Outcomes of Cadaveric Renal Transplantation in Patients using Mycophenolate Mofetil or Azathioprine

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Declaration

I, Linda Gathara, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature: _____.

Date: _____.

Dedication

I thank the Almighty God, for without Him I am nothing. He has seen me this far and it is through Him that I have been able to achieve this. I am grateful to God for my parents, George and Victoria Gathara. You have instilled morals, values and discipline in my life and have been a constant source of encouragement, unending love and support. I love you Mum and Dad. To my elder sister Juliet, I have always looked up to you. You are my rock! You have inspired me and taught me to believe in my abilities. You have challenged me to grow and attain new heights. I am proud of you. My twin sister Rita Gathara, for your kind words of wisdom, strength, support and kindness. You know me better than anyone.

Abstract

Introduction: Mycophenolate Mofetil (MMF) has replaced Azathioprine (AZA) in immunosuppressive regimens worldwide in the prevention of acute allograft rejection. Whether long term treatment with MMF is superior to treatment with AZA is a matter of debate. There are no studies in South Africa that have been done to show that MMF is superior to AZA.

Objectives: To describe the outcomes of cadaveric renal transplantation in patients using Mycophenolate Mofetil or Azathioprine over a five year period at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

Design: This was a retrospective comparative study.

Setting: The CMJAH Renal Transplant Unit in Johannesburg, Republic of South Africa.

Patients: A convenience sample of all eligible patients, 208 in total, was recruited from the Renal Transplant Unit at CMJAH.

Methods: The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (Protocol Number: **M130434**). The data source was clinical records of renal transplant recipients at the Renal Transplant Unit at CMJAH from 1985-2013, who were treated with one of the two immunosuppressive agents required for analysis in the study. The data were entered in Microsoft Excel Spreadsheets and transferred to STATA version 14 for statistical analysis.

Results: Of the 208 patients, 101 patients were treated with Azathioprine and 107 patients treated with Mycophenolate Mofetil, in addition to corticosteroids and cyclosporine. A total of 16 patients developed acute allograft rejection 12-52 weeks after cadaveric transplantation. Of these, 6% were in the AZA group and 10 % in the MMF group. There was a mortality rate of 1% in the MMF and 16.8% in the AZA group respectively (p= 0.0001). The serum creatinine at 3 months was found to be a strong predictor of allograft function in patients on Mycophenolate Mofetil ($p<0.001$). After adjusting for the confounders, serum creatinine at 3 months remained a strong predictor of outcome. Patients experiencing abdominal pain while on treatment with Mycophenolate Mofetil were 62% less likely to develop acute allograft rejection than patients on treatment with Azathioprine [unadjusted HR = 0.38(0.14-1.05)]. This finding however, is not statistically significant (p=0.06). After adjusting for the other confounders the association with abdominal pain was marginally significant (adjusted HR 0.18(0.01-1.02) p=0.05).

Conclusions: Patient survival was superior in the longterm in cadaveric renal transplant patients receiving Mycophenolate Mofetil therapy.

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List of abbreviations/ Acronyms

A	Adenine
AZA	Azathioprine
APCs	Antigen Presenting Cells
C	Cytosine
CD	Cluster of Differentiation
C3	Complement 3
C5a	Complement 5a
CNIs	Calcineurin Inhibitors
CP	Cyclophillins
CYA	Cyclosporine A
DAMPS	Damage Associated Molecular Patterns
DNA	Deoxyribonucleic acid
ESRD	End Stage Renal Disease
FK506	Tacrolimus
FKBP	FK506 Binding Protein
G	Guanine
GMP	Guanine Monophosphate
HLA	Human Leucocyte Antigen
Ig	Immunoglobulin
IL	Interleukin
IMP	Ionisine Monophosphate
LFL	Leflunomide
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid
mTOR	Mammalian Target of Rapamycin
MYSS	Mycophenolate Steroid Sparing
NFAT	Nuclear Factor of Activated T cells
RNA	Ribonucleic acid
T	Thiamine
TCR	T cell receptor
U	Uracil
US	United States of America
Vs	Versus

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Chapter 1: INTRODUCTION

1.1 Background

End stage renal disease (ESRD) impacts the lives of thousands of individuals in South Africa and globally. Due to the high cost of renal replacement therapy only a fraction of the patients are afforded the benefit of treatment in developing countries due to limited resources. The 2012 South African Renal Annual Registry Report showed that in the public sector, 3182 ESRD patients were on treatment of a population of 43.6 million. . The more recent 2013 report shows a decrease in ESRD patients on treatment (3150), for a population of 44.2 million [2]. . With an increasing ESRD population, it is vital to conserve and utilise the available resources cost effectively in order to cater for the growing demand for renal replacement therapy.

1.2 Literature review

1.2.1 Introduction

End stage renal disease (ESRD), advanced (or severe) irreversible impairment of kidney function, is associated with poor outcomes if it is untreated. It is also debilitating as it affects various aspects of the patient's life. Common problems associated with ESRD include lethargy, declining health, demanding treatment regimes, unemployment and depression [3, 4]. The most common causes of ESRD in the United States of America (USA) are diabetes mellitus and hypertension [5, 6]. In South Africa the most common cause of ESRD is glomerulonephritis (36.5%), followed by hypertensive renal disease (31.7%) and diabetic nephropathy (11.8%) [2]. Other causes in South Africa include unknown/ not stated (8.6%) and cystic kidney disease (3.1%) [2].

Renal transplantation is the most effective therapy for ESRD [7]. This therapy is achieved by a multidisciplinary team of expert nephrologists, transplant surgeons, pathologists, nurse coordinators as well as consultations from urology, vascular surgery, cardiology, infectious diseases and psychiatry. Immunosuppressive therapy is essential, even in patients with ideal tissue matches, in order to prevent allograft rejection [6]. Successful transplantation is associated with decreased mortality and an improved quality of life. It frees a patient from chronic dialysis, fluid and dietary restrictions. Anemia and infertility are also corrected. The donor can be a living related, non-related living or a cadaveric donor [4, 7].

1.2.2 History of kidney transplantation

Kidney transplantation is thought to have started as early as the 18th century with researchers performing experimental work and attempts at grafting [8]. The first kidney transplant was in 1902 when Emeriti Ullman, an Austrian surgeon, performed an autologous kidney transplant in the neck of a dog. He performed a xenograft the same year, which was a cross species transplant between a dog and a goat [9].

There was subsequently little advancement in the field until the 1950's. The first successful renal transplant was performed by Dr. Joseph Murray on identical twins in Boston in 1954. He went on to perform the first successful allograft, from a fraternal twin to his dizygotic brother in 1959 [10], and the first cadaveric renal transplant in 1962 [11].

In the 1940's, a British biologist named Peter Medawar demonstrated that organ rejection was an immunological event [12]. This discovery led to the concept of immunosuppression and need for pharmacological agents that would allow successful renal transplantation [13]. In the late 1950's, 6-Mercaptopurine (6-MP) and Azathioprine (AZA), were developed [14]. Azathioprine is one of the oldest agents still in use to date.

Prior to the availability of Azathioprine and corticosteroids, renal transplantation was a challenge except in identical twins. This regimen changed in the 1980's after trials showed improved allograft survival with cyclosporine therapy [15]. The subsequent introduction of Mycophenolate Mofetil (MMF), Tacrolimus and Sirolimus has permitted the use of combination immunosuppressive therapy for successful cadaveric transplantation [16]. These breakthrough discoveries as well as the advancement in organ preservation and tissue typing techniques have paved the way for modern transplantation.

In South Africa, there are approximately 8840 patients with ESRD on dialysis out of a population of 52.98 million [2]. More than half of this number are awaiting renal transplantation [2]. It is a challenge to offer renal replacement therapy to all of them due to the high cost and limited resources. The South African Organ Donor Foundation statistics indicate that there were 222 adult renal transplants performed in South Africa in 2013 [2]. This is an improvement from 213 adult renal transplants in 2012. However, there had been a decline in numbers, with 263 renal transplants done in 2009 and 234 in 2011.

1.2.3 Immunology of transplantation

The immunological threat to the renal graft begins before transplantation. This arises from the effects of donor brain death or perioperative ischemia-reperfusion injury [17]. This causes an upregulation of HLA antigens by the graft and thus release of chemokines, pro-inflammatory cytokines, and adhesion molecules within the graft. This intensifies the immune response and increases cellular infiltration of the graft, causing an increase in the risk of rejection [17-19].

1.2.4 Innate immunity

This refers to the older nonspecific immune system and is the first line of defense by the host. It involves recruitment of macrophages, neutrophils, natural killer cells and complement

components [20]. Pathways of inflammation upregulate the innate system. Following organ transplantation, the injured tissues express ligands of the toll-like receptors. These are damage associated molecular pattern molecules (DAMPS) that activate the immune response and promote acute rejection [19]. The complement system plays an active role in both acute and chronic allograft rejection by production of C3a and C5a which directly activate anti-graft T cells and antigen presenting cells (APCs) [21, 22].

Figure 1: Innate and acquired immunity

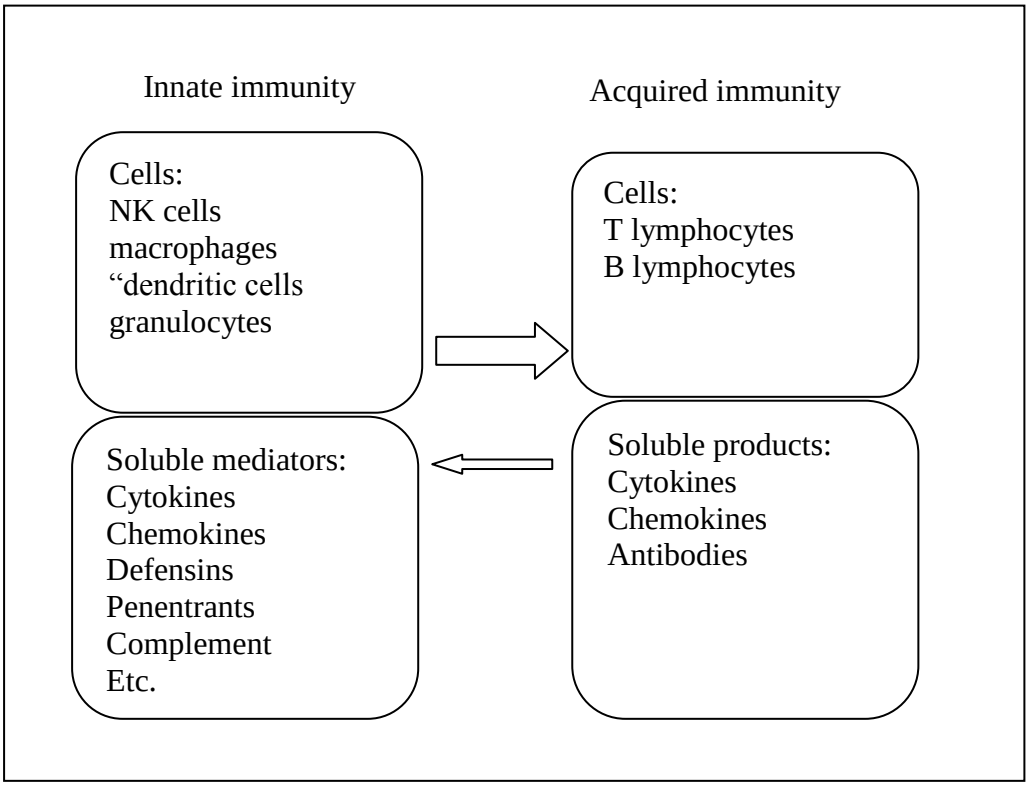


Figure adapted with permission from [23].

1.2.5 Adaptive immunity

Adaptive immunity is also known as acquired or specific immunity and involves recognition of specific antigens and confers both specificity and a memory effect by T and B lymphocytes. T cell mediated immunity involves the presentation of donor antigens to T cells by the APCs. Once activated, the T cells undergo clonal proliferation into antigen specific T cells [24]. Such cells are differentiated into CD 8 cytotoxic and CD 4 helper T cells. The CD

8 cytotoxic cells are responsible for induction of donor cell death while the helper T cells produce allo-antibodies and help macrophages induce delayed type hypersensitivity responses [25]. Both mechanisms are active in graft rejection [26].

Antibodies against the ABO blood group antigens, endothelial cell antigens and HLA molecules can mediate rejection [24]. Most transplant recipients do not have foreign HLA molecules prior to transplantation unless they are sensitised through blood transfusion, pregnancy or previous transplantation [17, 27]. This may be treated through desensitisation protocols in which plasmapharesis and aggressive immunosuppressive regimens result in the removal of these antibodies [28].

1.2.6 Allograft rejection

Graft rejection is one of the commonest causes of transplant failure. In the UK, 7-12% of renal transplants fail within the first year, with the rate increasing to approximately 20% within five years and 40% ten years post transplantation [4]. The transplanted allograft fails due to acute or chronic rejection. The nature and magnitude of the recipient's immune response determines the type of rejection.

1. Hyperacute rejection manifests within minutes to hours after transplant. It is mediated predominantly by IgG antibodies directed towards foreign molecules e.g. MHC molecules. It results from prior exposure to allo-antigens from blood transfusions, pregnancy or a previous transplant. The pre-existing host antibodies bind to antigens present in the graft and cause rapid thrombotic occlusions and hemorrhage from the graft vasculature [29]. Figure 2 shows the histology of hyperacute rejection.

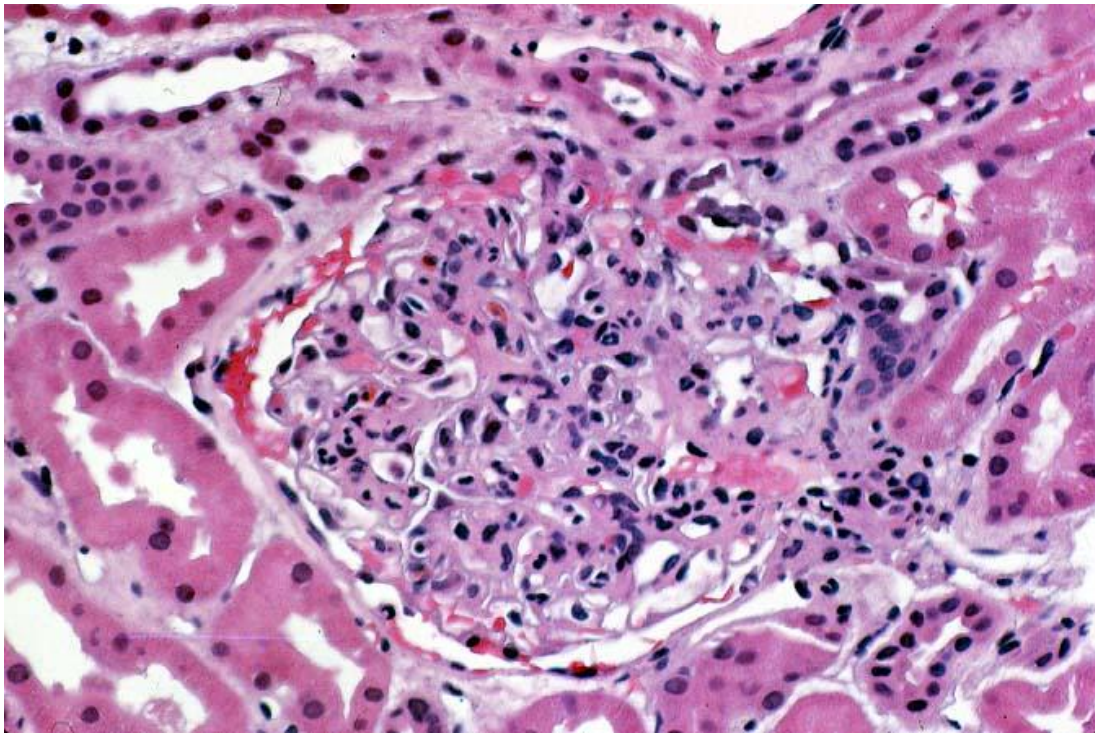


Figure 2: Histology of hyperacute rejection.

Figure 2 is an early post-transplant allograft biopsy showing a glomerulus with prominent neutrophil infiltration. A few neutrophils are also seen in the interstitium. These findings can be seen in early stages of hyperacute rejection, but are more frequently a manifestation of ischaemic injury [30].

2. Acute rejection generally refers to rejection occurring within the first year after transplantation [31]. It begins as early as one week after transplantation with the risk being highest with the first three months. It is seen in 10-30% of transplant recipients and usually presents with declining renal function within the first 3 months [27]. It is associated with specific pathologic changes in the graft. There are two main histological forms of acute rejection:

- Acute cellular rejection (Figure3), which is characterised by lymphocyte and inflammatory cell infiltration of the allograft [32].

- Acute antibody-mediated rejection (Figure 4), which is diagnosed by presence of donor specific allo-antibodies in the allograft, morphological evidence of acute tissue injury, and immunologic evidence of an antibody-mediated process (such as C4d deposition in the allograft) [33]. Cellular infiltrates may not be present.

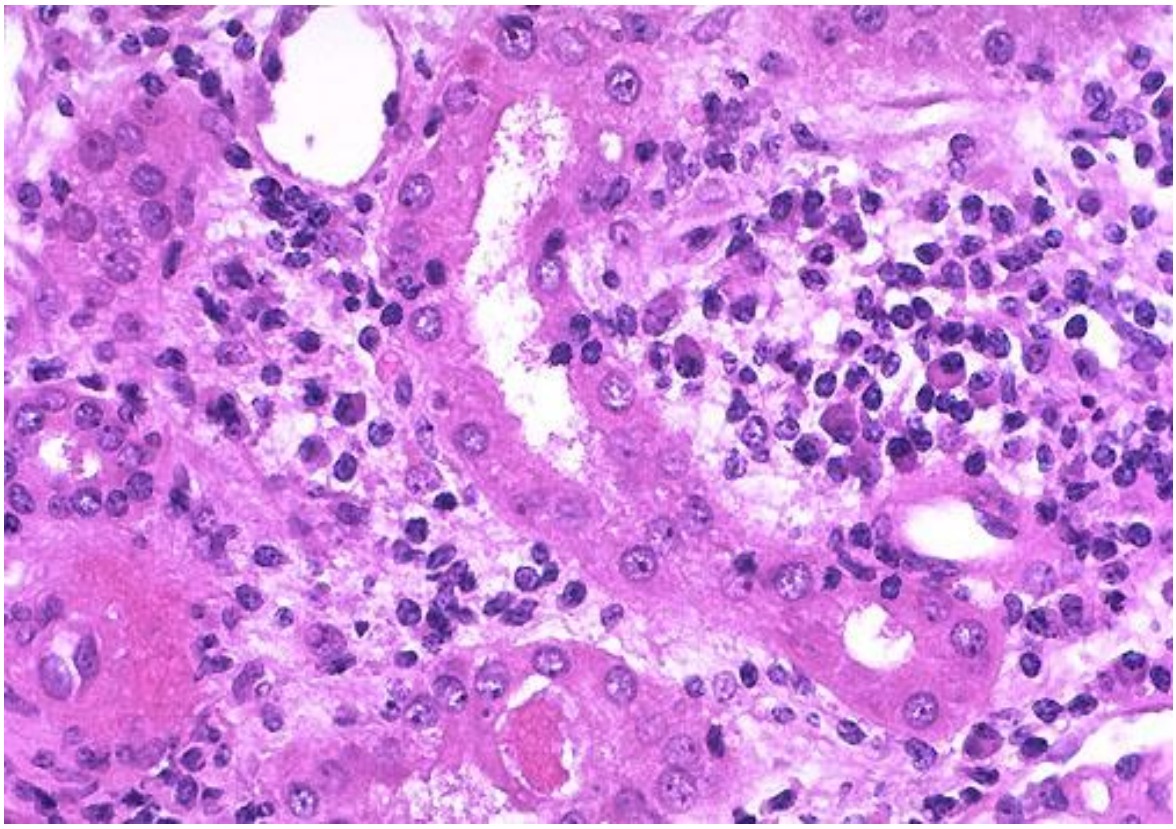


Figure 3: Histology of acute cellular rejection.

Lymphocytes and plasma cells are seen around a renal tubule in a renal transplant patient with acute cellular rejection in Figure 3 [30].

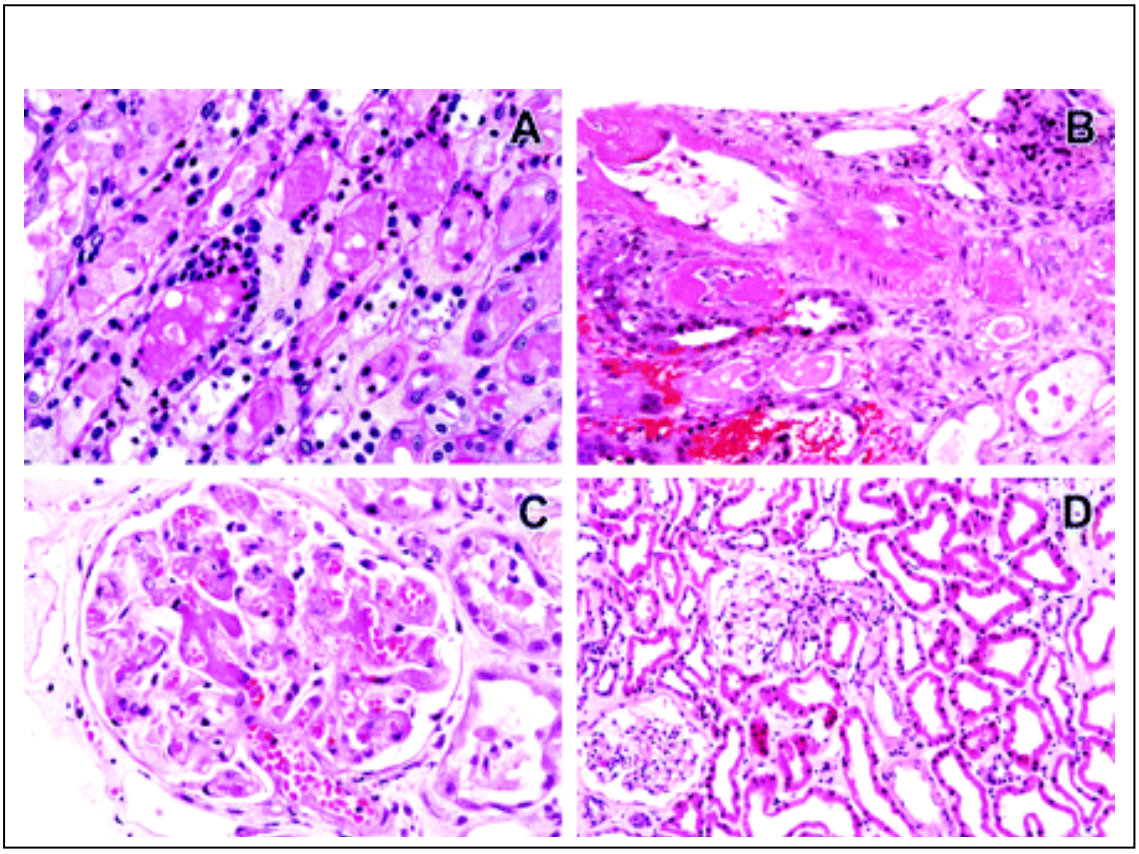


Figure 4: Histology of antibody-mediated rejection.

Figure 4 shows histologic expressions of acute antibody-mediated rejection (AMR). (A)Margination of neutrophils in peritubular capillaries.(B) Arterial fibrinoid necrosis. (C) Thrombotic microangiopathy. The glomerulus contains intracapillary fibrin thrombi and also shows red blood cell stasis, suggesting poor perfusion. (D) Acute tubular injury. The proximal tubules appear dilated with flattening of the epithelium. Glomeruli appear unremarkable, and there is no significant interstitial inflammation or leukocyte margination in peritubular capillaries [30].

Early episodes have a major effect on allograft survival and carry an increased relative risk for transplant failure [34]. Acute rejection episodes can also predict the occurrence of chronic allograft nephropathy [35]. Acute rejection is monitored by measuring the serum creatinine and by protocol (surveillance) biopsies.

3. Chronic rejection is the third type of rejection and occurs months to years following transplantation. It is characterised by a slowly progressive decline in renal function causing an increase in serum creatinine levels. Proteinuria also increases and hypertension worsens [36, 37]. The histological changes in chronic allograft nephropathy involve the entire renal parenchyma. This includes the glomeruli, tubules, and interstitium and blood vessels [38-40]. There is smooth muscle proliferation and collagen production by fibroblasts resulting in arterial graft occlusions. This process, known as accelerated graft arteriosclerosis, causes fibrosis which can lead to ischemia and cell death. The severity of chronic allograft nephropathy is graded using the Banff classification [41], as follows:

    - Grade I – Mild fibrosis of the interstitium and mild atrophy of the tubules either with or without specific glomeruli or vascular findings suggestive of chronic allograft nephropathy.

    - Grade II – Moderate interstitial fibrosis and moderate tubular atrophy with or without specific changes as in Grade I.

    - Grade III – Severe interstitial fibrosis and tubular atrophy.

1.2.7 Immunosuppressants for transplantation

Induction immunosuppressive therapy is essential in optimizing outcomes particularly in patients at high risk for poor short-term outcomes [42]. A high degree of immunosuppression is provided during the early post transplant period to prevent acute rejection [42]. Induction immunosuppressive strategies fall into one of two categories:

- High dose conventional agents, consisting of a calcineurin inhibitor, corticosteroid and an antimetabolite.

- Antibody induction, which consists of a reduced dose or delayed introduction of the conventional agents in addition to one of the following; rabbit antithymocyte globulin (rATG), Basiliximab or Alemtuzumab [43].

Maintenance immunosuppressive therapy is administered to almost all renal transplant recipients in order to prevent rejection and loss of the allograft. "*The risk of acute rejection is highest during the first three months after transplantation"*. This is the period during which the immunosuppressants should be at their highest doses.

Most adverse effects of immunosuppressive treatment correlate with the amount of immunosuppression and thus the dose should be tapered slowly over time. The most common agents can be divided into five classes:

- Corticosteroids,
- Antimetabolites- azathioprine and mycophenolic acid derivatives,
- The calcineurin inhibitors (CNIs)- cyclosporine and tacrolimus,
- Mammalian target of Rapamycin (mTOR) Inhibitors- sirolimus and everolimus,
- Co stimulation blockers- betalacept.

Most transplant centers currently utilise a maintenance triple therapy regimen consisting of a calcineurin inhibitor, an antimetabolite and a steroid [44].

1.2.7.1 Calcineurin inhibitors

Calcineurin catalyses intracellular processes associated with the activation of T lymphocytes. Cyclosporine and Tacrolimus are two of the most potent immunosuppressants currently in use and are a fundamental part of the current maintenance immunosuppressive therapy for

prevention of allograft rejection [45]. They "*share similar physicochemical properties and a common mechanism of action"*. They are termed calcineurin inhibitors because of their ability to suppress T cell activation through the inhibition of calcineurin [46].

Calcineurin inhibitors exert their cellular effects by binding to immunophilins [47]. Cyclophilins (CP) bind to cyclosporine and FK- binding proteins (FKBPs) bind tacrolimus. Cyclophilin A is the most abundant cyclophilin in T lymphocytes, and the predominant tacrolimus binding immunophilin is the FKBP12. Cyclophilins and FKBPs are structurally unrelated but both families have a cis- transproplyl- peptidase isomerase activity. "*The binding of cyclosporine or tacrolimus to its respective immunophilin enhances the immunophilin's affinity to calcineurin"*. Formation of this complex results in its binding to and inhibition of calcineurin. In the process of T cell activation, calcineurin associates and dephosphorylates inactive nuclear factor of activated T cells (NFAT). This leads to NFAT translocation to the nucleus and, in association with other transcription factors, initiation of downstream events involved in T- call activation. The drug-immunophilin complex forms an inhibitory association with calcium calmodulin- activated calcineurin, preventing binding and activation of NFAT [45], thereby decreasing the production of interleukin 2 and proliferation of T cells [48, 49].

1.2.7.2 Antimetabolites

Antimetabolites are purine or pyrimidine analogues. Prototypes include Azathioprine and Mycophenolate Mofetil (MMF). Purines and pyrimidines are the two types of bases used in DNA and RNA synthesis. Purines include guanine (G) and adenine (A); pyrimidines are cytosine (C), thymine (T), and uracil (U). Uracil (U) is found only in RNA, whereas thymine (T) is only in DNA. The bases are all attached to a single phosphate group, making them

monophosphates [50]. Ionisine monophosphate (IMP), the precursor to guanine monophosphate (GMP) is converted by IMP dehydrogenase [51]**.**

Mycophenolate is hydrolyzed to mycophenolic acid (MPA), which inhibits IMP dehydrogenase [52] and thereby prevents GMP production. GMP is a required base for both DNA and RNA, and without it, DNA and RNA synthesis is reduced. Mycophenolate reversibly "*inhibits the type II isoform of IMP dehydrogenase, which is preferentially expressed by activated lymphocytes"*. MMF is used in patients considered to be at high risk for rejection. It is teratogenic and thus contraindicated in pregnancy [53]. Other toxicities of MMF include gastrointestinal disturbances namely nausea, vomiting, diarrhea, abdominal pain and myelosuppresion mainly neutropenia. It is more potent than Azathioprine, with studies showing a reduction in acute rejection episodes [54].

Azathioprine is an imidazolyl derivative and prodrug of Mercaptopurine. It is converted to Mercaptopurine, which is then metabolized to 6-thio-GTP [55]. This antagonises purine metabolism by halting further DNA and RNA synthesis and subsequent protein synthesis. Reduced DNA synthesis results in reduced proliferation of cells. Reduced RNA synthesis results in reduced protein transcription and thus reduced functional activity of cells [55].

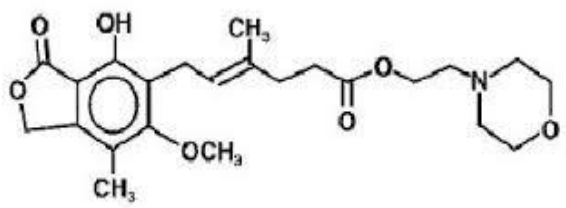
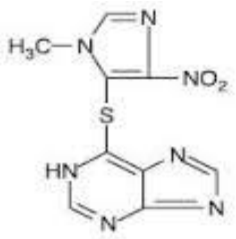
Mycophenolate Mofetil	Azathioprine
	

Figure 5: Structures of Mycophenolate Mofetil and Azathioprine

Both T and B lymphocytes depend on *de novo* synthesis of purines, whereas other cell types are able to use salvage pathways to obtain their DNA and RNA building blocks [56]. This makes lymphocytes more susceptible to antimetabolites than the other cell lines. Azathioprine inhibits the maturation of T cells and blocks delayed hypersensitivity reactions. It also has anti-inflammatory activity. Azathioprine is the preferred drug in low risk patients and in patients wanting to conceive while on immunosuppression. Dose related toxicities include nausea, vomiting, hepatoxicity and bone marrow depression [57, 58].

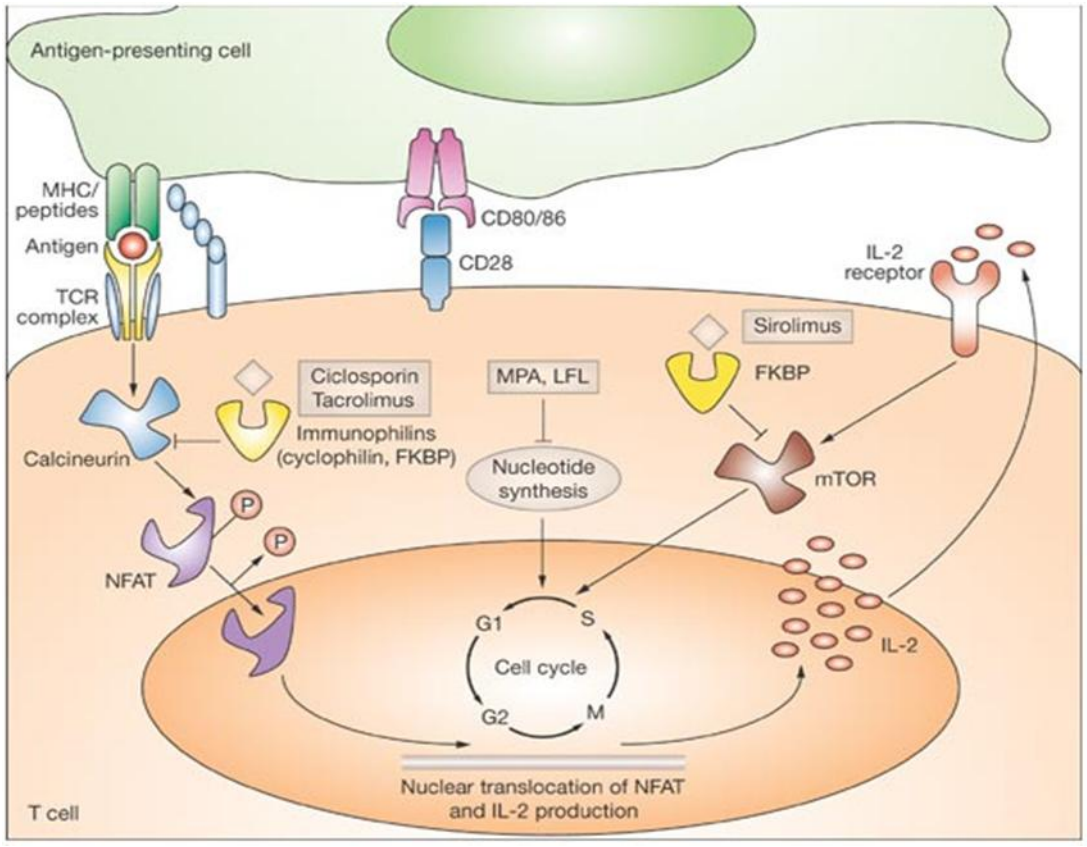


Figure 6: Mechanisms of action of maintenance immunosuppressive agents

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CNIs (cyclosporine and tacrolimus) bind to their respective immunophilins, and inhibit calcineurin. Calcineurin is then unable to dephosphorylate NFAT, which will prevent translocation of NFAT to the nucleus and thereby production of IL-2. Sirolimus is an mTOR inhibitor. It binds to FKBP and inhibits mTOR, which in turn inhibits transition of the cell cycle from G1 to S phase. MPA and LFL are also cell-cycle inhibitors, and act via inhibition of nucleotide synthesis. Abbreviations: CNI, calcineurin inhibitor; FKBP, FK506-binding protein; IL-2, interleukin-2; LFL, leflunomide; MHC, major histocompatibility complex; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; TCR, T cell receptor.

1.2.7.3 Corticosteroids

Prednisone is frequently used as part of the triple agent immunosuppressive regimen following renal transplantation. "*Corticosteroids have immunosuppressive, anti-inflammatory and lymphocytic effects"* [60], the net result of which is decreased cytokine production, lymphocyte proliferation and cellular mapping.

1.2.7.4 Outcomes with Mycophenolate Mofetil and Azathioprine

Multiple studies have been done looking at outcomes of patients treated with AZA and MMF after transplantation. MMF has been shown to decrease the occurrence of acute rejection and treatment failure during the first year after renal transplantation when it is added to a cyclosporine and steroid regimen [54, 61]. The TranCept Stay study showed that long term MMF immunosuppression preserves renal function and that higher doses were associated with better outcomes [62]. It was however postulated that the initial benefit might not be translated into improved graft function in the long term [63].

The Mycophenolate Mofetil versus Azathioprine for prevention of acute rejection in renal transplantation (MYSS) randomised trial compared acute rejections and adverse events of cadaveric transplants over a six month treatment with MMF or AZA. The trial showed that MMF was no better than AZA in limiting rejection episodes and that both drugs were equally well tolerated. It also postulated that use of AZA rather than MMF would give a yearly net saving of $77million in the US and 77 million Euros in Europe, as the cost for standard treatment with MMF exceeded AZA fifteen-fold [64].

The MYSS follow-up study, an extension of MYSS, showed that both drugs were similar in terms of long-term survival and suggested the inclusion of AZA rather than MMF in standard immunosuppressive regimes because of the remarkable difference in cost [65].

1.3 Problem statement

Mycophenolate Mofetil is a more expensive drug compared to Azathioprine. Despite this, it is still widely preferred. There are no local studies that have been done to show that it is a superior drug.

1.4 Motivation and rationale for this research

Mycophenolate Mofetil (MMF) has replaced Azathioprine (AZA) in immunosuppressive regimens worldwide to prevent acute graft rejection. In the US, it is currently more popular than AZA (87% versus 0.6%) in transplant patients before hospital discharge [66]. Whether long term treatment with MMF is better than treatment with AZA is a matter of debate [67].

1.5 Study aim and objectives

1.5.1 Study aim

To compare graft and patient survival in patients treated with MMF versus AZA after cadaveric renal transplantation.

1.5.2 Specific objectives

- To compare time to allograft rejection between MMF versus AZA.
- To establish factors associated with allograft rejection in the two groups.
- To compare the side effects of MMF versus AZA in transplant recipients.

Chapter 2: METHODS

2.1 Study design

This was a retrospective comparative study.

The original proposal was designed to cover all cadaveric transplant patients treated with an AZA or MMF containing regimen between 2002- 2012; with an AZA containing regimen between 2002- 2007 and patients treated with an MMF containing regimen between 2007- 2012. The study period was however extended to the 1980's and 1990's in order to recruit the required number of patients. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (Protocol Number: **M130434**).

2.2 Study population

The population consisted of renal transplant patients at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

2.3 Inclusion criteria

- First cadaveric renal transplant
- Patients on CYA, AZA, Prednisone
- Patients on CYA, MMF, Prednisone
- Patients on CYA, AZA, Prednisone up to time of change in regimen
- Induction therapy with Interleukin 2 receptor antagonist (Basiliximab/ Dacluzimab)

2.4 Exclusion criteria

- Patients on Tacrolimus
- Patients with graft failure < 3months after transplantation
- Living donor transplant

- Repeat renal transplants

2.5 Sample size

A convenience sample of all eligible adult patients, 208 in total, was recruited from the Renal Transplant Unit at CMJAH.

2.6 Data

The data source was a review of clinical records of renal transplant patients at CMJAH from 1985-2013 who were treated with one of the two immunosuppressive agents required for analysis in the study. Acute allograft rejection was diagnosed by a rise of 25% in the baseline serum creatinine and histologically via renal biopsy.

2.7 Variables

Outcomes include:

- Serum creatinine
- Number of acute rejections
- Graft survival
- Patient survival
- Independent variables
    - Socio-demographic characteristics (age at transplant, gender and race).
    - Risk factors for ESRD (hypertension, diabetes, glomerulonephritis, others).
    - Serum creatinine levels measured at intervals (at 3 months, 6 months then yearly) for a period of 60 months post transplantation.
    - Number of acute infections (classified as presence or absence of infections due to limitations of the data).
    - Adverse effects (e.g. nausea, vomiting, anemia, leukopenia, diarrhea).

2.8 Data management and analysis

Data was entered into Microsoft Office Excel Spreadsheets and transferred to STATA 14 (Statacorp, College Station, Texas) for statistical analysis. Strict confidentiality of the patients' records was observed. Names of patients were not used. The student's t test, Chi square and Fischer's exact tests were used for bivariate analysis. Kaplan Meier curves were used to assess survival for each group. Cox Regression Model was used to determine the risk factors associated with the acute rejection in each group of patients on different drugs. The goodness of fit of the model for the data was assessed using the Cox Snell residual and Nelson – Aalen cumulative Hazard test. The results of these two tests showed a good fit.

Chapter 3: RESULTS

3.1 Characteristics of the study population

A total number of 208 patient records from the Charlotte Maxeke Transplant Unit were reviewed for the study. The files are clinical records of patients followed up for a period of 5 years after undergoing successful cadaveric renal transplantation. Of these, 101 patients were treated with Azathioprine and 107 patients treated with Mycophenolate Mofetil. Patients who had lost their grafts <3 months after renal transplantation were excluded from this study.

Table 1 shows characteristics of the study population. The mean age was similar in both groups of patients: 38.1 years in the Azathioprine group and 39.7 years in the Mycophenolate Mofetil group. There were more males than females in both study groups, 64 (63.4%) in the Azathioprine and 81 (75.5%) in the Mycophenolate Mofetil groups. Hypertension was the most common risk factor for ESRD in both groups, with 54.5% in the Azathioprine and 36.5% in the Mycophenolate Mofetil groups respectively. Infections developed in 42.6% of the patients on AZA compared to 69.2% on MMF (p=0.01). A total of 16 patients developed acute allograft rejection during the study period, with 6 (6%) occurring in the Azathioprine group and 10 (10%) in the Mycophenolate Mofetil group (p=0.3). The median time to rejection was significantly longer in the MMF group compared to the AZA group (p=0.03). There was a mortality rate of 1% in the MMF and 16.8% in the AZA group respectively (p<0.0001).

Table 1: Characteristics of the study population

Variable	Azathioprine n (%)	Mycophenoate Mofetil n (%)	p- value
Age (years) Mean (SD)[a]	38.1 (11.0)	39.7 (10.7)	0.29
Gender			
Male	64 (63.4)	81 (75.7)	0.05
Female	37 (36.6)	26 (24.3)	0.05
Ethnicity			
Black	31 (30.7)	83 (77.5)	<0.01
White	64 (63.4)	19 (17.8)	<0.01
Indian	6 (5.9)	5 (4.7)	0.70
Hypertension	46(45.5)	68(63.5)	0.01
Diabetes	2 (2.0)	1 (0.9)	0.61
Both diabetes and hypertension	0 (0.0)	6 (5.6)	0.03
Glomerulonephritis	22 (21.8)	3 (2.8)	<0.01
Unknown cause of CKD	11 (10.9)	23 (21.5)	0.04
Other risk factor [b]	19 (18.8)	6 (5.6)	0.05
Graft failure/ rejection	6 (6.0)	10 (10.0)	0.30
Time to rejection (in weeks), Median (IQR)[c]	12 (12 – 16)	52 (44 – 52)	0.03
Infections	43(42.6)	74(69.2)	0.01
Mortality	17 (16.8%)	1 (1.0%)	<0.0001

[a] SD = Standard deviation.

[b] Other risk factors include; pyelonephritis, cortical necrosis, analgesic nephropathy, posterior urethral valves, post obstetric obstruction, obstructed ureter, medullary cystic disease, reflux nephropathy, poisoning.

[c] IQR = Inter-quartile range.

3.2 Kaplan-Meier survival curves for the Azathioprine and Mycophenolate Mofetil groups

Figure 6 shows the graft survival curves of Azathioprine and Mycophenolate Mofetil treated patients.

From the figure, there are two periods (0-7 & 14-40 months) where the survival curves are parallel. Another period is 8-15 months where the curves are close together. These two observations explain the high p- value (0.37) from the log rank test. From this, we conclude that graft survival is not different between the two groups over a long period of time (60 months)[1].

[1] Time to rejection measured in weeks in Table 1 showed statistical significance between the two groups over a short period of time.

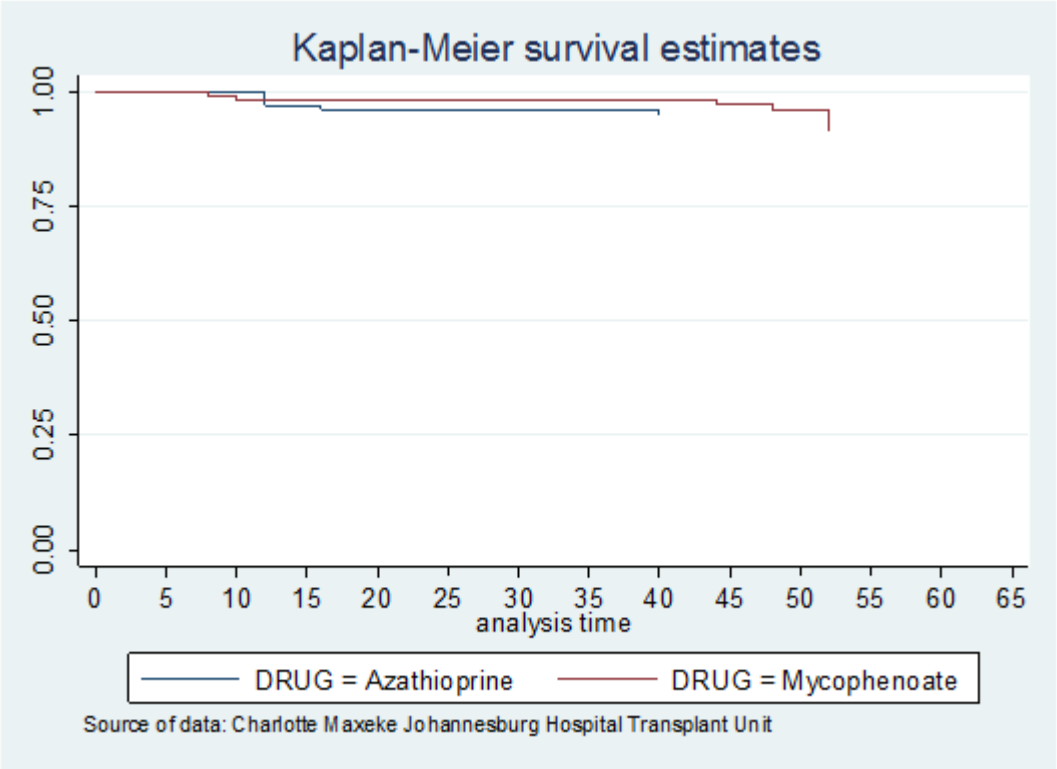

Figure 7: Kaplan- Meier graft survival curves on Azathioprine and Mycophenolate Mofetil

3.3 Serum creatinine levels in the study population

Table 2 (below) shows the distribution of serum creatinine levels in the study population for a period of five years of observation after renal transplantation. There is a steady improvement in renal function in both groups, with the Mycophenolate Mofetil cohort having lower serum creatinine levels at all time intervals (p-value < 0.05).

Table 2: Distribution of Serum Creatinine levels for Azathioprine and Mycophenolate Mofetil of the study population for a five year period of observation

Variable	Azathioprine (µmol/l)	Mycophenoate Mofetil (µmol/l)	Total (µmol/l)	p- value
Serum creatinine at 3 months Median (IQR)[a]	142 (120 – 198)	124 (99 – 153)	134 (108 – 166)	<0.01
Serum creatinine at 6 months Median (IQR)	132 (115 – 174)	120 (91 – 144)	126 (104 – 159)	<0.01
Serum creatinine at 12 months Median (IQR)	131 (113 – 172)	113 (92 – 146)	122 (98 – 154)	0.01
Serum creatinine at 24 months Median (IQR)	133 (109 – 165)	113 (96 – 141)	124 (102 – 148)	0.04
Serum creatinine at 36 months Median (IQR)	129(110.5–154)	121(93.5–143.5)	126(99.5– 148.5)	0.01
Serum creatinine at 48 months Median (IQR)	128 (111 – 155)	121 (94 – 142)	125.5(105 – 149)	0.05
Serum creatinine at 60 months Median (IQR)	129 (111 – 151)	115(93.5– 153.5)	126 (106 - 152)	0.15

[a] IQR = Interquartile Range

3.4 Adverse effects of Azathioprine and Mycophenolate Mofetil

Table 3 shows the distribution of the adverse effects of both drugs in the study population. 16.7% of patients on MMF experienced nausea compared to 5% in the AZA group. In addition, 11.8% of patients treated with MMF had vomiting compared to 4% in the AZA group. About 41% of the patients on Azathioprine had anemia. Neutropenia developed in 49% of patients treated with MMF compared to 0% on AZA ($p<0.01$). Leukopenia occurred in 9.9% of patients on AZA compared to 0% on MMF ($p<0.01$). Thrombocytopenia developed in 6.9% of patients on AZA compared to 0% on MMF ($p=0.01$). Hepatotoxicity was seen in 7.9% of patients on AZA compared to 0% on MMF ($p<0.01$). Abdominal pain was reported in 39% of patients treated with Mycophenolate Mofetil and 29.7% of patients in the same group experienced diarrhea as an adverse effect of treatment. Azathioprine treated patients had more hematological side effects (except for neutropenia), while MMF treated patients had more gastrointestinal side effects.

Table 3: Distribution of Adverse Effects of Azathioprine and Mycophenoate Mofetil in the Study Population

Variable	Azathioprine, N=101 n, (%)	Mycophenoate Mofetil, N=107 n, (%)	Total N 208 n (%)	p-value
Nausea	5 (5.0)	17 (16.7)	22 (10.8)	0.01
Vomiting	4 (4.0)	12 (11.8)	16 (7.9)	0.04
Anemia	41 (40.6)	0 (0.0)	41 (19.7)	<0.01
Neutropenia	0 (0.0)	49 (49.0)	49(24.4)	<0.01
Leukopenia	10 (9.9)	0 (0.0)	10 (4.8)	<0.01
Thrombocytopenia	7 (6.9)	0 (0.0)	7 (3.4)	0.01
Hepatotoxicity	8 (7.9)	0 (0.0)	8 (3.9)	<0.01
Diarrhea	0 (0.0)	30 (29.7)	30 (14.9)	<0.01
Abdominal pain	0 (0.0)	39 (39.0)	39 (19.4)	<0.01

3.5 Unadjusted and adjusted Cox hazard ratios (HR) of allograft rejection for Mycophenolate Mofetil

Table 4 illustrates the unadjusted and adjusted hazard ratios of allograft rejection across selected variables for Mycophenolate Mofetil. The serum creatinine at 3 months was found to be a strong predictor of graft function in patients on Mycophenolate Mofetil ($p < 0.001$). After adjusting for the confounders, serum creatinine at 3 months remained a strong predictor of outcome. Patients experiencing abdominal pain while on treatment with Mycophenolate Mofetil were 62% less likely to develop graft failure than patients on treatment with Azathioprine [unadjusted HR = 0.38(0.14-1.05)]. This finding, however, is not statistically significant (p=0.06). After adjusting for the other confounders, the association with abdominal pain was marginally significant (adjusted HR 0.18(0.01-1.02) p= 0.05).

Table 4: Unadjusted and adjusted hazard ratios (HR) of allograft loss across selected variables for Mycophenolate Mofetil

Variable	Unadjusted HR (95%CI)	p-value	Adjusted HR (95% CI)	p-value
Drug				
Azathioprine **(Ref)**	–	–	–	–
Mycophenolate Mofetil	1.58 (0.57-4.33)	0.38	1.16(0.15-8.77)	0.88
Gender				
Male **(Ref)**	–	–	–	–
Female	0.32 (0.07 – 1.40)	0.13	–	–
Ethnicity				
Black **(Ref)**	–	–	–	–
Other races	0.55(0.19-1.58)	0.27	–	–
Risk Factor				
All Other risk factors **(Ref)**	–	–	–	–
Hypertension	1.57(0.55-4.52)	0.40	–	–
Serum creatinine at 3 months	1.00(1.00-1.01)	<0.001	1.01(1.00-1.01)	<0.001
Serum creatinine at 12 months	0.98(0.96-1.00)	0.08	0.97(0.92-1.02)	0.26
Serum creatinine at 24 months	0.99(0.97-1.01)	0.16	1.03(0.97-1.03)	0.35
Serum creatinine at 36 months	0.98(0.96-1.00)	0.081	0.97(0.91-1.03)	0.34
Nausea				
Azathioprine **(Ref)**	–	–	–	–
Mycophenoate Mofetil	0.35(0.11-1.07)	0.07	–	–
Vomiting				
Azathioprine **(Ref)**	–	–	–	–
Mycophenoate Mofetil	0.35(0.10-1.24)	0.10	–	–
Anemia				
Azathioprine **(Ref)**	–	–	–	–
Mycophenoate Mofetil	1.73(0.39-7.59)	0.47	–	–
Leukopenia				
Azathioprine **(Ref)**	–	–	–	–
Mycophenoate Mofetil	0.73(0.10-5.54)	0.76	–	–
Abdominal pain				
Azathioprine **(Ref)**	–	–	–	–
Mycophenoate Mofetil	0.38(0.14-1.05)	0.06	0.18(0.01-1.02)	0.05

3.6 Goodness of fit of the model

Figure 7 shows goodness of fit of the final model (Table 4). From the graph, we see that the hazard function follows the 45 degree line very closely except for higher time values. Thus we can conclude that the final model fits the data quite well.

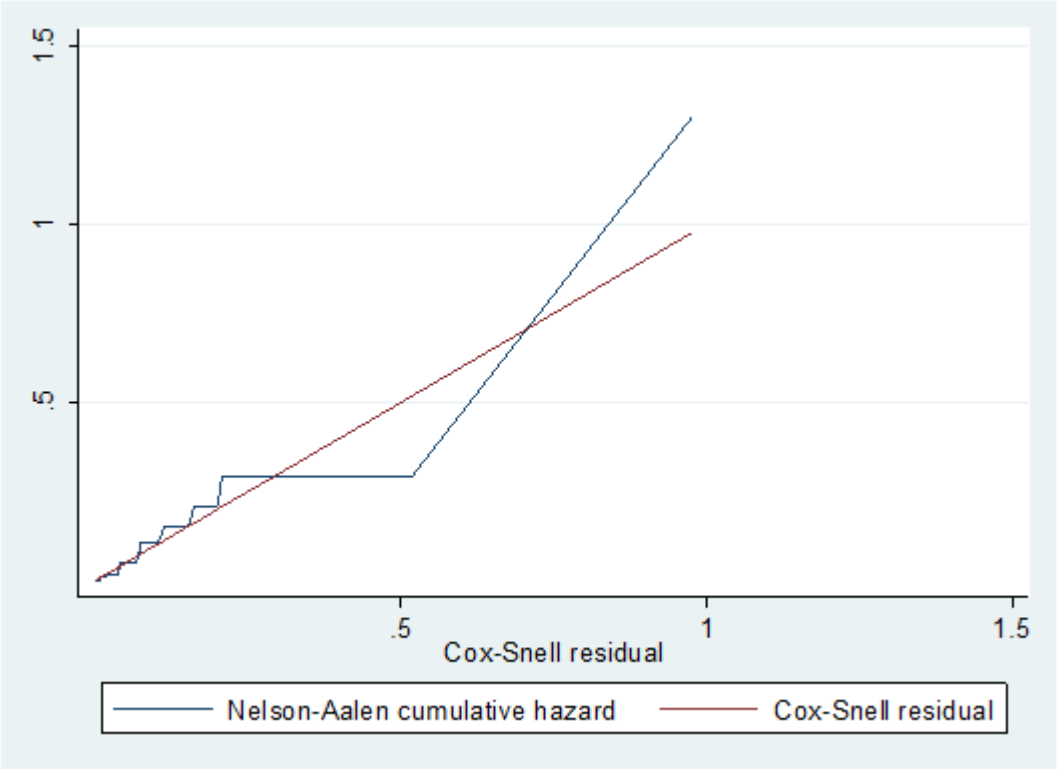

Figure 8: Goodness of fit of the Cox PH multivariate model

Chapter 4: DISCUSSION

4.1 Summary of main findings

A retrospective comparative study was carried out to assess the factors associated with allograft and patient survival in renal transplant patients treated with MMF and AZA at the Renal Transplant Unit at CMJAH. The mean age at transplant was similar in both groups with more male patients being transplanted than females. This gender disparity is consistent with studies done in the US that have shown that more male than female patients have access to hemodialysis and kidney transplantation [68]. Hypertension was the most common cause of ESRD in the study population. A similar finding was reported in a study conducted in the USA [5]. This study illustrated a steady improvement in serum creatinine after renal transplantation during the study period (60 months). The serum creatinine at 3 months was found to be a strong predictor of allograft function, the primary outcome of this analysis. Previous studies conducted elsewhere have reported on serum creatinine levels at 6 months and 12 months as significant predictors of long-term allograft survival [69, 70]. Both drugs were equally tolerated, with nausea and vomiting as the common adverse effects. The prevalence of gastrointestinal side effects was higher in the MMF group compared to the AZA group. This finding is consistent with previous studies which have also shown the same effect [71, 72]. The presence of abdominal pain was found to be protective of allograft function. This association was however marginally significant (p value 0.06) when adjusted for confounding factors. With regard to hematological side effects, anemia was commoner in the AZA group compared to the MMF group. This finding is also consistent with findings from the literature [73].

It is well known that there is an association between immunosuppressive drugs and infection. However, the specific contribution of different drugs to these infections has been poorly

analyzed [74]. In this study, we collected data on the type of infections. However, the data was not sufficient for statistical analysis. With this limitation, the only possibility was to dichotomise the data to the presence or absence of infections as shown in Table 1.

The Kaplan- Meier survival curves showed similar graft survival across both drugs groups (p value 0.23). While serum creatinine was lower in the MMF group, this study showed no evidence to suggest that MMF was better than AZA in preventing allograft loss over the study period.

Findings of this study are consistent with previous studies that have shown that treatment benefits and risks are similar in patients treated with Azathioprine and Mycophenolate Mofetil and that MMF therapy is not superior in preventing long term graft loss when compared to AZA [65, 75]. The effectiveness of both drugs was demonstrated in the study. However, patient survival was superior in the MMF group. However, the patient selection was different in this study with significant differences in ethnicity between the two groups. The AZA patients were recruited from the apartheid era where white patients, a group with lower immunological risk, formed the majority of transplant recipients in South Africa. This changed in the post-apartheid MMF study period where black patients were a majority; this group is known to have higher immunological risk [76]. However the outcomes with respect to graft and patient survival could not be adequately compared in this study because the selection of patients during the apartheid period was biased towards whites. Also, the Aza group had higher doses of steroids, which may have contributed to complications and outcomes.

4.1 Study limitations

The results of this study must be interpreted in the light of the following limitations. Initially, the study was designed to include a comparison of the cost profile of the MMF vs. AZA. It was not possible to do this comparison because of insufficient data. Another limitation is that we used a convenience sample since the sampling frame is not known. This often suffers from bias since the sample is not chosen at random. Despite these limitations, this is the first study to compare the cadaveric renal transplant recipients treated with Azathioprine and Mycophenolate Mofetil in a South African population.

4.2 Conclusion

This study has shown that patient survival was superior in the long term in cadaveric renal transplant patients receiving Mycophenolate Mofetil therapy. While both drugs are effective in the maintenance of allograft function in renal transplant patients in the long term, serum creatinine levels were lower in the MMF group. Whilst this evidence does not support the MYSS trial which concluded that the long term risk and benefit of MMF therapy was similar to AZA in renal transplantation [65], there is need for future research to evaluate the cost profile between the two drug Attachments

R14/49 Dr Linda W Gathara

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130434

NAME: (Principal Investigator)	Dr Linda W Gathara
DEPARTMENT:	Internal Medicine/Renal Transplant Unit CM Johannesburg Academic Hospital
PROJECT TITLE:	Outcomes of Cadaveric Renal Transplantation in Patients using Mycophenolate Mofetil or Azathioprine
DATE CONSIDERED:	26/04/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S Naicker
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 26/04/2013

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

DECLARATION OF INVESTIGATORS

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

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

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Data sheet

Pt. no	
Age	
Gender	
Ethnicity	
Comorbities	

Date of Transplant: _____

Immunosuppressive regimen:

Cya	3mo	6mo	1yr	2yr	3yr	4yr	5yr
Aza							
Pred							

Cya	3mo	6mo	1yr	2yr	3yr	4yr	5yr
MMF							
Pred							

Renal function:

	3mo	6mo	1yr	2yr	3yr	4yr	5yr
eGFR							
Serum Creatinine							

No. of Acute Rejection: _____

No. of Acute Infections

	3mo	6mo	1yr	2yr	3yr	4yr	5yr
No. of Infections							

Side Effects

MMF

Nausea	
Vomiting	
Diarrhea	

Abdominal pain	
Neutropenia	

AZA

Nausea	
Vomiting	
Anemia	
Leukopenia	
Thrombocytopenia	
Hepatotoxicity	

Hospitalization following rejection

	MMF	AZA
No. of Admissions		
Biopsy		

Mortality:
YES/NO : _____

Time after Transplant	
Cause of Death	

Costs:

Drug costs	
No. of rejected episodes	
No of hospital admissions	

Drug costs
Costs of treating rejection episodes
Costs of hospitalization