RISK FACTORS, MICROBIOLOGY, AND OUTCOMES OF CAPD-RELATED PERITONITIS AT HELEN JOSEPH HOSPITAL, SOUTH AFRICA

Midhu Mary Sunnyraj, MBChB

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Medicine.

Johannesburg, 2022

DECLARATION

I, Midhu Mary Sunnyraj, declare that this research report is my own, unaided work. It is submitted (in the submittable format with my protocol and extended literature review) for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Signature: _____ (date) 15/06/2022

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr Malcom Davies and Dr Zaheera Cassimjee, for their academic acumen, boundless patience, and expert guidance; this task would not have been possible if it were not for them. They have served not just as supervisors but rather as role models whom I have witnessed serve their patients with empathy, care, and unfailing dedication. I am privileged to have worked with them.

I would like to thank my husband, family, and close friends for their understanding and constant motivation.

ABSTRACT

Background:

In the resource-constrained public health care sector of South Africa, with limited availability for haemodialysis (HD) and long wait-list periods for renal transplantation, the longevity of the peritoneal dialysis (PD) modality is vital. We aimed to investigate the spectrum of causative organisms and associated sensitivity patterns of continuous ambulatory peritoneal dialysis (CAPD) related peritonitis cases. This study will further explore associated risk factors and outcomes among this patient population.

Methods:

This is a retrospective, open cohort study conducted at the Division of Nephrology, Helen Joseph Hospital, Johannesburg, South Africa for the period January 2013 – December 2018. During this period, 145 episodes of peritonitis were document in 149 patients.

Results:

The overall rate of peritonitis was 1 episode of peritonitis per 24.35 patient months. Of all peritonitis episodes, 43.45% (n=63) were due to gram-positive organisms, 30.34% (n=44) due to gram-negative organisms, and 21.38% (n=31) of episodes were culture- negative. Mycobacterium tuberculosis (2.07%, n=3) and fungal peritonitis (2.76%, n=4) were rare. The majority (55.55%, n=35) of gram-positive peritonitis episodes were methicillin-resistant. Extended-spectrum b-lactamase producing organisms caused 12.5% (n=2) of index and 15.90% (n=7) of all gram-negative peritonitis cases. All-cause modality failure was experienced in 28.10% (n=34) of study patients; the majority (76.47%, n=26) were peritonitis related. The risk of modality failure was higher among peritonitis patients (HR 2.79 95% CI 1.22 – 6.38, p =0.014), and modality survival was poorer in this group in comparison to those that were peritonitis-free (p = 0.0002). The time to the first episode of peritonitis showed a correlation with the duration of modality survival (p=0.000001). A 30.08% (n=37) all-cause mortality was documented on study completion.

Conclusion:

These findings have refined in-unit empiric antimicrobial protocols for the management of CAPD-associated peritonitis with the purpose of improving modality and patient survival. It has also provided insight into the risk factors and outcomes relative to this

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
CAPD	Continuous ambulatory peritoneal dialysis
CKD	Chronic kidney disease
CNS	Coagulase-negative *Staphylococcus*
ESKD	End stage kidney disease
HD	Haemodialysis
HIV	Human Immunodeficiency virus
HJH	Helen Joseph Hospital
HREC	Human Research Ethics Committee
IAPD	Intermittent automated peritoneal dialysis
IQR	Interquartile range
IP	Intraperitoneal
ISPD	International Society for Peritoneal Dialysis
NHLS	National Health Laboratory Service
PD	Peritoneal dialysis
RRT	Renal replacement therapy
TB	Tuberculosis

CHAPTER 1 – PROTOCOL WITH EXTENDED LITERATURE REVIEW

1. INTRODUCTION

The diagnosis of advanced chronic kidney disease (CKD), and end stage kidney disease (ESKD), carries with it the risk of significant morbidity and mortality [1]. Whilst renal transplantation remains the gold standard renal replacement therapy (RRT) for the management of ESKD, the chronic shortage of donor organs has resulted in long wait-list periods on dialysis [2]. A growing prevalence of ESKD has been reported world-wide; and this has led to an increased number of patients on dialysis awaiting a transplant [2]. In a resource-limited setting, peritoneal dialysis (PD) is an attractive renal replacement therapy for patients wait-listed for transplant. PD offers lower facility costs compared to haemodialysis (HD), with comparable survival rates and improved quality of life [3-6]. However, peritonitis is a leading cause of PD failure which may result in the need for modality change from PD to HD [7]. The treatment of peritonitis frequently requires admission leading to patient morbidity, and severe peritonitis may result in patient mortality.

Appreciation of the spectrum of causative organisms and the antibiotic susceptibility pattern thereof in the local context has the potential therefore to facilitate early prescription of appropriate empiric therapy, with the potential to shorten admission times, improve mortality rates, and decrease the likelihood of PD membrane failure [8].

2. BACKGROUND

2.1. Haemodialysis Versus Peritoneal Dialysis

Due to the chronic shortage of donor organs, many patients diagnosed with ESKD require bridging renal replacement therapy with dialytic modalities for a number of years before a kidney becomes available for transplantation [9, 10]. Haemodialysis or peritoneal dialysis may be offered to such patients, depending upon the local availability of these therapies.

In comparison to haemodialysis, peritoneal dialysis has been shown to have superior patient survival rates for the first 3 years after dialysis initiation, a benefit which is thought to derive partially from the more stable hemodynamic profile of this modality [3-6]. In addition, PD offers an improved quality of life than HD due to its out-patient setting. Home based dialysis also reduces facility costs which in turn contributes to PD being a more cost-effective dialysis modality than HD, as well as facilitating dialytic support for patients of lower socioeconomic status by reducing individual patient transport costs to dialysis units [3, 5, 6]. As a result of this latter consideration, PD is often the preferred therapeutic modality in developing countries. For example, PD is the dialytic therapy of choice for 80% of Colombia's dialysis population compared to 7% of the dialysis population of the USA [6, 7]. Similar considerations underlie the disparity in PD prescription between the state and private sector in South Africa, with 29% of the public sector dialysis population receiving PD in comparison to the 6% of the private sector dialysis population [11].

There are two main forms of PD which may be combined into a number of hybrid techniques. Continuous ambulatory peritoneal dialysis (CAPD) comprises the manual

installation and drainage of dialysate fluid by the patient into the peritoneal cavity through a Tenckhoff dialysis catheter and Y-drainage set. Typically, three diurnal exchanges (drainage of spent dialysate fluid from the abdominal cavity followed by installation of fresh fluid) are performed each day with the dialysate fluid remaining in the peritoneal space for a “dwell time” of 4 hours. A single dwell of 8 hours is performed overnight. In intermittent automated peritoneal dialysis (IAPD), automated nocturnal dwells are performed with the aid of a cycler, a device which facilitates the automatic exchange of dialysate whilst the patient sleeps.

The fundamental mechanism of dialysis in both CAPD and IAPD is the same, namely, diffusion of solutes across a semi-permeable membrane constituted by the peritoneal membrane and its capillary endothelium along a concentration gradient generated by the differences in concentration of these solutes in the patient’s blood and the instilled dialysate. There are theoretical advantages to IAPD in some patients for example, improved fluid removal or ultrafiltration in so-called “high transporter” patients in whom the dialysis gradient decays rapidly due to a peritoneal membrane ultrastructure which favours rapid translocation of oncotic solutes from the dialysate into the patient’s capillary bed. However, the cost of the Cycler machine to facilitate IAPD limits its use in the state sector, with the result that CAPD is the more commonly utilised PD modality [3].

Any potential benefit of CAPD over HD to the individual or to the health facility only remains extant as long as PD remains a viable option for the patient concerned. Loss of peritoneal membrane dialysis efficiency may develop from a variety of insults, of

which peritonitis is the leading cause [7]. In addition to PD failure, PD-associated peritonitis carries with it a 15% risk of mortality [12, 13].

2.2. Pathophysiology of Peritonitis

Peritonitis occurs as a consequence of the introduction of infectious organisms into the sterile peritoneal cavity. Various risk factors have been identified in the development of peritonitis, including black race, diabetes, obesity, hypoalbuminemia at PD initiation, the presence of pets in the dialysis setting, depression, preceding haemodialysis, staphylococcus carrier status, prior exit site or tunnel infection and lack of residual renal function [14-18].

Peritonitis may be classified as primary or secondary, depending on the route of introduction of the infecting pathogen into the peritoneal space.

Secondary peritonitis involves cases in which there is spread from an intra-abdominal source (bowel or pelvic organs) or dissemination from a systemic source. Cases of primary peritonitis include those in which there is contamination of the peritoneal space by pathogenic organisms resident on the skin surface via the Tenckhoff catheter or as a result of exit site or tunnel infections. Exit site and tunnel infections have proven to be the major route of infection leading to the development of peritonitis in PD patients [19]. As a result, exit site care with daily topical antibiotic creams and prompt treatment of exit site and tunnel infections is recommended by the International Society for Peritoneal Dialysis (ISPD) guidelines [8].

2.3. Micro-organisms Implicated in Peritonitis

PD-associated peritonitis may be caused by either bacteria or fungi; bacteria being the most commonly implicated micro-organisms with fungi accounting for only 7 – 15% of cases [20].

The risk of developing fungal peritonitis is higher in patients with recent exposure to antibacterial agents for the treatment of bacterial peritonitis or exit site and tunnel infections [21]. Candida albicans is the most common causative organism of fungal peritonitis [21]. Fungal peritonitis is associated with a higher risk of morbidity and mortality [17, 18].

The spectrum of bacterial micro-organisms implicated in PD-associated peritonitis varies between the developed and the developing world. In developed countries such as the USA, Canada, and Australia, gram-positive bacteria are the dominant isolate, accounting for 53-63% of cases compared to the 22-23% of cases attributable to gram-negative bacteria [22, 23]. The most common gram-positive bacteria cultured in this setting is coagulase-negative *Staphylococcus* (CNS) [23]. In contrast, in developing nations such as India [24], Malaysia [25], and Tunisia [26], gram-positive and gram-negative isolates account for a roughly similar number of cases (26 – 33% and 22 – 29%, respectively). Culture-negative, fungal, and tuberculoid peritonitis contribute a substantially higher proportion of cases than that seen in the developed world [24-26]. In this setting, coagulase-negative staphylococci, *Staphylococcus aureus*, and streptococcus species are the commonest gram-positive bacteria cultured [24-26]. Whereas *Escherichia coli,*

Pseudomonas aeruginosa, Acinetobacter species and *Klebsiella pneumoniae* are the commonest causes of gram-negative peritonitis [24-26].

Limited and contradictory data is available in the South African context regarding the microbiology of CAPD-related peritonitis. Two separate studies conducted in the more urban South African cities of Johannesburg and Durban; reported a gram-positive predominance of 31-42% and gram-negative isolates comprising 17% of peritonitis cases; *Staphylococcus aureus was* noted as the most typical cause of gram-positive peritonitis in both series [27, 28]. Furthermore, fungal peritonitis was documented in up to 31% of peritonitis episodes [28], and mycobacterial peritonitis was documented in 7% of cases in a separate study [29]. A single study in KwaZulu-Natal has reported a higher rate of gram-negative isolates (44%) compared to gram-positive isolates (33%), with low rates of culture-negative peritonitis [31]. A further study conducted in the city of Bloemfontein documented an alarming gram-positive predominance of 73%; CNS was found to be the most common causative organism in this audit [32]. The disparities in these studies may arise as a result of differences in the socio-economic conditions of sample populations and demonstrate the importance of establishing local CAPD-related peritonitis microbiology patterns for individual dialysis units, especially since South African data concurs with the global literature implicating peritonitis as the leading cause of modality failure amongst PD patients [30].

South Africa is home to the world's largest epidemic of human immunodeficiency virus (HIV) and Acquired immunodeficiency syndrome (AIDS); data indicates an increased risk of peritonitis associated with PD in such patients [31]. Other risk

factors within the South African context that have been associated with PD failure and poorer patient survival include: increased body mass index, serum albumin <30g/L, haemoglobin level <11g/dL and patients with >1 peritonitis episode [33]. Interestingly, some South African studies have failed to show a contribution of socio-demographic and socio-economic factors such as age, sex, ethnicity, marital status, employment status and low total household income in PD technique failure and patient survival [33]. However, other studies have shown that some socio-economic factors such as lower level of education, presence of running water and presence of electricity at home do correlate with poorer outcomes in regards to technique failure and patient survival [33].

2.4. Diagnosis and Empirical Treatment of Peritonitis

The 2016 ISPD guidelines require the presence of two or more of the following for the diagnosis of peritonitis [8]:

- Clinical features in keeping with peritonitis, the most specific being abdominal pain and a cloudy effluent.
- White cell count of > 100cell/μL with a > 50% polymorphonuclear predominance obtained on a peritoneal effluent after a dwell of at least 2 hours duration.
- A culture positive peritoneal effluent.

ISPD guidelines recommend that patients presenting with a cloudy effluent should have PD fluid sent for cell count and culture prior to the initiation of empiric treatment which should be continued until the diagnosis is confirmed on laboratory testing and pathogen sensitivity is determined [8]. The choice of empiric antimicrobial agents should be based on the known prevalence of centre-specific micro-organisms, and the antibiotic sensitivity and resistance patterns thereof [8].

Empirical antimicrobial agents should aim to provide cover for both gram-negative and gram-positive organisms. Current ISPD guidelines recommend gram-positive cover using vancomycin or a first-generation cephalosporin, and gram-negative cover using a third-generation cephalosporin or an aminoglycoside [8]. Vancomycin and cefazolin intraperitoneally have been shown to effect a similar cure rate in the treatment of gram-positive organisms, although vancomycin is the preferred empiric therapy in centres that are known to have high rates of methicillin-resistant organisms [34]. The use of gentamycin for the empiric treatment of gram-negative organisms has shown to be cost-effective and convenient, although the utilization of late generation cephalosporins such as ceftazidime or cefepime, or other beta-lactams such as the carbapenems have been found to be equally effective [35-37].

2.5. Definitive Treatment of Peritonitis

Once the causative organism and antimicrobial sensitivity pattern has been determined, antibiotic therapy should be adjusted accordingly and de-escalated where appropriate. Substantial clinical improvement is expected within 72 hours after initiation of appropriate antibiotic therapy. PD fluid should be resent for cell count, repeat microscopy, culture and sensitivity on day three to evaluate response to therapy [8]. A white cell count of ≥1090/mm³ on day three has high predictive value for treatment failure, and should provoke further radiological investigation for underlying loculated infection [38]. Peritonitis is defined as being refractory when the PD fluid has failed to clear by day five of culture directed antimicrobials at which point catheter removal is recommended [8].

2.6. Duration of Treatment

Gram-positive cocci should be treated for 14 - 21 days depending on the specific organism [8]. Gram-negative organisms usually require treatment with appropriate antibiotics for 21 days; peritonitis due to *Pseudomonas* requires treatment with synergistic antibiotics for 21 - 28days [8].

Polymicrobial cultures with multiple gram-negative organisms or a combination of gram-positive and gram-negative organisms has a poor prognosis and usually requires radiological investigation to exclude the presence of an intra-abdominal collections and treatment with oral metronidazole and intraperitoneal (IP) vancomycin together with IP ceftazidime or an IP aminoglycoside for 21days [8]. Polymicrobial cultures with multiple gram-positive organisms require treatment with appropriate antimicrobials based on sensitivity patterns for 21 days [8].

Culture-negative peritonitis is usually caused by gram-positive organisms. Patients with culture-negative peritonitis that manifest clinical improvement with reduction of white cell counts on day three repeat PD fluid culture should be continued on empiric vancomycin or a first-generation cephalosporin for 14 days. However, patients that manifest a poor clinical response with persistent high white cell count on repeat PD fluid specimen require investigation for other pathogens such as fungi or mycobacteria and consideration for catheter removal [8].

Fungal peritonitis requires the removal of the peritoneal dialysis catheter and treatment with sensitive anti-fungal therapy for two weeks thereafter [8]. Suspicion for tuberculous peritonitis should be raised with relapsing culture-negative peritonitis;

catheter removal may not always be required as these patients often manifest a good response to anti-tuberculous treatment [8].

Dosing and administration route of any antimicrobial should be prescribed in consideration of the bioavailability of the antimicrobial and the stability thereof in PD solutions. The decision for inpatient versus outpatient care is based on the severity of the peritonitis and the presence or absence of systemic sepsis.

2.7. Centre Specific Prevalence of Micro-organisms, Sensitivities and Resistance Pattern Considerations

The ISPD recommends that the identity of micro-organisms implicated in the development of PD-associated peritonitis and the antimicrobial resistance and sensitivity pattern thereof should be determined at the individual centre in order to adequately guide empiric treatment.

2.8. Centre Specific Incidence of Peritonitis, Terminology and Recommendations

The ISPD guidelines recommends that every centre monitor the incidence of peritonitis yearly at minimum [8]. This includes establishing the overall rate of peritonitis, rate of peritonitis specific to each causative organism and the percentage of patients that are free of peritonitis [8]. The peritonitis rate is represented as a fraction of peritoneal peritonitis episodes by the months of peritoneal dialysis at risk and expressed as intervals in months between episodes, which should not exceed 1 episode every 18 months [39]. The ISPD guidelines further classifies recurring peritonitis episodes as recurrent, repeat or relapsing. Recurrent peritonitis is defined

as an episode of peritonitis that occurs within four weeks of completion of treatment for a recent episode but has now cultured a different organism [8]. Relapsing peritonitis is defined as another episode of peritonitis that has occurred within four weeks of completion of treatment for a recent episode and has cultured the same organism or is culture negative [8]. A repeat episode is defined an episode of peritonitis that cultures the same organism after four weeks of the completion of treatment for a recent episode of peritonitis [8]. Relapsing peritonitis episodes are not to be counted as another episode of peritonitis when calculating the rate of peritonitis [8].

3. RATIONALE FOR RESEARCH

Peritoneal dialysis is an attractive bridging therapy to transplantation, offering superior patient outcomes compared to HD for the first three years of therapy and improved patient quality of life, whilst reducing facility and patient therapy-related costs. Peritonitis is a not-uncommon and feared complication of peritoneal dialysis, being the leading cause of modality failure and carrying a significant risk of patient mortality. In addition, the diagnosis often requires in-patient therapy which increases the cost of treatment to both the providing facility and the affected patient. The ISPD recommends early initiation of empiric antibiotic therapy in suspected cases of PD-associated peritonitis in order to improve patient and modality outcomes.

Prescription of an appropriate empiric antibiotic regimen requires knowledge of the causative micro-organisms and the sensitivity patterns in the local setting. This data has not been previously elucidated for Helen Joseph Hospital (HJH) and would greatly improve the standard of therapy and outcomes of patients with peritonitis.

4. AIMS AND OBJECTIVES

The primary objective of this study is to determine which micro-organisms are commonly implicated in the development of PD-associated peritonitis at Helen Joseph Hospital and the antimicrobial sensitivity patterns thereof, in order to develop an appropriate empiric antibiotic regimen as recommended by the ISPD guidelines.

Secondary objectives of this study comprise the following:

- To determine the overall rate of peritonitis;
- To determine the percentage of patients that are peritonitis-free;
- To determine the risk factors for the development of PD-associated peritonitis in the local setting;
- To determine the outcome of PD-associated peritonitis in terms of duration of hospitalization, survival of PD as dialytic modality, and patient survival.

5. METHODS

5.1. Study Design

This research project will be a retrospective non-interventional open cohort study.

5.2. Study Site

Division of Nephrology, Helen Joseph Hospital, Johannesburg, South Africa.

5.3. Study Population

The study population will be drawn from patients attending the Helen Joseph Hospital outpatient CAPD clinic.

5.4. Inclusion Criteria

All patients receiving outpatient peritoneal dialysis at Helen Joseph Hospital during the period 1 January 2013 – 31 December 2018 will be considered for inclusion in this study.

5.5. Exclusion Criteria

Patients will be excluded from this study on the basis of:

1. Age less than 18 years at the time of diagnosis of index episode of peritonitis
2. Incomplete or missing data

6. DATA COLLECTION

Patients receiving PD at Helen Joseph Hospital during the period of the study will be identified from PD clinic registers maintained in the unit. PD clinic files and the electronic database maintained by the National Health Laboratory Service (NHLS) at the hospital will be reviewed in order to identify all patients diagnosed with index and subsequent episodes of peritonitis during the course of the study period.

Baseline data (gender, age at dialysis initiation, race, aetiology of ESKD, distance of residence from the hospital, comorbidities including diabetes mellitus and HIV infection, and, where applicable, CD4 count at dialysis initiation) will be extracted for all patients included in the study. Time in months from dialysis initiation to first episode of peritonitis will be determined for all patients diagnosed with index episode of peritonitis during the study period. The identity and sensitivity of all organisms cultured during all episodes of peritonitis will be recorded. The duration of hospitalization for the treatment of all cases of peritonitis will be determined through retrospective review of clinical notes and electronic lab records. The total number of peritonitis episodes will be determined for all patients included in this study. In the

event of modality failure or patient death during the course of the study, the time to either outcome from both dialysis initiation and from index peritonitis episode (in months) will be determined and recorded. Where possible, the aetiology of patient death will be retrospectively reviewed and recorded.

A reference list containing the recipient hospital number and an assigned case number will be created. For the dataset, recipients included in this study will be identified only using the assigned case number. The reference list will be available only to the principal investigator of this study and will not be disseminated, thus maintaining patient anonymity and confidentiality.

7. STATISTICAL METHODOLOGY

The identity and sensitivity patterns for all episodes of peritonitis occurring during the study period will be described. Where ISPD diagnostic criteria are retrospectively validated but no organism is cultured from submitted fluid, the diagnostic category of “culture negative peritonitis” will be applied. Comparison of the category of causative organism (gram-positive, gram-negative, culture-negative, mycobacterial and fungal) will be made across the category of episode of peritonitis using the Fischer Exact Test.

Baseline data (gender, age at dialysis initiation, race, aetiology of ESKD, distance of residence from the hospital, comorbidities including diabetes mellitus and HIV infection, and, where applicable, CD4 count) will be compared between patients diagnosed with peritonitis during the course of the study and those not developing peritonitis, using the Mann Whitney U test and the Fischer Exact test for continuous and categorical data respectively. Cox regression analysis will be used to determine

the effect of these variables on the time to diagnosis with index episode of peritonitis. Duration of hospitalization will be compared by category of causative organism (gram-positive, gram-negative, culture-negative and fungal) using the Mann Whitney U test. Patients diagnosed with peritonitis during the course of the study will further be classified as experiencing a single episode or recurrent episodes. Baseline data will be compared between these categories using the Mann Whitney U and Fischer Exact test for continuous and categorical variables respectively.

The effect of the diagnosis of peritonitis on patient and modality survival after index diagnosis will be analysed in a three-step process. Firstly, the Fischer Exact test will be used to compare the frequency of the diagnosis of peritonitis between modality and patient survival groups. Thereafter, the Cox Mantel test will be used to compare modality and patient survival between those patients diagnosed with peritonitis and those not developing peritonitis during the course of the study, survival curves will be plotted using the Kaplan-Meyer method. Finally, Cox regression analysis will be used to compare the effect of multiple factors (age at dialysis initiation, gender, race, aetiology of ESKD, comorbidity, CD4 count where applicable, distance of residence from the facility, category of causative organism, and number of episodes of peritonitis) on modality and patient survival respectively from time of dialysis initiation.

A P value < 0.05 will be considered statistically significant for these analyses.

8. ETHICAL CONSIDERATIONS

Permission to conduct this study will be obtained from the Division of Nephrology at Helen Joseph Hospital, the Chief Executive Officer of Helen Joseph Hospital, the NHLS and the Human Research Ethics Committee of the University of the Witwatersrand.

9. FUNDING

The chief expenses of this study will comprise stationary expenses which will be borne by the investigator. Additional funding for this study is not anticipated.

10. LIMITATIONS

The following potential limitations may compromise the quality of this study:

1. Missing or incomplete data may compromise the number of patients included in the study resulting in bias. Non-parametric testing will be used to minimize the possible effect of a small sample

2. For patients initiated onto PD late in the study period, a lack of sufficient follow-up may introduce bias since it is possible that these patients may develop peritonitis after the close of data collection. To account for this possibility, Cox regression analysis censored for patient or modality survival (as determined by the respective analysis) at the time of close of data collection will be used.

11. REFERENCES

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351(13):1296-305.

2. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med. 1999;341(23):1725-730.

3. Yang F, Lau T, Luo N. Cost-effectiveness of haemodialysis and peritoneal dialysis for patients with end-stage renal disease in Singapore. Nephrology (Carlton). 2016;21(8):669-77.

4. Teixeira JP, Combs SA, Teitelbaum I. Peritoneal dialysis: update on patient survival. Clin Nephrol. 2015;83(1):1-10.

5. Morris PL, Jones B. Life satisfaction across treatment methods for patients with end-stage renal failure. Med J Aust. 1989;150(8):428-32.

6. Chaudhary K, Sangha H, Khanna R. Peritoneal dialysis first: rationale. Clin J Am Soc Nephrol. 2011;6(2):447-56.

7. Chen JHC, Johnson DW, Hawley C, Boudville N, Lim WH. Association between causes of peritoneal dialysis technique failure and all-cause mortality. Sci Rep. 2018;8(1):3980

8. Li PKT, Szeto CC, Piraino B, Arteaga JD, Fan S, Figueiredo AE, et al. ISPD Peritonitis recommendations: 2016 update on prevention and treatment. Perit Dial Int. 2016;36(5):481–508.

9. Muller E. Organ donation and transplantation in South Africa – an update. CME. 2013;31(6):220-22.

10. Stel VS, Kramar R, Leivestad T, Hoitsma AJ, Metcalfe W, Smits JM, et al. Time trend in access to the waiting list and renal transplantation: a comparison of four European countries. Nephrol Dial Transplant. 2012;27(9):3621–31.

11. Davids RM, Marais N, Jacobs JC. South African renal registry annual report 2015. AJN. 2017;20(1):201-13.

12. Fontan MP, Rodriguez-Carmona A, Garcia-Naveiro R, Rosales M, Villaverde P and Valdes F. Peritonitis-related mortality in patients undergoing chronic peritoneal dialysis. Perit Dial Int. 2005;25(3):274-84

13. Boudville N, Kemp A, Clayton P, Lim W, Badve SV, Hawley CM, et al. Recent peritonitis associates with mortality among patients treated with peritoneal dialysis. J Am Soc Nephrol. 2012;23(8):1398-405.

14. Nessim SJ, Bargman JM, Austin PC, Nisenbaum R and Jassal SV. Predictors of peritonitis in patients on peritoneal dialysis:results of a large, prospective Canadian database. Clin J Am Soc Nephrol. 2009;4(7):1195-120

15. Chow KM, Szeto CC, Leung CB, Kwan BC, Law MC and Li PK. A risk analysis of continuous ambulatory peritoneal dialysis-related peritonitis. Perit Dial Int. 2005;25(4):374-79.

16. Tian Y, Xie X, Xiang S, Yang X, Lin J, Zhang X, et al. Risk factors and outcomes of early-onset peritonitis in chinese peritoneal dialysis patients. Kidney Blood Press Res. 2017;42(6):1266-76.

17. McDonald SP, Collins JF, Rumpsfeld M and Johnson DW. Obesity is a risk factor for peritonitis in the Australian and New Zealand peritoneal dialysis patient populations. Perit Dail Int. 2004;24(4):340-6.

18. Cho Y and Johnson DW. Peritoneal dialysis-related peritonitis: towards improving evidence, practices, and outcomes. Am J Kidney Dis. 2014;64(2):278-89.

19. van Diepen AT, Tomlinson GA and Jassal SV. The association between exit site infection and subsequent peritonitis among peritoneal dialysis patients. Clin J An Soc Nephrol. 2012;7(8):1266-71.

20. Prasad N and Gupta A. Fungal peritonitis in peritoneal dialysis patients. Perit Dial Int. 2005;25(3):207-22.

21. Restrepo C, Chacon J and Manjarres G. Fungal peritonitis in peritoneal dialysis patients: successful prophylaxis with fluconazole, as demonstrated by prospective randomized control trial. Perit Dial Int. 2010;30(6):619-25.

22. Ghali JR, Bannister KM, Brown FG, Rosman JB, Wiggins KJ, Johnson DW, et al. Microbiology and outcome of peritonitis in australian peritoneal dialysis patients. Perit Dial Int. 2011;31(6):651-62.

23. Mujais S. Microbiology and outcomes of peritonitis in North America. Kidney Int Suppl. 2006;70(103):55-62.

24. Prasad KN, Singh K, Rizwan A, Mishra P,Tiwari D, Prasad N, et al. Microbiology and outcomes of peritonitis in northern India. Perit Dial Int. 2014;34(2):188-94.

25. Phui VE,Tan CHH, Chen CK, Lai KH, Chew KF, Chua HH, et al. Causative organisms and outcomes of peritoneal dialysis-related peritonitis in Sarawak General Hospital, Kuching, Malaysia: a 3-year analysis. Ren Replac Ther. 2017;3(35).

26. Lasfar LB, Guedri Y, Azzebi A, Sahtout W, Mrabet S, Fradi A, et al. Microbiology risk factors and outcomes of peritonitis in tunisian peritoneal dialysis patients.WJN. 2018;7(2):45-52.

27. Naidoo S, Naicker S, Malgas S, Paget G, Wadee S and Fabian J. Peritoneal dialysis in South Africa-a single centre experience. Indian J of Perit Dial. 2008;13:18-21.

28. Ikabu AS. A study of epidemiology and microbiology of peritonitis in continuous ambulatory peritoneal dialysis patients at Inkosi Albert Luthuli Central Hospital, Durban, South Africa. University of Kwazulu Natal. 2015.

29. Tamayo-Isla RA, de la Cruz MC and Okpechi IG. Mycobacterial peritonitis in capd patients in Limpopo: a 6-year cumulative report from a single center in South Africa. Perit Dial Int. 2016;36(2):218-22.

30. Mashiloane B, Moshesh FM and Mpe MJ. Peritonitis in patients with end-stage renal disease on continuous ambulatory peritoneal dialysis. SAMJ. 2008; 98(12):942-43.

31. Ndlovu KCZ, Sibanda W and Assounga A. Peritonitis outcomes in patients with HIV and end-stage renal failure on peritoneal dialysis: a prospective cohort study. BMC Nephrol. 2017;18(1):48.

32. Musoke J, Bisiwe F, Natverlal A, Moola I, Moola Y, Kajee U, et al. The prevalence and bacterial distribution of peritonitis amongst adults undergoing continuous ambulatory peritoneal dialysis at Universitas hospital. S Afr J Infect Dis. 2020;35(1), a104. https://doi.org/10.4102/sajid.v35i1.104

33. Isla RA, Mapiye D, Swanepoel CR, Rozumyk N, Hubahib JE and Okpechi IG. Continuous ambulatory peritoneal dialysis in Limpopo Province, South Africa: predictors of patient and technique survival. Perit Dial Int. 2014;34(5): 518-25.

34. Khairullah Q, Provenzano R, Tayeb J, Ahmad A, Balakrishnan R and Morrison L. Comparison of vancomycin versus cefazolin as initial therapy for peritonitis in peritoneal dialysis patients. Perit Dial Int. 2002;22(3):339-44.

35. Lui SL, Cheng SW, NG F, NG SY, Wan KM, Yip T, et al. Cefazolin plus netilmicin versus cefazolin plus ceftazidime for treating CAPD peritonitis: effect on residual renal function. Kidney Int. 2005;68(5):2375-80.

36. Wong KM, Chan YH, Cheung CY, Chak WL, Choi KS, et al. Cefepime versus vancomycin plus netilmicin therapy for continuous ambulatory peritoneal dialysis-associated peritonitis. Am J Kidney Dis. 2001l;38(1):127-31.

37. Kobayashi K, Nakamoto H, Okada S, Hoshitani K, Uchida K, Arima H, et al. Efficacy and safety of meropenem plus tobramycin followed by meropenem plus vancomycin for treating peritonitis in patients on continuous ambulatory peritoneal dialysis. Adv Perit Dial. 2006;22:65-8.

38. Chow KM, Szeto CC, Cheung KKT, Leung CB, Wong SSH, Law MC, el al. Predictive value of dialysate cell counts in peritonitis complicating peritoneal dialysis. Clin J Am Soc Nephrol. 2006;1(4):768-73.

39. Piraino B, Bailie GR, Bernadini J, Boeschoten E, Gupta A, Holmes C, et al. Peritoneal dialysis-related infectious recommendations: 2005 update. Peritoneal Dialysis International. 2005;25:107-31

CHAPTER 2 – SUBMISSIBLE ARTICLE

Title: Risk Factors, Microbiology and Outcomes of CAPD-related Peritonitis at Helen Joseph Hospital, South Africa

Authors:

1. **MM Sunnyraj [a]**
2. **M Davies [a,b]**
3. **Z Cassimjee [a,b]**

Affiliations:

a. **Department of Internal Medicine, Helen Joseph Hospital, School of Health Sciences, University of the Witwatersrand**
b. **Division of Nephrology, Helen Joseph Hospital**

Conflicts of Interest: Nil

Corresponding Author:
Midhu M Sunnyraj
Email: midhusrj@gmail.com
Telephone: +27766931679

Abstract word count: 308
Total word count: 4014

ABSTRACT

Background:

In the resource-constrained public health care sector of South Africa, with limited availability for haemodialysis (HD) and long wait-list periods for renal transplantation, the longevity of the peritoneal dialysis (PD) modality is vital. We aimed to investigate the spectrum of causative organisms and associated sensitivity patterns of continuous ambulatory peritoneal dialysis (CAPD) related peritonitis cases. This study will further explore associated risk factors and outcomes among this patient population.

Methods:

This is a retrospective, open cohort study conducted at the Division of Nephrology, Helen Joseph Hospital, Johannesburg, South Africa for the period January 2013 – December 2018. During this period, 145 episodes of peritonitis were document in 149 patients.

Results:

The overall rate of peritonitis was 1 episode of peritonitis per 24.35 patient months. Of all peritonitis episodes, 43.45% (n=63) were due to gram-positive organisms, 30.34% (n=44) due to gram-negative organisms, and 21.38% (n=31) of episodes were culture- negative. Mycobacterium tuberculosis (2.07%, n=3) and fungal peritonitis (2.76%, n=4) were rare. The majority (55.55%, n=35) of gram-positive peritonitis episodes were methicillin-resistant. Extended-spectrum b-lactamase producing organisms caused 12.5% (n=2) of index and 15.90% (n=7) of all gram-negative peritonitis cases. All-cause modality failure was experienced in 28.10% (n=34) of study patients; the majority (76.47%, n=26) were peritonitis related. The risk of modality failure was higher among peritonitis patients (HR 2.79 95% CI 1.22 – 6.38, p =0.014), and modality survival was poorer in this group in comparison to those that were peritonitis-free (p = 0.0002). The time to the first episode of peritonitis showed a correlation with the duration of modality survival (p=0.000001). A 30.08% (n=37) all-cause mortality was documented on study completion.

Conclusion:

These findings have refined in-unit empiric antimicrobial protocols for the management of CAPD-associated peritonitis with the purpose of improving modality and patient survival. It has also provided insight into the risk factors and outcomes relative to this.

Introduction

The diagnosis of advanced chronic kidney disease (CKD), and end-stage kidney disease (ESKD), carries with it the risk of significant morbidity and mortality [1]. While renal transplantation remains the gold standard for the management of ESKD, the chronic shortage of donor organs has resulted in long wait-list periods on dialysis [2-5].

The resource-limited public healthcare sector of South Africa, which provides dialytic support to patients of lower socio-economic status, offers a “peritoneal dialysis first” policy. PD offers lower facility costs than HD, with comparable survival rates and improved quality of life [6-9]. However, CAPD-associated peritonitis is a leading cause of PD failure, resulting in the need for modality change from PD to HD [10]. In addition to PD failure, PD-associated peritonitis carries a 15% risk of mortality [11,12].

Appreciation of the spectrum of causative organisms and the antibiotic susceptibility pattern thereof in the local context as recommended by the International Society of Peritoneal Dialysis (ISPD), has the potential to facilitate early prescription of appropriate empiric therapy, with the potential to shorten admission times, improve mortality rates, and decrease the likelihood of PD membrane failure [13].

A 2017 study highlighted an increased risk of peritonitis associated with PD in an human immunodeficiency virus(HIV) prevalent South African population [14]. Diabetes mellitus, African ethnicity, and recurrent episodes of peritonitis are

additional documented risk factors associated with poor PD modality and patient outcomes in South Africa [14-16].

In comparison to international data, there are wide disparities in reference to risk factors and peritonitis microbiology patterns in review of the limited number of similar studies conducted in South Africa [14-20]. This may be attributed to the differences in the socio-economic conditions among our populations. This further highlights the significance of attaining CAPD-related peritonitis microbiology patterns and associated risk factors for individual dialysis units [13]

This study investigates causative micro-organisms involved in CAPD-associated peritonitis at a South African tertiary-level facility and the antibiogram thereof to appropriately develop an empiric antimicrobial regimen as recommended by the ISPD guidelines and explores associated risk factors and outcomes of CAPD-associated peritonitis within this population.

Materials and Methods

Ethical Considerations

Study approval was obtained from the Human Ethics Research Committee (HREC) at the University of the Witwatersrand (M190703).

Study Population and Design

This study was a retrospective, non-interventional open cohort study undertaken at the Division of Nephrology, Helen Joseph Hospital (HJH), Johannesburg, South Africa. All patients that received outpatient PD during the period 1st January 2013 –

31st December 2018 were considered for inclusion. Those patients who were less than 18 years of age at the time of diagnosis of index episode of peritonitis and those with incomplete or missing data were excluded.

Baseline data (gender, age at dialysis initiation, race, aetiology of ESKD, distance of residence from the hospital, comorbidities including diabetes mellitus and HIV infection, and, where applicable, CD4 count at dialysis initiation) was obtained for all patients included in the study. Time in months from dialysis initiation to first episode of peritonitis was determined for all patients who developed peritonitis during the study period.

Peritonitis was defined as per the 2016 ISPD guidelines and required two or more of the following: (1) clinical features in keeping with peritonitis; (2) white cell count of > 100cell/μL with a > 50% polymorphonuclear predominance obtained on a peritoneal effluent after a dwell of at least 2 hours duration; (3) a culture-positive peritoneal effluent [13]. The identity and sensitivity patterns for all episodes of peritonitis occurring during the study period were documented. Where ISPD diagnostic criteria were retrospectively validated but no organism was cultured from submitted fluid, the diagnostic category of "culture-negative peritonitis" was applied. A recurrent peritonitis episode was defined as peritonitis that occurred within four weeks of completion of treatment for a recent episode but had cultured a different organism [13].

PD clinic files, in-unit peritonitis registers, and the electronic database maintained by the National Health Laboratory Service (NHLS) were cross-referenced to identify all patients diagnosed with index and subsequent episodes of peritonitis. The study's

initial cohort consisted of 149 patients who documented 145 episodes of peritonitis. For the comparative and survival analysis, 28 patients with incomplete or missing data were excluded. Patients who did not develop a single episode of peritonitis during this study were noted to be peritonitis-free.

Modality and survival outcomes were recorded for all patients in the peritonitis group and those who were peritonitis-free. Modality failure was defined as failure PD as a RRT requiring the conversion to HD on a continues basis at any time during the study period. Modality failure due to peritonitis were documented as such, and those not due to peritonitis were documented as due to other causes. PD modality survival was documented in patients still using PD as the method of RRT at the end of the study period. Patient mortality was documented as those that were directly related to peritonitis or due to other causes. Patient survival was documented in those patients included in the study that were alive at the end of the study period.

STATISTICAL DATA ANALYSIS

Data was collated from individual datasheets into a Microsoft Excel (Microsoft Corp, USA)® database and exported to Statistica v13 (TIBCO Software Inc, USA)™ for analysis. Since Shapiro Wilk W testing and visual inspection of the nomogram indicated non-parametric distribution, comparative analysis of continuous variables was undertaken using the Mann Whitney U test; categorical data was subjected to Fisher Exact testing.

Cox regression analysis was used to determine the effect of these variables on time to diagnosis of the index episode of peritonitis. Duration of hospitalisation was

compared by category of causative organism (gram-positive, gram-negative, and culture-negative) using the Mann Whitney U test; episodes of fungal and mycobacterial peritonitis were excluded from comparative analysis due to small numbers. Baseline data were compared between patients experiencing index and recurrent peritonitis episodes using the Mann Whitney U and Fischer Exact test for continuous and categorical variables, respectively.

The effect of the diagnosis of peritonitis on patient and modality survival after index diagnosis was analysed in three steps. Firstly, the Fischer Exact test was used to compare the frequency of peritonitis diagnosis between modality and patient survival groups. Thereafter, survival curves were plotted using the Kaplan-Meier method and the Cox Mantel F test was used to compare modality and patient survival between those patients diagnosed with peritonitis and those not developing peritonitis during the study. Finally, Cox regression analysis was used to compare the effect of multiple demographic and clinical factors on modality and patient survival from dialysis initiation.

A p-value of < 0.05 was considered statistically significant for these analyses.

RESULTS

One hundred and forty-nine patients were included in this study; baseline demographics and clinical characteristics of this cohort are tabulated in Table 1. The majority of the patients were male (51.01%, n=76), with a median age at PD initiation of 44.22 years (IQR 34.27 – 50.83 years, range 16.64 – 64.84 years). African patients (66.44%, n=99) were the predominant ethnic group within this cohort. The most

common causes of ESKD were hypertension (33.56%, n=50), diabetes (20.13%, n=30) and HIV-related kidney disease (16.78%, n=25). A total of 42 patients in this series (28.19%) were HIV positive; CD4 count at PD initiation was retrospectively available in 30 patients (71.43%). The median CD4 count at PD initiation in this subgroup was 339 (IQR 184 – 440, range 18 – 720). Forty patients (28.19%) within this cohort were known to be diabetic. Seventy-eight patients (52.35%) did not develop peritonitis during the study period; 71 patients (47.65%) had at least one episode of peritonitis. A total of 145 episodes of peritonitis were recorded over the 6 year study period. A general trend suggestive of a year-on-year increase in the number of annual peritonitis episodes in the unit was noted (Figure 1). The peritonitis rate calculated per ISPD definition for this study period was 1 episode of peritonitis per 24.35 patient-months.

Gram-positive organisms accounted for 43.45% (n=63) of all peritonitis episodes, whilst gram-negative organisms accounted for 30.34% (n=44); *Mycobacterium tuberculosis* (2.07%, n=3) and fungal peritonitis (2.76%, n=4) accounted for a minority of all peritonitis episodes. 21.38% (n=31) of peritonitis episodes were culture-negative. The most common gram-positive infections encountered were coagulase-negative staphylococcus species (30.70%, n=35), *Corynebacterium* species (9.65%, n=11) and *Staphylococcus aureus* (6.14%, n=7). The majority of gram-negative infections were accounted for by *Klebsiella pneumoniae* (8.77%, n=10), *Escherichia coli* (7.89%, n=9), and *Pseudomonas aeruginosa* (6.14%, n=7).

The majority (55.55%, n=35) of peritonitis episodes involving gram-positive organisms were methicillin resistant and only a minority (33.33%, n=21) sensitive to cefazolin,

which hitherto has been the empiric antibiotic of choice prescribed for PD-related peritonitis at this institute (Table 3). The antimicrobial sensitivities of gram-negative organisms causing peritonitis in this study are tabulated in Table 4.

The spectrum of organisms causing peritonitis is tabulated in Table 2. The majority (63.45%, n=92) of all peritonitis episodes required inpatient management. The median duration of hospital stay was 11 days (IQR 7 - 20days, range 4 - 91days). The duration of hospital stay of all index episodes of peritonitis compared by category of causative organisms (gram-negative, gram-positive and culture-negative episodes) was not found to contribute to the duration of hospital stay. Tuberculosis (TB) and fungal peritonitis episodes were excluded from this analysis due to their small contributory numbers.

Twenty-six patients with incomplete data were excluded from subsequent analysis. Comparative analysis was undertaken of the remaining 123 patients: comprising 58 patients (47.15%) who experienced at least 1 episode of peritonitis, and 65 patients (52.85%) who remained peritonitis-free during the study period. Demographic and clinical variables were assessed as possible risk factors in both peritonitis status groups (Table 1). Demographic factors such as age, gender, and clinical factors such as age at PD initiation, HIV status, and CD4 count in HIV seropositive patients were similar in both the peritonitis and non-peritonitis cohorts of this study. Somewhat surprisingly, the proportion of diabetic patients was found to be higher in the non-peritonitis cohort ($p = 0.044$). In keeping with these findings, univariate Cox proportional hazards modelling did not find a significant effect for patient age at PD initiation ($p = 0.689$), gender ($p = 0.674$), HIV infection status ($p = 0.243$), CD4 count

at dialysis initiation in HIV positive patients (p = 0.769), diagnosis of diabetes mellitus (p = 0.243), or distance of residence from the dialysis unit (p = 0.513) in predicting the time to first episode of peritonitis. There were 32 recurrent episodes (56.14%) and 25 single episodes (43.86%) of peritonitis within this cohort. Logistic regression analysis failed to demonstrate a significant role for age at PD initiation (p = 0.104), distance of residence from the dialysis unit (p = 0.733), age at first peritonitis episode (p = 0.099), gender (p = 0.094), diagnosis of diabetes mellitus (p = 0.689), or HIV infection status (p = 0.375) on the odds of patients experiencing recurrent episodes of peritonitis compared to a single episode.

A total all-cause modality failure of 28.10% (n = 34) was recorded for the cohort under study. The median time to modality failure was 11.50 months (IQR 5.00 – 27.00 months). The bulk of modality failures were directly attributable to peritonitis (n = 26, 76.47%) of modality failures. Reflecting this, Kaplan-Meier analysis using Cox's F test showed significantly poorer modality survival in those patients who developed peritonitis compared to those who did not (p = 0.0002) and, Cox proportional hazards modelling found an increased risk of modality failure in those developing peritonitis (HR 2.79 95% CI 1.22 – 6.38, p = 0.014)); no significant effect was demonstrated for age at dialysis initiation (p = 0.956), gender (p = 0.690), diagnosis of diabetes mellitus (p = 0.166) and, HIV infection status (p = 0.450). Amongst patients developing peritonitis, a later index episode of peritonitis was associated with reduced risk of modality failure (HR 0.952, 95% CI 0.918 – 0.988, p = 0.008. In this group age at PD initiation (p = 0.381), distance of residence from the dialysis centre (p = 0.275), gender (p = 0.358), diagnosis of diabetes mellitus (p = 0.892), HIV infection status (p = 0.419), and recurrent episodes of peritonitis (p = 0.769) had no discernible effect on modality

failure. Time to first episode of peritonitis showed correlation with duration of modality survival in those patients with modality failure during the course of this study (Spearman rank order R = 0.765, p < 0.000001) (Figure 2) . A non-significant trend (p = 0.113) towards poorer modality survival was observed amongst those patients with recurrent episodes of peritonitis compared to those with a single episode was observed; similarly, survival modelling suggested graded modality outcomes according to whether patients experienced recurrent, single, or no episodes of peritonitis during the study period (p = 0.058).

There was a 30.08% (n = 37) all-cause mortality rate during this study; 13.82% (n = 17) were a part of the peritonitis group compared to the 16.26%(n = 20) who did not experience any episode of peritonitis (p = 0.842 in Fisher two-tailed testing); Kaplan-Meier analysis found no difference in patient survival from PD initiation between the peritonitis and non-peritonitis survival (p = 0.320). A small majority of peritonitis related deaths were associated with gram-negative organisms (n = 5, 45.45%), followed by gram-positive organisms (n = 3, 27.28%), with culture-negative and mycobacterial infections contributing a smaller number of cases each (n = 2 and n=1 respectively). Multivariate Cox proportional hazards modelling did not identify a significant role for age at PD initiation (p = 0.605), gender (p = 0.393), diagnosis of diabetes (p = 0.196), HIV infection status (p = 0.699), or diagnosis of peritonitis (p = 0.421) in predicting patient death, Amongst patients developing peritonitis, increasing age at PD initiation (HR 1.102, 95% CI 1.030 – 1.180, p = 0.005) was associated with an increased risk of death, whereas PD vintage at index episode of peritonitis was associated with decreased risk of death (HR 0.958 95% CI 0.919 – 0.100, p = 0.047).

DISCUSSION

The peritonitis rate of 1 per 24.35 patient-months calculated for the period of this study is below the maximum rate of 1 per 18 patient-months recommended by the ISPD [22], and is reassuring evidence of the suitability of this modality as a dialytic therapy in areas of reduced socio-economic development. Gram-positive bacteria were the commonest isolates in cases of peritonitis in this series (43.45%), followed by gram-negatives (30.34%); a significant minority of cases were culture negative (21.38%). Previous studies from the developing world have reported similar rates of gram-positive and gram-negative bacteria [23-25], whereas reports from the developed world have noted a higher rate of gram-positive isolates than that found in the present study [18, 19]; similar isolate patterns to the present cohort have been reported in a single-centre study from a centre in close proximity to our institution [26]. The rate of culture negative peritonitis in this study of 21.38% is above the acceptable 10-20% recommended by the 2016 ISPD guidelines [13], modifiable factors such as PD effluent culture methods and delays in specimen processing at this institution will have to be assessed. *Mycobacterium tuberculosis* and fungal organisms were rare isolates in this study (2.07% and 2.76% respectively); similar rates of mycobacterial peritonitis have been reported from the developing world [23, 24], although higher rates of *Mycobacterium tuberculosis* of 7% have been noted from other South African centres [15, 17]. The incidence of fungal peritonitis in these latter studies has been reported to be similar to that of the present study [17, 20].

Coagulase-negative staphylococci were the causative organism in 30.70% of peritonitis cases, the dominance of these organisms in this study is comparable to other studies undertaken in Northern America [19], India [23], and Australia [18]; in

contrast, other studies from South Africa have reported *Staphylococcus aureus* to be the most commonly encountered gram-positive isolate in PD-related peritonitis [15,16,26]. Corynebacterium species were the second most common gram-positive isolates in this series at 9.65% of cases, an unusual cause of peritonitis in other studies. The three most common gram-negative causes of peritonitis in the present study were *K. pneumoniae (*8.77%) *E. coli (*7.89%), *and P. aeruginosa (*6.14%); rates which are keeping with international and local reports [15,18,19,23,24,25].

The majority (55.55%) of gram-positive isolates in this study were methicillin-resistant. Sensitivity patterns have been poorly reported in the local context; a previous report from South Africa noted minimal methicillin resistance within their unit [15]. Notably in the present cohort, 12.5% of index and 15.90% of all gram-negative peritonitis cases were caused by extended-spectrum b-lactamase producing organisms . Empiric antibiotic therapy for PD-related peritonitis at this institution has hitherto comprised intraperitoneal cefazolin and gentamicin; the findings of this study necessitate adaptation of this protocol as recommended by the ISPD.

Patient and disease-related risk factors contribute to an increased prevalence of ESKD in younger patients in the local context. In the South African state sector, resource constraints favour a “PD-first” policy for patients presenting with ESKD. This combination likely underlies the relative youth of patients included in the present study and reported by other South African studies [14-17]. In contrast; not only does ESKD in developed world populations more commonly affect older patients, but patients of advanced age are also more likely to be offered PD in consideration of the modality’s improved haemodynamic profile and home-based nature [11,12,18,19]. This cohort's

African ethnic predominance reflects the demographics of the population served by Helen Joseph Hospital. Hypertension was the leading presumed cause of ESKD in this cohort , consistent with other reports from this region [15,20]. The prevalence of HIV seropositivity in this cohort (23.19%,n=42) is higher than the national prevalence reported by Statistics South Africa (20% in 2013 and 19% in 2018) [21], possibly reflecting a degree of selection bias caused by the contribution of HIV-related kidney disease to the development of ESKD in the local setting.

Baseline demographic characteristics analysed in this study did not appear to increase the risk of PD-related peritonitis. Previous data from South Africa has similarly failed to find significant predictors of subsequent peritonitis [16]; these findings are at odds with other local studies which have reported an increased risk of peritonitis amongst patients of African ethnicity [15] and amongst patients living with HIV [14]. It seems likely that the formerly reported association of ethnicity with peritonitis risk arises due to confounding factors such as level of education and socioeconomic status, and it is likely that homogeneity in these factors between the ethnic groups accounts for the lack of significance of ethnicity in the present study. The lack of association in this study between peritonitis and HIV infection status is reassuring in view of the significant burden of HIV-related kidney disease in the local setting. It is possible that the lack of observed effect may arise from a form of selection bias: since initiation of PD in this institution requires that the individual patients is eligible for transplantation, it is likely that HIV positive patients included in this study were already initiated onto anti-retroviral therapy, so that immunological risk for peritonitis was somewhat ameliorated. Somewhat surprisingly, diabetics in this cohort were diagnosed less frequently with peritonitis that non-diabetics. Available literature suggests that

diabetes increases the development of peritonitis [27,28]. The retrospective nature of the present work precluded an evaluation of glycaemic control in diabetic patients in this cohort, which limits the interpretation of this finding, but it is tempting to speculate that education on sterile self-injection prior to dialysis initiation may have resulted in improved PD catheter exit site care in this group.

As has been reported by other centres, the majority (63.45%) of peritonitis cases at this institution required inpatient management [14,18,23]. Patients diagnosed with gram-negative peritonitis required a non-significantly longer period of inpatient treatment than either culture-negative or gram-positive peritonitis. However, this contrasts with other publications that document significantly longer hospitalization duration in gram-negative peritonitis cases [18, 23]. This discrepancy could be due to the smaller comparative population of this cohort and the larger proportion of culture-negative peritonitis cases. A significant proportion (56.90%) of peritonitis episodes in this study were recurrent. No significant risk factor for recurrent peritonitis was identified in this study. It is likely that un-assayed factors such as level of education, socioeconomic status, and patient satisfaction with PD may be better predictors of recurrent peritonitis than the patient and dialysis-related factors collected in this study.

CAPD-associated peritonitis has been documented in many previous studies as a significant risk factor for PD modality failure [14,18,19,23,24,25]. In this study, of the one-hundred and twenty-one patients included in the survival analysis, 28.10%%(n=34) experienced all-cause modality failure over the six-year study period; of those, 74.47%(n=26) were peritonitis-related; modality survival was poorer in those patients developing peritonitis compared to those who remained peritonitis-free, and

a higher dialysis vintage at index peritonitis was associated with a reduced risk of modality failure; a trend towards poorer modality survival was suggested for recurrent peritonitis

No significant effect was demonstrated in this study for the diagnosis of peritonitis on patient survival, consistent with other reports [16,18,19,20,25]. Other studies which have reported an effect for peritonitis on patient survival comprise older patient cohorts [11, 12]; it is noteworthy that in the present study increasing patient age was associated with an increased risk of patient death amongst those diagnosed with peritonitis. In comparison, increased PD vintage at index diagnosis of peritonitis was associated with a moderately decreased risk of patient death. It is likely that unsampled confounding variables may underlie this observed effect; in particular, it is tempting to speculate that delayed advent of peritonitis may evidence better general health and compliance with prescribed therapy, contributing to reduced risk of mortality. Consistent with this hypothesis, other investigators have reported early onset peritonitis to be a significant predictor of all-cause mortality [29].

The survival of patients living with HIV was no different to that of HIV negative patients in this series, consistent with a previous report from South Africa [14]. Access to PD in state sector units is restricted in accordance with transplant eligibility criteria which require demonstration of a stable suppressed viral load on antiretroviral therapy for at least 6 months prior to dialysis initiation. Effective selection for immunocompetency in HIV positive patients may underlie the lack of increased risk for peritonitis or for mortality in such patients in this study.

Application of transplant eligibility criteria requires the active exclusion of significant cardiovascular disease in patients initiating PD. Selection of patients of comparatively low cardiovascular risk may have ameliorated the effect of diabetes on patient survival outcomes in this study; longer duration of follow-up may have uncovered the cumulative contribution of diabetes to accelerated cardiovascular disease in this cohort.

CONCLUSION

The findings of this study emphasise the need for all peritoneal dialysis units to investigate their own peritonitis related microorganisms and sensitivity patterns to guide empiric antimicrobial guidelines specific to their population. Understanding the profile and antibiogram of the causative organisms commonly implicated in CAPD-associated peritonitis through the present study in our unit has resulted in a change in our empiric regimen. Peritoneal dialysis is an attractive modality in the South African state sector, reducing the financial strain on impoverished communities that in-centre haemodialysis otherwise imposes. Since HD availability is limited in the state sector, PD modality failure may nevertheless ultimately lead to patient loss in situations where haemodialysis is unavailable. The low peritonitis rate in this study confirms the suitability of this modality for patients resident in low socio-economic settings, and the lack of significant negative effect for either HIV infection or diabetes mellitus on modality and patient survival suggests the utility of PD in patients affected by these important causes of end stage kidney disease in the local context. Although PD-associated peritonitis is not a significant direct contributor to patient mortality, the advent of peritonitis may reduce modality survival. The greatest risk for peritonitis likely arises from patient adherence to antiseptic technique which is

difficult to predict from baseline demographic characteristics; as a result, PD centres must maintain a high level of surveillance for this complication in individual patients and should formulate empiric antibiotic regimens in accordance with the local microbiological sensitivity profile.

The authors have no conflicts of interest to declare.

Tables and figures

Table 1| Baseline demographic and clinical characteristics

Parameter	Initial cohort n=149(100%)	Comparative cohort n=123(100%)		
		Peritonitis n=58(47.15)	Peritonitis free n=65(52.86%)	P-value
Race				
African	99(66.44)	40(68.98)	41(63.08)	0.12[d]
Caucasian	23(15.44)	6(10.34)	13(20)	
Mixed:	17(11.41)	10(17.24)	5(7.70)	
Asian:	10(6.71)	2(1.63)	6(9.23%)	
Aetiology of ESKD				
Hypertension	50(33.56)	25(43.10)	17(26.15)	0.12[d]
Diabetes	30(20.13)	9(15.52	16(24.62)	
HIV-associated	25(16.78)	9(15.52)	13(20)	
Unclear/idiopathic/missing	17(11.41)	6(10.34)	2(3.08)	
Other [a]	16(10.74)[a]	4(6.90)	11(16.92)	
Glomerular disease	10(6.71)	5(8.62)	5(7.69)	
Obstructive/reflux	1(0.67)	0	1(1.54)	
Gender				
Male	76(51.01)	29 (50.88%)	36 (56.25%)	0.674[c]
Female	73(48.99)	28 (49.12%)	28 (43.75%)	
Prevalence of Diabetes				
Known diabetic	40(26.85)	11 (19.30%)	24 (37.50%)	0.243[c]
Known non-diabetic	98(65.77)	46 (80.70%)	40 (62.50%)	
Retrospectively unclear	11(7.38)			
Prevalence of HIV				
Known positive	42(28.19)	17 (29.82%)	13 (20.31%)	0.243[c]
Known negative	104(69.80)	40 (70.18%)	51 (79.69%)	
Retrospectively unclear	3(2.01)			
	Initial cohort Median (IQR)	**Peritonitis Median (IQR)**	**Peritonitis free Median (IQR**	
CD4 count in HIV positive(cells/mm^3) (n = 30)	339 (184-440)	348(250-424) (n=17)	321(129-443) (n=13)	0.769[b]
Age at PD initiation (Years)	44.22 (32.27-50.83)	42.33 (33.50-47.27)	45.76 (34.43-51.79)	0.689[b]
Distance of residence from PD unit(km)	11.95 (6.60-21.85)	10.30 (5.90-21.50)	11.25 (5.80-17.40)	0.513[b]

[a] Other cause: ADPCKD = 4, Hepatitis B = 2, Primary oxalosis = 1, Herbal intoxication / cortical necrosis = 1, Gout / NSAID abuse = 1; [b] Mann-Whitney U Test, 2-sided p value; [c] Fisher exact, two tailed, [d] Pearson Chi square

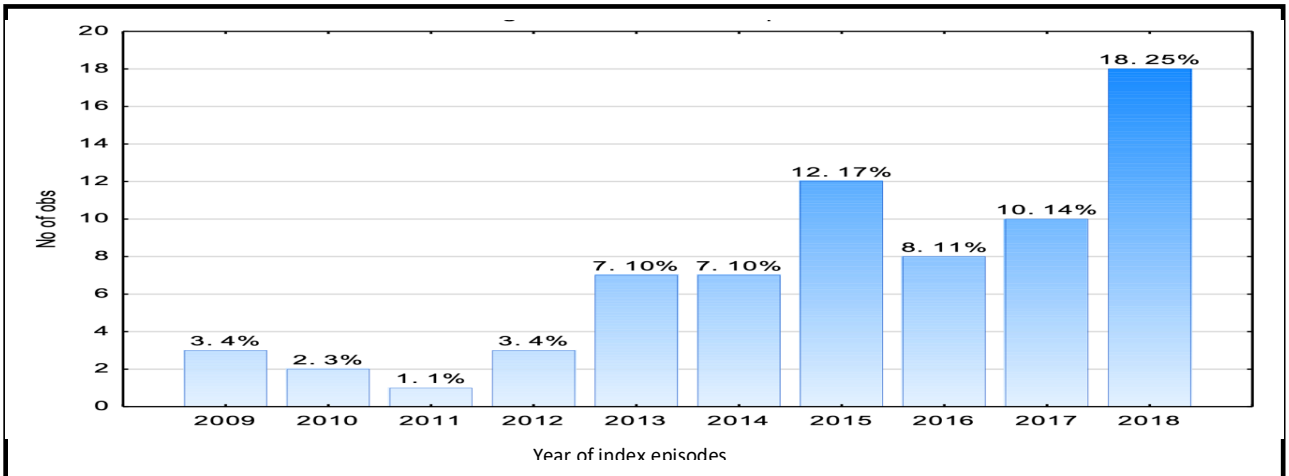

Figure 1| Histogram: Annual proportions of index peritonitis episodes

Table 2| Peritonitis: Spectrum of causative organisms			
Organism Identity	**Overall episodes** n=145(100%)	**Index episodes[a]** n=71(100%)	**2nd episodes[a]** n=41(100%)
Gram-positive organisms:			
CNS* Staph spp** NOS**	13(11.40)	11(21.57)	1(2.94)
S. epidermidis	19(16.67)	6(11.76)	4(11.76)
S. haemolyticus	3(2.63)	0	2(5.88)
S. aureus	7(6.14)	4(7.84)	2(5.88)
S. viridans	3(2.63)	2(3.92)	1(2.94)
S. pneumoniae	1(0.88)	1(1.96)	0
S. mitis	2(1.75)	1(1.96)	0
Corynebacterium spp	11(9.65)	5(9.80)	4(11.76)
E. faecium	1(0.88)	0	1(2.94)
Bacillus spp NOS	2(1.75)	2(3.92)	0
Total	63(43.45)	33(46.48)	15(36.59)
Gram-negative organisms:			
K. pneumoniae	10(8.77)	4(7.84)	4(11.76)
P. aeruginosa	7(6.14)	2(3.92)	3(8.82)
E. coli	9(7.89)	2(3.92)	4(11.76)
E. cloacae	4(3.51)	2(3.92)	1(2.94)
E. aerogenes	3(2.63)	1(1.96)	2(5.88)
C. koseri	1(0.88)	1(1.96)	0
P. stutzeri	1(0.88)	1(1.96)	0
C. amalonaticus	2(1.75)	1(1.96)	1(2.94))
S. paucimobilis	2(1.75)	1(1.96)	1(2.94)
S. marascens	1(0.88)	0	1(2.94)
Pantoea spp.	1(0.88)	0	0
O. ureolytica	1(0.88)	0	0
P. mirabilis	1(0.88)	0	1(2.94)
A. lwoffii	1(0.88)	1(1.96)	0
Total	44(30.34)	16(22.54)	18(43.90)
Fungal:			
C. parapsilosis	4(2.76)	1(1.41)	1(2.44)
Mycobacterium:			
M. tuberculosis	3(2.07)	1(1.41)	0
Culture-negative cause:	31(21.38)	20(28.12)	7(17.07)
Totals:	145(100)	71(100)	41(100)

*CNS: Coagulase negative *Staphylococcus*, **spp: species, ***NOS: not otherwise specified,
[a] 7 episodes in total, only the first 2 episodes are included here

Table 2 | Gram-positive peritonitis sensitivities by episode of peritonitis			
Antimicrobial	Overall episodes n(%)	First episode n(%)	Second episode n(%)
Vancomycin (MRSA)	35 (55.55)	17 (51.52)	7 (46.67)
Cefazolin	21 (33.33)	12 (36.36)	6 (40)
Amoxicillin	4 (6.35)	3 (9.09)	1 (6.67)
Ceftriaxone	1 (1.59)	0	1 (6.67)
Cefepime	1 (1.59)	0	0
Rifampicin	1 (1.59)	1 (3.03)	0
Piperacillin/ tazobactam	0	0	0
Total	63, 100%	33, 100%	15, 100%

Table 3 | Gram-negative peritonitis sensitivities by episode of peritonitis			
Antimicrobial	Overall episodes n(%)	First episode n(%)	Second episode n(%)
Carbapenem (ESBL)	7 (15.90)	2 (12.5)	2 (11.11)
Ceftriaxone	10 (22.27)	3 (18.75)	5 (27.78)
Cefepime	8 (18.18)	3 (18.75)	3 (16.67)
Cefepime	8 (18.18)	3 (18.75)	3 (16.67)
Ciprofloxacin	6 (13.64)	1 (6.25)	3 (16.67
Piperacillin/ tazobactam	6 (13.64)	4 (25)	2 (11.11)
Amoxicillin	3 (6.82)	2 (12.5)	1 (5.56)
Gentamicin	2, (4.55)	0	2 (11.11)
Total	44, 100%	16, 100%	18, 100%

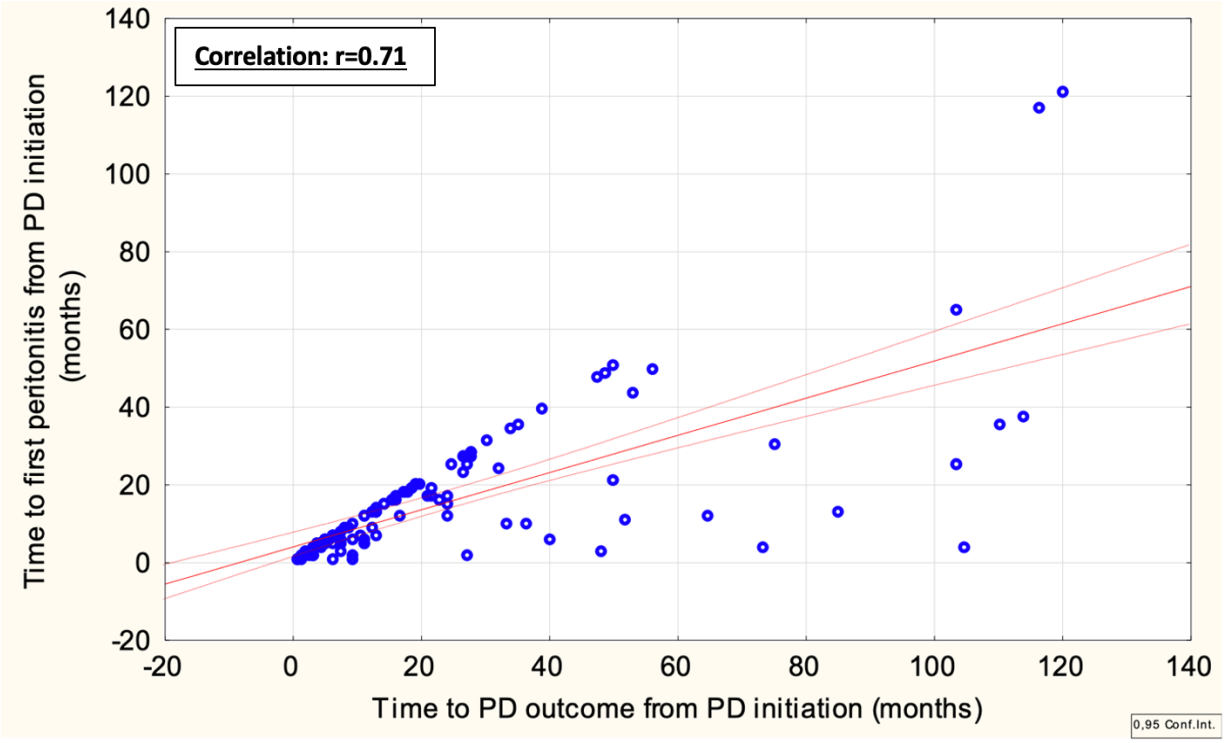

Figure 2| Pearson's rho correlation: The time to the index the episode of peritonitis from PD initiation and time to PD outcome.

References

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351(13):1296-305.

2. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med. 1999;341(23):1725-730.

3. Muller E. Organ donation and transplantation in South Africa – an update. CME. 2013;31(6):220-22

4. Davids RM, Marais N, Jacobs JC. South African renal registry annual report 2015. AJN. 2017;20(1):201-13.

5. Stel VS, Kramar R, Leivestad T, Hoitsma AJ, Metcalfe W, Smits JM, et al. Time trend in access to the waiting list and renal transplantation: a comparison of four European countries. Nephrol Dial Transplant. 2012;27(9):3621–31.

6. Yang F, Lau T, Luo N. Cost-effectiveness of haemodialysis and peritoneal dialysis for patients with end-stage renal disease in Singapore. Nephrology (Carlton). 2016;21(8):669-77.

7. Teixeira JP, Combs SA, Teitelbaum I. Peritoneal dialysis: update on patient survival. Clin Nephrol. 2015;83(1):1-10.

8. Morris PL, Jones B. Life satisfaction across treatment methods for patients with end-stage renal failure. Med J Aust. 1989;150(8):428-32.

9. Chaudhary K, Sangha H, Khanna R. Peritoneal dialysis first: rationale. Clin J Am Soc Nephrol. 2011;6(2):447-56.

10. Chen JHC, Johnson DW, Hawley C, Boudville N, Lim WH. Association between causes of peritoneal dialysis technique failure and all-cause mortality. Sci Rep. 2018;8(1):3980

11. Fontan MP, Rodriguez-Carmona A, Garcia-Naveiro R, Rosales M, Villaverde P and Valdes F. Peritonitis-related mortality in patients undergoing chronic peritoneal dialysis. Perit Dial Int. 2005;25(3):274-84

12. Boudville N, Kemp A, Clayton P, Lim W, Badve SV, Hawley CM, et al. Recent peritonitis associates with mortality among patients treated with peritoneal dialysis. J Am Soc Nephrol. 2012;23(8):1398-405.

13. Li PKT, Szeto CC, Piraino B, Arteaga JD, Fan S, Figueiredo AE, et al. ISPD Peritonitis recommendations: 2016 update on prevention and treatment. Perit Dial Int. 2016;36(5):481–508.

14. Ndlovu KCZ, Sibanda W and Assounga A. Peritonitis outcomes in patients with HIV and end-stage renal failure on peritoneal dialysis: a prospective cohort study. BMC Nephrol. 2017;18(1):48.

15. Ikabu AS. A study of epidemiology and microbiology of peritonitis in continuous ambulatory peritoneal dialysis patients at Inkosi Albert Luthuli Central Hospital, Durban, South Africa. University of Kwazulu Natal. 2015.

16. Isla RA, Mapiye D, Swanepoel CR, Rozumyk N, Hubahib JE and Okpechi IG. Continuous ambulatory peritoneal dialysis in Limpopo Province, South Africa: predictors of patient and technique survival. Perit Dial Int. 2014;34(5): 518-25.

17. Tamayo-Isla RA, de la Cruz MC and Okpechi IG. Mycobacterial peritonitis in capd patients in Limpopo: a 6-year cumulative report from a single center in South Africa. Perit Dial Int. 2016;36(2):218-22.

18. Ghali JR, Bannister KM, Brown FG, Rosman JB, Wiggins KJ, Johnson DW, et al. Microbiology and outcome of peritonitis in australian peritoneal dialysis patients. Perit Dial Int. 2011;31(6):651-62.

19. Mujais S. Microbiology and outcomes of peritonitis in North America. Kidney Int Suppl. 2006;70(103):55-62.

20. Mashiloane B, Moshesh FM and Mpe MJ. Peritonitis in patients with end-stage renal disease on continuous ambulatory peritoneal dialysis. SAMJ. 2008; 98(12):942-43.

21. South Africa. Department of Statistics South Africa. 2019. Mid-year population estimates. Statistical release no. P0302

22. Piraino B, Bailie GR, Bernadini J, Boeschoten E, Gupta A, Holmes C, et al. Peritoneal dialysis-related infectious recommendations: 2005 update. Peritoneal Dialysis International. 2005;25:107-31

23. Prasad KN, Singh K, Rizwan A, Mishra P,Tiwari D, Prasad N, et al. Microbiology and outcomes of peritonitis in northern India. Perit Dial Int. 2014;34(2):188-94.

24. Phui VE,Tan CHH, Chen CK, Lai KH, Chew KF, Chua HH, et al. Causative organisms and outcomes of peritoneal dialysis-related peritonitis in Sarawak General Hospital, Kuching, Malaysia: a 3-year analysis. Ren Replac Ther. 2017;3(35).

25. Lasfar LB, Guedri Y, Azzebi A, Sahtout W, Mrabet S, Fradi A, et al. Microbiology risk factors and outcomes of peritonitis in tunisian peritoneal dialysis patients.WJN. 2018;7(2):45-52.

26. Naidoo S, Naicker S, Malgas S, Paget G, Wadee S and Fabian J. Peritoneal dialysis in South Africa-a single centre experience. Indian J of Perit Dial. 2008;13:18-21.

27. Nessim SJ, Bargman JM, Austin PC, Nisenbaum R and Jassal SV. Predictors of peritonitis in patients on peritoneal dialysis:results of a large, prospective Canadian database. Clin J Am Soc Nephrol. 2009;4(7):1195-120

28. Chow KM, Szeto CC, Leung CB, Kwan BC, Law MC and Li PK. A risk analysis of continuous ambulatory peritoneal dialysis-related peritonitis. Perit Dial Int. 2005;25(4):374-79.

29. Tian Y, Xiea X, Xiang S, Yang X, Lin J, Zhang X, et al. Risk Factors and Outcomes of Early-Onset Peritonitis in Chinese Peritoneal Dialysis Patients. Kidney Blood Press Res. 2017 December; 42(6): p. 1266-76.

CHAPTER 3 – APPENDICES

Appendix A: Data Collection Sheet

Study number::……

Gender:	Male:	Female:

Race:	Black:	White:	Asian:	Mixed:

Age at PD initiation:…….. Date of PD initiation:…….

Aetiology of ESKD:	DM	HT	HIV-associated	GN	Obstructive / reflux	Other

If "Other", specify:…….

Comorbidities:	DM	HT	HIV	Other

If "Other", specify:……..

If "HIV positive", CD4 count at dialysis start:…….

Antecedent haemodialysis:	Yes	No

Peritonitis:	Yes	No

Classification of episode	**New**	**Relapsing**	**Recurrent**	**Repeat**

Episodes of peritonitis	1	2	3	4	5
Date of episode					

	Causative organism					
Episode	Gram pos	Gram neg	Culture neg	Fungal	TB	Specify
1						
2						
3						
4						
5						

		Episode number				
		1	2	3	4	5
Sensitivity	Cefazolin					
	Gentamycin					
	Methicillin					
	Vancomycin					
	Extended □ lactam					
	Diflucan					
	AmpB					

	Duration of hospitalization				
Episode	1	2	3	4	5
Duration (days)					

	Outcome			
	PD failure	PD survival	Pt death	Pt survival
Date				

Appendix B – Human Reasearch Ethics Council Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr MM Sunnyraj
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Helen Joseph Hospital

E-mail: midhusrj@gmail.com

CC: Supervisor: Drs M Davies and Z Cassimjee <Malcolm.Davies@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/03/23

REF: *R14/49*

PROTOCOL NO: **M190703** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Risk factors, microbiology and outcomes of the continuous ambulatory peritoneal dialysis related peritonitis at Helen Joseph Hospital, South Africa*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix C: Turnitin Originality Report

MMED Turnitin-1.docx

by Midhu Sunnyraj

Submission date: 24-Feb-2022 06:22AM (UTC+0200)
Submission ID: 1769664739
File name: MMED_Turnitin.docx (22.57K)
Word count: 3726
Character count: 21700

MMED Turnitin-1.docx

ORIGINALITY REPORT

9%	7%	6%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

5%

★ www.ncbi.nlm.nih.gov

Internet Source

Exclude quotes	On	Exclude matches	< 8 words
Exclude bibliography	On		