

MASTER OF MEDICINE IN THE SPECIALTY OF INTERNAL MEDICINE

**Mycophenolate Mofetil in Renal Transplant Recipients: Predisposition to
Gastrointestinal Intolerance**

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DECLARATION

I, Min-Shien Chen, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine (Internal Medicine) of the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

(Signature)

7th day of June, 2017

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Abstract

Objective

Renal transplantation is the ideal therapeutic option for patients that reach end-stage renal failure. However, patients require long term immunosuppression following surgical transplantation to prevent graft rejection [1,2,4]. Mycophenolate mofetil (MMF) had proven to be an effective immunosuppressant in transplant patients[8,9,10], although it is associated with an increase in gastrointestinal adverse effects, which may result in dose adjustment or termination of use [22]. There is a paucity of data regarding gastrointestinal side effects of MMF in South Africa. This study attempts to describe the incidence of gastrointestinal complications, incidence of dose adjustment and discontinuation of MMF due to side effects, to compare the incidence of GI complications between those that had prior gastrointestinal ailments and those that had no prior gastrointestinal ailments and finally to determine possible risk factors (age, gender, ethnicity, donor type, pre-transplant GI diagnosis, pre-transplant diabetes and combination of MMF with tacrolimus) of gastrointestinal adverse effects.

Method

Data was collected retrospectively from the file records of the renal transplant unit at CMJAH (Charlotte Maxeke Johannesburg Academic Hospital) on adult patients who had received kidney transplants between 1998 and 2010 and who had received MMF as part of the immunosuppressive regimen for at least the one year post-transplant. Relevant data

was captured in an anonymous fashion on a collection sheet. Descriptive analysis of the data was carried out. Time-to-event data were analysed by Kaplan-Meier survival analysis. The assessment of the effect of prior gastrointestinal ailments, as well as risk factors, was carried out by Cox Proportional Hazards regression to estimate the Hazard Ratios.

Results

A total of 188 patients were included in the study group, which comprised 65.4% males and 32.4% females (2.1% missing data). The mean age at transplant was 38.1 years. The patients were predominantly black (69.1%). Donors were predominantly deceased donors. Of the 24.5% of donors who were living donors, 76.1% were related living donors, while the rest were non-related living donors. The majority of patients (82%) were induced with MMF dose of 2 grams per day.

After 5 years, 13.8% of patients discontinued MMF while 86.2% of the patients were still on MMF. 48.1% had a dose adjustment due to gastrointestinal side effects. 61% of patients had had a diarrhoeal adverse event by 5 years. 21.8% of the patients had gastrointestinal side effects other than diarrhoea by 5 years. The combination of tacrolimus and MMF was found to be a significant risk factor for diarrhoeal adverse events (Hazard Ratio 1.82; 95% CI 1.21-2.73). Having a living donor graft reduced the chance of non-diarrhoeal gastrointestinal adverse event (Hazard Ratio 0.33; 95% CI 0.13-0.84, $p<0.02$). A trend towards significance was seen in living donors having less diarrhoeal events although it did not reach statistical significance (Hazard Ratio 1.32; 95% CI 0.87-2.00, $p=0.20$).

Conclusion

As far as the authors are aware, this is the first local study on MMF and GIT adverse effect. We found the combination of MMF and tacrolimus is associated with increased risk of having diarrhoeal adverse events, which is consistent with international data[34,35]. Living donor graft is associated with a lower risk of developing non-diarrhoeal gastrointestinal events. Although non-significant, data suggest the same trend favoring living donor graft with regards to diarrhoeal events.

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List of Abbreviations

AD	Anno Domini
ATG	Anti-lymphocyte globulin
AZA	Azathioprine
CI	Confidence Interval
CKD	Chronic kidney disease

CMV	Cytomegalovirus
CsA	Cyclosporine A
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GIT	Gastrointestinal Tract
HR	Hazard Ratio
MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid
NRLD	Non-Related Living Donor
RLD	Related Living Donor
RR	Relative Risk
SD	Standard Deviation
US	United States
USA	United States of America
USRDS	United States Renal Data System
USSR	Union of Soviet Socialist Republics

Definition of Terms

- Chronic Kidney Diseases
 - Kidney damage indicated by urine, imaging, and histologic findings, or a low eGFR, < 60 ml/min/1.73 m², for more than 3 months.
- Dose Adjustment
 - MMF dose reduction, dose interruption, dosing interval changes.

- Dose Termination
 - Any discontinuation of MMF for any period of time.
- Non-diarrhoeal gastrointestinal adverse events
 - Any gastrointestinal complaints that is not diarrhoea related, such as abdominal pain, cholelithiasis, constipation, colonic polyps, heart burn, haemorrhage, hepatitis, ulceration anywhere in the GI tract, pancreatitis and vomiting.
- Xenograft
 - A graft of tissue taken from a donor of one species and grafted into a recipient of another species.

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Chapter One

1.1 Introduction and Literature Review

1.1.1 History of Renal Transplantation

Renal transplantation has become the treatment of choice for modern day patients with end-stage renal disease. However, before the era of haemodialysis, death was the usual outcome for patients with end stage renal disease. It is thus little wonder that the kidney was the first organ to be transplanted in human history. Joboulay first attempted a xenograft transplant on two patients in 1906, by transplanting a goat kidney in one and a pig kidney in another, and attaching the graft onto the brachial vessels [1,2].

Retrospectively, it was obvious that with the primitive surgical technique, sub-optimal post-procedure care and without any immunosuppressive therapy, the graft failed and both patients died soon afterwards.

The first human cadaver renal transplant was pioneered by Voronoy in the former USSR in 1933. He harvested the graft from a donor that died due to brain injury and transplanted it into a 26 year old young female who was dying from acute renal failure due to mercury intoxication. The graft kidney was placed into the groin of the recipient. Despite the fact that there was no appreciation of ABO-compatibility and warm ischaemic time, the graft was harvested from the deceased donor 6 hours post-mortem, the graft produced urine after the procedure. The recipient died 4 days later with no thrombosis in the graft vasculature. Although for a very brief time period, the graft did function post transplantation. This was indeed a milestone in transplant medicine [1,3].

Several problems hampered the effort of these early transplant pioneers. One of the problems was the availability of suitable donor grafts, which still haunts modern day transplant units. During the 1950s, transplant units realized the importance of excessive ischaemic injury to grafts and there was a movement to utilize live donor grafts, either from relatives of the patient or live patients that had been declared brain dead. The positioning and vascular anastomosis technique also needed refinement. A kidney anastomosed to the arm or thigh vessels was not feasible long term. In 1951, a new technique was described in which the graft was placed in an extra-peritoneal position in the iliac fossa, whereby the external iliac vessels were easy to access. The bladder is in close proximity to implant the ureters. This has since then become the standard procedure [1,3].

The next hurdle was the immune response. Foreign agents induce a potent immune response that protects the host by eliminating the foreign agent. This is beneficial to the host in case of an infection or malignancy, but detrimental to the transplanted graft. Initial attempts to reduce this reaction in the forms of whole body irradiation did not yield satisfactory long term results. Patient often suffered bone marrow aplasia and died of infection afterwards [1,2]. The ideal would be a pharmacological agent that could suppress the immune system sufficiently to permit graft survival, but specific enough such that other protective immune responses remained functional, with acceptable adverse effects.

The first successful agent was azathioprine (AZA). It is a pro-drug of a more toxic substance 6-mercaptopurine, which then becomes incorporated into replicating DNA and inhibits purine nucleotide synthesis and metabolism. It also blocks the de novo pathway of purine synthesis and inhibits lymphocyte proliferation. Major adverse effects are also

linked to the haematological system, with leucopenia and bone marrow suppression being the most serious. Combined with prednisolone, azathioprine enabled the transplantation of unrelated donor kidneys with around 50% still functioning at 1 year [1].

The next step in chemical immunosuppression was the advent of cyclosporine. It allowed selective immune regulation of T cells without excessive toxicity. It was isolated from the fungus *Tolypocladium Inflatum*. Cyclosporine was first investigated as an anti-fungal antibiotic but its spectrum was too narrow to be of any clinical use. Borel discovered its immunosuppressive activity in 1976, which led to further investigations into its properties and structure. Cyclosporine A was the first of its kind to inhibit functional T lymphocytes specifically and reversibly. It inhibits the production of T cell growth factors like interleukin-2(IL-2) by binding with intra-cellular proteins called immunophilins, which then inhibit calcineurin. Inhibition of calcineurin blocks activation of early T-cell specific genes and ultimately results in inhibition of T-cell proliferation [1,4].

Due to its specific target, cyclosporine made immune suppression more tolerable and less toxic to the bone marrow. In 1983 cyclosporine was approved for clinical use to prevent graft rejection in transplantation. Cyclosporine dramatically improved the results of kidney transplantation. Today, 90–95% of kidney transplants on cyclosporine survive 1 year [1]. Since then, cyclosporine had been used with success in transplantation of various solid organs, such as heart, lung, pancreas and liver [1,4].

Tacrolimus is a newer agent that falls in the same class as cyclosporine, as both inhibit calcineurin albeit via slightly different mechanism. Tacrolimus binds to a different immunophilin, the FK506 binding protein (FKBP12). The combined complex then binds to

a different site on calcineurin to achieve the same effect as cyclosporine. It is more potent and shares a similar toxicity profile as cyclosporine. Well recognised adverse effects include neurotoxicity, post-transplant diabetes and nephrotoxicity [4]. Interestingly tacrolimus is also implicated in post-transplant diarrhoea, both in combination with MMF and AZA [5].

Three vital steps of an immune response should be targeted to achieve adequate immune suppression; first, T cell recognition of foreign antigen presented by antigen-presenting cells such as B lymphocytes, dendritic cells and macrophages, second, co-stimulatory interactions on the antigen-presenting cells and T lymphocytes, and lastly, when the above two steps are completed, activation of calcium-calcineurin pathway which activates and induces proliferation of T lymphocytes. Current immuno-suppressive therapy meets the challenge by using a triple drug regimen, which consists of corticosteroid, a calcineurin inhibitor (such as cyclosporine or tacrolimus) with an anti-metabolite (such as MMF or AZA) [3]. Triple therapy combines drugs that have different mechanisms of action and toxicity together to ensure maximal effectiveness with minimal adverse effects. The same principle is evident in cancer chemotherapy and anti-retroviral therapy [4].

A high level of immune suppression is required initially after transplant to prevent acute and hyper-acute rejection. This is achieved by using high doses of immune suppressants with or without combination of biologic antibodies against T cells. Thereafter, the dose can be reduced gradually to a lower maintenance level [1,4]. The most common regimen used today in kidney transplantation is a CD25 monoclonal antibody such as basiliximab, followed by a combination of tacrolimus, mycophenolate, and steroids [1].

1.1.2 What is Mycophenolate Mofetil (MMF)?

MMF is an ester pro-drug of mycophenolate, which was derived from the fungal species *Penicillium*. Discovered in 1893, it was the first antibiotic crystallized and purified from a mould. Although mycophenolate was not an effective antibiotic agent, there was interest in its potential as an anti-neoplastic and immunosuppressive agent. Mycophenolate was only recognized as an immunosuppressant in the 1970s by Anthony Allison, a South African born medical scientist who graduated from the University of Witwatersrand, for its powerful inhibition of inosine-monophosphate-dehydrogenase and subsequent role as an immunosuppressant. After several years of in vitro studies, he devised a more bio-available and tolerable pro-drug, mycophenolate mofetil which finally marketed as CellCept and was approved for use in renal transplant by the FDA in 1995[6].

The main mechanism of action of mycophenolate is the inhibition of inosine monophosphate dehydrogenase as mentioned before, which is a key enzyme in the de novo pathway of purine synthesis. This depletes guanine nucleotides, impairs DNA synthesis and thus arrests cell proliferation [4,5,7]. Different cells in the human body rely on the de-novo pathway for purine synthesis to a different degree. Neurons employ both the salvage pathway and the de novo pathway as compared to lymphocytes, which depends almost exclusively on the de novo pathway. Thus mycophenolate can selectively inhibit lymphocyte proliferation and suppress immune function [5,7].

After oral administration, MMF is absorbed and broken down to form the active metabolite, mycophenolic acid(MPA). The bioavailability of mycophenolic acid (MPA) following oral administration is approximately 90%. The half-life of MPA, including enterohepatic re-

circulation is 15.8 hours with a peak plasma concentrations occurring at 0.6 to 0.7 hours. UDP-glucuronosyl transferase (UDPGT) in the intestine and liver inactivates MPA by converting it into MPA-7-O-glucuronide (MPAG). MPAG then undergoes enterohepatic recirculation, enabling effective plasma concentrations of the drug to be sustained. 93% of the drug is eliminated in the urine, while 6% of the drug is eliminated in stool [4,5,7].

1.1.3 Rationale for MMF Use

MMF had been shown to decrease acute rejection rates significantly after renal transplantation in combination therapy [8,9,10]. All three referenced trials have similar designs. The US trial by Sollinger compared MMF 2g and 3g group to azathioprine (AZA) with the same follow-up period [8]. The European study compared MMF 2g and 3g group to placebo on the background of combination therapy with cyclosporine and corticosteroids during the first 6 months of transplantation [9]. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study also compared MMF at 2g and 3g dose to AZA and followed-up for 12 months post transplantation [10].

In the US renal transplant MMF group study, biopsy-proven acute rejection episodes or treatment failure occurred in 47.6% of patients in the AZA group compared with 31.1% in the MMF 2g treatment group ($p=0.0015$) and 31.3% in the MMF 3g group ($p=0.0021$). It can be deduced that significantly fewer acute rejection episodes or treatment failure occurred in the MMF groups. However, the cumulative incidence of graft loss and patient death was comparable amongst the three treatment groups with 10.4% (AZA group), 5.5%(MMF 2g group) and 8.5%(MMF 3g group). Graft rejection was the predominant cause of graft loss. A slight increase in patient death at 6 months in the MMF 3g group of

5.5% was noted, as compared to 3% in the AZA group and 3.6% in the MMF 2g group. This was due to the higher incidence of infection [8].

In the European MMF study, significantly more patients in the placebo group had acute graft failure confirmed on biopsy compared to either of the MMF groups. The addition of MMF was associated with 60-70% reduction in the frequency of acute rejection episodes compared with placebo group. The actual percentage for biopsy proven rejection was 46.4% in the placebo group, 17% in the MMF 2g group and 13.8% in the MMF 3g group ($p<0.001$). Greater efficacy was found in the 3g group than the 2g group, however this was offset by more treatment failure due to adverse events (13.3% vs 25%, $p<0.001$) [9].

Similar findings were reported in the Tricontinental trial. By 6 months, treatment failure, which included rejection, graft loss, death, and discontinuation of study drug occurred in 50% of patients in the AZA group, compared with 34.8% in the MMF 3g group and 38.2 % in the MMF 2g group. Pair wise comparison showed no difference between the MMF groups, but did show an important difference between both of these groups and the AZA group (MMF 3g vs. AZA: $p=0.0045$, MMF 2g vs. AZA: $p=0.0287$). Biopsy-proven rejection occurring in 15.9% of patients in the MMF 3g group and 19.7% in the MMF 2g group, compared with 35.5% in the AZA group. At 1 year after transplantation, the investigators found that graft survival in the MMF groups was marginally superior to that in the AZA group, although this difference was not statistically significant. The investigators further concluded that MMF is associated with a significantly lower rate of treatment failure compared with AZA during the first 6 months after renal transplantation and produces a clinically important reduction in the incidence, severity, and treatment of acute graft rejection. These differences persist throughout the first year of follow-up [10].

The above 3 studies had proven that MMF is an effective immunosuppressive agent in renal transplantation by reducing the incidence of biopsy-proven rejection. One noticeable trend is that although the higher MMF dose group have fewer rejection episodes, adverse events tend to increase with higher dose of MMF. In the European study, the investigators specifically mentioned the increase in leukopenia and GI adverse events in both the MMF groups [9]. The Tricontinental study also found that clinical benefit was greatest with a dose of MMF 3 g/day, but gastrointestinal effects, invasive cytomegalovirus infection, and malignancies were slightly more common at that dose [10].

All 3 of the above studies mostly looked at 6 months post-transplantation. The investigators hoped that the decrease in acute rejection in the first 6 months would translate into long term benefits as well. At the time, MMF had just been approved by FDA, thus it was impossible to obtain long term data. The investigators all did a 3-year follow-up on the original study. In the European study, 491 participants were included in the analysis. The 3-year patient survival was 88.9% in the placebo group, 92.7% in the MMF 2 g group, and 91.8% in the MMF 3 g group. The overall 3-year graft survival was 78.0% in the placebo group, 84.8% in the MMF 2 g group, and 81.2% in the MMF 3 g group. When compared with placebo, MMF 2 g had a significant difference in survival curves over the 3-year period [11].

In the Tricontinental follow-up at 3 years after transplant, both intent-to-treat and on-study analyses of graft and patient survival showed a trend toward advantage for MMF 2 g and 3 g vs. AZA, although this trend did not reach statistical significance. Gastrointestinal toxicity, leukopenia, and tissue-invasive cytomegalovirus disease were more common in the MMF

3 g group both during and after the first post transplant year. Mortality was comparable in all three groups (AZA, 8.6%; MMF 2 g, 4.7%; MMF 3 g, 9.1%) by 3 years of follow-up [12].

Furthermore, a review of the US renal transplant scientific registry suggested that MMF decreased the relative risk for development of chronic allograft failure by 27%, independent of its outcome on acute rejection [7,13]. Both 4-year patient survival (91.4% versus 89.9%) and graft survival (85.6% versus 81.9%) were better for those patients receiving MMF than for those receiving AZA [13]. Similar results were published by Merville et al, whereby the number of patients with chronic allograft nephropathy at 1-year post-transplantation was significantly reduced in the MMF group (46% versus 71%) compared with the AZA group [14].

It appears from the above that MMF preserves graft function effectively by preventing acute rejection as well chronic allograft nephropathy [7,13,14], which positions MMF as an ideal immunosuppressive agent in a combination regimen. It is not without disadvantages though, and the most common adverse effects, emerging from all 3 pivotal studies, are hematological and gastrointestinal. This study shall focus on the gastrointestinal adverse effects.

1.1.4 Gastrointestinal Adverse Effects of MMF and Post-Transplant Diarrhoea

Gastrointestinal toxicity is a major adverse effect of MMF [15]. In the European MMF study group trial, up to 52.5% of patients treated with MMF 3g daily developed gastrointestinal side effects [9]. In the US trial by Sollinger et al, 31.5% and 37.3% of patients treated with MMF 2g and 3g per day respectively developed diarrhoea as compared to 23.8% from

azathioprine group at 6 months [8]. A similar comparison is shown in the Tricontinental trial, whereby 28% and 31% of the MMF group developed diarrhoea, compared to only 17% from azathioprine group at 12 months [10]. The consistency of higher gastrointestinal adverse effects with higher MMF dosage suggest that this may be dose related [15].

While adverse gastrointestinal effects range from abdominal cramps, bleeding, nausea and vomiting, diarrhoea is the most common gastrointestinal side effect associated with MMF. Intestinal epithelial cells are about 50% dependent on the de novo pathway for purine synthesis. MMF inhibits this pathway and could potentially inhibit enterocyte replication and fluid absorption [15]. This may explain the high incidence of gastrointestinal adverse effects especially diarrhoea, observed in the trials [8,9,10]. Case reports have shown intestinal villous atrophy following MMF administration causing diarrhoea, which resolved following MMF withdrawal [16,17].

The etiologies of diarrhoea in transplant patients are often multi-factorial. They may be related to immune suppression, infection by various organisms, other medical co-morbidities or that of direct toxicities from the immune suppressive medication. The addition of MMF in a multi-drug regimen enhances immune suppression and increases susceptibility to various intestinal infections [15]. A case report from Guerard et al described two cases of microsporidiosis with chronic intractable diarrhoea and associated weight loss. Symptoms persisted despite treatment and only subsided with the substitution of MMF with azathioprine [18]. A series of 26 patients on MMF with persistent diarrhoea, by Maes et al, showed that all but one patient had enterocolitis on histology and that about 60% of these were due to infection. Again, reduction or cessation of MMF therapy was the only effective therapy [19].

However, adjusting the dose can have detrimental effects on the graft. Knoll et al in a study of 213 renal transplant recipients, found that the cumulative number of days with the MMF dose reduced below full dose was an independent predictor of acute rejection. The relative risk of rejection increased by 4% for every week that the MMF dose was reduced below full dose. However, there was no significant association observed between the number of days with MMF dropped below full dose and chronic allograft failure [20]. Pelletier et al, in a study of 721 recipients, found that those with a dose adjustment of MMF within the first transplant year had significantly higher incidence of acute rejection (23.3%) as compared to those without a dose adjustment (3.7%) [21]. This demonstrated that suboptimal MMF exposure in the early post-transplant period predisposes to higher risk of acute rejection.

In a retrospective study reviewing records of 772 patients, Tierce et al found that 49.7% of patients experienced at least one GI complication within the first 6 months post-transplant, out of which, 39% (n=149) experienced MMF dose adjustments or discontinuation of MMF therapy. Out of those that had MMF dose adjustments due to GI complications, a significantly increased incidence of acute rejections (30.2% vs 19.4%) was found as compared to those without [22].

Tacrolimus, in combination with MMF also increases the risk of post-transplant diarrhoea. The Symphony Study, which compared 4 treatment groups: standard dose cyclosporine, low dose cyclosporine, low dose tacrolimus and low dose sirolimus, all in combination with daclizumab, MMF and steroids, concluded that low-dose tacrolimus provided adequate immunosuppression with better renal function and less acute rejection. However, Kaplan–

Meier estimates for diarrhoea differed significantly between the groups ($P < 0.001$), with the lowest rates occurring in the two cyclosporine groups (15.6% and 13.0% respectively), while the highest rate was in the low dose tacrolimus group (25.3%). The higher rate of diarrhoea in the low dose tacrolimus group persists in the three-year follow-up study (33%), compared to the standard and low dose cyclosporine group (23% and 18 %, $p < 0.0001$) [23]. In the DIDACT study, which involved 16 transplant centers in Belgium, 108 patients with severe diarrhoea (≥ 3 stools/day for ≥ 7 consecutive days) irrespective of the time from transplant and immunosuppression, were enrolled. The majority of the patients were on MMF ($n=96$) and tacrolimus ($n=70$). The investigators found that MMF was associated with the largest number of dose reductions or termination ($n = 34$, 35%), followed by tacrolimus ($n = 12$, 17%) due to post-transplant diarrhoea [24].

From all of the above, one can conclude that GI complications are common in those that receive MMF as part of their regimen. GI complications themselves often leads to dose adjustment or termination, which may predispose patients to a higher risk of acute graft rejection. This also leads to higher health cost for the patient. Tierce et al found that mean incremental cost for patients experiencing GI complications was 3700 USD per patient during the 6 months post-transplant [22]. This is unaffordable in a resource limited setting such as South Africa. The aim of this study is to determine if there are any clinical criteria that one can easily use to predict which group of patients receiving MMF are likely to have GI complications, in order to assist clinicians choosing immunosuppressive regimens.

1.2 Research Objectives

To our knowledge, there has been no similar study conducted in South Africa,

investigating the gastrointestinal adverse effects of MMF. Research objectives are stated below:

1. Describe the incidence of discontinuation of MMF due to GI side effects.
2. Describe the incidence of dose adjustment of MMF due to GI side effects.
3. Describe the incidence of GIT complications in the study group.
4. Compare the incidence of GIT complications between those that had prior GI ailment and those that had no prior GIT ailments.
5. Determine the effect of possible risk factors (age, gender, ethnicity, donor type, diabetes, tacrolimus exposure) on GIT side effects.

Chapter Two

2.1 Methodology

2.1.1 Study Setting

The study was conducted in the renal transplant unit at Charlotte Maxeke Academic Hospital (CMJAH) in Johannesburg, Gauteng Province. The Unit provides renal transplant services for the City of Johannesburg as well as the southern part of Gauteng Province and part of North West Province.

2.1.2 Study Design

This was a retrospective observational study to determine any possible risk factors for transplant patients that are on mycophenolate mofetil (MMF) that develop gastrointestinal side-effects that lead to dose adjustment/discontinuation. Data was collected anonymously from patient records in the CMJAH renal transplant unit from January 1998 till December 2010.

2.1.3 Study Population

Bearing in mind the multitude of socio-economic factors in post-Apartheid South Africa, public hospitals serve wide geographical areas with variable socio-economic groups. The patient population studied comprised of a mixed racial group with lower socio-economic

status.

2.1.4 Inclusion Criteria

- All adult patients over the age of 18 years that received kidney allograft in CMJAH from 1998 till December 2010.
- Mycophenolate mofetil needed to be part of the immunosuppressive regime for at least a year.
- To exclude surgical causes of early graft failure, only those whose graft survived the first 6 months of transplant were included.

2.1.5 Exclusion Criteria

Incomplete patient file records were not included in the study.

2.1.6 Instruments

A data capture sheet was created to collect relevant epidemiological data (i.e. age, gender, race group, year of transplant, age at transplant) as well as variables such as prior gastrointestinal diagnosis, date of gastrointestinal events and date of change in immunosuppressive regimen. (Annexure 1) from patient file records. A study number on the data collection sheet was generated which could be matched to the patient's name and date of birth on a separate sheet (Annexure 2), and thus data could be accurately tracked while ensuring patient confidentiality.

2.2 Data Analysis

2.2.1 Statistical Analysis

A descriptive analysis of the data was carried out as follows: Categorical variables were summarised by frequency and percentage tabulation, and illustrated by means of bar charts. Continuous variables were summarized by the mean, standard deviation, median and interquartile range, and their distribution illustrated by means of histograms. Time-to-event data were analyzed by Kaplan-Meier survival analysis. The assessment of the effect of prior GI ailments, as well as risk factors, was carried out by Cox Proportional Hazards (PH) regression to estimate the Hazard Ratios (HRs). At least 10 events per estimated parameter were required. The Cox PH regression analysis was carried out for diarrhoeal and non-diarrhoeal complications separately. In each case, each risk factor was examined on its own, after which risk factors with $p < 0.20$ were combined in a multivariate regression. Confounding relationships between risk factors combined in a multivariate regression were assessed by determining the strength of the association between each pair of risk factors (phi coefficient or Cramer's V for pairs of categorical variables, or Cohen's d for categorical-continuous variable pairs). Data analysis was carried out using SAS. The 5% significance level was used. In other words, p-values < 0.05 indicate significant results.

2.2.2 Sample Size

Calculation of sample size requirements was based on the key research question to be answered, in this case, the hazard ratio for gastrointestinal complications amongst those who had prior gastrointestinal ailments versus those who did not. Assuming equal group

sizes, a hazard ratio of 1.5, a median survival time (to GI complication) of 1 year, a censoring rate of 10%, and an average follow-up duration of 5 years, a significance level of 5% and 80% power, a sample size of 216 is required. Thus, the actual sample size of 188 is adequate for a study of this nature.

2.3 Ethical Considerations

The researcher has no conflict of interest. Two separate data collecting sheets were used to ensure anonymity. The use of unrelated record numbers on the data sheets further ensure anonymity. No personal details except those pertaining to the study are recorded. Due to the retrospective nature of this study, no direct contact with patients was required. This study was approved by the University of Witwatersrand Human Research Ethics Committee, protocol reference number M141126. The use of hospital records was approved by the CEO's office of CMJAH. The relevant documentation is attached in Annexure 3 and 4.

Chapter Three

3.1 Descriptive Analysis of Study Group

A total number of 399 traceable records of transplant recipients were found. Out of which 41 (10%) had incomplete file records. A total of 188 (52.5%) out of 358 file records met the inclusion criteria and were included for the study. In the study group, 70.7% (n=133) of the patient survived without rejection past 5 years post-transplant, 11.1% (n=21) lost to follow up, 12.7% (n=24) rejected and 5.3% (n=10) demised.

Description of the study population are presented under the following heading,

- Year of Transplant
- Age of Transplant
- Gender Distribution
- Ethnicity
- Graft Type
- Combination of tacrolimus and MMF

3.1.1 Year of Transplant

The distribution of the study group with respect to the year of transplant are shown in the following graph.



Figure 1. Distribution of patient numbers over the study period in years AD.

3.1.2 Age at Transplant

The mean age at transplant was 38.1 years (SD=10.6 years; range 18.4-66.3 years). The distribution of the ages is shown below:

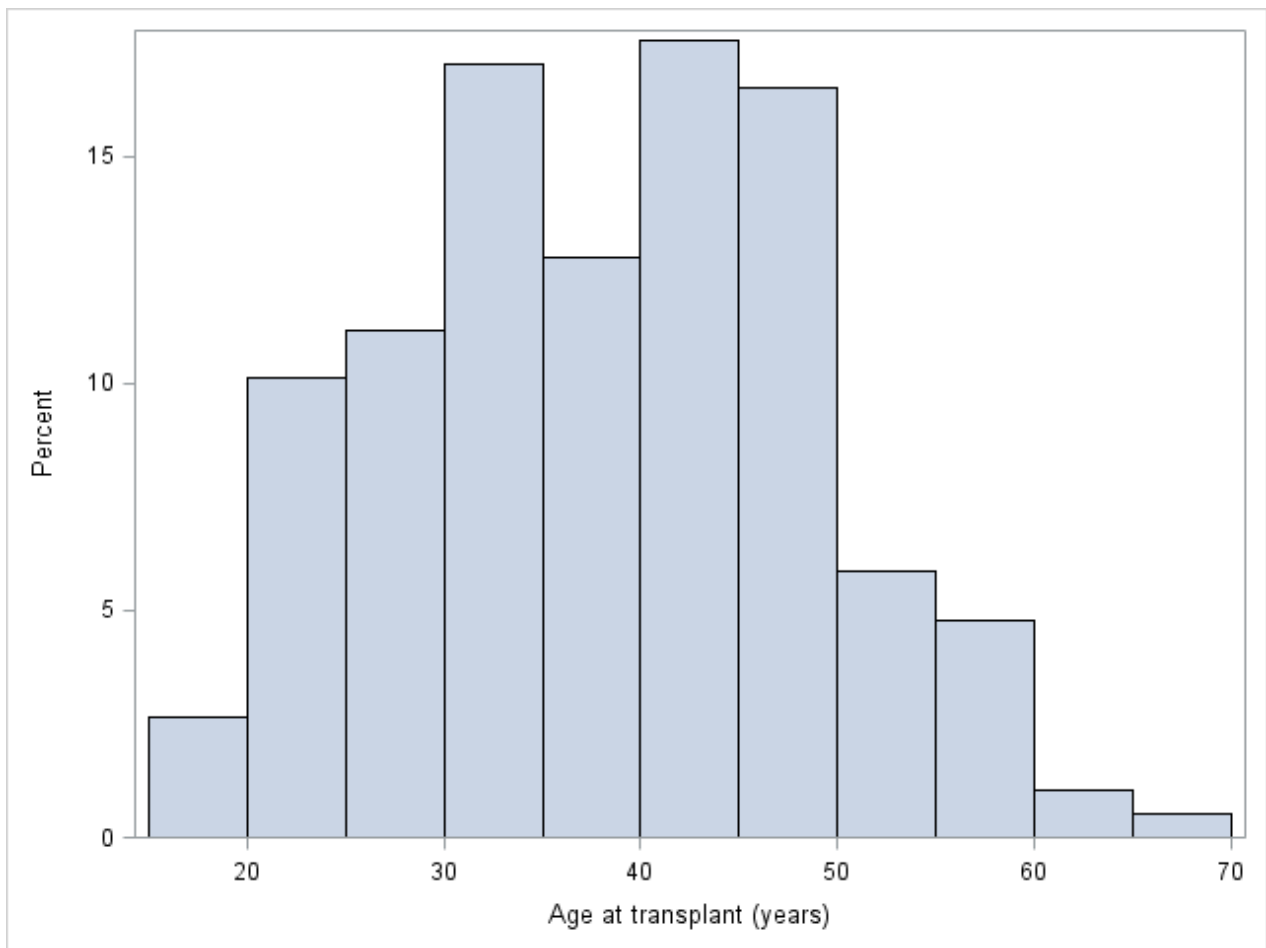


Figure 2. Age distribution of patients in the study

3.1.3 Gender Distribution

The study group comprised 65.2% males (n=123) and 32.2% females (n=61). 4 files (2.1%) have missing or unclear data with regards to gender. There is a preponderance of male gender in the study population.

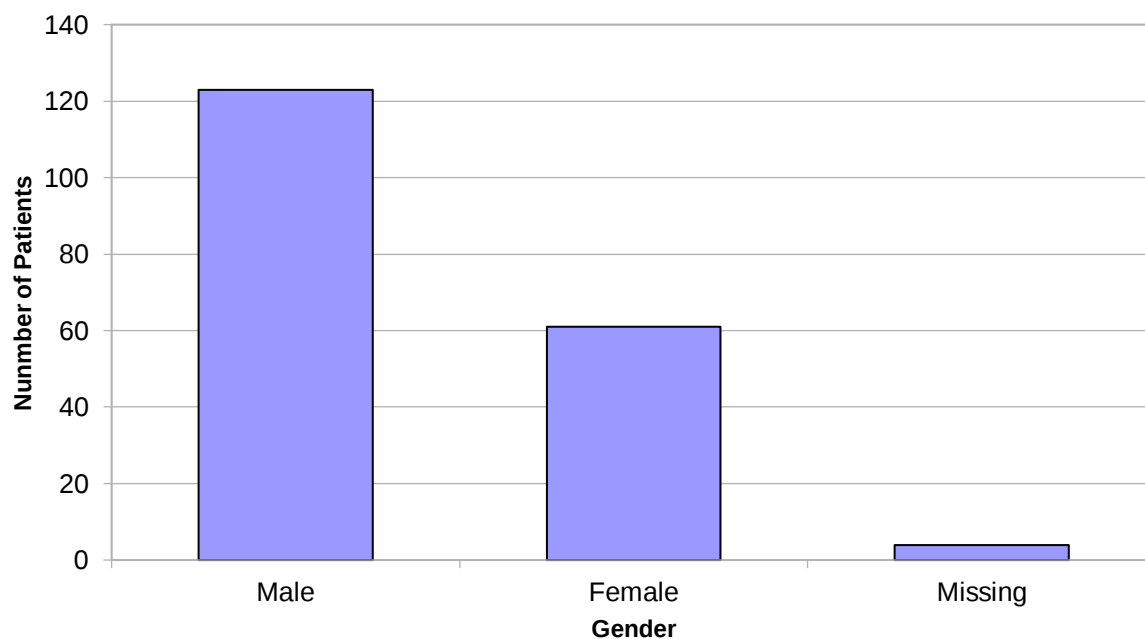


Figure 3. Gender distribution of the patients in the study group

3.1.4 Ethnicity

As reflected by the general population, majority (n=130, 69.1%) of the patients are black.

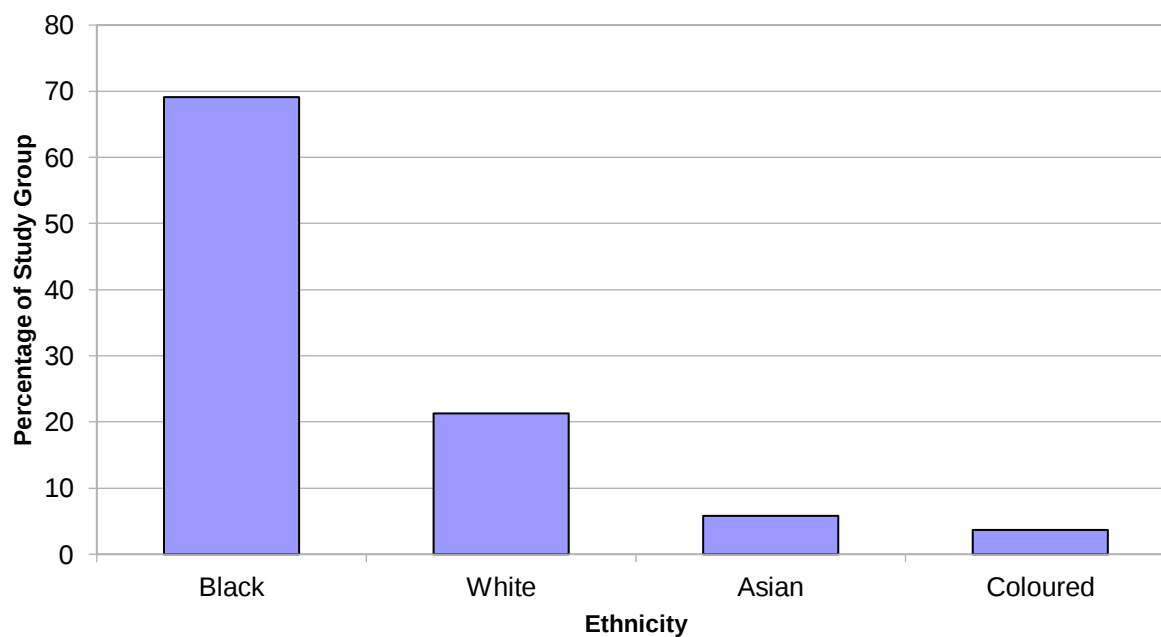


Figure 4. Ethnicity distribution of the study population.

3.1.5 Graft Type

There were 142 (75.5%) cadaveric donors. Of the 46 (24.4%) donors who were living donors, 35 (18.6%) were related living donors, while 11 (5.8%) were non-related living donors.

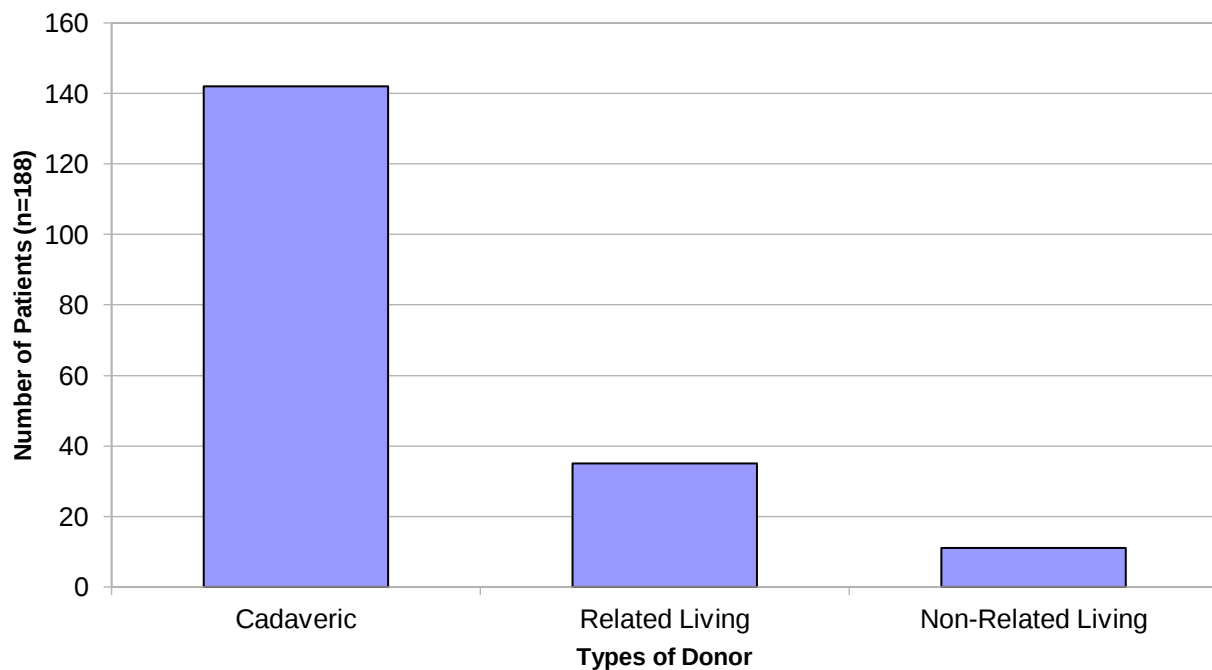


Figure 5. Bar chart demonstrating the three types of donor grafts: cadaveric, related living donor graft and non-related living donor graft.

3.1.6 Pre-transplant GI Diagnosis

The level of missing data for this variable is in excess of 30% (37.8%) which means that this variable cannot be analysed at all. This unfortunately means that we will not be able to address some of the study objectives.

3.1.7 Pre-transplant Diabetes

The level of missing data for this variable is in excess of 30% (37.8%) which means that this variable cannot be analysed at all. This unfortunately means that we will not be able to address some of the study objectives.

3.1.8 MMF Induction Dose

The distribution of the data is shown below. The majority of patients were initiated on a dose of 2 grams daily. 0 g means that the patient did not initiate on MMF, only changed to MMF later on.

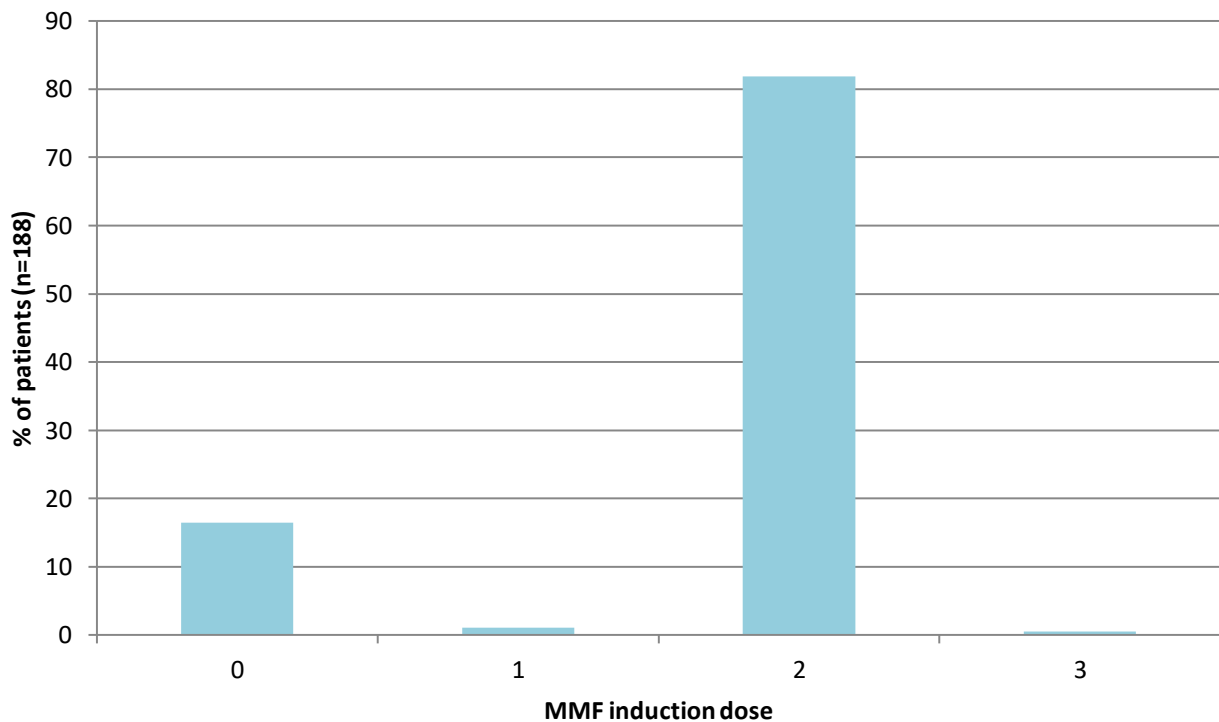


Figure 6. Bar chart showing MMF induction dose.

3.1.9 Combination of Tacrolimus and MMF

59 out of 188 patients enrolled had been exposed to combination of tacrolimus and MMF as part of the multi-drug immune suppression regimen, which translates to 31.4% of the total study population.

3.2 Statistical Analysis of Study Data

The aims of this study were to describe incidence of discontinuation and/or the incidence of dose adjustment of MMF due to GI side effects, the incidence of GIT complications in the study group and then, compare the incidence of GIT complications between those that had prior GI ailments and those that had no prior GIT ailments. Finally, we want to determine the effect of possible risk factors (age, gender, ethnicity, donor type, diabetes) on GIT side effects.

3.2.1 Discontinuation of MMF due to GI side effects

The median follow-up time for the study group (until MMF discontinuation due to GI adverse events, death, or loss to follow-up) was 4.6 years.

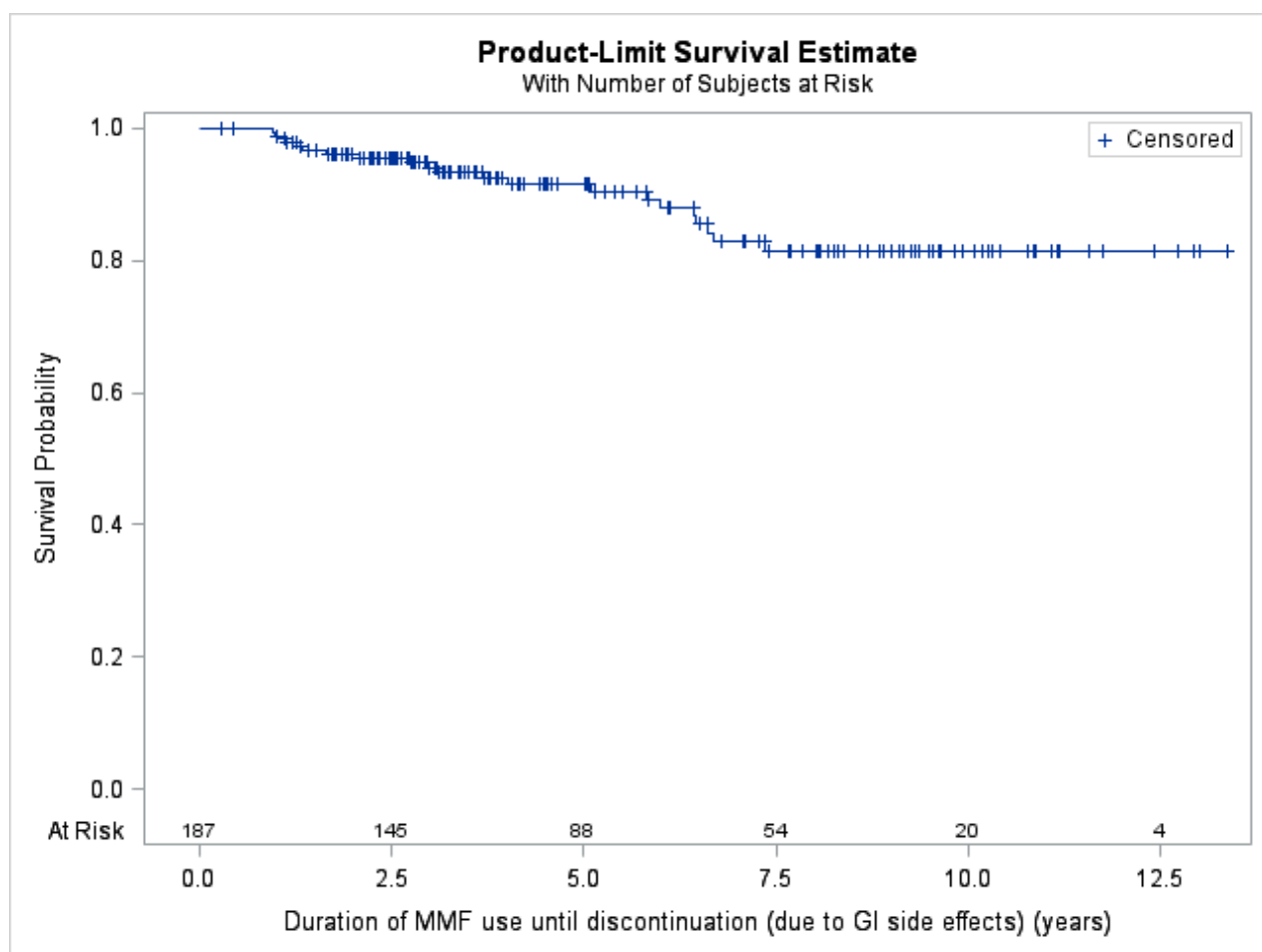


Figure 7. Kaplan-Meier survival curve for time until MMF discontinuation due to GI adverse events is shown. The data beyond 10 years should not be interpreted, due to the low number of subjects at risk.

The survival estimates (i.e. the percentages of cases who did not have MMF discontinued due to GI side effects) at 1, 3, 5, and 10 years after transplant are tabulated below. Thus, we can conclude that, after 5 years, 91.6% of the patients were still on MMF (and so on).

Time (years)	Survival (%)	95% CI for survival	
1	98.9%	95.7%	99.7%
3	94.1%	89.3%	96.8%
5	91.6%	85.8%	95.1%

10	81.3%	72.0%	87.8%
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Table 1. Percentage of patients that continued MMF over time

3.2.2 Dose adjustment of MMF due to GI side effects

The median follow-up time for the study group (until MMF dose adjustment, MMF discontinuation, death, or loss to follow-up) was 3.1 years. The Kaplan-Meier survival curve for time until MMF dose adjustment due to side effects is shown below. The data beyond 8 years should not be interpreted, due to the low number of subjects at risk.

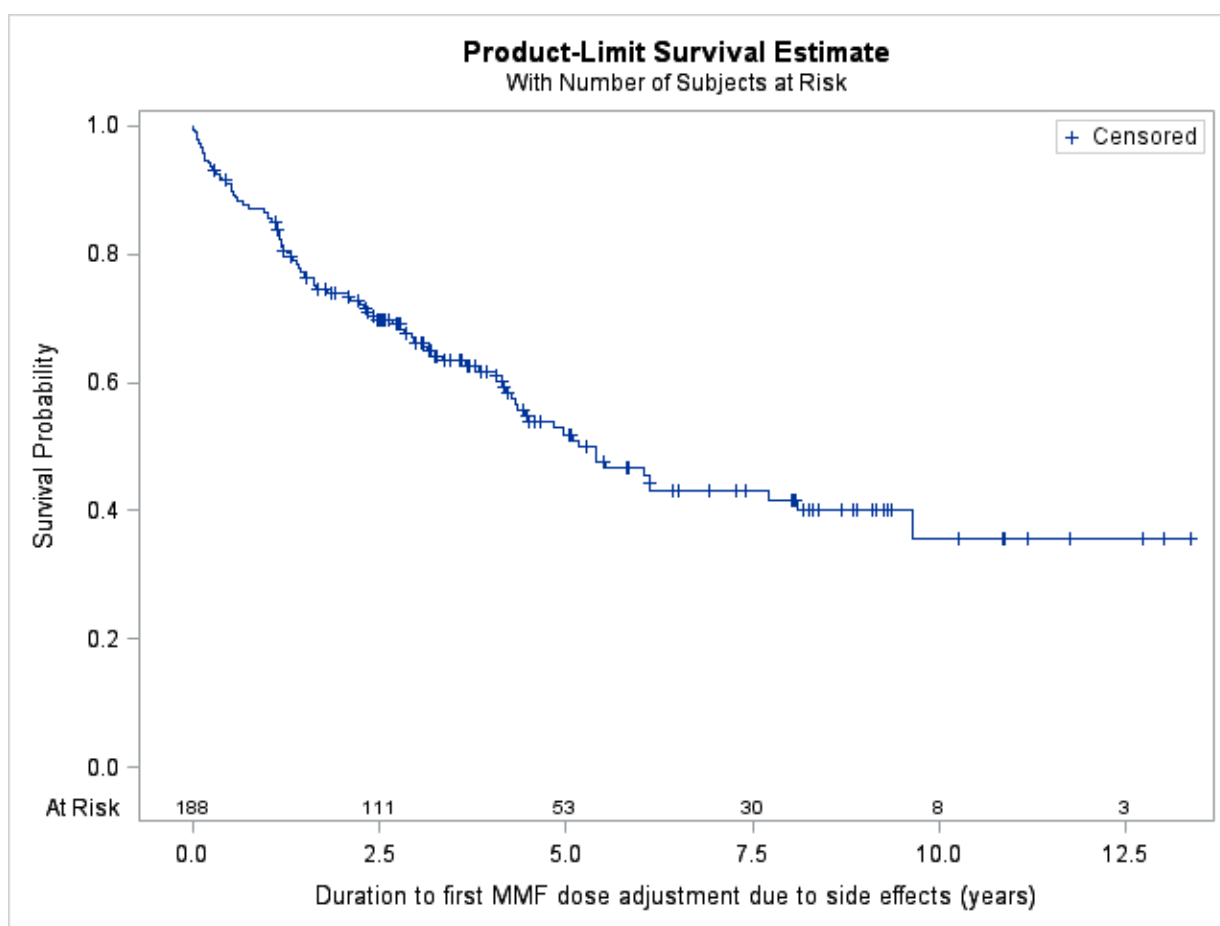


Figure 8. Kaplan-Meier curve showing patients that continued on induction dose of MMF over time.

The survival estimates (i.e. the percentages of patients who are on induction dose of MMF) at 1, 3, 5, and 8 years after transplant are tabulated below. After one year, 13.4% of patients have their MMF dose adjusted. After 5 years after transplant, 48.1% (n=90) had a dose adjustment due to GI adverse events.

Time (years)	Survival (%)	95% CI for survival	
1	86.6%	80.9%	90.8%
3	66.3%	58.7%	72.8%
5	51.9%	43.4%	59.8%
8	41.7%	32.6%	50.4%

Table 2. Table showing percentage of patients on induction dose of MMF over time.

3.2.3 Incidence of GI complications

The data is analyzed by dividing GI complications into diarrhoeal and non-diarrhoeal events.

3.2.3.1 Incidence of Diarrhoeal Complications

The median follow-up time for the study group (until first episode of diarrhoea, MMF discontinuation, death, or loss to follow-up) was 2.6 years. The Kaplan-Meier survival curve for time until the first episode of diarrhoea is shown below. The data beyond 8 years should not be interpreted, due to the low number of subjects at risk.

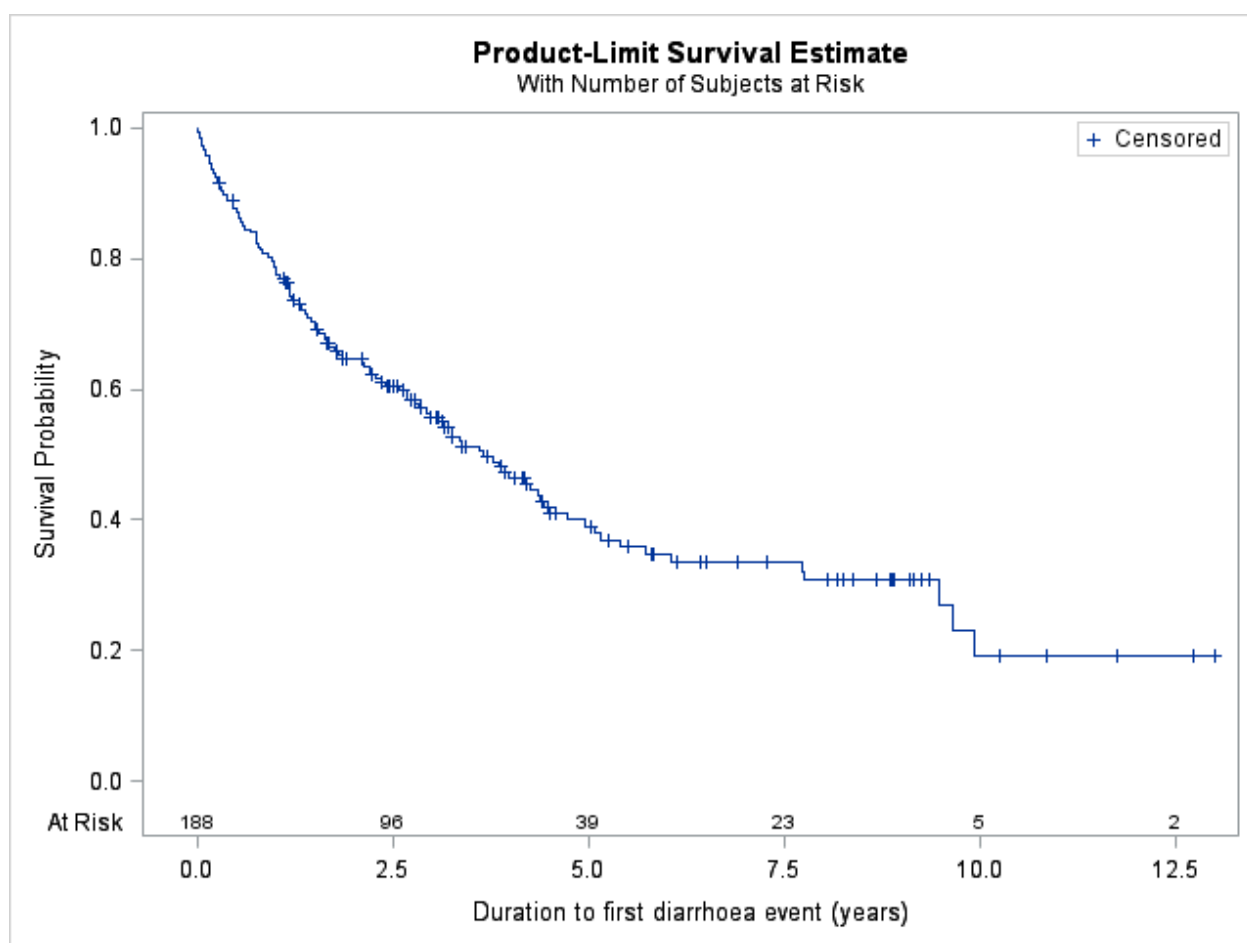


Figure 9. Kaplan-Meier graph depicting patients that did not have diarrhoea over time

The survival estimates (i.e. the percentages of cases who did not have diarrhoea) at 1, 3, 5, and 8 years after transplant are tabulated below. Thus, one can conclude that, after 5 years, 39.0% (n=73) of the patients had not experienced diarrhoea as an adverse event, while 61% (n= 115) had.

Time (years)	Survival (%)	95% CI for survival	
1	78.6%	72.0%	83.8%
3	55.7%	48.0%	62.7%
5	39.0%	30.9%	47.0%
8	30.7%	22.5%	39.3%

Table 3. Table depicting percentage of patients who did not have diarrhoea over time.

The median time to the first diarrhoeal event was 3.6 years (95% CI: 2.8-4.4 years). Of the 109 patients who had a diarrhoeal event, 22 subsequently discontinued MMF (20.2%), and 80 had a dose adjustment (73.4%).

3.2.3.2 Incidence of Non-diarrhoeal GI complication

The median follow-up time for the study group (until first episode of non-diarrhoeal GI complication, MMF discontinuation, death, or loss to follow-up) was 3.6 years. The Kaplan-Meier survival curve for time until the first episode of non-diarrhoeal GI complication is shown below. The data beyond 8 years should not be interpreted, due to the low number of subjects at risk.

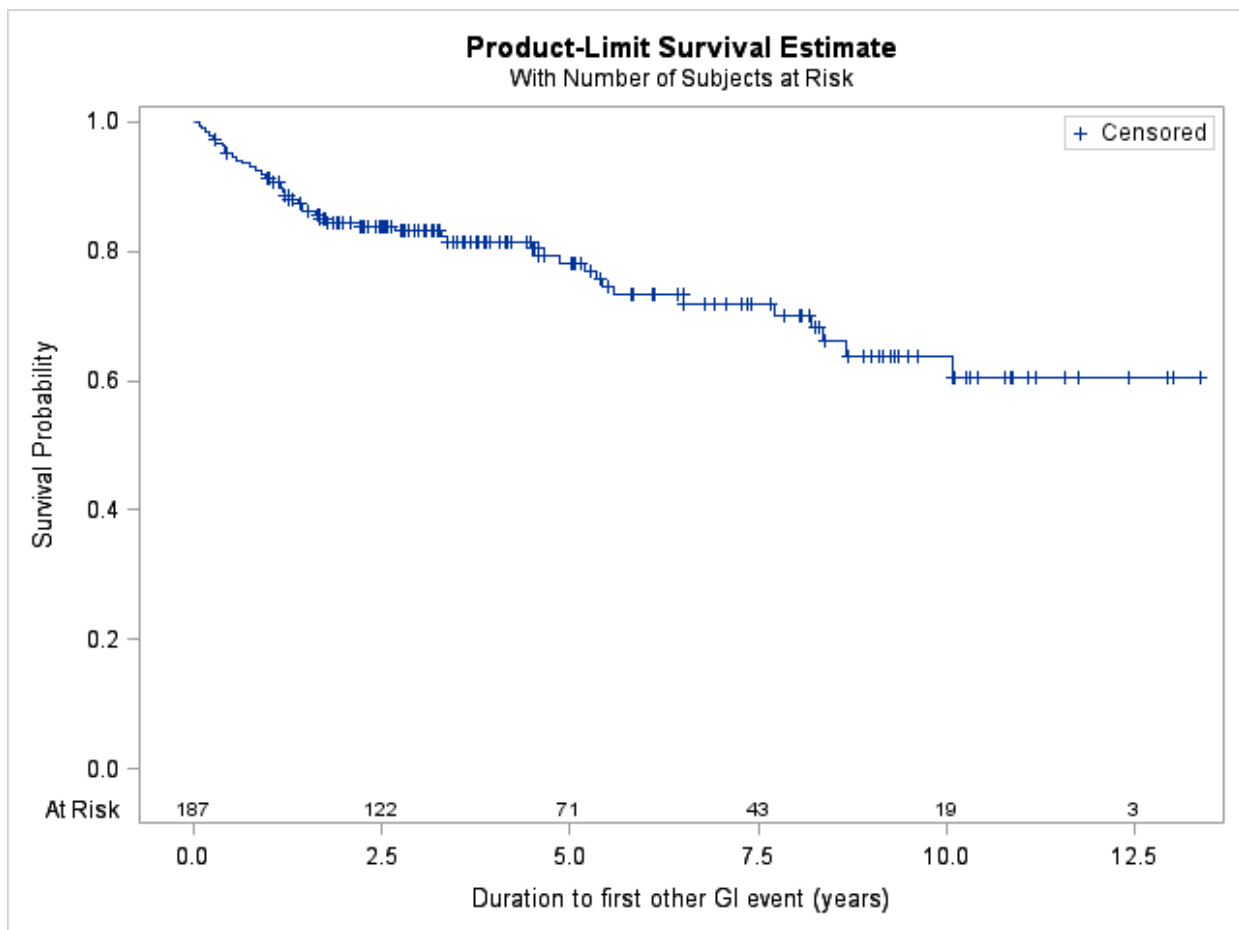


Figure 10. Kaplan-Meier graph showing patients that are free of non-diarrhoeal complications over time.

The survival estimates (i.e. the percentages of cases who did not have a non-diarrhoeal gastrointestinal complication episode) at 1, 3, 5, and 8 years after transplant are tabulated below. Thus, one can conclude that, after 5 years, 78.2% (n=147) of the patients had not had a non-diarrhoeal gastrointestinal complication, while 21.8% (n=41) had a non-diarrhoeal gastrointestinal complication.

Time (years)	Survival (%)	95% CI for survival	
1	91.4%	86.3%	94.6%
3	83.1%	76.7%	87.9%

5	78.2%	70.7%	84.1%
8	70.2%	60.6%	77.9%

Table 4. Percentage of patients that did not have non-diarrhoeal complication over time

Of the 46 patients who had a non-diarrhoeal complication, 11 subsequently discontinued MMF (23.9%), and 13 had a dose adjustment (28.3%).

3.2.4 Comparing the incidence of GI complications between patients that had prior GI ailments and those that had no prior GI ailments

This objective cannot be addressed due to the large amount of missing data in the pre-transplant GI diagnosis variable as mentioned previously.

3.2.5 Determining Possible Risk Factors of GI Adverse Events

There are enough cases in the smallest group (either event or non-event) for each of the outcome variables to allow analysis.

- Diabetes was excluded from this analysis due to the high levels of missing data (as discussed earlier).
- Ethnicity: Coloured and Asian were combined due to their small group sizes.
- Donor type: RLD and NRLD were combined due to the small group size for NRLD.
- The following reference categories were used for the categorical risk factors:
Gender: male; Ethnicity: Black; Donor type: cadaver; Combination of tacrolimus and MMF. Age at transplant was used as a continuous variable.

3.2.5.1 Risk Factors for Diarrhoeal Adverse Event

Parameter	Reference category	Category	p-value	Hazard Ratio	95% CI for Hazard Ratio	
Age at transplant	-	-	0.50	0.99	0.98	1.01
Sex	M	F	0.50	1.15	0.77	1.71
Ethnicity	B	A/C	0.34	0.73	0.39	1.39
		W	0.43	1.20	0.77	1.88
Graft type	CD	RLD/NRLD	0.20	1.32	0.87	2.00
Combination of tacrolimus and MMF	No	Yes	0.0038	1.82	1.21	2.73

Table 5. Table depicting risk factors for diarrhoeal adverse event

Combination of tacrolimus and MMF was a significant risk factor. It increases the risk of a diarrhoeal event (Hazard Ratio 1.82; 95% CI 1.21-2.73) as illustrated below:

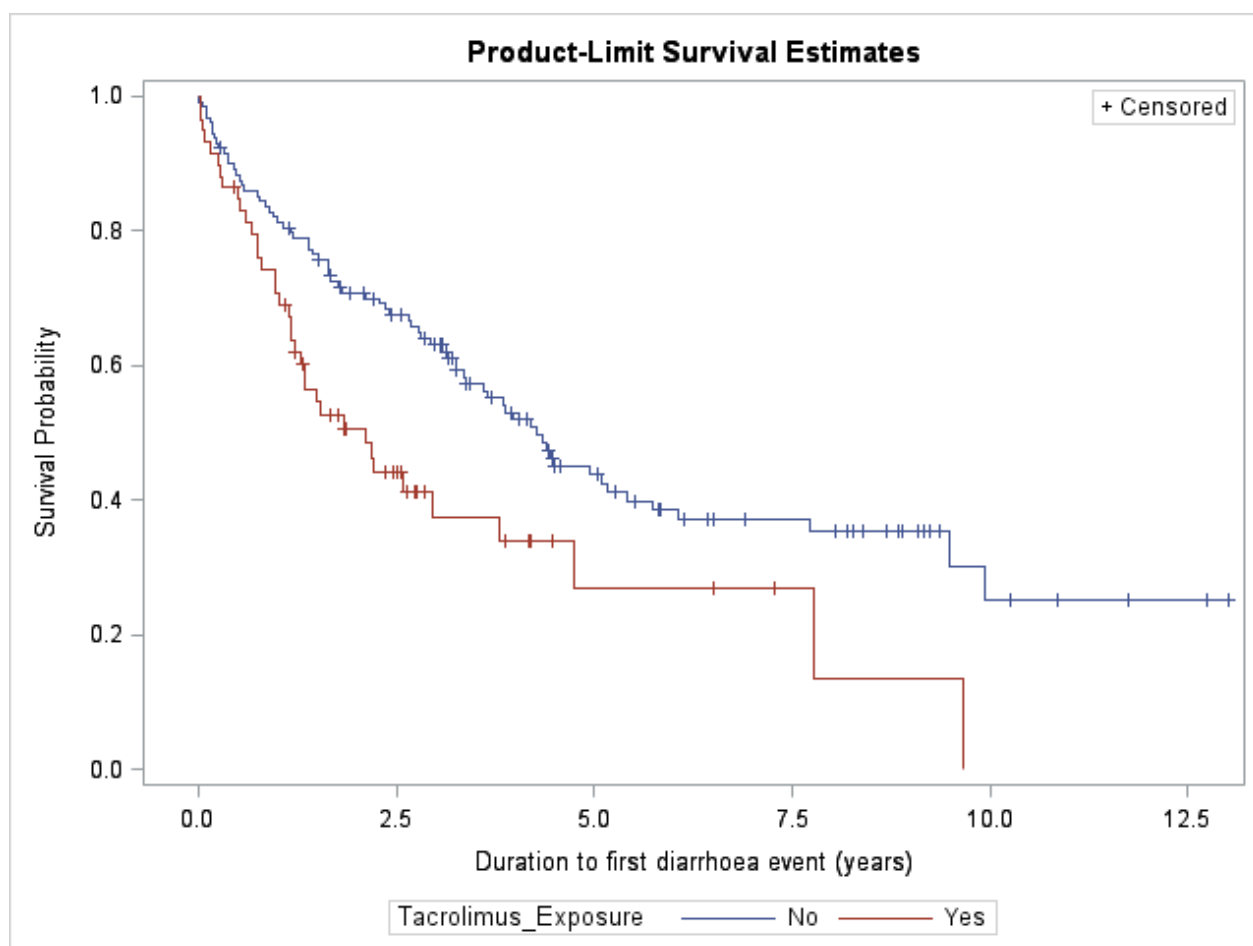


Figure 11. Kaplan-Meier survival curve comparing tacrolimus exposure to non-tacrolimus exposure in patients already on MMF therapy.

None of the other risk factors was significant when considered individually. No p-values were below 0.20, thus no multivariate analysis was carried out.

3.2.5.2 Risk Factors for Non-Diarrhoeal GI Adverse Events

Parameter	Reference category	Category	p-value	Hazard Ratio	95% CI for Hazard Ratio	
Age at transplant	-	-	0.47	0.99	0.96	1.02
Sex	M	F	0.49	1.24	0.67	2.29

Ethnicity	B	A/C	0.55	0.75	0.29	1.94
		W	0.26	0.63	0.28	1.42
Graft type	CD	RLD/NRLD	0.020	0.33	0.13	0.84
Combination of tacrolimus and MMF	No	Yes	0.83	1.08	0.55	2.10

Table 6. Table depicting risk factors for non-diarrhoeal gastrointestinal adverse event

Graft type was a significant risk factor. Having a living donor graft (compared to a cadaveric graft) reduced the risk of a non-diarrhoeal event (Hazard Ratio 0.33; 95% CI 0.13-0.84) as illustrated below:

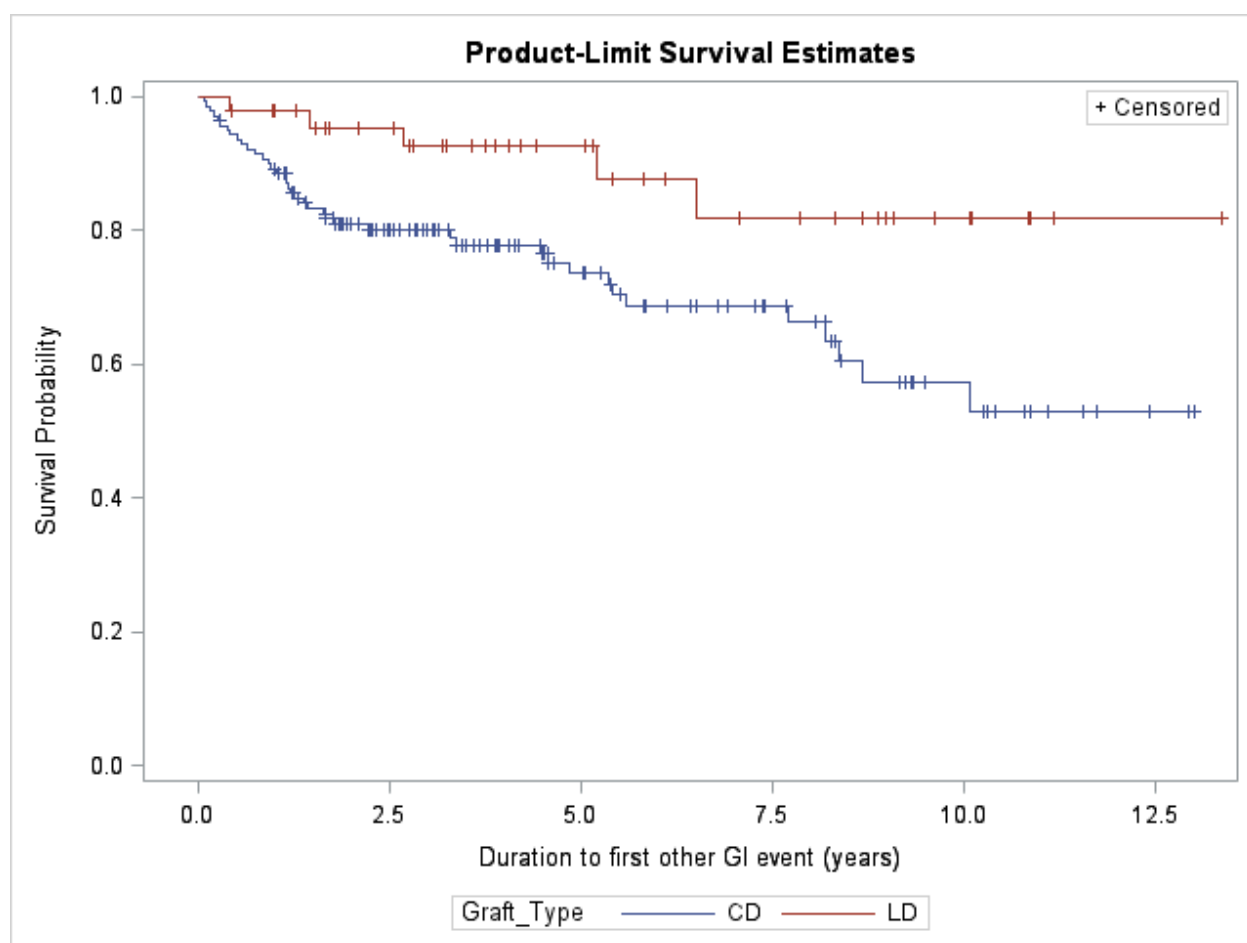


Figure 12. Kaplan-Meier survival curve comparing cadaveric (CD) versus living donor (LD) graft with regards to other non-diarrhoeal gastrointestinal adverse events.

No other risk factors had p-values below 0.20, thus no multivariate analysis was carried out.

Chapter Four

4.1 Discussion of Descriptive Analysis

4.1.1. Gender

The study population were predominantly males (65.2%). The same trend can be seen in the US 2011 transplant data, whereby 16,816 renal transplants were performed with 10,235 (60.8%) male recipients and 6,581 (39.1%) female recipients [25]. One may argue that females are traditionally disadvantaged in the South African society thus have less resources to seek health care. However, if the above hypothesis stands to reason, then in a more developed and sophisticated first-world country such as USA, one expects a more balanced gender representation of renal transplant recipients. This clearly is not the case, suggesting perhaps an underlying biological reason for the apparent gender discrepancy.

Goldberg et al, after examining all the recent literature in a review article, concluded that the prevalence of CKD tends to be higher in females, whereas the disease is more severe with a higher progression rate in males [26]. Neugarten suggested a possible hormonal mechanism to explain the gender discrepancy. Various renal benefits of estrogen include; reduction in the expression of renin, ACE, and angiotensin II on the renin-angiotensin system; inhibition of endothelin (a potent vasoconstrictor and promotes sodium retention) synthesis; suppression of superoxide anion generation and decrease apoptosis of kidney podocytes; all of which have a renal protective effect [27]. These effects may contribute to alterations in kidney haemodynamics and slows kidney disease progression in females.

Hence it may explain the difference in gender distribution in the transplant population.

4.1.2 Age

Mean age of the study population is 38.2 years. In the 2011 US transplant data, the majority of the recipients are between the age of 50-64 (39.7%) [25]. Since age is a well-known risk factor for chronic renal disease, our study population is relatively young. This could be a reflection of the general younger population and shorter life expectancy in South Africa [28].

4.1.3 Type of Graft

The majority of transplanted grafts are deceased donor grafts (75.5%). This largely corresponds with US 2011 data (65.6%) [25]. Deceased donor grafts are usually more readily available than living donor grafts and that is reflected in this study.

4.1.4 Ethnicity

Black Africans are the major racial group in this study population, which reflects the national population of South Africa [27]. In the USA and Europe, there is a predominance of Caucasian recipient population which also reflects the general population there [25]. Since most of the published data are from USA and Europe, our rather different racial profile makes our data unique.

4.2 Discussion of Statistical Analysis

4.2.1 Discontinuation and Dose Adjustment of MMF due to GI adverse effects

GI side effects and effects on dosage have been investigated extensively in renal transplant recipients, since the earliest three pivotal trials [8,9,10]. We shall discuss dose adjustment and termination together for convenience.

Knoll et al in a study of 213 renal transplant recipients, found 59% (n=126) of the patients had at least one MMF dose reduction. A total of 176 MMF dose reductions were recorded during the course of the study. The most frequent cause for a dose reduction was leukopenia (55.1%). MMF dose was reduced because of gastrointestinal symptoms such as nausea, vomiting, or diarrhoea (22.2%), infection (7.4%), malignancy (1.1%), and unknown reasons (14.2%) in the remainder of cases. MMF was permanently discontinued in 16 patients [20].

Pelletier et al, in a single center retrospective analysis of 721 patients, found that dose adjustments were common in the first post-transplant year, with 70.3% (n=507) of patients having at least one dose adjustment. The majority of the indications for dose adjustments were hematological and infectious. GI complications accounted for 21% of the dose adjustments. Of the 507 dose-adjusted patients, 102 (20.1%) patients discontinued MMF within the first post-transplant year [21].

Tierce et al, in a record review of 768 renal transplant patients who were initiated on MMF therapy, reported 49.7% (n=382) patients who experienced GI complications and 39.0% (n=149) underwent MMF dose adjustments or MMF discontinuation within the first 6

months of post-transplant [22].

In the Diarrhoea Diagnosis Aid and Clinical Treatment (DIDACT) study by Maes et al, whereby 108 renal transplant patients with severe diarrhoea were enrolled, MMF was associated with the largest number of dose reductions (24%) and termination (11%). Interestingly, >50% of patients diarrhoea started 2 years or more after transplantation. This correlates with our study which found a median time to the first diarrhoeal event was 3.6 years (95% CI: 2.8-4.4 years) [24].

Hardinger et al in a retrospective review of 6400 MMF treated patients from USRDS, found that 27.3% (n= 1753) had GI adverse events, and MMF was discontinued in 17.5% (n=1117) of patients after one year. The frequency of MMF discontinuation was significantly higher in patients with GI complications than in those without such complication (21.3% vs 16.0%, odds ratio 1.33, $p<0.0001$) [29].

Bunnapradist et al analyzed file records of 3675 patients on MMF therapy and had a GI complication. Only 45.7% (n=1681) of the patients remained on full-dose MMF. MMF was discontinued in 33.7% (n=1240) of the patients, with a further 12% (n=455) requiring a dose reduction >50%. In terms of time to onset of GI complications, 69% of the patients had a first diagnosis within the first year post-transplant (median time 166 days) [30].

The data in some of the studies did not distinguish between dose adjustment and termination of MMF. However, the studies points to a discontinuation rate of between 11% to 20%, with various follow-up period. Our overall MMF discontinuation rate due to GI complications is 18.7%, which largely correlates with other studies [20-22,24,29,30]. The

dose adjustment rates among the studies were 22%-54.3% with various follow-up periods [20-22,24,29,30]. Our study showed that after one year, 13.4% of patients had their MMF dose adjusted. After 3 years, this increased to 33.7% and finally, 5 years after transplant, 48.1% (n=90) had a dose adjustment due to GI adverse events. Although the follow-up period varies from 6 months to 3 years, the result of our study is reasonably consistent with the above quoted studies [20-22,24,29,30].

The minimal one year MMF duration inclusion criteria may have biased our result, as that patients who had GI complications and discontinued MMF before one year may be excluded. The number of patients excluded by this criterion may be as high as 39% as suggested by Tierce et al [22]. Our study was conducted in a later time period when physician may have had more knowledge and experience with MMF which may lead to less termination. The population is also different both in racial, gender and socio-economical background. The difference in racial composition of our population compared to developed countries like USA, where majority of the data came from, deserves further scrutiny.

As mentioned previously, the majority of our study population are black Africans. In a subset analysis of the US clinical trial of MMF in renal transplantation [8], Nyelan found that dose-dependent prevention of acute rejection in African-Americans is most effective at a dosage of MMF at 3g/day, which is more than the 2g/day dose for non-African-Americans. At 3g/day, African-Americans experienced the same incidence of reported adverse events (9.1%) compared with non-African Americans (9.8%) [31]. In a later study investigating racial difference in MMF dosing, outcomes and adverse effects in pediatric kidney transplant patients, Jensen et al found that African-American pediatric patients

receive higher MMF doses within the first three-year post-transplant. The African-American population also have a lower incidence of any adverse event compared to non-African-Americans (44% vs. 51%). Although statistically non-significant, the incidence of GI adverse events in African-Americans compared to non-African-Americans was lower (13% vs. 26%) [32].

This suggests there are some differences in pharmacokinetics in African-Americans. In a study of MMF pharmacokinetics in 53 renal transplant patients, Tornatore et al MPA clearance in African American males was longer (26.5 ± 14.4 L/h versus 17.9 ± 6.1 L/h in Caucasian males $p=0.035$) with no difference noted in MPA troughs. Enterohepatic circulation occurred less frequently in African American males (23%) compared with Caucasian males (42%). A racial difference was noted with more rapid MPA clearance in African American males compared with Caucasians [33].

Thus, African-Americans have a higher clearance rate of MMF, which is due to less enterohepatic circulation of MPA and MPAG. This may account for the higher dose of MMF required and less GI adverse events. The difference in racial composition may explain the discrepancy between the incidence of GI adverse events found in our study compared with international data, especially those from USA.

Another flaw in our dose adjustment is that we derive the data by calculating patients that remained on induction dose of MMF. Thus, those that had MMF discontinuation, death, or loss to follow-up were indirectly included in the statistical analysis. Similar flaw also applies to dose termination data.

4.2.2 Incidence of GI complications

The earliest trials such as Sollinger et al, reported 31.5% of patients in the 2g daily MMF group had diarrhoea as compared to AZA group at 6-months post transplantation [8]. The Europe MMF Cooperative Study group reported 45.5% of patients in the 2g MMF treatment group had GI adverse event. Out of those that had GI adverse events, 28% had diarrhoea [9]. The Tri-continental group concurred that diarrhoea was more common among patients receiving MMF than among those receiving AZA and occurred with highest frequency in those receiving the higher dose of MMF (MMF 3g=31%, MMF 2g=28%, AZA=17%) [10].

Later studies also showed a wide range of incidences of GI adverse events. Tierce et al demonstrated 49.7% had GI adverse event within the first 6 months post-transplant [22]. Hardinger et al found that 27.3% (n=1753) of MMF treated patients had GI adverse events in the first year post transplant [28]. Both of these studies did not distinguish diarrhoeal with non-diarrhoeal GI adverse events.

In another retrospective analysis of 41 442 patient records, Bunnapradist et al reported 17.1% (n=7103) of patients had post-transplant diarrhoea in the 3-year study period. The authors sub-categorised diarrhoea according to etiologies. Compared with all other categories of diarrhoea, the cumulative incidence of unspecified non-infectious diarrhoea was the highest during the whole study period. Almost half the cases first occurred in the first post-transplantation year [34]. The majority of patients (60%, n=25 014) were on a MMF containing regimen, however the author did not report the incidence of diarrhoea on the MMF treated patient group. The use of Medicare claim data for the diagnosis of

diarrhoea, which often only included severe disease meant that mild diarrhoeal episodes may be under-reported.

In our study, the prevalence of diarrhoeal complications is 21.4% in the first year post-transplantation, which, although different, is comparable with other studies quoted above despite a relatively smaller sample size. This increases as follow-up lengthens, resulting 44.3% at 3 years and 61% at 5 years post-transplant. Overall, 57.9% of the study population (n=109) experienced a diarrhoeal adverse event.

However, it is difficult to compare results from these trials to our study, since some studies did not distinguish between diarrhoeal and non-diarrhoeal GI adverse events. The wide spread of GI adverse event incidence may also be due to lack of clear, uniform definition of GI adverse events across the studies. A lot of the GI symptoms relied on patient self-reporting which is difficult to be verified independently. Our study did not record and sub-classify the diarrhoeal adverse events according to etiology, which could lead to bias.

The prevalence of non-diarrhoeal GI events is much less: 8.6% at one-year post-transplant, 16.9% at 3 years and 21.8% at 5 years. The prevalence of non-diarrhoeal GI events also increases as follow-up period lengthens.

4.2.3 Risk Factors Contributing Towards GI Adverse Events

Five variables were investigated for possible risk factors contributing towards adverse GI events, namely gender, ethnicity, age at transplant, graft type and combination of tacrolimus and MMF therapy. Once again, distinction between diarrhoeal and non-

diarrhoeal events were made when determining risk factors. The combination of tacrolimus and MMF was found to be a significant risk factor for diarrhoeal events (Hazard Ratio 1.82; 95% CI 1.21-2.73), but not non-diarrhoeal events. A living donor graft reduced the risk of non-diarrhoeal event (Hazard Ratio 0.33; 95% CI 0.13-0.84).

Bunnapradist et al, in a retrospective analysis of 41 442 Medicare patient records, found that female gender is associated with increased risk of all types of diarrhoea. Also, regimens based on MMF and tacrolimus were associated with a greater incidence of infectious (HR=1.44 (95% CI =1.14-1.81), $p<0.05$) and unspecified noninfectious diarrhoea (HR=1.37 (95% CI =1.28-1.46), $p<0.05$) [34]. Although our study did not distinguish between types of diarrhoea, both studies concurred on tacrolimus being a significant risk factor for diarrhoea in MMF treated patients. However, this study did not find gender to be a predisposing factor towards post-transplant diarrhoea in patients with MMF containing regimen (HR=1.15; 95% CI 0.77-1.71, $p=0.50$).

A more recent study by Zhao et al, investigating late, non-infectious diarrhoea in renal transplant patients receiving MMF with either cyclosporine A or tacrolimus. 541 patients were enrolled and followed up for at least 36 months. None of the cyclosporine treated patients presented with late, severe, noninfectious diarrhoea compared with tacrolimus treated group ($n=301$), which had 7% ($n=21$) of late, severe, noninfectious diarrhoea [35]. This further supports the result of our study.

The Symphony study was a landmark prospective study in de novo solid organ transplant patients. In the 1-year Symphony study, the regimen including daclizumab induction, MMF 2g daily, low-dose tacrolimus and steroids resulted in better renal function, less acute

rejections and less graft losses compared to the other regimens [23]. The benefit continued in the subsequent 3-year follow-up study. Low-dose tacrolimus continued to be the group with the best outcomes [36]. The Symphony trial had demonstrated that combination of MMF and tacrolimus is superior to combination of MMF and cyclosporine A. Unfortunately, the combination of MMF and tacrolimus is associated with increased risk of post-transplant diarrhoea as mentioned previously.

While Bunnapradist did not theorize on possible mechanisms of the combination of MMF and tacrolimus giving rise to more post-transplant diarrhoea, Zhao et al postulated that it is due to drug interactions between the two, although no MPA (the active metabolite of MMF) levels were done in that particular study [35]. However, there is strong evidence that MPA trough concentrations are influenced by tacrolimus. A dose-dependent inhibition of UDP-glucuronosyl transferase (an enzyme responsible for inactivation of MPA) by tacrolimus, leading to an increase in MPA and reduction in MPAG concentrations [7]. Therefore, when MMF was co-administered with tacrolimus, the area-under-curve(AUC) values for MPA increased with time, such that by 3 months the AUC values were 20–30% higher. Tacrolimus co-administration with MMF shows a higher MPA level compared with cyclosporine. The pharmacokinetics of tacrolimus are unchanged in the presence of MMF [37]. The increase in MPA level when MMF is co-administered with tacrolimus may explain the increased risk of diarrhoea associated with co-administration.

This study found that graft type contributed significantly towards the occurrence of non-diarrhoeal adverse GI events (HR=0.33; 95% CI 0.13-0.84, p=0.02). The result is somewhat expected. It is well-known that living donor grafts have better graft survival compared with deceased donor grafts [38]. Deceased donor grafts usually have more

ischaemic damage, less matching HLA and may require more aggressive immuno-suppression [39]. In a retrospective cohort study of 218 patients, Nemati et al found that although there are no significant differences in one-year survival rates, three-years survival rates of patient and graft were significantly longer in living donor kidney transplants in comparison with the deceased donor kidney recipients [39]. It is logical to deduce that living donor grafts would have less complications than deceased donor grafts.

4.3 Limitations of The Study

The inclusion criteria may be too strict and thus limit the sample size. The inclusion criteria was originally thought to exclude acute surgical causes of graft failure. Only 52.5% of complete patient records were included after the application of the inclusion criteria, which although statistically adequate, is still a small sample compared to other studies. It may not be a representative distribution of the general population and thus may introduce bias.

Another important factor is that of incomplete data, especially in the pre-transplant work-up file. Missing data resulted in the exclusion of the pre-transplant GI diagnosis from statistical analysis, which limits the findings in this study. Since MPA undergoes extensive enterohepatic re-circulation, this may be crucial in determining susceptibility to GI complications.

There are no clear definitions for diarrhoeal events. Diarrhoeal events relied largely on patient reporting, and can vary in severity. The incidence of diarrhoeal event can thus be biased, as it cannot be objectively verified. The etiology of the diarrhoea was often not investigated in the files and this study did not include the etiology of diarrhoeal event,

which may again influence the result. The design of the study also did not include further classification of non-diarrhoeal gastrointestinal events. Further study is needed to include a clear definition of diarrhoea and investigate possible causes.

According to Hardinger et al, the greatest risk associated with GI complications was in patients with CMV infection (RR= 1.85; $p<0.0001$). Other factors associated with a heightened risk of GI complications were hyperlipidemia (RR=1.29; $p<0.0001$), post-transplant diabetes (RR=1.20; $p=0.0002$), the number of post-transplant hospitalizations (RR=1.20; $p<0.0001$), pre-transplant maintenance peritoneal dialysis (RR=1.21; $p=0.0002$), pre-transplant maintenance haemodialysis (RR=1.39; $p<0.0001$), and hypertension as the cause of ESRD. This study did not include all of the above as possible risk factors to GI adverse events. This would be important for future studies.

Some of the other studies also investigated outcomes related to GI adverse events and , such as graft loss, death, acute rejection episodes [11,12,13,20,21,22] and financial cost-effectiveness [22]. This study did not include these parameters into its design. Future studies can investigate further the link between GI adverse events and patient outcomes in a South African context.

Chapter 5

5.1 Conclusion

End-stage renal disease was a terminal illness until the advancement in surgical techniques, peri-operative care and medical immunosuppressive therapy made renal transplantation a reality. Immuno-therapy had progressed rapidly from the rather primitive idea of whole body irradiation to the combination chemotherapy we have today [1,2]. At the same time, we are demanding not only better patient survival, but also more tolerable side effects and more cost effectiveness.

MMF is one of the many drugs available in the arsenal of immune-suppressive therapy. Not only has it proven to be an effective agent at preventing acute graft rejections in large randomised control trials [8,9,10], but also in preventing chronic graft failure [7,13,14]. Unfortunately, MMF does have side effects that physicians need to be aware of. One of the common adverse effects are gastrointestinal. Since the very first pivotal trials, GI adverse events were documented as the major side effects in MMF treated renal transplant recipients [8,9,10]. These GI adverse effects often lead to dose adjustment or discontinuation of the drug, which is linked to more acute rejection episodes and poorer patient outcome [20-22].

As far as the authors are aware, this is the first study in South Africa that investigates MMF and gastrointestinal adverse events using local data. Our study population is younger and made up of more black African population as contrary to most of the published data, which

consists of a much older population and more Caucasian racial distribution. Despite this, our results in terms of dose adjustment and termination is still reasonably consistent with international data. This study found that combination therapy of MMF and tacrolimus is a significant risk factor for post-transplant diarrhoea in renal transplant recipients, which concurs with Bunnapradist and Zhao [34,35]. This is most likely due to drug interaction. Tacrolimus increases MPA level and thus may be associated with more diarrhoeal events [37]. Living donor graft is associated with less non-diarrhoeal GI complication in renal transplant recipients using MMF as part of the immunosuppressive regime. This could be due to less ischaemic injury, better HLA match and less aggressive immunosuppression associated with living donor graft [39].

5.2 Recommendation

Future studies in the field of post-transplant diarrhoea, especially in the South African setting, should have a more accommodating inclusion criteria to achieve a bigger sample size. There should be a clear definition of diarrhoea and perhaps sub-classify diarrhoea into categories according to etiology (eg. infectious vs. non-infectious). Patient and graft outcomes can also be included in future studies to determine the consequence of GI adverse events in renal transplant patients. Other risk factors such as CMV infection, post-transplant diabetes and length of dialysis before transplant can also be investigated in future studies.

References

1. Watson CJ, Dark JH. Organ transplantation: historical perspective and current practice. *Br J Anesth* 2012;108(S1):i29-i42
2. Morris JP. Transplantation - A Medical Miracle of the 20th Century. *NEJM* 2004;351;26:2678-2680
3. Squifflet JP. The History of Kidney Transplantation: Past, Present and Future (with Special References to the Belgian History). In: Ortiz J, editor, *Understanding the Complexities of Kidney Transplantation*. InTech Publishing. 2011:3-40
4. Lin S. Immunosuppressive strategies in solid organ transplantation. In: *Pharmacotherapy self-assessment program, 5th Edition*. American College of Clinical Pharmacy.
5. Aulagnon F, Scemla A, De Wolf S, Legendre C and Zuber J. diarrhoea After Kidney Transplantation: A New Look at a Frequent Symptom. *Transplantation* 2014; 98(8):806-816
6. Sollinger HW. Obituary of Anthony Allison. 2014
<http://www.crippenandflynnchapels.com/sitemaker/memsol_data/911/1251558/docs/5023_AnthonyAllisonObit-SollingerAJT.pdf>
7. Behrend M. Mycophenolate Mofetil: Suggested Guidelines for Use in Kidney Transplantation. *BioDrugs* 2001;15(1):37-53
8. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. *Transplantation* 1995;60:225–232
9. European Mycophenolate Mofetil Cooperative Study Group: Placebo-controlled study of mycophenolate mofetil combined with cyclosporine and corticosteroids for

- prevention of acute rejection. *Lancet* 1995;345:1321–1325
10. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group: A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. *Transplantation* 1996;61:1029–1037
 11. Srinivas T, Kaplan B, Schold J and Meier-Kriesche HU. The Impact of Mycophenolate Mofetil on Long-Term Outcomes in Kidney Transplantation. *Transplantation* 2005;80:S211–S220
 12. Matthews T. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. *Transplantation* 1998;65(11):1450-1454
 13. Ojo AO, Meier-Kriesche HU, Hansen JA et al. Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. *Transplantation* 2000;69(11):2405-2409
 14. Merville P, Berge F, Deminiere C, et al. Lower incidence of chronic allograft nephropathy at 1 year post-transplantation in patients treated with mycophenolate mofetil. *Am J Transplant* 2004;4:1769-1775
 15. Behrend M. Adverse gastrointestinal effects of mycophenolate mofetil: etiology, incidence and management. *Drug Safety* 2001;24(9):645-663
 16. Ducloux D, Ottignon Y, Semhoun-Ducloux S et al. Mycophenolate mofetil induced villous atrophy. *Transplantation* 1998;66(8):1115-6
 17. Kamar N, Faure P, Dupuis E, et al. Villous atrophy induced by mycophenolate mofetil in renal transplant patients. *Transplant International* 2004;17(8):463-7
 18. Guerard A, Rabodonirina M, Cotte L, et al. Intestinal microsporidiosis occurring in two renal transplant recipients treated with mycophenolate mofetil. *Transplantation*

1999;68:699-707

19. Maes BD, Dallel, Geboes K, et al. Erosive enterocolitis in mycophenolate mofetil treated renal transplant recipients with persistent afebrile diarrhoea. *Transplantation* 2003;75(5):665-672
20. Knoll GA, MacDonald I, Khan A and Van Walraven C. Mycophenolate Mofetil Dose Reduction and the risk of acute rejection after renal transplantation. *J Am Soc Nephrol* 2003;14:2381-2386
21. Pelletier RP, Akin B, Henry ML, Bumgardner GL, Elkhammas EA, Rajab A and Ferguson RM. The Impact of Mycophenolate Mofetil Dosing Patterns on Clinical Outcome After Renal Transplantation. *Clin Transplant* 2003;17:200-235.
22. Tierce JC, Porterfield-Baxa J, Petrilla AA, Kilburg A, Ferguson RM. Impact of Mycophenolate Mofetil (MMF)-related Gastrointestinal Complications and MMF Dose Alterations on Transplant Outcomes and Healthcare Costs in Renal Recipients. *Clin Transplant* 2005;19:779.
23. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *NEJM* 2007;357:2562–2575.
24. Maes B, Hdaya K, De Moor B, Cambier P, Peeters P, De Meester J, Donck J, Sennesael J and Squifflet J. Severe diarrhoea in Renal Transplant Patients: Results of the DIDACT Study. *Am J of Transplantation* 2006; 6: 1466–1472
25. U.S. Department of Health & Human Services, Organ Procurement and Transplantation Network, National Data. <https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/>
26. Goldberg I and Krause I. The Role of Gender in Chronic Kidney Disease. *EMJ* 2016;1(2):58-64
27. Neugarten J and Golestaneh L. Gender and the Prevalence and Progression of

- Renal Disease. *Adv in Chronic Kidney Dis* 2013;20(5):390-395
28. Stats SA. Mid-Year Population Estimates 2014. Statistics South Africa
29. Hardinger KL, Brennan DC, Lowell J, Schnitzler MA. Long-term outcome of gastrointestinal complications in renal transplant patients treated with mycophenolate mofetil. *Transpl Int* 2004;17(10):609-616
30. Bunnapradist S, Lentine KL, Burroughs TE, Pinsky BW, Hardinger KL, Brennan DC and Schnitzler MA. Mycophenolate Mofetil Dose Reductions and Discontinuations after Gastrointestinal Complications Are Associated with Renal Transplant Graft Failure. *Transplantation* 2006;82(1):102-107
31. Neyan JF. Immunosuppressive Therapy in High Risk Transplant Patients: Dose-Dependent Efficacy Of Mycophenolate Mofetil In African-American Renal Allograft Recipients. *Transplantation* 1997;64(9):1277-1282
32. Jensen CJ, Shrivastava S, Taber DJ, Weimert NA, Shatat IF, Orak J, Chavin KD and Baliga PK. A Critical Analysis of Racial Difference with Mycophenolate Mofetil (MMF) Dosing, Clinical Outcomes and Adverse Effects in Pediatric Kidney Transplant Patients. *Clin Transplant* 2011;25:534–540
33. Tornatore M, Sudchada P, Dole K, DiFrancesco R, Leca N, Gundroo AC, Danison RT, Attwood K, Wilding GE, Zack J, Forrest A and Venuto RC. Mycophenolic Acid Pharmacokinetics During Maintenance Immunosuppression in African American and Caucasian Renal Transplant Recipients. *The J of Clin Pharmacology* 2011;51(8):1213-1222
34. Bunnapradist S, Neri L, Wong W, Lentine K, Burroughs TE, Pinsky BW, Takemoto SK and Schnitzler MA. Incidence and Risk Factors for diarrhoea Following Kidney Transplantation and Association with Graft Loss and Mortality. *Am J of Kidney Diseases* 2008;51(3):478-486

35. Zhao YJ, Wen JQ, Cheng K, Ming YZ, She XG, Liu H, Liu L, Ye QF, and Ding BN. Late, Severe, Noninfectious diarrhoea After Renal Transplantation: High-Risk Factors, Therapy, and Prognosis. *Transplant Proceed* 2013;45:2226-2232
36. Ekberg H, Bernasconi C, Tedesco-Silva H, Vitko S, Hugo C, Demirbas A, Reyes Acevedo R, Grinyó J, Frei U, Vanrenterghem Y, Daloze P and Halloran PF. Calcineurin Inhibitor Minimization in the Symphony Study: Observational Results 3 Years after Transplantation. *Am J of Transplant* 2009;9:1876–1885
37. Undre NA. Pharmacokinetics of tacrolimus-based combination therapies. *Nephrol Dial Transplant* 2003;18(S1):i12–i15
38. Agarwal D, Mandal S and Sagar G. An Overview of Renal Transplantation. *J, Indian Acad of Clin Med* 2007;8(1):81-92
39. Nemati E, Einollahi B, Lesan Pezeshki M, Porfarziani V, Fattahi MR. Does Kidney Transplantation with Deceased or Living Donor Affect Graft Survival? *Nephro-urology Monthly*. 2014;6(4):e12182.

Record Number:		Age
	DOT	Transplant Age
Graft Type:	Cadaver / Related Living / Non-Related Living	
Ethnicity:	Caucasian / Black / Indian / Coloured	

Annexure 1

Data Collection Sheet

Pre-transplant

GI Diagnosis

--

Gastroscopy Results

--

Diabetic Pre-transplant

Yes/No

Acid Suppressive Therapy

Yes/No

Medication and Dose

Post Transplant

Induction Therapy

Agent	Dose

Maintenance Therapy

Agent	Dose and Frequency

MMF Dose Adjustment

Date	Amount	Possible Reason

--	--	--

Rejection Episode

Date	Biopsy Result

Steroid Pulse

Dates
Total Number
Over how many years

GI Complication

Diagnosis	Method	Date

Annexure 2

Record Number	Patient Name	Date of Birth

Annexure 3



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Min-Shien Chen

CLEARANCE CERTIFICATE

M1211100

PROJECT

Mycophenolate Mofetil in Renal Transplant
Recipients: Predisposition to GIT Intolerance

INVESTIGATORS

Dr Min-Shien Chen.

DEPARTMENT

Internal Medicine/Nephrology

DATE CONSIDERED

30/11/2012

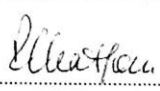
DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/11/2012

CHAIRPERSON


(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr Graham Paget

DECLARATION OF INVESTIGATOR(S)

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

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES..

Annexure 4



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries: Ms. Lefentse Mapaisa
Office of the Clinical Executives
Tel: (011)488-3710
Fax: (011)488-3947
03 December 2014

Dr. Min-Shien Chen
Registrar
Department of Medicine
University of the Witwatersrand

Dear Dr. Chen

RE: Mycophenolate Mofetil in Renal Transplant Recipients: Predisposition to GIT Intolerance

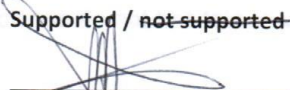
Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic hospital will not in any way incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the Head of Department and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

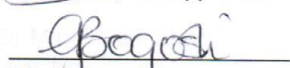
Kindly forward this office with the results of your study on completion of the research.

~~Supported / not supported~~


Dr. M.I. Mofokeng
Clinical Director

DATE: 3/12/2014

Approved / not approved


Ms. G. Bogoshi
Chief Executive Officer

DATE: 5/12/2014