney injury on admission stratified by TDF exposure. TDF, tenofovir disoproxil fumarate.

($p = .522$). Furthermore, in patients with severe AKI, the OR of vomiting [OR–1.14 (1.00–1.30; 95% CI; $p = .046$)] and diarrhea [OR–1.20 (1.05–1.30; 95% CI; $p = .007$)] was significantly increased (Table 2).

Noninfectious comorbidities: hypertension (22%) and diabetes (6%) were reported at similar rates in both groups. Receipt of traditional medication was infrequently self-reported, but high rates of nonsteroidal anti-inflammatory drug (NSAID) use, within the month before admission, were reported by both groups. A greater number of TDF-exposed patients were virally suppressed than ART-treated patients from the non-TDF group ($p = .017$); and in those not suppressed, the median viral load was lower in the TDF group ($p = .002$).

Treatment and outcomes

The overwhelming majority of patients received intravenous fluid therapy (96%) and only seven received RRT.

In those who received RRT, the admission SCr was significantly higher than those treated with intravenous fluid therapy: median SCr 553 μmol/l; IQR 471–623 vs. 216 μmol/l; IQR 156–438, $p = .021$; furthermore, all seven patients presented with severe AKI, $p = .037$. However, there were no statistical differences in the two groups in: TDF exposure and risk factors for AKI.

Eighty-five percent of all patients recovered sufficiently to be discharged from hospital. Even though the median length of hospital stay was the same in both groups (9 days), the TDF group had delayed renal recovery with higher SCr on discharge: median SCr 120 μmol/l; IQR 87–240 vs. 97 μmol/l; IQR 75–137; $p = .032$ (Fig. 2). Moreover, a smaller proportion of TDF exposed patients had complete renal recovery at the 3-month postdischarge follow-up visit (61% vs. 78%; $p = .043$) (Table 3).

Although TDF was stopped on admission in all patients, at 3-month follow-up, 55 patients (31%) had been restarted on full dose TDF based ART without apparent adverse effects, 38 (22%) of whom had been TDF exposed on admission. Only one patient who was reinitiated on TDF was subsequently changed to non-TDF based ART. No significant difference in renal recovery ($p = .114$) or mortality ($p = .105$) at 3-month follow-up was found in those reinitiated on TDF compared to those where non-TDF based ART was used following discharge.

Furthermore, among the TDF exposed group, no significant difference was noted in mortality or renal recovery at discharge or at 3-month follow-up when stratifying for duration of TDF use (Table 3).

In those who died during their hospital stay, no difference was noted in either SCr values measured before death or in renal recovery when comparing the TDF and non-TDF groups (Table 3). Fifty-five percent of deaths occurred in

Table 2. Univariable Analysis to Determine Risk of Severe Acute Kidney Injury and Mortality at 3-Month Follow-Up

	Severe AKI		*Mortality after 3-month follow-up*	
	OR (95% CI)	p	*OR (95% CI)*	p
TDF exposure	1.20 (1.02–1.40)	.013	1.00 (0.86–1.14)	.993
HIV viral load suppressed	1.10 (0.95–1.27)	.196	1.07 (0.90–1.26)	.763
TB coinfection	1.05 (0.90–1.22)	.516	1.21 (1.06–1.38)	.003
Vomiting	1.14 (1.00–1.30)	.046	1.14 (0.99–1.30)	.073
Diarrhea	1.20 (1.05–1.30)	.007	1.12 (0.96–1.30)	.164
NSAID use	1.06 (0.92–1.22)	.409	0.97 (0.83–1.14)	.706
Severe AKI	N/A	N/A	1.10 (0.97–1.26)	.144

95% CI, 95% confidence interval; AKI, acute kidney injury; OR, odds ratio.

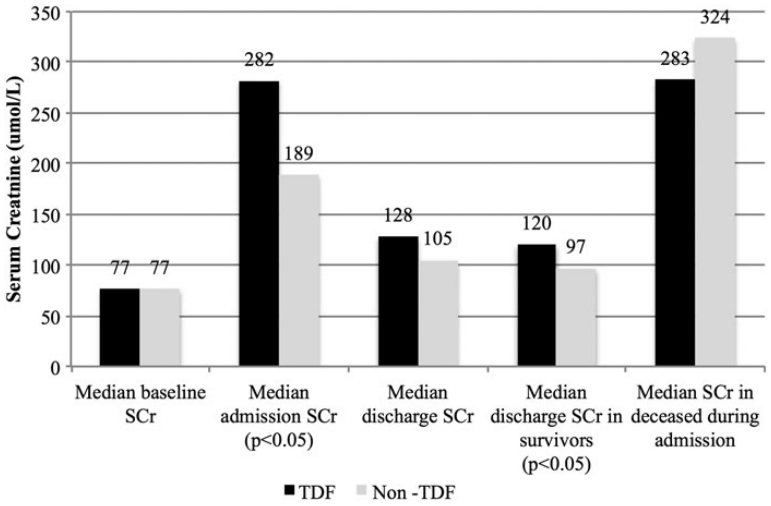

FIG. 2. Comparison of median SCr values stratified by TDF exposure at various time intervals: baseline before admission; on admission; on discharge; in survivors to discharge; and in deaths during hospital stay. SCr, serum creatinine.

hospital and they had a higher median SCr before death compared to the median SCr at discharge in survivors ($p < .001$). Furthermore, most patients who died in hospital had severe AKI immediately before death ($p < .001$).

Sixteen (9.1%) patients were lost to follow-up after discharge. In those who died following discharge, similar mortality rates were found in both groups ($p = .383$). When assessing overall outcome, from admission to 3-month follow-up, the mortality rate in both groups was 27%, but the duration from admission to death and discharge to death was significantly shorter in the TDF group (Table 3). Seventy percent ($n = 33$) of those who died were newly diagnosed with TB compared to 42% of survivors ($p = .001$), and the OR of mortality at 3-month follow-up in those newly diagnosed with TB was 1.21 (1.06–1.38; 95% CI, $p = .003$) compared to those without TB (Table 2). Similarly, elevated C-reactive protein and anemia were more frequently found in patients who died in this cohort.

Discussion

This prospective cohort suggests that HIV-infected patients with AKI who also received TDF-based ART had more severe AKI and a higher presenting median SCr compared to those on non-TDF-based ART. Moreover, the renal recovery was slower in those taking TDF. In-hospital outcomes, duration of admission, and overall mortality, however, were similar in both TDF exposed and unexposed groups. AKI in HIV-infected individuals has a poor prognosis, as more than a quarter of the patients in this cohort died.

In our study, TDF exposed HIV-infected patients who develop AKI have a similar etiology and rate and range of nephrotoxic risk factors that we measured as those not receiving TDF. However, our data suggest that TDF has an added nephrotoxic effect in patients with AKI causing: a more rapid worsening of renal function; a higher proportion with proteinuria and acidosis; and delayed renal recovery.

The data on TDF use and AKI in Southern Africa are limited; previous studies in ambulatory patients report a frequency of TDF-associated nephrotoxicity of just less than 3% and risk factors for nephrotoxicity with TDF use include: preexisting renal dysfunction, anemia, and immunosuppression.[24,25] This study is the first we are aware of that reports AKI, stratified by TDF exposure, in hospitalized HIV-positive patients in South Africa, and is particularly relevant due to the massive increase in prescriptions of TDF as part of first line ART in the country.[33]

RRT use was limited in this cohort, despite increasing SCr in over a third of those who died during admission and despite no formal restrictions or eligibility criteria for the initiation of RRT in this setting. We postulate that RRT may have prevented some deaths. Moreover, the substantial mortality following discharge from hospital suggests that more intensive monitoring and follow-up after discharge from hospital are necessary in all patients with AKI. Further studies are required to assess the likely causes of and contributors to death and to better identify potential interventions to prevent mortality.

Consistent with our findings, international studies have reported the duration of TDF exposure before onset of decline in renal function to be 6–9 months,[18,23] although studies of ambulatory patients in South Africa report a shorter median time to nephrotoxicity (3.6 months).[24,25]

Current recommendations suggest that TDF dose adjustment is necessary in those with renal injury while on TDF.[34]

Although published literature reports male gender and lower body weight as risk factors associated with AKI,[18,19,23,25] the high proportion of women with AKI that we report is likely due to higher seroprevalence of HIV among South African women and national policy to initiate ART in all HIV-positive pregnant women irrespective of CD4 count.[33,35]

The apparent role of TB, which was frequently diagnosed in this cohort, in contributing to both morbidity and mortality following discharge is substantial. The association between AKI and TB is described in a retrospective cohort from Johannesburg, in which 26% of HIV-positive patients with AKI were diagnosed with TB, almost half of what we report.[8] However, over half of the cases of TB we report were not laboratory confirmed.[36]

Fifty-three percent of our cohort had laboratory findings suggestive of interstitial nephritis, which could be at least

Table 3. Outcomes of Mortality, Renal Recovery, and Comorbidities Measured at Discharge and 3-Month Follow-Up Stratified by Both Tenofovir Disoproxil Fumarate Exposure and Duration of Tenofovir Disoproxil Fumarate Use ($N = 175$)

				Duration of TDF use			
	TDF	*Non-TDF*	p	*<6 Weeks*	*6–24 Weeks*	*>24 Weeks*	p
	93 (53)	82 (47)		20 (22)	25 (27)	48 (51)	
Mortality at discharge							
Deceased, *n* (%)	16 (17)	10 (12)	.353	4 (20)	5 (20)	7 (15)	.787
Fluid therapy alone, *n* (%)	88 (95)	80 (98)	.322	19 (95)	25 (100)	44 (92)	.324
Deceased, *n* (%)	13 (15)	9 (11)	.499	3 (16)	5 (20)	5 (11)	.617
Fluid and renal replacement therapy, *n* (%)	5 (5)	2 (2)	.322	1 (5)	0 (0)	4 (8)	.324
Deceased, *n* (%)	3 (60)	1 (50)	.809	1 (100)	0 (0)	2 (50)	.361
Duration of stay (days), median (IQR)							
If survived	9 (7–15)	9 (7–14)	.722	8 (7–20)	11 (7–15)	9 (7–15)	.949
If deceased	10 (6–16)	13 (9–16)	.286	11 (10–15)	10 (7–37)	6 (5–16)	.666
Mortality after 3-month follow-up							
Deceased, *n* (%)	25 (27)	22 (27)	.921	6 (30)	8 (32)	11 (23)	.665
Duration to death (days), median (IQR)							
Admission to death	30 (13–40)	64 (57–129)	.049	35 (30–40)	28 (13–125)	25 (13–88)	.783
Discharge to death	24 (18–30)	52 (47–122)	.016	22 (18–25)	23 (5–97)	28 (6–30)	.895
Renal parameters on discharge							
Renal recovery among deceased, *n* (%)							
Complete creatinine recovery	3 (20)	1 (10)	.504	0 (0)	1 (25)	2 (29)	.501
Partial creatinine recovery	7 (47)	5 (50)	.870	2 (50)	3 (75)	2 (29)	.328
Worsening creatinine	5 (33)	4 (40)	.734	2 (50)	0 (0)	3 (42)	.248
Renal recovery among survivors, *n* (%)							
Complete creatinine recovery	37 (48)	39 (54)	.456	9 (69)	9 (47)	19 (51)	.438
Partial creatinine recovery	32 (42)	19 (26)	.051	4 (31)	10 (53)	18 (49)	.438
Creatinine and eGFR (CKD-EPI) at death by renal recovery							
By creatinine (μmol/l), median (IQR)							
Deceased and complete recovery	79 (78–83)	92 (92–92)	.5	—	83 (83–83)	79 (79–79)	.667
Deceased and partial recovery	283 (131–368)	271 (139–513)	1	326 (283–368)	131 (116–147)	574 (311–836)	.095
Deceased and worsening creatinine	572 (527–627)	388 (366–577)	.413	577 (527–627)	—	572 (227–852)	1
By eGFR (ml/min per 1.73 m^2), median (IQR)							
Deceased and complete recovery	77 (76–78)	63 (63–63)	.5	—	76 (76–76)	78 (78–78)	.667
Deceased and partial recovery	13 (8–41)	13 (5–38)	1	10 (8–13)	41 (33–48)	6 (2–9)	.095
Deceased and worsening creatinine	4 (3–5)	7 (7–12)	.413	4 (4–5)	—	3 (2–18)	1
Renal parameters after 3-month follow-up							
Renal recovery among survivors, *n* (%)							
Complete creatinine recovery	37 (61)	40 (78)	.043	8 (62)	7 (54)	22 (63)	.849
Partial creatinine recovery	9 (15)	2 (4)	.055	0 (0)	2 (15)	7 (20)	.221

partially attributed to TB and/or NSAID use, although this finding is limited by the absence of renal biopsies in this cohort.[37,38] While the immune reconstitution inflammatory syndrome (IRIS) is a known cause of an interstitial nephritis, the duration of ART exposure in the majority of our patients was for a longer period than that associated with the onset of IRIS; moreover, some patients were ART naive.[38,39]

This study has several limitations. We restricted our sample to patients admitted to a single regional hospital in South Africa and we are unable to report the overall rate of AKI in TDF and other ART recipients. Tubular dysfunction using urine beta-2 microglobulin and fractional excretion of phosphate was not measured, and in some patients, not every study parameter was measured, but all had sufficient measured data to be eligible for inclusion.

Baseline SCr values were not available in all patients and missing baseline values were inferred using the MDRD formula (calculated using an eGFR of 75 ml/min/1.73 m^2); this may have biased the classification of AKI severity in both the TDF, but particularly in the non-TDF group where baseline SCr levels were less readily available as ART-naive patients infrequently had a measured SCr before admission or on follow-up.[28,40]

Furthermore, no renal biopsies were performed in this cohort, particularly in those with partial renal recovery; however, in most instances, the etiology of AKI was clinically evident. Finally, the small sample size of the study and missing data values limited the regression analysis of this cohort.

Comparison of the two groups suggests that risks factors, etiology, and mortality of AKI in HIV-infected in-patients, irrespective of TDF use, are similar, but that coexisting TDF use appears to result in more severe and longer lasting AKI. Our findings suggest that a lower threshold for RRT may be needed in those with limited or no SCr recovery and intensive follow-up after discharge from hospital may reduce mortality in all HIV-infected AKI patients, and especially in those with concomitant TB.

Acknowledgments

The authors express gratitude to the clinicians and staff of the Klerksdorp/Tshepong Hospital Complex for their assistance and care provided to the patients and thank the staff of the Perinatal HIV Research Unit (PHRU) for their support. The project was cofunded by PHRU and Council for Scientific and Industrial Research (CSIR).

Authors' Contributions

F.S., N.M., E.V., and D.M. conceived the idea and designed the study. K.M. and P.A. contributed to data collection and data interpretation. F.S. drafted the article, with assistance from N.M., E.V., and S.N. All authors approved the final article.

Author Disclosure Statement

No competing financial interests exist.

References

1. Wyatt CM, Arons RR, Klotman PE, Klotman ME: Acute renal failure in hospitalized patients with HIV: Risk factors and impact on in-hospital mortality. AIDS 2006;20:561–565.
2. Kalim S, Szczech LA, Wyatt CM: Acute kidney injury in HIV-infected patients. Semin Nephrol 2008;28:556–562.
3. Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG: Long-term clinical consequences of acute kidney injury in the HIV-infected. Kidney Int 2010;78:478–485.
4. Islam FM, Wu J, Jansson J, Wilson DP: Relative risk of renal disease among people living with HIV: A systematic review and meta-analysis. BMC Public Health 2012;12:234.
5. Franceschini N, Napravnik S, Eron JJ, Jr, Szczech LA, Finn WF: Incidence and etiology of acute renal failure among ambulatory HIV-infected patients. Kidney Int 2005;67:1526–1531.
6. Roe J, Campbell LJ, Ibrahim F, Hendry BM, Post FA: HIV care and the incidence of acute renal failure. Clin Infect Dis 2008;47:242–249.
7. Lopes JA, Melo MJ, Viegas A, Raimundo M, Camara I, Antunes F, *et al.*: Acute kidney injury in hospitalized HIV-infected patients: A cohort analysis. Nephrol Dial Transplant 2011;26:3888–3894.
8. Vachiat AI, Musenge E, Wadee S, Naicker S: Renal failure in HIV-positive patients—A South African experience. Clin Kidney J 2013;6:584–589.
9. Lopes JA, Melo MJ, Raimundo M, Fragoso A, Antunes F: Long-term risk of mortality for acute kidney injury in HIV-infected patients: A cohort analysis. BMC Nephrol 2013;14:32.
10. Miro JM, Cofan F, Trullas JC, Manzardo C, Cervera C, Tuset M, *et al.*: Renal dysfunction in the setting of HIV/AIDS. Curr HIV/AIDS Rep 2012;9:187–199.
11. Wikman P, Safont P, Del Palacio M, Moreno A, Moreno S, Casado JL: The significance of antiretroviral-associated acute kidney injury in a cohort of ambulatory human immunodeficiency virus-infected patients. Nephrol Dial Transplant 2013;28:2073–2081.
12. DeChristoforo R, Penzak SR: Tenofovir: A nucleotide analogue reverse-transcriptase inhibitor for treatment of HIV infection. Am J Health Syst Pharm 2004;61:86–98;quiz 99–100.
13. South African National Department of Health. Clinical guidelines for the management of HIV & AIDS in adults and adolescents. Pretoria: Department of Health, 2010. Available at www.sahivsoc.org, accessed April 14, 2015.
14. Schooley RT, Ruane P, Myers RA, Beall G, Lampiris H, Berger D, *et al.*: Tenofovir DF in antiretroviral-experienced patients: Results from a 48-week, randomized, double-blind study. AIDS 2002;16:1257–1263.
15. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K: Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 2003;37:e41–e43.
16. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, *et al.*: Tenofovir-associated renal and bone toxicity. HIV Med 2009;10:482–487.
17. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M: Systematic review and meta-analysis: Renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 2010;51:496–505.
18. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, *et al.*: The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: The first 4 years. AIDS 2007;21:1273–1281.
19. Young B, Buchacz K, Baker RK, Moorman AC, Wood KC, Chmiel J, *et al.*: Renal function in tenofovir-exposed and tenofovir-unexposed patients receiving highly active antiretroviral therapy in the HIV Outpatient Study. J Int Assoc Physicians AIDS Care (Chic) 2007;6:178–187.

20. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J, *et al.*: Tenofovir nephrotoxicity: 2011 Update. AIDS Res Treat 2011;2011: 354908.
21. Hall AM, Hendry BM, Nitsch D, Connolly JO: Tenofovir-associated kidney toxicity in HIV-infected patients: A review of the evidence. Am J Kidney Dis 2011;57:773–780.
22. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L, *et al.*: Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. J Acquir Immune Defic Syndr 2010;53:62–69.
23. Madeddu G, Bonfanti P, De Socio GV, Carradori S, Grosso C, Marconi P, *et al.*: Tenofovir renal safety in HIV-infected patients: Results from the SCOLTA Project. Biomed Pharmacother 2008;62:6–11.
24. Brennan A, Evans D, Maskew M, Naicker S, Ive P, Sanne I, *et al.*: Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. AIDS 2011;25:1603–1609.
25. Kamkuemah M, Kaplan R, Bekker LG, Little F, Myer L: Renal impairment in HIV-infected patients initiating tenofovir-containing antiretroviral therapy regimens in a primary healthcare setting in South Africa. Trop Med Int Health 2015;20:518–526.
26. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group: KDIGO clinical practice guideline for acute kidney injury. Kidney Inter Suppl 2012;2:1–138.
27. Siew ED, Ikizler TA, Matheny ME, Shi Y, Schildcrout JS, Danciu I, *et al.*: Estimating baseline kidney function in hospitalized patients with impaired kidney function. Clin J Am Soc Nephrol 2012;7:712–719.
28. Wald R: Predicting baseline creatinine in hospitalized patients. Clin J Am Soc Nephrol 2012;7:697–699.
29. Okusa MD, Davenport A: Reading between the (guide)lines—The KDIGO practice guideline on acute kidney injury in the individual patient. Kidney Int 2014;85:39–48.
30. Gaiao S, Cruz DN: Baseline creatinine to define acute kidney injury: Is there any consensus? Nephrol Dial Transplant 2010;25:3812–3814.
31. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P: Acute renal failure – Definition, outcome measures, animal models, fluid therapy and information technology needs: The Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204–R212.
32. Kashani K, Kellum JA: Novel biomarkers indicating repair or progression after acute kidney injury. Curr Opin Nephrol Hypertens 2015;24:21–27.
33. South African National Department of Health. The South African antiretroviral treatment guidelines. Pretoria: Department of Health, 2013. Available at www.sahivsoc.org, accessed April 14, 2015.
34. Jafari A, Khalili H, Dashti-Khavidaki S: Tenofovir-induced nephrotoxicity: Incidence, mechanism, risk factors, prognosis and proposed agents for prevention. Eur J Clin Pharmacol 2014;70:1029–1040.
35. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Zungu N, *et al.*: *South African National HIV Prevalence, Incidence and Behaviour Survery, 2012*. HSRC Press, Cape Town, 2014, pp. 1–198.
36. Hoffmann CJ, Hoffmann JD, Kensler C, van der Watt M, Omar T, Chaisson RE, *et al.*: Tuberculosis and hepatic steatosis are prevalent liver pathology findings among HIV-infected patients in South Africa. PLoS One 2015;10:e0117813.
37. Parkhie SM, Fine DM, Lucas GM, Atta MG: Characteristics of patients with HIV and biopsy-proven acute interstitial nephritis. Clin J Am Soc Nephrol 2010;5:798–804.
38. Zaidan M, Lescure FX, Brocheriou I, Dettwiler S, Guiard-Schmid JB, Pacanowski J, *et al.*: Tubulointerstitial nephropathies in HIV-infected patients over the past 15 years: A clinico-pathological study. Clin J Am Soc Nephrol 2013;8:930–938.
39. Leone S, Nicastri E, Giglio S, Narciso P, Ippolito G, Acone N: Immune reconstitution inflammatory syndrome associated with *Mycobacterium tuberculosis* infection: A systematic review. Int J Infect Dis 2010;14:e283–e291.
40. Siew ED, Peterson JF, Eden SK, Moons KG, Ikizler TA, Matheny ME: Use of multiple imputation method to improve estimation of missing baseline serum creatinine in acute kidney injury research. Clin J Am Soc Nephrol 2013;8:10–18.

Address correspondence to:
Faheem Seedat
Department of Internal Medicine
Faculty of Health Sciences
University of Witwatersrand
P.O. Box 96630
Brixton 2019
Johannesburg
South Africa

E-mail: faheem@global.co.za