Tuberculosis In Paediatric Kidney Transplant Recipients – A Single Centre Experience

UNIVERSITY OF CAPE TOWN

Degree: Master's degree of Philosophy In Paediatric Nephrology

By

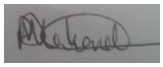
PRIVILAGE D. MAKANDA-CHARAMBIRA

MKNPRI015

Contents

Declaration

This research reported is based on independent work performed by the candidate (P.D. Makanda-Charambira) and neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. This work has not been reported or published prior to registration for the above mentioned degree.



Privilage D. Makanda-Charambira

Abstract

Background: Tuberculosis remains a major challenge in transplantation particularly in endemic countries. This study aims to describe the incidence, clinical presentation and outcomes of tuberculosis in paediatric kidney transplant recipients and to assess the impact of Isoniazid prophylaxis. Methods: Single-centre retrospective descriptive analysis of children who received kidney transplants from 1995-2019. The cohort was stratified according to receipt of isoniazid prophylaxis which began in 2005. Results: 212 children received a kidney transplant during the study period. Median age at transplantation was 11.2 years (IQR: 2.2 – 17.9) and 56% were males. Tuberculosis was diagnosed in 20 (9%) children, with almost two thirds (n=12) occurring within the first year. Most infections were pulmonary. The main presenting symptoms included fever (n=13/20), weight loss (n=12/20) and cough (n=10/20). Tuberculin skin test was positive in four of 20 children. Coinfection with Ebstein Barr virus, Cytomegalovirus or Staphylococcus was found in five children. Due to drug interactions, an up to three fold increase in calcineurin inhibitor dose was required to maintain therapeutic blood levels. Isoniazid prophylaxis was protective against development of tuberculosis (p=0.04). Gender, age and type of allograft were not significant risk factors. Graft and patient survival were 100% upon completion of TB treatment Conclusion: Kidney transplant recipients in endemic countries have a high risk of developing tuberculosis. Diagnosis remains a challenge. Frequent and meticulous monitoring of immunosuppression drug levels during treatment of TB is required to avoid loss of patient or graft. Isoniazid prophylaxis protects against development of TB in this population.

Acknowledgements

This work has been made possible through an ISN-IPNA funded Fellowship awarded to Dr Makanda-Charambira. The International Society of Nephrology (ISN) is a global professional association dedicated to advancing worldwide kidney health. The International Pediatric Nephrology Association (IPNA) is a global, non-profit charitable organization that works to disseminate knowledge about kidney disease in children in the areas where care is needed most.

The author is grateful to Prof M.McCulloch, Prof V.Luyckx, Dr P.Nourse and Dr A.Coetzee who supervised this work, and Kelvin Charambira and Collins Timire for assisting with statistical analysis.

List of tables

List of figures

Abbreviations

AFB, acid fast bacilli; Aza, azathioprine; CMV, *cytomegalovirus;* CNI, calcineurin inhibitors; CSA, cyclosporine; EBV, *Epstein Barr Virus;* FK, tacrolimus; IGRA, interferon gamma release assay; IL2, interleukin 2; INH, isoniazid; LTB, latent tuberculosis; MMF, mycophenolate mofetil; MTB/RIF, mycobacteria tuberculosis/Rifampicin; OKT3, muromonab CD3; Pred, prednisolone; RAPA, rapamycin; SDG, sustainable development goals; Staph, *staphylococcus;* TB, tuberculosis; TNF, tumor necrosis factor; TST, tuberculin skin test.

Chapter 1: INTRODUCTION

1.1 Context

Tuberculosis (TB) is a highly communicable disease and is the leading cause of death from a single infectious agent globally [1] after SARS-COV2 infection . It is estimated that 10.0 million (range, 9.0–11.1 million) people globally fell ill with TB in 2018 [1]. The highest TB incidence rates in the world occur in sub-Saharan Africa. South Africa is among the top 20 countries in the world with a high burden of TB [2]. The World Health Organisation (WHO) 2019 statistics give an estimated incidence in South Africa of 615 per 100 000 population [2]. With such a global burden of TB, sustainable development goal (SDG)3 target 3.3, explicitly states that efforts should be made to end the epidemics of TB and other communicable diseases by 2030 [3].

Mycobacteria tuberculosis, the bacteria that causes TB, is spread from person to person via droplets. It commonly affects the lungs, although it can spread to become multisystemic with a myriad of presentations depending on the organ involved. Diagnosis is made by a suggestive history (cough, weight loss, night sweats, contact), examination (lymphadenopathy, wasting, and pleural effusion) and the presence of acid-fast-bacilli (AFB) on sputum microscopy or other specimens or detection using geneXpert technology. Clinical investigations of childhood TB are however hampered by the paucibacillary nature of the disease and the difficulties in obtaining specimens. Molecular technology such as the geneXpert MTB/RIF improves TB diagnosis in the paediatric population, and increases the diagnosis of bacteriologically confirmed TB two-fold compared to microscopy [4].

The incidence, clinical manifestations, and optimal investigations for TB specifically in the post-transplant population have not yet been adequately studied. Globally, the median time of onset of TB is estimated at nine months post transplantation[5], with an incidence estimated to be 20 to 70 times higher than that in the general population, and a mortality rate of up to 30% [6]. Immunosuppression exacerbates the risk and clinical course of the disease. Classical manifestations of TB including cough, haemoptysis, and shortness of breath are nonspecific[7]. TB presents diagnostic challenges due to its atypical presentation in this subgroup of patients. Co-infection with other organisms such as cytomegalovirus (CMV) has been reported in about 19% of TB cases [8]. Management of TB post-transplant is complicated by greater toxicity of treatment and important drug interactions with immunosuppressive medications[9].

Few studies have been conducted on tuberculosis in the paediatric population after kidney transplant [10]. A previous study from our centre conducted between 1996 – 2004 found a TB incidence of 9.7% (7/72) in children post-kidney transplant [11]. TB prophylaxis was not being used routinely during this time. All cases were successfully treated with meticulous monitoring of immunosuppression drug levels, with no loss of patient or graft. Results of this study led to a change in policy in the Western Cape where all children now receive daily isoniazid prophylactically after kidney transplantation to prevent TB infection.

Important gaps therefore remain in our understanding of the burden of TB in children with kidney transplants:

1. Clinical risk factors associated with TB infection in our setting must be determined as INH prophylaxis alone does not prevent all infections.
2. Clinical presentation of TB in the paediatric transplant population and utility of various new diagnostic possibilities must be understood to improve diagnosis.
3. The complications (clinical, medication interactions, outcomes) of TB in children with kidney transplants must be better described.
4. More studies are required to identify preferred treatment modalities which minimize drug-drug interactions and reduce risk of graft loss.
5. Evaluation of the impact of routine INH prophylaxis on TB incidence and outcomes in our centre has not yet been conducted.
6. There is no clarity on the duration of INH prophylaxis in the paediatric post-transplant population, especially when the background infection risk remains high, and re-initiation of INH during episodes of high risk such as treatment of rejection.

Given the high prevalence of TB in our setting, the recent change in practice regarding INH prophylaxis, and the paucity of data from paediatric transplant centres in low and middle income countries, we conducted a retrospective review of tuberculosis in recipients of kidney transplants at out centre to determine the incidence and factors associated with TB infection in paediatric kidney transplant recipients.

1.2 Ethical considerations

This study complied with the ethical guidelines and principles of the Helsinki Declaration of 2008, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research and was approved by the Humah Research Ethics committee of the University of Cape Town (Ref 463/2020).

Medical records of all children below 18 years of age, who received kidney transplants were retrieved. To protect study participant privacy and confidentiality, patient names were used to access files but only study numbers were used on the electronic database. De-identified data was extracted into a case report form and imported into Excel. Data entry was re-checked by an independent investigator. A data key list including patient identification and study numbers was stored separately from the de-identified data set and was accessible exclusively to the Principle Investigator. All paper records were stored in a locked cupboard until data entry and study completion, after which paper records were destroyed. Electronic data were stored on a password protected computer, only accessible to the research team.

1.3 Authors guidelines

Pediatric Transplantation (PETR) is the official journal of the International Pediatric Transplant Association (IPTA). PETR seeks to provide its readership with literature of the highest quality and impact through a process of careful peer review and editorial comment. All papers, including those invited by the Editorial Board, are subject to peer review (see appendix 2).

References

1. GraphicExecutiveSummary.pdf. Accessed June 22, 2020. https://www.who.int/tb/publications/global_report/GraphicExecutiveSummary.pdf?ua=1

2. TB Statistics for South Africa | National & provincial. TBFacts. Accessed March 7, 2020. https://tbfacts.org/tb-statistics-south-africa/

3. Sustainable Development Report 2019. Accessed June 2, 2020. https://www.sustainabledevelopment.report

4. Raizada N, Sachdeva KS, Nair SA, et al. Enhancing TB Case Detection: Experience in Offering Upfront Xpert MTB/RIF Testing to Pediatric Presumptive TB and DR TB Cases for Early Rapid Diagnosis of Drug Sensitive and Drug Resistant TB. *PLOS ONE*. 2014;9(8):e105346. doi:10.1371/journal.pone.0105346

5. Singh N, Paterson DL. Mycobacterium tuberculosis infection in solid-organ transplant recipients: impact and implications for management. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 1998;27(5):1266-1277. doi:10.1086/514993

6. Muñoz P, Rodríguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2005;40(4):581-587. doi:10.1086/427692

7. Sundaram M, Adhikary SD, John GT, Kekre NS. Tuberculosis in renal transplant recipients. *Indian J Urol IJU J Urol Soc India*. 2008;24(3):396-400. doi:10.4103/0970-1591.42625

8. Boubaker K, Gargah T, Abderrahim E, Ben Abdallah T, Kheder A. Mycobacterium tuberculosis Infection following Kidney Transplantation. BioMed Research International. doi:https://doi.org/10.1155/2013/347103

9. Ram R, Swarnalatha G, Prasad N, Dakshinamurty KV. Tuberculosis in renal transplant recipients. *Transpl Infect Dis*. 2007;9(2):97-101. doi:10.1111/j.1399-3062.2006.00182.x

10. El-Husseini AA, Sobh MA, Ghoneim MA. Complications of pediatric live-donor kidney transplantation: a single center's experience in Egypt. *Pediatr Nephrol*. 2008;23(11):2067-2073. doi:10.1007/s00467-007-0669-x

11. McCulloch M, Gajjar P, Spearman W, et al. Overview of a paediatric renal transplant programme. *S Afr Med J*. Published online 2006. doi:10.7196/SAMJ.1294

Chapter 2: PUBLICATION-READY MANUSCRIPT

Tuberculosis In Paediatric Kidney Transplant Recipients – A Single Centre Experience

Authors: Makanda-Charambira PD[1*], Nourse P[1], Luyckx VA[2], Coetzee A[1], McCulloch MI[1]

[1]Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa

[2]Paediatric Nephrology Department, University Children's Hospital Zurich, Zurich, Switzerland

[*]**Corresponding author:** Makanda-Charambira PD, Department of paediatric nephrology, Red Cross War Memorial Children's Hospital, Rondebosch, Cape Town, South Africa Email:pmakanda@psmi.co.zw

ABSTRACT

Background: Tuberculosis remains a major challenge in transplantation particularly in endemic countries. This study aims to describe the incidence, clinical presentation and outcomes of tuberculosis in paediatric kidney transplant recipients and to assess the impact of Isoniazid prophylaxis.

Methods: Single-centre retrospective descriptive analysis of children who received kidney transplants from 1995-2019. The cohort was stratified according to receipt of isoniazid prophylaxis which began in 2005.

Results: 212 children received a kidney transplant during the study period**.** Median age at transplantation was 11.2 years (IQR: 2.2 – 17.9) and 56% were males. Tuberculosis was diagnosed in 20 (9%) children, with almost two thirds (n=12) occurring within the first year post-transplant. The main presenting symptoms included fever (n=13/20), weight loss (n=12/20) and cough (n=10/20). Tuberculin skin test was positive in four of 20 children. Coinfection with *Ebstein Barr virus, Cytomegalovirus* or *Staphylococcus* was found in five children. Due to drug interactions, an up to three fold increase in calcineurin inhibitor dose was required to maintain therapeutic blood levels. Isoniazid prophylaxis was protective against development of tuberculosis (p=0.04). Gender, age and type of allograft were not significant risk factors for developing tuberculosis. All the tuberculosis infections were successfully treated. Graft and patient survival was 100% when assessed one month after completion of TB treatment

Conclusion: Kidney transplant recipients in endemic countries have a high risk of developing tuberculosis. Diagnosis remains a challenge. Frequent and meticulous monitoring of immunosuppression drug levels during treatment of TB is required to avoid loss of patient or graft. Isoniazid prophylaxis protects against development of TB in this population.

KEY WORDS

Tuberculosis, kidney, transplant, allograft, infection, Isoniazid prophylaxis

Abbreviations

AFB, acid fast bacilli; Aza, azathioprine; CMV, *cytomegalovirus;* CNI, calcineurin inhibitors; CSA, cyclosporine; EBV, *Epstein Barr Virus;* FK, tacrolimus; IGRA, interferon gamma release assay; IL2, interleukin 2; INH, isoniazid; LTB, latent tuberculosis; MMF, mycophenolate mofetil; MTB/RIF, mycobacteria tuberculosis/Rifampicin; OKT3, muromonab CD3; Pred, prednisolone; RAPA, rapamycin; SDG, sustainable development goals; Staph, *staphylococcus;* TB, tuberculosis; TNF, tumor necrosis factor; TST, tuberculin skin test.

Acknowledgements

This work has been made possible through an ISN-IPNA funded Fellowship awarded to Dr Makanda-Charambira. The International Society of Nephrology (ISN) is a global professional association dedicated to advancing worldwide kidney health. The International Pediatric Nephrology Association (IPNA) is a global, non-profit charitable organization that works to disseminate knowledge about kidney disease in children in the areas where care is needed most.

The authors are grateful to Kelvin Charambira and Collins Timire for assisting with statistical analysis.

INTRODUCTION

Tuberculosis (TB) is a highly communicable disease and is the leading global cause of death from a single infectious agent after SARS-COV2 infection[1]. It is estimated that 10.0 million (range, 9.0–11.1 million) people globally fell ill with TB in 2018 [1]. The highest TB incidence rates in the world occur in sub-Saharan Africa. South Africa is among the top 20 countries in the world with a high burden of TB [2]. The World Health Organisation (WHO) 2019 statistics give an estimated incidence in South Africa of 615 per 100 000 population [2].

Mycobacteria tuberculosis, the bacteria that causes TB, is transmitted from person to person via droplet spread. It commonly affects the lungs; although it can be multisystemic with a myriad of presentations, depending on the organ involved. Diagnosis is made by a suggestive

history (cough, weight loss, night sweats, and contact), examination (lymphadenopathy, wasting, and pleural effusion) and/or the presence of acid-fast-bacilli (AFB) on sputum microscopy, gastric washings or other specimens; or detection using GeneXpert technology. Clinical investigation of childhood TB is hampered by the paucibacillary nature of the disease and the difficulties in obtaining specimens. Molecular technology such as GeneXpert MTB/RIF improves TB diagnosis within the paediatric population, and increases the diagnosis of bacteriologically confirmed TB two-fold compared to microscopy [4]. First-line treatment of TB involves use of a standard combination of drugs (Isoniazid, Ethambutol, Pyrazinamide and Rifampicin) given over a prolonged period. The incidence, clinical manifestations, and optimal investigations for TB specifically in the paediatric post-transplant population have not been adequately studied. Globally, the median time of onset of TB post transplantation is estimated at nine months[5], with an incidence estimated to be 20 to 70 times higher than that in the general population. Immunosuppression exacerbates the risk and complicates the clinical course of the disease.

TB presents diagnostic challenges due to its atypical presentation in the transplant population. Classical manifestations of TB including cough, haemoptysis, and shortness of breath are nonspecific[6]. In addition, co-infection with other organisms such as cytomegalovirus (CMV) has been reported in about 19% of TB cases [7]. Management of TB post-transplant is complicated by greater toxicity of treatment and important drug interactions with immunosuppressive medications[8]. There is variability in dose and duration of treatment regimens used in different centres[6]. Randomized controlled studies are lacking. Isoniazid (INH) prophylaxis has been used to prevent post-transplant TB in endemic areas without significant adverse effects [9,10].

Few studies have been conducted on TB in the paediatric population after kidney transplant [11]. A previous study from our centre conducted between 1996 – 2004 found a TB incidence of 9.7% (7/72) in children post-kidney transplant [12]. TB prophylaxis was not routinely used during this time. All patients were successfully treated, with meticulous monitoring of immunosuppression drug levels, with no loss of patient or graft. Results of this study led to a change in policy in the Western Cape where all children now receive daily isoniazid prophylactically for at least a year after kidney transplantation to prevent TB infection.

Given the high prevalence of TB in our setting, the recent change in practice regarding INH prophylaxis, and the paucity of data from paediatric transplant centres in low and middle

income countries, we conducted a retrospective review of tuberculosis in recipients of kidney transplants at out centre, to answer the following questions :

1. Describe the clinical presentation of TB and the utility of new diagnostic tools.
2. Determine clinical risk factors associated with TB infection, in the paediatric kidney transplant population.
3. Describe the outcomes and complications (clinical, medication interactions, outcomes) of TB infection in the paediatric kidney transplant population and strategies to minimize drug-drug interactions and reduce the risk of graft loss.
4. Evaluate the impact of routine INH prophylaxis on TB incidence and outcomes, consider duration of therapy and use during higher-risk times such as treatment or rejection.

PATIENTS AND METHODS

The study was conducted at Red Cross War Memorial Children`s Hospital (RCWMCH), located in Cape Town, Western Cape Province, South Africa. RCWMCH is a tertiary stand-alone children's hospital providing highly sub-specialised paediatric care, including paediatric nephrology, to a broad catchment area. Children from all nine provinces of South Africa and from all over Africa are referred to the Hospital by outside hospitals and clinics.

Clinical approach at RCWMCH

Diagnosis of tuberculosis

TB was classified according to WHO definitions outlined in ***Guidance for national tuberculosis programmes on the management of tuberculosis in children (Second edition)***[13]:

Tb contact – A close contact is defined as living in the same household or in frequent contact with a source case with sputum smear- positive TB.
Presumptive TB - a patient who presents with symptoms or signs suggestive of TB (previously known as a TB suspect).
A bacteriologically confirmed TB cases - one from whom a biological specimen is positive by smear microscopy, culture or WHO-approved rapid diagnostics (such as GeneXpert MTB/RIF).

A clinically diagnosed TB case - is one which does not fulfil the criteria for bacteriological confirmation but which has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation.

Pulmonary TB (PTB) - any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree.

Extrapulmonary TB (EPTB) - any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges.

Routine pre-transplant screening and prophylaxis regimen

Pre-transplant workup in recipients at our centre includes routine TB screening which includes history of symptoms and TB contact or previous TB, skin testing (TST), gastric washings or induced sputum for AFB and chest X-ray. If screening is positive, TB treatment is initiated and transplantation delayed. A positive TST is defined as an induration of ≥10mm, or ≥5mm in those with severe acute malnutrition. Living related donors are routinely screened for TB using chest X-rays and induced sputum for AFB. In our setting, tuberculin skin test (TST) is used to identify latent TB (LTB) since interferon gamma release assay (IGRA) is too costly. Since 2005 routine INH prophylaxis has been administered to each child for at least 1 year post kidney transplant. INH is administered for longer durations in those who stay in communities with very high TB burden. Routine re-initiation of INH during periods of intensified immunosuppression was also started in 2005. In South Africa Bacille Calmette-Guerin (BCG) vaccination is given at birth or at first health care contact in the first year of life. No repeat doses are given

Study methods

Retrospective descriptive analysis of TB infections in paediatric kidney transplant recipients at RCWMCH. This study complied with the ethical guidelines and principles of the Helsinki Declaration of 2008, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research and was approved by the Humah Research Ethics committee of the University of Cape Town (Ref 463/2020).

Data collection

Medical records of all children below 18 years of age who received kidney transplants were retrieved. All post kidney transplant patients who were followed up between 1995 and 2019 and who had a diagnosis of TB, were included in the study. Patients who had TB infection were identified from the Clinicom ICD coding, kidney transplant database and pharmacy anti-TB medication registry. To protect study subject privacy and confidentiality, patient names were used to access files but only study numbers were used on the electronic database. De-identified data was extracted into a case report form and imported into Excel. Data entry was re-checked by an independent investigator. A data key list including patient identification and study numbers was stored separately from the de-identified data set and was accessible exclusively to the Principle Investigator. All paper records were stored in a locked cupboard until data entry and study completion, after which paper records were destroyed. Electronic data were stored on a password protected computer, only accessible to the research team.

Demographic information, TB diagnosis and treatment outcomes were recorded. Transplant data included date of transplant and period between transplant and diagnosis of TB, indication for transplant, type of transplant – living related donor or cadaveric donor, immunosuppression used, presence or absence of rejection at TB diagnosis and calcineurin dose at time of TB diagnosis and highest dose used during TB treatment. Blood culture to detect bacteraemia was done and viral loads for *Ebstein Barr Virus (EBV)* and *Cytomegalovirus (CMV)* were assessed.

Statistical analysis

Statistical Package for Social Sciences (SPSS) was used for statistical analysis. Data were tested for normality using Shapiro Wilks W test. Normally distributed data was presented as means (standard deviations and range) and non parametric data as medians (interquartile range or range). Continuous data was compared using t-tests for independent samples or Mann Whitney U tests for nonparametric data. Categorical variables were compared using Chi square tests (or corrected chi-square where cell variables are <10). Categorical data was expressed as percentages or proportions with 95% confidence intervals. A significance level of $p < 0.05$ was used. Risk factors for TB such as gender, residence, type of transplant (living related/cadaveric), INH prophylaxis use and years after transplant were analyzed. The crude

odds ratios was adjusted for some measures of effects by fitting the significant and variables of interest into logistic regression models.

RESULTS

A total of 212 children (including 72 children reported previously[12]) received a kidney transplant between 1995 and 2019. Males constituted 56.1 % (n=119). The median age at transplantation was 11.2 years (IQR: 2.2 – 17.9) and 44% (n=94) were aged between 10 and 14 years. The majority of the patients were from the Western Cape Province followed by the Eastern Cape Province (Table 1). Only two were from outside South Africa. Of the 212 transplant recipients, 142 (67%) received cadaveric donor allografts. The three leading known causes of end stage kidney disease were nephrotic syndrome (19% n=41), kidney dysplasia (15% n=31) and glomerulonephritis (12% n=26) (Table 1). All children had received BCG vaccination at birth.

Demographics of children with TB.

Twenty (9%) children were diagnosed with tuberculosis (including 7 children from the previous study[12]), of which 2 were de novo Rifampicin resistant TB infections. The causes of end stage kidney failure among children who were diagnosed with TB infection included posterior urethral valves (PUV), glomerulonephritis, polycystic kidney disease (PCKD), Alports syndrome, kidney dysplasia, vasculitis, nephrotic syndrome and bilateral nephroblastoma. No patient had a history of previous TB treatment. Fourteen (70%) children were males. Age at transplantation ranged from 5 to 15.9 years. TB was diagnosed during the first 2 years post-transplant in 14 children (70%), with 12(60 %) of these being diagnosed in the first year (Fig 1). The median interval between kidney transplantation and TB diagnosis was 2.8 years (IQR 0.9 to 4.3 yrs.). Thirteen (65%) children diagnosed with TB had received a living related allograft. No demographic factors were associated with the risk of TB infection as outlined in Table 1.

Diagnosis of TB.

Tuberculin skin test was performed in all cases and was positive in 4 (20%) (Table 2). TB was confirmed bacteriologically in 12 (60%) children (positive TB culture from sputum in 2, gastric aspirates in 3, bronchoalveolar lavage in 1, tissue from the kidney in 4, lung in 1 and

tonsils in 1). The remaining 8 children were diagnosed clinically – 4 had a positive TB contact and a positive TST, 2 had a positive TST but no TB contact, and 2 had no TB contacts but a suggestive chest X-ray. All children treated empirically on the basis of a clinical diagnosis had marked improvement of symptoms after receiving TB treatment consistent with a correct diagnosis. A rise in *Ebstein Barr Virus (EBV)* viral load was observed in 3 children, *Cytomegalovirus (CMV)* in 2 children and *Staphylococcus* was isolated in a further 2 children (Table 2).

Symptoms of TB.

The main reported symptoms at presentation were fever (n=13, 65%), weight loss (n=12, 60%) and cough (n=10, 50%) (Table 2). Pericardial effusions were present in 2 children (10%) and 3 children (15%) had sterile pyuria. A history of a TB contact was elicited in 6 (30%) children.

Pulmonary TB was the commonest site of infection with abnormalities suggestive of TB being present on chest radiography in 16 (80%) children (Table 2). Graft dysfunction had occurred within 3 months prior to the TB diagnosis in 15 (75%) children, all of whom underwent kidney biopsy. Acute rejection was confirmed in 9 of these 15 children (Table 2). Immunosuppression was intensified with pulsed intravenous steroids in 8 (40%) of these patients within 3 months preceding the diagnosis of TB. Three of these 8 children had received routine INH prophylaxis, 5 had not. Of the 3 children who developed TB infection in the era after introduction of INH prophylaxis, 2 developed the infection during the first year of transplantation while still receiving INH prophylaxis. In the third child TB occurred 3 years post transplant and this child received INH prophylaxis when immunosuppression was intensified. Among those who underwent kidney biopsy, TB foci were observed in the graft kidney in 3 children, 2 of whom also had lung involvement (Table 3). All children with TB isolated in the graft had graft dysfunction but only one had concomitant acute rejection.
TB was also found on histology of the native kidney in 1 child with end stage kidney disease secondary to posterior urethral valves who required a nephrectomy at the time of transplantation to make room for the graft kidney. This child had a negative TST and clear chest x-ray on transplant work up. There was no history of TB contact or previous TB treatment in this child.

TB treatment and outcomes.

All patients, at the time of TB diagnosis, were receiving immunosuppression comprising a calcineurin inhibitor (Cyclosporine/CSA or Tacrolimus/FK), prednisolone and an antiproliferative agent (Rapamycin/RAPA, Mycophenolate mofetil/MMF or Azathioprine/Aza) (Table 2).

TB in transplant recipients is treated using the same protocol as for the general population [14]. The standard treatment includes a 2 month initiation phase and a 4 month continuation phase. Those with genitourinary TB receive 2 months initiation and 7 months continuation. The specific drugs used depend on age and presence of extrapulmonary TB. During the 2 month initiation phase of TB treatment 10 patients (i.e. ≥8 years of age and those with extrapulmonary TB) received 4-drug combination therapy including isoniazid, rifampicin, ethambutol, and pyrazinamide, while eight (i.e. below 8 years of age) received a 3 drug combination of INH, rifampicin and pyrazinamide as per national TB treatment guidelines. In the 2 patients with drug resistant TB, one received moxifloxacin and the other ofloxacin in place of rifampicin. During the 4 or 7 month continuation phase all patients without drug resistant TB received INH and rifampicin. No patient developed hepatotoxicity. Due to drug interactions, an increase in the dose of calcineurin inhibitor was anticipated and needed in all patients. An up to three fold increase in cyclosporine or tacrolimus dose was required to maintain therapeutic blood levels. At the end of treatment, recovery from TB infection was achieved in all cases. Graft and patient survival was 100% when assessed one month after completion of TB treatment.

Impact of isoniazid prophylaxis.

Of the 129 children who received INH prophylaxis 8 (6.2%) developed TB compared with 12 of 83 (14.5%) patients who received no prophylaxis (Table 1). INH was protective against development of TB overall (p=0.04). INH prophylaxis was associated with fewer infections within the first year of transplantation although this was not statistically significant (p = 0.3) (Table 4). The introduction of INH prophylaxis during periods of immunosuppression intensification resulted in a reduction of TB during these periods from 6% of all TB infections pre-INH era to 2% post universal INH prophylaxis (table 4). Drug resistance against INH was not identified.

Comparison of TB incidence before and after universal INH prophylaxis.

Subsequent to the prior study of TB in kidney transplant recipients at our centre[12] INH is given universally for at least 1 year post transplant to prevent TB infection. A total of 72 transplant recipients were included in the first study [12] and a further 11 received kidney transplants before the roll out of universal INH after the study (n = 83). The incidence of TB since initiation of universal INH prophylaxis decreased significantly from 14.5% to 6.2% (p=0.04). In addition, the time to TB diagnosis shifted from 75% of cases occurring within the first year of transplantation before INH prophylaxis to 37.5% occurring within the first year since INH prophylaxis was instituted. The main presenting symptoms remain fever, cough and weight loss, and majority still have pulmonary TB.

DISCUSSION

TB was diagnosed in almost 1 in 10 paediatric kidney transplant recipients between 1995 and 2019 at our centre. During this time, institution of universal INH prophylaxis, instituted in 2005 led to a reduction in the incidence of post-transplant TB. Overall the majority of TB infections were diagnosed within the first year after transplant, although this shifted to beyond the first year after institution of universal INH prophylaxis. The most frequent symptoms on TB infection at presentation were fever, cough and weight loss. Pulmonary TB was the most frequent site of infection. TST was not sensitive in identifying patients with TB. Age, sex and type of donor allograft were not associated with development of TB. During treatment of TB an up to 3 fold increase in calcineurin inhibitor dose was required in order to maintain therapeutic levels. Although graft dysfunction was present in 75% of patients at presentation, graft and patient survival was 100% one month after completion of treatment.

Incidence of post-transplant TB is directly associated with the incidence in the general population. It is as low as 0.35% - 1.2% in USA were there are 2.7 TB cases per 100 000 population [15] and as high as 5% to 15% in India were there are 199 TB cases per 100 000 population [16]. The high proportion (9.4%) of renal transplant recipients who developed TB over the 25 years in our study reflects the high burden of TB in our country[2]. Furthermore, our centre is in the Western Cape province where a third of all TB cases in South African children during the 2018/2019 period originated [2], thus making our patients a high risk population.

As reported by others, most TB infections developed before the end of the

second year post transplantation [5,7,17]. The common assumption is that TB infection follows reactivation of old foci, enhanced by immunosuppressive therapy, although whether this holds true in children is less certain [18–20]. The high risk of TB infection in the first year post transplant is however likely exacerbated by the high levels of immunosuppression required during this period when the risk of rejection is highest.

Receipt of a deceased donor graft has been independently associated with an increased risk for developing TB following liver or kidney transplantation [21]. It has been postulated that stronger immunosuppression used in recipients of organs from deceased donors, when compared to living related donors, may lead to higher rates of donor-derived transmission of TB. In contrast, we found no association between receipt of a deceased donor graft and increased risk of post-transplant TB, possibly because we use the same immunosuppressive protocol for all kidney recipients. Age and gender have not been described as risk factors for TB in kidney transplant recipients in other studies [7,21].

Immunosuppression increases risk of TB [22]. As in our study, Hall et al. found that intensification of steroid for treatment of rejection preceded development of TB in a third of patients [23]. A study comparing development of TB post kidney transplant on CSA/Aza/Pred and other combinations of immunosuppressants found that immunosuppression with one or both of Tacrolimus and MMF was associated with the development of TB at a younger age and at a higher frequency during the first 6 months after transplantation when compared with CSA/Aza/Pred [24]. This increased risk may be related to the higher potency of immunosuppression with these drugs. Consistent with this, the majority (n=13: 65%) of those who developed TB in our study were on regimens containing Tacrolimus or MMF.

The clinical features of TB can be atypical and may be masked by the blunted response to infection due to immunosuppression. Fever was the most common symptom. This finding is in agreement with other studies which showed fever in 64-93% of cases [5,7,18,25]. These studies also showed, as did we, that other common presenting symptoms included weight loss and cough. TB should be suspected in all solid organ transplant recipients with fever of unknown origin [25].

Diagnosis of TB post transplantation remains a challenge. After transplantation, TST testing has a low utility [25]. TST has been found to have a sensitivity of 50%, positive predictive value of 5%, and a specificity of 52% for post-transplant TB [26], whereas in our study TST

showed an even lower sensitivity of 20%. Interferon gamma release assays (IGRA) have been shown to be more sensitive and specific than TST with regard to the diagnosis of LTB in the transplant candidates, but we did not have access to this and could not asses the utility on our setting [27]. The World Health Organization (WHO) also recommends chest X-ray as a screening tool and diagnostic aid for TB [28]. In the lung, radiographic findings may vary among focal or diffuse interstitial infiltrates, nodules, pleural effusion, or cavitary lesions. The majority (80%) of children diagnosed with TB in our study had chest X-rays suggestive of active TB which is consistent with other reports [22].

Another factor that makes diagnosis of TB in transplant recipients difficult, is its frequent association with other infections, reported to occur in up to a fifth of cases [17]. We found a high co-infection rate of 25%. These co-infections not only make the diagnosis of TB difficult but also increase the risk of acquiring TB. CMV was found to independently increase the risk for developing post- renal transplant TB by 2.25 fold [29]. Patients suffering from other opportunistic infections concomitant with TB have a higher risk of mortality, compared with patients without this complication [18].

Pulmonary TB was observed in 75% of children in our study, and is the most common form of TB described in kidney transplant recipients. Genitourinary TB occurring after kidney transplantation is uncommon [22]. Three of our recipients had infection localised to the allograft. None of them presented with symptoms specific to the genitourinary system, since these are more likely to be found in immunocompetent patients [2230]. The occurrence of TB localized to the graft with absence of any pulmonary involvement suggests donor derived transmission, highlighting that despite our careful attempts to exclude TB in kidney donors, where the background prevalence is high it is very difficult to completely exclude TB in the kidney of a donor. Similarly, we report on a patient where TB was isolated on the native kidney after routine nephrectomy but was asymptomatic. TB can also not be completely excluded during recipient work-up.

The treatment of TB in kidney transplant recipients should be the same as in the general population [20,22]. However rifampicin increases catabolism of corticosteroids and calcineurin inhibitors, making it difficult to maintain therapeutic levels despite massive dose increases. We and others have shown that higher doses of calcineurin inhibitors are required during TB treatment, making close monitoring of drug levels crucial in order to avoid acute graft

rejection [31,32]. In settings such as ours, the higher doses required to maintain therapeutic drug levels have cost implications in terms of medications, more frequent drug level monitoring and more frequent hospital visits. Acute rejection during TB treatment was not observed in our study while other studies have shown acute rejection of up to 30% during TB treatment , which may be due to medication interactions but also possibly to inability of some patients to sustain the associated additional costs[20]. Hepatotoxicity, although not observed in our study, is another complication that occurs during TB treatment due to interaction of INH and immunosuppressive drugs [20,22]. Vigilant follow-up is therefore required in patients with TB post-transplant.

Prevention of TB post-transplant is an area of great interest. INH has been shown to be up to 60% to 90% effective in preventing latent TB infection from progressing to clinically apparent disease in immunocompetent individuals [33,34] and also in people living with HIV [35]. Given the difficulties in diagnosing and treating established TB infection in the immunocompromised transplant recipient, together with the high risk of graft dysfunction it poses, there is interest in similar chemoprophylaxis regimens in this population. Prophylaxis against TB in transplant recipients is still controversial and deserves further investigation. Some observational studies have reported benefit [36,37] while others did not find any benefit from chemoprophylaxis in kidney transplant recipients [38,39].
A meta-analysis of studies carried out in India and Pakistan, including 771 patients, did find that the relative risk of TB infection was significantly reduced with INH prophylaxis in kidney transplant recipients (4 studies, 771 patients, RR 0.31, 95% CI 0.19–0.51) [9]. Other studies also point to a benefit in preventing post-transplant TB in endemic areas without significant adverse effects [9,10]. We observed a reduction in clinical TB infection and a marked decrease in TB diagnosis during the first year post transplant (Table 4)

Indications for prophylaxis in some centres include a history of TB contact before transplantation, patients who have been newly infected with *M. tuberculosis* (i.e., those with a recent TST conversion), and recipients of transplants from donors with a history of untreated TB [5]. However in TB endemic regions like ours where resources are limited, a balance of the cost of providing INH prophylaxis against the extra cost of maintaining immunosuppression in a recipient needing to take rifampicin for treatment of active TB is of paramount importance. This is exemplified by Pakistan were the average cost of immunosuppression and

drug monitoring in a patient without TB is 2000 US$ annually, which becomes 5000 US$ annually for a renal transplant recipient who has developed TB [40].
The debate on when to initiate INH prophylaxis, i.e. before or after transplantation is also still ongoing. Some recommend starting INH pre-transplant since the patient on dialysis already has a degree of immunosuppression [41].
The duration of INH prophylaxis also varies. The European Best Practice Guidelines for Renal Transplantation [42] and the American Society of Transplantation [41] recommend a 9-month course. Some randomized controlled studies used a 1 year course [9].More research is required on the ideal duration of INH prophylaxis in endemic areas such as ours in order to standardize management.

The mortality rate among patients with solid organ transplants and TB may reach 30% in both adults and children [25] with a similar rate of graft loss [5]. Factors associated with mortality in these patients are graft rejection, receipt of steroid therapy, antilymphocyte antibody treatment, and presence of other opportunistic infection concomitant with TB [25]. Graft loss is attributed to chronic rejection in most of these patients [5].

We did not observe any short-term graft loss or mortality, however the long term graft and patient survival was not evaluated. Meticulous monitoring of drug levels probably resulted in the good graft and patient survival, over the short-term.

Our study has several strengths and some limitations. The retrospective nature of the study has inherent limitations and only quantitative data was available for analysis. We were unable to assess adherence to INH, which would impact interpretation of its effectiveness. The small number of participants in our study may limit power to detect true differences. The study is from a single centre, however, which adds the significant strength that treatment is protocolized and therefore standard across all patients. The RCWMCH is one of the largest paediatric transplant programmes in a middle income country, patients are carefully followed up, and therefore we are confident that the data quality was adequate for analysis. Although we did not get bacteriological proof of TB in a few cases due to challenges in TB diagnosis, all patients improved after treatment reflecting that the diagnosis was likely correct. Due to challenges in TB diagnosis some cases may however have been missed. Our study reflects a South African experience and may not be generalizable to a wider community, but serves to

raise awareness of the clinical problem, and the need for meticulous follow-up, which will become more and more relevant as lower income countries begin to start transplantation. The findings reported here will inform design of future studies, including the need to collect qualitative data to better understand patient experiences and barriers to care.

CONCLUSION

Kidney transplant recipients at our centre face a high risk of TB because of their immune compromised state and the high prevalence of the disease locally. A high index of suspicion is required due to the atypical presentation of TB in these patients and the frequent association with other infections. TB should be strongly suspected if fever, weight loss and cough are presenting features. Optimal diagnosis of TB post transplantation remains a challenge and requires further study especially in resource limited settings. Chest X-ray is a sensitive TB screening tool and diagnostic aid in this population. Chemoprophylaxis with INH in populations with high prevalence of TB is desirable and feasible. Treatment of TB must take into consideration the drug toxicity and important interactions with immunosuppressive medications. With vigilant care and close monitoring of drug levels there can be both good patient and graft outcomes.

REFERENCES

1. GraphicExecutiveSummary.pdf. Accessed June 22, 2020. https://www.who.int/tb/publications/global_report/GraphicExecutiveSummary.pdf?ua=1

2. TB Statistics for South Africa | National & provincial. TBFacts. Accessed March 7, 2020. https://tbfacts.org/tb-statistics-south-africa/

3. Sustainable Development Report 2019. Accessed June 2, 2020. https://www.sustainabledevelopment.report

4. Raizada N, Sachdeva KS, Nair SA, et al. Enhancing TB Case Detection: Experience in Offering Upfront Xpert MTB/RIF Testing to Pediatric Presumptive TB and DR TB Cases for Early Rapid Diagnosis of Drug Sensitive and Drug Resistant TB. *PLOS ONE*. 2014;9(8):e105346. doi:10.1371/journal.pone.0105346

5. Singh N, Paterson DL. Mycobacterium tuberculosis infection in solid-organ transplant recipients: impact and implications for management. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 1998;27(5):1266-1277. doi:10.1086/514993

6. Sundaram M, Adhikary SD, John GT, Kekre NS. Tuberculosis in renal transplant recipients. *Indian J Urol IJU J Urol Soc India*. 2008;24(3):396-400. doi:10.4103/0970-1591.42625

7. Boubaker K, Gargah T, Abderrahim E, Ben Abdallah T, Kheder A. Mycobacterium tuberculosis Infection following Kidney Transplantation. BioMed Research International. doi:https://doi.org/10.1155/2013/347103

8. Ram R, Swarnalatha G, Prasad N, Dakshinamurty KV. Tuberculosis in renal transplant recipients. *Transpl Infect Dis*. 2007;9(2):97-101. doi:10.1111/j.1399-3062.2006.00182.x

9. Currie AC, Knight SR, Morris PJ. Tuberculosis in renal transplant recipients: the evidence for prophylaxis. *Transplantation*. 2010;90(7):695-704. doi:10.1097/TP.0b013e3181ecea8d

10. Naqvi R, Naqvi A, Akhtar S, et al. Use of isoniazid chemoprophylaxis in renal transplant recipients. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc*. 2010;25(2):634-637. doi:10.1093/ndt/gfp489

11. El-Husseini AA, Sobh MA, Ghoneim MA. Complications of pediatric live-donor kidney transplantation: a single center's experience in Egypt. *Pediatr Nephrol*. 2008;23(11):2067-2073. doi:10.1007/s00467-007-0669-x

12. McCulloch M, Gajjar P, Spearman W, et al. Overview of a paediatric renal transplant programme. *S Afr Med J*. Published online 2006. doi:10.7196/SAMJ.1294

13. WHO. Guidance for nationaltuberculosis programmeson the managementof tuberculosisin childrenSecond edition. Published online 2014.

14. Guidelines for the management of tuberculosis in children, 2013. :58.

15. Data & Statistics | TB | CDC. Accessed November 14, 2020. https://www.cdc.gov/tb/statistics/default.htm

16. TB Statistics India - latest numbers. TBFacts. Accessed November 14, 2020. https://tbfacts.org/tb-statistics-india/

17. Canet E, Dantal J, Blancho G, Hourmant M, Coupel S. Tuberculosis following kidney transplantation: clinical features and outcome. A French multicentre experience in the last 20 years. *Nephrol Dial Transplant*. 2011;26(11):3773-3778. doi:10.1093/ndt/gfr156

18. Aguado JM, Herrero JA, Gavaldá J, et al. Clinical presentation and outcome of tuberculosis in kidney, liver, and heart transplant recipients in Spain. Spanish Transplantation Infection Study Group, GESITRA. *Transplantation*. 1997;63(9):1278-1286. doi:10.1097/00007890-199705150-00015

19. Aguado JM, Torre-Cisneros J, Fortún J, et al. Tuberculosis in solid-organ transplant recipients: consensus statement of the group for the study of infection in transplant recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2009;48(9):1276-1284. doi:10.1086/597590

20. Korzeniewska A, Dyła T, Kosacka M, Jankowska R. [Tuberculosis after renal transplantation]. *Pneumonol Alergol Pol*. 2009;77(1):61-65.

21. Liu J, Yan J, Wan Q, Ye Q, Huang Y. The risk factors for tuberculosis in liver or kidney transplant recipients. *BMC Infect Dis*. 2014;14(1):387. doi:10.1186/1471-2334-14-387

22. Boubaker K, Gargah T, Abderrahim E, Ben Abdallah T, Kheder A. Mycobacterium tuberculosis Infection following Kidney Transplantation. BioMed Research International. doi:https://doi.org/10.1155/2013/347103

23. Hall C, Willcox P, Swanepoel C, Kahn D, Smit R. Mycobacterial infection in renal transplant recipients. *Chest*. 1994;106:435-439. doi:10.1378/chest.106.2.435

24. Atasever A, Bacakoglu F, Toz H, et al. Tuberculosis in renal transplant recipients on various immunosuppressive regimens. *Nephrol Dial Transplant*. 2005;20(4):797-802. doi:10.1093/ndt/gfh691

25. Muñoz P, Rodríguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2005;40(4):581-587. doi:10.1086/427692

26. Ms S, An A, Pm S, et al. The prevalence of tuberculin sensitivity and anergy in chronic renal failure in an endemic area: tuberculin test and the risk of post-transplant tuberculosis. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc*. 2005;20(12):2720-2724. doi:10.1093/ndt/gfi141

27. Nasiri MJ, Pormohammad A, Goudarzi H, et al. Latent tuberculosis infection in transplant candidates: a systematic review and meta-analysis on TST and IGRA. *Infection*. 2019;47(3):353-361. doi:10.1007/s15010-019-01285-7

28. WHO | Chest radiography in tuberculosis detection. WHO. Accessed December 1, 2020. http://www.who.int/tb/publications/chest-radiography/en/

29. John GT, Shankar V. Mycobacterial infections in organ transplant recipients. *Semin Respir Infect*. 2002;17(4):274-283. doi:10.1053/srin.2002.36445

30. Andre Avarese FigueiredoÆAntonio Marmo LuconÆRenato Falci Ju´niorÆDiego Shiguemi IkejiriÆWilliam Carlos NahasÆMiguel Srougi. Urogenital tuberculosis in immunocompromised patients. *22 July 2008*. (Int Urol Nephrol (2009)).

31. Cassidy MJ, Van Zyl-Smit R, Pascoe MD, Swanepoel CR, Jacobson JE. Effect of rifampicin on cyclosporin A blood levels in a renal transplant recipient. *Nephron*. 1985;41(2):207-208. doi:10.1159/000183583

32. Langhoff E, Madsen S. rapid metabolism of cyclosporin and prednisone in kidney transplant patient receiving tuberculostatic treatment. *the lancet*. 1983;322(8357):1031. doi:10.1016/s0140-6736(83)91019-x

33. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. *Bull World Health Organ*. 1982;60(4):555-564.

34. The Use of Preventive Therapy for Tuberculous Infection in the United States Recommendations of the Advisory Committee for Elimination of Tuberculosis. Accessed November 30, 2020. https://www.cdc.gov/mmwr/preview/mmwrhtml/00001643.htm

35. Pape JW, Jean SS, Ho JL, Hafner A, Johnson WD. Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. *Lancet Lond Engl*. 1993;342(8866):268-272. doi:10.1016/0140-6736(93)91817-6

36. Spence RK, Dafoe DC, Rabin G, et al. Mycobacterial Infections in Renal Allograft Recipients. *Arch Surg*. 1983;118(3):356-359. doi:10.1001/archsurg.1983.01390030086014

37. Yildiz A, Sever MS, Türkmen A, et al. Tuberculosis after renal transplantation: experience of one Turkish centre. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc*. 1998;13(7):1872-1875. doi:10.1093/ndt/13.7.1872

38. Agarwal SK, Gupta S, Dash SC, Bhowmik D, Tiwari SC. Prospective randomised trial of isoniazid prophylaxis in renal transplant recipient. *Int Urol Nephrol*. 2004;36(3):425-431. doi:10.1007/s11255-004-6251-6

39. Sayiner A, Ece T, Duman S, et al. Tuberculosis in renal transplant recipients. *Transplantation*. 1999;68(9):1268-1271. doi:10.1097/00007890-199911150-00009

40. Naqvi A, Rizvi A, Hussain Z, et al. Developing world perspective of posttransplant tuberculosis: morbidity, mortality, and cost implications. *Transplant Proc*. 2001;33(1-2):1787-1788. doi:10.1016/s0041-1345(00)02680-4

41. Mycobacterium tuberculosis in Solid Organ Transplant Recipients | Ovid. Accessed April 17, 2021. https://oce.ovid.com/article/00024798-200912004-00009

42. EBPG Expert Group on Renal Transplantation. European best practice guidelines for renal transplantation. Section IV: Long-term management of the transplant recipient. IV.1. Organization of follow-up of transplant patients after the first year. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc*. 2002;17 Suppl 4:3-4.

Table 1 Characteristics of children with kidney transplants at Red Cross War Memorial Children's Hospital from 1995 to 2019.

	All children n (%) or median	TB positive	P value
Demographics			
Age in yrs at transplant			
<5	29 (14)	3	
5 – 9	58 (27)	7	0.81
10 – 14	94 (44)	8	0.76
15+	31 (15)	2	0.59
Range	2.2 – 17.9	5-15.9	
Male	119 (56)	14	0.25
Female	93 (44)	6	
Province of origin			
Western Cape	144 (68)	16	0.21
Other	68 (32)	4	
Causes of end stage kidney disease			
Other or unknown	71 (34)	7 (35)	
Nephrotic syndrome	41 (19)	0	
Dysplastic kidneys	31 (15)	2 (10)	
Glomerulonephritis	26 (12)	5 (25)	
PUV	23 (11)	3 (15)	
PCKD	9 (4)	3 (15)	
Reflux nephropathy	7 (3)	0	
Cystinosis	4 (2)	0	
Allograft			
Deceased donor	142 (67)	7 (35)	
Living related	70 (33)	13 (65)	0.73
INH prophylaxis			
Yes	129 (61)	8 (40)	0.04
No	83 (39)	12 (60)	

Key: PUV, posterior urethral valves; PCKD, polycystic kidney disease; INH, isoniazid.

Table 2 Characteristics of children who developed TB post-transplantation

	Patients N (%) or median
Immunosuppressive drugs	
CSA/Aza/Pred	7 (35)
CSA/MMF/Pred	3 (15)
FK/Aza/Pred	3 (15)
FK/MMF/Pred	5 (25)
FK/RAPA/Pred	2 (10)
% CNI dose increase	125 – 330
Steroid intensification	8 (40)
Symptoms	
Fever	13 (65)
Weight loss	12 (60)
Cough	10 (50)
LRTI	7 (35)
Abdominal pain	3 (15)
Diarrhoea	3 (15)
Pyuria	2 (10)
Pleural effusion	3 (15)
Pericardial effusion	2 (10)
Graft dysfunction	15 (75)
Acute rejection	9 (45)
Positive TST	4 (20)
Bacteriological confirmation	12 (60)
Site of infection	
Isolated Pulmonary	13(65)
Isolated graft	1(5)
Pulmonary and graft	2(10)
Pulmonary and tonsils	1(5)
TB pericarditis	2(10)
Native kidney	1(5)
Raised viral load or bacteraemia:	
EBV	2 (10)
CMV + EBV	1 (5)
EBV + Staph	1 (5)
CMV + Staph	1 (5)

Key: CSA, cyclosporine; Aza, azathioprine; Pred, prednisolone; FK, tacrolimus; MMF, mycophenolate mofetil; RAPA, rapamycin; CNI, calcineurin inhibitor; LRTI, lower respiratory tract infection; TST, tuberculin skin test; EBV, *Ebstein Barr Virus;* CMV, *cytomegalovirus;* Staph, *staphylococcus*.

Table 3 Characteristics of Children with TB isolated in the graft kidney.

Patient	Gender	Type of graft	Time after transplant (in years)	Pyuria	Suggestive chest x-ray	TST test positive	Graft dysfunction	Acute rejection
1	F	DD	10.8	No	Yes	No	Yes	Yes
2	M	LRD	1.2	No	Yes	No	Yes	No
3	M	LRD	3	No	No	No	Yes	No

Key: DD, deceased/cadaveric donor; LRD, living related donor.

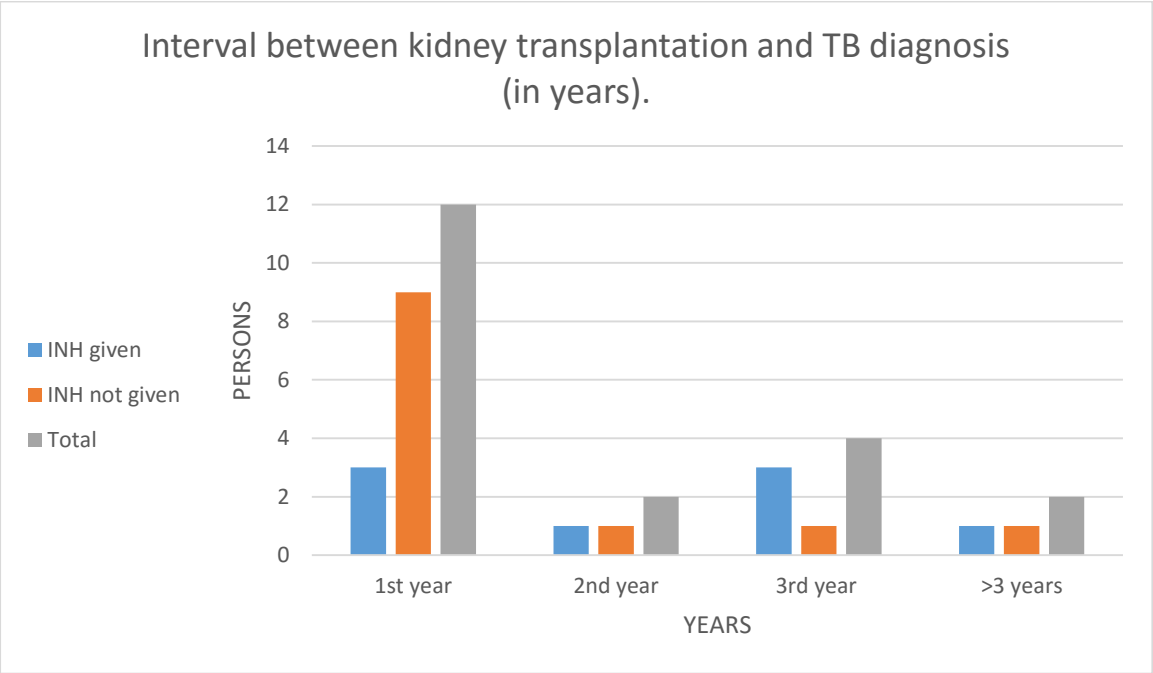
Table 4 Comparison of TB cases before and after initiation of universal INH prophylaxis.

	Pre universal INH prophylaxis[12] N (%)	Post universal INH prophylaxis N (%)
Total transplant recipients	83	129
TB cases	12(14.5)	8(6.2)
TB diagnosis	Acid-fast bacilli using a combination of sputum, nasogastric aspirate and bronchial lavage	Acid-fast bacilli using a combination of sputum, nasogastric aspirate and bronchial lavage, and gene X- pert
Type of graft in TB cases LRD DD	 6 6	 7 1
Time to TB after transplant	10m – 8 years	2 months – 10.8 yrs.
Cases diagnosed in first year post transplant	9 (75.0)	3(37.5)
Intensification of immunosuppression within 3 months prior to TB diagnosis	5	3
Bacteriologically confirmed cases	6	6
INH prophylaxis given Total Diagnosed TB	 0 0	 129 8
Common presenting symptoms	Fever , cough , weight loss	Fever , cough , weight loss
Site of TB infection Pulmonary Graft Other extra pulmonary sites	 10 1 1 (native kidney)	 6 2 0

Drug resistant TB cases		
Rifampicin	0	2
INH	0	0
Other	0	0

Key: DD, deceased/cadaveric donor; LRD, living related donor; INH, Isoniazid.

Figure 1: Time to TB diagnosis in children who received INH prophylaxis and those who did not.



APPENDIX

1. Data collecting tool / Case report form

Study number

Gender M F

Age in years

Weight in kgs

Transplant history

Age in (yrs) at transplant

Indication for transplant

Date of transplant

Type of transplant LR/DD

Immunosuppression meds

Recent steroid intensification Y/N

Cyclosporin / Tacrolimus dose at diagnosis of TB

Highest Cyclosporin / Tacrilimus dose during treatment of TB

TB history

Time after transplant (in years)

BCG Y/N Reactive mantoux Y/N Previous hx of TB Y/N

Sputum positive Y/N/NA Gastric aspirate positive Y/N/NA BronchoAlveolar lavage positive Y/N/NA

TB culture positive Y/N/NA

Site of TB

Tissue histology

TB medication

Successful completion of TB treatment Y/N

Symptoms

Fever Y/N Cough Y/N Weight loss Y/N

Diarrhea Y/N Pyuria Y/N Abdominal pain Y/N

Examination

Pleural effusion Y/N Pericardial effusion Y/N

Investigations

ESR WBC Creatinine rise Y/N

Co-infection EBV/CMV/NON

Suggestive CXR Y/N

Ultrasound scan report

MRI scan report

CT scan report

Outcome

Graf survival Y/N Patient survival Y/N

Graft rejection - Biopsy done Y/N Proven rejection on biopsy Y/N/NA

TB treatment outcome:

Cured	
Treatment completed	
Treatment failed	
Died	
Lost to follow up	
Not evaluated	
Treatment success	

Complications 

2. Instructions to authors – Pediatric Transplantation Journal

File upload

1. **Cover letter** (optional)
2. **Main body document** is the most important file that you will upload. It must be double spaced. For most manuscript types, the main body should contain the following:

- Title Page
    - Title
    - Author's full names
    - ORCIDs
    - Affiliations
    - Corresponding author email
    - Abbreviations
- Abstract
- Main Body Text
- Acknowledgements / Funding
- Disclosure
- Figure Legends
- Description of Supporting Information
- References

3. **Tables and Figures** can be included at the end of the main body document or uploaded separately. Do not embed them within the text. Each table and figure should appear on its own page, and tables and figures should not be mixed.

	Text word limit	Abstract word limit	Reference limit	Figure limit
Original Article	5000	250	N/A	N/A

Original Articles should be original factual observations important to the practice of pediatric transplantation or its related science. Original Articles must follow the standard presentation format as detailed in the Manuscript Preparation section below, and they must present results in sufficient detail for their merit to be judged by the authors' peers and for the experiments to be repeated by others. The use of supplemental information is acceptable in cases where the word limit constrains the authors' abilities to provide sufficient detail. The Discussion should convey the significance of the findings with minimal extrapolation or hyperbole, allowing the empirically tested facts to support the commentary.

Parts of the Manuscript

The manuscript should be submitted in separate files: main text file; figures.

Main Text File

The text file should be presented in the following order:

1. A short informative title containing the major keywords. The title should not contain abbreviations (see Wiley's best practice SEO tips);
2. A short running title of less than 40 characters;
3. The full names of the authors;
4. ORCIDs: ORCIDs are unique identifiers authors can use to be sure their work is linked. Get more information at orcid.org.
5. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
6. Corresponding author email: Provide the email address of the author to whom correspondence should be directed after publication.
7. Acknowledgments;
8. Abbreviations: Include an alphabetical list of all abbreviations used. All terms should be spelled out at first mention in the text.
9. Abstract and keywords;

10. Main text;
11. References;
12. Tables (each table complete with title and footnotes);
13. Figure legends;
14. Appendices (if relevant).

Figures and supporting information should be supplied as separate files.

Authorship

Please refer to the 'Editorial Policies and Ethical Considerations' section for details on author listing eligibility.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Editorial Policies and Ethical Considerations' section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

Pediatric Transplantation requires **structured** abstracts. Please provide an abstract of no more than 250 words containing the major keywords. Abstracts should clearly describe the purpose, methods, findings, and conclusions of the study.

Abstracts should be structured and labeled as follows:
Background: The problem being addressed in the study.
Methods: How the study was performed.
Results: The salient results.
Conclusions: What the authors conclude from the results.

Abbreviations are discouraged. Brand names are not allowed in the abstract. For prospective randomized trials, include the clinical trial registration number at the end of the abstract.

Keywords

Please provide relevant keywords.

Main Text

Introduction - Present the background briefly, but do not review the subject extensively. Give only pertinent references. State the specific questions you want to answer.

Patients and Methods/Materials and Methods - Describe selection of patients or experimental animals, including controls. Do not use patients' names or hospital numbers. It is the authors' responsibility to have the research study approved by the proper institutional review board (IRB). The submission must state the IRB approval if the paper describes human subjects or experimental animals. For human studies, the IRB approval number and protocol number should be listed. In the case of a multi-center study, the statement may be a global statement reinforced with supplementary material listing the individual approval numbers. Identify methods, apparatus (manufacturer's name and address), and procedures in sufficient detail to allow other workers to reproduce the results. Provide references and brief descriptions of methods that have been published. When using new methods, evaluate their advantages and limitations. Identify drugs and chemicals, including generic name, dosage, and route(s) of administration. Provide a brief but complete description of the statistical methods

used, including which tests were used to analyze which variable.

Results - Present results in logical sequence in tables and illustrations. In the text, explain, emphasize or summarize the most important observations. Units of measurement should be expressed in accordance with Système International d'Unités (SI Units).

Discussion - Do not repeat in detail data given in the Results section. Emphasize the new and important aspects of the study. Relate the observations to other relevant studies. On the basis of your findings (and others'), discuss possible implications/conclusions. When proposing a new hypothesis, clearly label it as such.

References

All references should be numbered consecutively in order of appearance and should be as complete as possible. In text citations should cite references in consecutive order using Arabic superscript numerals.

For more information about this reference style, please see the **AMA Manual of Style**.

Reference examples follow:

Journal article

1. King VM, Armstrong DM, Apps R, Trott JR. Numerical aspects of pontine, lateral reticular, and inferior olivary projections to two paravermal cortical zones of the cat cerebellum. *J Comp Neurol* 1998;390:537-551.

Book

1. Voet D, Voet JG. *Biochemistry*. New York: John Wiley & Sons; 1990. 1223 p.

Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

Internet Document

1. American Cancer Society. Cancer Facts & Figures 2003. http://www.cancer.org/downloads/STT/CAFF2003PWSecured.pdf. Accessed March 3, 2003.

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Tables must be cited, but not embedded, in the text and numbered according to their order of appearance. Each table should appear on its own page with self-explanatory titles and footnotes as needed. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes.

Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

Figures

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

Click here for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

3 Ethics approval letter.

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

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

15 September 2020

HREC REF: 463/2020

Dr M McCulloch
Division of Paediatric Nephrology
ICH Building, 3rd Floor Room 3.21
Red Cross War Memorial Children's Hospital
Rondebosch
Email: mignon.mcculloch@uct.ac.za
Student: pmakanda@psmi.co.zw

Dear Dr McCulloch

PROJECT TITLE: TUBERCULOSIS IN PAEDIATRIC KIDNEY TRANSPLANT RECIPIENTS - A SINGLE CENTRE EXPERIENCE (MPHIL CANDIDATE: DR PD MAKANDA-CHARAMBIRA)

Thank you for your response letter, addressing the issues raised by to the Faculty of Health Sciences Human Research Ethics Committee (HREC).

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

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 September 2021.

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

The HREC acknowledge that the student: - Dr Privalage Makanda-Charambira will also be involved in this study.

Please quote the HREC REF in all your correspondence.

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

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007