The burden of tuberculosis in patients with stage 5 chronic kidney disease undergoing dialysis therapy at Livingstone hospital, Port Elizabeth

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Siviwe Ndamase (NDMSIV001)

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Co-supervisor: Professor Ikechi Okpechi, University of Cape Town

DECLARATION

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

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Date: February 2019

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The burden of tuberculosis in patients with stage 5 chronic kidney disease undergoing dialysis therapy at Livingstone hospital, Port Elizabeth

Investigator: Dr Siviwe Ndamase, University of Cape Town, South Africa

Supervisor: Dr R.Freercks, University of Cape Town and Livingstone Hospital Renal Unit, Port Elizabeth, South Africa

Co-Supervisor: Professor I. Okpechi, Division of Nephrology and Hypertension, and Kidney and Hypertension Research Unit, University of Cape Town, Cape Town, South Africa

ABBREVIATIONS

CKD – chronic kidney disease

CKD 5 - chronic kidney disease stage 5

CKD-5D - chronic kidney disease stage 5 on dialysis

TB - tuberculosis

HD - haemodialysis

PD - peritoneal dialysis

CAPD- continuous ambulatory peritoneal dialysis

WHO - world health organization

INH - isoniazid

AFB - acid fast bacilli

VBP- vitamin D binding protein

25(OH)vitD3 – 25 hydroxyvitamin D3

1,25(OH)2vitD3- 1,25 dihydroxyvitamin D3

FGF-23 – fibroblast growth factor -23

MTB- mycobacterium tuberculosis complex

TST- Tuberculin skin test

IGRA- interferon gamma release assay

PART A: Abstract

Background

Tuberculosis (TB) now ranks as the leading cause of death from a single infectious agent worldwide. Patients on dialysis are particularly vulnerable to TB infection due to immune dysfunction. Despite this, there is a paucity of incidence data on TB in dialysis patients in high burden settings such as South Africa. The aim of this study was to determine the incidence of TB in chronic kidney disease stage 5 patients on dialysis (CKD-5D) at a single centre in the Eastern Cape, South Africa and to identify risk factors associated with TB infection.

Methods

We conducted a retrospective cohort study of all consenting prevalent CKD-5D patients between April 2010 and March 2014 at the Livingstone Tertiary Hospital Renal Unit in the Eastern Cape, South Africa. TB was defined as "definite" or "probable" according to WHO criteria.

Results

One hundred and eleven patients were enrolled: they were predominantly black African (73%) and female (53%); mean age was 42 years (SD ±9years). The prevalence of HIV infection was 11%: all were on antiretroviral treatment and all had suppressed viral loads. Sixty eight patients were on haemodialysis (HD) and 43 patients were on peritoneal dialysis (PD). Nineteen patients were diagnosed with 20 episodes of TB; 14 cases were pulmonary and 6 cases extrapulmonary. Of the patients with TB, 2 were HIV infected. Of the 20 TB cases, 7 (35%) were definite TB cases and 13 (65%) had probable TB. The calculated incidence rate was 4505 per 100 000 patient years. Only informal housing and a history of hospitalization were significantly associated with a diagnosis of TB.

Conclusion

Dialysis patients in the Eastern Cape region of South Africa are at extremely high risk for the acquisition of TB with an incidence rate that is 4.1 times that of the local Nelson Mandela Bay population and over 5 times that reported in the general population for the country as a whole. Only informal housing and a history of hospitalization were identified as positive risk factors in

this young population with a low HIV prevalence. Isoniazid prophylaxis in this high risk group might be of benefit but further studies are required to inform such treatment.

PART B: Structured Literature review

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a. Introduction and worldwide epidemiology of tuberculosis

Tuberculosis remains a major health burden and is now the leading cause of death from an infectious agent worldwide[1] . In 2017, the world health organization reported an estimated 10.0 million new TB cases worldwide, of which 5.8 million cases were among men, 3.2million cases among women and 1.0 million cases among children[1]. People living with HIV accounted for 9% of all new TB cases[1]. The estimated number of TB deaths in 2017 was 1.3 million among HIV negative people, and an additional 300 000 deaths resulting from TB disease among people living with HIV[1]. Although deaths from TB are still unacceptably high, it has been reported that the number of TB deaths is declining every year with an estimated fall of TB deaths by 29% among HIV negative patients and 44% among HIV positive patients between year 2000 and 2017[1].

South Africa has an estimated tuberculosis incidence of 567 per 100 000 population[1]. The Eastern Cape Provincial Department of Health has reported cure rates of 77% which is below the targeted 90% recommended by the WHO[2]. The South African national treatment cure rates vary between 62% and 82% depending on whether it's a new case or a retreatment case[1].

b. Incidence and clinical presentation of tuberculosis in CKD patients on dialysis

The incidence of tuberculosis is higher in dialysis patients with a reported incidence of active TB among prevalent dialysis patients of 6.9 to 52.5 times higher than the general population depending on regional factors[3,4,5,6].High income countries report a lower incidence than that mentioned above but the incidence of TB is still increased in dialysis patients compared to the general population and also carries a high mortality[7]. Dobler et al, in a large population based cohort study involving 19.9 million Australians reported that people on dialysis have a seven to eight-fold increased risk of developing TB compared with the general population ,the overall incidence of TB among patients on dialysis was 66.8 per 100,000 person-years compared to the incidence of TB of 5.7 per 100,000 in the general population over their study period[5].

The incidence and prevalence of TB in dialysis patients in sub Saharan Africa is largely undocumented. However, one study from Limpopo, South Africa reviewed the outcomes of CAPD patients who developed tuberculous peritonitis and reported that TB peritonitis carries a high mortality rate in patients on CAPD even when they are treated for the infection[8]. Their cohort had a 7.1% of diagnosed and treated cases of TB peritonitis[8].

The increased incidence of tuberculosis in dialysis patients is thought to be a result of impaired cell mediated immunity[6].The uremic state of CKD-5 is associated with granulocyte and lymphocyte dysfunction and this is particularly significant for the development of tuberculosis as granulocytes and lymphocytes are the cells responsible for killing intracellular organisms like mycobacterium tuberculosis[9].

The diagnosis of TB among dialysis patients can be challenging because the presentation is often insidious and patients present with non-specific symptoms including malaise, fever, weight loss and adenopathy[10]. Extra pulmonary TB is more common in patients with CKD-5 and caseating necrosis is often absent owing to their immunosuppressed state[10]. The diagnosis of TB in CKD-5D therefore requires a very index of suspicion and tissue biopsies and cultures are frequently required to make the diagnosis. This non-specific symptomatology and difficulty in ascertaining

drug sensitivity contributes to the delay in diagnosis and initiation of effective treatment[3]. Tamayo-Isla et al. recently emphasized the difficulties encountered with making the diagnosis of TB in CAPD patients, and that a combination of biochemical, microbiological and radiological assessments need to be employed to prove the diagnosis[8]. In their cohort, TB was isolated in only 9/12 (75%) of the patients and the diagnosis of TB in the other 25% was made based on chest radiograph and abdominal ultrasound features suggestive of TB[8]. Vikrant also reiterated the difficulty with making a definite diagnosis of TB in dialysis patients and only 50% were confirmed cases in his cohort and the other 50% was treated based on clinical grounds[11].

c. Risk factors for tuberculosis in dialysis patients

Risk factors for TB in the general population include: host related risk factors including conditions which cause immunosuppression-HIV, diabetes and use of immunosuppressive drugs,[12] plus environmental and socioeconomic related risk factors such as overcrowded households, use of biomass fuel and poor ventilation[12]. Health care workers are also at increased risk of TB because of occupational exposure to TB patients[12].

Risk factors for development of TB in dialysis patients can be divided into modifiable and non-modifiable risk factors. The non-modifiable risk factors include advanced age, primary cause of chronic kidney disease (such as diabetic nephropathy) and ethnicity[13]. These non-dialysis related risk factors are themselves associated with depressed cell mediated immunity and anergy of effector lymphocytes thereby increasing risk of TB infection, and further serve as confounding factors to the high incidence of tuberculosis in CKD[7,13].

Modifiable risk factors include, malnutrition, smoking and the mode of dialysis[7]. Haemodialysis patients are at increased risk of TB compared to PD patients[7]. The frequent hospital visits, lack of isolation facilities and close proximity of patients to each other during the dialysis sessions are thought to contribute to this increased risk[7]. Klote et al. showed in their study that age ($p<0.001$), haemodialysis (vs peritoneal dialysis, $p=0.019$), unemployment ($p<0.001$), reduced BMI ($p<0.001$), ischaemic heart disease ($p=0.032$), smoking history ($p=0.010$), illicit drug use ($p=0.018$), reduced serum albumin ($p<0.001$), and African American ($p=0.001$) and Asian

(p=0.002) race were independently statistically significant risk factors for the development TB infection[7].

HIV as a modifiable risk factor is particularly significant, and poses a great challenge for our CKD population in the African context given that Sub-Saharan Africa already has a huge burden of the HIV and TB co-epidemic. A significant number of the TB cases are diagnosed within the first year of starting dialysis[14] and Unsal et al., reported that in their retrospective review 54.2% of their TB cases were diagnosed in their first year of dialysis[14]. This may be because of the general poor state of health and seriously impaired immune host defense mechanism at the start of their dialysis treatment[14].

d. Effect of chronic kidney disease on the immune system

The key function of the immune system includes detecting and destroying invading microbes[15]. The immune defense against invading microbes is accomplished through a complex interaction between the innate and adaptive immune system[15]. The innate immune system comprises of cells and processes that culminate in a non-specific and rapid response to invading microorganisms. The innate immune system includes circulating monocytes and their tissue counterparts, macrophages, neutrophilic polymorphonuclear leukocytes, dendritic cells, natural killer cells, mast cells, eosinophils, basophils, and nearly all other cells in the body[15]. The adaptive immune system on the other hand has a more complicated well developed capacity for host defense and plays a role in the host recognizing and remembering specific pathogens and enables the host to mount stronger attacks on re-encounter with the same pathogen[16] . The adaptive immune system includes T and B lymphocytes which express cell specific receptors with which they recognize specific antigens[16].

While immune dysfunction in CKD is complex and not fully understood, a number of mechanisms have been suggested including: (a)decreased granulocyte and monocyte/macrophage phagocytic function, (b) defective antigen-presenting capacity of antigen-presenting cells, (c) depletion of the antigen-presenting dendritic cells, (d) reduced numbers and antibody producing capacity of B cells, (e) increased T cell turnover and apoptosis leading to

depletion of naive and central memory CD 41 and CD81 T cells and impaired cell-mediated immunity[15].

e. Immunopathogenesis and classification of TB disease

TB is a communicable infectious disease caused by the mycobacterium tuberculosis (MTB) complex and is transmitted via droplet spread through person to person contact[17]. After inhalation the MTB complex is taken up by dendritic cells and alveolar macrophages where it proliferates and sets off an intracellular signaling cascade resulting in the production of various cytokines[18]. MTB complex then gets transported by dendritic cells to mediastinal lymph nodes where antigen presenting cells activate T cells resulting in activation of adaptive immune response and production of more cytokines resulting in the formation of an immune granuloma comprised of macrophages, neutrophils, monocytes, dendritic cells, and T cells[18].

Tuberculosis can be classified according to the anatomical site of the disease as either pulmonary or extra-pulmonary TB[19]. Pulmonary TB refers to TB disease involving the lung parenchyma. Included in this category are miliary TB, tuberculous hilar lymphadenopathy and pleural effusion with lung parenchymal changes. Extra-pulmonary TB includes TB in the following sites, pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges[20]. Extra-pulmonary TB accounts for 15-20% of TB in populations with low HIV prevalence,[19] but in high HIV prevalence areas, the proportion of cases is much higher[19].

Tuberculosis can also be classified bacteriologically as either ‘smear positive’ or ‘smear negative’. A patient is considered smear positive when one or more initial sputum smear examinations by direct smear microscopy is AFB- positive, or one sputum examination is AFB-positive in association with radiographic abnormalities consistent with active pulmonary TB as determined by a clinician or one sputum sample positive for AFBs and culture positive for AFBs[20]. Smear-positive cases are the most infectious[19].

Smear negative TB includes at least two sputum AFB-negative smear examinations but with one or more of the following: a positive culture result, radiographic abnormalities consistent with active pulmonary TB, histologic evidence of caseating granulomas or no response to a course of broad-spectrum antibiotics plus a decision by a clinician to treat with a full course of anti-TB chemotherapy[19].

Table 1: WHO Tuberculosis clinical classification[1,21]

Case classification	Definition
Definite TB[1]	A patient with mycobacterium TB identified on a clinical specimen either by culture or new molecular line probe assay specimens. For countries which lack facilities to routinely identify mycobacterium TB, one or more initial sputum samples positive for acid fast bacilli (AFB) is considered definite TB case, provided there is a functional external quality assurance protocol for blind rechecking.
Probable TB[21]	Any patient who does not fit the 'definite' TB case defined above but has clinical symptoms including one/more of the following: 1. Cough more than two weeks plus other respiratory symptoms (shortness of breath, chest pain, haemoptysis) 2. Constitutional symptoms such as loss of weight, loss of appetite, drenching night sweats. PLUS one or more of: 1. Radiological abnormalities consistent with TB. 2. Histology of biopsy tissue showing caseating granulomas. 3. No response to broad spectrum antibiotics and a decision by a clinician to treat with a full course of anti-TB

	therapy.
No TB	TB disease has been activity excluded.

f. Latent TB infection in dialysis patients

Getahun et al. define latent TB infection as a state of persistent bacterial viability, immune control without evidence of clinically manifested active tuberculosis[22]. An estimated 2 billion people worldwide have latent TB and are at risk of reactivation[23]. The annual rate of reactivation of latent TB in South Africa is estimated at 4.2%[22]. CKD-5 confers an increased risk of reactivation of latent TB because of immune dysfunction[22]. The risk of reactivation of latent TB infection is 10-25 times higher in CKD-5 patients compared to the general population[24],[25]. There are two tests currently used for screening for latent TB infection. Tuberculin skin test (TST) which uses PPD (purified protein derivative) containing many antigens and the antigen specific interferon gamma release assays (IGRA's) that detect the release of interferon γ by T cells in response to tuberculosis specific antigens. The usefulness of tuberculin skin testing in assessing latent tuberculosis infection in dialysis patients is questionable because of very low sensitivity[26] with false negatives of up to 50% due to impaired host immune response and anergy have been reported[27]. False positives are not infrequent because of the cross reactivity of TST with Bacillus Calmette–Guérin (BCG) antigens, especially in countries where BCG is part of the national immunization programme. However the effect of BCG on the results of TST is expected to not last more than 15 years and the induration of the TST as a result of BCG immunization should not be more than 15mm[28]. The usefulness of TST in areas where TB is endemic is therefore questionable. The new IGRA can be used together with the tuberculin skin test to screen for latent tuberculosis infection. The IGRA tests serve to complement and not substitute TST and results in increased sensitivity for detecting latent TB infection[29].

The current recommendations in low tuberculosis incidence areas are to screen only selected patients within a dialysis programme such as those who are on the transplant waiting list, young patients with a long life expectancy and those who have immigrated from tuberculosis endemic

areas[27]. Screening of all patients in these areas is not recommended as it may not be cost effective and the risk of hepatitis from chemoprophylaxis may outweigh that of development of tuberculosis[27].

The above recommendations may not necessary be applicable to tuberculosis endemic areas. One randomized clinical trial done in India demonstrated significant protection against development of tuberculosis with Isoniazid (INH) prophylaxis given for a period of 1 year to CKD-5D patients. INH has been found to be reasonably safe and well tolerated[30,31]. Although there may be concerns of emergence of drug resistant TB with use of INH prophylaxis, a recent South African cohort study done in a high TB prevalence area showed that active tuberculosis infection following recent isoniazid prophylaxis had treatment outcomes and prevalence of drug resistance similar to that of the general population[30].

There are currently no guidelines in South Africa to inform us on INH prophylaxis in dialysis patients. The only widely available data on INH chemoprophylaxis is for HIV infected patients[32]. A recent published trial in South African gold mine workers showed no reduction in either the incidence or prevalence of tuberculosis or the rate of death from any cause after 9 months of INH chemoprophylaxis at 18 months follow up. Although the incidence of TB was reduced during chemoprophylaxis, protection from infection was rapidly lost on discontinuation of INH[33].

Tuberculosis tends to behave as an opportunistic infection in transplant patients and the current recommendations[34] from the European Best Practice Guidelines for Renal Transplantation[35] and the American Society of Transplantation[36] are for a 9-month course of INH prophylaxis. A meta-analysis by Currie A.C et al recommends prophylaxis in renal transplant recipients in endemic areas or in recipients in non-endemic countries who are at risk including immigrants from TB endemic countries[34].

g. Vitamin D metabolism and deficiency in TB

According to current international guidelines, vitamin D deficiency can be defined as a 25-hydroxyvitamin D level of <30nmol/L and insufficiency as 30-75nmol/L[37]. CKD5 is associated with low levels of vitamin D as a result of deficiency of 25 hydroxyvitamin D3 as well as impaired hydroxylation of 25 hydroxyvitaminD3 to the active metabolite 1, 25 dihydroxyvitamin D3 by the enzyme 1 alpha hydroxylase in the kidneys and elsewhere in the body[38]. A number of mechanisms in which CKD 5 results in vitamin D deficiency have been suggested, including the loss of functional renal mass leading to decreased production of 1 alpha hydroxylase[39]. Other suggested mechanisms including suppression of enzyme activity due to metabolic acidosis, hyperphosphatemia, and accumulation of other uraemic toxins associated with CKD have been suggested[39]. In addition to the mechanisms stated above, one other main physiological regulator of vitamin D metabolism in CKD is fibroblast growth factor-23 (FGF-23)[39].

In the pre antibiotic era, vitamin D was used in the treatment of tuberculosis[40]. Vitamin D facilitates monocyte-macrophage activity in the body and plays a role in human innate immunity to certain infectious agents[41]. This role is thought to be important in the body's defense against tuberculosis. Vitamin D acts by binding to nuclear receptors in target cells. Therefore, both abnormalities in vitamin D receptor structure and function and low serum levels of vitamin D may result in impairment in hosts handling of the tubercle bacillus[41]. First, the active form of vitamin D enhances the ability of macrophages to suppress the intracellular growth of Mycobacterium tuberculosis[41,42]. Secondly, on triggering of toll-like receptors by molecules of the TB bacillus, production of microbiocidal cathelicidin is impaired in the absence of adequate serum vitamin D[41,42].

Several studies assessing the association of active tuberculosis and vitamin D status have been done[41] with most reporting an increased prevalence of vitamin D deficiency in tuberculosis[43]. The highest prevalence of vitamin D deficiency in an African study was reported in a South African study done on HIV infected and HIV uninfected black patients in Cape Town, which showed a prevalence of 62,7% as defined by a 25-Hydroxyvitamin D level of <50 nmol/L[44]. One

study done in prevalent dialysis patients in Cape Town revealed a combined prevalence of vitamin D insufficiency/deficiency of 57%[45].

Studies have shown an increased risk of TB in patients with vitamin D deficiency. Therefore, patients with advanced kidney disease may have a compounded risk of TB as a result of their depressed cellular immunity in conjunction with vitamin D deficiency.

Although observational studies have shown an association between vitamin D deficiency and increased prevalence of tuberculosis, randomized controlled studies have demonstrated conflicting results with most studies to date failing to show any benefit from vitamin D supplementation to non-CKD TB patients [40].

h. TB in Renal Transplant patients

Renal transplant recipients are reported to be at an increased risk of TB infection owing to their immunosuppressed state. Reis-Santos et al in their meta-analysis, reported an increased combined TB prevalence rate of 2.51% which is 14 times greater than the prevalence rate of 0.18% seen in the general population[46].

i. Treatment of TB in CKD and transplantation

The pharmacodynamics of antituberculosis drugs determine how their levels are likely to be influenced by renal failure, clearance during dialysis and also their interaction with immunosuppressive drugs used in patients undergoing renal transplantation[47]. The British Thoracic Society recommends for active TB cases with CKD to be treated with 4 drugs(with drug dosage modifications) in the first 2 months followed by 2 drugs for a further 4 months with the exception of central nervous infections which requires a prolonged treatment duration of up to 12 months[47]. No drug dose adjustments are required for isoniazid and rifampicin as both drugs are extensively metabolized by the liver[48]. Pyrazinamide dosages need to be adjusted as it accumulates and it's thought to interfere with uric acid metabolism with resultant hyperuricaemia and increased risk for gout arthropathy[48]. Its dosage adjustment is as shown in Table 2[47]. Ethambutol and aminoglycosides are renal eliminated and thus require dose adjustments to avoid

toxicity as shown in Table 2[47]. If a major concern for ethambutol toxicity exists, then ethambutol can be substituted with moxifloxacin during the first 2 months of the initiation phase. For patients on haemodialysis the drugs can be given immediately post dialysis or alternatively 4-6 hours before dialysis to avoid premature drug elimination[47]. It is advisable to monitor peak and trough levels of drugs including ethambutol and aminoglycosides where possible to avoid under and over dosing[47].

In transplant patients anti-TB drug interactions with immunosuppressive treatment poses a major challenge and can lead to acute graft rejection[47]. Rifampicin is the drug most likely to interfere with immunosuppressive therapy as it is a strong inducer of multiple enzyme systems and many drug adjustments need to made including doubling the dose corticosteroids and ciclosporin drug levels need to be closely monitored and dose adjusts employed accordingly[47].

Table 2 : British Thoracic Society's recommended doses of first line drugs in CKD[47]

	Stage 1-3 CKD	Stage 4 and 5 CKD	Renal transplant recipients
Isoniazid	300mg daily	300mg daily or 15mg/kg maximum 900mg 3*/week	300mg daily
Rifampicin	<50kg :450mg daily	<50kg :450mg daily	<50kg :450mg daily
	≥50kg :600mg daily	≥50kg : 600mg daily	≥50kg :600mg daily
Pyrazinamide	<50kg :1.5g daily	25-30mg/kg 3*week	<50kg :1.5g daily
	≥50kg :2.5g daily		≥50kg :2g daily
Ethambutol	15mg/kg daily	15-25mg/kg 3*weekly maximum(2.5g)	15mg/kg daily
Moxifloxacin	400mg daily	Not suitable for 3*weekly regimen	400mg daily

j. Relevance and rationale of the study

Tuberculosis still remains an important cause of morbidity and mortality despite employed strategies to improve diagnosis and treatment success rates[1]. The burden of tuberculosis is reportedly higher in CKD-5D patients owing to impaired cell mediated immunity[6].

Given that there is limited published data regarding the burden of tuberculosis in CKD-5D patients in South Africa and that the real extent of the problem is therefore unknown, we sought to highlight the burden of tuberculosis and identify any modifiable risk factors that can be addressed in the CKD-5D population.

On completion of the study we hope to make recommendations that will inform and ultimately improve the care of end stage kidney disease patients in the Eastern Cape.

k. Study objectives

Primary Objective

1. To determine the incidence of TB in the Livingstone renal unit patients with CKD-5D over the study period.

Secondary objectives

1. To identify variables associated with an increased risk for TB in CKD-5D.
2. To compare vitamin D deficiency in the dialysis patients diagnosed with tuberculosis with those who do not develop TB.
3. To compare the incidence of TB in Livingstone Hospital CKD-5D patients with the Eastern Cape Province as a whole.

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PART C: Journal Ready Manuscript

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Abstract

Background

Tuberculosis (TB) now ranks as the leading cause of death from a single infectious agent worldwide. Patients on dialysis are particularly vulnerable to TB infection due to immune dysfunction. Despite this, there is a paucity of incidence data on TB from dialysis patients in high burden settings.

Objective

To determine the incidence of TB in chronic kidney disease stage 5 patients on dialysis (CKD-5D) at a single centre in the Eastern Cape, South Africa and to identify risk factors associated with TB infection.

Methods

We conducted a retrospective cohort study of all consenting prevalent CKD-5D patients between April 2010 and March 2014 at the Livingstone Tertiary Hospital Renal Unit in the Eastern Cape, South Africa. TB was defined as "definite" or "probable" according to WHO criteria and the cohort was split into those who developed TB (TB+) and those that did not (TB-).

Results

One hundred and eleven patients were enrolled: they were predominantly black African (73%) and female (53%); mean age was 42 years (SD ± 9years). The prevalence of HIV infection was 11%: all were on antiretroviral treatment and all had suppressed viral loads. Sixty eight patients were on haemodialysis (HD) and 43 patients were on peritoneal dialysis (PD). Nineteen patients were diagnosed with 20 episodes of TB; 14 cases were pulmonary and 6 cases extrapulmonary. Of the patients with TB, 2 were HIV infected. Of the 20 TB cases, 7 (35%) were definite TB cases and 13 (65%) had probable TB. The calculated incidence rate was 4505 per 100 000 patient years. Only informal housing (30% in TB+ versus 12% in TB-, $p = 0.042$) and a history of hospitalization (90% versus 76% respectively, $p = 0.042$) were significantly associated with a diagnosis of TB.

Conclusion

Dialysis patients in the Eastern Cape region of South Africa are at extremely high risk for the acquisition of TB with an incidence rate that is 4.1 times that of the local Nelson Mandela Bay population and over 5 times that reported in the general population for the country as a whole. Only informal housing and a history of hospitalization were identified as positive risk factors in this young population with a low HIV prevalence. Isoniazid prophylaxis in this high risk group might be of benefit but further studies are required to inform such treatment.

a. Introduction

Tuberculosis (TB) remains a major global health burden, responsible for ill health among millions of people each year and now ranks as the leading cause of death from a single infectious agent worldwide [1]. South Africa has a reported TB incidence of 567 cases per 100 000/year [1]. The reported incidence of TB in the Nelson Mandela Bay district, Eastern Cape Province, South Africa is 1009 per 100 000/year[2], much higher than the national average where it is reported to be the leading cause of death in ages 25-64 years[2]. Unfortunately, South Africa is still failing at a national level to reach the proposed target of 90% treatment cure rate as set by the World Health Organization, and currently sits at 82%[1].

Patients on dialysis are particularly vulnerable to active mycobacterium TB infection (hereafter referred to as TB) compared to the general population due to immune dysfunction induced by the uraemic state[3,4,5]. In other lower middle income countries (LMIC) , the incidence of TB among prevalent dialysis patients is reported to be 6.9 to 52.5 times higher than it is in the general population, depending on regional factors and background prevalence[6,7,8]. Despite this, there is a paucity of epidemiological and incidence data on TB from dialysis patients in South Africa. We are only aware of one published single-centre study by Tamayo-Isla et al. conducted in Polokwane, Limpopo Province wherein they describe a high mortality associated with TB peritonitis in CAPD patients[9].

b. Objective

To determine the incidence of TB in chronic kidney disease stage 5 patients on dialysis (CKD-5D) at a single centre in the Eastern Cape, South Africa and to identify modifiable and non-modifiable risk factors associated with TB.

c. METHODS

d. Study design and ethics approval

The study was approved by the Research Ethics Committee of the University of Cape Town (HREC/REF 127:2014), South Africa and permission was obtained from local authorities. We

conducted a retrospective cohort study of all consenting prevalent CKD-5D patients between April 2010 and March 2014 in the Livingstone Hospital Renal Unit, Eastern Cape Province, South Africa. A total of 111 consenting prevalent dialysis patients were enrolled in the study.

e. Data collection

TB was defined as "definite" or "probable" according to WHO criteria[11]. Definite TB cases were defined as cases where mycobacterium tuberculosis complex was identified from a clinical specimen either by culture or newer methods, including line probe assays. Probable TB cases were those cases with constitutional symptoms, radiological features suggestive of TB and caseating granulomas on histological tissue, but with no positive culture identification of mycobacterium TB[11]. A medical questionnaire with all the required data was used. Patient demographics including dialysis vintage, age, gender, race (self-reported), employment status, smoking, alcohol use, history of hospitalization during the study period but prior to enrollment, housing type and TB household contact status were recorded. Details concerning the diagnosis of tuberculosis, mean time between initiation of dialysis and diagnosis of TB, HIV status, Vitamin D level, comorbidities and medications were also recorded.

f. Statistical analysis

The estimated incidence was calculated as a percentage of patients surveyed and then converted into an incidence per 100 000 divided by 4 (for the 4 years) to give an estimated incidence per 100 000 per year of study period.

Normality was determined with the Shapiro-Wilk test.Continuous variables were expressed as mean ± SD while categorical variables were presented as frequencies and percentages. Univariate analysis was performed using the independent student's *t*-test, chi-squared test or the Wilcoxon rank sum test as appropriate. All analyses were conducted using Stata 12.0 Statistical Software (College Station, TX, USA).

g. Results

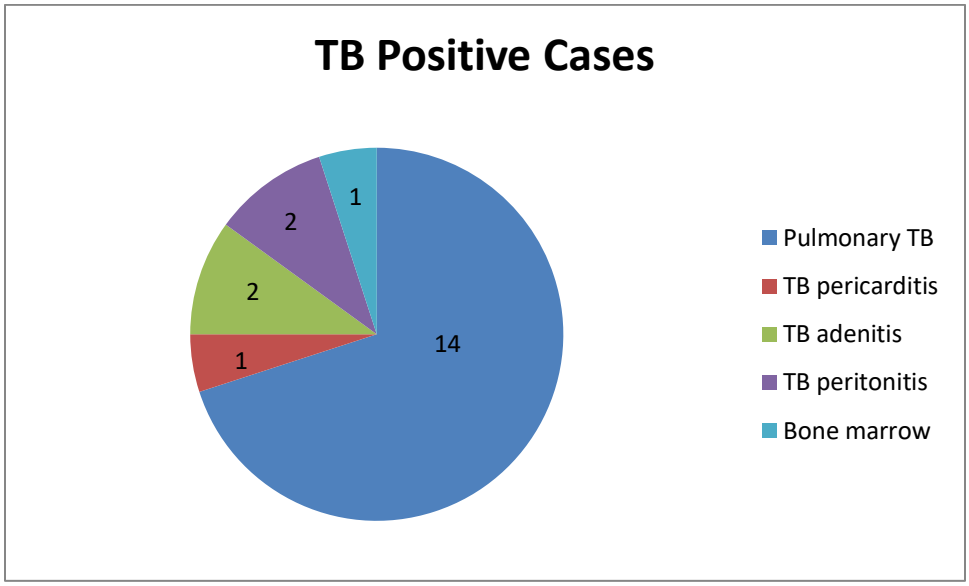
Baseline characteristics of the patients including gender, HIV status, prior immunosuppression use, employment, smoking, known TB contact status and dialysis modality are presented in Table 1. One hundred and eleven patients were enrolled: they were predominantly Black African (73%) and female (53%); mean age was 42.1 (± 9.8) years. The prevalence of HIV infection was 11%: all were on antiretroviral treatment. Sixty eight patients were on haemodialysis (HD) and forty three patients were on peritoneal dialysis (PD); 93% of the patients were unemployed.

Table 1: Baseline characteristics assessed between the TB+ and TB- dialysis patients. . Abbreviations: HD, haemodialysis; PD, peritoneal dialysis; TB, tuberculosis.

Table 1:Baseline characteristics assessed between the TB+ and TB- dialysis patients.(N=111)				
Variables	**ALL (%) (n=111)**	**TB+(%) (n=19)**	**TB-(%) (n-92)**	**P-value**
Variable	**Value**	**TB+**	**TB-**	**p-value**
Age, mean (years)	42	42.4	41.8	0.868
Gender (%)				
Female	53	45	55	0.396
Employment Status (%)				
Unemployed	93	90	93	0.584
Smoking Status (%)				
≥10 Pack years	16	10	18	0.405
< 10 pack years	84	90	82	0.405
Hospitalisation (%)	79	90	76	0.042
Housing (%)				
Brick	85	70	88	0.042
Shack	15	30	12	0.042
Use of biomass fuel (%)	8	5	8.7	0.582
TB contact (%)	28	40	25	0.174
HIV status (%)				
Positive	11	10	11	0.909
Use of immunosuppression (%)	8	1.4	6.6	0.171
Vitamin D (nmol/L) %				
(Deficiency and insufficiency)	45.7	20.8	79.2	0.800
Dialysis modality				
HD	61	18	82	0.898
PD	39	19	81	0.898

The spread of TB diagnosis is shown in Figure 1.

Figure 1 : Site of TB diagnosis



The characteristics of patients with TB are shown in table 2. Nineteen patients (18%) were diagnosed with TB over the 4 year study period, with one patient treated for TB on two occasions (patient #6 and 7 on table 2),giving a total of 20 TB cases; 14 cases were pulmonary, and 6 extrapulmonary as shown in figure 1. Of the 20 TB cases, 7 (35%) were definite TB cases and 13 (65%) had probable TB. Of the 7 definite cases: 1 (#5) had TB peritonitis with dialysate effluent culture positive for drug sensitive mycobacterium TB; 2 cases (#7 and 11) had TB adenitis and culture confirmed drug sensitive mycobacterium TB; 1 case (#1) had disseminated mycobacterium TB with a miliary pattern on chest X ray, a bone marrow biopsy that showed caseating granulomas and a mycolytic blood culture that confirmed a drug sensitive mycobacterium TB; 1 case (#9) had drug resistant mycobacterium TB(MDR) on culture; 1 case (#12) of pulmonary TB had positive acid fast bacilli on sputum microscopy but no culture was available and 1 case (#13) was pulmonary TB with a suggestive chest radiograph (CXR) and was positive for drug sensitive TB on sputum culture.

Of the thirteen probable TB cases, 10 were diagnosed based on clinical features and lymphocytic pleural effusion, 2 had pericardial effusions with stranding on echocardiogram and a pericardial tap on both patients revealed a lymphocyte-predominant picture and 1 had a miliary pattern on CXR but negative culture.

Patient	Gender	Age (years)	Race	Cause of CKD	Time on dialysis* (months)	Housing	HIV status	Dialysis modality	Location of TB Infection	TB culture and smear results	Definite/probable	Drug sensitivity**
1	Female	52	Black	HPT	4	Brick	Negative	HD	Bone marrow	Positive	Definite	Sensitive
2	Female	44	Black	HPT	63	Shack	Negative	HD	Pleural effusion	Negative	Probable	Not available
3	Female	39	Black	Unknown	3	Brick	Positive	HD	Pleural effusion	Negative	Probable	Not available
4	Female	35	Mixed	HPT	0	Shack	Negative	PD	Pleural effusion	Negative	Probable	Not available
5	Male	34	Black	Chronic GN	16	Brick	Negative	PD	Peritoneal fluid	Positive	Definite	Sensitive
6	Female	59	Black	HPT	12	Brick	Negative	HD	Pulmonary	Negative	Probable	Not available
7	Female	59	Black	HPT	12	Brick	Negative	HD	TB adenitis	Positive	Definite	Sensitive
8	Male	38	Black	Unknown	56	Brick	Positive	HD	Pleural effusion	Negative	Probable	Not available
9	Female	53	Black	HPT	38	Shack	Negative	HD	Pulmonary	Positive	Definite	MDR TB
10	Female	50	Mixed	HPT	50	Brick	Negative	PD	TB pericarditis	Negative	Probable	Not available
11	Male	34	Mixed	Unknown	72	Brick	Negative	PD	TB adenitis	Positive	Definite	Sensitive
12	Male	45	Black	HPT	35	Brick	Negative	HD	Pleural effusion	AFB on smear	Definite	Not available
13	Male	56	Black	HPT	32	Shack	Negative	HD	Pulmonary	Positive	Definite	Sensitive
14	Male	43	Black	HPT	19	Brick	Negative	PD	Pleural effusion	Negative	Probable	Unknown
15	Male	31	Black	Chronic GN	2	Brick	Negative	PD	TB pericarditis	Negative	Probable	Unknown
16	Male	28	Mixed	HPT	13	Brick	Negative	PD	Milliary	Negative	Probable	Unknown
17	Female	41	Mixed	Chronic VUR	44	Brick	Negative	HD	Pleural effusion	Negative	Probable	Unknown
18	Male	35	Black	Unknown	37	Shack	Negative	HD	Pleural effusion	Negative	Probable	Unknown
19	Male	38	Black	HPT	59	Shack	Negative	HD	Pleural effusion	Negative	Probable	Unknown
20	Male	41	Black	HPT	49	Brick	Negative	PD	Pleural effusion	Negative	Probable	Unknown

Table 2 : Demographics and clinical characteristics of the patients who had TB.

Abbreviations: CKD, chronic kidney disease; HPT, hypertension; GN, glomerulonephritis; INH, isoniazid; HD, haemodialysis; PD, peritoneal dialysis; MDR-TB, multidrug resistant tuberculosis; TB, tuberculosis;,VUR, vesicoureteral reflux, AFB – Acid fast bacilli

*Represents duration on dialysis before diagnosis with Tb

**Represents drug sensitivity to first-line anti-tuberculous agents

The calculated incidence of TB in our unit was 4505 per 100 000 per year (see Figure 2 for a comparison to local incidence).

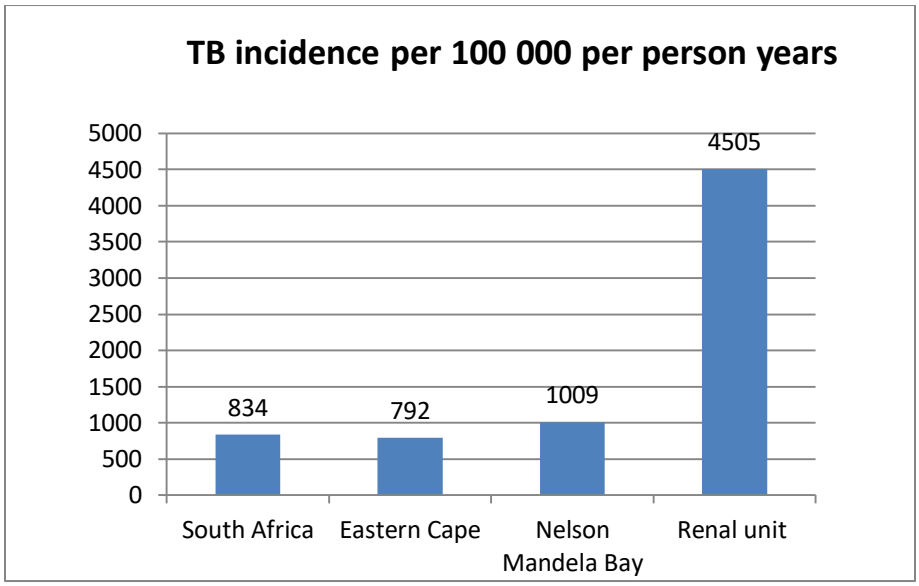


Figure 2: Incidence of tuberculosis in the study population compared to the local, regional and national incidence in 2015[12,13].

h. Discussion

This single-centre retrospective cohort study reveals an extraordinarily high incidence of TB in dialysis patients in the Eastern Cape Province of South Africa, with a calculated incidence of TB 4.1 times that of the local population and over 5 times that reported in the general population for the country as a whole. This ratio is consistent with the previously reported results from other areas in the world [8]. The patients are predominantly young and HIV negative, reflecting the selection bias that exists within the South African public sector dialysis programme. Moosa et al have previously reported on rationing of dialysis treatment in South Africa and that patients are screened and accepted onto the dialysis programme based on favourable medical and socioeconomic criteria[14].

Several studies have shown that the risk of TB in dialysis patients is increased with incidence rates reported at 6.9-52.5 times higher than the general population [7, 8, 15]. This observed increase in the incidence of TB in dialysis patients may be related to impaired cellular immunity in uraemic patients [3,16,17]. Other factors which might contribute to the decreased immunity are malnutrition, vitamin D deficiency and hyperparathyroidism [18].

Known TB risk factors assessed in this study included age, employment status, smoking, alcohol use, average number of days admitted to hospital per year, housing type and TB contact status, HIV and Vitamin D levels[19,20]. However, of all these risk factors, only informal housing and a history of hospitalization were significantly associated with a diagnosis of TB ($p < 0.05$). In South Africa, informal housing (shack dwelling) is associated with overcrowding and unhygienic conditions which would favour the transmission of TB in the community. While a history of hospitalization may represent a sicker cohort prone to TB, the lack of adequate isolation facilities in our hospital during the study period may well have contributed to nosocomial transmission of TB to our patients, although this is speculative. Interestingly, HIV was not predictive of TB in our study, but this may be confounded by the low overall prevalence of HIV in our unit (11%). This low HIV prevalence reflects a selection bias for non-HIV positive patients in the public sector, despite a higher background HIV prevalence of 19.9% among the age group15-49

years[20]. Moreover, all HIV positive patients on dialysis have undetectable viral loads and relatively preserved CD4 counts as per dialysis selection criteria in the public sector. More patients with TB had a history of known contact (40% vs 25%), but this was not statistically significant (p= 0.174). The combined vitamin D deficiency/insufficiency prevalence for our cohort of patients was 45.7%. Vitamin D deficiency was not shown to be a significant risk factor for the development of TB in this cohort. However, the vitamin D levels were not measured at the time of TB diagnosis since the assay has not always been available at our study centre.

The incidence of extrapulmonary TB is very high at 30% in our study and is consistent with reports from previous studies of CKD patients [8,11,22-25]. The increased tendency for extra pulmonary tuberculosis in chronic kidney disease is thought to be due to impaired cellular immunity[24]. In dialysis patients, TB symptoms are often non-specific and difficult to distinguish from uraemic symptoms which can result in delayed diagnosis [8, 24]. This may also facilitate transmission to other patients in the unit, particularly in haemodialysis units where patients spend much time in close proximity to each other. Of the 20 TB cases, 7 (35%) were definite cases with positive microscopy or culture. Tamayo-Isla et al, in their retrospective TB peritonitis cohort study in South Africa, reported that mycobacterium tuberculosis was isolated in only 75% of their cohort[9]. In our cohort, 13 of the 20 (65%) patients with TB were culture negative, reflecting the difficulty of definitive TB diagnosis in dialysis patients.

Isoniazid (INH) chemoprophylaxis in HIV positive individuals has been found to be efficacious in a number of large scale randomized controlled trials with up to 74% reduction in TB incidence in select groups with a positive tuberculin skin test[26]. Clear guidelines exist for the HIV positive population, but data on INH chemoprophylaxis in CKD patients is lacking. A few randomized clinical trials have assessed INH chemoprophylaxis in both pre and post-transplant patients and demonstrate benefit in administering chemoprophylaxis to transplant recipients for up to 1 year [27,28,29]. Further large randomized clinical studies in the dialysis setting are required to help inform best practice on when to commence INH chemoprophylaxis and the most efficacious duration of therapy.

There were some limitations to the study. Due to the retrospective nature of the study, we were unable to include dialysis patients who had died during the study period, due to health record unavailability. Another limitation is that we cannot make assumptions about hospitalization as a risk factor for TB since we cannot prove causality, and details regarding the reason for admission, length of stay and timing in relation to onset of TB are not available. Strengths include the use of a single laboratory with standardized methodology as well as the availability of accurate clinical notes for determination of diagnosis. While the overall numbers are relatively low for this cohort, a further strength is that the study included all patients alive on dialysis in the public sector for the entire Western part of the Eastern Cape and is therefore inclusive.

i. Conclusion

Dialysis patients in the Eastern Cape region of South Africa are at extremely high risk for the acquisition of tuberculosis, with a calculated incidence of 4505 per 100 000 population per year. There is a need for improved health care structures including the provision of adequate isolation facilities for patients with tuberculosis in our hospital and renal unit in order to reduce transmission. Further research on outcomes and response to TB treatment of CKD patients in South Africa is required. Whether INH prophylaxis is a reasonable option for TB prevention in this population remains unknown and should be the subject of further study.

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PART D: Supporting Documents

a. Consent Form for Participation in a Research Study

STUDY TITLE: The study of the burden of tuberculosis in patients with chronic kidney disease stage 5 who are undergoing renal replacement therapy with dialysis (CKD-5D) at Livingstone hospital renal unit in Port Elizabeth

WHY IS THIS STUDY BEING DONE?

You are invited to participate in a research study conducted by Dr Siviwe Ndamase. South Africa has a huge burden of tuberculosis and patients with chronic kidney disease are at increased risk of contracting tuberculosis compared to the general population. This study is done to determine burden of tuberculosis in dialysis patients at Livingstone hospital renal unit.

Benefit of the study?

The research will help inform us of the burden of tuberculosis in chronic kidney disease patients on dialysis at Livingstone hospital. With the information gained from this research we hope to make recommendations that will inform and ultimately improve the care of dialysis patients in the Eastern Cape. We also hope to identify modifiable risk factors that we can address.

How and when will the study take place?

Your participation will involve answering a few questions and will take approximately 5 minutes. The questions will be answered in an interview format during one of your dialysis sessions. Questions will be explained in a language that is easy to understand for the interviewee. The interviewer is Xhosa and English speaking and will conduct the interview in Xhosa where required.

Risks

There are no known risks associated with this research.

Informed consent and confidentiality

Your names will not be recorded on the questionnaires and only the folder number will be used. The information obtained from the interviews will only be accessible to the individuals involved in the study. Your participation in this research study is voluntary. You may choose not to participate and you may withdraw your consent to participate at any time. You will not be penalized in any way should you decide not to participate or to withdraw from this study. Your HIV status will also be requested and the solely for the research purposes and will be kept completely confidential.

Your privacy will be respected and only folder numbers and no patient names will be used.

This consent form has been explained to me and I have been given the opportunity to ask questions. I fully understand the details and implications of the study and hereby give my consent to participate in this study.

Participant's signature_____ Date:_____

A copy of this consent form will be given to you.

b. Data record form

Data record form	
Demographics	
Case number	
Hospital number	
Consent signed	
Date of Birth:	
Age in years:	

Race(self-reported) :	□Black □ White □Mixed □ Asian
Gender:	□Male □Female
Employment status:	□Employed □unemployed
Smoking:	□Never □ current □ previous use If so: number of pack years
Do you ever drink alcoholic beverages?	□Yes □ No If yes, what is your approximate intake of these beverages (units per week)?
What type of house do you live in?	□Brick-house □shack
How many people do you live with in the house?	
What fuel appliances do you use to cook at home:	□Electrical □gas □wood
	Dialysis history
Dialysis day:	
Which year did you start dialysis	
Months on peritoneal dialysis:	
Months on haemodialysis	
Total months on any dialysis:	
Total months on any dialysis prior to TB diagnosis (where applicable)	
Date of dialysis cessation (where applicable) and why (example Tx/death etc)	Date: Why?
Have you been admitted to hospital between 1 April 2010 and 31st March 2014?	□Yes □ No If yes number of days:_____

Tuberculosis history	
Did you get diagnosed TB between April 2010 and March 2014?	□Yes □ no If yes when----------
Is there anyone at home who has been diagnosed with tuberculosis?	□Yes □No If yes, when?
Details to get from file	
What type of TB were you diagnosed with	□Pulmonary □ extra-pulmonary
How was the diagnosis made?	□Definite (mycobacterium TB identified on a clinical specimen either by culture or new molecular line probe assay specimen/acid fast bacilli) _____ □Probable (AFB'S/TB culture negative for tuberculosis but patient with clinical features, histological and radiological abnormalities consistent with TB) :_____
Drug sensitivity where known	□Drug sensitive □mono-resistance □MDR □XDR
Other medical history	
Have you been diagnosed with diabetes?	□yes □ no
Have you ever been on any immunosuppressive drugs e.g. prednisone, cyclosporine, azathioprine, and	□ Yes □ No

cyclophosphamide:	
Primary diagnosis and cause of CKD if known	1._____ 2._____ 3._____
Laboratory Results (Last Known Blood Results)	
Test	**Date**
Albumin	
Haemoglobin	
25,hydroxyVitamin D3	
HIV status	
Ferritin	
%transferrin sats	

c. UCT ethics approval document

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • Facsimile [021] 406 6411
Email: Sumayah.arieff@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

17 March 2014

HREC/REF: 127/2014

Dr R Freercks
Nephrology & Hypertension
28 Wares Road
Mill Park
Port Elizabeth 6001

Dear Dr Freercks

Project Title: THE BURDEN OF TUBERCULOSIS IN PATIENTS WITH CHRONIC KIDNEY STAGE 5 WHO ARE UNDERGOING RENAL REPLACEMENT THERAPY WITH DIALYSIS AT LIVINGSTONE HOSPITAL RENAL UNIT IN PORT ELIZABETH-MMed- Dr Sivlwe Ndamase

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

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

Approval is granted for one year until the 28 March 2015.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:- Dr Sivine Ndamase is also involved in this study.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator

Please quote the HREC REF in all your correspondence.

Yours sincerely

Signature removed to avoid exposure online

PROFESSOR M BLOCKMAN

Hrec/ref:127/2014

CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

d. Livingstone Hospital approval letter

Province of the
EASTERN CAPE
HEALTH

Office of the Medical Superintendent- 1st Floor .Nurses Home- Livingstone Hospital
. Stanford Road- Korsten- Port Elizabeth
PO Korsten - Port Elizabeth - 6014. REPUBLIC OF SOUTH AFRICA
Tel: +27 (0)41 405 2100/2102 . Fax: +27 (0)41 405 2103
E-mail: robyn.may@impilo.ecprov.gov.za: francis.gelderblaem@impilo.ecprov.gov.za

26 February 2014

Dr Siviwe Ndamase
ICU
Livingstone Hospital
Korsten
6014

APPLICATION TO CONDUCT A STUDY IN THE RENAL UNIT

Your application to conduct a study in the Renal Unit under Dr Freercks' supervision is supported in principle. Approval will be granted based on the following conditions:

1) The proposal must be approved by the Human Research Ethics Committee at UCT.
2) You must observe and respect the rights of your research participants and ensure that confidentiality is maintained.
3) At the completion of your study you are expected to provide a copy of your findings and recommendations to this hospital.
4) Your results will not be presented anywhere unless you have first shared them with the Department of Health as indicated above.

signature removed to avoid
exposure online

SENIOR MANAGER: MEDICAL SERVICES
/fg

e. Acceptance of abstract for moderated poster presentation at world congress of nephrology 2015

Brussels, 28/11/2014

Dear Dr Ndamase,

Original Abstract Number: WCN15-1194
Abstract Title: HIGH BURDEN OF TUBERCULOSIS IN SOUTH AFRICAN DIALYSIS PATIENTS: THE REPORT OF A SINGLE CENTRE IN THE EASTERN CAPE, SOUTH AFRICA.
Number of your Poster Board: SUN-041

Thank you for submitting your abstract(s) to the World Congress of Nephrology 2015. We are pleased to inform you that your abstract as listed above has been selected by the Scientific Program Committee for Moderated Poster Presentation at the Congress.

Please find below important instructions for the preparation of your presentation at the congress. We recommend you to carefully read through all information provided.

Registration
To present your poster abstract, you must be registered to the Congress. WCN 2015 will provide Early Bird Registration for all poster presenters provided they register before **14 December 2014**. If you are not yet registered, you can do so by simply clicking on the following link and follow the instructions.http://www.wcn2015.org/registration-home
Kindly register using the same login details you have used for submitting your abstract, otherwise we will not be able to link your abstract to your registration. For your convenience find your logon details below:

User name: Ndamase.Siviwe
Password: 77225

After completion of your registration you will receive a confirmation notification per email. Two weeks prior to the congress you will receive a personalized barcode letter, which you must bring with you to the Congress when picking up your badge and Congress material in the registration area. If you applied for travel grant, please do not register before receiving your travel grant notification (by December 5, 2014).
Accommodation
After registering we remind you to also secure your accommodation in one of the designated Congress hotels. Note that demand on hotels in Cape Town is high so booking early is strongly advised. Bookings can be made through the official housing agency via http://www.wcn2015.org/accommodation .

Poster Area

Posters will be located in the WCN 2015 Exhibition Hall situated on the ground floor of the Cape Town International Convention Centre (CTICC).
Posters should be the following size: AO-size (1189 mm High x 841 mm Wide), portrait orientation.
Important note: Larger posters in a different orientation will not fit on the poster boards.
Your poster will be scheduled for presentation for one day only. Please see below for the scheduled day of your poster. The Exhibition Hall opens daily for poster presenters to mount their posters between 09.00 am – 10.30am. Your poster must be removed between 03:40pm and 04:30pm latest. Posters that are not removed by 04:30pm will be removed by the organizers and can, unfortunately, not be recovered.

Poster Presentation Date: 15/03/2015
Number of your Poster Board: SUN-041

During the time of the moderated poster session you are expected to be physically present at your poster as it will be viewed and moderated. Please have your poster ready for viewing no later than 10:30am on the day of your presentation. The times of the moderated sessions are as follows:

Saturday, 14 March	11:00am – 12:00pm
Sunday, 15 March	11:00am – 12:30pm
Monday, 16 March	11:00am – 12:30pm

Online ePoster Library
In addition to your physical poster, the WCN 2015 will feature an online ePoster library. This library will be accessible onsite at electronic poster kiosks, as well as via the WCN web site during and for 12 months after the Congress. The library will allow all those who visit the congress to view your scientific work and interact with you through the email address displayed on the website and as such will increase the visibility of your scientific work.
The WCN 2015 is grateful to Roche for its support in making the online ePoster library possible.
Additional information on how to access the online ePoster Library, including login and customer service details will be provided separately after 8 January. For any queries about the online poster library, please contact societyposter@learnersdigest.com.

Poster Printing Service
The World Congress of Nephrology 2015 has selected Call4Posters® as their preferred poster printing service for WCN 2015. This service is the simple and most convenient way to print your poster and pick it up on-site at the congress in Cape Town.

Additional information in this service will be sent after 8 January in a separate email. Please take note there will be no onsite poster printing service. For any queries about the online poster library, please contact societyposter@learnersdigest.com.
We congratulate you again on having your abstract selected for presentation at the ISN World Congress of Nephrology 2015. We appreciate your participation and look forward to seeing you in Cape Town.
Sincerely,

Markus Ketteler
WCN 2015 Abstract Co-Chair

Mohammed Rafique Moosa
WCN 2015 Abstract Co-Chair

f. SAMJ Manuscript preparation guidelines

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
    - **Background:** why the study is being done and how it relates to other published work.
    - **Objectives:** what the study intends to find out
    - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
    - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
    - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.

- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

From submission to acceptance

Submission and peer-review

To submit an article:

- Please ensure that you have prepared your manuscript in line with the SAMJ requirements.
- All submissions should be submitted via Editorial Manager
- The following are required for your submission to be complete:
  - Anonymous manuscript (unless otherwise stated)
  - **Author Agreement form**
  - Manuscript
  - Any supplementary files: figures, datasets, patient consent form, permissions for published images, etc.
- Once the submission has been successfully processed on Editorial Manager, it will undergo a technical check by the Editorial Office before it will be assigned to an editor who will handle the review process. If the author guidelines have not been appropriately followed, the manuscript may be sent back to the author for correcting.

Sponsored supplements

Contact claudian@hmpg.co.za for information on submitting ad hoc/commissioned supplements, including guidelines, conference/congress abstracts, Festschrifts, etc.

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. Named authors consent to publication and meet the requirements of authorship as set out by the journal.
2. The submission has not been previously published, nor is it before another journal for consideration.
3. The text complies with the stylistic and bibliographic requirements in **Author Guidelines**.
4. The manuscript is in Microsoft Word document format. The text is single-spaced, in 12-point Times New Roman font, and contains no unnecessary formatting.
5. Illustrations/figures are high resolution/quality (not compressed) and in an acceptable format (PDF or jpeg). These must be submitted individually as 'supplementary files' (not solely embedded in the manuscript).
6. For illustrations/figures or tables that have been published elsewhere, the author has obtained written consent to republication from the copyright holder.
7. Where possible, references are accompanied by a digital object identifier (DOI).
8. An abstract has been included where applicable.
9. The research was approved by a Research Ethics Committee (if applicable)
10. Any conflict of interest (or competing interests) is indicated by the author(s).