The retrospective analysis of patients with Lupus Nephritis at Helen Joseph Hospital

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A research report submitted to the University of the Witwatersrand in fulfilment of the requirements of the degree of Master of Medicine 2021.

DECLARATION

I, Lindokuhle Felicity Qwabe, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine (in the submissable format) to the department of Internal Medicine, University of the Witwatersrand, Johannesburg. It has not previously been submitted for any degree or examination purposes at this or any other university.

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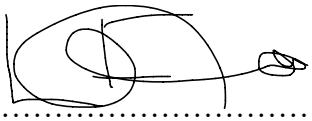
Signed:  ……..

Date:……16…….day….of …march….. 2021

DEDICATIONS

This work is dedicated to my loving parents, both educators by profession, who have taught me the importance of education, and that it is never impossible to reach ones' goals. A huge thank you to my friends, that supported me and encouraged me to not give up. To my son Mpilenhle Qwabe, I did this for us, and to show you nothing is impossible.

ACKNOWLEDGEMENTS

I am grateful to my supervisors Drs Hlanjwa Maepa and Kagiso Motse for their invaluable support, patience and inspiration during this journey. A big thank you to the staff at Helen Joseph Hospital, Renal unit, for their support and encouragement whilst collecting data for my study.

ABSTRACT

Background

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with multisystem involvement. The exact aetiology remains unknown. The kidney is the most involved visceral organ in SLE and is termed Lupus Nephritis (LN).

Objectives

The aim of this study is to determine the demographic profile, prevalence of LN and its histological subtypes, and to evaluate the clinical response to treatment. The study also aimed to identify common complications amongst patients with biopsy proven LN receiving immunosuppressive therapy, following up at Helen Joseph Hospital (HJH), South Africa.

Methods

A retrospective analysis of patient records from 2010-2018, kept in the Renal Unit at HJH was performed. Data was collected from all files of patients with biopsy proven LN following up at the unit. Lupus Nephritis histological subtypes observed; blood and urinary laboratory results; and immunosuppressive agents used during induction and maintenance therapy phases were evaluated.

Results

A total sample of 45 renal biopsies confirming LN was evaluated. This was comprised of 75.6% *(n=34)* females, and 24.4% *(n=11)* males, with a male to female ratio of 1:3.1. There was an African patient predominance of 68.9% (*n=31*) with a mean age of 32 years. Proliferative LN type III *(*26.7%) ,class IV (24.4%) and mixed classes accounted for (26,7%)

were the most common histological subtypes observed. Induction therapy in the form of Mycophenolate Mofetil (MMF) was given in 75.5% *(n=34)* of patients, whilst the remaining study population received cyclophosphamide (CYC). Seventy percent *(n=24)* of the MMF group achieved complete remission versus 18.2% *(n=2)* in the CYC group over a period of six months. Twenty seven percent *(n=12)* of patients in the cohort developed infection during the course of their treatment. These patients commonly presented with Pulmonary Tuberculosis, Urinary Tract Infection, and skin related infections. Other complications such as gastro-intestinal disease, steroid induced diabetes and osteoporosis were also observed.

Conclusion

Lupus Nephritis is a common complication seen in patients with SLE, with a strong female predominance. Most patients present with proliferative LN, warranting treatment with immunosuppressive therapy. Due to paucity of African and South African studies pertaining to LN; treatment regimens are extrapolated from European and American studies. This study aims to educate treating physicians on the clinical course of LN, and the best treatment modalities available, and their outcomes, in our South African setting.

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ABREVIATIONS

- ACR — American College of Rheumatology
- AZA — Azathioprine
- APOL1 — Apo lipoprotein 1
- CKD — Chronic Kidney Disease
- CD4 T — Cluster of Differentiation 4 T
- CHBAH — Chris Hani Baragwanath Academic Hospital
- CYC — Cyclophosphamide
- DNA — Deoxyribonucleic Acid
- EBV — Epstein Barr Virus
- ESRD — End Stage Renal Disease
- HLA II — Human Leucocyte Antigen II
- HJH — Helen Joseph Hospital
- HIV — Human Immunodeficiency Virus
- IFM — Immunofluorescence Microscopy
- ISN/RPS Society — International Society of Nephrology /Renal Pathology
- KDIGO — Kidney Disease Improving Global Outcomes
- LN — Lupus Nephritis
- LFA-1 — Lymphocyte function associated antigen: I
- MMF — Mycophenolate Mofetil
- SLE — Systemic Lupus Erythematosus
- SLICC — Systemic Lupus International Collaboration Clinics

CHAPTER ONE: PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1 Introduction and background

Systemic Lupus Erythematosus (SLE) is a debilitating chronic autoimmune disease, with incidence and prevalence greatly influenced by gender, ethnicity, and geographic region.[1] The exact epidemiology, incidence and prevalence of SLE, and its disease manifestations remains undetermined in Africa. The pathogenesis of disease remains poorly understood with several factors such as genetic predisposition, environmental, certain viruses namely Epstein Barr Virus and drugs, reported to influence the development of the disease.[2-6]

The loss of tolerance against nuclear auto antigens, with resultant immune complex formation is the key feature in patients developing SLE.[1,3] The formed immune complexes are then deposited in the different organ systems, causing chronic inflammation.[1] The disease therefore manifests itself in different organ systems, with 95% of patients commonly complaining of arthralgia or arthritis.[1,5] This is followed by dermatological, neuropsychiatric and renal manifestations.[5] The kidney is the most commonly involved visceral organ in SLE, and is known as Lupus Nephritis (LN).[2-4]

1.2 Prevalence and Incidence of Lupus Nephritis

Studies have shown that 40-50% of patients with SLE will develop renal involvement within the first 10 years of diagnosis.[6-8] International studies have shown a significantly higher incidence of LN in the African American population compared to the Caucasian population.[9-12] The overall incidence reported is 1.8 to 7.6 cases per 100,000 people with SLE.[10,13]

1.3 Pathogenesis of Lupus Nephritis

The pathogenesis of LN is complex; involving different immunological pathways that result in the development of autoimmunity and immune complex deposition.[2,14] The mainstay of diagnosis of LN is the renal biopsy.[15] Visualisation of the deposited autoantibodies namely Immunoglobulin A, M, and G on microscopy, is diagnostic of LN.[10]

These visualised antibodies can be deposited in the mesangium, subendothelial and subepithelial spaces, or the peritubular capillaries. Deposition of the auto-antibodies activates the innate immune system, which in turn activates the T and B lymphocytes. [1]Adaptive immune cells are then activated and a continued release of pro-inflammatory cells (Neutrophils) and cytokines ensues.[1,11] This in turn results in impaired clearance of apoptotic debris, due to increased exposure to the auto-antigens.[1,10,11]The autoantibodies then bind to the circulating antigens, forming immune complexes. These bind and deposit at the renal glomerular membrane and vessel wall, thus initiating an inflammatory and cytotoxic reaction causing podocyte damage.[11]

1.4 The role of genetic susceptibility in Lupus Nephritis

SLE develops in genetically susceptible individuals, with studies showing a variety of genetic abnormalities that predispose individuals to the disease. Human Leucocyte antigen (HLA) II gene polymorphism, HLA II DR2 and HLA II DR3, are commonly found in patients with SLE.[11,15-17] HLA II DR2 subtype has been identified in the black population and is linked with worsening renal disease.[5,18] A local study done in Cape Town, with a predominant coloured population, demonstrated HLA DR2 association in patients with LN.[17]

The Apolipoprotein 1 (APOL1) gene that has been implicated in the development of End Stage Renal Disease (ESRD) amongst the black population, has also been associated with

disease progression to ESRD in patients with LN.[11] The APOL 1 genetic variant (alleles G1and G2) in SLE patients, confer increased susceptibility to develop LN in black South Africans.[11,19] FcγRII A polymorphism has also been observed more in African American and Korean populations with LN.[6,11]

Identification of the different genetic mutations would aid in early detection of LN and commencement of appropriate therapy, however, these genetic tests are costly and almost inaccessible to the African continent. Their use at this stage, needs further research and cannot be generalised.

1.5 The renal biopsy and the histo-pathology classification

Lupus Nephritis is a histo-pathological diagnosis and a renal biopsy is the gold standard in making the diagnosis.[3,20] It provides pertinent information regarding the extent of disease activity, chronicity, and informs treating physicians on the best treatment options for each individual patient.[10,20] In addition, the diagnosis of LN is made on the following clinical and biochemical findings: the presence of persistent proteinuria of more than 0.5g/day, or greater than 3+ proteinuria by urinalysis; active urinary sediment comprised of more than five red or white blood cells; granular, tubular and or mixed casts associated with an unexplained increase in serum creatinine in patients with SLE.[20-22]

Patients with SLE may have several different types of renal manifestations. [23] The most common finding being immune-mediated glomerular disease often associated with tubulo-interstial changes.[23]Involvement of the renal vasculature is also common, ranging from indolent vascular immune deposits to fibrinoid necrosis and thrombotic micro-

angiopathy.[10,13,15,24] The first World Health Organization (WHO) classification of LN was done in 1974 by *Pirani et al*.[10] In 2003, the International Society of Nephrology (ISN) /Renal Pathology Society (RPS) formulated a revised classification of LN that is currently in practice.[10,13,15] In this classification, the acceptable renal biopsy should include a minimum of 10 glomeruli to exclude the existence of a focal lesion; must be studied by immunofluorescence microscopy (IFM) including IgA, IgG, IgM isotypes; Kappa and Lambda light chains; as well as C3 and C1q complement components.[8,10,25]

• Class I Minimal Mesangial LN • Class II Mesangial Proliferative LN • Class III Focal Proliferative LN: ➢ III A: Purely active lesions. This is a focal proliferative LN ➢ III A/C: Active and chronic lesions. This is a focal proliferative and sclerosing LN ➢ III C: Chronic inactive lesions with glomerular scars. A focal sclerosing LN.	• Class IV Diffuse Proliferative LN: ➢ IV A: Purely active lesions. This is a diffuse segmental or global proliferative LN. ➢ IV A/C: Active and chronic lesions. A diffuse segmental or global proliferative and sclerosing LN. ➢ IV C: Inactive with glomerular scars. This is a diffuse segmental or global sclerosing LN. • Class V Membranous LN • Class VI Advanced Sclerosing LN[7]

Table :1 International Society of Nephrology (ISN) /Renal Pathology Society (RPS) Lupus Nephritis classification

The National Institute of Health (NIH) developed guidelines to assess LN activity, chronicity and prognosis. Poor LN disease outcomes are associated with higher activity and chronicity indeces. [8,27-29] Glomerular lesions that define activity on light microscopy include: endocapillary hypercellularity with or without leukocytes with substantial luminal reduction;

karyorrhexis or fibrinoid necrosis; glomerular basement membrane rupture; crescents, cellular or fibro-cellular sub-endothelial deposits.[23]

Intraluminal immune aggregates (hyaline thrombi); and tubular interstitial inflammation or infiltration are also used to characterise glomerular activity. Each lesion is scored zero to three (0-3+).[13] Severe lesions of crescents and fibrinoid necrosis are given double weight. The total sum of the six components yields a total of zero to 24.[13] Chronic lesions are characterised by glomerular sclerosis (segmental, global); fibrous crescents; tubular atrophy and interstitial fibrosis.[13] A chronicity index of zero to 12 is obtained from the total sum of lesions found, and each is scored zero-three.[13]

Several studies have shown an increased frequency in the proliferative forms of LN (class III, class IV and mixed class V) which are associated with poorer outcomes.[29,30]A cohort of 315 patients in Tygerberg Hospital revealed Class IV LN as the most common pattern seen in the past three decades.[31]

1.6 Clinical characteristics of the different histological types of Lupus Nephritis

1.6.1 Class I and Class II

The histological finding of mesangial immune deposits with or without mesangial proliferation under light microscopy is characteristic of these classes.[7,14] They are predominantly asymptomatic with mild urinary abnormalities such as sub-nephrotic proteinuria or microscopic haematuria.[14,32]

1.6.2 Class III and Class IV

Studies have shown class III to have a heterogenous clinical presentation ranging from nephrotic syndrome, renal dysfunction and active urinary sediments.[14] Class IV is the most active and severe subtype of LN with 50% of patients presenting with nephrotic range proteinuria and renal dysfunction.[9,14,32]

1.6.3 Class V and Class VI

Renal impairment is uncommon in class V LN, with 59-70% of patients presenting with nephrotic range proteinuria.[14,32] Class VI is associated with end renal stage disease presenting with severe renal impairment with high levels of serum creatinine.[32]

1.7 Management of Lupus Nephritis

Treatment of LN is dependent on the histopathological classification of LN as per ISN/RPN classification, and the degree of renal impairment. The main objectives of treatment in LN are to reduce urinary excretion of protein and delay progression of renal pathology to End Stage Renal Disease (ESRD).[11,33]

1.7.1 Non- Proliferative Lupus Nephritis

Class I and II LN are described as non-proliferative subtypes of LN and are associated with a good clinical outcome. They do not require specific immunosuppressive therapy directed to the kidney.[7,14] The KDIGO guidelines recommend that management should include aggressive treatment of co-existing extra-renal lupus manifestations to improve outcomes.[7,14]

1.7.2 Proliferative Lupus Nephritis

Lupus nephritis subtypes greater than class II are termed as proliferative LN. They follow a more aggressive course requiring early treatment to improve outcome. Treatment of the proliferative sub-types of LN is comprised of induction and maintenance therapy. The use of immunosuppressive agents in proliferative LN has improved the overall survival rate to about 80% in 5 years.[11]

1.7.3 Induction Therapy

The KDIGO and ACR guidelines recommend the use of high dose corticosteroids in combination with cyclophosphamide (CYC) or mycophenolate mofetil (MMF) for proliferative LN.[6] Steroid therapy consists of an intravenous methylprednisolone pulse of 250mg-1000mg for three days, followed by oral prednisone.[6,14] The oral prednisone dose is initiated at a dose of 0.5-1.0mg/kg/day (with a maximum dose of 80 mg/day) for a few weeks and then tapered off to the lowest effective dose.[6,22]

The NIH suggests a course of intravenous CYC at a dose of 500-1,000mg/m^2 of body surface area (BSA) given over six to seven months.[34,35] This is the recommended regimen compared to the extended course of oral CYC that was associated with significant adverse events and higher cumulative doses of CYC. The KDIGO guidelines recommend a lifetime maximum dose of 36g for CYC.[14]The alternative is to use the Euro Lupus regimen which consists of CYC 500mg given every two weeks for three months in combination with oral steroids.[7] A CYC containing regimen is the gold standard in inducing renal remission and preventing renal flares in patients with proliferative LN.[6,9] This regimen has a response rate of more than 80% in the Caucasian population with poorer response in the African American and Hispanic population.[6] It is however, associated with severe drug related complications such

as bone marrow suppression, premature gonadal failure, opportunist infections, hemorrhagic cystitis, and an increased risk of malignancy.[6,7,9,36]

Mycophenolate Mofetil is an alternative drug for induction therapy. It is a reversible inhibitor of inosine monophosphate dehydrogenase.[9,34]It mainly inhibits B and T cell proliferation as well as antibody formation.[34] The initial dose may be started at 500mg twice a day and built up to the maximum tolerated dose.[14] A targeted maximum dose of 3g/day may be used.[9,14,15,22] Recent studies have demonstrated a good response rate with MMF of up to 80%.[9,12,34] The most common adverse event encountered with the use of MMF is gastro-intestinal intolerance. This agent is mostly well tolerated with fewer side effects such as severe infection, alopecia and amenorrhea seen with the use of CYC.[34]

1.7.3.1 Mycophenolate Mofetil verses Cyclophosphamide

Studies evaluating the remission rates of patients with proliferative LN on MMF versus CYC during induction therapy have concluded that both agents have similar efficacy.[13]An Asian study showed that 82.6% of patients placed on MMF regimen achieved remission over 24 weeks.[6] Of these patients almost 35% had complete remission; whilst 48.4% had partial remission in the stated period.[40] This implies that patient ethnicity has a role to play in drug response and achieving disease remission. Careful patient selection when choosing between MMF and CYC remains important.

1.7.3.2 Mycophenolate Mofetil and the African patient with Lupus Nephritis

MMF has been shown to be an effective agent during the induction and maintenance therapy phases, in the non-Caucasian population with severe LN.[6] Genetic factors in metabolism of drugs (particularly MMF) have been implicated in contributing to how individual patients respond to immunosuppressive therapy. [38] Miura *et al* identified SLCO1B1 and SLO1B3 polypeptide polymorphism as the main cause for poor response to MMF therapy in Japanese transplant patients.[38] Mok *et al* attributed the ethnic influence to treatment to be based on socioeconomic factors such as level of education, treatment compliance and health care availability which are relevant in the African population.[9,28]

1.7.4 Adjuvant Therapy

Supportive therapy in all classes of LN is aimed at treating co-existing disorders and other clinical manifestations of SLE. Angiotensin Converting Enzyme inhibitors (ACEi), or Angiotensin Receptor Blockers (ARBs) are first line anti-hypertensive agents in the management of hypertension with proteinuric kidney disease.[7,8,12,14,22,29,34,39]These agents can be safely used simultaneously with immunosuppressive agents in induction and maintenance therapy phases of LN. Diuretic therapy is important for symptomatic relief of oedema in patients with overt nephrotic syndrome.

KDIGO guidelines also recommend that all patients with LN, unless contra-indicated, must be treated with antimalarial therapy.[12,14,22,39] Research has shown that lack of antimalarial therapy use may be associated with an increase in LN treatment failure.[29]Anti-malarial drugs such as chloroquine and hydroxychloroquine have been proven to have anti-inflammatory, antithrombotic and anti-lipidaemic effect.[14,29,33,40] This results in better disease control and reduced number of renal flares.[37]The aim of antimalarial drugs in LN is to prevent disease progression to ESRD and to prolong renal survival.[14,29]

1.7.5 Maintenance Therapy

Azathioprine (AZA) and MMF are currently the treatment options for the maintenance phase of LN. In the MAINTAIN Nephritis Trial, MMF 2g/day was compared to AZA 2mg/kg/day as maintenance therapy after induction with low dose intravenous CYC, which was 500mg of CYC every 2 weeks for 3 months i.e. Euro- lupus regimen.[6,41] The study showed that both drugs were equally effective in preventing renal flares.[39] The Aspreva Lupus Management Study (ALMS) showed MMF to be more superior than AZA regardless of the induction therapy used, particularly in black and Hispanic patients.[41] Azathioprine used in high doses is associated with more adverse effects such as macrocytosis and leucopenia, when compared to MMF.[7]

In view of both studies, MMF is the drug of choice to use for maintenance therapy in LN. Oral prednisone, up to a permitted dose of 10mg/day, can be used in combination with AZA or MMF during the maintenance phase of LN. Immunosuppressive therapy may be tapered to the most effective minimum dose once the disease is clinically and serologically inactive for at least one year. The average duration as per KDIGO guidelines for immunosuppressive agents is 3.5 years.[14]

1.7.6 Therapeutic options for refractory Lupus Nephritis

Disease progression despite optimal immunosuppressive therapy is termed refractory LN. This indicates treatment failure requiring intensification of therapy or re-induction of immunosuppressive agent. The ACR guidelines advocate switching from CYC to MMF oe vice versa in cases of refractory LN.[39,40] A repeat renal biopsy is indicated in patients who show a poor response to initial induction therapy. The KDIGO guidelines recommend a

review of therapy every three months for early detection of refractory disease. Patients who have failed both CYC and MMF, are to be initiated on Rituximab (RTX), tacrolimus or cyclosporine (a calcineurin inhibitor).[12,33,39,40]

Rituximab is a monoclonal antibody, which depletes auto-reactive CD20 B cells, thereby reducing the production of autoantibodies necessary for disease manifestation of LN.[37,40] The ACR guidelines have promoted the use of Rituximab as mono-therapy, or to be added to another immunosuppressive agent. In contrast, The Lupus Nephritis Assessment with Rituximab (LUNAR) trial has reported no additional benefit to adding an agent such as RTX in the treatment of refractory LN.[37,40] This implies that, the use of RTX in refractory LN, should be individualized and that benefit of using this drug cannot be generalized at present.

Calcineurin inhibitors such as cyclosporine A and Tacrolimus exert a potent anti-proteinuric effect required in LN. [40] They block the release of inflammatory cytokines, T cell activation, thereby decreasing the overall inflammatory response. These above-mentioned drugs may be used as monotherapy or in combination with MMF as part of a multi-targeted approach in the treatment of refractory LN.[40]

1.7.7 Assessment of response to therapy

Studies have revealed that patients with LN who showed more than 25 percent reduction in proteinuria, and normalization of the serum C3 and/or C4 after eight weeks of induction therapy were likely to have a better prognosis.[22,32] Similarly, after 6 months of treatment, a decrease in serum creatinine level , as well as proteinuria levels less than 1gram/ 24 hours, predicted a good long-term outcome.[15,39,42]

Several criteria have been used to define and monitor clinical response to immunosuppressive therapy. In a randomized control trial of 370 patients with nephrotic and sub-nephrotic range LN, they defined treatment response as the following: (1) a decrease in urine protein / creatinine ratio (UPCR) of less than 3grams in 24 hours in the nephrotic range LN group; (2) a more than 50% reduction in urine PCR (in the case of sub-nephrotic LN); and (3) stabilization or improvement in serum creatinine at 24 weeks.[39] A reduction in UPCR therefore correlates with improved renal survival, and decrease in progression of renal disease to ESRD.[31,39]

1.8 Determining factors affecting prognosis in Lupus Nephritis

In developing countries, such as South Africa, SLE is associated with increased mortality and morbidity when compared to developed countries.[17] Multiple aetiological factors have been identified cause for progression of LN to ESRD requiring renal replacement therapy (RRT).[43] These patients have poor overall survival when compared to other SLE patients with no renal involvement.[3,12] Black and Hispanic population have been reported to have the worst outcome.[18,22,35,37,43,44] It is with this data in mind, that one can extrapolate that African survival rates would also be reduced.This has been hypothesized to be due to poor socio-economic conditions commonly found in developing countries.[27]

Barr *et al* noted an increased risk of doubling serum creatinine when adjusting for socio-economic status, which was statistically significant in the Hispanic population.[18,45] Okpechi *et al* observed pregnancy was associated with an increase in disease activity; as well as pregnancy related complications such as pre-eclampsia, preterm labour and foetal mortality.[20]

In another study, hypertension was noted to significantly influence the degree of renal function decline in patients with proliferative LN, thereby conferring a poor outcome.[13,39]

An increase in the number of renal flares is strongly associated with the development of CKD, as well as progression to ESRD in patients with already established CKD.[46] Houssiau *et al* in Euro-Lupus Nephritis Trial demonstrated that a decrease in serum creatinine level, and proteinuria of less than 1gram/24 hours at six months, was the best predictor of good long-term renal outcome.[35]

Even with treatment improvement over the past decades; LN is still associated with a high mortality and morbidity. This can be related to immunosuppressive therapy or disease progression. Several complication have been reported by both international and local studies; the commonly reported complications are seen with CYC; i.e. bone marrow suppression presenting with cytopenia's, haemorrhagic cystitis and infertility. Infection has been reported more with patients taking CYC when compared to those taking MMF.[34] LF *et al* study reported a 7% bacterial infections rates amongst their cohort. Severe infections such as pneumonia and sepsis was noted and viral infections such as herpes zoster was also seen in 7 of their patients.[34] Mycophenolate Mofetil has been reported have less side effects and less infection rates, with gastro-intestinal symptoms being the most common. Other infections that can be seen in patients with LN are those associated with the chronic use of systemic steroids[9,12,34].

1.9 Conclusion

Lupus Nephritis is a critical manifestation of SLE, with higher mortality and morbidity observed in the African-American, Hispanic and Asian populations. The proliferative histo-

pathological subtypes are most commonly observed at presentation and treatment should be followed diligently to prevent disease progression to ESRD, and death.

The treatment of LN has evolved in the past few decades with the availability of immunosuppressive therapy that can be used during both induction and maintenance therapy phases. The use of MMF seems to be ideal for our predominant black African population, with fewer side effects when compared to CYC. Patients with LN are prone to relapsing, and therefore need close monitoring to prevent refractory LN with treatment failure and resultant disease progression to ESRD. Several studies have suggested monitoring of proteinuria to be the strongest predictor of response to treatment.

Azathioprine remains a good alternative therapy to MMF in the maintenance phase of treatment. Newer agents such as Calcinurin inhibitors and Rituximab have been used in cases of refractory LN with treatment failure. The major challenge in Africa is lack of access and high costs associated with these drugs.

There is paucity of research pertaining to LN in the African population, with most treatment regimens guided by American and European population studies. Clinicians in Africa, are therefore obligated to research SLE and Lupus Nephritis more aggressively to improve disease outcome and ensure successful management strategies. In conclusion, this study was conducted to evaluate LN in our unique population at Helen Joseph Hospital (HJH), Renal Unit. Disease prevalence, response to immunosuppressive therapy, and complications were

evaluated during our study. This study will aid in educating treating physicians on disease manifestations, and best treatment options for patients with LN in our African population.

1.10 Study aims and Objectives

1.10.1 Aims

This study aims to profile LN in a mixed demographic urban African population receiving treatment at Helen Joseph Hospital (HJH), Johannesburg, South Africa.

1.10.2 Study objectives

- To determine the demographic profile of patients with biopsy proven Lupus Nephritis at Helen Joseph Hospital.
- To determine the prevalence of the different histo-pathological subtypes of Lupus Nephritis at Helen Joseph Hospital.
- To determine the response to treatment used during the induction and maintenance phases of therapy, in patients treated for Lupus Nephritis at the renal outpatient clinic at Helen Joseph Hospital**.**

1.11 Methods

1.11.1 Study Design

This study will be a retrospective analysis of all biopsy proven Lupus Nephritis patients attending the renal clinic at Helen Joseph hospital (HJH). Helen Joseph Hospital is one of the tertiary hospitals in Gauteng, South Africa. This hospital provides medical services to a low to medium income population of approximately one million people. The hospital has 21

wards, the majority of which are occupied by medical patients. It has a fully functional intensive care unit (ICU) , high care and a renal dialysis unit.

1.11.2 Study Sample

In the study, a total of 74 biopsy records were found in the renal unit records. Only 45 patient records fulfilled all criteria to be included in this study, and were therefore, further evaluated.

Inclusion Criteria

- Biopsy proven Lupus Nephritis
- Patients over the age of 18 years.

Exclusion criteria

- End Stage Renal Disease (ESRD) on dialysis at initial presentation.
- Inadequate clinical records

1.11.3 Data Collection

All data was collected from the renal patient records, kept at the renal clinic file room. This data was cross referenced with the biopsy records file kept in the renal unit. The names of the patients and file numbers were used to identify appropriate histology and blood results. Data was recorded and stored using a numerical system in a password protected excel data sheet.

Data to be collected included:

- Demographics
- Comorbidities
- Lupus Nephritis histo-pathological subtype observed on renal biopsy.
- Adjunct serum investigation results
- Other SLE clinical manifestations
- Induction therapy and Maintenance therapy agents used.
- Disease remission and relapses
- Lupus Nephritis associated complications.

Assessment of clinical response variable used

- Complements C3 and C4
- Hemoglobin
- UPCR
- Renal function

1.11.4 Statistical Analysis

All data collected was recorded on a data collection sheet, and digitally captured on a Microsoft Excel spreadsheet. Findings from the data collected were analysed using the latest STATA MP software, version 15.0 (StataCorp, Texas, USA). Categorical variables were described using frequencies and percentages; the relationship between categorical variables was assessed using a chi squared test. The continuous data was assessed for normality and where appropriate, presented as means and standard deviations. The differences in continuous variables and non-parametric equivalents between groups was assessed using a T test. All analysed data was performed at 95% confidence interval. A p value < 0.05 was defined as being statistically significant. The population group in HJH is diverse but predominantly an African ethnic group. This allowed for a comparative analysis of the treatment regimen currently used.

1.12 Definitions

- Complete Response (CR): This was defined as proteinuria <0.2grams/day with a stable estimated Glomerular filtration rate (eGFR), or an improvement of eGFR by >25% from baseline.
- Partial Response (PR): Defined as a reduction in proteinuria between 0.2-0.29grams/day, stable eGFR if normal at baseline, or an increase >25% from baseline if abnormal.
- Hypertension: Patients with systolic blood pressure of >140mmHg and diastolic blood pressure >90 mmHg, or if patient is on anti-hypertensive agents.

- Refractory disease: This is defined as persistent proteinuria of >3grams/day or progressive or worsening renal function.

1.13 Limitations

This was a retrospective study, with anticipated missing patient clinical records and laboratory investigation results due to lack of standardised record keeping. There were inconsistencies in interpretation of patients' clinical condition by different treating physicians, as observed on clinical notes. In those instances; blood, urine and histology results were used versus physician assessments on patients condition. Additionally, some patients were lost to follow up, reducing the sample size significantly. Therefore, the researcher has considered that the sample size obtained may not be truly reflective of LN in this population. This implies, research findings cannot at present be generalised.

1.14 Ethical consideration

The study was granted clearance on the 30th June 2017 by the Human Research Ethics Committee (medical) (clearance certificate No. M170643).

1.15 Schedule

	Research protocol	Protocol submission	Ethics Application Submission	Data Collection	Data Analysis	Research Report	Research Submission
April – May 2017							
May 2017							
June 2017							
July-August 2018							
September-October 2018							
October 2018-September 2020							
September 2020							

1.16 Funding

Stationery, photocopying, printing, and binding were anticipated and paid for by the researcher.

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CHAPTER 2: SUBMISSIBLE ARTICLE

TITLE: THE RETROSPECTIVE ANALYSIS OF PATIENTS WITH LUPUS NEPHRITIS AT HELEN JOSEPH HOSPITAL IN GAUTENG.

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Conflict of interest: Nil

Key words: Systemic lupus erythematosus Lupus nephritis Human Immune Deficiency Virus Mycophenolate Mofetil chronic kidney disease

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Total word count: 7587

Abstract word count: 381

ABSTRACT

Background

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with multisystem involvement. The exact aetiology remains unknown. The kidney is the most involved visceral organ in SLE and is termed Lupus Nephritis (LN).

Objectives

The aim of this study is to determine the demographic profile, prevalence of LN and its histological subtypes, and to evaluate the clinical response to treatment. The study also aimed to identify common complications amongst patients with biopsy proven LN receiving immunosuppressive therapy, following up at Helen Joseph Hospital (HJH), South Africa.

Methods

A retrospective analysis of patient records from 2010-2018, kept in the Renal Unit at HJH was performed. Data was collected from all files of patients with biopsy proven LN following up at the unit. Lupus Nephritis histological subtypes observed; blood and urinary laboratory results; and immunosuppressive agents used during induction and maintenance therapy phases were evaluated.

Results

A total sample of 45 renal biopsies confirming LN was comprised of 75.6% *(n=34)* females, and 24.4% *(n=11)* males, with a male to female ratio of 1:3.1. There was an African patient predominance of 68.9% (*n=31*) with a mean age of 32 years. Proliferative LN type III *(*26.7%) ,class IV (24.4%) and mixed classes accounted for (26,7%) were the most common histological subtypes observed. Induction therapy in the form of Mycophenolate Mofetil (MMF) was given in 75.5% *(n=34)* of patients, whilst the remaining study population received cyclophosphamide (CYC). Seventy percent *(n=24)* of the MMF group achieved complete remission versus a minority 18.2% *(n=2)* in the CYC group over a period of six months. Twenty seven percent *(n=12)* of patients in the cohort developed infection during the course of their treatment. These patients commonly presented with Pulmonary Tuberculosis, Urinary Tract Infection, and skin related infections. Other complications such as gastro-intestinal disease, steroid induced diabetes and osteoporosis were also observed.

Conclusion

Lupus Nephritis is a common complication seen in patients with SLE, with a strong female predominance. Most patients present with proliferative LN, warranting treatment with immunosuppressive therapy. Due to paucity of African and South African studies pertaining to LN; treatment regimens are extrapolated from European and American studies. In this study it can be extrapolated that the use of MMF is more superior than CYC; as our population is dominated by black Africans.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a debilitating chronic autoimmune disease with a prevalence greatly influenced by gender, ethnicity, and geographic region.[1]The exact epidemiology and prevalence of SLE, and its disease manifestations remain undetermined in Africa. The pathogenesis of the disease remains poorly understood with several factors such as genetic predisposition, environmental, certain viruses namely Epstein Barr Virus, and drugs, reported to influence the development of the disease.[1]

The loss of tolerance against nuclear auto antigens, with resultant immune complex formation is the key feature in patients developing SLE.[1] The formed immune complexes are then deposited in the different organ systems causing chronic inflammation.[1] SLE is a multi-organ disease, with a vast majority (95%) of patients commonly complaining of arthralgia or arthritis.[2] This is followed by dermatological, neuropsychiatric and renal manifestations respectively.[2] The kidney is the most commonly involved visceral organ, with 40 – 50% of SLE patients developing Lupus Nephritis (LN). [1,3-4]American studies have consistently reported a significantly higher incidence of LN in the African-American population compared to the Caucasian population.[3-5] The overall incidence reported is 1.8 to 7.6 cases per 100,000 people with SLE.[1-2,4-5]

The Pathogenesis And Diagnosis Of Lupus Nephritis

The mainstay of diagnosis of LN is the renal biopsy.[3] It is on renal biopsy that immune complex deposition (IgA, IgM, and IgG), due to different immunopathological pathways, is visualised at the glomerulus, mesangium, subendothelial and subepithelial spaces, and/or around the peritubular capillaries.[3-4] The immune complexes deposited initiate an

inflammatory, cytotoxic reaction, that if left untreated, will continue to damage the renal micro-architecture, with resultant End Stage Renal Disease (ESRD).[4]

Genetic abnormalities including Human Leucocyte antigen (HLA) II gene polymorphism, apolipoprotein 1 (APOL1) gene mutations, and FcγRII A polymorphism have been observed in African patients with LN.[6,7] These genetic mutations are hypothesised to be the contributing factors in the development of LN, with its presence predicting a severe form of LN.[6,7] Genetic testing in developing countries such as South Africa is not readily available and therefore cannot be implemented as a standardised

The diagnosis of LN can be challenging in early disease and asymptomatic patients who show no clinical features of kidney disease. The evaluation of urine for sediment, urine dipstick testing and laboratory examination of urea and electrolyte (U&E) may be a useful initial screening tool.[1] The diagnosis of LN is made by the presence of persistent proteinuria of more than 0.5g/day, or greater than 3+ proteinuria by urinalysis; active urinary sediment comprised of more than five red or white blood cells; granular, tubular and or mixed casts associated with an unexplained increase in serum creatinine in patients with SLE.[1,3-5,7-9]

Although serum and urinary biomarkers are primarily used to assist make the diagnosis of LN, they are further used to measure disease activity, evaluate risk of renal flares , and predict disease outcomes. The most commonly used serum biomarkers are Anti-dsDNA and complements C3 and C4.[8-10],[13,16]A positive level of anti-dsDNA with a low level of complement can be used as a marker for renal flares and disease activity.[4,9,10,16]Elevated Anti-C1q levels in patients with LN and confer an increased risk for renal flares .[4,7] Anti -C1q

antibodies have been shown to have a sensitivity and specificity of 76% and 80% respectively, differentiating between active and inactive nephritis.[8,11-13] Other serological biomarkers used to demonstrate ongoing kidney injury are vascular cell adhesion molecule-1(VCAM-1) and tumour necrosis factor like weak inducer of apoptosis (TWEAK).[4,11,13] The novel urine biomarkers MCP-1, OPN N-half, SCD163 can be used to demonstrate early kidney injury in patients with LN.[4,11]

Classification of Lupus Nephritis

In 2016 the International Society of Nephrology / Renal Pathology Society (ISN/RPS) revised the 2003 histological classification of LN *(refer to Table 1*).[14] Research evaluating the clinico-pathological features of LN is still being conducted to substantiate and affirm the use of the proposed new classification. The histo-pathological classification of LN is solely based on the location and extent of immune complex deposition within the glomeruli. The pattern of injury may be reported as acute inflammation or sclerosis (indicating chronic disease).[4] There are six histological subtypes described in the new classification, broadly divided into proliferative (Class III-VI) and non-proliferative types (Class I-II).[4-5,9] Several studies have shown an increased frequency in the proliferative forms of LN (class III, class IV and mixed class V) which are associated with poorer outcomes.[9,15] This was correlated by a South African study in Tygerberg Hospital[16],that revealed class IV LN as the most common histological subtype in their cohort of 315 patients.[16] Immune mediated glomerular disease with resultant tubulo-interstitial changes; renal vasculature damage in the form of fibrinoid necrosis, and thrombotic microangiopathy can also be observed on microscopy.[17]

Class I and II LN are characterised by mesangial immune deposits with or without mesangial proliferation under light microscopy.[7,18] They are predominantly asymptomatic with mild urinary abnormalities such as sub-nephrotic proteinuria or microscopic haematuria.[4,7,15]Class III and Class IV LN have a heterogenous clinical presentation ranging from nephrotic syndrome, renal dysfunction and active urinary sediments.[4,7,15] Class IV is the most active and severe subtype of LN with 50% of patients presenting with nephrotic range proteinuria and renal dysfunction.[4,7,15] Renal dysfunction is uncommon in class V LN, with 59-70% of patients presenting with nephrotic range proteinuria.[4,7] Class VI is characterised by a lesion of more than 90% fibrosis and sclerosis, often progressing to ESRD.[4,7,18]

Treatment of Lupus Nephritis

Treatment of LN is dependent on the histo-pathological classification of LN as per ISN/RPN classification, and the degree of renal impairment.[15]The main objectives of treatment in LN are to reduce urinary excretion of protein and delay progression of renal disease to ESRD.[7-8,19] Classes I and II LN do not require specific immunosuppressive therapy directed to the kidney, and are associated with good clinical outcome.[4,20] The KDIGO guidelines recommend that management should include aggressive treatment of co-existing extra-renal SLE manifestations.[15,21]

The proliferative subtypes of LN follow a more aggressive disease course requiring early treatment to improve outcomes. Treatment of the proliferative sub-types of LN is comprised of induction and maintenance therapy.[15] The use of immunosuppressive agents in proliferative LN has improved the overall survival rate to about 80% in 5 years.[17]The Kidney Disease Improving Global Outcomes (KDIGO) and American College of Rheumatology

(ACR) guidelines recommend the use of high dose corticosteroids in combination with cyclophosphamide (CYC) or mycophenolate mofetil (MMF) for proliferative LN.[10,15] Steroid therapy consists of an intravenous methylprednisolone pulse of 250mg-1000mg mg for three days, followed by oral prednisone.[15] The oral prednisone dose is initiated at a dose of 0.5-1.0mg/kg/day (with a maximum dose of 80 mg/day) for a few weeks and then tapered off to the lowest effective dose.[15]

The KDIGO guidelines suggest that a CYC containing regimen is the gold standard in inducing renal remission and preventing renal flares in patients with proliferative LN.[15] However, African, Asian and Hispanic patients fare better with MMF.[4-5] Mycophenolate Mofetil, an alternative drug for induction therapy, is a reversible inhibitor of inosine monophosphate dehydrogenase, thereby inhibiting B and T cell proliferation and antibody formation.[19] The targeted dose of MMF in severe LN is 3g/day.[15,19] Both MMF and CYC have been found to have similar efficacy in achieving remission when used as induction therapy in patients with proliferative LN.[4,15] Careful patient selection when choosing between MMF and CYC remains important.

Azathioprine (AZA) and MMF are currently the treatment options for the maintenance phase of LN. Azathioprine is a purine analogue which inhibits DNA synthesis, thereby strongly blocking rapidly dividing cells.[10] Both drugs are equally effective in preventing renal flares. Landmark trials such as The Aspreva Lupus Management Study (ALMS), showed MMF to be more superior than AZA irrespective of the induction therapy used, particularly in black and Hispanic patients.[15,21] Immunosuppressive therapy may be tapered to the most effective minimum dose once the disease is clinically and serologically inactive (in remission) for at

least one year.[5,21] The average duration as per KDIGO guidelines for immunosuppressive agents is 3.5 years.[15]

The goal of this treatment is to achieve complete remission, which is defined as a return of serum creatinine to previous baseline, and a decline in the urine protein creatinine ratio (uPCR) to <500 mg/mmol as per KDIGO guidelines.[8,15] Partial remission is defined by stabilisation (± 25%) or improvement of serum creatinine, but not to normal, and a greater than50% decrease in uPCR.[5,8-10,15] A reduction in uPCR therefore correlates with improved renal survival and decrease in progression of renal disease to ESRD.[15]

Supportive therapy in all classes of LN is aimed at treating co-existing disorders and other clinical manifestations of SLE. Angiotensin Converting Enzyme inhibitors (ACEi), or Angiotensin Receptor Blockers (ARBs) are first line anti-hypertensive agents in the management of hypertension with proteinuric kidney disease.[3-5,9,15,21]These agents can be safely used simultaneously with immunosuppressive agents in induction and maintenance therapy phases of LN. Diuretic therapy is important for symptomatic relief of oedema in patients with overt nephrotic syndrome.

Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that all patients with LN, unless contra-indicated, must be treated with antimalarial therapy.[9,15] Research has shown that lack of antimalarial therapy use may be associated with an increase in LN treatment failure.[5] Anti-malarial drugs such as chloroquine and hydroxychloroquine, have been proven to have anti-inflammatory, antithrombotic and anti-lipidaemic effects.[4-5,8,10]

This results in improved disease control as well as reduced number of renal flares.[4-5,8,10]Antimalarial drugs in LN are used to prolong renal survival and preventing disease progression to ESRD.[4-5,8,15]

Treatment of Refractory Lupus Nephritis

Disease progression despite optimal immunosuppressive therapy is defined as refractory LN. The European League against Rheumatism in combination with European Renal Association -European Dialysis and Transplant Association (EULAR/ERA-EDTA) has defined refractory LN as failure to achieve a partial response to immunosuppressive therapy in 6-12 months.[8,22]This is further defined by ACR as worsening nephritis in 3 months after commencement of immunosuppressive therapy.[8-9] Treatment failure therefore, requires intensification of therapy or re-induction of the immunosuppressive agent.[3,8]

The ACR guidelines advocate switching from CYC to MMF in cases of refractory LN.[22] A repeat renal biopsy is indicated in patients who show a poor response to initial induction therapy. The KDIGO guidelines recommend a review of therapy every three months for early detection of refractory disease.[22]Studies have shown that patients with LN who have more than 25% reduction in proteinuria, and normalization of the serum C3 and/or C4 after eight weeks of induction therapy were likely to have a better prognosis.[15] Similarly, after 6 months of treatment, a decrease in serum creatinine level , as well as proteinuria levels less than 1gram/ 24 hours, predicted a good long-term outcome.[23] Patients who have failed both CYC and MMF, are to be initiated on Rituximab (RTX), tacrolimus or cyclosporine (calcineurin inhibitors).[5,25]

Rituximab, is a monoclonal antibody, which depletes auto-reactive CD20 B cells, thereby reducing the production of autoantibodies necessary for disease manifestation of LN. The ACR guidelines recommend the use of Rituximab as monotherapy, or to be added to another immunosuppressive agent, in the treatment of refractory disease. [4-5,8,12,22] In contrast, The Lupus Nephritis Assessment with Rituximab (LUNAR) trial has reported no additional benefit to adding RTX in the treatment of refractory LN.[12-13,21,22] The use of RTX in refractory LN therefore remains unsubstantiated by research and cannot be generalized as routine practice. Patient selection for its use is therefore imperative.

Calcineurin inhibitors (CI) such as cyclosporine A and Tacrolimus exert a potent anti-proteinuric effect required in LN. [7,17]They block the release of inflammatory cytokines required for T cell activation, thereby decreasing the overall inflammatory response.[7] These above-mentioned drugs have been studied extensively as monotherapy or in combination with MMF as part of a multi-targeted approach in the treatment of refractory LN.[7,15,,17,21,22] Novel Calcineurin Inhibitors such as Voclosporin in combination with MMF(2g/daily) and prednisone were studied in the Aurinial Lupus Nephritis trial. This study showed 25% reduction in urine PCR as well as achieving high complete remission rates.[24]

Factors Affecting prognosis and disease outcomes in Lupus Nephritis

Multiple factors contribute to the progression of LN to ESRD requiring Renal Replacement Therapy (RRT).[16-17]Patients with ESRD have the worst overall survival rates when compared to other SLE patients with no renal involvement.[11,16] Black and Hispanic population have been reported to have the worst outcome.[15,17]

Okpechi *et al* observed pregnancy was associated with an increase in disease activity of LN ; as well as pregnancy related complications such as pre-eclampsia, preterm labour and foetal mortality.[9] In another study, hypertension was noted to significantly influence the degree of renal function decline in patients with proliferative LN, thereby inferring a poorer outcome.[15,16] An increase in the number of renal flares is strongly associated with the development of CKD, as well as progression to ESRD in patients with already established CKD.[15,17]

This study was conducted to evaluate LN in our African population at Helen Joseph Hospital (HJH)- Renal Unit. Disease prevalence, response to immunosuppressive therapy, and complications were evaluated during this study. This study aims to educate physicians on the clinical course of LN, and the best treatment options available for patients with LN in the African population.

MATERIALS AND METHODS

This was a cohort retrospective study; of all biopsy proven Lupus Nephritis patients attending the renal clinic at Helen Joseph hospital (HJH). The follow up period was over 12 months. Helen Joseph Hospital is one of the tertiary hospitals in Gauteng, South Africa. This hospital provides medical services to a low to medium income population of approximately one million people. The hospital has 21 wards, the majority of which are occupied by medical patients. It has a fully functional intensive care unit (ICU) , high care and a renal dialysis unit.

Study Sample

In the study, a total of 74 biopsy records confirming LN were found in the renal unit records. Only 45 patient records fulfilled all criteria to be included in this study, and were therefore, further evaluated. All biopsies confirming Lupus Nephritis in patients over the age of 18 years were included in this study. Incomplete or inadequate clinical records, as well as patients who presented with ESRD were excluded from the study.

Data Collection

All data was collected from the renal patient records, kept at the renal clinic file room at HJH. This data was cross referenced with the biopsy records file kept in the renal unit. The names of the patients and file numbers were used to identify appropriate histology and blood results. Data was recorded and stored using a numerical system in a password protected excel data spreadsheet.

Statistical Analysis

Data was analysed using the STATA MP software, version 15.0 (StataCorp, Texas, USA). Categorical variables were described using frequencies and percentages; the relationship between categorical variables was assessed using a chi squared test. The continuous data was assessed for normality and where appropriate, were presented as means and standard deviations. The differences in continuous variables and non-parametric equivalents between groups was assessed using a T test. All analyzed data was performed at 95% confidence interval. A p value < 0.05 was defined as being statistically significant.

RESULTS

Sample size

A total of 74 patients were found to have biopsy proven LN over the past 8 years at the renal clinic in Helen Joseph Hospital. Of these patients 10 were following up at rheumatology clinic, six were lost to follow up, and 13 were not treated with immunosuppressive therapy. The remaining 45 patient clinical records could therefore be evaluated for this study.

Demographics

Of the forty-five patients that were included in our study, 75% *(n=34)* were female and 24.4% *(n=11)* were male, with a male to female ratio of 1:3.1. The mean age was 32 years. This study was comprised of 68.9% *(n=31)* Black patients, 17.8% *(n=8)* Coloured, 8.9 % *(n=4)* Caucasian and 4.4% *(n=2)* Indian patients.

Co-morbidities

Hypertension was the leading comorbidity observed with 51.1 % of the patients suffering from the disease. HIV infection was the least associated comorbidity. Almost 35% of patients with class IV LN, and 21% of patients with membranous LN had established hypertension at presentation. There was only a total of four HIV infected patients.

Table 2: Lupus Nephritis Subtypes and association with Hypertension

Biopsy	Hypertension		Total N (%)	P-value
	No N (%)	Yes N (%)		
Class II	2 (9.1)	1 (4.3)	3 (6.7%)	0.173
Class III	9 (40.9)	3 (13)	12 (26.7)	
Class III + IV	3 (13.6)	1(4.3)	4 (8.9)	
Class III + V	0	1(4.3)	1 (2.2)	
Class IV	3 (13.6)	8 (34.8)	11 (24.4)	
Class IV + V	3 (13.6)	4 (17.4)	7 (15.6)	
Class V	2 (9.1)	5 (21.7)	7 (15.6)	

Extra-renal Manifestations of SLE

A majority of the patients studied also exhibited other SLE associated clinical manifestations. Sixty one percent of patients had arthritis; whilst 50% had SLE cutaneous features in the form of a malar/discoid rash. Chronic nasal or oral ulcers were seen in 30% *(n=14)* of our study sample. Seizures, chronic headaches and aseptic meningitis were observed in 11%(*n= 5)* of patients.

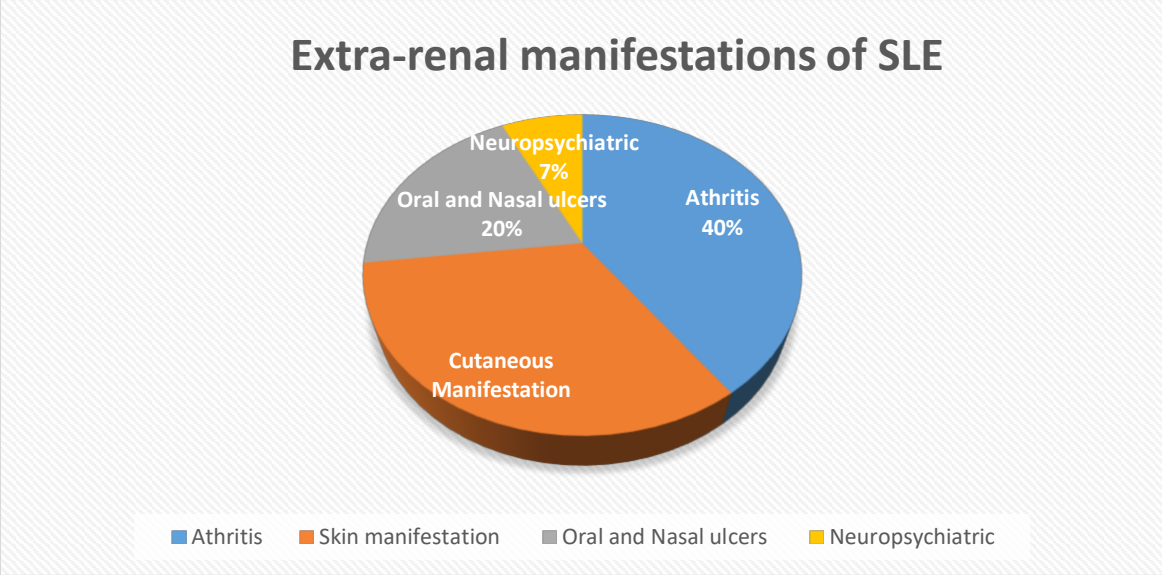

Figure 1: Extra-renal SLE manifestations. ***SLE: Systemic Lupus Erythematosus.***

Serological biomarkers at presentation

The results showed that 80% of the study population had a positive Anti-nuclear antibody (ANA) test. Anti-smith antibodies were found in 55.6%of the study population, whilst28% had a positive result for anti-ds DNA. C3 and C4 compliment deficiency was found in 35.6% *(n=16)* and 31.1% *(n=14)* respectively.

Table 3: **Comparison of biomarkers by gender**

	Female	Male	Total	P-value
Age (years)	32.23 ± 8.55	32.27 ± 13.42	32.24 ± 9.9	0.991
uPCR baseline	0.59 ± 0.57	0.39 ± 0.32	0.54 ± 0.53	0.298
uPCR 6months	0.18 ± 0.19	0.14 ± 0.18	0.17 ± 0.18	0.604
uPCR 12 months	0.09 ± 0.12	0.35 ± 0.74	0.16 ± 0.38	0.444
ANA	568.15 ± 396.15	604.44 ± 429.16	577.22 ± 398.66	0.817
ALB	22.68 ± 8.64	24.6 ± 10.37	23.18 ± 9.02	0.570
HB	9.84 ± 2.74	12.54 ± 2.02	10.56 ± 2.81	0.011
PLT	228.04 ± 149.29	307.33 ± 153.82	249.03 ± 152.33	0.185

*** mean-standard deviation**

uPCR: mg*/24 hours* , **ANA:** *Antinuclear antibodies,* **ALB:** *albumin,* **HB:** *Haemoglobin*, **PLT:** *Platelet count*

Female study participants were found to have more severe proteinuria of 0.59 mg/24 hours as compared to males population which had 0.39 mmol at presentation. This was statistically insignificant with a p-value of 0.298.

Table 4: Lupus Nephritis subtypes and occurrence of renal failure at presentation

	Biopsy								
Renal Function at presentation	Class II n=3	Class III n=12	Class III + IV n=4	Class III + V n=1	Class IV n=11	Class IV + V n=7	Class V n=7	Total n=45	P-value
Normal	2 (66.7)	10 (83.3)	2 (50)	1 (100)	4 (36.4)	4 (57.1)	5 (71.4)	28 (62.2)	0.053
Stage 3 CKD	0	1 (8.3)	0	0	6 (54.5)	2 (28.6)	2 (28.6)	11(24.4)	
Stage 4 CKD	0	1 (8.3)	2 (50)	0	0	0	0	3 (6.7)	
Stage 5 CKD	1 (33.3)	0	0	0	1 (9.1)	1 (14.3)	0	3 (6.7)	

***n= number, (percentages)**

In this study population, 62.2% of the patients studied had a normal renal function at presentation. Class III LN accounted for the majority of patients that had normal renal function at presentation. Twenty four percent of patients presented with stage 3 CKD; a majority of which (54,5%) had evidence of class IV LN.

Table 5: Comparative analysis of degree of proteinuria between proliferative and membranous subtype of LN.

Variables	Proliferative classes (class III/IV/mixed)	Class V (Membranous)	Total	P-value
uPCR 0	0.55 ± 0.52	0.46 ± 0.57	0.54 ± 0.53	0.685
uPCR 6 months	0.16 ± 0.19	0.22 ± 0.16	0.17 ± 0.18	0.546
uPCR 12 months	0.18 ± 0.41	0.02 ± 0.01	0.16 ± 0.38	0.432
ANA level (0 months)	558.67 ± 383.45	670 ± 497.31	577.22 ± 398.66	0.540

Units: urine PCR *mg/24 hours , mean standard deviation,* **ANA:** *Antinuclear antibodies,*

The proliferative subtypes of LN presented with significant proteinuria with a mean level of 0.55mg/24hours as compared to the membranous subtype with a proteinuria mean of 0.46 mg/24 hours.

Renal Biopsy results

Almost 80% of the renal biopsies studied showed proliferative LN. Only 15% *(n=7)* of the study population had membranous LN. Two of the samples were recorded to have micro-angiopathic changes.

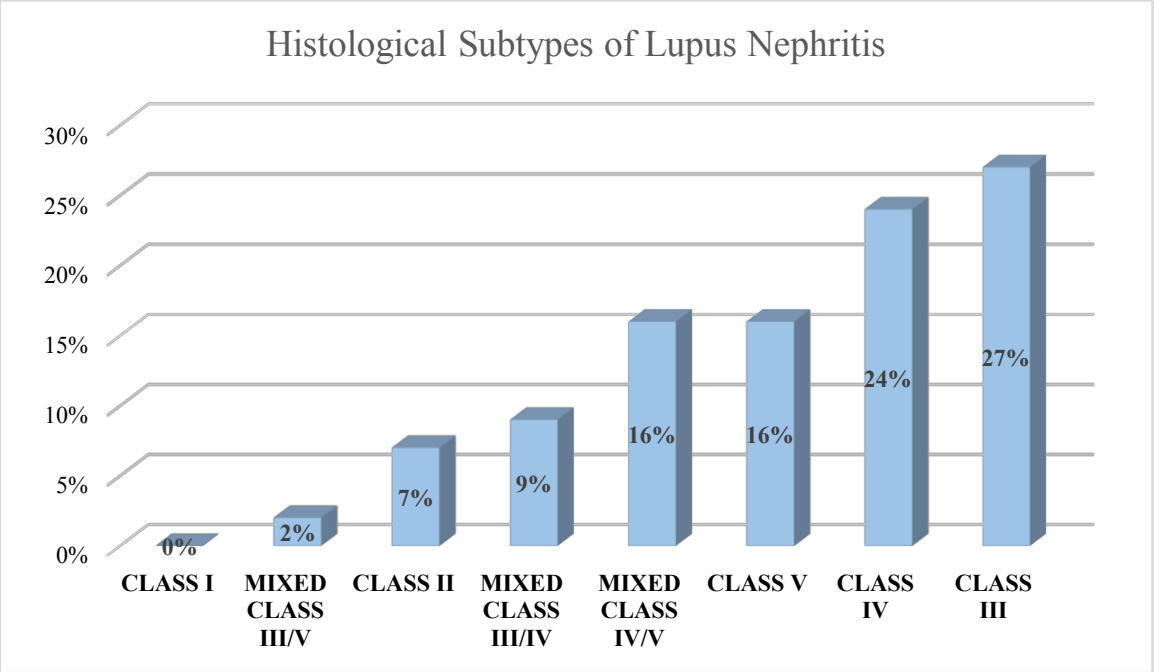

Figure 2: Histological subtypes of LN

Induction Therapy

Out of 45 patients in this study, 75.5% *(n=34)* received MMF as induction therapy. The remaining eleven patients (24.4% of study group) received the CYC based induction regimen, with the majority *(n=10)* receiving the NIH regimen, and only one receiving the Euro-Lupus regimen CYC regimen. High dose Solumedrol at a dose of 1g daily for three days was given as part of induction therapy in patients given CYC and MMF group. Only one patient with class II LN presented with stage 5 renal dysfunction whilst the rest had significant persistent proteinuria of more than 3grams/24 hours.

Response to Induction Therapy

Mycophenolate Mofetil (MMF) was given in 75% *(n=34)* of the study population with over 70% of patients achieving complete remission at the end of 6 months; and a further 17% *(n=6)* achieving complete remission at the end of 12 months. This was attributed to better treatment compliance and the African predominance in the MMF group. Only one patient in the MMF group was noted to have CKD over a 12 month period. Twenty four percent of patients received the CYC regimen, with only 18.2%*(n=2)* achieving complete remission at 6 months, and the remaining18.2% *(n=2)* at 12 months. Three of these patients were subsequently lost to follow up and 36.4% *(n=4)*of these patients did not achieve remission.

Table 6: Response to Induction Therapy agents

	Drug used		Total n=45 (%)	P-value
Time to remission	MMF n=34 (%)	CYC n=11(%)		
6 months	24 (70)	2 (18.2)	26 (57)	0.005
12 months	6 (17.6)	2 (18.2)	7 (15.5)	
Progressed to ESRD	1 (3)	4 (36.4)	5 (11.1)	0.005
Lost to Follow up	2 (6)	3 (27.3)	3 (6.6)	
Partial in 6 months	1 (3)	0	1 (2.2)	0.005

***ESRD:** *end stage renal disease*, **MMF :** *Mycophenolate mofetil,* **CYC:** *cyclophosphamide*

In the study 76.3% *(n=29)* of patients with proliferative LN were able to achieve remission, and only 15.8% of these had no remission, with 7.9% of them lost to follow up. Seventy percent of membranous LN (class IV LN) achieved complete remission post induction therapy.

Table 7: Comparison of patients with membranous Lupus Nephritis and proliferative classes to response to treatment.

Outcome	Proliferative subclasses n=38 (%)	Class V n=7 (%)	Total Number n=45(%)	
Remission	29 (76.3)	5 (71.4)	34 (75.6)	
Partial remission	0	1 (14.3)	1 (2.2)	
Refractory disease	5 (13.2)	0	6 (13.3)	0.014
Lost to Follow-up	3 (7.9)	0	3 (6.7)	
No record	0	1 (14.3)	1 (2.2)	

All HIV infected patients *(n=4)* achieved complete remission. HIV status had no influence in the remission rate of patients in this study *(p-value=0.025)*.

Maintenance and Supportive therapy

The reviewed clinical records showed that patients induced with MMF, who achieved complete remission, were maintained with MMF and oral steroids. Only four of the CYC group were maintained with Azathioprine, whilst the rest received MMF as maintenance. As part of the treatment guidelines all patients in the study were also treated with Chloroquine, with only one reported to have developed Chloroquine maculopathy. Eighty-six percent *(n=20)* of the patients with hypertension were treated with a combination of Angiotensin-converting enzyme inhibitors (ACEi)and diuretics. Only three of these patients were only treated with Loop diuretics.

Complications

Twenty seven percent *(n=12*) of patients in the study group developed infections during the course of their treatment. Of these patients 16% *(n=7)* had Pulmonary Tuberculosis, 6.6% *(n=3)* had urinary tract infections, 2% *(n=1)* had *Clostridium difficile* gastroenteritis, and only one patient in the study had a varicella skin infection. Sixteen percent *(n=9)* of patients

on MMF complained of gastrointestinal symptoms. Other minor complications observed included chloroquine maculopathy, osteoporosis and steroid induced Diabetes Mellitus.

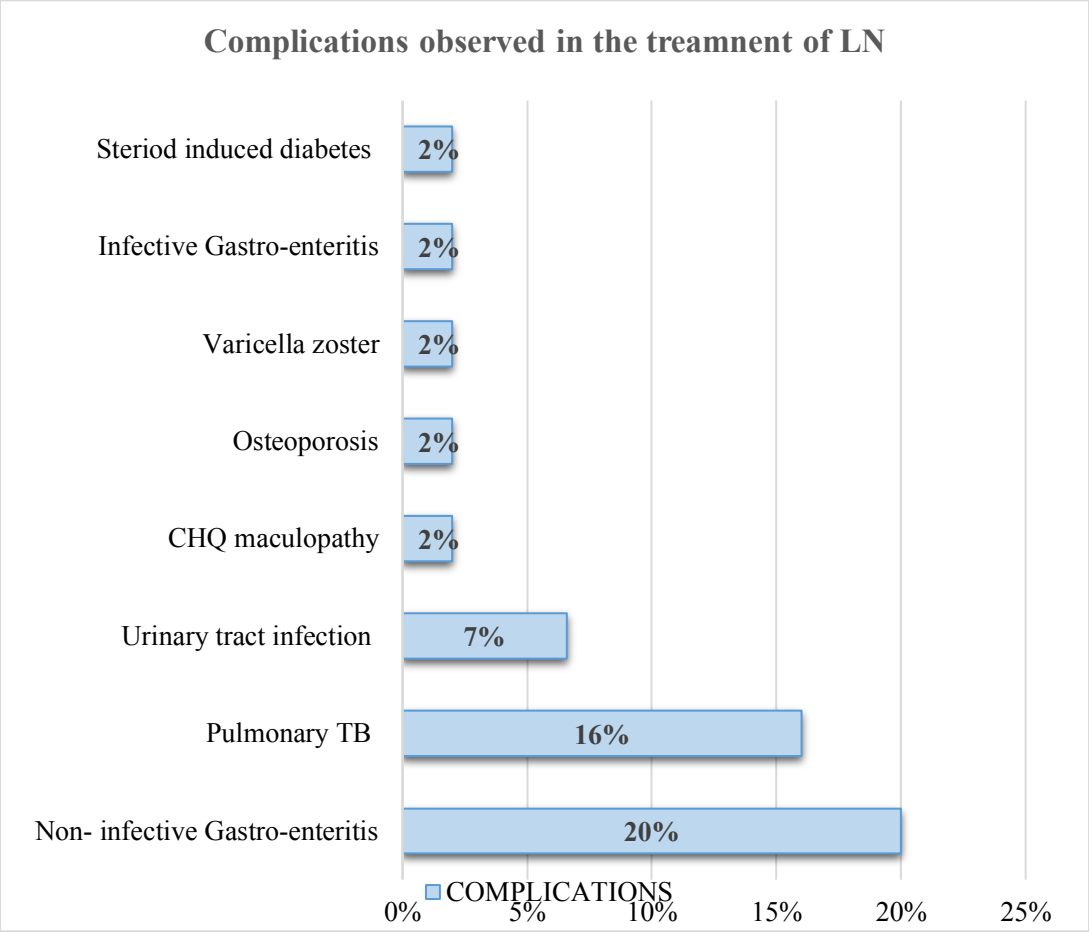

Figure 3:Complications observed in the treatment of Lupus Nephritis *CHQ: Chloroquine*

Renal Flares

In this study, 18.4% *(n=7)* of patients with proliferative subtypes of LN, had both nephrotic and nephritic flares.

Table 8: Renal flares observed in patients with Proliferative vs Membranous Lupus Nephritis.

FLARES	PROLIFERATIVE SUBTYPES N (%)	MEMBRANOUS N (%)	TOTAL N (%)	P-VALUE
Nephritic	2 (5.3)	1 (14.3)	3 (6.7)	0.281
Nephrotic	1 (2.6)	1 (14.3)	2 (4.4)	
Mixed	7 (18.4)	0	7 (15.6)	
No flares	28 (73.7)	5 (71.4)	33 (73.3)	

DISCUSSION

The immense burden of infectious disease, and communicable diseases such as hypertension in Sub-Saharan Africa, has influenced governments to focus their limited recourses on aggressive health campaigns in this area, neglecting the rising spectrum of autoimmune diseases such as SLE and LN.

The prevalence of SLE continues to rise in Africa, with females of child bearing age mostly affected. This is hypothesized to be due to abnormal oestrogen metabolism, hyperprolactinaemia, and the females' heightened antibody response when compared to males. Our study comprised mostly of females (75.6%) verses males (24.4%) with a mean age of 32 years. Helen Joseph Hospital services a large community mostly comprised of a Black African population; followed by Coloured, Caucasian and Indian African populations respectively. The ethnic distribution of our study population concurred this, with 68.9% *(n=31)* of our study population being Black, followed by 17.8% *(n=8)* Coloured, 8.9% *(n=4)*

Caucasian and 4.4% *(n=2)* Indian. The above demographic distribution is similar to international and current African studies specifically those that were done in Cape town.

Over 1 billion people worldwide suffer from hypertension, with its prevalence as high as 60% in South Africa. According to the World Health Organisation (WHO) approximately 27.4% of men, and 26.1% of women in south Africa have hypertension.[25] Fifty one percent *(n=23)* of our study participants suffered from hypertension; a majority of which had proliferative LN. Almost 35% of patients with class IV LN, and 21% of patients with membranous LN had established hypertension at presentation. In this study; hypertension with a higher serum creatinine and proteinuria at presentation; was identified as a poor prognostic marker. These patients were noted to have persistent proteinuria and progressed to ESRD. Optimum blood pressure control is therefore important to prevent poor disease outcomes such as disease progression to ESRD.[16]

Lupus Nephritis can present alone, or in concert with other SLE conditions. Arthritis was seen in 61% of our study participants, with the malar/discoid rash seen in 50% of our patients. Serological biomarkers such as antinuclear antibody (ANA) and anti-double stranded DNA (anti-ds DNA) tests are primarily used to diagnose SLE, aided by urinary biomarkers to clinch the diagnosis of LN. In our study, more than 80% of study participants had a positive ANA test; 55.6% had a positive anti-smith antibody; and 28% had a positive anti-dsDNA test. In those patients where the diagnosis of LN was not confirmed by serological and urinary laboratory results, the renal biopsy remained the gold standard for making the diagnosis.

Although a renal biopsy is mandatory to make the diagnosis of LN, the technical equipment and the skillset required to perform a renal biopsy, is not easily accessible in resource limited countries such as South Africa. It is with this in mind that researchers are now exploring whether titre levels of serological and urinary biomarkers can be used to diagnose specific histological subtypes of LN.[26]A study by Mavragani *et al* sought to do this by formulating a predictive score, which uses both clinical features and biomarker laboratory results, to make the diagnosis attributable to a specific histological subtype of LN.[26] Based on this, appropriate immunosuppressive therapy can be commenced timeously without the need of a renal biopsy. A predictive tool of this nature, can be very useful in diagnosing and treating more patients with LN, in our setting.

International studies have shown that proliferative LN subtypes are the most common, and follow a more severe clinical course. Class IV LN remains the most prevalent (40-70%) in local and international studies. A study in Cape Town; with a large cohort of 315 patients; also found class IV LN to be the most common.[16] In our study; class III and Class IV LN were the most common with 26.7% *(n=12)* and 24.4% *(n=11)* respectively. Twenty four percent of the total study participants presented with stage 3 CKD; a majority of which (54.5%) had class IV LN. Membranous LN has been cited as the least common LN by international studies, with some reporting prevalence between 10-20%. In this study, Membranous LN (Class V LN) accounted for 15.6% *(n=7)* of the total study population; with a majority presenting with normal renal function. This implies that patients with SLE, presenting with CKD are likely to have Proliferative LN, and a diagnosis of LN must be made expediently.

The presence of proteinuria in patients with Lupus is suspicious of LN, and can be used to monitor response to immunosuppressive therapy. In our study, heavy proteinuria was found more in females (0.59 mg/24hrs) verses males (0.39mg/24hrs). In this female predominant study population, gender played no role in the extent of proteinuria, and was statistically insignificant. The proliferative LN study participants; showed a baseline urine PCR of 0.55mg/24hrs at presentation; which gradually reduced to 0.18mg/24hrs by the end of 12 months with the continuance of immunosuppressive therapy. The above findings highlight the need for routine serological and urinary tests for close monitoring of this disease, and prevention of disease progression to ESRD.

In recent decades, the evolution of the treatment approaches to LN has been remarkable. Studies have shown a tremendous improvement in the morbidity and renal outcomes of patients treated with immunosuppressive agents. There's been a growing comparable efficacy and a better side effect profile with the use of MMF as an induction therapy when compared to CYC. Despite CYC being the drug of choice in patients with severe and rapidly worsening nephritis; its side effect profile has encouraged researchers to explore safer alternative agents. Cyclophosphamide still has an important role in induction therapy, especially in patients with severe renal dysfunction.

The benefits of using MMF as an induction and maintenance agent have been demonstrated more in the African population group.[26] Genetic factors pertaining to the metabolism of drugs such as MMF, are key in determining patients' response to immunosuppressive therapy, and consequently their outcome. Ginzler *et al* reported a remission rate of 52% in patients induced with MMF.[27] In this study, 75.5% *(n=34)* of the study group was induced with

MMF. Out of these, 70% achieved complete remission at the end of six months, and 17.6% showed complete remission at the end of a 12 month period. The remaining study participants (23%) were induced with CYC, with 18.2% of the CYC group achieving complete remission within six months, and 18.2% at the end of 12 months. The evaluation of efficacy of MMF vs CYC as an induction agent was not possible due to the small sample size of this study. There was also no identifiable treatment protocol used for drug selection in our study participants; with a significant number of patients being allocated to MMF as their induction and maintenance therapy agent. Treatment protocols, with clear clinical criteria are needed to accurately guide treating physicians on the best immunosuppressive agent to be used on each individual patient.

Studies evaluating the remission rates of patients with proliferative LN on MMF versus CYC during induction therapy concluded that both agents have similar efficacy. An Asian study showed that 82.6% of patients placed on a MMF regimen, achieved remission over 24 weeks.[6] Of these patients, almost 35% had complete remission; whilst 48.4% had partial remission in the stated period.[6] Our study showed good outcomes in those where MMF was used, and therefore supports its use in non-Caucasian patients with LN. The role of ethnicity in patients' response to immunosuppressive therapy, needs to be further evaluated in South Africa. The findings of our small study may not be fully representative of the treatment response in our unique ethnically diverse population. distribution.

The use of adjuvant therapy like anti-malarial drugs and ACE inhibitors play an important role in the management of patients with LN. Both drug groups have been proven to halt disease progression to ESRD.[15] Angiotensin Converting Enzyme inhibitors (ACEi), or

Angiotensin Receptor Blockers (ARBs) are first line anti-hypertensive agents in the management of hypertension with proteinuric kidney disease.[15,19,26] The use of ACEi and ARBs was not documented in every hypertensive patient with proteinuric disease. Chloroquine, as an anti-malarial, was used in every patient we evaluated.[15] There was no standardized protocol for facilitation of ophthalmology assessments to evaluate Chloroquine induced Maculopathy and other associated complications in these patients. These complications may have been missed or identified at an advanced stage.

The simultaneous use of statins, anti-diabetic agents, and other adjunct therapies used was not routinely recorded in the patient files, and therefore could not be further evaluated. It is our recommendation that all treating clinicians should adhere to strict recording of all drugs used, to identify and avoid potential unwanted drug-drug interactions, as well as drug adverse reactions.

During the course of managing LN, Renal flares are frequently encountered. These may present as Nephrotic or nephritic flares. In this study, patients with Proliferative LN commonly had nephritic and nephrotic renal flares interchangeably. Each individual renal flare differed in severity, and time to resolution. The clinical significance of these renal flares could not be established in this study, as biomarkers used to diagnose and monitor this disease entity were not routinely performed. International studies have associated the number of renal flares to be one of the poor prognostic determinants of LN. A study by Moroni *et al* found that only nephritic flares are associated with a poor outcome.[28] Findings from the research of Parikh *et al* , in contrast, suggested that nephritic flares are the most common renal flares observed and are associated with the worst outcome.[29] In this study, patients who

eventually developed ESRD, were found to have had recurrent renal flares in the course of their disease. This finding concurred with international studies, that recurrent renal flares conferred a significant risk in developing ESRD.

Tuberculosis and HIV/AIDS remain a huge burden on the Sub-Saharan health system, with increasing morbidity and mortality rates. Only four of the study participants were recorded to be HIV infected on anti-retroviral therapy; with a majority (75.6%) of the study participants being recorded as HIV negative. The HIV status of the remaining 15.6% of the study participants was unknown , and nil explanation was provided. In this study, 27% *(n=12)* of the study population suffered from infection, commonly in the form of Pneumonia, and urinary tract infections. The immunosuppressed state of patients being treated for LN, is primarily due to the disease itself as well as the immunosuppressive agents used. In this study we found that 16% *(n=7)* of patients were treated for pulmonary tuberculosis. Infection, by means of Mycobacterium Tuberculosis is associated with increased morbidity and mortality.

Drug adverse reactions was also found to be a common occurrence in our study. A total of nine patients (20%) treated with MMF, developed MMF induced gastro-intestinal disturbance. This was of no clinical significance, and use of an alternative immuno-suppressive agent such as CYC was not required. Only 2% *(n=1)* of the entire study group was found to have a *Clostridium difficile* infective diarrhoea. Chloroquine induced maculopathy was found in one patient. There was no other ocular complications in our study participants. Due to lack of routine ophthalmology assessments, this may be well under represented. It is the recommendation of the authors that ophthalmology assessments are mandatory in patients being treated with chloroquine.

Only one study participant developed steroid induced diabetes and osteoporosis respectively. These findings highlight the need for a thorough physical examination of patients with LN at every follow-up visit , to identify disease and treatment related complications early.

Studies report that 10% of patients with LN will naturally progress to ESRD despite optimum immunosuppressive therapy.[15] Renal involvement in patients with SLE has been associated with a reduction in survival rates from 92% in 10 years after diagnosis; to approximately 88% in 10 years. Non-Caucasians have even lower survival rates at the same time periods, when compared to their Caucasian counterparts. A comparative analysis of survival rates is beyond the scope of this study. However, one can concede that to improve survival ; early diagnosis, strict monitoring and full adherence to treatment protocols is paramount.

CONCLUSION

In conclusion; early diagnosis, and management of LN remain a huge challenge in a resource limited setting like ours. The disease still carries high morbidity and mortality despite advances in treatment over the decade. Strict adherence to treatment protocols and thorough patient evaluations will aid in effective treatment of these patients. This study highlights the need for individualised treatment strategies for patients; expedient identification and prevention of poor disease outcomes.

We have shown that MMF is the agent of choice in our predominantly black population, with high remission rates, and a much more tolerable drug side effect profile. Although efficacy of CYC was not evaluated in this same population, larger studies are needed to evaluate this further. Lupus Nephritis in the African context needs to be researched more, with the aim to

obtain a better understanding of this disease, and facilitate the development of new immunosuppressive agents best suited for our unique population.

LIMITATIONS

This was a retrospective study with a propensity for missing data and poor record keeping. A few of the clinical parameters were not uniformly and routinely recorded by clinicians and this made it difficult to assess patients' treatment response; complications of therapy and other disease manifestations. Some of the patients were lost to follow up, and this affected the true statistical significance of the data collected.

Although this was a single centred, small sampled study, it was encouraging that the findings of this study correlated well to the international quoted studies, and aided in understanding the clinical course of the disease in our unique setting.

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CHAPTER3: APPENDICES

APPENDIX A

CLASSES OF LUPUS NEPHRITIS AND CLINICOPATHOLOGICAL BIOPSY FINDINGS (14, 29, 49)

Class I and class II

Class 1 is Minimal mesangial immune deposits on immunofluorescence (IF) with normal light microscopy. Class II mesangial hypercellularity or matrix expansion on light microscopy with immune deposits confirmed to mesangium on IF. These two classes do not usually present with nephritic syndrome and heavy proteinuria; thus do not need any immunosuppressive therapy.

The treatment in these two classes is based on extra renal manifestations of SLE.

CLASS III

Class III has subendothelial immune deposits and proliferative glomerular changes involving less than 50% of glomeruli. Common clinical findings: micro haematuria, hypertension, active Lupus serology, proteinuria >1gram. A portion of patients may present with nephrotic syndrome; 25% have deranged renal function at presentation.

CLASS IV

Class IV has subendothelial immune deposits and proliferative glomerular changes involving more than 50% of glomeruli. Clinically patients have high serological activity, active urinary sediments, hypertension, reduced Glomerular Filtration Rate (GFR), heavy proteinuria , almost 50% have nephrotic syndrome.

CLASS V

Membraneous LN; subendothelial immune deposits and membraneous thickening of glomerular capillaries. The clinical presentation of these lesions, involves heavy proteinuria, nephrotic syndrome, hypertension, renal dysfunction can be present. Mebraneous LN is associated with risk of thrombotic complications.

CLASS VI

This histological class is has >90% sclerosis of the glomeruli and these patients need to be prepared for renal replacement therapy and there is no role of immunosuppressive therapy.

APPENDIX B: DATA COLLECTION SHEET

APPENDIX B: DATA COLLECTION SHEET

PATIENTS STUDY NUMBER							
DATE OF FIRST CONSULTATION							
	BASELINE	**3 MONTHS**	**6 MONTHS**	**12 MONTHS**	**2 YEARS**	**3 YEARS**	**4 YEARS**
DEMOGRAPHIC							
Age							
Gender							
Ethnicity							
Weight							
CO-MORBIDITIES							
Hypertension							
Hypercholesterolemia							
Diabetes Mellitus							
SLE CLINICAL MANIFESTATIONS							
Neuropsychiatric disease							
Arthritis							
Serositis							
Malar/ discoid rash							
Chronic sores (nasal/oral)							
URINALYSIS							
uPCR							
Urine Dipstix							
Heamaturia							
Proteinuria							
Urine sediments							
White cell cast							
Red cell casts							
Dysmorphic cells							

PAGE 1

Histology Result	BASELINE	3 MONTHS	6 MONTHS	12 MONTHS	2 YEARS	3 YEARS	4 YEARS
Class III C							
Class IV A							
Class IV A/C							
Class IV C							
Class VI							
Class VI							
Microangiopathy changes							
INDUCTION THERAPY							
Date of commencement:							
Date of completion:							
Drugs used							
SOLUMEDROL PULSE							
MMF							
CYC							
RE-INDUCTION THERAPY							
Date of commencement:							
Date of completion:							
Drugs Used							
SOLUMEDROL PULSE							
MMF							
CYC							
OTHER IMMUNOSUPPRESSIVE AGENTS USED							
Date of commencement:							
Date of completion:							
Drugs Used							
RITUXIMAB + MMF							
CYCLOSPORINE							
TACROLIMUS							

Histology Result	BASELINE	3 MONTHS	6 MONTHS	12 MO
Class III C				
Class IV A				
Class IV A/C				
Class IV C				
Class VI				
Class VI				
Microangiopathy changes				
INDUCTION THERAPY				
Date of commencement:				
Date of completion:				
Drugs used				
SOLUMEDROL PULSE				
MMF				
CYC				

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	BASELINE	3 MONTHS	6 MONTHS	12 MONTHS	2 YEARS	3 YEARS	4 YEARS		
RENAL FUNCTION									
Stage 1									
Stage 2									
Stage 3									
Stage 4									
Creatinine Level									
AUTOIMMUNE SEROLOGY									
ANA									
DS DNA									
ANTI-SM									
ANCA									
Lupus Anticoagulant									
Beta 2 glycoprotein Ab									
ACLA									
C3/C4									
OTHER SEROLOGY									
HB									
PLT									
Albumin									
HIV STATUS									
Year of Diagnosis									
CD4 Count									
Viral Load									
RENAL BIOPSY									
Year of biopsy									
Histology Result									
Class I									
Class II									
Class III A									
Class III A/C									
									PAGE 2

	BASELINE	3 MONTHS	6 MONTHS	12 MONTHS	2 YEARS	3 YEARS	4 YEARS
MAINTANANCE THERAPY							
Date of commencement:							
Date of completion:							
Drugs Used							
Azathioprine + oral steroids							
MMF + oral steroids							
OTHER DRUGS							
ARB/ACEi							
Diuretic							
OTHER DRUGS							
Antimalarial							
Statin							
TYPES OF REMISSION							
Partial Remission							
Complete Remission							
Refractory disease							
COMPLICATIONS							
Septicaemia							
Tuberculosis							
Diarrhoea							
Haemorrhagic Cystitis							
Cushingoid Appearance							
Steroid Induced Diabetes							
Amenorrhoea							
Osteoporosis							
Cataract							
RENAL RELAPSES							
Nephritic							
Proteinuric							
DISEASE PROGRESSION REQUIRED DIALYSIS							
Date of commencement							

APPENDIX C: GLOSSARY TO DATA COLLECTION SHEET

1. **uPCR:** Urine Protein Creatinine Ratio
2. **Renal Function:** This is determined by the Estimated Glomerular Filtration Rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) Formula:

   eGFR(ml/min/1.73m^2) =175 x (serum Creatinine(μmol/l) x 0.0113)$^{-1.154}$ x Age (years)$^{-0.203}$ (x0.742 if female)

Stage 1	eGFR (> 90ml/min) normal function
Stage 2	eGFR (60-90 ml/min) mild reduction
Stage3a Stage3b	eGFR (45-59ml/min) moderate reduction eGFR (30-44ml/min)moderate reduction
Stage 4	eGFR (15-29ml/min) severe reduction

3. **ANA:** Anti-Nuclear Antibody
4. **DS DNA:** Anti-Double Stranded DNA
5. **ANTI-SM:** Anti-Smith Antibody
6. **ANCA:** Anti-Neutrophil Cytoplasmic Antibody
7. **LA :** Lupus Anticoagulant
8. **Beta 2 glycoprotein Ab:** Beta 2 Glycoprotein Antibody
9. **ACLA:** anticardiolipin antibodies
10. **C3:** Complement Component 3
11. **C4:** Complement Component 4
12. **HB:** Haemoglobin
13. **PLT:** Platelet Count
14. **ARB:** Angiotensin Receptor Blocker

15. **ACEi:** Angiotensin Converting Enzyme Inhibitor

16. **Renal biopsy Histology Results:** The International Society of Nephrology and the Renal Pathology Society (ISN/RPS) has classified Lupus Nephritis into six different histopathological categories.

Class I Minimal Change
Class II Mesangial Proliferative
Class III Focal LN (<50%) • (A): active lesion • (A/C): active and chronic • (C) : chronic lesions
Class IV Diffuse LN (>50%)Diffuse segmental (IV-S)/ Diffuse global (IV-G) • (A): active lesion • (A/C): active and chronic • (C) : chronic lesions
Class V Membranous LN
Class VI Advanced sclerosing (>90%)

R14/49 Dr LF Qwabe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170643

NAME: Dr LF Qwabe
(Principal Investigator)
DEPARTMENT: School of Clinical Medicine
Department of Internal Medicine
Charlotte Maxeke Johannesburg Academic Hospital

PROJECT TITLE: The retrospective analysis of patients with Lupus Nephritis in Helen Joseph Hospital

DATE CONSIDERED: 30/06/2017

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Drs HM Maepa & K Motse

APPROVED BY: Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 11/10/2017

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 Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised 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 to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case the study was initially reviewed in **June** and will therefore be due in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES