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AUDIT OF ACUTE REJECTION IN RENAL ALLOGRAFTS

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

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Abstract

Acute graft rejection is acknowledged to have a negative impact on graft survival in renal transplantation. South Africa provides for limited renal transplantation amidst the increasing burden of chronic kidney disease in the local context. Despite this suboptimal provision and limited resources, amongst many other concerns, the role of acute graft rejection on graft survival has not been characterized in the context of South Africa, as well as the African continent. This study is an audit, characterising acute graft rejection diagnosed at the Charlotte Maxeke Johannesburg Academic Hospital over a ten-year period (2003-2012).

The study revealed the incidence of acute rejection in renal transplants to be 34.5%, similar to that reported in international studies. The majority of acute rejections occurred within the first year of transplantation (53.8%), which was lower than that reported in other studies, with 40% of patients having recurrence of acute rejection. The main form of rejection diagnosed was acute cellular rejection (predominantly BANFF grades 1A and 1B), followed by Borderline acute cellular rejection, the combination of which comprised the majority (86.9%) of all rejections diagnosed.

This population was found to be a male dominant and Black African dominant study group, in keeping with the racial distribution of the dialysis population of South Africa, commonly influenced by treatment-seeking behaviour. Cadaveric donor grafts were engrafted in 77.7% of this population and 77.8% of the population had less than 40% of HLA antigens in common with their donor. Delayed graft function was observed in 22.4% of recipients with a significant association with more severe acute graft rejection. Hypertension was the most dominant primary aetiology leading to chronic kidney disease of native kidneys in this population. Immunosuppressive regimen, including cyclosporin, mycophenolate mofetil and prednisone, was used in 80% of recipients, with 97.6% of recipients on mycophenolate mofetil and prednisone.

The five-year survival of grafts developing acute rejection was 61.7%. Graft function deteriorated more dramatically amongst recipients who progressed to graft loss, with recovery of graft function observed to be more prominent amongst recipients with surviving grafts.

This study adds to the literature on this topic, and also describes the characteristics and outcomes of this entity.

Declaration

I, Riju Mathew Thomas, do hereby declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine in the Department of Internal Medicine. I further declare that this work has not been submitted for any other examination or degree at this or any other University.

(Signed) **Riju Mathew Thomas** *on this day, 27*th of August, 2019

Ethical Considerations

Permission for this retrospective study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (clearance certificate number M140667).

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List of Acronyms and Abbreviations

ACR	Acute Cellular Rejection
AGR	Acute Graft Rejection
AHR	Acute Humoral Rejection
ALG	Antilymphocyte Globulin
ANOVA	One-way Analysis of variance
APC	Antigen-Presenting Cells
APOL1	Apolipoprotein L1
ATG	Antithymocyte Globulin
CD	Cadaveric donor
CIT	Cold Ischaemic Time
CKD	Chronic Kidney Disease
CMJAH	Charlotte Maxeke Johannesburg Academic hospital
CMV	Cytomegalovirus
Cr	Creatinine
CsA	Cyclosporin
DGF	Delayed Graft Function
DSA	Donor Specific Antibodies
ESKD	End Stage Kidney Disease
HIV	Human Immunodeficiency virus
HLA	Human leucocyte Antigen
HR	Hazard ratio
IFTA	Interstitial fibrosis and tubular atrophy
IL2-RA	Interleukin-2 Receptor Antagonist
IQR	Interquartile range
IVIG	Intravenous Immunoglobulins
MHC	Major Histocompatibility Complex
MMF	Mycophenolate mofetil
NHLS	National Health laboratory Services
NRLD	Non-related living donor
OKT3	Muromonab-CD3

PE	Plasma exchange
Pmp	Patients per Million Population
RLD	Related living donor
RRT	Renal Replacement Therapy
RTx	Renal Transplantation
SSA	Sub Saharan Africa
USA	United States of America

1 Introduction

Kidney transplantation is an important modality in renal replacement therapy (RRT) for patients with end stage kidney disease (ESKD). Transplantation has several advantages over long term dialysis, being more economical in the long term and offering improved morbidity and mortality rates (1). Despite these advantages, it has been estimated that only 1% of patients with ESKD in Sub Saharan Africa (SSA) are offered renal transplant (RTx), as compared to 30% in first world countries – the major limiting factors being availability of donors and cost (1). In such circumstances, renal allografts represent a scarce resource, and extension of allograft survival through appreciation of factors which result in allograft injury is an important area of research. In this regard, acute graft rejection (AGR) has been shown to be an important limiting factor in renal allograft survival (2–7).

This study was undertaken in an effort to review the contribution of AGR to allograft survival in our environment and to expand the local literature. The risk factors, incidence, and outcomes of AGR in renal allograft recipients attending Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) over a 10-year period (2003 – 2012) were analysed.

2 Literature Review

2.1 History of transplantation and rejection

2.1.1 History of transplantation

Attempts at renal transplantation predate the development of haemodialysis by some four decades. The development of techniques which facilitated vascular anastomosis by Jaboulay and Carrel at the beginning of the twentieth century were followed by unsuccessful attempts at xenotransplantation of animal (goat and pig) kidneys in 1906 (8,9). In 1936, Voronay performed six unsuccessful human renal allotransplants in patients suffering from mercury poisoning (8,9). The cause of this failure was thought to be mainly due to prolonged ischaemic time, which led to attempts to optimise the surgical procedure, resulting in 1951 in the development of the still-in-use Kuss technique (placing the kidney extra-peritoneally in the iliac fossa in close proximity to the external iliac vessels and the bladder) (8,9). Such refinements resulted in the first successful syngeneic renal transplant between identical siblings in 1954 by Joseph E Murray (8,9). The first renal transplant in Africa was performed in Johannesburg, South Africa, in 1966 (10).

2.1.2 History of rejection

In 1944 Medawar, through his work with skin grafts, demonstrated that rejection was an immunological event (8,9). This resulted in attempts to suppress the immune system, initially using total body irradiation, which had unsatisfactory outcomes for both the renal graft and the recipient (8,9). This gave rise to the introduction of chemical immunosuppression: in 1960, Willard Goodwin described the effects of methotrexate and cyclophosphamide in extending graft survival; however, these improvements came at the cost of potential bone marrow suppression (9). Greater success was achieved through the use of combined azathioprine and prednisone, which resulted in functional grafts at 1 year in approximately half of non-related renal transplants (8). Later, drugs with improved side-effect profiles were introduced (eg cyclosporine (CsA), tacrolimus, sirolimus, and mycophenolate mofetil (MMF)) which further extended allograft survival rates (8).

2.2 The burden of Chronic Kidney Disease worldwide and in Sub-Saharan Africa

Chronic kidney disease (CKD) remains a worldwide health problem with increasing incidence and prevalence. Between the 1990 and 2010, the mortality of CKD nearly doubled, and in 2010 it was the 18th highest cause of death worldwide (11). In 2005, the incidence of ESKD was reported to be increasing worldwide at an rate of 8% yearly, which exceeded the population growth rate of 1.3% (12).

Several studies have described a high incidence of CKD among Black Americans (13,14). However, due to the lack of functioning registries in Sub-Saharan Africa (SSA), the exact prevalence of CKD in the African continent is not clear and may be underestimated (12,14,15). Chronic kidney disease in SSA may be 3–4 times more frequent than in more developed countries (14).

There is marked variation in the epidemiological characteristics of CKD in SSA compared to other regions (15). In SSA, CKD affects mainly young adults aged 20–50 years in comparison to the older population afflicted in more developed regions (14–16). Chronic kidney disease in SSA is primarily due to hypertension and glomerular disease, whereas in developed countries CKD is predominantly due to diabetes mellitus and hypertension (14–16); in South Africa, the burden of CKD is exacerbated by the prevalence of Human Immunodeficiency virus (HIV) (15–17). The mortality of CKD is higher in SSA due to late presentation and associated co-morbidities (14,16,18). This combination of a younger affected population with a higher risk of mortality has a significant impact on population life-span as well as the long-term economic burden of CKD in SSA.

2.3 Renal Replacement Therapy

2.3.1 Global versus Africa

Grassman et al. reported that of the 1.8 million people worldwide undergoing RRT for ESKD at the end of 2004, 77% were on dialysis treatment, with the remaining 23% having a functioning renal graft (19). The 122 countries surveyed for this report accounted for 92% of the 2004

world population (19). However, approximately half of all these dialysis patients resided in North America and Europe, and approximately three-quarters of all patients living with a renal transplant were located in these two continents (19). The global contribution of patients on dialysis in SSA is less than 5% of the worldwide dialysis population, while less than 1% of ESKD patients in SSA receive renal transplants (1,14). It is clear that SSA and Africa severely lags behind the developed world in achieving a 70/30 split in RRT modalities.

A more recent systematic review by Liyanage et al., which included 123 countries, representing 93% of the world population and 81% of the African population in 2010, reported an increased total of 2.618 million people receiving RRT, with proportions of dialysis and RTx similar to that reported by Grassman et al. in 2004 (20). High-income and upper-middle income countries contributed to 92.8% of RRT recipients, while only 7.2% of recipients were from lower-middle income and lower income countries (20). The highest prevalence of RRT was reported in North America at 1840 per million population (pmp) while the lowest was reported in Africa at 80 pmp, once again highlighting the great disparity that exists between Africa and the rest of the world (20). Africa remained the region with the greatest deficit in RRT, when comparing the difference between patients needing RRT and patients receiving RRT.

2.3.2 Dialysis in Africa

Haemodialysis is the main form of RRT for the majority of African countries (15,16). In 2013, the dialysis treatment rates were described as below 20 pmp for most of SSA, except for Sudan at 120 pmp and South Africa at 150 pmp (16); some SSA countries are not able to provide dialysis treatment at all (16). The rate of treatment of ESKD in South Africa in 1994 was approximately 17 pmp per year, at a point where the number of patients requiring treatment was estimated to be 100-150 pmp (21); in 2009, the rate of dialysis treatment was reported as 70 pmp (14). The most recent South African Renal Registry Annual Report of 2016 indicated the prevalence of RRT to be 183 pmp, with great discrepancies reported between the private and the public sector (22); the reported rate of RRT in 2015 was 72 pmp in the public sector in comparison to 799 pmp in the private sector (10). This trend of an increasing rate of treatment of ESKD is partly driven by the increasing burden of disease, but other factors

include an increasing life expectancy in the general population as well as increased survival of treated ESKD patients, and improved accessibility to treatment via private funding in South Africa (19,23).

2.3.3 Renal transplantation (RTx) in SSA

Renal transplantation is only available in 7 of the 45 countries In the SSA region, namely: South Africa, Sudan, Nigeria, Mauritius, Kenya, Rwanda, and Ghana (15,16). As discussed previously, less than 1% of ESKD patients in SSA receive transplants (14). The main factors limiting transplantation in SSA are the costs of the procedure, the lack of donor organs, and the paucity of infrastructure and expertise in the local context:

- The cost of renal transplantation cannot be afforded by the majority of the general population and hence becomes a burden for the state. Setting up and maintenance of facilities, as well as funding of expensive consumables (immunosuppressive drugs) contribute to these costs (1,16,23).
- Donation is rare due to cultural beliefs and lack of brain-death laws (1,16). Most countries in SSA depend on living donor transplants, except South Africa which has a preponderance of deceased donor transplants, with the source of renal donor organs being 60% deceased donors and 40% living donors (10,16).
- The lack of suitably qualified specialists in Africa hampers the provision of expert care to the growing number of patients considered for renal transplantation (16,23). A 2008 survey showed that there were 1154 nephrologists for a population of approximately 900 million in Africa, whereas in the United States a population of about 300 million was served by over 5000 nephrologists (23).

Some of these limitations and concerns have been raised in a recent local report that has described a declining overall renal transplant rate in South Africa, over the last 25 years, with this decline most prominent in the public sector (10).

2.3.4 Benefits of renal transplantation

Renal transplantation is the most economical treatment for patients with ESKD (10). Whilst the cost of dialysis remains relatively constant throughout dialysis, the majority of the cost of RTx is incurred at initiation of transplantation and for a short period thereafter, following

which the cost of treatment is ameliorated (1,23,24). Renal transplantation provides better quality of life across all aspects of patients' well-being (physical, social and emotional) (1,24,25). It has also been shown that when compared to patients remaining on dialysis, RTx has better long-term outcomes, despite the increased short-term risk of death after transplantation (25,26).

2.4 Acute Graft Rejection

The economic and health benefits of renal transplantation remain extant only as long as the graft survives; appreciation of factors such as acute graft rejection (AGR) which limit allograft survival is therefore vital in capitalizing on the potential benefit of transplantation.

2.4.1 Definition of AGR and its significance

Acute graft rejection occurs when a recipient's immune response is triggered and targets the alloantigens of the donor graft, resulting in inflammation and damage to the graft (27).

Although AGR is often associated with deterioration in renal function (manifested, for example, by increasing serum creatinine), the process of rejection may also be subclinical, in which case there is no associated clinical deterioration in renal function (27).

Renal grafts are lost at a rate of 2-3% per year after the first year post RTx and AGR has been shown to be one of the strongest factors affecting long-term graft survival (2–7).

2.4.2 Appreciation of the effects AGR on graft survival over the years

Following the development of successful engraftment techniques in the 1950s, focus shifted in the latter half of the 20th century to overcoming the negative effect of AGR on graft survival. The rate of AGR was estimated at greater than 50% in the 1980s, resulting in the surgical removal of many grafts within months of transplantation (28) in response to an acute clinical picture of rejection manifesting with fever and graft tenderness. This clinical picture has disappeared almost completely with the development of new immunosuppressive drugs and

better immunologic matching of recipient and donor (27). By 2010, the overall risk for AGR was thought to be less than 15% within the first year of transplant (27). In 2004, Meier-Kriesche et al. retrospectively reviewed episodes of AGR between 1995 and 2000 which demonstrated a significant decrease in the episodes of AGR rates annually within the first two years of transplantation (5).

However, these authors also noted that overall graft survival had remained relatively unchanged during the same period, despite the significant reduction in rate of AGR (5). Similarly Lamb et al. in describing long term graft survival in terms of half-lives, reported living donor transplants to have half-lives of 11.4 years in 1989 and 11.9 years in 2005, with the half-life of deceased donor transplants being 8 years in 1995 and 8.8 years in 2005 (29).

The discordance between trends in AGR rates and trends in long-term graft survival has been observed in several recent clinical trials (5,28–30). Possible explanations underlying these observations include:

- In 1972, Silcott et al. reported a 93% graft failure rate and 47% mortality rate if recovery to within 20% of pre-rejection serum creatinine levels did not occur; in comparison, a 27% graft failure rate was reported for those grafts which showed recovery of creatinine to within 20% of baseline serum creatinine (31). In 2004, Meier-Kriesche et al. reported similar findings that suggest that the degree of recovery of allografts after an episode of AGR has a crucial impact on graft survival (5), with poor recovery of allograft function following an acute rejection episode increasing the risk of subsequent late rejection (5). Madden et al. similarly described no effect on long-term allograft survival of AGR provided that graft function recovers after the AGR episode (4); their observations were carried out over two consecutive years in a 5-8 year follow-up period, with full recovery of graft function post-AGR observed in all (100%) recipients who maintained good graft function, in comparison to 32% of recipients with declining baseline graft function ($p<0.001$) (4). Therefore a possible explanation for persistent poor long-term allograft survival despite improved AGR rates may be that the recent findings showing a declining rate of AGR reflect mainly a decrease of milder episodes of AGR with associated graft recovery, whilst the rates of

survival-limiting, more severe episodes with poorer functional recovery remain unchanged (5).

- Alternatively, newer, more potent drugs with increased immunosuppressive effect may result in fewer episodes of AGR, but increase the frequency of opportunistic infections and drug nephrotoxicity which may compromise long-term graft survival (5,29,30).
- It has also been suggested that statistical bias may have contributed to the apparent disconnect between improved AGR rates and lack of improvement in allograft survival. Data from the ANZDATA registry indicates that under 4% of graft losses in the first 12 months of engraftment are attributed to AGR; improvement in the AGR rate would therefore only produce a small change in graft survival (30).
- It is also conceivable that recent trends in including higher risk donor kidneys and recipients may affect the overall long-term statistics (5,32).

It is also worth noting that repeated episodes of AGR have been shown to be a significant negative predictor of graft survival and graft function. Heaf and Ladefoged described the 5-year graft survival in recipients as 62% with no rejection episode, 34% with one episode, 26% with two episodes and 19% with three episodes ($p<0.001$) (2). Similar finding were reported by Mateu et al. (6).

Acute graft rejection has also been shown to be a strong risk factor for chronic rejection (4,33). The risk ratio of chronic rejection in patients with one or more episodes of AGR has been estimated to be 6.45 to 7.7 times higher than those with no AGR (33). Madden et al. reported that incompletely reversed AGR further increased the risk of chronic rejection as compared to completely reversed AGR; in this study, grafts were lost to chronic rejection in 82% of patients with incompletely reversed AGR as compared to 8% in patients with completely reversed AGR (4).

In describing the causes of allograft loss, Stegall et al. (28) in 2011 reported on a cohort of 1317 renal allografts in which 330 grafts were lost (25%) between 1996 and 2008. Of these lost grafts, 113 were due to death censored graft losses occurring in the first 5 years of transplantation, of which 32 occurred in the first year and 81 in the subsequent 4 years. Acute

graft rejection (both clinical and subclinical) was attributed as the cause of 18.8% of graft losses (6 out of 32 cases) in the first year and 12.3% of graft losses (10 out of 81 cases) between 1 and 5 years. Interstitial fibrosis and tubular atrophy (IFTA) was a significant cause of graft loss, especially between 1 and 5 years at 32% (26 out of the 81 cases); a further 45% (18/40) of graft losses after 5 years was attributable to IFTA. Although the aetiology of this histological fibrosis is likely multifactorial, chronic inflammation was considered to be a common contributor; the impact of subclinical and borderline rejection in the development of this chronic inflammation is still unclear and should therefore not be underestimated. Interestingly, when the outcomes of patients with 1 year protocol biopsies were reviewed, approximately 40% of grafts with subclinical inflammation resulted in graft failure or significant loss of function between 1 and 5 years. (28). Similar findings were reported by Cosio et al., describing the negative impact of inflammation and fibrosis on graft survival (34).

In comparison to these findings, one of the largest studies of ultra-long term graft survival (that is, kidney transplants surviving 20 years) (32) reviewed 1174 transplants and found 255 (21.7%) grafts that survived 20 years. Surprisingly, 49% of the 255 survivors had exposure to early (within the first 3 months) acute rejection episodes. The study cohort, however, comprised recipients with low immunological risk and comorbidities and the nature and severity of the rejection episodes and recovery of graft function after treatment thereof is unclear (32). However, such findings may suggest that long term graft survival is possible despite the diagnosis of AGR, if diagnosed and managed appropriately.

2.4.3 Clinical picture and diagnosis

The clinical presentation of AGR may vary between asymptomatic with stable graft function, clinically asymptomatic with increasing creatinine, or deteriorating renal function with clinical symptoms (such as fever, oliguria and graft tenderness) (35). Renal biopsy is the gold standard for diagnosing AGR (35). Renal biopsy permits diagnosis, prognostication, and planning of treatment; in the case of protocol biopsy, this intervention also allows identification of subclinical AGR which does not exhibit any clinical deterioration or renal dysfunction, but may inflict harm to the graft in the long term(27).

2.4.4 Clinical AGR versus Subclinical AGR

Subclinical rejection is detected in up to 30% of patients with stable renal function, within the first 3 months of transplantation (3,36,37) and may affect long term graft function (3,27,36). Subclinical AGR is identified at protocol biopsy; in such cases there is no clinical deterioration; the accepted definition of subclinical rejection requires less than 10% increase in creatinine in the two weeks prior to the biopsy with a histological grading by the Banff classification of type 1A or greater (37). Although the severity of inflammation may be greater in clinical AGR, subclinical AGR cannot be morphologically differentiated from clinical AGR (3). Grimm et al. compared the phenotype of the cellular infiltration on renal biopsy of allografts with normal histology, subclinical AGR and clinical AGR and reported an increasing presence of interstitial infiltrates in the order of normal (lowest), subclinical AGR (intermediate) and clinical AGR (highest) respectively (3). Further comparison of markers of activity of infiltrating cells across these groups found the degree of expression of macrophage-associated allograft inflammatory factor-1 to be significantly different with the highest expression thereof being detected in allografts with clinical AGR (3). This suggests that the severity of the interstitial infiltrate determines the severity of clinical presentation through the release of allograft damaging intermediaries such as allograft inflammatory factor-1 and macrophage thromboxane synthase (3).

2.4.5 Classification of Acute graft rejection

Acute graft rejection can be classified according to a number of different factors (27):

- pathophysiological mechanisms - cell-mediated, antibody-mediated, or mixed
- severity – the extent of histological inflammation and injury as graded using the Banff classification
- presence or absence of allograft dysfunction - clinical or subclinical rejection
- response to treatment – presence or absence of steroid resistance
- timing of episode - hyperacute (occurring within minutes of engraftment), acute (occurring within days to weeks of engraftment), late acute (occurring after 3 months), or chronic (occurring months to years after transplantation).

2.4.6 Mechanism of AGR

The entity of AGR has its origins in the immunological threat resulting from the effects of the donor's death and perioperative ischaemia on the donor kidney (27). Ischaemia and reperfusion cause up-regulation of the expression of human leucocyte antigen (HLA) by the donor kidney, as well as the release of inflammatory molecules (27). The major histocompatibility complex (MHC) antigens (HLA in humans), are major targets in AGR (38). The increased expression of HLA facilitates recipient immunological recognition and response to the allograft increasing the risk of rejection (27). Engagement of the recipient's immune system with donor tissue is facilitated by the degree of incompatibility of recipient and donor HLA types (HLA "mismatching"), and the functionality of the recipient's immune system; the latter being subject to factors such as recipient age (with older age being associated with altered immunological function due to immunosenescence), genetics, and immunosuppression protocol prescribed (27,39–41).

There are 2 principal forms of AGR - acute cellular rejection (ACR) and acute humoral rejection (AHR) (27,35,42). Differentiating between the two is important, as it impacts on therapy and prognosis. The clinical presentation of both processes are similar; however AHR, has a higher incidence in the first few weeks after transplantation (43). Mauiyyedi et al. have demonstrated disparate outcomes between these two major categories of AGR, with the overall graft loss at 1 year being 4% for ACR and 30% for AHR (44).

2.4.6.1 Acute cellular rejection (ACR)

Acute graft rejection of renal allografts is the commonest form of AGR and is primarily a T cell–mediated process (27,44). Figure 2.4.6.1 describes the process and histology of ACR. The development of ACR involves the presentation of donor alloantigen by antigen-presenting cells (APCs) to recipient T lymphocytes. These APCs may be of donor or recipient origin. Initially, donor APCs play the major role in alloantigen presentation (direct pathway); with time these cells diminish in number, leaving the recipient APCs (indirect pathway) to play the dominant role in the long-term immune-mediated injury to the graft. Activation of recipient T cells by APCs leads to their differentiation into various subgroups, such as T helper 17 cells, T

helper 1 cells, regulatory T cells, CD4 T cells and ultimately damage to the allograft through subsequent activation of cytotoxic CD8 T cells (27,45).

T cells enter the graft with aid of adhesion molecules, which also facilitate T cell migration across peritubular capillaries, leading to infiltration and proliferation within the interstitial space and further invasion of the renal tubules, causing tubulitis, a histological hallmark of ACR (27).

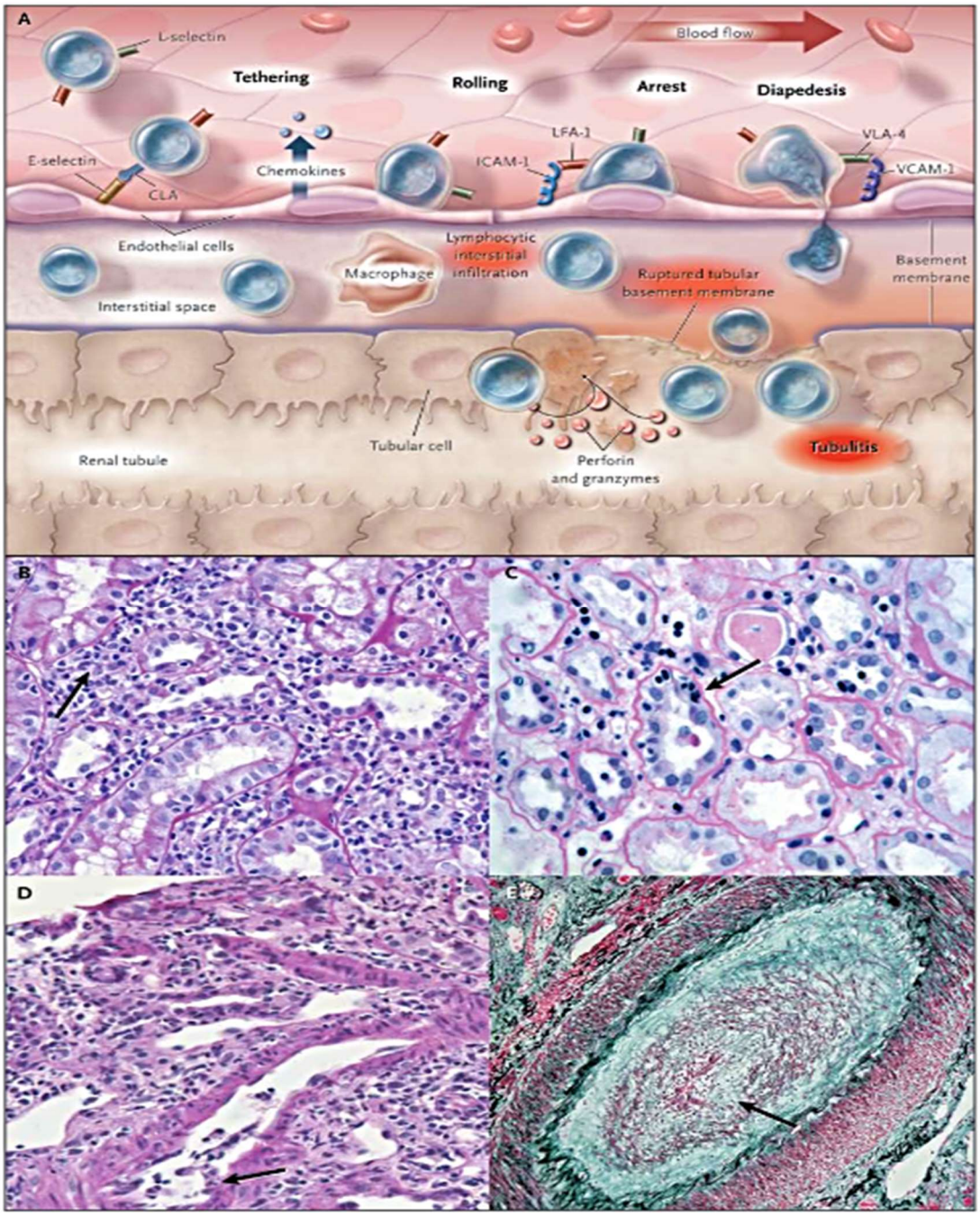

Figure 2.4.6. 1 Rejection Process and Histology of Acute Cellular Rejection (26)

"Cellular rejection and transport of cells into the transplant are shown (Panel A). After the initial tethering, rolling, and arrest of effector T lymphocytes (which bind selectins and integrins on endothelial cells), lymphocytes and other immune cells enter the interstitial compartment and invade tubules, causing local tissue destruction. The histologic features of T-cell–mediated rejection include a dense interstitial lymphocytic infiltration (Panel B, arrow; periodic acid–Schiff stain), with mononuclear cells crossing the tubular basement membrane (pink) into the renal tubules, resulting in tubulitis (Panel C, arrow; periodic acid–Schiff stain). In acute vascular rejection, mononuclear cells adhere to the endothelium of small muscular arteries (Panel D, arrow; hematoxylin and eosin). In chronic vascular rejection, neointimal thickening (Panel E, arrow; Masson trichrome stain) due to myofibroblasts leads to complete vascular occlusion. ICAM-1 denotes intercellular adhesion molecule 1, LFA-1 leukocyte-function–associated antigen, VCAM-1 vascular-cell adhesion molecule 1, and VLA-4 very late antigen 4." (27)[1]

[1] Reproduced with permission from Nankivell BJ, Alexander SI. Rejection of the Kidney Allograft. N Engl J Med. 2010;363(15):1451–62, Copyright Massachusetts, Medical Society.

CD4 T cells produce inflammatory cytokines interferon- γ and interleukin (IL)-2, which drive a cellular response, and IL-4, IL-5, and IL- 13, which produce a humoral response. Inflammatory cytokines also activate tubular epithelial cells, in turn attracting further T lymphocytes through the secretion of chemotractants. CD8 T cells mediate an allograft specific cytotoxic effect, crossing the basement membrane of the tubule, where they proliferate and induce apoptosis of tubular cells. The necrosis of tubular epithelial cells and basement membrane rupture results in urinary leak, culminating in progressive tubular atrophy and graft dysfunction.(27)

2.4.6.2 Acute Humoral Rejection (AHR)

Acute humoral rejection occurs in 5-7% of all renal transplants; 30% of all biopsies undertaken for acute rejection manifest AHR, which carries a substantially worse prognosis than ACR (44,46). Acute humoral rejection often manifests as acute vascular injury / rejection and is a major limiting factor in carrying out xenotransplantation successfully (47).

The development of AHR depends upon the elucidation of donor specific antibodies (DSAs) against HLA molecules, endothelial-cell antigens, and ABO blood-group antigens (27,46,47). These antibodies may either be pre-formed or may develop de novo after transplantation (46). Anti-HLA antibodies may develop pre-engraftment due to sensitization following pregnancy, blood transfusion, viral molecular mimicry, or previous transplantation (27,46). The contribution of both preformed and de novo antibody to reduced allograft survival through vascular injury has been documented in a number of studies (43,48,49).

Antibody-mediated vascular injury targets particularly the peritubular and glomerular capillaries (43), possibly due to decreased anti-complement protective pathways in the peritubular capillaries (50). The resultant histopathological features of AHR include the presence of neutrophils in peritubular capillaries, glomerulitis, fibrin thrombi, interstitial oedema, infarction, severe vasculitis and fibrinoid necrosis in vessels walls (46,49,50). C4d is a fragment of complement component C4 released during activation of the classical complement pathway by antigen-antibody complexes (44) which binds covalently to tissue locally (endothelium and basement membrane collagen), thereby serving as an in situ

pathological marker of antibody mediated injury; as a result, C4d immunofluorescence positivity has been proposed as a reliable indicator for AHR (44,49,50). However, not all biopsies of AHR show typical features of AHR (including C4d positivity); and some cases may show acute tubular necrosis only whilst others may coexist with ACR. This is complicated by the fact that no morphological feature has been described to be pathognomonic for AHR (43,44,46,50). As a result, histological diagnosis of AHR is sometimes challenging. At present, AHR is diagnosed in the presence of two of the following three parameters: morphological evidence of acute tissue injury, immunopathological evidence of C4d deposition in the peritubular capillaries, and serological evidence of circulating DSA (43,44). Figure 2.4.6.2 below depicts the histology of AHR.

Differentiating ACR from AHR is crucial since prognosis and therapy differs between the two. Acute humoral rejection commonly occurs in the first few weeks after transplantation, but can also develop years after transplantation (commonly due to inadequate immunosuppression – whether due to iatrogenic minimization protocols, noncompliance with prescribed drugs, or malabsorption of ingested drugs) (43). Though the overall prognosis of AHR is worse than ACR, those that recover from the acute episode of AHR may have comparable long-term outcomes (50).

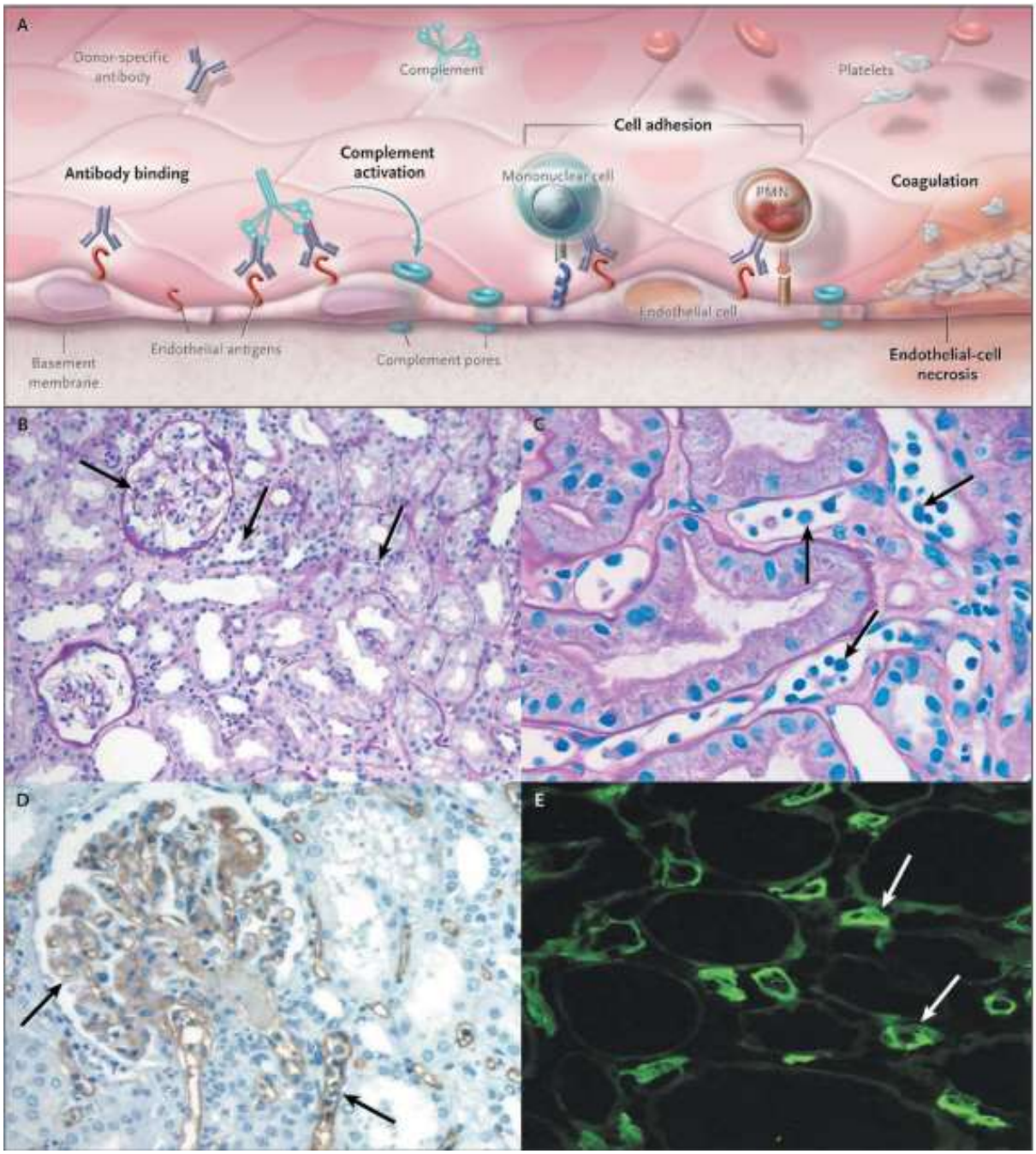

Figure 2.4.6. 2 Histology of Acute Humoral Rejection (26)

"Antibodies against donor antigens bind to antigens expressed on endothelial cells in the graft vessel (Panel A). The subsequent complement activation and cell adhesion result in endothelial-cell necrosis, followed by platelet deposition and coagulation. PMN denotes polymorphonuclear cell. The corresponding histologic changes are shown in Panels B through E. Mononuclear cells adhere to the endothelium of the glomeruli (Panel B, arrows; periodic acid–Schiff stain) and the peritubular capillaries (shown at higher magnification in Panel C, arrows; periodic acid–Schiff stain). This process is accompanied by C4d deposition in the glomeruli and peritubular capillaries (Panel D, arrows; C4d immunohistochemical stain) and in the peritubular capillaries between ghost outlines of the renal tubules (Panel E, arrows; C4d immunofluorescent stain)" (27).[2]

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2.4.7 Banff classification of Renal Transplant Pathology

The Banff Classification was introduced in an attempt to standardise the pathological interpretation of renal allograft biopsies and to facilitate accurate interpretation of the mechanism of graft injury, thus directing targeted therapy (51,52). The first version of the Banff Classification was published in 1993 (52); since then the Banff classification has undergone multiple reviews reflecting evolving understanding of the pathology of allograft injury (51,52).

The Banff classification, as depicted in Table 2.4.7.1, provides criteria for diagnosis of different entities of rejection observed in renal allografts, which includes antibody mediated rejection (C4d staining without evidence of rejection, acute humoral rejection (AHR) and chronic antibody mediated rejection), Borderline ACR and T-cell mediated rejection (acute cellular rejection (ACR) and Chronic T-cell mediated rejection). Over time, histopathological, molecular and serological parameters have been incorporated into the Banff classification to describe the type and severity of AGR (53). Common histopathological lesions include interstitial inflammation (i), tubulitis (t), intimal arteritis (v), glomerulitis (g) and peritubular capillaritis (ptc) all of which are graded in increasing severity, denoted by numerical values ranging between 0 and 3 (53).

The Banff classification for ACR comprises of the milder grade 1 which reflects interstitial inflammation and tubulitis, and is further divided into two subgroups – 1A ($i \geq 2$ and $t = 2$) and 1B ($i \geq 2$ and $t = 3$) (53). Grade 2 and 3 includes increasing degrees of intimal arteritis regardless of severity of interstitial inflammation and tubulitis – 2A (v1), 2B (v2) and 3 (v3) (53).

As described earlier, AHR is diagnosed in the presence of two of the following three parameters: morphological evidence of acute tissue injury ($g>0$ or $ptc>0$ or $v>0$, all in the absence of T-cell mediated rejection) immunopathological evidence of C4d deposition in the peritubular capillaries, and serological evidence of circulating DSA (43,44).

Table 2.4.7. 1 Banff classification of Renal allograft pathology (53)

Category 1 : Normal biopsy of nonspecific changes	
Requires exclusion of any diagnosis from the Banff diagnostic categories 2 – 4, 6 below.	
Category 2 : Antibody-mediated rejection (AMR)/Humoral rejection Use the diagnostic criteria groups (right column) to reach 1 diagnosis (left column)	
Diagnoses	**Diagnostic criteria groups**
C4d staining without evidence of rejection Banff lesion score C4d > 1 (IF on fresh frozen tissue) OR C4d > 0 (IHC on paraffin-embedded tissue) AND Banff lesion scores t0, v0, no arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima, no criterion from group 1 (AMR activity), no criterion from group 4 (histologic features of AMR chronicity), no increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR	Criteria group 1 AMR activity: – Banff lesion score g > 0 in the absence of glomerulonephritis and/or Banff lesion score ptc > 0 in the absence of T cell mediated rejection (TCMR) or Borderline – Banff lesion score v > 0 – Acute thrombotic microangiopathy in the absence of any other cause – Acute tubular injury in the absence of any other apparent cause
Active AMR No criterion of AMR chronicity (Criteria group 4) AND At least 1 criterion from Criteria group 1 (AMR activity) AND At least 1 criterion from Criteria group 2 (antibody interaction with tissue) AND At least 1 criterion from Criteria group 3 (DSA or equivalents)	Criteria group 2 antibody interaction with tissue: – Banff lesion score C4d > 1 (IF on fresh frozen tissue) or C4d > 0 (immunohistochemistry (IHC) on paraffin-embedded tissue) – At least moderate microvascular inflammation (g + ptc > 1) in the absence of recurrent or de novo glomerulonephritis; Borderline (Diagnostic category 3) or acute TCMR (Diagnostic category 4). If Borderline, acute TCMR, or infection are present, (Banff lesion scores g + ptc) > 1 is not sufficient and Banff lesion score g > 1 is required. – Increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR
Chronic active AMR At least 1 feature of AMR chronicity (Criteria group 4) AND At least 1 criterion of antibody interaction with tissue (Criteria group 2) AND At least 1 criterion of DSA or equivalents (Criteria group 3)	Criteria group 3 DSA or equivalents: – DSA (anti-HLA or other specificity) – Banff lesion score C4d > 1 (IF on fresh frozen tissue) or C4d > 0 (IHC on paraffin-embedded tissue) – Increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR
Chronic AMR Banff 2017 permits the use of this term for biopsy specimens showing transplant glomerulopathy and/or peritubular capillary basement membrane multilayering in the absence of criterion of current/recent antibody interaction with the endothelium (Criteria group 2) but with a prior documented diagnosis of Active or Chronic active AMR or documented prior evidence of DSA	Criteria group 4 histologic features of AMR chronicity – Banff lesion score cg > 0 (by LM or EM, if available), excluding biopsies with evidence of chronic thrombotic microangiopathy – 7 or more layers in 1 cortical peritubular capillary and 5 or more in 2 additional capillaries, avoiding portions cut tangentially by EM, if available – Arterial intimal fibrosis of new onset, excluding other causes; Leukocytes within the sclerotic intima favour Chronic AMR if there is no prior history of biopsy-proven TCMR but are not required
Category 3 : Suspicious (Borderline) for Acute T cell mediated rejection (TCMR)/Cellular rejection	
Foci of Banff lesion score t > 0 AND Banff lesions score i ≤ 1 (retaining the Banff lesion score i1 threshold from Banff 2005 is permitted but it must be made transparent in the methods section of reports and publications) OR Foci of Banff lesion score t1 AND Banff lesion score i ≥ 2	
Category 4 : T cell medicated rejection (TCMR)/Cellular rejection	
Acute TCMR IA Banff lesion score i ≥ 2 AND Banff lesion score t2 Acute TCMR IB Banff lesion score i ≥ 2 AND Banff lesion score t3	

Acute TCMR IIA
Banff lesion score v1 regardless of Banff lesion scores i or t
Acute TCMR IIB
Banff lesion score v2 regardless of Banff lesion scores i or t
Acute TCMR III
Banff lesion score v3 regardless of Banff lesion scores i or t
Chronic active TCMR grade IA
Banff lesion score ti ≥ 2
AND
Banff lesion score i-IFTA ≥ 2, other known causes of i-IFTA (eg, pyelonephritis, BK-virus nephritis etc.) ruled out
AND
Banff lesion score t2
Chronic active TCMR grade IB
Banff lesion score ti ≥ 2
AND
Banff lesion score i-IFTA ≥ 2, other known causes of i-IFTA ruled out
AND
Banff lesion score t3
Chronic active TCMR grade II
Arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima

Important limitations of the Banff Classification are the reproducibility thereof and the orphan category of Borderline rejection (51). Reproducibility may be affected by biological variability and by the experience of the examining pathologist (51). The Borderline category was developed for cases in which the threshold for diagnosis of rejection is not reached in order to prevent overtreatment; however, this may compromise sensitivity (51). It has been suggested that the incorporation of molecular and genomic diagnostic technologies will result in greater accuracy of the classification system; however this may be difficult in resource limited areas (51).

2.4.8 Factors influencing graft survival

2.4.8.1 Age

The effects of recipient and donor age on graft survival remain controversial. In many developed countries, an aging population and improved transplant success rates have resulted in an increase in transplantation amongst elderly recipients. In the United States of America (USA), almost half of the candidates on the waiting list for a transplant are over 50 years of age, substantially more than during the preceding decade (54).

Although graft survival is decreased in elderly recipients, censoring data for death with functioning grafts has shown that increasing recipient age is associated with decreased episodes of AGR, despite poorer organ matching criteria in this group (40,55). This may be a

function of immunosenescence in older patients (40). Similar findings have been reported in the local South African context by Moosa et al., demonstrating improved allograft survival with increasing age when censored for death with functioning grafts (56).

Other studies have reported that increasing donor age is associated with increased frequency of AGR and worse long term outcomes (40,55). The impact of increased donor age in these studies on outcomes is independent of the recipient age (55) and likely reflects the use of suboptimal donor organs, in the form of expanded criteria donor kidneys (55).

2.4.8.2 Ethnic origin

African patients have been reported to be at increased risk of AGR (57). Studies have also reported poorer graft function and lower graft survival in black recipient populations (57–59), independent of donor type and HLA mismatch (59).

These poorer outcomes of renal transplantation in black recipients are thought to be the effect of immunological and non-immunological factors. Black recipients are considered to carry an increased immunological risk due to greater degree of HLA polymorphisms, a hyperimmune response (requiring more immunosuppression), and altered pharmacokinetics of immunosuppressants (57–59). Non immunological factors which have been implicated include higher dialysis vintage in this population, underlying ESKD aetiologies which may carry a poorer prognosis for the allograft and / or recipient (for example, diabetes mellitus), increased risk of post-transplant hypertension and diabetes mellitus, and lower socio-economic status leading to poorer access to healthcare (57–59). Interestingly, a study by Moosa et al. reported comparable outcomes of renal transplants in black, white and coloured recipients in Cape Town (56). Of note, however, black patients only contributed 10.3% of patients included in this study. A study undertaken at Wits Donald Gordon Medical Centre has reported a significantly worse patient and graft survival amongst black recipients; in this study the proportion of black patients was 35.1% (60).

More recent reports have raised growing interest in the Apolipoprotein L1 (APOL1) gene and its impact in renal disease. APOL1 has been reported to play a significant role in renal pathology associated with hypertension, HIV, primary glomerular disease amongst others aetiologies (61). The greater proportion of this gene has been found to be prominent in people

of recent African ancestry, and this has been partly attributed to its protective traits over African Trypanosomes (61). The greatest threat for kidney disease is observed by the presence of a homozygous high risk genotype (G1/G1; G2/G2; G1/G2) and it is estimated that 13% of African Americans have these APOL1 high risk genotypes (61,62) which most likely contributes to the four fold increased risk of ESKD in the African population.

Literature on APOL1 in kidney transplantation has suggested that APOL1 of renal origin is primarily responsible for renal disease. This stems from studies that have confirmed shorter graft survival in patients receiving donor kidneys with high risk APOL1 genotype (61–65), while no allograft survival difference was noted when analysing a recipient population with high risk APOL1 genotype (66). Due to the prominence of APOL1 amongst patients of African origin and its association with diseases that constitute significant burden in our local setting, like hypertension and HIV, this is definitely an area of interest in the African context.

2.4.8.3 Cold Ischaemic Time (CIT)

Cold ischaemic time (CIT) is the time between harvesting and transplanting during which the donor organ is exposed to hypothermic preservation. During this period, the organ suffers a degree of tissue injury due to lack of perfusion; on reperfusion there is an amplified expression of HLA antigens by the graft, which facilitates engagement by the recipient immune system (27,67,68), increasing the risk for AGR (27).

A number of studies have demonstrated a negative effect of CIT on graft survival (27,67–69). However, the duration of CIT which confers increased risk for AGR is not known. Furthermore, the contribution of CIT to AGR has been questioned by a large study by Kayler et al. comparing paired grafts from 7115 donors transplanted into 15230 recipients which reported no significant association between CIT and AGR at 1 year post transplantation, and no significant association of CIT with graft survival (69). A strong association between CIT and delayed graft function (DGF) was, however, noted (69).

2.4.8.4 Delayed Graft Function (DGF)

Delayed graft function (DGF) is defined as the need for dialysis within the first seven days post transplantation (68). Numerous factors have been implicated in the development of DGF, including donor graft quality, donor age, donor sex, mechanism of donor brainstem death (such as head injury), surgical technical issues, recipient blood pressure and the presence of panel reactive antibodies; however, CIT is amongst the most frequently implicated risk factors for DGF (69–72). Delayed graft function has been implicated as a risk factor for AGR and decreased graft survival (68,71,72), although this is subject to some controversy (2); some data suggests that although DGF may increase the risk of AGR this does not translate into poorer allograft survival (70). It is postulated that DGF may lead to increased expression of graft antigen facilitating the recipient immune response (68). Alternatively, it has been suggested that early loss of nephrons due to DGF may lead to later allograft glomerular hyperfiltration and glomerular hypertension resulting in nephrosclerosis (71).

2.4.8.5 Human Leucocyte Antigen (HLA)

Major histocompatibility complex antigens are the major target for allorecognition (38); HLA mismatched transplants have poorer survival than HLA matched transplants (41).

Activation of B cells by APCs presenting donor HLA in the context of T cell help results in maturation to plasma cells producing alloantibody (38,41). Interaction of recipient T cells with class I antigens results in the activation of cytotoxic T cells through the direct mechanism of antigen presentation, and interaction with class II antigens through the indirect mechanism results in activation of helper T cells that release cytokines, facilitating the cellular and humoral immune response (41).

Amatya et al. have shown a significantly increased incidence of acute rejection in HLA mismatched recipients, regardless of donor type (39). In these HLA mismatched recipients, the development of AGR was further shown to negatively affect allograft survival; in HLA matched recipients the development of AGR has no effect on long-term outcomes (39).

The use of potent immunosuppressive drugs may ameliorate the effect of HLA mismatching on allograft survival (39,41). In addition, the concept of "mismatch permissibility" suggests that some HLA mismatches may not be immunologically significant (41). These developments have allowed the relaxation of HLA matching over time from "identical HLA match" to "phenotypical matching" to "zero mismatch kidneys" (39). Allocation by minimization of HLA mismatching in the United States may have previously favoured Caucasian recipients over African Americans (39,41) due to the Caucasian origin of many donors, and the presence of a greater diversity of HLA within the African American population (39,41). The increased availability of HLA mismatched grafts occasioned by the advent of potent immunosuppressants thus circumvents a potential ethical conundrum.

2.4.8.6 Deceased donor vs living donors

The lack of available living donor kidneys in SSA (16,73) and elsewhere (54) has been ameliorated by increased use of deceased donor organs. In South Africa, 60% of renal allografts are obtained from deceased donors compared to 40% from living donors (16).

Living donor allografts have been shown to have better long term survival than deceased donor grafts (39,54,73–75). It is likely that a number of factors underlie this disparate outcome, including a reduced CIT in living donation, the optimal health of the living donor (as compared to the deceased donor where the mechanisms of and conditions surrounding brain stem death may affect the quality of the donated kidney), better HLA compatibility between donor and recipient (due to higher probability of genetic similarity between related individuals) and the planned surgical approach facilitated by living donation (39,74). As noted previously, CIT and the nature of donor death have an effect on the expression of HLA antigens and pro-inflammatory signals by the allograft, which may translate into subsequent risk for AGR and graft loss (73,74). Indeed, living donor allografts have been shown to have a reduced risk of AGR (75), which is not fully explained by the improved HLA matching achievable with this form of donation (39).

Beyond effects on the allograft, the mode of donation may affect recipient outcomes. Deceased donor recipients have been found to require more hospital admissions after engraftment (73). In the local context, Fabian et al. have reported a better recipient survival in living donor transplantation in Johannesburg (60).

2.4.8.7 Infections

Infection remains an important contributor to morbidity and mortality in renal transplantation and is thought to be the second most common cause of death with functioning grafts (76). This mostly comprises of nosocomial and surgery associated infections very early in transplantation; thereafter donor-derived infections and opportunistic infections come into play, all these are amidst immunosuppressive therapy (76).

The most common opportunistic infection in renal transplantation is cytomegalovirus (CMV), where CMV disease is reported to affect 8% of recipients (76). Risk factors associated with CMV infection are donor seropositivity, donor age greater than 60 years, induction immunosuppressive agents such as T lymphocyte depleting antibodies, simultaneous kidney-pancreas transplantation, allograft rejection and concurrent infections from other viral infections (76). The effects of CMV infection, apart from the direct effects of the disease, include its association with atherosclerosis, chronic rejection and reduced patient and graft survival (76,77). Cytomegalovirus disease has been reported to be an independent risk factor for biopsy proven AGR within the first 12 months of transplantation (78). These concerns around CMV in renal transplantation have strengthened the practice of appropriate donor and recipient screening, along with appropriate chemoprophylaxis early in transplantation, especially where T lymphocyte depleting antibodies are used.

2.4.9 Immunosuppression therapy during renal transplantation

Immunosuppressive therapy has evolved since the adoption of the first protocols in the 1960s. Current protocols may be categorised as:

1) **Induction therapy** – prescribed during the engraftment period in order to prevent hyperacute or accelerated acute rejection
2) **Maintenance therapy** – prescribed after induction to prevent rejection during the lifetime of the graft

2.4.9.1 Induction therapy

Induction of immunosuppression is achieved through inhibition of recipient T-cell interaction with donor HLA expressed on the allograft (79). Two strategies are available in this regard: depletion of T cells, and inhibition of T cell activation on stimulation by donor HLA through blockade of the costimulatory signal.

T lymphocyte-depleting agents include Antithymocyte globulin (ATG), antilymphocyte globulin (ALG) and Muromonab-CD3 (OKT3), and are mainly used in patients considered to be of high immunological risk or on a steroid sparing immunosuppressive regimen (38,79,80). Antithymocyte globulin is the only depleting agent currently in use at CMJAH.

Co-stimulatory blockade is commonly achieved through the use of IL2 receptor antagonists (IL2-RA), which include Basilixumab and Daclizumab (38,79,80); Basilixumab is the only IL2-RA in use at CMJAH. IL2-RAs have been shown to be non-inferior to depleting agents in low immunological risk recipients with fewer side effects; lymphocyte depleting therapies may reduce the risk of AGR but do not appear to improve allograft survival in comparison to IL2-RA (79). Current KDIGO guidelines recommend IL2-RA as first line induction therapy in this setting (79), although lymphocyte-depleting agents remain the preferred induction therapy in the USA (80).

High dose corticosteroids are commonly used as part of induction therapy with rapid dose tapering to minimize steroid side effects (38,79). Combination oral immunosuppressants are initiated during the induction phase in order to improve efficacy and minimise drug toxicity. Triple therapy consisting of a calcineurin inhibitor such as Cyclosporin or Tacrolimus, an anti-proliferative agent such as MMF or Azathioprine, and oral prednisone is recommended with dose reduction when stable (38,79). The current KDIGO guideline recommends Tacrolimus and MMF as first-line therapy since this combination has been shown to reduce the risk of rejection, decrease incidence of subclinical rejection, and improve graft survival (79).

2.4.9.2 Maintenance therapy

Following induction, immunosuppression is maintained through prescription of triple combination oral agents initiated during the induction phase, the dose of which is gradually reduced in order to reduce the risk of side-effects. The recommended triple regimen includes a calcineurin inhibitor, an anti-proliferative agent and oral corticosteroids; a mammalian target of Rapamycin inhibitor is sometimes substituted for a calcineurin inhibitor.

- Oral corticosteroids (prednisone)
    - Glucocorticoids diffuse passively into cells and bind to intracellular glucocorticoid receptors, forming a complex that translocates into the nucleus. Interaction with specific genetic sequences results in enhancement or suppression of the transcription of susceptible anti-inflammatory and pro-inflammatory genes, as well as alterations to translational processes.(81)
    - Immunosuppressive actions of corticosteroids include:
        - Blocking transcription of proinflammatory genes such as IL-1 (38,79,81).
        - Recruiting of transcription factors that promote transcription of anti-inflammatory genes coding for I-kappa-B-alpha, IL-1 receptor II, lipocortin- 1, IL-10 and alpha-2-macroglobulin(81).
        - Blocking the function of transcription factors such as nuclear factor-kappa-B and activator protein-1, which are required for transcription of pro-inflammatory mediators. The increased synthesis of I-kappa-B-alpha mentioned earlier, also inactivates nuclear factor-kappa-B(81).
        - Inhibiting the secretion of inflammatory cytokines by affecting post-translational events, resulting in instability of the messenger RNA coding for IL-1, IL-2, IL-6, IL-8, tumour necrosis factor and granulocyte-macrophage colony-stimulating factor(81).
    - Concern exists as to the potential for side-effects due to long-term steroid use in transplant recipients. To reduce this risk, it has been suggested that steroids may be withdrawn within 1 week of transplantation in recipients who have low

immunological risk of rejection and have received induction therapy (79). However, other studies have shown increased episodes of acute rejection with steroid withdrawal (79). The current KDIGO guidelines recommend continued use of steroids if steroids are used past the first week (79).

- Calcineurin inhibitors (CsA and Tacrolimus) bind to cytoplasmic proteins (cyclophillins and FK binding proteins respectively) and thereafter as a complex, competitively bind to and inhibit calcineurin (82). This leads to suppression of transcriptional activity of cytokine genes for IL-2, tumour necrosis factor-alpha, IL-3, IL-4, granulocyte-macrophage colony-stimulating factor and interferon-gamma (82). Ultimately, T cell proliferation is suppressed by inhibiting the expression of T cell activation in response to antigen presentation (38,79,82,83).
    - Some data suggests that Tacrolimus offers superior immunosuppression over CsA (84). Tacrolimus may prevent the development of subclinical rejection more effectively than CsA (79) and has been associated with reduced rates of AGR with improved allograft survival (84); in addition, Tacrolimus may be prescribed as part of a therapeutic regimen to re-establish operational tolerance in cases of rejection occurring in patients receiving CsA (38). Furthermore, it has been suggested that Tacrolimus is less nephrotoxic than CsA which may contribute to improved allograft survival with this drug (85).

- Anti-proliferative agents (Mycophenolate Mofetil (MMF) / Azathioprine) – inhibit purine synthesis and therefore selectively inhibit proliferation of T and B lymphocytes, resulting in dampened proliferation of cytotoxic and helper T lymphocytes, and reduced antibody formation (38,83,86).
    - MMF has been reported to delay the onset and reduce the risk of AGR in comparison to Azathioprine; in recipients treated with MMF, episodes of AGR appear to be more responsive to therapeutic intervention (86). MMF has also been reported to increase allograft survival in some studies (79).

- Mammalian target of Rapamycin inhibitors (mTORi, Sirolimus and Everolimus), bind to FK binding protein to inhibit the mTOR complex to arrest the cell division cycle, preventing lymphocyte proliferation (38,83).
    - Rapamycin has been associated with an increased risk of nephrotoxicity in combination with calcineurin inhibitors and is not used in the early transplant phase due to inhibitory effects on wound healing (79).
    - Metanalysis has not shown benefit for Rapamycin in terms of graft or recipient survival over the calcineurin inhibitors or the anti-proliferative agents (79).

Dose and choice of immunosuppressant components of the triple drug regimen may be adjusted based on individual recipient immunological risk and side-effect profile. Use of a triple drug protocol may reduce the risk of AGR; one-year graft survival on this regimen has been reported to be greater than 85% (38).

2.4.9.3 Treatment of acute rejection

Allograft rejection can be conceptualised as a loss of operational tolerance; treatment of AGR therefore involves achieving rapid control of the alloresponse using a short course of intensive immunosuppression superimposed on maintenance therapy augmented to re-establish tolerance to the graft (79,87).

- Acute cellular rejection is treated using either high dose methylprednisolone or lymphocyte depleting therapies as the intensive immunosuppression phase.
    - Pulse intravenous methylprednisolone is recommended as first-line therapy, however the dose and duration has not been well defined (38,79).
    - Lymphocyte depleting therapies (ATG or ALG or OKT3) may be used in steroid-resistant rejection or recurrent rejection (38,79,87).
    - No evidence exists to suggest an advantage for either protocol, and most studies assessing their efficacy were undertaken in historical cohorts under older immunosuppression regimes (87); the impact of these protocols on allograft

survival after the diagnosis of AGR in the context of modern immunosuppression is not well elucidated.

- Augmented maintenance therapy after intensive phase treatment consists of optimised MMF and Tacrolimus with low dose oral prednisolone (79). This has been shown to reduce incidence of subsequent rejection episodes(79).

- KDIGO guidelines recommend treatment of both subclinical and borderline rejection episodes (79). Treating subclinical rejection has been shown to be of benefit in allograft survival (36,79); however, there is a lack of evidence for benefit of treatment in borderline rejection (79).

- Intensive phase therapy in the setting of antibody-mediated rejection involves the rapid depletion of alloantibody using plasmapheresis assisted by the anti-idiotype / immunomodulatory effect of intravenous immunoglobulin (IVIG) (79,88); B-lineage cell depletion therapies (Rituximab, a monoclonal antibody to CD20 which depletes immature B cells, and Bortezomib, a proteasome inhibitor which depletes plasma cells) have been theorised to facilitate long-term alloantibody suppression by decreasing the cells responsible for antibody production; and T-cell depletion therapies (ATG) have been suggested to be of benefit by reducing T-cell elucidated cytokines which facilitate B cell maturation and alloantibody production (79).
  - Currently, there is no consensus on the management of AHR (79,88,89). In addition, recent studies have failed to demonstrate the efficacy of Rituximab and Bortezomib therapy in this setting (89).
  - There is similarly a paucity of data on the optimal maintenance therapy augmentation in AHR, although Tacrolimus and MMF may be of benefit based on small series (90).

2.5 Justification for study

This study was undertaken to better understand and characterise acute rejection in renal allografts in the local setting. The primary objective of this study was to determine the incidence of confirmed AGR in all renal transplantations undertaken during the period 01/01/2003 to 31/12/2012.

Secondary objectives were:

- To describe the histological patterns of injury as outlined in the Banff classification, with specific reference to rejection subtypes.
- To determine the contribution of various risk factors to the development of AGR, including:
    - Demographic factors (age, sex and race)
    - Type of donor (related living versus cadaveric)
    - Degree of HLA matching
    - Delayed graft function
    - Cold ischaemic time
    - Primary aetiology of CKD
- To describe the management of AGR in the local setting and assess the response to therapy.
- To determine the five-year outcome of patients diagnosed with AGR in relation to the following factors:
    - Type of rejection
    - Histological features as outlined in the Banff classification
    - Timing of acute rejection episode
    - Number of episodes of acute rejection
    - Degree of recovery

3 Methods

3.1 Study Population

This study was conducted at the renal transplant unit at CMJAH, Johannesburg, Gauteng, South Africa. This unit provides transplant services to the greater Johannesburg area as well as to neighbouring provinces including North-west province, Mpumalanga and Limpopo. The population served is ethnically diverse, including patients of African, Asian, Indian, Caucasian and Coloured descent. The study population included all adult recipients of a renal allograft during the period 01/01/2003 to 31/12/2012 in whom biopsy confirmed a diagnosis of AGR. This included screening protocol biopsies carried out routinely at 3 months and 1 year after transplantation. A total of 130 biopsy-proven episodes of AGR occurring in 85 patients were identified out of a total of 509 biopsies undertaken in this cohort. Among the 85 patients experiencing AGR, 34 had 2 episodes, 10 had 3 episodes, and one patient had 4 episodes of AGR.

3.1.1 Data collection

Data collection commenced after receipt of ethical approval from the Human Research Ethics Committee of the Faculty of Health Sciences, University of the Witwatersrand (certificate number M140667). Allograft recipients were identified by review of the transplant register maintained by the renal transplant unit for the period 01/01/2003 – 31/12/2012, with a total of 263 recipients identified. Review of the National Health Laboratory Service (NHLS) electronic database for this cohort identified a total of 509 biopsies undertaken on these recipients. Biopsy reports were retrospectively reviewed to identify cases of AGR; 105 patients experienced at least one episode of biopsy-confirmed AGR. Of these 105 recipients, 20 were excluded from this study. These 20 recipients included 6 repeat transplants and 7 patients who did not have continuous follow-up after engraftment; an additional 7 patients were excluded due to inadequate clinical records. A total of 85 first-time renal allograft recipients with at least one episode of AGR and adequate follow-up at CMJAH were therefore included in this study; 130 episodes of AGR were identified in these 85 recipients. Relevant data was then extracted from the NHLS electronic database and patient clinical files and electronically captured in an Excel database; patient anonymity was maintained in this study database

through recording data using a study number. The process of data collection is summarised below in Figure 3.1.1.

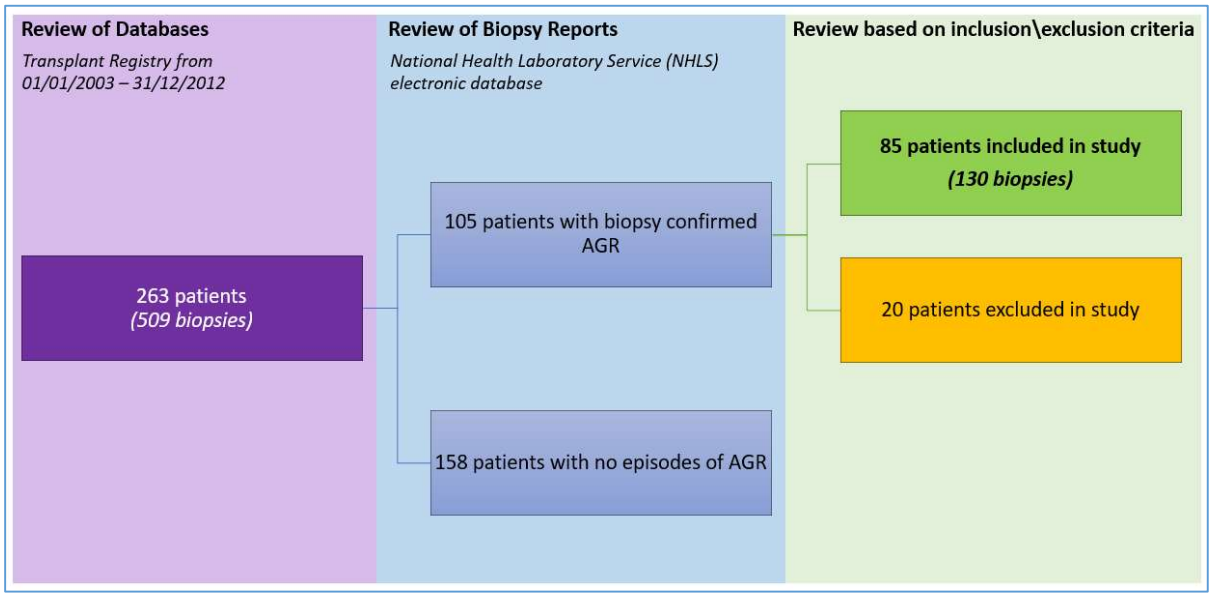


Figure 3.1.1 Data collection process

3.1.2 Inclusion criteria

Patients were considered for inclusion in this study if the following criteria were met:

- Being the recipient of a first renal transplant during the period 01/01/2003 – 31/12/2012
- Biopsy-confirmed AGR occurring during the life-span of the first renal transplant
- Continuous follow-up at CMJAH from the time of transplantation until the point of data collection (2014)

Both protocol biopsies and indicated biopsies were included in this study in order to facilitate inclusion of subclinical rejection.

3.1.3 Exclusion criteria

Patients were excluded from the study if:

- Retrospective review indicated missing patient files, incompleteness of data or the patient having been lost to follow up

3.1.4 Statistical analysis

Data was captured in Microsoft Excel and exported into SPSS (Statistical Product and Service Solutions) software for analysis.

Distribution of data was analysed using the Shapiro Wilk W test and through visual inspection of the histogram plot. Central tendency and data spread were described using means and standard deviations respectively for normally distributed data; medians and interquartile ranges respectively were used for non-parametric variables. Pie charts, bar graphs, and box and whisker plots were used to present a pictorial view of frequencies and distribution of samples.

The Chi-square test was used to assess categorical variables. One-way analysis of variance (ANOVA) was undertaken for analysis of continuous variables across multiple categories; the independent sample Student t-test and Mann Whitney U test were used to analyse continuous variables across binary categories as appropriate. The paired Wilcoxan rank sum test was used to assess dependent samples. The Kaplan–Meier estimator was used to analyse graft survival; Spearman's rank correlation coefficient was used to assess the relationship between two variables. A p value of 0.05 was considered statistically significant.

4 Results

4.1 Incidence of biopsy confirmed AGR amongst renal transplant recipients

Of the 263 patients who received first renal transplants in the study period, 105 had biopsy-confirmed acute rejection (Table 4.1.1). The overall incidence of AGR during the course of this series at CMJAH, amongst index transplantations, was 38.5%; after exclusion of patients with poor follow up and inadequate records, the incidence of AGR in this study was 34.9%.

Table 4.1.1 depicts the number of engraftments completed each year, along with the total number of biopsies and confirmed AGR episodes observed over the study period according to the year of engraftment.

Table 4.1. 1 Annual distribution of the number of transplants, biopsies, and episodes of AGR

Year of transplantation	Total number of transplants	Total number of biopsies performed	Total number of patients with biopsy confirmed AGR	Total number of patients with biopsy confirmed AGR included in this study
2003	38	93	25	19
2004	30	74	15	11
2005	29	61	13	8
2006	36	62	14	13
2007	19	48	11	10
2008	21	26	2	2
2009	22	55	11	9
2010	26	36	7	7
2011	23	27	4	3
2012	19	27	3	3
Total	263	509	105	85

Table 4.1.2 and figure 4.1.1 depicts the number of AGR episodes confirmed in each year reviewed, irrespective of the year of engraftment.

Table 4.1. 2 Annual rate of AGR diagnosis in study cohort

Year	Number of biopsy-confirmed AGR episodes in the study cohort (n=85)	Annual Percentage contribution of AGR episodes in the study cohort
2003	5	3,8%
2004	17	13,1%
2005	18	13,8%
2006	20	15,4%
2007	22	16,9%
2008	5	3,8%
2009	7	5,4%
2010	14	10,8%
2011	10	7,7%
2012	6	4,6%
2013	4	3,1%
2014	2	1,5%
Total	130	100,0%

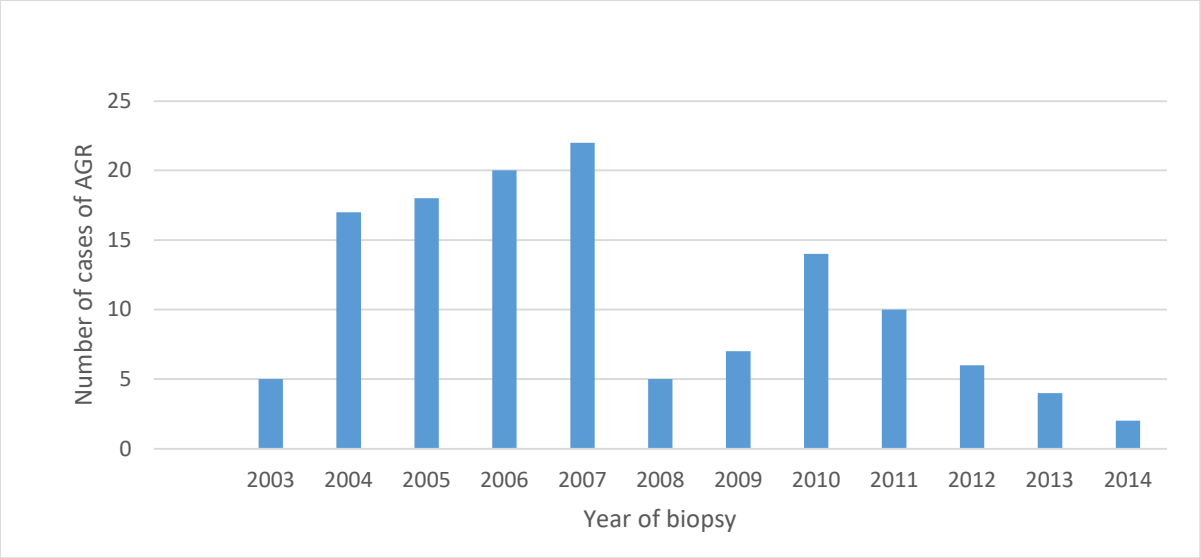


Figure 4.1. 1 Annual frequency of AGR episodes included in the study (n =130 AGR episodes)

Since the above analysis appeared to suggest that the number of recipients diagnosed with AGR declined during the course of the study period, further interrogation of the cohort was undertaken in order to determine whether this apparent trend might be explained by a decline in the annual number of transplants and biopsies performed during the study period. Figure 4.1.2 below shows the declining trends in the number of recipients with AGR and the number of transplants and biopsies undertaken on a year-by-year basis.

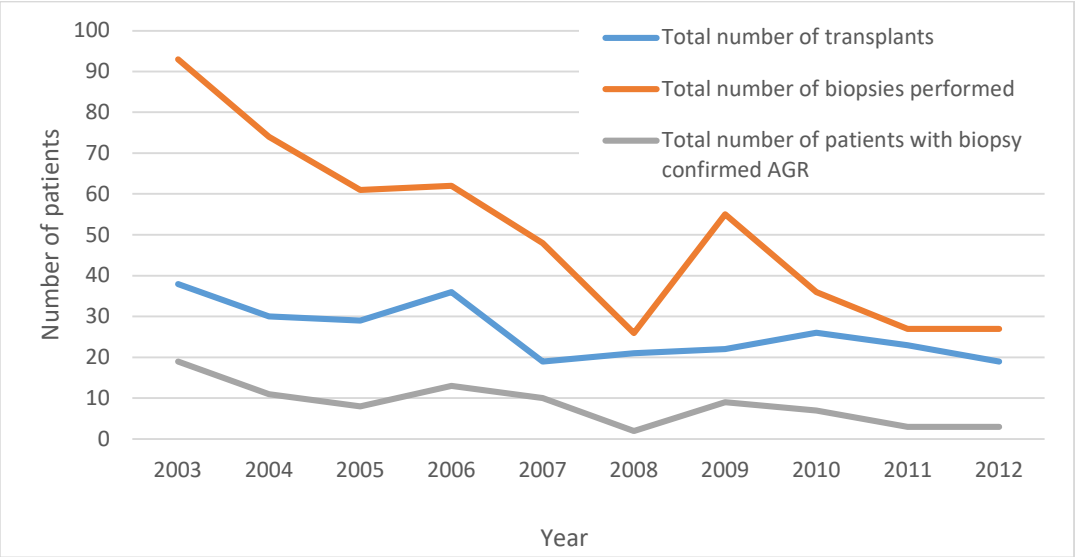


Figure 4.1. 2 Line graph depicting the trends of the number of transplants, the number of biopsies performed, and the number of patients diagnosed with AGR (2003-2012)

Spearman rank order correlation testing between the number of transplants and the number of patients with confirmed AGR showed a statistically significant association, R = 0.7, p = 0.0327 (the scatterplot of this analysis is shown in Figure 4.1.3 below).

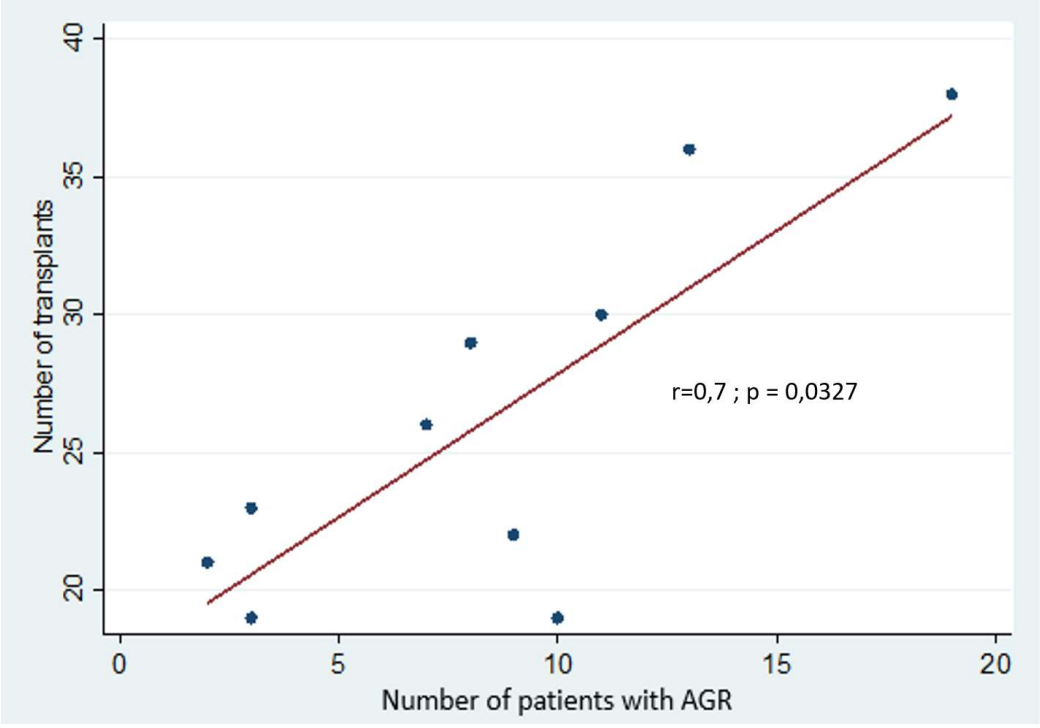


Figure 4.1. 3 Spearman's rank correlation between the number of transplants and the number of patients diagnosed with AGR (2003-2012)

A similar spearman rank order correlation between the number of biopsies performed and the number of patients with confirmed AGR showed a statistically significant association, R = 0.9, p < 0.001 (the scatterplot of this analysis is shown in Figure 4.1.4 below).

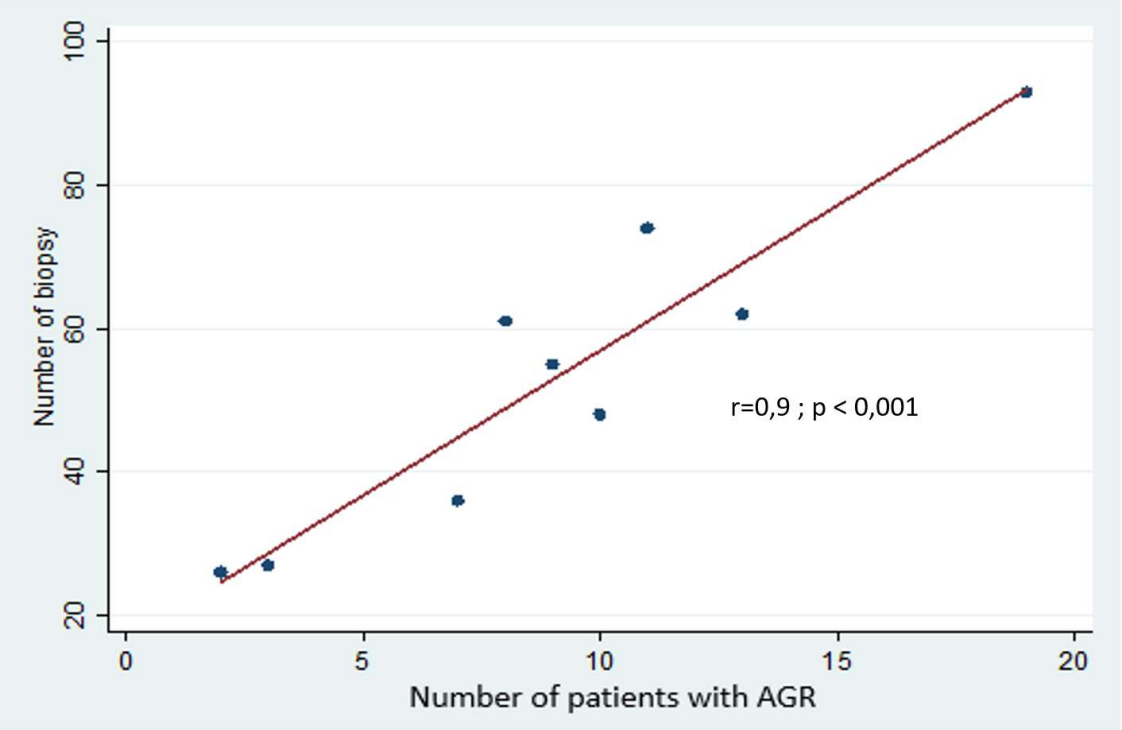


Figure 4.1. 4 Spearman's rank correlation between the number of biopsies performed and the number of patients diagnosed with AGR (2003-2012)

To further describe the correlations observed above, between the two independent variables (number of transplants and number of biopsies performed) and the number of confirmed AGR, a linear regression analysis was performed as depicted in Table 4.1.3. This model confirmed that both independent variables can explain the variation in the number of patients with confirmed AGR to a significant degree (R^2=0.89, F(2,7)=27.45, p<0.001). However, when analysing the correlation of each independent variable with the number of patients with confirmed AGR in this regression model, the number of biopsies performed was observed to be of statistical significance (R coefficient = 0.2, p<0.01), while the number of transplants did not reach statistical significance.

Table 4.1. 3 Linear regression model testing the association of the number of transplants and the number of biopsies on the number of AGR episodes

Number of obs.	10				
F(2, 7)	27,45				
p value	0,0005				
R-squared	0,8869				
Adjusted R-squared	0,8546				
Number of AGR	**Coefficient**	**Std. of Error**	**p value**	**[95% Confidence interval]**	
Number of transplants	0,09	0,16	0,6	-0,3	0,48
Number of biopsies	0,2	0,05	0,006	0,08	0,31
Constant	-3,86	2,8	0,21	-10,49	2,76

This study included 130 episodes of biopsy-proven AGR which were diagnosed in 85 recipients, comprising of 85 first episodes, 34 second episodes, 10 third episodes and 1 fourth episode of AGR. Table 4.1.4 illustrates the distribution of recipients by number of episodes of AGR. Another description would be 40% (34/85) of recipients were exposed to recurrent AGR episodes, while the greater proportion of recipients, 60% (51/85), had a single AGR episode.

Table 4.1. 4 Distribution of AGR episodes in the sample population

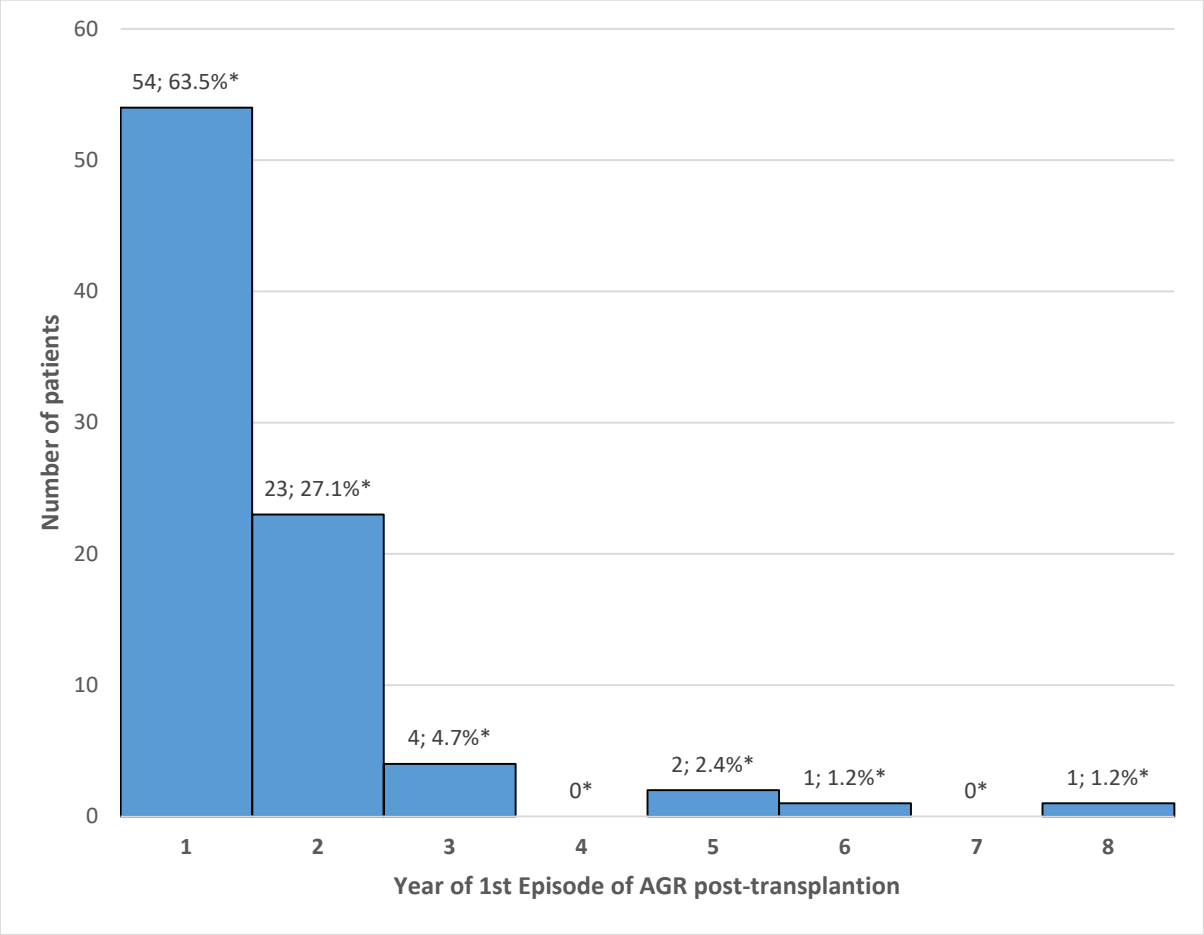
Episode of AGR	Number of patients	Percentage of patients (n = 85)	Percentage of total number of episodes (n = 130)
1st episode	85	100	65.4
2nd episode	34	40,00	26.2
3rd episode	10	11,76	7.7
4th episode	1	1,18	0.8

The timing of all AGR episodes included in this study is depicted in Table 4.1.5; 53.8% occurred within the first year after engraftment, 30.8% in the second year and 15.4% occurred in grafts older than 2 years.

Table 4.1. 5 Timing episodes of AGR (n=130)

Timing of AGR	number of AGR	Percentage of AGR
within 1 month	10	7,7%
within 6 months	48	36,9%
within 12 months	70	53,8%
12-24 months	40	30,8%
>24 months	20	15,4%

The timing of index AGR episodes (n=85) is depicted in Figure 4.1.5 below. A total of 63.5% of recipients were diagnosed with index AGR within the first year of transplantation; 27.1% were diagnosed in the second-year post transplantation; only 9.4% of first episode of AGR was diagnosed in grafts older than 2 years. Forty four of the 54 patients diagnosed with AGR in the first year were diagnosed within the first 6 months. The longest AGR free period was 8 years.



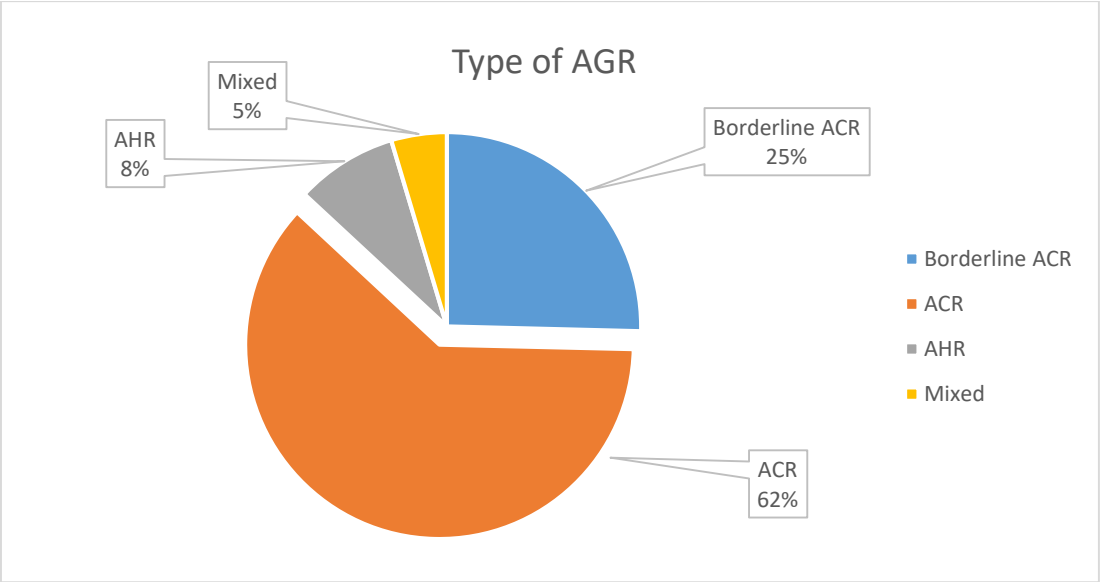
*N; % - number; percentage

Figure 4.1. 5 Histogram plot of the timing of index episodes of AGR (n=85 patients)

The majority (59.2%) of AGR episodes were diagnosed on clinically indicated biopsy, 37.7% of cases were diagnosed on protocol biopsy; AGR was diagnosed in nephrectomy specimens in 3.1% of cases. Thus, 62.3% of AGR episodes in this study comprised clinical AGR with 37.7 % of episodes being subclinical.

4.2 The incidence of ACR and AHR, and histological patterns according to the Banff classification

The most common type of AGR was ACR, constituting 61.5% (80 episodes) of all cases. This was followed by Borderline ACR (25.4%), AHR (8.5%) and mixed rejection (4.6%) (Figure 4.2.1). The AHR group included two episodes of hyperacute rejection.



AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Figure 4.2. 1 Graph describing different proportions of the type of AGR amongst all the episodes

The distribution of the type of AGR amongst the repeated episodes is presented in Table 4.2.1 below.

Table 4.2. 1 The distribution of the type of AGR by episode of biopsy

		Episode of AGR (n; %)*				Total (n; %)*
		1st Episode	2nd Episode	3rd Episode	4th Episode	
Type of AGR	ACR	49; 57.6%	23; 67.6%	7; 70.0%	1; 100.0%	80; 61.5%
	Borderline ACR	27; 31.8%	5; 14.7%	1; 10%	0; 0%	33; 25.4%
	AHR	8; 9.4%	3; 8.8%	0; 0%	0; 0%	11; 8.5%
	mixed	1; 1.2%	3; 8.8%	2; 20.0%	0; 0%	6; 4.6%
Total		85	34	10	1	130
Chi-Square						14.53
p-value						0.105

*n; % - number; percentage

AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Acute cellular rejection was the commonest form of histology observed in all repeat AGR episode groups; however, no significant difference was observed in the types of AGR between these groups (p = 0.105).

Analysis of the Banff grade of ACR is shown in Table 4.2.2. The most commonly reported ACR grade was 1A (45.5% of all episodes), followed by 1B (37.7%) and 2A with 11.7%; grades 2B and 3 were reported in 2.6% of episodes each. The Banff grade was not reported in 3 episodes of ACR. The most common ACR Banff grade amongst first episodes of rejection was 1A; in second episode cases, the most common Banff grade was 1B. However, no statistically significant difference was found in the frequency of ACR Banff grades by repeated episodes of rejection (p = 0.720).

Table 4.2. 2 Banff ACR grade and number of episodes of AGR

		Episode of AGR (n; %)*				Total (n; %)*
		1st Episode	2nd Episode	3rd Episode	4th Episode	
ACR BANFF classification	1A	22; 46.8%	8; 36.4%	4; 57.1%	1; 100.0%	35; 45.5%
	1B	17; 36.2%	10; 45.5%	2; 28.6%	0; 0%	29; 37.7%
	2A	6; 12.8%	2; 9.1%	1; 14.3%	0; 0%	9; 11.7%
	2B	2; 4.3	0; 0%	0; 0%	0; 0%	2; 2.6%
	3	0; 0%	2; 9.1%	0; 0%	0; 0%	2; 2.6%
Total		47	22	7	1	77
Chi-Square						8.80
p-value						0.72

*n; % - number; percentage

Among the 9 documented episodes of AHR, 55.6% were C4d positive, with the remaining 44.4% of cases having been diagnosed before the availability of C4d immunofluorescence staining at CMJAH.

The distribution of AGR type between biopsy categories (clinically indicated and protocol biopsies, and nephrectomy specimen) was analysed and is shown in Table 4.2.3.

Table 4.2. 3 Type of AGR by biopsy type

		Type of Biopsy (n; %)*			Total (n; %)*
		Protocol	Indicated	Nephrectomy	
Type of AGR	ACR	31; 63.3%	49; 63.6%	0; 0%	80; 61.5%
	Borderline ACR	18; 36.7%	15; 19.5%	0; 0%	33; 25.4%
	AHR	0; 0%	9; 11.7%	2; 50.0%	11; 8.5%
	Mixed	0; 0%	4; 5.2%	2; 50.0%	6; 4.6%
Total		49	77	4	130
Chi-Square					**40.52**
p-value					**<0.001**

*n; % - number; percentage
AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

In this series, protocol biopsy is considered analogous to subclinical rejection. ACR was the most common type of AGR observed amongst the 49 protocol biopsies included in this study (63.3%). Borderline ACR was also reported in this group (36.7%); no cases of AHR or mixed rejection were reported by the histopathologist. Amongst the 77 indicated biopsies (clinical AGR), 63.6% were reported as being indicative of ACR, 19.5% as Borderline ACR, 10.4% as AHR and 5.2% as mixed rejection. The four nephrectomy specimens demonstrated either AHR (50%) or mixed (50%), with no ACR or Borderline ACR being reported by the histopathologist. The difference in frequency of AGR category by allograft specimen type was statistically significant ($p < 0.001$).

Since ACR was the dominant type of AGR in the study cohort, the frequency of the various ACR Banff grades were compared between the clinical (indicated biopsy) and subclinical (protocol biopsy) rejection groups (Table 4.2.4).

Table 4.2. 4 ACR grade episodes by rejection type

		Type of Biopsy (n; %)*		Total (n; %)*
		Clinical	Subclinical	
ACR BANFF classification	1A	19; 40.4%	16; 53.3%	35; 45.5%
	1B	20; 42.6%	9; 30.0%	29; 37.7%
	2A	5; 10.6%	4; 13.3%	9; 11.7%
	2B	1; 2.1%	1; 3.3%	2; 2.6%
	3	2; 4.3%	0; 0%	2; 2.6%
Total		47	30	77
Chi-Square				**2.93**
p-value				**0.57**

*n; % - number; percentage

A non-significant trend toward a higher frequency of milder ACR grade in subclinical rejection (53.3% of biopsies in this group being grade 1A) was observed; all cases of grade 3 ACR occurred in the clinical rejection group.

4.3 Potential factors in the development of AGR

Factors reported to play a role in the development of AGR were observed with the aim of describing them in this study population. The distribution of these factors were analysed amongst the different types of AGR episodes, as well as that amongst patients exposed to a single AGR episode and patients with recurrent episodes.

4.3.1 Demographics

The mean age of the cohort was 45.4 years ± 11.8 years (range 21 - 69 years). The age distribution is summarised in Figure 4.3.1.1 below.

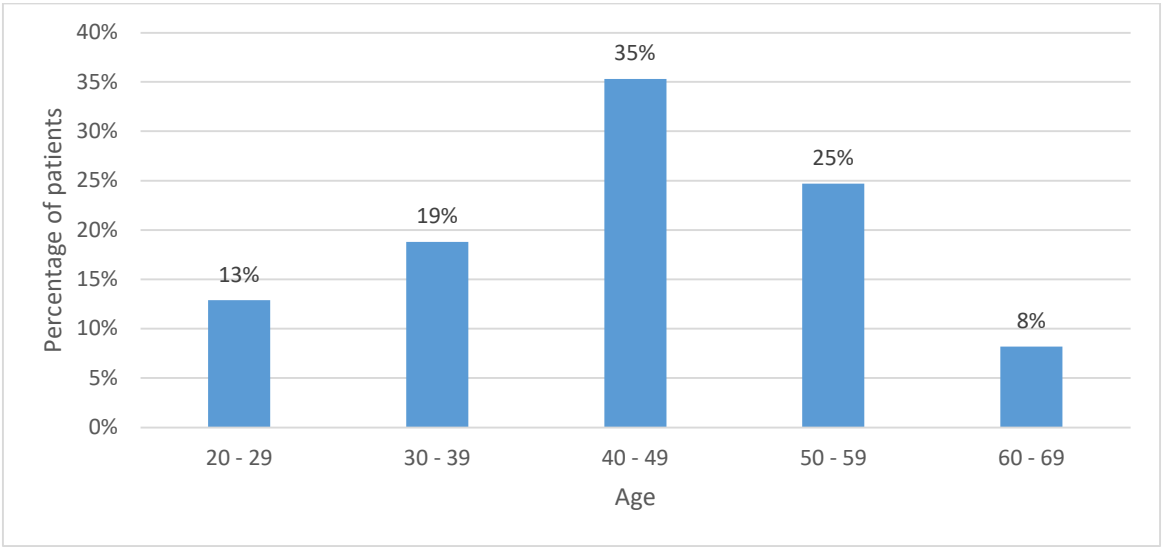
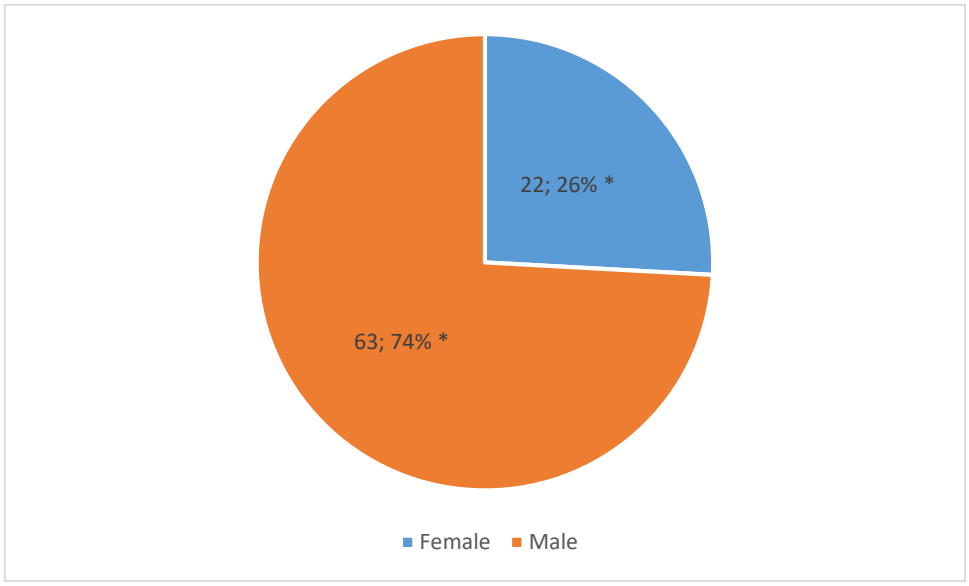


Figure 4.3.1. 1 Graph depicting the age distribution amongst the sample population

A male dominance was observed in this population, with males contributing to 74.1% of the cohort and females contributing to 25.9% as depicted in Figure 4.3.1.2.



*n; % - number; percentage

Figure 4.3.1. 2 The gender distribution of the sample population

No significant difference in gender ratios was observed between AGR histological type or the frequency AGR episode groups (Tables 4.3.1.1 and 4.3.1.2 respectively)

Table 4.3.1. 1 Proportion of type of AGR among males and females

	Type of AGR (n; %)*				Total (n; %)*
	ACR	Borderline ACR	AHR	Mixed	
Males	59; 73.8%	22; 66.7%	10; 90.9%	6; 100%	97; 74.6%
Females	21; 26.3%	11; 33.3%	1; 9.1%	0; 0%	33; 25.4%
Total	80	33	11	6	130
Chi-Square					**4.72**
p-value					**0.19**

*n; % - number; percentage
AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Table 4.3.1. 2 Frequency of repeated AGR episodes amongst males and females

	Episode of AGR (n; %)*		Total (n; %)*
	Single episode	Recurrent episodes	
Males	38; 74.5%	25; 73.5%	63; 74.1%
Females	13; 25.5%	9; 26.5%	22; 25.9%
Total	51	34	85
Chi-Square			**0.0102**
p value			**0.919**

*n; % - number; percentage

It is probable that the lack of significant difference in gender ratios in this analysis is in part due to sampling bias arising from the preponderance of male recipients in this cohort.

The cohort comprised 77.6% African, 10.6% Caucasian, 8.2% Asian, and 3.5% Coloured recipients (Table 4.3.1.3). The percentage of rejection episodes considered by ethnic group was similar to the percentage of total recipients in each ethnic group, with no statistical significance (p=0.978), indicating a lack of clustering of rejection within any particular ethnic group.

Table 4.3.1. 3 Ethnic distribution of patients and episodes of rejection

	Number of patients	Percentage of total patients (n=85)	Number of episodes of rejection	Percentage of total episodes of rejection (n=130)
African	66	77.6	102	78.5
Caucasian	9	10.6	15	11.5
Asian	7	8.2	9	6.9
Coloured	3	3.5	4	3.1
Total	85	100.0	130	100.0
Chi-Square				**0.197**
p value				**0.978**

Analysis of the effect of ethnicity of recipients on type of AGR and recurrence of AGR is depicted in Table 4.3.1.4 and Table 4.3.1.5 respectively below

Table 4.3.1. 4 Proportion of type of AGR amongst different ethnic groups

	Type of AGR (n; %)*				Total (n; %)*
	ACR	Borderline ACR	AHR	Mixed	
African	59; 73.8%	29; 89.9%	8; 72.7%	6; 100%	102; 78.5%
Caucasian	12; 15%	2; 6%	1; 9%	0; 0%	15; 11.5%
Asian	7; 8.8%	1; 3%	1; 9%	0; 0%	9; 6.9%
Coloured	2; 2.5%	1; 3%	1; 9%	0; 0%	4; 3.1%
Total	80	33	11	6	130
Chi-Square					**6.601**
p value					**0.679**

*n; % - number; percentage
AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Table 4.3.1. 5 Frequency of repeated AGR episodes amongst different ethnic groups

	Episode of AGR (n; %)*		Total (n; %)*
	Single episode	Recurrent episodes	
African	40; 78.4%	26; 76.5%	66; 77.6%
Caucasian	4; 7.8%	5; 14.7%	9; 10.6%
Asian	5; 9.8%	2; 5.9%	7; 8.2%
Coloured	2; 3.9%	1; 2.9%	3; 3.5%
Total	51	34	85
Chi-Square			**1.3540**
p value			**0.716**

*n; % - number; percentage;
AGR-Acute graft rejection

In both analyses, recipients of African origin comprised the dominant ethnic group, reflecting the ethnic distribution of the cohort as a whole. ACR was the most dominant type of AGR in all ethnic groups. No significant difference in histological type of AGR or recurrence of AGR by ethnic group was observed in either analysis.

4.3.2 Type of donor

The distribution of donor types in this series is shown in Table 4.3.2. 1, which includes cadaveric donor (CD) graft, related living donor (RLD) grafts and non-related living donor (NRLD) grafts.

Table 4.3.2. 1 Donor types of the study cohort

	Number	Percent
CD	66	77.65
NRLD	3	3.53
RLD	16	18.82
Total	85	100.0

CD – Cadaveric donor; RLD – Related living donor; NRLD - Nonrelated living donor

An analysis of the distribution of AGR type and recurrence of AGR episodes in different donor type categories is depicted in Table 4.3.2.2 and Table 4.3.2.3.

Table 4.3.2. 2 Proportion of type of AGR amongst different donor types

	Type of AGR (n; %)*			Total
	CD	RLD	NRLD	(n; %)*
ACR	61; 61%	15; 60%	4; 80%	80; 61,5%
Borderline ACR	26; 26%	7; 28%	0; 0%	33; 25,4%
AHR	8; 8%	3; 12%	0; 0%	11; 8,5%
Mixed	5; 5%	0; 0%	1; 20%	6; 4,6%
Total	100	25	5	130
Chi-Square				**6.211**
p value				**0.400**

*n; % - number; percentage

AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection; CD – Cadaveric donor; RLD – Related living donor; NRLD- Nonrelated living donor

Table 4.3.2. 3 Proportion of different donor types amongst recipients exposed to a single and recurrent AGR episode.

	Episode of AGR (n; %)*		Total (n; %)*
	Single episode	Recurrent episodes	
CD	41; 80.4%	25; 73.5%	66; 77.6%
RLD	9; 17.6%	7; 20.6%	16; 18.8%
NRLD	1; 2%	2; 5.9%	3; 3.5%
Total	51	34	85
Chi-Square			**1.1064**
p value			**0.575**

*n; % - number; percentage
CD – Cadaveric donor; RLD – Related living donor; NRLD- Nonrelated living donor

The type of AGR diagnosed revealed similar frequencies in CD and RLD grafts. ACR contributed 61% and 60% of AGR episodes in CD and RLD engraftments respectively. Borderline ACR was the second commonest rejection type (CD 26%; RLD 28%), followed by AHR (CD 8%; RLD 12%). The proportion of different donor types amongst recipients with a single AGR episode and recurrent AGR were similar; these proportions were in keeping with the distribution of donor type within the whole study population. Both analyses were not observed to be of statistical significance

4.3.3 Degree of HLA matching

The degree of HLA matching between donor and recipient is known to influence AGR risk; however, advances in modern immunosuppression protocols have facilitated increased use of HLA mismatched allografts as a means to overcome the paucity of living donors and the diversity of HLA types in African recipient populations.

HLA matching data was obtainable in 71 recipients included in this series (83.5%). During the course of this series, advances in HLA typing techniques and appreciation of immunological risk resulted in an increase in the number of HLA antigens assayed. For purposes of comparison, the percentage of HLA matching between recipient and donor was used for analysis.

Table 4.3.3. 1 describes the degree of HLA matching in recipient-donor pairs included in this study. The majority (78.8%) of patients diagnosed with AGR in this study had less than 40% of HLA antigens in common with their donor. All 9 patients with HLA matching greater than 60% were RLD recipients. 41.1% of recipients with HLA matching of less than 40% experienced at least one episode of AGR recurrence.

Table 4.3.3. 1 HLA matching amongst 71 recipients with Acute graft rejection (AGR)

Percentage HLA matching (%)	Number of recipients	Percentage	Number of recipients with recurrent AGR	Percentage of recipients with recurrent AGR (%)
0.0 - 20	27	38.0%	11	40.7
20.1 - 40	29	40.8%	12	41.4
40.1 - 60	6	8.5%	1	16.7
60.1 - 80	4	5.6%	1	25
80.1 - 100	5	7.0%	3	60

4.3.4 Cold ischaemia time (CIT) and delayed graft function (DGF)

Data on CIT was documented in only 15 patient files. Acute graft rejection diagnosed in these 15 patients comprised of 13 cases of ACR, 1 case of Borderline ACR and 1 case of AHR. From the data available, 4 recipients had CIT lasting less than 12 hours, while the remaining 11 recipients had CIT between 12 and 24 hours. Due to this small sample size and the degree of ACR predominance among this sample, statistical analysis was not carried out.

DGF in this study was defined as requirement for dialysis within the first seven days of transplantation. Among the 85 engraftments included in the study, 22.4% met the definition of DGF, as depicted in Figure 4.3.4.1.

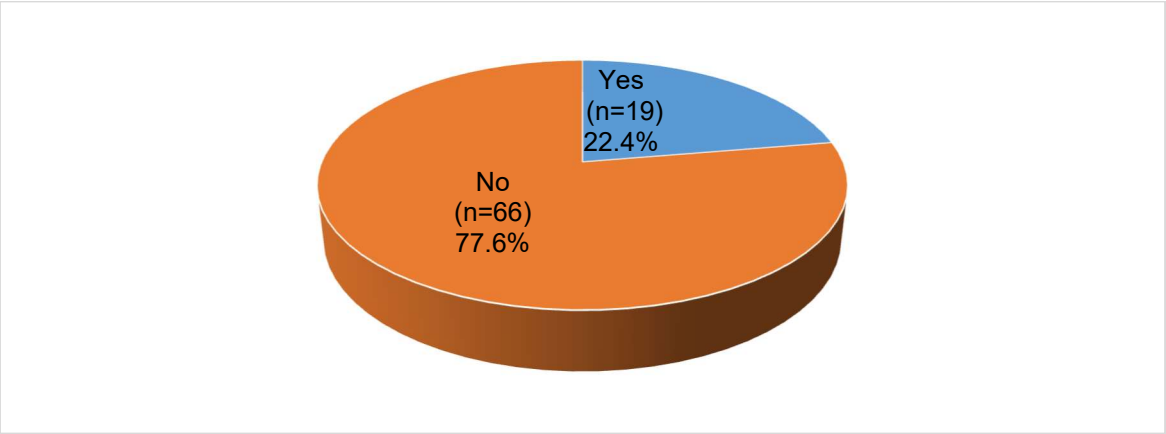


Figure 4.3.4. 1 Proportion of patients with and without delayed graft function

Twenty-seven episodes of AGR were documented amongst the 19 patients with DGF. An analysis of the frequency of DGF in the various histological categories of AGR, as well as in recipients exposed to a single AGR episode as opposed to recurrent AGR episode is depicted in Table 4.3.4.1 and Table 4.3.4.2 below.

Table 4.3.4. 1 Exposure to Delayed graft function (DGF) amongst the various histological categories of AGR.

	Type of AGR (n; %)*				Total (n; %)*
	ACR	Borderline ACR	AHR	Mixed	
DGF	11; 13.8%	9; 27.3%	4; 36.4%	3; 50%	27; 20.8%
No DGF	69; 86.3%	24; 72.7%	7; 63.6%	3; 50%	103; 79.2%
Total	80	33	11	6	130
Chi-Square					**7.985**
p value					**0.046**

*n; % - number; percentage
AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Table 4.3.4. 2 Exposure to Delayed graft function (DGF) amongst recipients confirmed with single and recurrent AGR episodes.

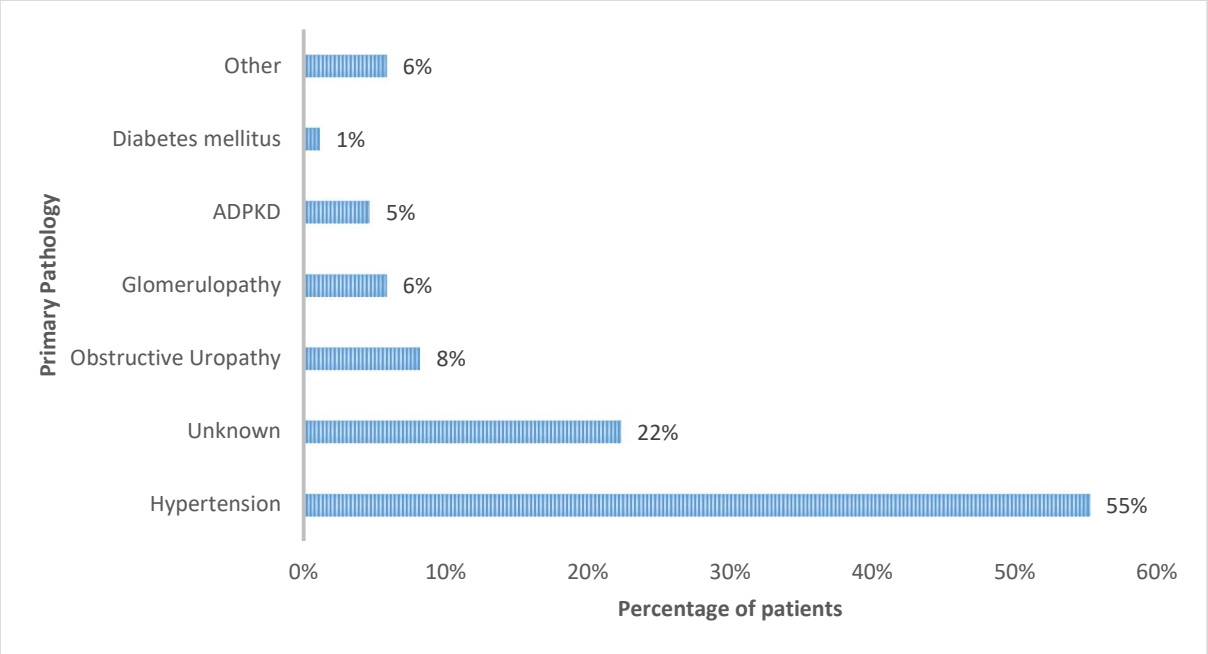
	Episode of AGR (n; %)*		Total (n; %)*
	Single episode	Recurrent episodes	
DGF	14; 27.5%	5; 14.7%	19; 22.4%
No DGF	37; 72.5%	29; 85.3%	66; 77.6%
Total	51	34	85
Chi-Square			**1.9092**
p value			**0.167**

*n; % - number; percentage

The presence of DGF was documented in 22.4% of recipients developing subsequent AGR included in this series; it is possible that the resultant small exposed population affected statistical analysis. Nevertheless, it is noteworthy that analysis demonstrated a statistically significant difference (p = 0.046) in the presence of DGF amongst the various histological categories of AGR, and that AGR types traditionally associated with adverse prognoses (AHR and mixed rejection) were observed to have an increasing frequency in recipients with documented DGF. A lower proportion of recipients with DGF was observed amongst those exposed to recurrent AGR episodes when compared to recipients only exposed to a single AGR episode; however, this was not statistically significant.

4.3.5 Primary aetiology of CKD

Hypertension (55%) was the most common cause of ESKD in this cohort followed by obstructive uropathy (8%), glomerulopathy (6%) and autosomal dominant polycystic kidney disease (5%). The cause of ESKD was unknown in 22% of recipients included in this study. 6% of recipients included in this study were assigned to the category of 'other', consisting of varied diagnosis such as vasculitis, thrombotic microangiopathy, trauma, aplastic kidneys. This data is summarised in Figure 4.3.5.1.



ADPKD – Autosomal dominant polycystic kidney disease

Figure 4.3.5. 1 Primary aetiology for ESRD amongst patients with renal transplantation exposed to AGR.

4.4 The management of ACR/AHR in this study

Maintenance immunosuppressive treatment in this cohort included calcineurin inhibitors such as CsA and Tacrolimus, anti-proliferative agents such as MMF and Azathioprine, Rapamycin and Prednisone. The majority of patients in this study (80%, n=68) were maintained on a regimen consisting of CsA, MMF and Prednisone. A smaller proportion of patients (15.3%, n=13) were on a regimen consisting of Tacrolimus, MMF and Prednisone, while the remaining patients (4.7%, n=4) were on combinations including Rapamycin, Everolimus, or Azathioprine. MMF and prednisone were consistently used at the point of transplantation in 83 of the 85 patients (97.6%) included in this study population. Acute induction management of AGR episodes in this series of patients included Methylprednisolone pulsing, increased maintenance immunosuppression, Plasma exchange with or without IV immunoglobulins (PE/IVIG) and Antithymoglobulin (ATG). The management of the different types of AGR was analysed and is tabulated in Tables 4.4.1, 4.4.2 and 4.4.3 below.

Table 4.4. 1 Management of Acute cellular rejection (ACR)

Management (ACR)	Number	Percentage
Methylprednisolone	46	57,5
ATG	4	5,0
Changes to maintenance therapy	71	88,8

The management of the 80 episodes of ACR diagnosed in this series is described in Table 4.4.1. Methylprednisolone was administered in 57.5% of cases, with changes to maintenance therapy made in 88.8% of cases. ATG was used in 5% of cases, in the setting of steroid-resistant rejection or recurrent rejection. Only 6 cases (7.5%) amongst the ACR episodes required supportive dialysis.

Table 4.4. 2 Management of Acute humoral rejection (AHR)

Management (AHR)	Number	Percentage
Methylprednisolone	0	0,0
PE/IVIG	3	27,3
Rituximab	0	0,0
Change to Maintenance therapy	7	63,6

There were 11 cases of AHR diagnosed and managed during the course of this study (Table 4.4.2). PE/IVIG was administered in 3 cases (27.3%) and change to maintenance therapy was undertaken in 63.6% of cases. Supportive dialysis was required in 6 cases (54.5%) of cases, with 3 cases progressing to graft failure requiring nephrectomy.

Table 4.4. 3 Management of Borderline Acute cellular rejection (ACR)

Management (Borderline ACR)	Number	Percentage
Methylprednisolone	3	9,1
ATG	2	6,1
Change to Maintenance therapy	22	66,7

Borderline ACR contributed significant numbers to the episodes of AGR (n=33) in this series, and Table 4.4.3 describes the management strategies used in these cases. Methylprednisolone was administered in 3 cases (9.1%); ATG was given in 2 cases. Change to maintenance therapy was undertaken in 66.7% of cases. Only 3 cases (9.1%) required supportive dialysis; 1 case presented with graft dysfunction requiring dialysis, while the other two cases deteriorated requiring dialysis.

Adjustments to the baseline maintenance therapy were made in 79.2% of all rejection episodes regardless of histological category. Further breakdown of this group revealed that a third of all the rejections (44 episodes - 33.8%) were managed solely by adjusting the maintenance immunosuppression regimen; this group comprised 28.8% of ACR episodes, 54.5% of Borderline ACR episodes and 27.3% of AHR episodes.

Six patients (7 % of the study population) with AGR developed graft failure requiring nephrectomy. Half of these nephrectomies (3 cases) were performed as a result of first episodes of rejection, all of which were subsequently diagnosed as AHR. The remaining three nephrectomies were undertaken during subsequent episodes of rejection, all of which were histologically diagnosed as mixed AGR.

First episodes of subclinical rejection (n=42) consisted only of cases of histologically diagnosed ACR and Borderline ACR. These episodes were managed with Methylprednisolone pulsing

and/or with changes to maintenance therapy as shown in Table 4.4.4. Methylprednisolone was administered in the majority (63%) of subclinical ACR cases, while being only minimally used in subclinical Borderline ACR cases (7% of such cases). Similar management strategies were deployed in patients with clinical episodes of Borderline ACR and ACR (59% and 9%, respectively). Changes to maintenance therapy were implemented in both subclinical ACR and Borderline ACR groups, in 81% and 67% of cases respectively. However, both were lower than their respective clinical counterparts, 95% and 83% respectively.

Table 4.4. 4 Therapeutic strategies in clinical and subclinical AGR groups

Subclinical episodes of rejection (Protocol biopsies)			
	ACR (n=27)	AHR (n=0)	Borderline ACR (n=15)
Methylprednisolone	63%	-	7%
ATG	0%	-	0%
Change to Maintenance therapy	81%	-	67%

ACR-Acute cellular rejection; AHR-Acute Humoral rejection

4.5 Response to AGR and treatment

In order to compare graft function between rejection types, serum creatinine (Cr) concentration at three time points (immediately preceding AGR-diagnosing biopsy, at highest level within one month of AGR diagnosis, and at three months after AGR diagnosis) was compared between AGR histological categories diagnosed on clinically indicated biopsy. To limit the effect of preceding episodes of AGR on this analysis, comparison was made using data from first AGR episodes only. This data is presented in Table 4.5.1 below.

Table 4.5 1 Comparison of serum creatinine concentration at different time points between AGR types

	Type of AGR	N	Mean ± SD (95% CI)	P (One Way ANOVA)
Cr at biopsy (µmol/L)	ACR	22	127.86 ± 28.34 (115.30 – 140.43)	0.042
	AHR	4	398.75 ± 497.13 (0 – 1189.80)	
	Borderline ACR	12	256.83 ± 270.94 (84.69 – 428.98)	
	Total	38	197.11 ± 225.21 (123.08 – 271.13)	
Highest Cr within 1 month of AGR (µmol/L)	ACR	22	303.41 ± 256.15 (189.84 – 416.98)	0.258
	AHR	4	568.00 ± 435.70 (0 – 1261.29)	
	Borderline ACR	11	348.64 ± 302.43 (145.46 – 551.81)	
	Total	37	345.46 ± 293.40 (247.63 – 443.29)	
Cr at 3 months after diagnosis (µmol/L)	ACR	21	209.57 ± 153.68 (139.62 – 279.53)	0.239
	AHR	5	297.40 ± 303.92 (0 – 674.77)	
	Borderline ACR	11	151.18 ± 33.83 (128.46 – 173.91)	
	Total	37	204.08 ± 160.57 (150.54 – 257.62)	

AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection; Cr-creatinine

Mean serum creatinine in AHR was consistently higher at all three points of measurement; however this sample was very small with wide confidence intervals. A significant difference was noted in Cr at biopsy between categories of AGR; no significant difference was observed for highest Cr within one month of diagnosis or Cr at three months post-AGR.

Multiple comparison ANOVA testing was undertaken to further analyse the statistically significant difference observed for serum creatinine at diagnostic biopsy. Creatinine at biopsy was significantly higher in recipients diagnosed with AHR than in recipients diagnosed with ACR (mean 398.75µmol/L versus 127.86µmol/L, p = 0.024).

Analysis was undertaken for serum creatinine across type of AGR in the subclinical rejection group and is presented in Table 4.5.2 below.

Table 4.5 2 Serum creatinine concentration across AGR type in subclinical rejection

	Type of AGR	N	Mean ± SD (95% CI)	P (One Way ANOVA)
Cr at biopsy (µmol/L)	ACR	27	126.48 ± 48.95 (107.12– 145.84)	0.173
	Borderline ACR	15	107.13 ± 30.01 (90.51 – 123.75)	
	Total	42	119.57 ± 43.76 (105.94– 133.21)	
Highest Cr within 1 month of AGR (µmol/L)	ACR	27	153.30 ± 63.59 (128.14 – 178.45)	0.272
	Borderline ACR	15	133.27 ± 37.24 (112.65– 153.89)	
	Total	42	146.14 ± 55.97 (128.70 – 163.58)	
Cr at 3 months after diagnosis (µmol/L)	ACR	27	128.78 ± 38.49 (113.55 – 144.00)	0.635
	Borderline ACR	15	121.47 ± 60.64 (87.89 – 155.05)	
	Total	42	126.17 ± 46.99 (111.52 – 140.81)	

AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection; Cr-creatinine

Of note, mean creatinine measurements were lower than that seen in clinical episodes of rejection. Although statistically not significant, serum creatinine was higher in cases of subclinical ACR compared to subclinical Borderline ACR at all time points of creatinine measurement.

4.6 Analysis of graft outcomes at five years post AGR

Data from a subpopulation of patients diagnosed with AGR during the period 2003 - 2007 was extracted for five-year outcome analysis of grafts developing rejection. Following exclusion of those recipients lost to follow-up (n = 6) or death from non-graft related causes (n = 2) within 5 years of diagnosis, a total of 47 of a possible 55 recipients were included in this analysis. Patient survival amongst patients that were followed up at CMJAH over 5 years was 95.9%.

4.6.1 Graft survival

The outcome of grafts at 5 years after the first episode of AGR in this subgroup of recipients was determined and is described in Table 4.6.1.1 below.

Table 4.6.1. 1 Five-year graft survival after AGR diagnosis

5 Year outcome	Number	Percent
Surviving graft	29	61.7%
Failed grafts	18	38.3%
Total	47	100.0%

Allograft survival after AGR diagnosis is depicted using the Kaplan-Meier method in figure 4.6.1.1 and Table 4.6.1.2 below. The Kaplan-Meier plot suggests that 25% of graft loss occurred in 2.3 years.

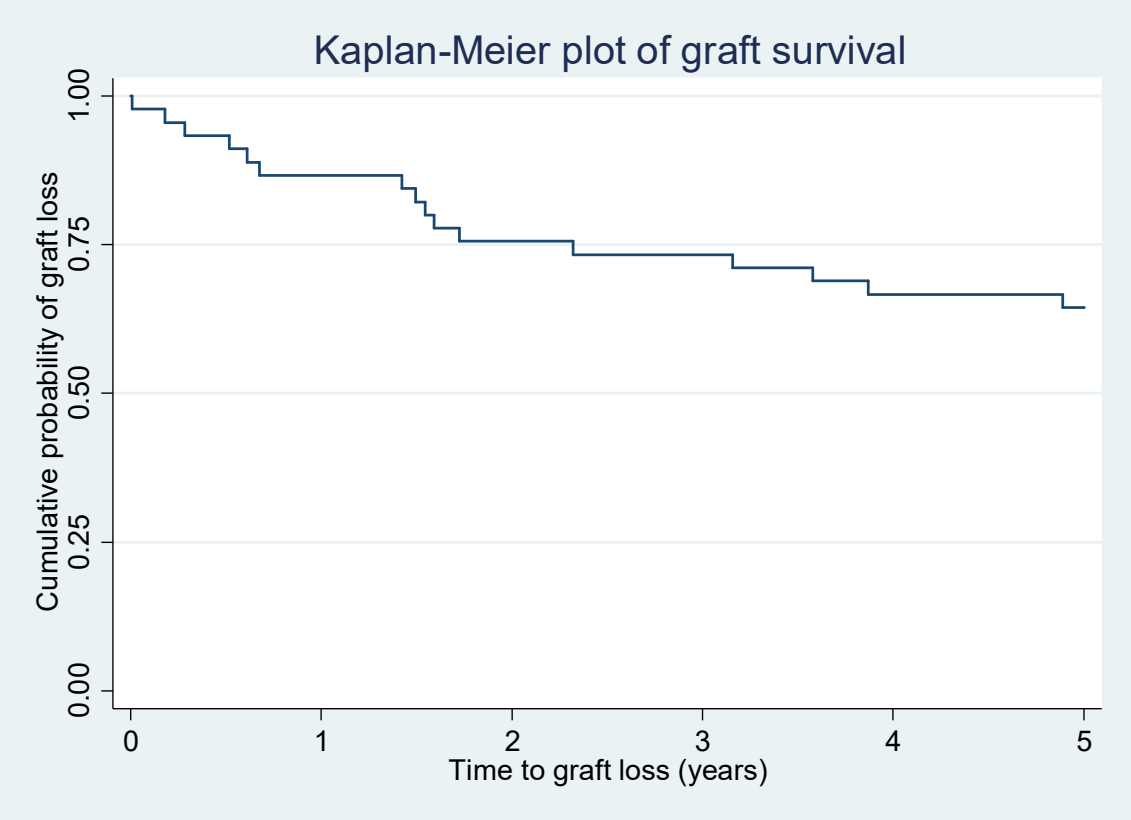


Figure 4.6.1. 1 Five-year graft survival after AGR diagnosis

Table 4.6.1. 2 Annual rate of allograft loss after AGR diagnosis

Year post AGR	Failed grafts	Surviving grafts at year end	Annual percentage failure of grafts
1	8	39	17.0
2	5	34	10.6
3	1	33	2.1
4	3	30	6.4
5	1	29	2.1
Total	**18**		**38.2**

The highest rate of allograft loss was within the first 2 years of diagnosis of AGR. 17% of recipients lost allograft function within 1 year of diagnosis, 11% within 2 years, 2% after 2 years, 6% after 3 years and 2% after 4 years. The mean time period for graft failure from the first episode of AGR amongst graft loss group over the 5-year period was 1.5 years.

4.6.2 Effect of type of AGR on five-year graft survival

Analysis of the type of first episode AGR is presented in Table 4.6.2.1 below.

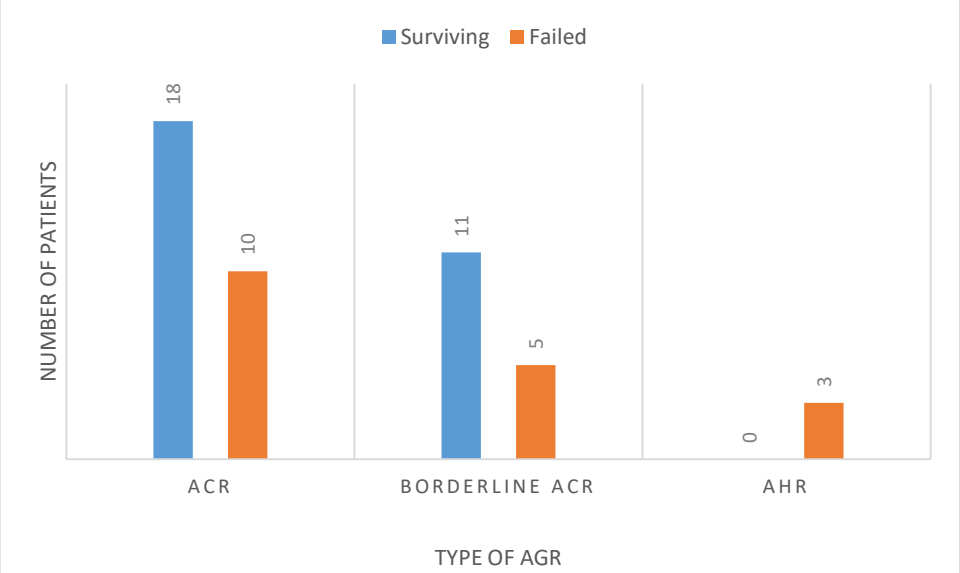
Table 4.6.2. 1 Type of AGR among failed and surviving graft at 5 years after index AGR

		Type of AGR (n/%)*			Total (n/%)*
		ACR	Borderline ACR	AHR	
5-year outcome	Graft survival	18; 64.3%	11; 68.8%	0; 0%	29; 61.7%
	Graft loss	10; 35.7%	5; 31.2%	3; 100%	18; 38.3%
Total		28	16	3	47
Chi-Square					5.249
p value					0.072

*n; % - number; percentage
AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Acute cellular rejection was the most common type of AGR in this analysis (59.6% of all rejection types). Amongst the 28 patients who had an episode of ACR, 10 patients lost their graft (35.7%) and 18 patients had grafts that survived 5 years or more (64.3%). Borderline ACR was the second common type of AGR in this group (16 patients - 34%); 5 patients with Borderline ACR developed graft loss (31.2%) and 11 had surviving grafts at 5 years (68.8%). The distribution of the type of AGR within the two outcome groups revealed that amongst those progressing to graft loss, 55.6% (10/18) were diagnosed at index presentation with ACR, 27.8% (5/18) with Borderline ACR, and 16.7% (3/18) with AHR; while amongst patients whose graft survived 5 years, 62.1% (18/29) were diagnosed with ACR, and 37.9% (11/29) with Borderline ACR; there were no episodes of AHR amongst patients whose graft survived 5 years post AGR. A Chi-square test did not identify any significant difference between the type of

AGR and allograft survival at 5 years (p = 0.072), probably due to small sample size. The results are illustrated in figure 4.6.2.1 below.



AGR-Acute graft rejection; ACR-Acute cellular rejection; AHR-Acute Humoral rejection

Figure 4.6.2. 1 Graft survival amongst the different histological types of index AGR episodes.

Since ACR was the most common histological type of rejection, sub-analysis of the 5-year graft outcome between the various Banff grades of ACR was undertaken (Table 4.6.2.2).

Table 4.6.2. 2 ACR Banff grading and graft survival.

		ACR Banff classification (n/%)*				Total (n/%)*
		1A	1B	2A	2B	
5-year outcome	Graft survival	9; 75%	5; 45.5%	3; 100%	1; 50%	18; 64.3%
	Graft loss	3; 25%	6; 54.5%	0; 0%	1; 50%	10; 35.7%
Total		12	11	3	2	28
Chi-Square						4.143
p value						0.246

*n; % - number; percentage

In this sub-analysis, graft survival was observed in 75% of grafts with Banff 1A ACR and 25% progressed to graft loss; 45.5% with Banff 1B ACR survived and 54.5% progressed to graft loss; all grafts with Banff 2A ACR survived, while the two grafts confirmed with Banff 2B ACR were equally represented in both graft outcome groups. The greater proportion of the surviving

grafts 9/18 (50%) were exposed to a milder grade, 1A ACR, while a greater proportion of failed grafts 6/10 (60%) were diagnosed with grade 2A ACR. Within each ACR Banff grade, the proportion of grafts surviving 5 years or more was better within the milder grades, however a Chi-square test did not reveal any significant difference (p = 0.246).

4.6.3 Analysis of recurrent episodes of AGR

The 47 patients included in this analysis underwent a total of 77 biopsies confirming AGR; 45 biopsies were undertaken in grafts that survived 5 years and 32 biopsies in grafts that were subsequently lost. For purposes of analysis, as higher degrees of repeated AGR episodes (like 3rd and 4th episodes) were of small sample size, survival was assessed between recipients diagnosed with a single episode of AGR and those diagnosed with recurrent episodes as depicted in Table 4.6.3.1 below. The overall occurrence of grafts diagnosed with a single episode and multiple episode of AGR were almost equal in proportion (51.1% and 48.9% respectively).

Table 4.6.3. 1 Repeat episodes of Acute graft rejection (AGR) amongst failed grafts and surviving grafts at 5 years post index AGR episode

	5-year outcome		Total (n/%)*
	Graft survival (n/%)*	Graft loss (n/%)*	
Single AGR episode	16; 55.2%	8; 44.4%	24; 51.1%
Recurrent AGR episode	13; 44.8%	10; 55.6%	23; 48.9%
Total	29	18	47
Chi-Square			0.5115
p value			0.474

*n; % - number; percentage

Two thirds of grafts (66.7%) with single AGR episodes were functioning at 5 years compared to 56.5% of grafts with repeated AGR episodes. The majority of grafts that survived 5 years or more from the index rejection episode were only diagnosed with a single episode of AGR (55.2%), while the majority, similar in proportion, among grafts that failed were diagnosed with recurrent episodes of AGR (55.6%). This was not found to be of significant difference (p = 0.474), possibly due to the small population size.

4.6.4 Timing of AGR

The timing of the first episode of AGR post transplantation was compared between the patients with graft loss and patients with surviving grafts, depicted in Table 4.6.4.1. The majority of AGR episodes in all the 47 recipients included in the 5-year follow-up analysis occurred in the first 6 months after engraftment (53.2%). In both allograft outcome groups, the majority of the episodes of AGR occurred in the first 6 months (55.6% of AGR in the group with graft loss and 51.7% in the group with surviving grafts). Amongst recipients diagnosed with AGR in the period 7 – 12 months after engraftment, those with grafts surviving more than 5 years showed a higher frequency of AGR episodes over the group with grafts progressing to failure (27.6% vs 11.1%). The incidence of late AGR episodes (AGR after first year post transplantation) was higher in those recipients progressing to graft failure as compared to those with graft survival (33.4% and 20.6% respectively).

Table 4.6.4. 1 Timing of diagnosis of first episode of AGR by allograft outcome at 5 years

		5-year outcome		Total (n/%)*
		Graft survival (n/%)*	Graft loss (n/%)*	
Timing of AGR	0 - 6 months	15; 51.7%	10; 55.6%	25; 53.2%
	7 - 12 months	8; 27.6%	2; 11.1%	10; 21.3%
	13 - 24 months	5; 17.2%	5; 27.8%	10; 21.3%
	25 - 36 months	1; 3.4%	0; 0%	1; 2.1%
	37 - 48 months	0; 0%	1; 5.6%	1;2.1%
Total		29	18	47

*n; % - number; percentage

A comparison of the time to first AGR episode in days by allograft outcome at 5 years was performed and is depicted in figure 4.6.4.1. The medians of both groups were similar, with 162 days in the group with surviving grafts and 169.5 days in the group with graft loss. A Mann-Whitney U test showed no significant difference between the timing to the first AGR episode and the 5-year graft outcome (p=0.6936).

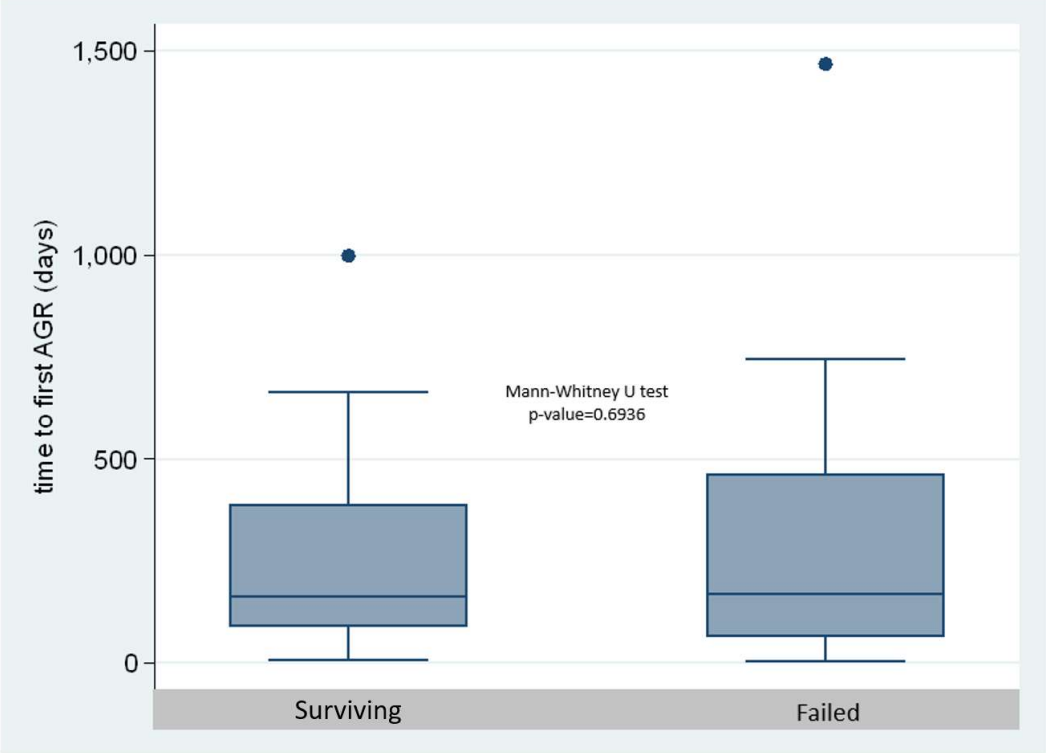


Figure 4.6.4. 1 Box and whisker plot, timing of first AGR episode by allograft outcome at 5 years

4.6.5 Graft function

Graft function was assessed through analysis of serum creatinine measurements at different points during an AGR episode, as well as at subsequent follow-up 5 years after diagnosis. Failed grafts were excluded from the analysis at 5 years. The results of these analyses are shown in Table 4.6.5.1.

Table 4.6.5. 1 Comparison of creatinine measurements and changes in creatinine in surviving and failed graft groups

	Graft Surviving	Graft loss
Pre AGR Cr (µmol/L)	119 (107-137)*	113.5 (91-173)*
Highest Cr within 1 month of AGR diagnosis (µmol/L)	145 (125-190)*	194 (127-352)*
Cr at 3 months after AGR diagnosis (µmol/L)	115 (103-130)*	160 (110-266)*
Cr at 5 year after AGR diagnosis (µmol/L)	120 (98-151)*	N/A
Mean percentage deterioration in creatinine within 1 month of AGR from pre AGR level (%)	32.9	79.72
Mean percentage improvement in creatinine at 3 months after AGR diagnosis from highest creatinine within 1-month diagnosis (%)	23.2	7.87
*Median (Interquartile range); AGR-Acute graft rejection; Cr-creatinine		

Median creatinine preceding AGR was similar amongst those recipients with persistent graft survival at 5 years after diagnosis and those in whom graft function was lost (119µmol/L and 113.5µmol/L); a Mann-whitney U test confirmed no significant difference (p = 0.962) as depicted in figure 4.6.5.1.

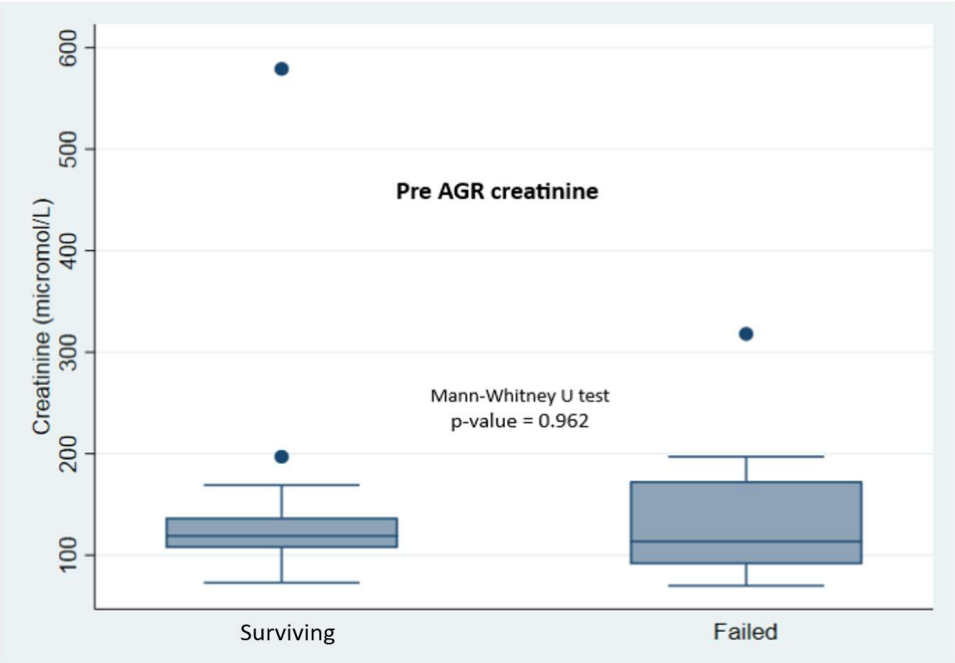


Figure 4.6.5. 1 Box and whisker plot, creatinine preceding AGR diagnosis by graft outcome

The median highest creatinine within 1 month of AGR diagnosis was non-significantly elevated in those allografts which progressed to loss (194µmol/L) in comparison to those allografts with continuing survival at follow-up at 5 years (145µmol/L) (p = 0.137) as depicted in figure 4.6.5.2.

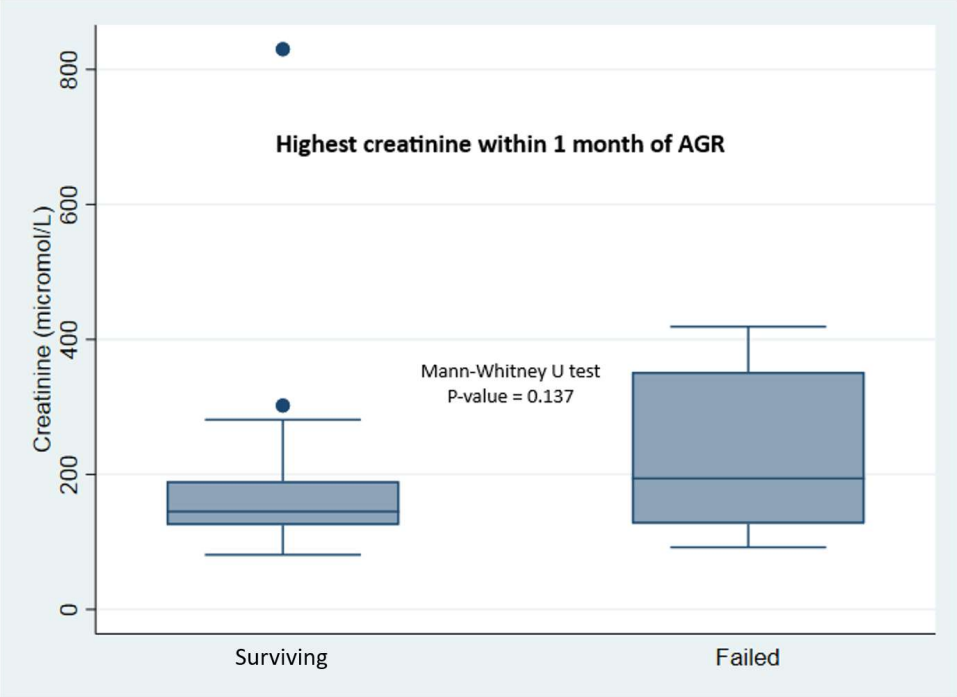


Figure 4.6.5. 2 Box and whisker plot, highest creatinine within 1 month of AGR diagnosis by graft outcome

The median creatinine measurements at 3 months post AGR was significantly higher in the group with graft loss (160mmol/L vs. 115mmol/L, p = 0.021), depicted in figure 4.6.5.3.

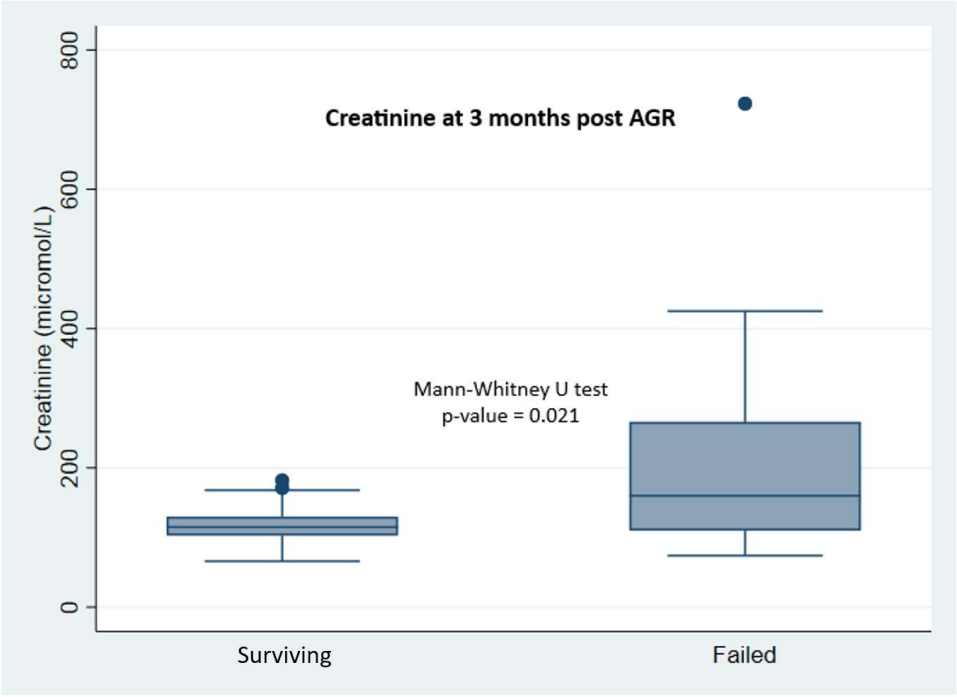


Figure 4.6.5.3 Box and whisker plot, creatinine at 3 months after AGR diagnosis by graft outcome

The average percentage increase in creatinine from measurements preceding AGR diagnosis to the highest measurement within 1 month of AGR was 79.7% in the graft loss group, which was higher than the percentage increase in these measurements in the graft survival group (32.9%). The percentage improvement of creatinine from the highest measurement within 1 month of AGR to that measured at 3 months after AGR diagnosis, was 7.9% in the graft loss group, compared to a 23.2% improvement in the graft survival group. There has been a great degree of variability in how response to treatment, in terms of graft function, has been reported, primarily due to application of different definitions (91). Such definitions include an improvement of at least 25% of the peak creatinine in response to treatment (91); applying this to this subpopulation revealed that 34.5% (10 out of 29 patients) of surviving grafts achieved this, in comparison to 16.7% (3 out of 18 patients) of grafts that progressed to failure. Another definition described is the recovery of graft function to 125% of baseline creatinine by 3 months after an AGR episode (91); this was achieved in 96.6% of surviving grafts, while only 50% of grafts lost achieved this.

The significance of the change in creatinine over the different points of measurement within each graft outcome group was further analysed using a paired Wilcoxan signed-rank test, which is depicted in Table 4.6.5.2. Although the change in creatinine from that measured before AGR diagnosis and that measured within 1 month after diagnosis was significantly different across the cohort as a whole ($p = 0.0483$), this significance did not persist when analysis was restricted to graft outcome groups ($p = 0.1268$ in the graft survival group and $p = 0.1838$ in the graft loss group). A statistically significant difference was detected for change in creatinine from that measured within 1 month and that measured at 3 months following AGR diagnosis for the cohort as a whole ($p = 0.0015$) and for those patients progressing to graft loss ($p = 0.0052$); this difference did not achieve statistical significance in those patients with allograft survival at 5 years after diagnosis ($p = 0.0569$). No significant difference was observed in creatinine from those levels recorded preceding AGR diagnosis and those recorded at 5 years of follow-up ($p = 0.1628$).

Table 4.6.5. 2 Paired Wilcoxon signed-rank test and creatinine measurements at various points of follow-up

Serum creatinine (Cr)	Paired Wilcoxan signed-rank test (p-value)		
	Total (n=47)	Graft survival (n=29)	Graft loss (n=18)
Pre AGR Cr and Highest Cr within 1 month of AGR diagnosis	**0.0483**	**0.1268**	**0.1838**
Highest Cr within 1 month of AGR diagnosis and Cr 3 months after AGR diagnosis	**0.0015**	**0.0569**	**0.0052**
Pre AGR Cr and Cr at 5 years after AGR diagnosis	-	**0.1628**	-

AGR-Acute graft rejection; Cr-creatinine

5 Discussion

5.1 Incidence of confirmed AGR among renal transplant recipients at CMJAH

Over the 10-year period of this study, a total of 263 renal transplants were carried out at the CMJAH renal transplant unit, a mean of 2.19 engraftments per month; 34.9% of these grafts subsequently developed AGR. In other studies, the incidence of AGR has been reported to be between 25% and 60% (2,4,6,7). However, the reported data may not be completely comparable to the current study due to variations in inclusion criteria (for example, inclusion of clinically-diagnosed AGR) and differences in follow-up periods (with other series limiting analysis to the first year following engraftment). The incidence of AGR occurring in renal grafts within one year of transplantation at CMJAH was 28.2%. The incidence of AGR at CMJAH falls within the lower end of the range reported in the international literature for both the overall and the one-year post engraftment analyses. However, AGR treated on clinical grounds, without biopsy-confirmation, were not included in this audit and may underestimate the true incidence of AGR. Renal histology remains the gold standard for the diagnosis of AGR.

Analysis of the CMJAH cohort appears to show a decrease in the annual incidence of AGR over the course of the series. Other studies have reported a declining trend in the annual occurrence of AGR, although this has not been associated with improved graft survival overall (5). Of note in the CMJAH series, the annual number of engraftments and the number of biopsies performed showed a concomitant decrease during the study period. Analysis involving both these variables suggests that the declining trend in the annual occurrence of AGR may be related to the declining number of biopsies performed during the study period ($p<0.01$). These trends and association may not take into consideration possible diagnosis and treatment of AGR without histological confirmation, which by the design of this study could not be analysed. However, the findings above may suggest that performing more renal biopsies may be beneficial for the renal unit at CMJAH in diagnosing and managing AGR.

The rate of AGR reported above includes repeated episodes of AGR; previous AGR episodes may increase the risk of subsequent episodes and may compromise long-term allograft

survival (2,4,6). Of the 130 episodes of AGR diagnosed during the course of this series, approximately two thirds were first AGR episodes, a quarter were second AGR episodes, and less than 10% were third and fourth AGR episodes combined. Amongst the 85 patients with confirmed AGR, 40% experienced a second episode of AGR, 11.8% experienced a third episode and 1.2% a fourth episode. Previous studies have reported a lower rate of second AGR episodes (20-30%) amongst recipients exposed to AGR and 5-17% in recipients diagnosed with more than two AGR episodes (2,4,6). The higher rates of recurrent AGR episodes observed in this cohort may reflect the impact of a black African dominant population, who have been reported to have increased risk for AGR (57). Other possible contributors to the higher rates of recurrent AGR may be due to minimisation of immunosuppression in this cohort as a result of infections (eg. CMV) or drug side effects (eg MMF-induced diarrhoea), however these factors were not investigated in this study.

The majority of all AGR episodes occurred within the first year (53.4%) after engraftment, 68.6% of these episodes occurring within 6 months of transplantation. 63.5% of index AGR episodes occurred within the first year of engraftment; only 10% of recipients experienced a first episode of AGR after two years post transplantation. Other studies have reported higher proportions of AGR confirmed within the first year of transplant at 76.8% (92).

63.3% of all AGR episodes were diagnosed on clinically indicated biopsy (biopsy undertaken due to clinical evidence of graft dysfunction); 37.7% of AGR cases were diagnosed on protocol biopsy. The role of clinically undetected, low levels of rejection has been described as an important factor limiting improvement in long-term graft survival, despite the decreased rate of overt clinical AGR in the recent past (37). Earlier studies have reported subclinical rejection to be detected in up to 30% of protocol biopsies undertaken within the first 6 months of transplantation (93–95). It is not possible to determine the rate of AGR diagnosed on protocol biopsy in the current study by design, as only AGR confirmed biopsies were included in this study.

5.2 The incidence of ACR and AHR, and analysis of the histological findings according to the Banff classification

Analysis of this cohort demonstrated that ACR was the most common category of rejection diagnosed (61.5% of all cases), followed by Borderline ACR (25.4%), and AHR (8.5%). Acute cellular rejection remained the dominant rejection type in recurrent episodes of AGR, demonstrating an increasing frequency with subsequent AGR episodes (comprising 57%, 67.6%, 70% and 100% of first, second, third and fourth AGR episodes respectively). Borderline ACR was the second most common type of AGR, comprising 31.8% of first episode AGR group. The combination of ACR and Borderline ACR contributed 86.9% of all AGR episodes in this study. An audit from Pennsylvania in 2015 documented a similar rate of ACR and Borderline ACR in their series with a combined proportion of 83% (96). The rate of AHR was relatively constant, contributing 7.1% to first AGR episodes and 8.8% to second episodes, and with no observed cases of AHR in the third and fourth AGR episode groups. C4d positivity was reported in 55.6% of AHR episodes; the remaining 44.4% of cases were diagnosed before C4d staining was available at CMJAH. Other studies have described AHR to occur in 5-7% of all transplants and to comprise 30% of all rejections diagnosed, which is significantly higher than the findings in CMJAH cohort (43,44,46). This may be due to the challenges faced in diagnosing AHR and the subsequent availability of C4d staining at CMJAH over this period. Late onset of AHR may also be misdiagnosed due to the slower progression of graft dysfunction which may result in missed biopsy opportunity, as well as misdiagnosis due to overlapping finding of ACR (46,97). The description of the different categories of rejection amongst subsequent episodes of rejection is not well reported in the literature.

Most ACR episodes in this study were of mild severity with grade 1A (43.8%) and grade 1B (36.3%), comprising a combined 80.1% of ACR episodes; this proportion remained relatively constant during the first, second and third episodes of AGR. The overall frequency of Banff grade 2A was 11.3%, grade 2B was 2.5% and grade 3 was 2.5%. No significant difference was observed in the grades of ACR between AGR episode number. Available literature suggests that the findings of this study are not unusual, with the majority of the ACR episodes being of milder grading (grade 1A comprising 44.2% and grade 1B 46.5%) (96).

The frequency of ACR was similar in those with clinical and subclinical AGR presentations (63.6% and 63.3% respectively). Traditionally, subclinical rejection has been thought to occur due to a T-cell driven process resulting in ACR; recent studies, however, have described AHR during subclinical rejection episodes (98). Previous studies have reported the incidence of subclinical ACR to be 42.7%, lower than that observed in the present study (98); this may due to higher immunogenicity reported in black African recipients, resulting in increased risk of AGR (57). The differences in AGR categories between the various types of biopsies analysed, which includes protocol biopsies, indicated biopsies and nephrectomy specimens, was statistically significant (p-value <0.001); however this most likely reflects nephrectomy specimens only confirming more severe types of AGR (AHR and mixed AGR) and the absence of these severe AGR types amongst protocol biopsies.

5.3 Factors which may have influenced the development of AGR in this series

A number of factors may contribute to the development of AGR in the individual recipient. The mean age of the sample population was 45.4 years ± 11.8 years. The youngest patient was 21 years old while the oldest was 69 years old, with the largest proportion (35%) of patients being between 40 and 49 years of age. Two thirds (67%) of patients fall within 20-49 years of age, with a third (33%) over the age of 50. The larger proportion of patients, below 50 years of age in this population, is in keeping with studies that have reported a decreasing incidence of AGR with increasing recipient age, which is thought to be explained by immunosenescence in older patients (40,55,56) and possibly differences in compliance with treatment. More specifically, studies have reported increased incidence of AGR in recipients below 18 years of age (not included in this study) as well as recipients between 40 and 55 years of age (99,100).

The majority (74.1%) of recipients in this series were male. Whether this majority reflects a greater risk of AGR among males or whether more males are recipients of kidney transplantation cannot be determined from this series of patients; however, the latter is more likely. The effects of gender on risk of AGR are not completely clear. There have been reports suggesting a 10% increased risk of AGR within 6 months of transplantation among female

recipients; however, there are also studies failing to demonstrate any significant difference between male and female recipients (101,102). A report from Tygerberg Academic Hospital in 2003 reported a male/female ratio for graft recipients of 1.2, while a more recent report from Wits Donald Gordon Medical centre in 2016, reported a male dominant population of recipients with a ratio of 1.8, similar to other international reports (56,60,102). This male predominance has been considered to be due to the increased incidence of ESKD amongst males, and the suggestion that females may be less inclined to accept transplantation (103). In this study, similar proportions of both genders were observed in recipients exposed to a single AGR episode and those exposed to recurrent episodes. Acute cellular rejection, followed by Borderline ACR, were the dominant types of AGR in both genders. No significant difference in the frequency of AGR type was detected between the genders.

The present study population comprised 77.6% black African; 11% Caucasian, 8% Asian and 3% Coloured recipients. The relative contribution of each ethnic group is comparable to the demographics of South Africa, which was estimated in 2016 to be 80.7% black African, 8.1% Caucasian, 8.8% Coloured and 2.5% Asian (22). No significant difference in the type of AGR or in recurrence of AGR episodes were detected between ethnic groups in this cohort. The contribution of ethnic origin to the outcome of renal transplantation is of interest in the local context, as international studies have reported African patients to have a fourfold increased risk of ESKD, resulting in a greater need for transplantation in this population; but also having an increased risk of AGR and lower rates of long-term graft survival (57).

The type of donor graft has been shown to play a role in the development of AGR and hence in long-term allograft survival. Living donor grafts are known to have superior outcomes over CD grafts (39,54,73–75). However, the increasing ESKD prevalence has resulted in a growing demand for renal transplantation which has not been associated with a concomitant increase in the number of living donors (16,54,73); at the same time, advances in immunosuppression have improved short-term allograft survival through more efficient inhibition of allosensitisation, facilitating the use of grafts with greater HLA mismatch (39,41). This has in turn widened the donor pool to include non-related donors, facilitating the use of anonymous unrelated cadaveric donors. Amongst SSA countries, South Africa is a leader in the utilisation

of CD grafts, with CD graft accounting for 60% of renal engraftments (16). In this study of AGR in renal allograft recipients, 78% of the cohort received a CD graft; non-CD grafts comprised a minority of the engraftments during the study period with RLD grafts comprising 19% and NRLD grafts contributing the remaining 3% of engraftments.

The proportion of various AGR categories demonstrated similar ratios between CD and RLD grafts. ACR contributed 61% and 60% of AGR episodes in CD and RLD engraftments respectively. Borderline ACR was the second most common rejection type (CD 26%; RLD 28%), followed by AHR (CD 8%; RLD 12%). No statistically significant difference in AGR types was demonstrated across donor type. A lower rate of AGR has been reported amongst living donor grafts (75); however a comparable description of the different types of AGR amongst the different types of donor grafts is not well described in the literature. The distribution of the different types of donor grafts amongst recipients exposed to a single AGR episode in comparison to those exposed to recurrent episodes revealed no significant difference.

The majority of recipients (78.8%) in this series of AGR had an HLA match with their donors of less than 40%. Forty percent of recipients with an HLA match of less than 40% experienced recurrent episodes of AGR.

The effects of delayed graft function on AGR incidence and graft survival are controversial. Although several international reports have implicated DGF as a risk factor for AGR and decreased graft survival (68,70–72), the exact mechanism is not well understood, and other reports have failed to show a relationship between DFG and rejection (2). In this study, 22.4% of recipients with AGR were associated with DGF. Analysis suggested an increasing proportion of DGF-affected engraftments associated with AGR types of increasing severity and the opposite trend amongst DGF-free engraftment. These proportions were observed to be of statistical significance ($p < 0.05$). Acute cellular rejection was the dominant AGR type in both the DGF-affected and the DGF-free group, followed by Borderline ACR. No significant difference was observed in the occurrence of recurrent AGR episodes between DGF-affected and DGF-free engraftments.

Hypertension was the most common aetiology (55%) of ESKD documented amongst patients in this population, which is in keeping with reports confirming hypertension as the leading cause of CKD in SSA (14,22). Of note, the proportion of hypertension reported above may be the presumed cause since native kidney biopsies were infrequently undertaken. Consideration should also be given to whether the hypertension observed was ESKD-related, rather than the primary aetiology. Other documented causes (diabetes mellitus, polycystic kidney disease, glomerulopathy, and obstructive uropathy) each contributed less than 10%. Surprisingly, the low proportion of diabetes as an aetiology observed (1%) in this population does not reflect the real proportion of patients diagnosed with CKD/ESKD due to diabetes mellitus (15.2%) in South Africa (22); this discrepancy may be the result of exclusion of patients with diabetes mellitus from transplantation on the basis of cardiac pathology.

5.4 Analysis of the management of ACR/AHR in the population and the response to therapy

Triple drug immunosuppressive therapy is the preferred regimen in use at CMJAH in view of data showing superior one-year graft survival rates and reduced rates of acute rejection (38). The majority of patients in this study (80%, n=68) were maintained on a regime consisting of CsA, MMF and prednisone. A smaller proportion of patients (15.3%, n=13) were on a regimen consisting of tacrolimus, MMF and prednisone. The remaining patients (4.7%, n=4) were on combinations including sirolimus, everolimus, or azathioprine. Further analysis was not done as the larger proportion of patients were on a standard regimen including CsA, tacrolimus, MMF and prednisone. Prednisone and MMF was used in 97.6% of the patient in this study population.

The therapeutic options used in categories of AGR including ACR, Borderline ACR and AHR were reviewed. First-line therapy for ACR (pulse methylprednisolone), was administered in 57.6% of ACR and 9.1% of Borderline ACR cases. The low rate of pulse methylprednisolone in Borderline ACR is probably a reflection of the uncertainty regarding optimal treatment of this entity (79). Antithymocyte globulin was administered in similar rates in the categories of ACR and Borderline ACR (5% and 6.1% respectively), perhaps reflecting restriction of the use

thereof to steroid-resistant or recurrent rejection episodes. Increased immunosuppression by adjusted maintenance therapy was prescribed in 88.8% of ACR and 63.6% of Borderline ACR cases. Amongst the 11 cases of AHR (including 3 cases diagnosed on nephrectomy), 3 cases received PE/IVIG while 7 of the cases were treated only with adjustments to maintenance therapy. It is likely that these disparities in the provision of a standardised therapeutic protocol in cases of AHR reflect the restriction of potent but costly treatments such as plasmapheresis and IVIG to cases deemed likely to respond to intervention, with alterations to maintenance immunosuppression being restricted to cases with significant, irreversible allograft injury.

A total of 44 cases of AGR (33.8%) in this study were managed with adjustments to maintenance therapy only. This consisted of 28.8% of all ACR episodes, 54.5% of Borderline ACR episodes and 27.3% of AHR episodes. This cohort consisted of both subclinical and clinical episodes of rejection, 40.9% and 59.1% respectively. A number of factors may have resulted in the decision to opt for conservative treatment in this group, including allograft prognosis and anticipated response to augmented therapy (as discussed above), severity of allograft dysfunction, and recipient biological fitness for augmented immunosuppression.

Supportive dialysis was required in 7.5% of ACR episodes, 9.1% of Borderline ACR episodes and 54.5% of AHR episodes. The higher frequency of AHR cases requiring dialysis may be explained by the tendency of this rejection category to be associated with more severe allograft dysfunction at the time of diagnosis (97). The observation that more cases of Borderline ACR required dialysis is counter-intuitive to the expectation that this category of allograft injury would be associated with milder graft dysfunction. Three possible scenarios may explain the surprisingly high rate of dialysis therapy prescribed in the Borderline ACR cohort. Firstly, other patterns of allograft injury may have combined with Borderline ACR (eg. missed AHR, especially during the period where C4d staining was not available) to cause a more marked deterioration in allograft function than is usually encountered in this rejection type. Secondly, dialysis may have been instituted prior to allograft biopsy; it is likely that in these cases empiric therapy for suspected ACR may have been administered, so that the ultimate diagnosis of “Borderline ACR” may instead represent partially treated ACR. Thirdly, it is possible that this observation highlights the limitations of biopsy as a diagnostic

methodology and that the “keyhole” nature of the procedure may have resulted in an underdiagnosis of the severity of rejection occurring in these cases.

The management of first-episode subclinical rejection comprised pulse methylprednisolone in 63% of subclinical ACR and 7% of subclinical Borderline ACR cases. A lower rate of adjusted maintenance therapy was observed in the subclinical group of ACR episodes when compared to the clinical group, 81% and 95% respectively, and similarly for the subclinical and clinical Borderline ACR groups, 67% and 83% respectively. The lower rates of intervention in the subclinical Borderline ACR episodes are probably due to the histologically confirmed low level injury manifested by a lack of significant allograft dysfunction prompting conservative management on a background of uncertainty around treating this entity. Whilst some studies have demonstrated a negative effect of subclinical rejection on graft survival (3,27,36), no consensus treatment strategy has been described (104).

Changes in graft function as determined by serum creatinine were analysed in an effort to describe response to therapeutic intervention. In cases of ACR, the mean serum creatinine at biopsy was 127.86µmol/L, with deterioration to 303.41µmol/L within 1 month, followed by a recovery to a mean of 209.57µmol/L by 3 months. Both AHR and Borderline ACR, although also demonstrating this trend, were found to have higher mean creatinine at all points of measurement when compared to ACR. This observation is not unexpected in the case of AHR which has been shown to be associated with an insidious progressive injury and a poorer prognosis than ACR; as a result, AHR may be associated with more severe graft dysfunction at presentation and may demonstrate persistently poor levels of allograft function despite therapy (46,97). These mechanisms likely underlie the observed statistically significant difference in creatinine level at AGR diagnosis between the clinical ACR and clinical AHR cohorts (p value = 0.024). The observation that the Borderline ACR in the present series is associated with more severe allograft dysfunction at presentation and at subsequent follow-up is, however, surprising given the interpretation of this histological category of injury as a “milder” pattern of injury than other forms of ACR. Possible explanations for this observation have been discussed above; in brief, the possibility of unsampled or partially treated ACR or

other categories of rejection, or sample error as evidence by smaller sample sizes and a wider 95% confidence interval in this group.

Lower creatinine levels were observed in cases of subclinical rejection compared to cases of clinical rejection, reflecting the use of deteriorating graft function, as indicated by serum creatinine, as an indication for biopsy in the clinical rejection cohort. Sub-analysis of the subclinical rejection cohort found no difference in serum creatinine between the histological categories of rejection (ACR and Borderline ACR) within this group. Whilst the available literature analysing the presentation of subtypes of subclinical rejection is scarce, it is likely that the lack of statistical difference observed in this analysis arises as a result of the restriction thereof to cases with normal allograft function only in accordance with the definition of subclinical rejection.

5.5 Analysis of the five-year outcome of allografts diagnosed with acute graft rejection

Five-year outcomes after AGR diagnosis were analysed in 47 patients with appropriate duration of follow-up, comprising 55.3% of the total study cohort. In this analysis, the overall 5-year allograft survival was 61.7%. Previous studies have reported a 6-year survival of 72.7% in patient with AGR in whom graft function returns to baseline, and 50.4% in patients with AGR in whom graft function does not return to baseline (5); other studies from the developing world have reported lower 5-year survival rates (42.7%) amongst recipients confirmed with AGR (105). Patient survival was observed to be 95.9%. Twenty five percent of graft loss occurred by 2.3 years. The largest proportion of graft loss occurred within the first year following AGR diagnosis (17% of the cohort lost graft function within the first year), with 10.6% lost in the second year and thereafter a tapering of graft loss to 2.1% in the fifth year. The higher rate of graft loss in the first two years after index AGR is reflected in the median time to graft loss of 1.5 years. The rate of graft loss within the first year in this study is higher than other studies reporting rates of 11% or less (29,30). Graft loss has been reported to be 2-3% annually after the first year of transplantation, with AGR being a major contributor (2–7).

Acute cellular rejection was the most common rejection type in grafts with persistent function at 5 years and in grafts progressing to loss of function within that period (62.1% vs. 55.6%); AHR was not detected in any graft with persistent function but accounted for 16.7% of rejection diagnoses in grafts progressing to loss. This data may suggest poorer outcomes for AHR although analysis did not reach statistical significance (p = 0.072). The 5-year survival outcome of patients diagnosed with a first episode of ACR was 64.3% and that of patients diagnosed with Borderline ACR was 68.8%.

In sub-analysis of grafts with evidence of ACR, the majority of episodes in both the 5-year surviving and graft loss cohorts were of Banff grade 1 severity (77.8% and 90% respectively) with the remaining episodes being of grade 2 severity; no grade III episodes were diagnosed in the course of this study. Graft survival was 75% amongst graft confirmed with Banff grade 1A ACR and 45.5% with Banff grade 1B ACR was 45.5%. Although suggesting a trend toward poorer outcome with more severe rejection grade, analysis of ACR grades 2A and 2B failed to show a similar association with outcome, most likely due to the small sample size (n=5). Risk of graft loss after the first year of transplant in patients diagnosed with Borderline ACR within the first year, has been reported to be 3.06 times that of patients who do not develop AGR (106). The risk of graft loss appears to vary with AGR histological category with AHR carrying a particularly high risk of graft failure (HR 11.2) (106). Somewhat surprisingly, ACR grade 2 has in previous reports been associated with a lower risk of graft loss compared to ACR grade 1 (HR of 1.95 vs. 2.86), although this may have reflected the small sample size of the study cohorts (106).

A higher rate of recipients (55.6%) who subsequently developed allograft loss were exposed to recurrent episodes of AGR, while the greater proportion (55.2%) of recipients with grafts surviving 5-years were exposed to a single AGR episode. Although not achieving statistical significance in this study, the trend toward poorer allograft outcomes in the setting of recurrent AGR episodes is consistent with other studies (2,4,6), including reports observing a 50% reduction of graft half-life with the occurrence of multiple episodes of AGR (107).

The mean time to first episode of AGR was similar between those grafts with persistent function at 5 years and those progressing to graft failure (162 vs. 169.5 days, p = 0.6936). A higher proportion of patients with allograft survival at 5 years after transplant were diagnosed with first episode AGR within the first year of engraftment compared to those who progressed to graft loss (79.3% vs. 66.7%, respectively); consequently, 33.3% of patients in whom AGR resulted in graft loss were diagnosed with first episode AGR at late follow-up (more than one year after engraftment), compared to 20.7% of those with graft survival at 5 years. Although not achieving statistical significance in this study, these findings are similar to other reports demonstrating a higher frequency of late AGR episodes in grafts with poorer outcomes (4,92,107). There is a lack of consistency in reports of the effects of timing of AGR on graft survival or outcome, which is thought to be due to variability in studies with regards to methods, population and definitions (92).

Although mean baseline serum creatinine levels were similar in the surviving and graft loss cohorts (119 and 113.5 μmol/L respectively), graft function demonstrated a higher percentage of deterioration within 1 month of AGR diagnosis in the failed graft cohort (79.7% increase in creatinine vs. 32.9% in those with graft survival); in the graft loss cohort, graft function demonstrated poorer response to intervention at 3 months of follow-up (7.9% improvement in creatinine level compared to 23.2% in the graft survival cohort). In consequence, the median creatinine measurement at 3 months post AGR diagnosis was 115μmol/L in surviving grafts and 160μmol/L in grafts subsequently progressing to failure (p = 0.021). This data illustrates the progression of AGR towards graft loss if unchecked by therapeutic intervention. In this regard, literature on the optimal minimal recovery of graft function which should be targeted in order to prevent later progression to graft loss remains subject to controversy (91). It has been suggested by some studies that recovery to within 125% of baseline creatinine by one month of treatment is associated with improved long-term allograft outcomes (91); in the present study, 96.6% of grafts with persistent function at 5 year follow-up demonstrated this degree of recovery by 3 months after diagnosis, compared to 50% in grafts progressing to failure.

6 Limitations

The following limitations are identified in respect of this retrospective study:

- Missing patient files data
    - These files could not be located amongst the archives of files stored in the renal unit, resulting in the exclusion of 20 patients confirmed with AGR from this study.
- Lack of CIT data
    - This information was not consistently recorded in the renal unit's patient files. The database of the transplant surgical team was extracted from the same files and therefore had incomplete data as well. This resulted in a very small sample size, which was confirmed with ACR predominantly, and therefore statistical analysis was not carried out.
- Small number of patients with 5-year follow-up and AHR episodes
    - These small sample sizes may influence the statistical analysis undertaken in this study and may be considered to account for discrepancies observed in comparison to other studies.
- This study by design, did not account for transplant recipients not exposed to AGR (control group)
    - Interesting comparisons can be made with regards to graft survival and description of potential factors that may influence the risk of AGR, which may better define our findings.
- The overall small transplant population may have limited sub-analysis in the different categories of AGR.

7 Recommendations

- Although the majority of AGR episodes in this study were diagnosed in the first year after engraftment, this proportion was lower than reports from other studies. Therefore, a high index of suspicion for AGR within the first year of engraftment, with potentially a lower threshold for biopsy during this period may be beneficial.
- Subclinical rejection diagnosed on protocol biopsies contributed to a significant proportion of AGR in this study, therefore supporting the continued practice of screening for rejection with protocol biopsy.
- Further focus on protocols for the management of ACR and Borderline ACR which formed the majority of AGR episodes diagnosed and managed at CMJAH.
- Expansion of this study to include a control group (recipients not exposed to AGR), which would further characterise potential factors influencing the risk for AGR.
- DGF was observed to be associated with more severe AGR types (AHR and mixed rejection). This would suggest more attention should be allocated to this group of recipients, with a lower threshold for renal biopsy.
- AHR was only observed amongst recipients who progressed to graft loss, which highlights the negative impact of this rejection type on graft survival. Further studies on this entity should be considered.
- Recovery of graft function at 3 months after AGR was more prominent amongst recipients with surviving grafts and suggests its use as a relative marker for predicting 5-year graft survival.

8 Conclusions

The overall incidence of AGR of 34.9% at CMJAH falls well within the ranges of international reports. Approximately two thirds of patients were diagnosed with AGR within the first year of engraftment. Recurrence of AGR was not uncommon, with 40% of patients experiencing a second episode of AGR, 11.8% a third episode, and 1.2% a fourth episode. Subclinical rejection contributed significantly to the number of cases of early AGR, reaffirming the value of protocol biopsy.

ACR was the most common histological type of AGR diagnosed in this series, with an overall incidence of 61.5%. The overall incidence of AHR was 8.5%. Potential factors which might contribute to the occurrence of AGR (age, gender, ethnic distribution, donor type, HLA matching, CIT and DGF) were not found to show statistically significant association with AGR, other than increased severity of AGR with the presence of DGF.

The 5-year outcomes revealed an overall graft survival rate of 61.7%. The annual rate of loss of grafts revealed a peak loss of 17% in the first year, with a steady decline in this rate in subsequent years. The 5-year survival outcome of grafts diagnosed with a first episode of ACR was 64.3% and of Borderline ACR was 68.8%, while all grafts diagnosed with AHR progressed to graft loss within 5 years.

Creatinine measurement at 3 months after AGR diagnosis and treatment was observed to be significantly elevated in those cases which subsequently progressed to graft failure. Almost all recipients with surviving grafts (96.6%) achieved recovery of graft function to 125% of baseline by 3 months of AGR diagnosis.

This study adds to the South African literature which has hitherto been scarce in this field. The consistency between some of the findings and trends observed in this study with previously published reports are reassuring. The differences observed between this study and these previously published reports may indicate subtle disparities in the immunobiology of the local recipient population which warrant further future study.

9 References

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10 Appendix

10.1 Human Research Ethics Committee (Medical) Clearance Certificate

R14/49 Dr Riju Mathew Thomas

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140667

NAME: (Principal Investigator)	Dr Riju Mathew Thomas
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Audit of Acute Rejection in Renal Allograft
DATE CONSIDERED:	27/06/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S Naicker & Dr M Davies
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/06/2014

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

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

25 July 2019

Internal Medicine

Sent by email to: mthomas85@gmail.com

Dear Dr Thomas

Re: **Protocol Ref No: M140667**
Protocol Title: Audit of acute rejection in Renal Allografts
Principal Investigator: Dr Riju Thomas
Protocol Amendment: Extension of time for submission

This letter serves to confirm that the Chairperson of the Human Research Ethics Committee (Medical) has approved the request for the above-mentioned protocol, as detailed in your letter, dated 14 July 2019.

The following documents were received:

- Summary Letter
- Clearance certificate

Thank you for keeping us informed and updated.

Yours Sincerely,

.....

Mr Joshua Ndlangamandla
Administrative Officer
Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE
25 JUL 2019
RESEARCH O.
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10.2 Turnitin Report

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