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RETROSPECTIVE COMPARISON OF CYCLOPHOSPHAMIDE AND MYCOPHENOLATE MOFETIL IN LUPUS NEPHRITIS AT GROOTE SCHUUR HOSPITAL NEPHROLOGY UNIT

Dissertation for the degree of

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DECLARATION

I, ***Phelisa Sogayise*** hereby declare that the work on which this dissertation/thesis is based on my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part has been, is being, or is to be submitted for another degree in this or any other university.

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TABLE OF CONTENTS Page

CHAPTER 2: JOURNAL READY MANUSCRIPT

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Figures and Tables

List of Abbreviations:

ANCA	Anti-neutrophil cytoplasmic antibody
CNIs	Calcineurin inhibitors
CsA	Cyclosporin A
CYC	Cyclophosphamide
DNA	Deoxyribonucleic acid
DOC	Daily oral dose
GN	Glomerulonephritis
GSH	Groote Schuur Hospital
IgAN	Immunoglobulin A nephropathy
IV CYC	Intravenous cyclophosphamide
KDIGO	Kidney disease improving global outcomes
LN	Lupus nephritis
MMF	Mycophenolate mofetil
IVCYC	Pulse intravenous cyclophosphamide
SLE	Systemic Lupus Erythematosus
RCT	Randomized controlled trial

TAC	Tacrolimus
TBAH	Tygerberg Academic Hospital

ABSTRACT

Background: Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus (SLE) and is associated with end-stage renal disease, need for renal replacement therapy and death. There is no African data comparing the efficacy or outcome of patients managed with Mycophenolate mofetil (MMF) or intravenous (IV) cyclophosphamide (CYC) during induction of proliferative LN.

Objectives: The study aimed to assess treatment response after induction therapy with MMF or IV CYC in patients with proliferative LN at a single centre in South Africa.

Methods: We performed a retrospective review of records of patients with biopsy proven proliferative LN diagnosed and treated with either MMF or IV CYC at Groote Schuur Hospital (GSH), Cape Town, South Africa. We assessed remission status, adverse events and death between the two groups.

Results: Patients treated with IVCYC and MMF had a mean age of 30.3 ± 11.2 and 27.0 ± 6.9 years respectively, (p=0.23). At baseline, the IVCYC group had significantly higher mean arterial pressure, serum creatinine and glomerular crescents (p<0.05). After completion of induction therapy, there was no significant difference in remission status (76.0% vs 87.5%, p=0.33) or relapse status (8.1% vs 10.3%; p=0.22) for the IVCYC and MMF groups, respectively. There was no significant difference in occurrence of infective complications IVCYC 20(30.8%) vs MMF 3(15.7%) p=0.20 or mortality IVCYC 14(21.5%) vs MMF 1(5.4%) between both groups. Estimated GFR at baseline was the only predictor of death (1.0 [0.9 – 1.0]; p=0.001).

Conclusion: Our study shows similar outcomes following induction treatment with MMF or IVCYC in patients with biopsy proven proliferative LN in South Africa. However, a prospective and randomized study is needed to adequately compare these outcomes.

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CHAPTER 1: LITERATURE REVIEW

1.0 The Objectives of the literature review

- To give a broad overview of what is known about Systemic Lupus Erythematosus (SLE) and lupus nephritis (LN) from published literature and international society guidelines.
- To put into context what is already known about LN globally and from an African perspective.

2.0 Broad overview of SLE

2.1 Epidemiology

Systemic lupus erythematosus (SLE) is the most common autoimmune disease, with peak incidence and prevalence reported to be about 23.2/100 000 person-years and 24/100 000 people respectively(1). A multinational and multiracial study showed that the burden of SLE is significantly higher in non-Caucasian populations(2). More specifically, populations of African ancestry have the highest incidence of SLE, while Caucasians have the lowest. Furthermore, SLE is far more prevalent in females than males irrespective of age and ethnic groups(1). The ethnic difference also exists for SLE severity as African-American and Afro-Caribbean's in Europe and West Indies express the most serious forms of the disease(1). SLE accounts for substantial morbidity, despite ongoing therapeutic advances, the mortality remains high as documented by the standardised mortality ratio of 2.66 found in the current meta-analysis of complete cause-specific mortality in SLE(3). The leading cause of this

increased mortality are infections, renal disease and cardiovascular diseases while the risk of death due to malignancy is not increased(4).

In Sub-Saharan Africa (SSA), SLE was regarded as a rare condition until the 20th century (5). However, the conclusions from reviews were limited by small numbers of studies included. Daily clinical experience and ever-increasing growth of the African literature on SLE suggest that the idea of SLE scarcity is a myth. The seeming low incidence rate in Africa may be the result of underdiagnoses due to poor access to health care, low disease recognition within primary health care settings, inadequate access to diagnostic tools and inadequate numbers of specialist physicians(6).

2.2 Clinical presentation

Patients who have SLE often present in numerous ways and may go on undiagnosed for many years due to non-specific symptoms such as fever and malaise.

Table 1: shows the various clinical presentations in a systematic review and meta-analysis of SLE patients in SSA(5).

Table 1: Prevalence of clinical features in patients with SLE(5)

SLE manifestations	SLE in SSA, prevalence of clinical features (%)
Rheumatology	5 – 99
Dermatological	4 – 100
Haematological	1 – 87
Constitutional symptoms	6 – 79
Renal	6 – 80
Cardiovascular	2 – 46
Serosal	6 – 31
Neuropsychiatry	5 -47
Pleuropulmonary	6 -25

SLE – systemic lupus erythematosus; SSA – sub-Saharan Africa

2.3 Diagnosis of SLE

The diagnosis of SLE is based on a composite of clinical manifestations, as well as haematological, immunological and histological (mainly of the skin and kidneys) features. SLE is a complex challenging condition that presents exclusive issues in diagnosis and management. Patients present in many different ways and various clinical symptoms do not frequently occur concurrently and may ensue at any stage of the disease. Fever, fatigue and arthralgia are the most often occurring non-specific symptoms at disease onset. Additionally, joint swelling or a “butterfly” rash – predominantly in women of child bearing age should prompt consideration for SLE(7).

SLE is diagnosed based on the Systematic Lupus International Collaborating Clinics (SLICC) criteria(8), which involves:

1) Fulfilment of at least four criteria, with at least one clinical criterion and one immunological criterion.

2) Lupus nephritis as the sole clinical criterion in the presence of anti-nuclear antibodies (ANA) or anti-double stranded (Anti-DsDNA) antibodies.

Table 2: SLICC CRITERIA(8)

"**Clinical Features**	**Immunological features**
1. Acute cutaneous lupus	ANA
2. Chronic cutaneous lupus	Anti- dsDNA
3. Oral or nasal ulcers	Antiphospholipid antibodies
4. Arthritis	Low complement (C3, C4,CH50)
5. Serositis	Direct Coombs' test (do not count in the presence of haemolytic anaemia)
6. Non-scarring alopecia	Anti-Sm antibodies
7. Renal	
8. Neurological	
9. Haemolytic anaemia	
10. Leukopenia	
11. Thrombocytopenia"	

"ANA- antinuclear antibody, anti-dsDNA- anti-double-stranded deoxyribonucleic acid, Anti-Sm – anti smith antibodies".

3. Lupus nephritis

3.1 Definition of lupus nephritis

Lupus nephritis (LN) should be considered in any SLE patient with impaired kidney function, proteinuria, hypertension or an active urine sediment according to the KDIGO guidelines(9). An active sediment includes presence of haematuria, leukocytes, red blood cell casts and white cell casts(9). Kidney involvement is specifically defined as persistent proteinuria (>0.5g/24h) or presence of cellular red cell casts by the SLICC criteria(8). However, LN must be confirmed by kidney biopsy because histological findings provide the basis for treatment recommendation(9).

The clinical features of LN is often present in conjunction with other extra-renal manifestations of SLE, including joint pain, malar rash, oral ulcers and photosensitivity. A previous study from our centre found joint and skin involvement to commonly occur along with kidney involvement(10). However, patients with LN will present with asymptomatic urinary involvement (mild proteinuria and/or haematuria alone), nephritic syndrome (oliguria, minimal proteinuria, haematuria, hypertension and azotaemia) or with signs of nephrotic syndrome (anasarca, heavy proteinuria, hypalbuminaemia)(11).

Routine performance of urinalysis (dipstick and microscopic examination) offer the best chance for early detection of LN (12).

3.2 Burden of lupus Nephritis in Africa and globally

LN is a common and major manifestation of SLE, occurring in 40-60% of SLE patients(13). LN is a huge contributing factor of morbidity and mortality in SLE patients. One study from South

African has shown that over half of 226 SLE patients from a lupus clinic had either died or been lost to follow up at 55 months from the time of diagnosis and kidney involvement (LN) was a major predictor of mortality with a 5 year survival rate of 60%(14).

LN is the commonest secondary cause of glomerular disease and a frequent cause of nephrotic range proteinuria globally (12). A systematic review on the prevalence of histologically proven glomerular diseases in Africa showed that LN was the most common reported both from North Africa and sub-Saharan Africa despite the high prevalence of HIV in the region(15). Up to 50% of SLE patients have derangements of renal function or urine (proteinuria, haematuria or cellular cast) early on in the disease course, while approximately 80% may later develop overt aberrations of the renal function(13). The prevalence of LN in the Euro-lupus cohort was 39 %(16), in South Africa the prevalence was slightly higher at 43%(17).

3.3 Lupus nephritis classification

LN is classified using the International Society of Nephrology (ISN) / Renal Pathology Society (RPS) classification of lupus nephritis 2003. It allows standardisation of definitions and emphasizes clinically relevant lesions making it easier to compare between centres(18)(19)(20). It is worth stating that the classification of LN refers to only glomerular changes that are a result of immune complex deposition. Other features can still be found, including tubulointerstitial inflammation and vasculopathy or thrombotic microangiopathy (usually in association with antiphospholipid antibodies). The degree of tubulointerstitial scarring is also prognostic and should be documented(21). A minimum of 10 glomeruli is a

prerequisite to logically exclude focal disease and the biopsy should be examined by light microscopy, immunofluorescence and if feasible by electron microscopy(20).

Table 3: Abbreviated ISN/RPS Classification of lupus nephritis(18)

Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal lupus nephritis (<50 involvement)
Class IV	Diffuse segmental or diffuse global lupus nephritis (≥50% involvement)
Class V	Membranous lupus nephritis
Class VI	Advanced sclerosing lupus nephritis

The association between the histological class of LN and the clinical course of the disease is well documented. Patients with class II and class V (pure membranous LN)- non proliferative LN disease frequently have a slow decline in renal function over long periods of surveillance. In contrast, patients with class III and class IV (or those mixed with class V)- proliferative LN disease predominantly have a more aggressive course of disease(11).

In SSA there are limited studies on biopsy confirmed LN mostly due to lack of expertise in performing a renal biopsy and frequently the lack of pathology support for handling of tissue or reporting of histologies (22)(23). Worldwide, several studies show that proliferative classes of LN are more frequently encountered than non-proliferative types(24)(25)(26)(27)(28)(29). In a study of 251 patients assessing the clinocopathological features of LN in South Africa, 63% were shown to have proliferative classes of LN(12).Factors that were related with proliferative LN were : “male gender, haematuria on dipstick, proteinuria, low serum albumin, low complement and a positive anti-DsDNA”(12).

3.4 The Role of renal biopsies in lupus nephritis

Guidelines support performing a renal biopsy when there is suggestion of renal involvement, since clinical and laboratory features do not accurately predict the histological class.(30)

The criteria for suspicion of renal involvement includes(9)(31):

- Unexplained decrease in renal function
- Proteinuria of > 500mg/day
- Active urinary sediment (granular casts, white blood cell (WBC) casts, red blood cell (RBC) casts.

4. TREATMENT OF LUPUS NEPHRITIS

Treatment of LN is dictated by the class of disease and degree of activity and chronicity indices and the 2003 ISN/RPS classification of LN allows for standardization of treatment(18).

4.1 Adjunctive therapies in Lupus Nephritis

Adjunctive therapies are initiated during the induction phase of treatment, a few may need to be halted after completing this therapy, and others will need to be continued throughout the maintenance phase.

Frequently recommended adjunctive therapies in LN include (30):

- Antimalarials: chloroquine or hydroxychloroquine is advocated for all patients (if not contraindicated e.g. visual disturbances). This is based on clinical data showing a reduced occurrence of nonrenal and renal flares, diminished organ damage and better survival in patients with SLE treated with antimalarial drugs(32). Screening

with an ophthalmologist for retinopathy at baseline and yearly after 5 years is advised (30).

- Renin-angiotensin-aldosterone system (RAAS) inhibition for proteinuria and blood pressure control (target blood pressure <130/80)
- Low dose acetylsalicylic acid in patients with antiphospholipid syndrome
- Anticoagulation in those with albumin of less than 20g/l.
- Tuberculosis prophylaxis with isoniazid (for those in high endemic areas)
- Contraception is necessitated for all patients on Mycophenolate Mofetil (MMF) or Cyclophosphamide (CYC), and pregnancy must be avoided until remission is sustained for a minimum of six months.

4.2 Immunosuppressive treatment of lupus nephritis

The therapeutic approach to lupus nephritis consist of 2 phases: induction and maintenance.

The aim of induction is to promptly control kidney inflammation, reduce residual scarring and conserve renal mass. Therapeutic options have extended from the use of single corticosteroids to the addition of wide variety of chemotherapeutic drugs and other adjunctive treatment. In a systematic review and meta-analysis by Singh et al. there was significant difference in the effectiveness of corticosteroids (CS) and immunosuppressive drugs. Substantially lower risk of end stage renal disease (ESRD) was seen in the CYC and CYC + azathioprine (AZA) group compared with the CS only, [OR: 0.18 to 0.48] (33).

The routine use of IVCYC in addition to glucocorticoids for the treatment of LN began after the ground breaking NIH trial(16). Since the 1980s, corticosteroids plus cyclophosphamide has been standard therapy for proliferative LN, clinical trials showed that cyclophosphamide

and corticosteroids was more efficient than corticosteroids alone in sustaining disease remission and preventing ESRD(34)(35)(36). The adverse events associated with CYC (including leukopenia, alopecia, susceptibility to infections, gonadal toxicity, haemorrhagic cystitis, uroepithelial tumors and increased occurrence of other malignancies) have led to approaches that lessened cyclophosphamide exposure(16)(37).

The Euro- Lupus Nephritis trial compared high- dose and low dose intravenous CYC, the results showed similar rates of improvement in serum creatinine, proteinuria, disease activity score and similar failure rates; interestingly both groups had no significant differences in the incidence of adverse effects(16). However, 76 of the 90 participants in the trial were Caucasians-who had mild to moderate severity of LN, and the Euro-Lupus regimen is usually not adopted in black patients, who frequently have severe disease that might not respond well to standard therapy and data for effectiveness of this regimen is lacking(38)(39)(40).

The Aspreva Lupus management study (ALMS) trial compared the efficacy of MMF and IV CYC as induction therapy. For the induction phase, 370 patient with III, IV or V lupus nephritis were randomly assigned to treatment with corticosteroids plus either monthly intravenous cyclophosphamide (0.5- 1.0g/m^2) or MMF (2.5-3.0g/day). The primary outcome was the proportion of patients who responded to treatment after 24 weeks. The results were not remarkably different between the two 2 groups, complete remission rates were comparable in both groups, achieved by 8.6% of the patients in the MMF group and 8.1% of the CYC group after 6 months. The response rates were comparable in Asian and Caucasian patients, the response rates were superior with MMF than cyclophosphamide in black patients(41).

Current induction protocols utilize high dose corticosteroids with either IVCYC or MMF. The maintenance phase uses either MMF or azathioprine with low dose corticosteroids to

augment responses and avoid disease flares. Many trials have been conducted in the past 40 years resulting in the publication of six guidelines in 2012 on the management of lupus nephritis(9)(42)(43)(44)(45)(46). Patients with proliferative LN should be pulsed with steroids and commenced on CYC or MMF as part of induction treatment. This is then ensued by maintenance treatment with MMF or Azathioprine with low doses of steroid(11)(23). At present there is no agreement on the duration of maintenance therapy. In South Africa the resolution to withdraw maintenance immunosuppression should be guided by sustained complete clinical response of at least 2 years(11).

In South Africa the modified National Institute of Health regimen (NIH) is used for induction, it involves the use of 3 consecutive pulse doses of intravenous(IV) methylprednisolone (500-750mg daily) together with immunosuppressive agent: CYC 0.5 -1g/m^2 monthly for 6 months or MMF of 2-3g per day(11). The lifetime cumulative CYC exposure should not exceed 36g(9).IV methylprednisolone is followed by prednisone (1mg/kg/day) tapered over 6 to 12 months depending on the clinical response(11).

Table 4: KDIGO guidelines on management of lupus nephritis: (reference)
Class I- Minimal- mesangial LN Treatment as dictated by the extrarenal clinical manifestations.
Class II- mesangial-proliferative LN Proteinuria <1g/dl as dictated by the extrarenal manifestations Proteinuria >3g/dl be treated with corticosteroids or CNIs

Class III- Focal LN and Class IV Diffuse LN Induction therapy: - corticosteroids combined with either MMF or cyclophosphamide, worsening LN(rising serum creatinine, worsening proteinuria) during the first three months of treatment, a change can be made to an alternative recommended initial therapy or a kidney biopsy is performed. Maintenance therapy: after induction therapy is complete, patients with class III and IV LN receive maintenance with low dose corticosteroids and azathioprine or MMF.
Class V- Membranous LN Patients with normal kidney function and non-nephrotic range proteinuria should be treated with antiproteinuric and antihypertensive agents. Persistent nephrotic proteinuria should be treated with corticosteroids plus additional immunosuppressive agents CYC, MMF, CNI or Azathioprine.
Class VI- Advanced sclerosis Treatment with corticosteroids and immunosuppressive agents only as dictated by the extrarenal manifestations of lupus.

The oral CYC regimen is not a preferred suggestion due to its associated prolonged treatment duration, greater cumulative doses and additional serious leukopenia. When oral CYC is given, the dose is 2.0 to 2.5 mg/kg daily(47). IVCYC based induction regimen is extensively used in Africa and is supported by various guidelines(30), particularly for patients with proliferative lupus nephritis. Cyclophosphamide is cheaper and widely available in Africa than MMF, although few African countries have shown data on its usage. Recent availability of MMF generic formulations has led to lowered cost and increased usage in South Africa(23).

4.3 Clinical course of lupus nephritis

Significant innovations in early diagnosis, classification and chemotherapy for patients with LN have resulted in a substantial improvement in renal and patient survival(48). Despite this improvement in survival, SLE patients with LN have poorer outcomes than those without renal involvement, signifying that LN is an exhibition of a more severe form of SLE(17).

The mortality rate from SLE is high in South Africa – the 5 year survival of 226 patients with SLE that were followed up for 55 months showed estimated survival rates between 57% and 72%; infections, nephritis, neuropsychiatric diseases and hypocomplementia were coupled with increased mortality, but multivariate analysis indicated nephritis as the key prognosticator of mortality(14).

The predictors of poor outcomes in lupus nephritis has been studied in several cohorts and the following are shown to be associated with poor outcomes(49)(50)(38)(51)(52):

- Systemic hypertension
- Raised serum creatinine at the onset of LN
- Diffuse proliferative glomerulonephritis (WHO –class IV)
- Failure of remission in the first year following lupus therapy
- Interstitial inflammation
- Massive proteinuria
- Black /Hispanic race

A South African study conducted by Ayodele et al showed that on multivariate analysis baseline creatinine, hypertension and failure to attain complete remission at initial treatment were substantial predictors of development of chronic kidney disease in lupus nephritis patients(49).

5.0 Gaps in literature

There is evidence that MMF may be equally effective as CYC for induction. The ALMS trial found similar response rates for MMF and CYC (56.2% vs 53%; p =0.58). However, response

rates in the ALMS, in a group labelled “other” (mainly patients of African descent and mixed race), was notably higher in the MMF group (60.4% vs 38.5%; p= 0.033). This could imply better response to MMF in blacks and Hispanics(53). There are few African studies that have shown data with MMF use and LN outcomes mainly due to the cost or unavailability of MMF(23).

Treatment of LN in Africa is challenged by lack of and very expensive chemotherapeutic agents, and by the lack of laboratory monitoring of patients. There are few published studies of patients with LN in Africa. An IVCYC- based induction regimen is commonly used, the high cost of MMF has limited its availability(22)(23). Recent accessibility of generic formulations of MMF in South Africa has led to reduced cost and increased usage for patients with LN, however data comparing the efficacy and outcomes of MMF and IVCYC are still not available.

6.0 Motivation of the study

The motivation to carry out this study is borne from the absence of data in Africa comparing the efficacy and outcome of patients treated with MMF or IVCYC during induction therapy for proliferative LN.

Hence, the primary aim of this study is to assess treatment response (after induction therapy with MMF or CYC) in patients with proliferative LN (class III, IV, V+III and V+IV).

Secondary objectives of this study will include;

- To compare the adverse effects of treatment between both groups
- To compare the rate of relapse post induction therapy between both groups

(Word count 3022)

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JOURNAL READY MANUSCRIPT

Title:

Comparison of induction treatment response in patients with Lupus Nephritis treated with intravenous cyclophosphamide or mycophenolate mofetil: A single centre retrospective analysis

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ABSTRACT

Background: Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus (SLE) and is associated with end-stage renal disease, need for renal replacement therapy and death. There is no African data comparing the efficacy or outcome of patients managed with Mycophenolate mofetil (MMF) or intravenous (IV) cyclophosphamide (CYC) during induction of proliferative LN.

Objectives: The study aimed to assess treatment response after induction therapy with MMF or IV CYC in patients with proliferative LN at a single centre in South Africa.

Methods: We performed a retrospective review of records of patients with biopsy proven proliferative LN diagnosed and treated with either MMF or IV CYC at Groote Schuur Hospital (GSH), Cape Town, South Africa. We assessed remission status, adverse events and death between the two groups.

Results: Patients treated with IVCYC and MMF had a mean age of 30.3 ± 11.2 and 27.0 ± 6.9 years respectively, (p=0.23). At baseline, the IVCYC group had significantly higher mean arterial pressure, serum creatinine and glomerular crescents ($p<0.05$). After completion of induction therapy, there was no significant difference in remission status (76.0% vs 87.5%, p=0.33) or relapse status (8.1% vs 10.3%; p=0.22) for the IVCYC and MMF groups, respectively. There was no significant difference in occurrence of infective complications IVCYC 20(30.8%) vs MMF 3(15.7%) p=0.20 or mortality IVCYC 14(21.5%) vs MMF 1(5.4%) p=0.17 between both groups. Estimated GFR at baseline was the only predictor of death (1.0 [0.9 – 1.0]; p=0.001).

Conclusion: Our study shows similar outcomes following induction treatment with MMF or IVCYC in patients with biopsy proven proliferative LN in South Africa. However, a prospective and randomized study is needed to adequately compare these outcomes.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic multi-system autoimmune disorder with a predilection for young females(1). The involvement of the kidneys or lupus nephritis (LN) is a major cause of morbidity and mortality in SLE patients since up to 60% of adults with SLE develop LN (2). In South Africa, presence of LN was the only factor associated with mortality in 226 SLE patients, with a 5-year survival rate of 60%(3).

Management of LN requires a timely and coordinated use of immunosuppressive therapy, which consists of induction and maintenance phases. One of the goals of the management of LN is to achieve the best possible clinical efficacy with renal remission and minimal toxic effects from immunosuppressive agents. The treatment is dictated by the class of the disease and degree of activity and chronicity indices(4). Use of aggressive immunosuppressive therapy has improved the prognosis of SLE patients, but up to 5-20% still progress to end-stage renal disease (ESRD) within ten years following the diagnosis of LN(5)(6). The progression to renal failure in patients with LN is higher in patients of African-American descent(7). Clinical trials indicated that the combination of Cyclopshosphamide(CYC) with steroids was more efficacious in sustaining disease quiescence and improving renal outcomes than corticosteroids alone; however there were concerns of adverse effects with CYC. (8)(9)(10).

The demand for therapies which are less toxic with equal or better efficacy has motivated studies for novel medications. Several randomized controlled trials (RCT) comparing Mycophenolate Mofetil (MMF) and CYC as induction agents in LN have shown that MMF is as effective as cyclophosphamide and may offer some advantages with regards to lowered adverse events (11)(12)(13)(14). Given the high cost of MMF over CYC in Africa, very few

studies have shown report on MMF use for LN in Africans(15). The recent availability of generic formulations of MMF has led to reduced cost and increased usage in patients with LN in African settings. We assessed response following induction therapy in patients with proliferative LN diagnosed and treated with MMF or IVCYC at a single centre in South Africa.

METHODS

Study population

This study had a retrospective design and was carried out at the Division of Nephrology and Hypertension, Groote Schuur Hospital (GSH), Cape Town, South Africa. GSH is a tertiary hospital in Cape Town, Western Cape. The study included all patients with biopsy-proven proliferative LN and who received induction treatment with either IVCYC or MMF from January 2010 to December 2014 at the Division of Nephrology and Hypertension, GSH, and University of Cape Town.

Patients included in the study were patients with biopsy-confirmed proliferative lupus Nephritis (class III, IV, V mixed type) classified according to the ISN/RPS 2003 criteria(16). The study protocol was approved by the Ethics Committee of the University of Cape Town HREC 159/2016.

Data collection

We collected relevant data including demographic, clinical (e.g. blood pressure, use of ACE-inhibitors, induction with IVCYC or MMF), biochemical (serum creatinine, estimated glomerular filtration rate, urine protein-creatinine ratio, autoimmune biomarkers) and histological features of included patients. The estimated glomerular filtration rate was

calculated using the abbreviated Modification of Diets in Renal Disease (MDRD)(17). These data were recorded at time of biopsy, at 6 months, 12 months and at time of last follow-up visit. Information from biopsy reports including number of glomeruli, presence of interstitial fibrosis, number of crescents if present, and histology class were extracted. The side effects during treatment and any episodes of infection were recorded.

Variable measurement

Treatment response was measured by assessment of proteinuria and serum creatinine at regular intervals. Response to therapy in LN was assessed as complete response (CR), partial response (PR) or no response, ESRD, or death. Occurrence of these outcomes were measured at time of last follow up visit. Outcome measures were defined as per the Kidney Disease Improving Global Outcomes guidelines (KDIGO) as follows(18):

- Complete remission (CR): Return of serum creatinine to the previous baseline, plus a decline in the urinary protein to creatinine ratio (uPCR) to < 0.5g/24 hours
- Partial remission (PR): stabilisation (25%) or improvement of serum creatinine, but not to normal, plus a > 50% decrease in uPCR. If there was nephrotic range proteinuria (uPCR> 0.3g/24 hours), the improvement required a 50% reduction in the uPCR.
- Deterioration was defined as a sustained 25% increase in the serum creatinine.
- Relapse was defined as clinical manifestations indicating activity, namely, active urinary sediments, increasing proteinuria with or without serological reactivation in a patient who was previously in complete or partial remission

Statistical analysis

Statistical analysis was performed using Stata 15.1 (Stata Corp, TX, USA). The Student t-test was used to compare means between the two groups if normally distributed while the Mann-

Whitney U test was used for comparison in cases of deviation from normality. The Chi-square was used for the statistical comparison of proportions between the two groups. Univariable and multivariable logistic regression models were used to identify independent associations with remission. Kaplan-Meier graphs were used to show the time-to-mortality experience of different groups within the cohort with the log-rank test used to compare survival experiences statistically. Cox proportional hazard models were used to identify independent predictors of mortality in the cohort. All differences in baseline characteristics between the two study groups were controlled for in both multivariable models. A significant p-value was taken as $p<0.05$.

RESULTS

Baseline features:

Eighty-four patients with proliferative LN were included in the analysis. Overall, the mean age was 29.6±10.4 years with a female preponderance (88.1%). There was also a preponderance of patients of mixed ancestry (67.8%) but there was no significant difference in racial distribution of patients (p=0.86). Although mean systolic and diastolic blood pressures were higher in those treated with IVCYC than in those treated with MMF, only the mean arterial pressure was noted to be significantly higher in the IVCYC group (P=0.04) (Table 1). Other baseline features (for the groups and overall) are shown in Table 1. Overall, the median estimated glomerular filtration rate (eGFR) was noted to be 69.7 (IQR: 33.5 – 99.2 ml/min/1.73m); this was significantly lower in the IVCYC group (p=0.02). (Table 1).

Histological features and complications of treatment:

Overall, there was a median of 14 (10–18) glomeruli per biopsy taken. The IVCYC group had a significantly higher proportion of crescents than the MMF group (3.8 [0.0 – 32.4] vs 0.0 [0.0 –

6.2]; p = 0.03). Presence of sclerosed glomeruli or any degree of interstitial fibrosis was not significantly different between the groups.

Although infections (upper respiratory tract and urinary tract infections) occurred more frequently in the IVCYC group, there was no significant difference for infections between both treatment groups (p=0.20) (Table 2). Other complications that were reported including steroid induced diabetes and tuberculosis were also more frequent in the IVCYC group, but were not significantly higher than in the MMF group.

Remission and Relapse

At six months follow up, overall, remission (either complete or partial) occurred in 52 (78.8%, 95% CI: 67.0-87.9%) patients with no significant difference between both treatment groups, p=0.33 (Table 3). The relapse rate at 12 months of therapy was also not significantly different between both groups; p=0.22 (Table 3). No independent predictor of remission emerged to be statistically significant from multivariable analysis (Table 4).

Mortality

A total of 15 deaths were reported; most (14/15) were in the IVCYC group (Table 2). The median time to death was 105 days (IQR 45-267 days) from the date of biopsy. During a total of 32,159 person-days of follow up, the mortality rate for the CYC group was 5.5 per 10,000 person-days of follow up compared to 1.5 per 10,000 person-days of follow up for the MMF group. Figure 1 shows overall survival and survival between the two treatment groups. In all, no significant difference in mortality was found in any of the comparison pairs. Multivariable Cox-proportional hazards model showed eGFR at baseline as the only independent predictor of mortality in this group of patients (Table 5). For every 1 ml/min/1.73m^2 increase in eGFR at baseline there was a 5% reduced risk of mortality in this cohort.

DISCUSSION

An unmet need in the treatment of patients with LN in Africa is finding cheap and potent chemotherapeutic agents with minimal adverse effects. Combination IVCYC and high dose steroids have remained the cornerstone of treatment of LN in Africans leading to various side effects including infections, metabolic derangements and death(15)(19) (20). Minimal data exists on the spectrum, treatment, and outcome of LN in sub-Saharan Africa(21). We report a retrospective comparison of MMF and IVCYC in patients with proliferative LN treated in a single Centre in Cape Town, South Africa. The important findings from our study include: (i) after 6 months of induction therapy with either IVCYC or MMF, there was no difference in the proportion of patients attaining complete or partial remission between both groups, (ii) there was no significant difference in reported infections-related adverse events between both groups at the last follow-up visit and (iii) there was no significant difference in mortality between the two treatment groups (p 0.11).

The Aspreva Lupus Management study (ALMS) was the first international RCT to compare the efficacy and safety of MMF with IVCYC for the induction therapy of patients with confirmed LN(22). In the ALMS study, 370 patients with classes III through V LN were randomized to open-label MMF (target dosage 3 g/d) or IVC (0.5 to 1.0g/m2 in monthly pulses) in a 24-wk induction study. Both groups received prednisone, tapered from a maximum starting dosage of 60 mg/d. The primary end point was a prespecified decrease in UPCR and stabilization or improvement in serum creatinine. Secondary end points included complete renal remission, systemic disease activity and damage, and safety. Overall, there was no significantly different

response rate between the two groups: [MMF - 56.2% vs IVCYC - 53.0%; p = 0.58]. Secondary end points were also reported to be similar between both treatment groups(22). Further analysis of the trial results however, showed that in patients with “other” as racial group (mainly Hispanics and African Americans), there was a significantly higher proportion of patients who responded to MMF (60.3% vs 38.5%; p = 0.033).

Several studies comparing MMF and IVCYC for induction in patients with LN have since been published showing varied results but mainly that there is no difference in response rate between MMF and IVCYC treatments for induction(23)(24)(25)(26). A study from India randomized (equally) 100 patients to IVCYC or MMF for a 24 week induction treatment of LN(26). Baseline characteristics were similar between both groups; however, proteinuria was significantly higher in the IVCYC group. At 24 weeks, the complete remission rate was 50% in CYC and 54% in MMF group (p=0.91)(26). Our study, conducted in a predominantly African population (black Africans and Africans with mixed ancestry) did not find any difference in response as the complete and partial remission rates were similar between the IVCYC and MMF groups. However, we are aware that this might be a sample size effect given a smaller sample size for the MMF group.

The complications associated with treatment were infections (mainly upper respiratory and urinary tract types), pulmonary tuberculosis and steroid induced diabetes. Although our study did not find any significant difference in complications rate between both treatment groups, most occurred in the IVCYC group. This could be an indication of the severity of disease in this group (evidenced by lower eGFR at treatment initiation) or toxicity related to this treatment. For similar reasons, we also found higher mortality in the IVCYC group. Our findings are similar

to a study conducted by Thong et al. in which MMF was associated with lower infection risk than CYC in Non-Asians(14).

Our study has a number of limitations. First, this was a retrospective analysis of those treated in a single centre in South Africa and therefore has all the biases associated with retrospective reviews including inadequate sampling and inability to adequately assess and document all adverse events as they occurred. Second, compliance to treatment was only certain in those who were receiving IVCYC as they had to come in to the clinic to receive the monthly pulse intravenous treatment. Compliance could, however, not be ascertained in the MMF group. Thus, what role this might have played regarding our results is not known. Also, given that lupus disease activity indices are not routinely checked in the Nephrology clinic, we were unable to obtain these scores which we could have correlated with response and relapse rates between both groups. Finally, the choice of induction therapy was solely based on physician preference given that those with more severe presentation, evidenced by significantly lower eGFR, were more likely to have been treated with IVCYC. Since it's discovery, IVCYC has been the mainstay of therapy for LN (especially in Africa) given that it is readily available and cheap. The low use of MMF in our study is related to high cost. However, with availability of generic formulations and ability to monitor MMF levels in blood, it is expected that use of MMF will likely increase for the treatment of LN. Despite these limitations, our study is the first of its kind in South Africa where the prevalence of SLE (and LN) is highest amongst all sub-Saharan African countries. Therefore, our results provide evidence to clinicians for use of either MMF or IVCYC in treating patients with proliferative LN. We still recommend adequate monitoring of patients for infections and other side effects related to these therapies.

CONCLUSION

In South Africans with proliferative LN, our study has found no difference in response rate, relapse rate or occurrence of adverse effects to induction therapy with MMF or IVCYC. However, prospective studies to test our findings are still needed.

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CONFLICT OF INTEREST

None

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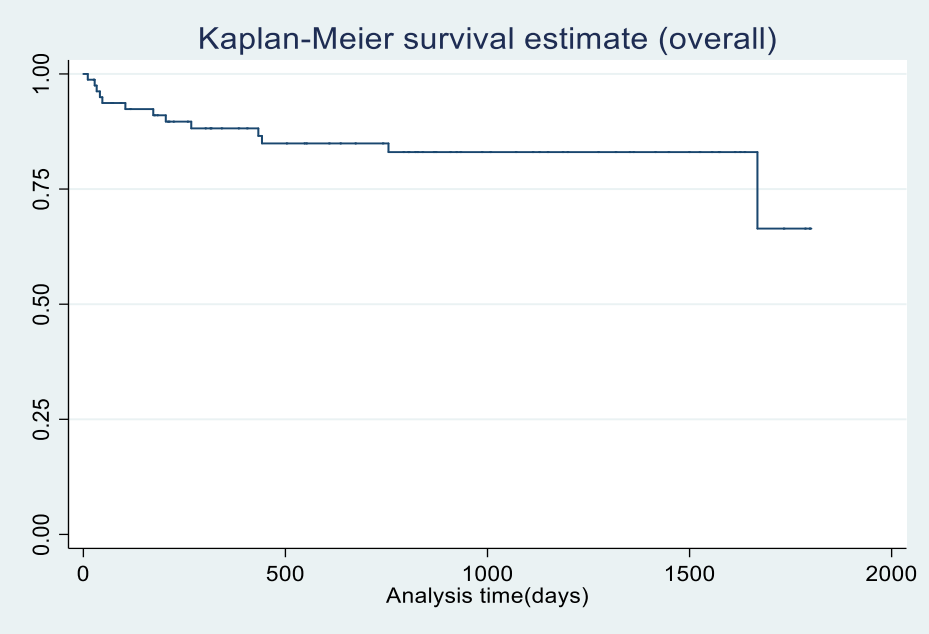

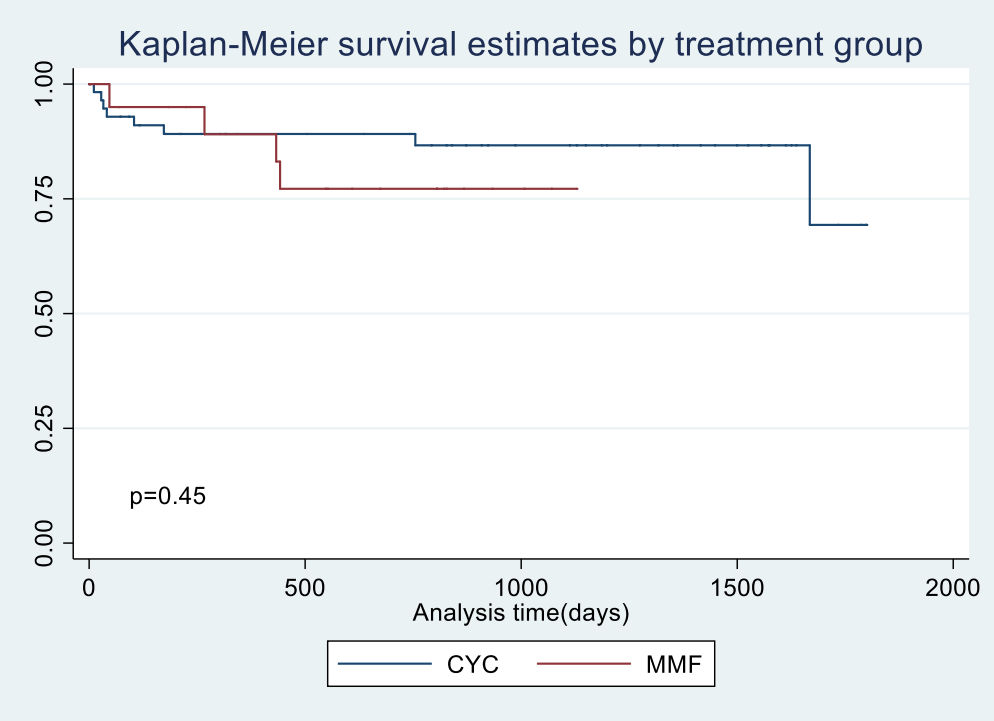

Figure 1: compares (a)shows overall survival and (b) survival between the IVCYC and MMF. In all, no significant difference in mortality was found in any of the comparison pairs.

Tables:

Table 1: Baseline demographic and clinical characteristics of study participants

	CYC group (n=65)	MMF group (n=19)	Total24 (N=84)	*p*-value
Mean age± SD (years)	30.3±11.2	27.0±6.9	29.6±10.4	0.23
Female sex (%)	74.3	25.7	88.1	0.07
Race (%)				
Black	30.7	31.5	30.9	
Coloured	67.6	68.4	67.8	0.86
White	1.5	0.0	1.1	
Mean SBP (at biopsy) (mmHg)	131.7±20.5	122.9±13.6	129.7±19.4	0.08
Mean DBP at biopsy (mmHg)	80.1 (14.2)	73.2±10.7	78.5±13.7	0.05
mABP (at biopsy) (mmHg)	97.4±15.0	89.7±10.9	95.7±14.5	**0.04**
Use of ACE-I/ARB (%)	89.2	94.7	90.5	0.47
Hb at biopsy (g/dl)	9.9±2.8	10.6±2.0	10.1±2.6	0.36
WBC at biopsy (x10^9/L)	8.1±4.5	6.7±3.5	7.8±4.3	0.23
Low C3 (%)	87.5	78.9	85.5	0.35
Low C4 (%)	69.8	79.0	72.0	0.44
Positive ANA (%)	86.9	88.9	87.3	0.82
Positive dsDNA(%)	90.3	84.2	88.9	0.46
Serum albumin (g/L)	28.2 ±6.9	29.5±9.59	28.5±7.5	0.51
Median SCr (IQR) (µmol/L)	96 (65-192)	70 (58-84)	84.5 (62-174)	**0.02**
Median eGFR (IQR) (ml/min/1.73m^2)	61.91 (27.9-94.5)	87.2 (69.6-106.8)	69.7(33.5-99.2)	**0.02**
uPCR (mg/mmol)	320(185-585)	290(170-670)	310 (170-590)	0.92

CYC=*Cyclophosphamide;* ***MMF=****mycophenolate mofetil;* ***SBP****=systolic blood pressure;* ***DBP****=diastolic blood pressure;* ***mABP****=mean arterial blood pressure;* ***ACE-I****=angiotensin converting enzyme inhibitor* ***ARB****=angiotensin receptor blocker;* ***Hb****=haemoglobin;* ***WBC****=white blood cell count;* ***ANA****=anti-nuclear antibodies;* ***dsDNA****=double stranded DNA;* ***SCr****=serum creatinine;* ***eGFR****=estimated glomerular filtration rate;* ***uPCR****=urine protein-creatinine ratio*,**SD**: *standard deviation.*

Table 2: Histopathological data and complications associated with both treatment groups

	CYC group	MMF group	Total	p-value
Number of glomeruli (median) (IQR*)	13(9-16)	18(12-26)	14(10-18)	0.01
Crescents %(IQR)	3.8(0.0-32.4)	0.0 (0.0-10.0)	0.0(0.0-28.6)	**0.03**
%Sclerosed glomeruli(IQR)	0.0(0.0-5.5)	0.0(0.0-10.0)	0.0(0.0-5.5)	0.59
Interstitial fibrosis n(%)	32(50.0)	7(36.8)	39(47.0)	0.31
IgG deposits(n=82)				
0	15(23.8)	4(21.1)	19(23.2)	
1	12(19.1)	2(10.5)	14(17.1)	0.80
2	23(36.5)	8(42.1)	31(37.8)	
3	13(20.6)	5(26.3)	18(21.9)	
IgM deposits				
0	11(17.6)	6(31.6)	17(20.7)	
1	13(20.6)	1(5.3)	14(17.1)	**0.04**
2	24(38.1)	3(15.8)	2732.9)	
3	15(23.8)	9(47.4)	24(29.3)	
IgA deposits				
0	34(54.0)	10(52.6)	44(53.7)	
1	18(28.6)	2(10.5)	20(24.4)	0.21
2	7(11.1)	4(21.1)	11(13.4)	
3	4(6.4)	3(15.8)	7(8.5)	
C3 deposits				
0	5(7.8)	2(10.5)	7(8.4)	
1	10(15.6)	4(21.1)	14(16.9)	0.61
2	20(31.3)	3(15.8)	23(27.7)	
Complications (%) :				
Infection	20(30.8)	3(15.8)	23(27.3)	0.20
Diabetes Mellitus	4(6.15)	0(0.0)	4(4.76)	0.57
TB	2(3.1)	0(0.0)	2(2.4)	0.22
Death	14(21.5)	1(5.3)	15(17.9)	0.17

CYC=*Cyclophosphamide;* ***MMF=****mycophenolate mofetil*
*IQR =Interquartile range

Table 3: Complete, partial remission and relapse in the two groups after therapy

	CYC group (95%CI)	MMF group (95%CI)	Total (95% CI)	P value
CR at 6 months	18.0 (8.6-31.4)	25.0 (7.3-52.4)	19.7 (10.9-31.3)	0.54
PR at 6 months	58.0 (43.2-71.8)	62.5 (35.4-84.8)	59.1 (46.3-71.0)	0.75
CR + PR	76.0 (61.8-86.9)	87.5 (61.6-98.4)	78.8 (67.0-87.9)	0.33
Relapse at 12 months	8.1 (1.7-21.9)	10.3 (4.3-48.1)	11.5 (4.3-23.4)	0.22

***CR**= complete remission; **PR**=partial remission; **CYC**=Cyclophosphamide; **MMF**=mycophenolate mofetil*

Table 4: Predictors of Remission

	Univariable models Odds ratio (95% CI)	P value	Multivariable model Odds ratio (95% CI)	P value
Induction regimen				
Cyclophosphamide	1		1	
Mycophenolate mofetil	2.2 (0.4-11.1)	0.34	3.2 (0.5-18.0)	0.20
Sex				
Female	1		1	
Male	1.7 (0.2-15.4)	0.64	2.4 (0.2-28.3)	0.48
Age (years)	1.0 (0.9-1.1)	0.77	1.0 (0.9-1.1)	0.43
Ethnicity				
Black	1		1	
Coloured	1.2 (0.3-4.5)	0.78	1.5 (0.3-6.5)	0.62
Baseline eGFR	1.0 (0.98-1.0)	0.85	1.0 (0.97-1.0)	0.43
Baseline uPCR	1.0 (0.99-1.00)	0.45	1.0 (0.99-1.0)	0.26
Interstitial fibrosis	1.0 (0.3-3.4)	0.95	1.7 (0.4-6.7)	0.46
% Crescents	1.7 (0.1-20.4)	0.68	3.0 (0.1-68.2)	0.49
% Sclerosed glomeruli	0.6 (0.0-9.4)	0.72	0.5 (0.0-14.6)	0.69

***eGFR**= estimated glomerular rate*; ***uPCR**=urine protein creatinine ratio*

Table 5: Predictors of mortality

	Hazard ratio (95% CI)	P value
Treatment Regimen		
CYC	1	
MMF	0.07 (0.003-1.87)	0.11
Age (years)	1.02 (0.94-1.09)	0.69
Sex		
Female	1	
Male	0.60 (0.06-5.87)	0.66
Histology type		
Non-proliferative	1	
Proliferative	0.41 (0.06-2.72)	0.35
Ethnicity		
Black	1	
Coloured	0.66 (0.14-3.01)	0.59
eGFR at baseline	0.95 (0.92-0.98)	0.001
uPCR at baseline	0.99 (0.99-1.00)	0.15
Mean arterial blood pressure	0.97 (0.93-1.02)	0.15
Interstitial fibrosis	0.34 (0.09-1.24)	0.10
% Sclerosed glomeruli	0.99 (0.07-12.99)	0.99
% Crescents	0.83 (0.08-8.37)	0.87

Q 1. Demographics	Sex			Race			Age		
Q2. Biopsy	First biopsy			Biopsy year			SBP biopsy		
Q3.Systolic blood pressures	SBP Biopsy	SBP 1ST visit	SBP 2ND visit	SBP 3rd visit	SBP 4th visit	SBP 5th visit	SBP 6th visit	SBP 12months	SBP Last visit
Q4. Diastolic blood Pressures	DBP biopsy			DBP 6months			DBP 12months		DBP Last FU
Q5. Haemoglobin levels	Hb at Biopsy			Hb 6months			Hb 12months		Hb Last FU
Q 6. White cell count	WCC at Biopsy			WCC 6months			WCC 12months		WCC Last FU
Q7. albumin	Albumin at Biopsy			Albumin 6months			Albumin 12months		Albumin Last FU
Q8. Serum Creatinine	creatinine at Biopsy			creatinine 6months			creatinine 12months		creatinine Last FU
Q9. eGFR	eGFR at Biopsy			eGFR 6months			eGFR 12months		eGFR Last FU
Q10. U PCR	U PCR at Biopsy			U PCR 6months			U PCR 12months		U PCR Last FU
Q11. Immunological results	C3 at Biopsy			C4 at Biopsy			ANA at Biopsy		Anti –Ds DNA at Biopsy
Q12. Biopsy results	Histology Class			# of glomeruli			# of crescents		# of sclerosed glomeruli
	Interstial fibrosis(Y/N)		Ig A deposits		Ig M		Ig G		C 3

Appendix 2: <u>Data Collection Sheet</u> **<u>Folder Number:</u>**

Treatment given								
Q 13. Treatment for induction	ACE/ ARB	CHLOROQUINE	SOLUMEDROL	ORAL PREDNISOLONE	CYC (GM IN MONTHS	CYC MONTHS GIVEN	MMF (gm FOR MONTHS)	MMF MONTHS GIVEN

Q 14. Treatment maintenance	Prednisolone (Y/ N)	Azathioprine (Y/N)	MMF (Y/N)	CYCLOSPORINE (Y / N)

Q 15. Treatment complications	Infections	TB	Diabetes

Q16 .DEATH (Y/ N)	IF YES CAUSE OF DEATH	No Death

Appendix 3: Ethics approval letter

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

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: nosi.tsama@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

18 March 2016

HREC REF: 159/2016

A/Prof I Okpechi
Nephrology & Hypertension
E13, NGSH

Dear A/Prof Okpechi

PROJECT TITLE: RETROSPECTIVE COMPARISON OF CYCLOPHOSPHAMIDE AND MYCCHENOLATE IN LUPUS NEPHRITIS (MMed-candidate P Sogayise)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

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

Approval is granted for one year until 30 March 2017.

Please add our contact details to the information sheet.

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

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr P Sogayise will also be involved this study.

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

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

Yours sincerely

Signature removed to avoid exposure online

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 159/2016

Appendix 3: Instructions to Authors South African Medical Journal (SAMJ)

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, and Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.

- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.