Biopsy proven lupus nephritis: A 5-year clinico-pathologic insight from a single centre

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Declaration

I, Pulane Mosiane, hereby declare that this research report is my own work. It is being submitted in partial fulfilment for the degree of Master of Medicine in Anatomical Pathology. It has not been submitted before for any degree or examination at this or any other university.

Pulane Mosiane

09 September 2022

Dedication

This research report is dedicated to my parents Daniel Gagoresepe and Alidah Mosiane for teaching me the importance of education. My two boys Rebaone and Atlehang Mofokeng for showing me unconditional love.

ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is a multi-organ, relapsing and remitting autoimmune disease in which auto-antibodies are produced against self-antigens, resulting in widespread inflammation and tissue damage in involved organs. Renal involvement is a major cause of morbidity and mortality. In this research report we aim to describe the epidemiologic features, clinical presenting features and histologic findings in patients with lupus nephritis.

Methods: A 5-year retrospective analysis of clinical presentation, serological data and histological findings was performed on patients with biopsy proven lupus nephritis.

Results: A total of 354 renal biopsies with a diagnosis of lupus nephritis were identified from January 2014 to December 2018. The mean age of participants was 30(22-37) years. The mean age at biopsy for children was 14(13-16) and adults 31(25-38). The most common lupus class in children was III and adults was V. Proteinuria was the commonest clinical presenting feature noted in 96/354 (72.1%) followed by nephrotic syndrome 80/354 (22.6%) and nephritic syndrome 17/354 (4.8%).

Conclusion: This report confirms that lupus nephritis is more common in females than in males and that sub-nephrotic and nephrotic range proteinuria are the most common clinical presenting features. Vascular lesions are uncommon in renal biopsies from patients with lupus nephritis. The most common lupus nephritis class in this study is V.

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List of Abbreviations

Abbreviation:	Expansion:
ACR	American College of Rheumatology
ANA	Antinuclear antibody
Anti-ds DNA	Anti-double stranded DNA
ATN	Acute tubular necrosis
CDC	Centers for Disease Control and Prevention
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
EM	Electron microscopy
FSGS	Focal segmental glomerulosclerosis
HIV	Human immunodeficiency virus
HJH	Helen Joseph Hospital
IMF	Immunofluorescence
ISN	International Society of Nephrology
LN	Lupus nephritis
MCTD	Mixed connective tissue disease
NHLS	National Health Laboratory Service
RPS	Renal Pathology Society
SA	South Africa
SLE	Systemic lupus erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
TMA	Thrombotic microangiopathy
USA	United States of America

CHAPTER 1:

1 Background and Literature Review:

Systemic Lupus Erythematosus (SLE) is a multi-organ relapsing and remitting autoimmune disease in which auto-antibodies are produced against self-antigens, resulting in widespread inflammation and tissue damage in involved organs, with significant resultant morbidity and mortality.[1] The disease presents with a variety of clinical manifestations based on organ involvement. Commonly affected organs include joints, skin, kidneys, blood vessels and central nervous system.[1] Less commonly, involvement of the lungs, lymph nodes and spleen is noted. The disease presents in early adulthood, with a striking female preponderance (80-90%)[2,3] and results in significant compromise to peoples' physical and emotional well-being.[4] A South African study based in Soweto found a male to female ratio of 1:18 and a mean age of presentation of 34 years.[5] The exact pathogenesis of SLE remains poorly understood. However, a complex interplay between genetic and environmental factors have been postulated. Environmental factors such as smoking, hormonal abnormalities, Vitamin D deficiency, exposure to UV light and certain infections are thought to play a part in the development of SLE.[1] Genetic predisposition is also thought to be significant, as shown by familial aggregation of SLE. The exact genetic mechanisms remain elusive. Loss of immune tolerance, increased antigenic load, and defective T- and B-cell immunologic responses have been postulated.[5] Previous reports indicate that SLE is uncommon in patients of African descent living in the tropics, but is common in immigrants of African descent who migrate to other parts of the world, particularly Europe.[6] International research indicates that SLE is more common in Africans, Hispanics and Asians when compared to Caucasians.[6] In this latter group, SLE has a poor prognosis.

In the USA, the prevalence of SLE varies widely by state, with figures ranging from 5.8-130 /100000 in different studies; the state of Minnesota has the highest prevalence rates.[7] In a recent meta-analysis of CDC National Lupus Registry data, the overall prevalence of SLE was 72.8/100 000 with females nine times more affected than males.[8] Black and Hispanic females were most affected. A similar race hierarchy of prevalence was noted for men. Another population-wide study in the United States found an SLE prevalence of 143.7/100 000

population, with a striking female (six times) and African American (double) predominance.[9] Just over 20% of SLE patients in this study had lupus nephritis(LN) (30.9/100 000).[9]

In comparison, a South African study found the prevalence of LN in SLE patients to be significantly higher (34%).[5] A local study from the western Cape supports a Non-Caucasian predominance.[2] Prevalence information on LN in Africa is scarce in the literature. This is due to the paucity of publications on this subject from Sub-Saharan Africa. Okpechi et al.[10] published data on 251 cases of biopsy proven LN. This is, to date, the largest published study from South Africa. A 30-year retrospective study carried out by Vermeulen et al.[11] at Chris Hani Baragwanath Academic Hospital in Soweto, South Africa found that LN was the commonest form of secondary glomerulonephritis in patients on whom a renal biopsy was performed.

A study from the United Kingdom found that the incidence rate of SLE is 4.91/100 000 over a 13 year period and a prevalence of 97.04/100 000 , with females of Black Caribbean ethnicity predominating. The peak age of incidence was 50-59 years. [12]

People suffering from SLE have an overall reduced lifespan with mortality 2-3 times higher than the general population. The most common causes of death are infections and cardiovascular disease.[1]

The variable multi-organ involvement in SLE results in protean clinical manifestations of disease, adding complexity to the diagnosis. The diagnosis of SLE depends on a combination of clinical and serological findings. Diagnostic criteria are set out in the Systemic Lupus Collaborating Clinics (SLICC); these are a validated update of the American College of Rheumatology (ACR) criteria.[13] In this classification system, four criteria need to be met, including one clinical and one serologic criterion. According to SLICC, the presence of biopsy proven lupus nephritis together with positive serology for SLE i.e. antinuclear antibody (ANA) or anti-double stranded DNA positivity, irrespective of other clinical and serologic findings, is adequate for the diagnosis of SLE.[13]

Between 40 and 80% of people with SLE have impaired renal function, which ranges from heamaturia and proteinuria to abnormal urinary sediment to end stage renal failure. The renal manifestation of SLE, referred to as lupus nephritis, occurs in 50% of people with SLE, usually presenting in young adulthood. LN is a poor prognostic indicator and has varied clinical and morphologic features.[5]

Clinical features range from microscopic haematuria to nephrotic syndrome to rapidly progressive glomerulonephritis and renal failure.[14] Renal involvement, when present, manifests early, most often within the first year of disease onset; however, it may develop years after initial diagnosis. The renal course of the disease may be characterised by remissions and exacerbations that may occur spontaneously or after treatment. Progression to chronic kidney disease occurs in a significant number of patients.[14] Lupus nephritis and renal failure are a common cause of death in people with SLE in South Africa, second only to infection.[5]

The presence of blood on urine analysis coupled with positive double-stranded DNA on serology in a patient with SLE should raise strong concerns for LN. Persistent proteinuria (.0.5grams/24 hour) or cellular red cell casts are indicative of renal disease.[10]

Several studies found nephrotic syndrome to be the commonest clinical presentation of LN irrespective of class.[10,15] In contrast, nephritic syndrome was the commonest clinical presentation in a Western Cape study with only 13% of patients having nephrotic syndrome.[2] This illustrates a poor correlation between clinical presentation and biopsy findings in LN.

A renal biopsy is essential for diagnosis, classification, treatment and prognostication of lupus nephritis. According to a publication from Cape Town, indications for biopsy in a South African setting include the following:

1) Persistent decline in renal function
2) Proteinuria (≥ 1.0g/24hr) or proteinuria (≥ 0.5g/24hr) if associated with haematuria
3) Active urinary sediment (granular casts, white blood cell casts and red blood cell casts).[16]

The initial classification system of lupus nephritis was developed in 1974 by a group of renal pathologists and nephrologists under the auspices of the WHO. This classification system was the most widely used, and stratified patients according to morphologic changes noted on biopsy. It was updated in 1982 and again in 1995. In 2003, a consensus conference was held at Columbia University under the auspices of the International Society of Nephrology /Renal Pathology Society (ISN/RPS), where a revised classification system was promulgated. This 2004 ISN/RPS classification system is currently the most widely used.[17]

Lupus nephritis is diagnosed by a combination of clinical and serological parameters coupled with histological evaluation of renal tissue in which immunofluorescence, immunohistochemistry and electron microscopy, in varying combinations, are used to assess

for the presence of immune complex deposits. Positive cases are assigned a class or combination of classes based on the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2004 classification (See Table 1).[17] Evaluation of LN class guides treatment regimens. Assessment of glomerular pathology is accompanied by evaluation of presence and severity of tubulo-interstitial disease and presence of vascular disease.

Tubulo-interstitial lesions are included in the National Institute of Health (NIH) activity and chronicity scores. The NIH scores include glomerular and tubulo-interstitial changes ranging from acute interstitial nephritis to interstitial fibrosis.[17] Hsieh C et al.[18] found interstitial inflammation to be an independent predictor of progression to end stage renal disease. The amount of tubulo-interstitial damage was strongly associated with progression, independent of glomerular injury. These authors were also able to demonstrate that interstitial damage was associated with poor prognostic markers such as elevated serum creatinine at the time biopsy, low GFR and high chronicity scores.[18] To date, as far as I am aware, there are no South African studies looking at interstitial changes, including inflammation and fibrosis, and their association with different classes of LN.

Table 1.1: Classification of lupus nephritis[17]

Class	Definition
I	**Minimal mesangial lupus nephritis:** Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence microscopy
II	**Mesangial proliferative lupus nephritis:** Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
III	**Focal lupus nephritis:** Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal

	subendothelial immune deposits, with or without mesangial alterations
IV	**Diffuse lupus nephritis:** Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations.
V	**Membranous lupus nephritis:** Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations
VI	**Advanced sclerotic lupus nephritis:** ≥90% of glomeruli globally sclerosed without residual activity
III+V, IV+V	Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed

Classes I, II and V demonstrate slower clinical progression whilst classes III and IV and mixed class V are associated with more rapid clinical progression.[19] Whilst class distribution varies somewhat in different studies, overall, the proliferative lesion characterising Class IV especially followed by class III predominate and are associated with the worst outcomes.[2,10,19] Western Cape studies found class IV to predominate (58%;55%) associated with the lowest survival,[2,19,20] followed by classes III and V.[2] This latter study of 30-year data found no cases of either class I or VI lupus nephritis.[2] Somewhat at variance with this observation, Wadee et al[5] found classes III and V to predominate (37 and 28%) followed by class IV (17%); Modi[21], in a study based in Natal, found class V to predominate and Ayodele[22] in the Western Cape – class IV followed by class V. The variance in class dominance observed in these South African studies may be explained to some degree by the dominant populations

involved – Mixed race in the Western Cape, Indian in Natal and Black African in Soweto. Surprisingly, class V alone was the most frequent class found in a local study by Vermeulen.[11] Lupus podocytopathy is a rare form of lupus nephritis characterised by nephrotic syndrome, normal glomeruli or focal segmental glomerulosclerosis by light microscopy, an absence of immune complex deposits and diffuse foot process effacement ultrastructurally.[23] Whilst it is not included within the 6 classes of LN, it forms a distinct but rare category of lupus-related renal pathology, accounting for approximately 1% of all LN biopsies. This lesion was first reported in 1994 by Hickman et al.[24] They reported a patient with SLE who presented with nephrotic syndrome. Biopsy findings from this patient revealed what was then referred to as idiopathic FSGS.

Five types of vascular lesions have been described in LN:[25,26]

1. Arteriosclerosis (AS)
2. Vascular immune-complex deposits(ICD)
3. Thrombotic microangiopathy(TMA)
4. Non-inflammatory necrotising vasculopathy(NNV)
5. True renal vasculitis(TRV)

To my knowledge, studies on prevalence and subtypes of vascular lesions in LN have not been undertaken in South Africa. In a Mexican study, vascular lesions were found in 53.4% of biopsy proven lupus nephritis with AS being the commonest lesion at 42%, followed by TMA (5.6%).[27] In some instances, the latter occurred in association with anti-phospholipid antibody syndrome. Necrotising vasculitis was uncommon, (2.6%) and associated with a dismal prognosis. Lupus vasculopathy was the least common (1.4%) of cases. Similar findings were noted by Huang et al,[28] who found arteriosclerosis to be the commonest vascular lesion(50/ 79 -63%). Another Chinese study found renal vascular lesions to be much commoner (81%), with up to 40% having 2 vascular lesions. Vascular immune complex deposits were the commonest (74%) followed by atherosclerosis (24%)[29] These authors note a positive correlation between vascular lesions and disease activity and chronicity, with TMA(18%) being associated with the poorest clinical outcomes. An Italian study found the prevalence of vascular lesions to be much lower at 27.7% with lupus vasculopathy at 9.5%, HUS/TTP at 8.4%, lupus vasculitis 2.8% and atherosclerosis 7%. These authors note a higher prevalence of hypertension and poorer kidney survival marks a greater likelihood of progression to end stage

renal failure.[30] Other authors have found vascular lesions to resolve with treatment, and be commoner in men.[31]

Histological evaluation of LN includes calculation of an activity and chronicity index to document active inflammation, disease progression and prognosis. The poor outcome of SLE patients with LN may be related to late presentation. 23% of patients in a Western Cape study had advanced renal failure at presentation, an overall 5 year survival of 67% and a mean survival of 10 years.[2] Highest mortality was noted in class IV. Similar findings were noted by Ayodele et al.[22] with 5- and 10-year survivals of 54% and 41 % respectively. In a Soweto study, 78% of SLE patients had established renal dysfunction at the time of diagnosis and a 5 year survival rate of 60%.[5] These local survival statistics differ significantly with those of Asian countries. Yap et al.[32] in China indicate a 99.5 % survival at 5 years and 98% at 10 years and Cheng[33] have shown survival of 93% at 5 years and 88% at 10 years.

Most studies indicate that higher activity and chronicity scores are associated with advanced disease and poor outcomes;[2] some studies find activity a predictor of poor outcome, but not chronicity.[20] Hypertension, LN class IV[20,22] and low C3 levels[2] are additional predictors of poor survival.

From a South African perspective, lupus nephritis was the most frequent form of secondary glomerulonephritis seen in a 30-year biopsy review at Baragwanath hospital according to an MMed thesis by Vermeulen. In this study, lupus nephritis was seen in 15.3% of all the renal biopsies. This accounted for 31% of all cases of secondary glomerulonephritis. The study found lupus nephritis to be more common in females in the 20-39-year age group. [11]

A recent publication from the Western Cape provides the only available data on SLE in children in South Africa. Of 72 participants with SLE, the majority were coloured(68%) followed by blacks at 24% and whites at 5%.[34] 70% of children with SLE enrolled in an Egyptian study developed lupus nephritis; the majority were girls, with a mean age of 12.9(+-2.5).[35] In children in the United States, the incidence of SLE is estimated to be 10 per 100 000. Like their adult counterparts, there is a female predominance (84%). An American study by Hiraki et al on SLE in children found 40% to be of African American origin and 21% Hispanic, a prominence in children of colour. Their study found a LN prevalence of 37% , indicating a prevalence of 3.64/100 000 children.[36]

One of the most important differential diagnosis of lupus nephritis on biopsy is Human Immunodeficiency Virus (HIV) associated immune complex glomerulonephritis with lupus-like features. In this entity, the histologic findings are identical to the those found in lupus nephritis, including full house staining on immunofluorescence microscopy. These patients, however, do not fulfil the clinical or immunologic criteria for SLE and are HIV positive. Although ANA may be positive, it is of low titre and anti- double stranded DNA is negative. The biopsy findings of these patients range from diffuse proliferative to membranous glomerulonephritis. Some biopsies may show subendothelial deposits with wire loops. This entity was first described by Nochy et al in 1993. [37]

A pilot study conducted at this institution and presented as a poster at USCAP(United States and Canadian Academy of Pathology) 2019, analysed 80 biopsy proven cases of lupus nephritis over 1 year. The preliminary data generated by that analysis indicated interesting and unique findings, prompting this larger 5-year analysis, the largest study of LN to date in South Africa. It is also the first to look at the frequency of vascular lesions of lupus nephritis in South Africa.

1.1 Aims and Objectives

1.1.1 Aim

To describe the clinical and pathologic features of biopsy proven lupus nephritis diagnosed at the National Health Laboratory Service, Johannesburg, over a 5-year period.

1.1.2 Objectives

The objectives of this study are:

1. To determine the number of patients with lupus nephritis in a 5-year period;
2. To describe the epidemiologic features, clinical presenting features and serological findings of patients with lupus nephritis;
3. To determine the frequency of the ISN/RPS lupus nephritis classes in these cases;
4. To determine if clinical presentation correlates with the histologic class of lupus nephritis;
5. To determine the percentage of biopsy diagnosed lupus nephritis cases with corroborating serology (positive ANA, anti dsDNA or both);

6. To determine if serologic findings (i.e. ANA titre where stated) correlate with histologic findings on biopsy; and
7. To assess the frequency (proportion) of vascular lesions in patients with biopsy proven lupus nephritis, to classify them and determine their association, if any, to class of lupus nephritis.

CHAPTER 2:

2 Materials and Methods:

2.1 Study Population

All cases of biopsy proven lupus nephritis diagnosed by the CMJAH laboratory from 1st January 2014 to 31st December 2018.

2.2 Study Design

This is a retrospective, non-interventional observational study.

2.3 Study Site

The site of the study is the Charlotte Maxeke Johannesburg Academic Hospital: NHLS Anatomical Pathology Laboratory.

2.4 Study Methods

Renal biopsy reports within the study period were retrieved from the NHLS database, using the current Labtrack system. All kidney biopsies received at Charlotte Maxeke Johannesburg Academic Hospital laboratory are given a laboratory number as well as a unique renal number. These details are entered in a logbook in the laboratory. The information from this logbook was used to retrieve cases meeting criteria for inclusion into the study. Data was abstracted from the pathology reports and also from the NHLS results database (routinely collected laboratory results). See attached data collection sheet for details of abstracted data (Appendix 1). If the patient had more than one biopsy in the study period, the first biopsy with sufficient material for histologic diagnosis was included in the study.

2.5 Sample Size

A pilot study conducted at this institution and presented as a poster at USCAP 2019 identified 80 biopsy proven cases of lupus nephritis in a period of one year. Our 5-year analysis revealed a total of 354 cases meeting criteria for inclusion into the study, as described below.

2.6 Inclusion Criteria

All cases of biopsy proven lupus nephritis, (meeting the SLICC 2012 criteria[13] diagnosed by the Charlotte Maxeke Johannesburg Academic Hospital NHLS laboratory in the study period were considered for inclusion in this study.

2.7 Exclusion Criteria

The following exclusion criteria were applied:

1. Biopsies from patients with clinical suspicion of lupus nephritis, but inadequate biopsy material for accurate histologic diagnosis.
2. Biopsy reports with inadequate, incomplete or unclear information.
3. Repeat biopsies from the patient within the 5-year period.
4. Cases that did not meet the SLICC 2012 criteria for SLE.
5. Post-transplant biopsies.
6. Human Immunodeficiency Virus (HIV) associated immune complex glomerulonephritis with lupus-like features (HIVIC).

2.8 Statistical Analysis

The data has been derived from a total of 354 biopsies. These have been subdivided into 2 two categories for analysis: Adults category - ≥ 18-years, (N=317) and children i.e. younger than 18-years old (N=37). In both subsets mean (standard deviation), median (Interquartile range), minimum and maximum values were computed for continuous variables,. For discrete variables, frequencies were computed (percentage) for each category and a p-value estimated using Fisher's exact test for the children's data and Chi-square test for the adult's data. For both datasets (children and adults), the variables representing demographic, clinical and histological measures were stratified by sex and HIV status of the participants. Lastly, cross-tabulations between clinical presentation and lupus class and between ANA serology and histology findings were conducted for both children and adults using Fisher's exact and chi-square tests for the p-value estimations, respectively. Statistical analysis was conducted in SAS Enterprise Guide 7.15 assuming a 5% level of significance.

CHAPTER 3:

3 Results:

3.1 Number of patients with a histological diagnosis of lupus nephritis in a 5-year period

The search identified a total of 367 biopsies in the 5-year period under review. Eleven participants had two biopsies in this period; the second was excluded from the analysis. Two cases were suboptimal for histological assessment and were also excluded, This yields a study sample of 354 biopsy proven cases of Lupus Nephritis.

3.2 Epidemiological features, clinical presenting features and serological findings of participants with lupus nephritis

3.2.1 Epidemiology

The cases were predominantly referred from the three major tertiary hospitals in Johannesburg, namely Chris-Hani Baragwanath Academic Hospital (CHBAH) - 165 (46.61%), Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) - 94 (26.55%), and Helen Joseph Hospital (HJH) - 59 (16.67%), with a small number of cases from Polokwane Provincial Hospital in Limpopo Province - 36 (10.17%).

All cases had gender and age recorded. 303 (85.59%) were female and 51 (14.41%) male. The median age at diagnosis was 30-years (IQR:22-37) with a range from 4-74 years. (See Figure 3.1) There were 317 adults(89.55%) and 37 children(10.45%).

Of the adults, the majority were female- 272 (85.80%); 45 (14.20%) were male. The median age at presentation for adults was 31-years(IQR:25-38) and the mean age 33-years. The age range was 18-74 years. Stratification by sex in adults revealed a median age of 30 in males(IQR:23-40) and 31 in females (IQR:25-38). Stratification of age at biopsy by HIV status in adults showed a higher median age of 36 (IQR:30-43) in HIV positive participants compared to their negative counterparts – 30 years(IQR:23-38)(p:0.0049).

Of 37 children in the study, there were 31 females (83.78%) and 6 males (16.22%). The median age was 14 years (IQR:13-16) with a range from 4-17 years. Stratification by sex revealed a median age of 16 in boys (IQR:13-16) and 14 in girls (IQR:11-16).

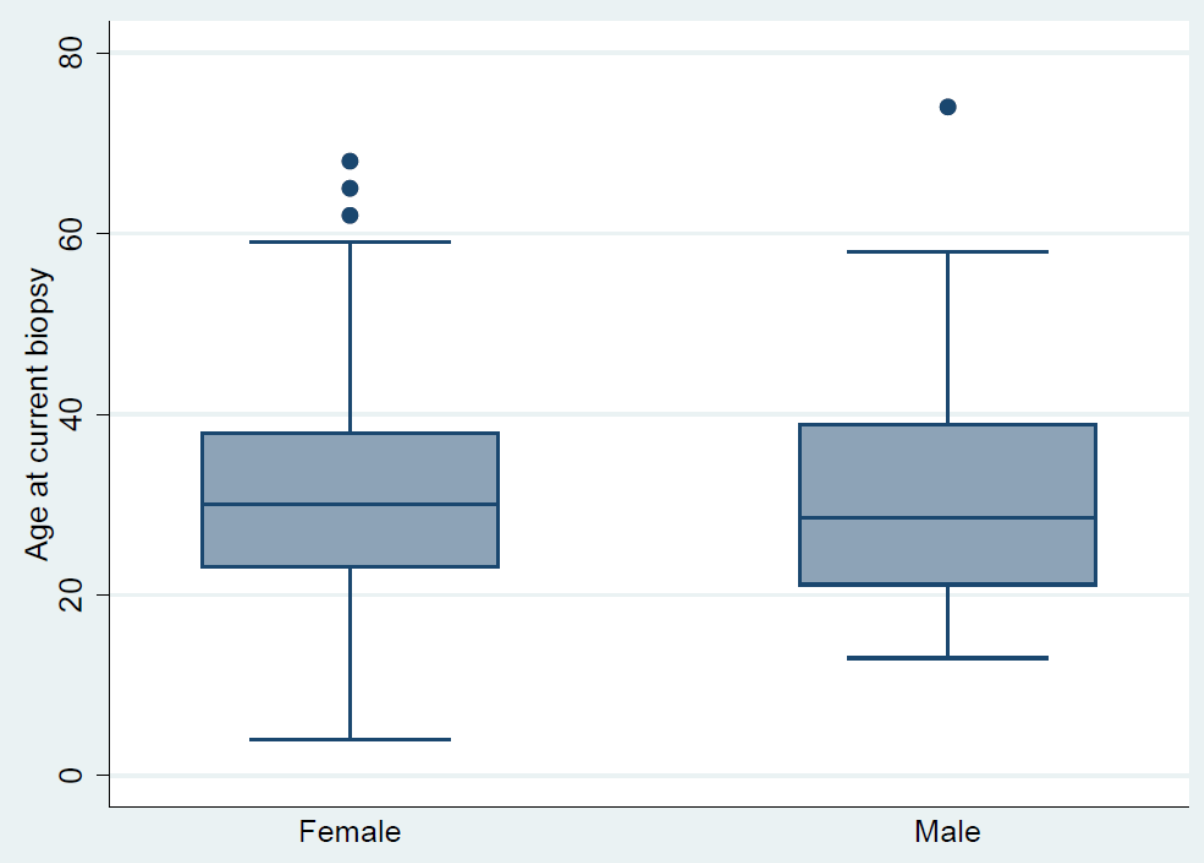

Figure 3.2: Age at current biopsy

3.2.2 HIV-status

The HIV status was known in 236 of 354 participants (66.67%). 37/236 (15.68%) were positive; 199 (84.32%) negative. There was no significant sex difference in HIV prevalence in adults. 2/37(5.41%) HIV positive participants were children (both female); of the 27 children with known HIV status, this corresponds to an HIV prevalence of 7.41% in children with lupus nephritis.

3.2.3 Clinical Presenting Features

107 biopsies (30.23%) were accompanied by a history indicating a clinical diagnosis of SLE. The most common clinical presentation was that of sub-nephrotic range proteinuria -

96(27.12%) followed by nephrotic syndrome – 80(22.60%). Nephritic syndrome and hypertension were less common at 4.80% and 3.11%. Acute and chronic renal failure and isolated haematuria were uncommon clinical presentations (see table 2).

There were no overall statistically significant differences in clinical presenting features between adults and children and between male and female children. However, hypertension in children at 8.11 % when compared to that in adults (2.52%) approached significance (p: 0.0639). Similarly, hypertension in adult males (6.67%) when compared to adult females (1.84%) approached statistical significance (p:0.056). Pre-nephrotic range proteinuria was found in 80/272 adult females (29.41%), significantly higher than in adult males, with a prevalence of 3/45 (6.67%) (p:0.0013). There were no differences in clinical presentation for adults stratified by HIV status.

Table 3.1: Epidemiological and clinical features of all participants; adults stratified by sex and HIV status.

Variable	**All participants** n=354	**Adults** n=317	**Adult females** n=272	**Adult males** n=45	**HIV-positive adults** n=35	**HIV-negative adults** n=174	**Children <18 years** n=37
Median age in years (IQR)	30 (22-37)	31 (25-38)	31 (25-38)	30 (23-40)	36 (30-43)	30 (23-38)	14 (13-16)
Age range (yrs)	4-74	18-74	18-68	18-74	22, 59	18-74	4-17
History of SLE (%)	107 (30.23)	97 (30.60)	86 (31.62)	11 (24.44)	8 (22.86)	54 (31.03)	10 (27.03)
Pre-nephrotic proteinuria (%)	96 (27.12)	83 (26.18)	80 (29.41)	3 (6.67)	7 (20.00)	40 (22.99)	13 (35.14)
Nephrotic syndrome (%)	80 (22.60)	73 (23.03)	59 (21.69)	14 (31.11)	13 (37.14)	42 (24.14)	7 (18.92)
Nephritic syndrome (%)	17 (4.80)	14 (4.42)	12 (4.41)	2 (4.44)	1 (2.86)	10 (5.75)	3 (8.11)
Hypertension (%)	11 (3.11)	8 (2.52)	5 (1.84)	3 (6.67)	1 (2.86)	6 (3.45)	3 (8.11)
Chronic renal failure (%)	5 (1.41)	4 (1.26)	3 (1.10)	1 (2.22)	0 (0.00)	2 (1.15)	1 (2.70)
Isolated haematuria (%)	2 (0.56)	2 (0.63)	2 (0.74)	0 (0.00)	0 (0.00)	1 (0.57)	0 (0.00)
Acute renal failure (%)	1 (0.28)	1 (0.32)	1 (0.37)	(0.00)	0 (0.00)	1 (0.57)	0 (0.00)

3.2.4 Serological Findings:

Serological testing for ANA was available in 238 participants (67.23%), 217(91.18%) of which were positive (See Table 3). A statistically significant difference in positivity for ANA was noted in adult females -172/186 (92.47%) when compared to adult males - 24/30 (80.00%) (p:0.0077). HIV status in adults had no impact on ANA serology status. There were no significant differences in ANA seroprevalence between adults and children.

ANA staining pattern was not reported in the vast majority of cases (345 – 97%). In the 9 reported cases, a speckled pattern was noted.

Anti-double-stranded DNA test results were available in 104 cases (28.38%). Of these, 65(62.50%) were positive. There were no significant differences in serological findings between adults and children, between adult males and females and between HIV positive and HIV negative adults (See Table 3.2)

For the purposes of this study, race was not documented as it was not consistently recorded on the request slip.

Table 3.2: Serological findings for adults and children; adults stratified by sex and HIV status.

Variable	All Participants n=354	Adults n=317	Adult Females n=272	Adult Males n=45	HIV-Positive Adults n=35	HIV-Negative Adults n=174	Children <18 years n=37
ANA status not available (%)	116 (32.77)	101 (31.86)	86 (31.62)	15 (33.33)	10 (28.57)	35 (20.11)	15 (40.54)
ANA Status Available (%)	**n=238** (67.23)	**n=216** (68.14)	**n=186** (68.38)	**n=30** (66.67)	**n=25** (71.43)	**n=139** (79.88)	**n=22** (59.46)
ANA positive (%)	217 (91.18)	196 (90.74)	172 (92.47)	24 (80.00)	23 (92.00)	122 (87.77)	21 (95.45)
ANA negative (%)	21 (8.82)	20 (9.26)	14 (7.53)	6 (20.00)	2 (8.00)	17 (12.23)	1 (4.55)
Anti-Ds DNA status not available (%)	250 (70.62)	222 (70.03)	187 (68.75)	35 (77.78)	25 (71.43)	113 (64.94)	28 (75.68)
Anti-Ds DNA status available (%)	104 (29.38)	95 (29.97)	85 (31.25)	10 (22.22)	10 (28.57)	61 (35.06)	9 (24.32)
Anti-Ds DNA positive (%)	65 (62.50)	58 (61.05)	51 (60.00)	7 (70.00)	6 (60.00)	38 (62.30)	7 (77.78)
Anti-Ds DNA Negative (%)	39 (37.95)	37 (38.95)	34 (40.00)	3 (30.00)	4 (40.00)	23 (37.70)	2 (22.22)

3.3 Frequency of the ISN/RPS lupus nephritis classes

Overall, the most common Lupus classes in descending order of frequency were III, V and IV respectively, followed by the combined classes IV+V and III+V (refer to Table 3.3 and Figure 3.2). Classes I and II were the least common classes. Lupus podocytopathy was extremely uncommon, accounting for 1.41% of cases. There was no statistically significant difference in class distribution by sex or HIV status in adults. This distribution changed significantly when stratified by age (p:0.0315)(See Figure 3.3). In adults the three dominant classes were ordered differently – V, then IV and III, but in children, class II replaced class V in the top three diagnoses in the following descending order: III, II and IV. For this study, proliferative lesions were defined as classes III, IV and mixed III+V and IV+V. Classes I, II,V and lupus podocytopathy were classified as non-proliferative. Class VI was analysed as a class but was excluded from the proliferative versus non-proliferative evaluations as the underlying lesion was not declared histologically.

When the prevalence of proliferative lesions(see Figure 3.5) was compared to non-proliferative lesions(see Figure 3.4), the former predominated (64.12%) even after stratification by age.

Lupus podocytopathy was found in 5 cases.

Table 3.3: Frequency of ISN/RPS lupus nephritis classes in adults and children; adults stratified by sex and HIV status.

Lupus Class	**All Participants** n=354	**Adults** n=317	**Adult Females** n=272	**Adult Males** n=45	**HIV - Positive Adults** n=35	**HIV- Negative Adults** n=174	**Children <18 years** n=37
I	16(4.52)	12(3.79)	11 (4.04)	1 (2.22)	1 (2.86)	6(3.45)	4(10.81)
II	28(7.91)	23(7.26)	18 (6.62)	5 (11.11)	5(14.29)	8(4.60)	5(13.51)
III	67(18.93)	55(17.35)	46(16.91)	9 (20.00)	7(20.00)	33(18.97)	12(32.43)
IV	63(17.80)	58(18.30)	47(17.28)	11(24.44)	4(11.43)	41(23.56)	5(13.51)
V	64(18.08)	63(19.87)	57(20.96)	6(13.33)	6(17.14)	31(17.82)	1(2.70)
VI	14(3.95)	13(4.10)	11 (4.04)	2 (4.44)	0 (0.00)	6(3.45)	1(2.70)
III + V	46(12.99)	41(12.93)	38(13.97)	3 (6.67)	3 (8.57)	26(14.94)	5(13.51)
IV + V	51(14.41)	48(15.14)	41(15.07)	7(15.56)	7(20.00)	21(12.07)	3(8.11)
Lupus podocytopathy	5(1.41)	4(1.26)	3 (1.10)	1 (2.22)	2 (5.71)	2(1.15)	1(2.70)
Non-proliferative GN(I,II,V, podocytopathy)	113 (33.24)	102 (33.55)	89 (32.72)	13 (28.89)	14 (40.00)	47 (27.01)	11 (30.56)
Proliferative GN (III,IV,III+V,IV+V)	227 (66.76)	202 (66.45)	172 (63.24)	30 (66.67)	21 (60.00)	121 (69.54)	25 (69.44)

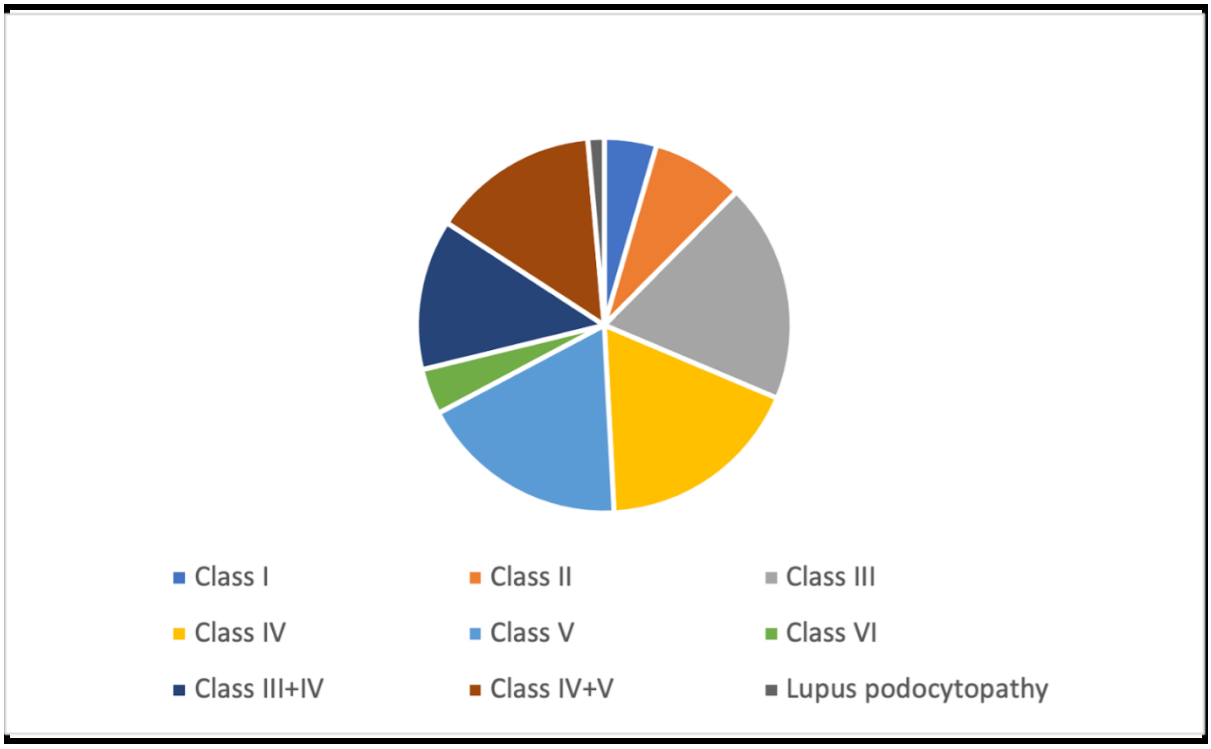

Figure 3.2: Pie Chart illustrating frequency of lupus nephritis classes

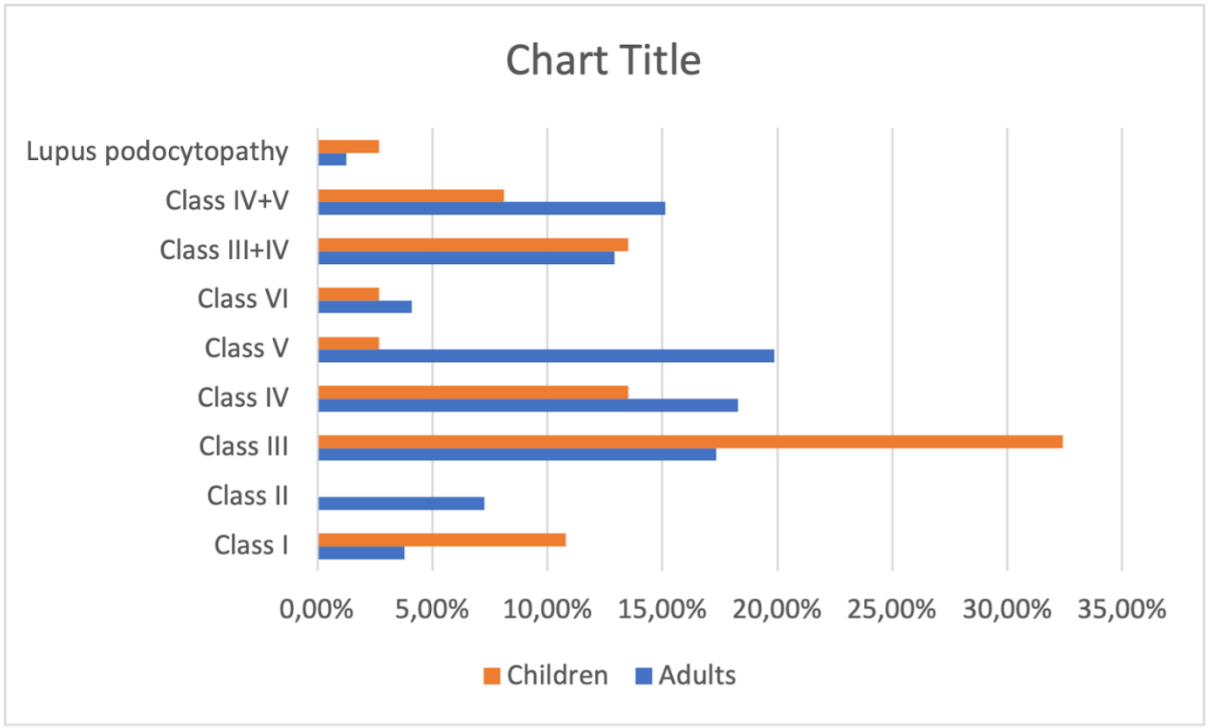

Figure 3.3: Comparison of lupus class prevalence in adults and children

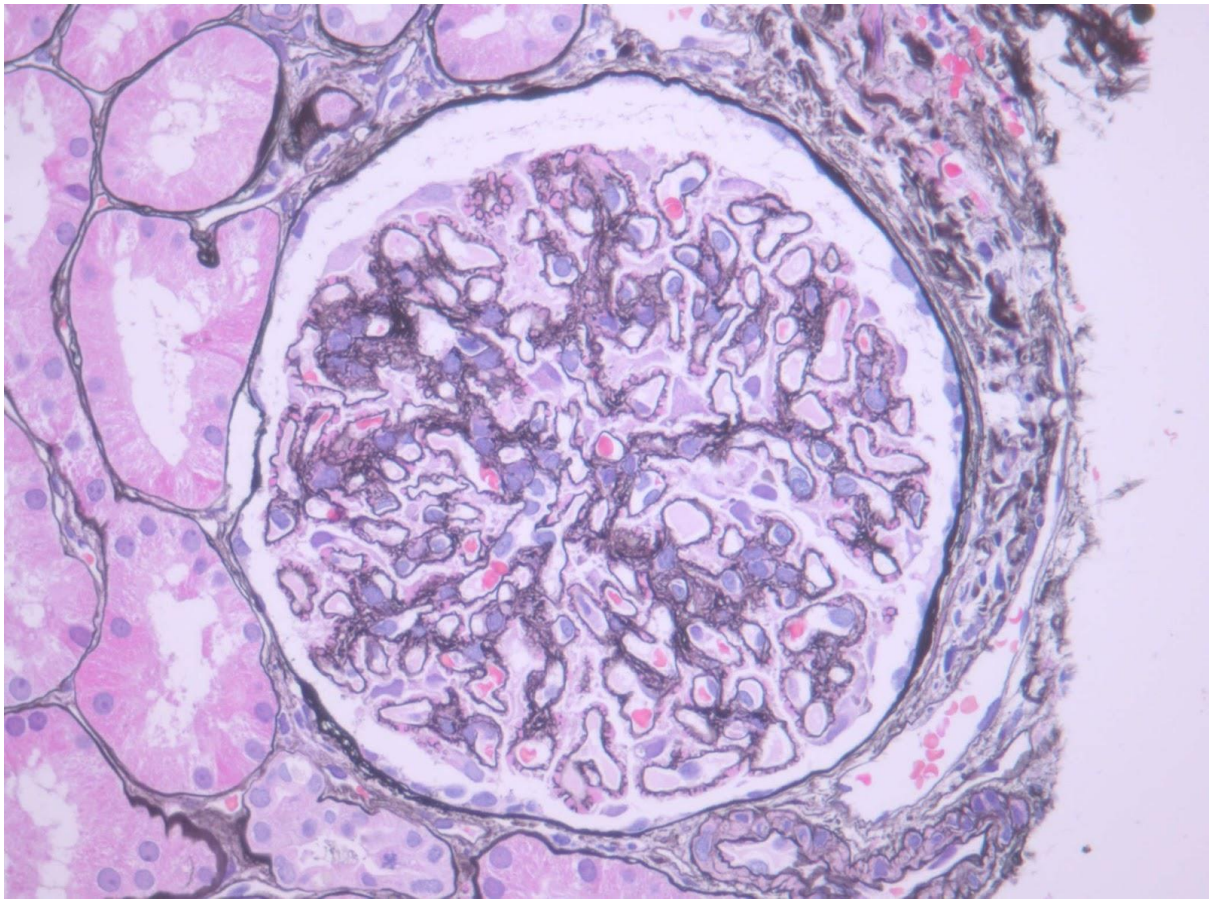

Figure 3.4: Non-proliferative lupus nephritis. A glomerulus with subepithelial immune complex deposits, consistent with class V lupus nephritis. Silver methenamine stain X400

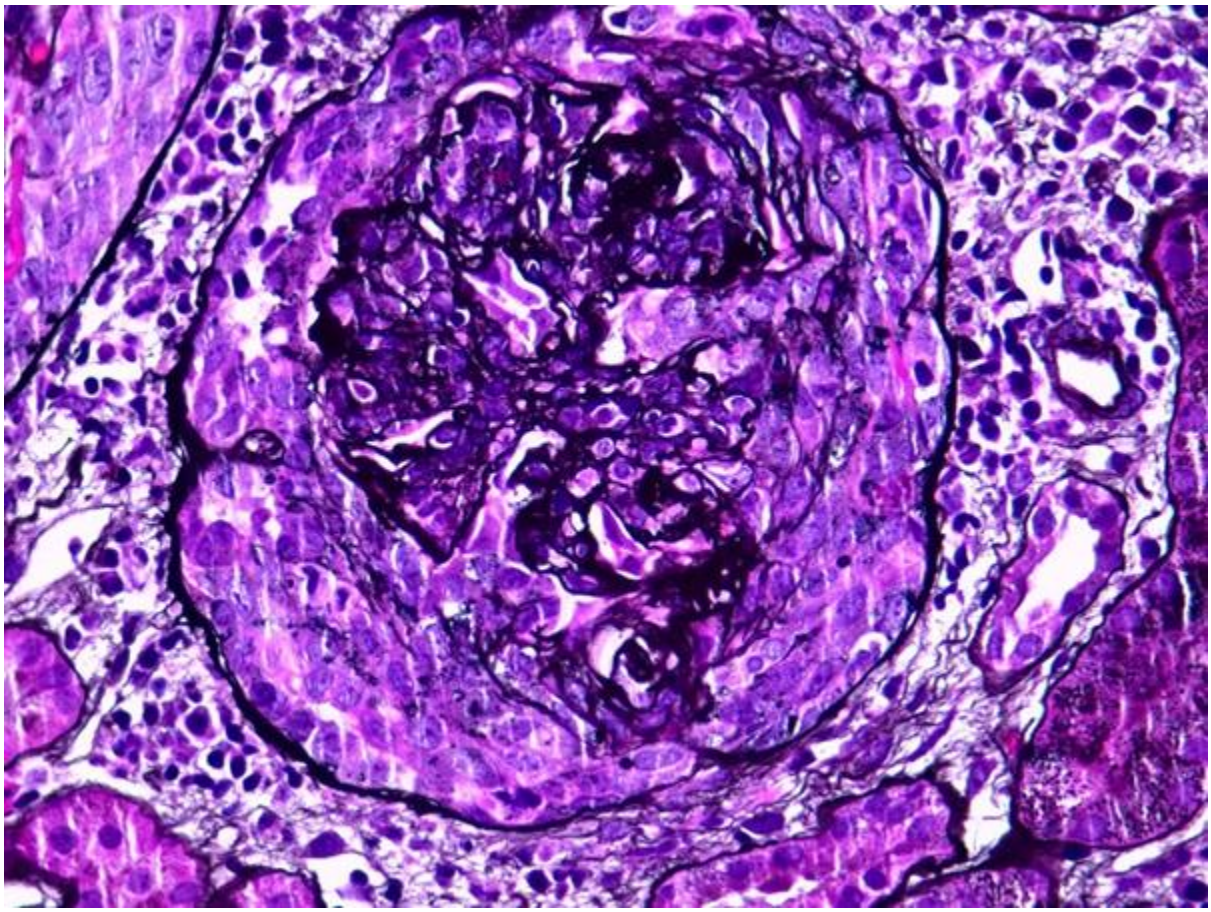

Figure 3.5: Proliferative glomerulonephritis. There is a circumferential cellular crescent associated with endocapillary hypercellularity, consistent with lupus nephritis class IV. Silver methenamine X400.

3.4 Vascular lesions in participants

The presence or absence of vascular lesions was stated in the majority of cases (345/354 – 97.46%). There were no vascular lesions in 240 (69.57%) of cases(See Table 3.4, Figure 3.6). The commonest lesion overall was atherosclerosis (82 - 23.77%) followed by thrombotic microangiopathy (20 – 5.80%) and non-inflammatory lupus vasculopathy (6 – 1.74%). 2 cases of true lupus vasculitis were noted(0.58%). There were no reported cases showing vascular immune complex deposits. When stratified by age, atherosclerosis was less prevalent in children (10.81%) than adults(24.61%), approaching statistical significance (p:0.0598). There were no cases of non-inflammatory lupus vasculopathy in children.

Table 3.4: Prevalence of Vascular Lesions

Vascular lesions	**All Participants n=354**	**Adults n=317**	**Adult Females n=272**	**Adult Males n=45**	**HIV-Positive Adults n=35**	**HIV-Negative Adults N=174**	**Children< 18 years N=37**
Not reported	9 (2.54)	8 (2.52)	7 (2.57)	1 (2.22)	1 (2.86)	7 (4.02)	1 (2.70)
Atherosclerosis	82 (23.16)	78 (24.61)	72 (26.47)	6 (13.33)	11 (31.43)	40 (22.99)	4 (10.81)
Thrombotic microangiopathy	20 (5.65)	17 (5.36)	12 (4.41)	5 (11.11)	1 (2.86)	10 (5.75)	3 (8.11)
Lupus vasculopathy	6 (1.69)	6 (1.89)	3 (1.10)	3 (6.67)	0 (0.00)	2 (1.15)	0 (0.00)
Lupus vasculitis	2 (0.58)	1(0.32)	1 (0.37)	0 (0.00)	0 (0.00)	1(0.57)	1(2.70)
Vascular immune complexes	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Vascular lesion absent	240 (67.80)	212 (66.88)	181 (66.54)	31 (68.89)	22 (62.86)	118 (67.82)	28 (75.68)

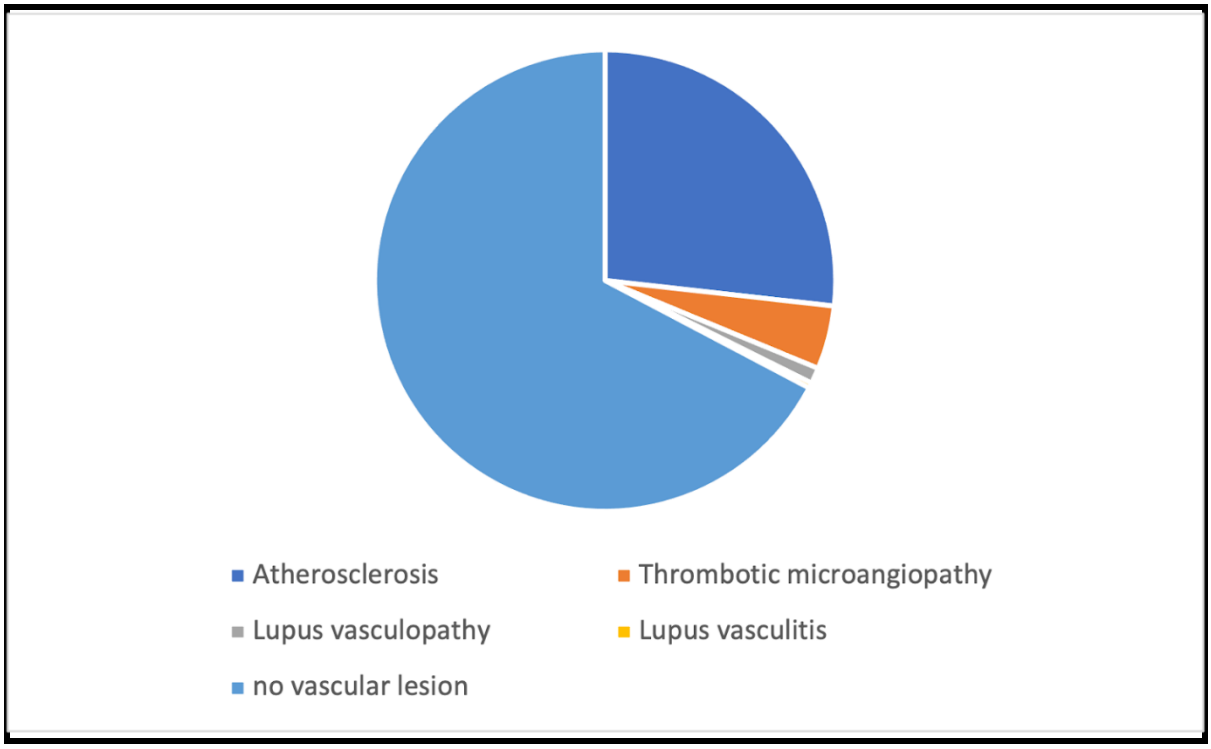

Figure 3.6: Pie chart illustrating prevalence of vascular lesions in all participants.

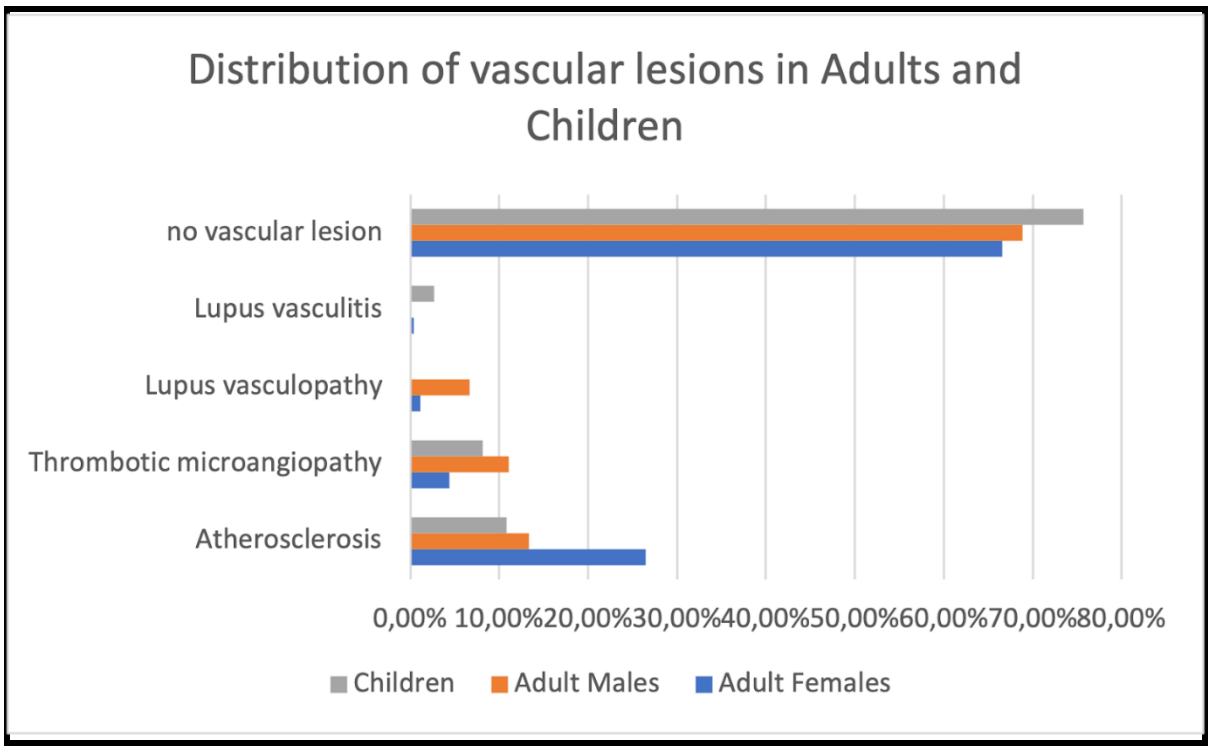

Figure 3.7: Bar graph comparing distribution of vascular lesions in adults and children

The evaluation of vascular lesions in adults, stratified by sex, (see Figure 3.7) showed significantly greater prevalence of non-inflammatory lupus vasculopathy in males (6.67%) than in females(1.10%)(p:0.0112). Atherosclerosis was more common in adult females(26.47% vs 13.33%) but did not reach significance (p:0.0584). There was no statistically significant difference in vascular lesions in adults stratified by HIV status.

3.5 Tubulo-interstitial lesions in participants

Tubulo-interstitial lesions were not reported in 55/354 cases (15.54%). Of 299 cases with reporting, 200 (66.89%) showed features of chronic interstitial nephritis, and 99 (33.11%) of acute interstitial nephritis. There were no statistically significant differences between adults and children and between adults stratified by sex and HIV status. However, a trend toward more acute disease and less chronic disease was noted in children compared to adults.

Severity of tubulo-interstitial disease was reported in all 299 cases. The majority (230/299-76.92%) had mild interstitial nephritis. 18.06% had moderate, and 5.02% severe interstitial nephritis. There was no difference in severity in adults stratified by sex and HIV status.(See Table 3.5)

Table 3.5: Tubulo-interstitial lesions

	All Participants n=354	**Adults** n=317	**Adult Females** n=272	**Adult Males** n=45	**HIV-Positive Adults** n=35	**HIV-Negative Adults** n=174	**Children<18 years** n=37
Interstitial nephritis not reported	55 (15.54)	48 (15.14)	43 (15.81)	5 (11.11)	5 (14.29)	25 (14.37)	7 (18.92)
Interstitial nephritis reported	**299** (84.46)	**269** (84.86)	**229** (84.19)	**40** (88.89)	**30** (85.71)	**149** (85.63)	**30** (81.08)
Acute interstitial nephritis	99 (33.11)	85 (31.60)	72 (31.44)	13 (32.50)	8 (26.67)	49 (32.89)	14 (46.67)
Chronic interstitial nephritis	200 (66.89)	184 (68.40)	157 (68.56)	27 (67.50)	22 (73.33)	100 (67.11)	16 (53.33)
Severity - mild	230 (76.92)	206 (76.58)	173 (75.54)	33 (82.50)	19 (63.33)	114 (76.51)	24 (80.00)
Severity - moderate	54 (18.06)	51 (18.96)	46 (20.09)	5 (12.50)	9 (30.00)	28 (18.79)	3 (10.00)
Severity - severe	15 (5.02)	12 (4.46)	10 (4.37)	2 (5.00)	2 (66.67)	7 (4.70)	3 (10.00)

3.6 Correlation of clinical presentation and serological findings to histologic class of lupus nephritis

The clinical history and serological findings were evaluated against the following parameters:

- Class of lupus nephritis
- Proliferative vs non-proliferative lesions
- Vascular lesions including type of vascular lesions
- Acute and chronic interstitial nephritis

The following significant associations were found:

3.6.1 Proteinuria:

A clinical history of proteinuria was present in 96 participants(27.12%). It was most common in class V lesions[28/64(43.75%)] followed by class IV+V[16/51(31.37%)] and Class I lesions(5/16(31.25%)]. There were significant differences in prevalence, with the lowest noted in class VI[1/14(7.14%)](p:0.0416). When analysed by lesion type, non-proliferative lesions showed a significantly higher prevalence of proteinuria[42/113(37.17%)] compared to proliferative lesions [53/227(23.35%)] (p:0.0416). A history of proteinuria was commoner in participants with thrombotic microangiopathy (p:0.0767) but was not associated with other vascular lesions or chronic and acute interstitial nephritis.

3.6.2 Nephrotic Syndrome(NS):

80 participants (22.6%) had a clinical history of NS. The presence of NS was highest in lupus podocytopathy[4/5(80%)] followed by LN classes VI (4/14(28.57%)(p=0.0516) and III [18/67(26.87%)](p=0.0135). However, it showed no difference in prevalence between proliferative and non-proliferative lesions (p:0.6321).

NS was not associated with the presence of vascular lesions (p:0.1952) and there was no relationship between NS and types of vascular lesions present.

NS was not correlated with the presence of acute or chronic interstitial nephritis (p:0.3200).

3.6.3 Nephritic Syndrome (NiS):

NiS was an uncommon clinical presenting symptom[17/354(4.8%)], being the most common in lupus class VI [2/14(14.29%)] followed by lupus class IV+V. None of the 92 participants in classes II and V presented with NiS. This is highlighted by the significant differences in NiS prevalence between proliferative[14/227(6.17%)] and non-proliferative lesions[1/113(0.88%)] (p:0.0255).

NiS was not correlated with the presence or type of vascular lesions, or with acute and chronic interstitial nephritis.

3.6.4 Isolated Haematuria(IH):

There were 2 cases of isolated haematuria(IH), both in proliferative lesions. Its presence was not related to vascular lesions, or acute and chronic interstitial nephritis.

3.6.5 Acute Renal Failure(ARF):

A single participant had a clinical history of ARF, in a class IV lupus nephritis. There was no relationship with lupus class or type or with acute and chronic interstitial nephritis. However, ARF was significantly associated with thrombotic microangiopathy and lupus vasculopathy (p:<0.001).

3.6.6 Chronic Renal Failure(CRF):

A history of CRF showed no association with lupus class, or with acute or chronic interstitial nephritis. However, a history of CRF was more likely to be present in cases with vascular lesions (p:0.0216) in particular, atherosclerosis(p:0.0501), and thrombotic microangiopathy(p:0.0008).

3.6.7 Hypertension (HT):

A history of HT was not associated with Lupus class or type, or acute or chronic interstitial nephritis. However, participants with a history of HT were significantly more likely to demonstrate a vascular lesion (p:0.0035), in particular, atherosclerosis(p:0.0123) and lupus vasculitis (<0.0001).

3.6.8 Clinical SLE:

A clinical history of SLE was associated with a higher prevalence of mild acute interstitial nephritis compared with chronic interstitial nephritis (p:0.0308) but was not associated with lupus class or presence of vascular lesions.

3.6.9 HIV Status:

HIV status was unrelated to lupus class, the presence of vascular lesions, and acute or chronic interstitial nephritis.

3.6.10 Serology: ANA and anti-double stranded DNA:

Positive ANA or anti-double stranded DNA serology was unrelated to lupus class, the presence of vascular lesions, and acute or chronic interstitial nephritis.

CHAPTER 4:

4 Discussion

Table 4.1 - Summary of results

Variables:	Value (%) n=354
Age years	
0-17	37(10.5%)
>18	317(89.5%)
Gender	
Female	303(85.59%)
Male	51(14.41%)
Clinical presenting features:	
Nephrotic syndrome	80(22.6%)
Nephritic syndrome	17(4.8%)
Proteinuria	96(27.1%)
Acute renal failure	1(0.6%)
Chronic renal failure	5(1.4%)
Hypertension	11(3.1%)
SLE	107(30.2%)
Other	43(12.1%)
HIV status:	
Positive	37(10.5%)
Negative	199(56.2)
Not stated	118(33.3%)
ANA serology	
Pos	217(61.3%)
Neg	21(5.9%)
Unknown	116(32.8%)
Histology: Lupus class	
I	16(4.5%)
II	28(7.9%)
III	67(18.9%)

IV	63(17.8%)
V	64(18.1%)
VI	14(4.0%)
III+V	46(13.0%)
IV+V	51(14.4%)
Lupus podocytopathy	5(1.4%)
Vascular lesions	
Arteriolosclerosis	82(23.2%)
Lupus vasculopathy	6(1.7%)
Lupus vasculitis	2(0.6%)
TMA	20(5.6%)
None	240(67.8%)
Not reported	9(2.5%)

Our 5-year study identified a total of 354 patients with biopsy proven lupus nephritis. To our knowledge, this is the largest described case series from South Africa. A similar study from Cape Town found 251 cases over a 10-year period.[10] In comparison to the Cape Town study, ours has a significantly higher number of membranous lupus nephritis. In the Cape Town study, class V was noted in 14.7% compared to 18.1% in our study. The mixed classes in our study were noted in 27,4% whilst the mixed classes were noted in 21.6% in the Cape Town study. In study from comparison Kwa-Zulu Natal, in South Africa, class V was found to be the commonest lupus class in a cohort of 105 patients over a period of 9 and half years. Class V was found in 34.9% followed by class IV and then III.[21]

The striking female predominance noted in our study [303 (85.59%)] is in keeping with local and international published literature, with adult females predominating (272/354(76.84%).[2,3,5,9,10,21] Our findings are similar to those of Okpetchi et al, who identified a female prevalence of 84.1% in their study. This is also similar to Mody et al who found a female predominance. [10,21]

In our study, proliferative lupus nephritides, with and without a membranous component were significantly commoner than non-proliferative forms of lupus nephritis. A surprising finding of our study is the significantly higher proportion of membranous LN compared to previous

reports, including reports from South Africa such as the study from the Western Cape where class IV was most common lupus class. [10] A study from Kwa-Zulu Natal found class V to be the most common lupus class similar to our study. The reason for this may require further investigation.[21]

The commonest lupus class in children in our study was class III (32.3%) followed by class IV (13.5%), II and mixed class III and V. These findings are in keeping with most reports on lupus nephritis in children and accords with a publication from South Africa where the commonest lupus classes in children were classes III and IV (63%).[34] A similar pattern has been noted in reports from Poland and Romania. In these publications, children tend to present with proteinuria and the most common lupus class is either a class III or IV. [34,38]

A study from Western Cape, South Africa, found nephrotic range proteinuria and asymptomatic urinary abnormalities to be significantly associated with lupus class.

In a study from Iran, there were 144 participants with lupus nephritis, the majority of patients were female (84.7%), with a mean age at biopsy was 25.6+-10.3 which is similar to our study. In this study, the most common lupus class was IV in keeping with most of the literature. In this study, presentation with oedema was found to correlate with biopsy findings, however, the most significant clinical presenting features associated with lupus class was that of an elevated creatinine (p=0.001) and elevated urea (p=0.010). [39]

Clinical presentation in our study was grouped into 7 categories. The most common clinical presentation is that of proteinuria including nephrotic syndrome and subnephrotic proteinuria. This accounted for approximately 50% of the patients. A significant number of patients had a kidney biopsy for clinical concern for lupus nephritis in a known SLE patient. The category of other included a miscellaneous group of clinical presentations, where the presentation could not be grouped into the 6 predetermined categories. Included in this group was a myriad of clinical presentations such as active urinary sediment, pancytopenia, jaundice, mouth ulcers, pericardial effusions. In these patients, the criteria for SLE was fulfilled and a kidney biopsy was performed. This miscellaneous group was noted in 43 cases. In the study from the Western Cape 90% of the patients were hypertensive and 79% of the patients had proteinuria.[10] This is unlike our data, where only 3.1% of patients had hypertension.

In this study, ANA serology was stated in 234 cases, where stated, the majority of cases were positive (214 cases/ 91%) . This is in keeping with the literature as ANA serology forms part of the SLICC criteria for diagnosis of LN. In the cases where the ANA serology was negative, the diagnosis of SLE was made on the basis of other clinical criteria. The most prevalent class showing ANA positivity was lupus podocytopathy, were all 5 cases had a positive ANA serology. This was followed by class III (45, 20.7%) and then by IV (42, 19.4%) and V (36, 16.6%). Of all the cases with positive serology, 144 of them had a proliferative form of lupus nephritis, with class IV being the most common. In the publication from Cape Town, positive ANA serology did not correlate with biopsy findings.[10]

In our study vascular lesions were uncommon. As these are not included in the ISN/RPS classification system, we used the classification system proposed by Appel et al. to classify vascular lesions.[26] The most common vascular lesion encountered was arteriosclerosis, seen in 78 adults (24.6%), this was followed by thrombotic microangiopathy (17, 5.4%) and lupus vasculopathy (6, 1.9%), necrotizing lupus vasculitis was noted in only one patient. (1, 0.3%). The vast majority of patients had no vascular lesions. (212, 66.9%). The patient with a necrotising vasculitis had a class IV lupus nephritis. The infrequency of lupus vasculitis is expected as these lesions are unusual in patients with lupus nephritis. Arteriosclerosis was seen more commonly in adults than in children and this may be because arteriosclerosis may appear later in life irrespective of the presence or absence of lupus nephritis. Arteriosclerosis is an aging related vascular lesion.

Thrombotic microangiopathy was noted in 5% of the adults in our study. This is in keeping with the other published studies.[25] The frequency of vascular lesions is in keeping with the literature.[27,29]

In children, vascular lesions were noted in 8 patients. 4 children had arteriosclerosis, 3 children had thrombotic microangiopathy and lupus vasculopathy and vasculitis were noted in 1 child each. Unlike in the adult, the child with lupus vasculitis had class III lupus nephritis.

There are no published reports on vascular lesions in the South African context. Our study is the first to report on vascular lesions in lupus nephritis. Further research is required in the South African setting, looking at the prevalence of vascular lesions and its correlation with prognosis.

4.1.1 Limitations

In many of the biopsy reports, the indication for biopsy was stated as clinical suspicion for lupus nephritis without elaboration of what the presentation that prompted the biopsy was. This limited our analysis of clinical presentation and may have had an impact on our results.

In this study race was not included as it was not stated on the request forms. This hampered epidemiologic assessment of the study population.

Missing or incomplete data was a limitation in this study as we relied on the information provided by the clinicians on the request slips. As the biopsies were reported by different pathologists, incomplete information in the histology report was a limitation.
Very small numbers had anti ds-DNA status available. The pattern of ANA positivity was stated in a very limited number of cases.

4.1.2 Suggestions for Future Research

Further research is required correlating the diagnosis of lupus nephritis with survival rates and possible treatment outcomes. Possible biomarkers in the urine in patients with SLE to establish if renal involvement and type of lesion can be predicted without a renal biopsy.

In this study we found 5 cases of lupus podocytopathy. This is an uncommon form of lupus nephritis that may require further research to ascertain if this is related to Apol 1 risk variants.

CHAPTER 5:

Conclusion

This is amongst the largest series of biopsy proven lupus nephritis reported from South Africa. This study contributes to the understanding of the epidemiology of lupus nephritis in South Africa.

In this study the following pertinent findings were noted:

1. Lupus nephritis is significantly more common in females than males. This is in keeping with the known literature.
2. In our cohort, a diagnosis of class V lupus nephritis with or without a concomitant class III or IV is surprisingly more common than in previously published literature.
3. This study confirms that vascular lesions of lupus nephritis are uncommon.
4. Concomitant SLE and HIV infection is not uncommon.
5. Demographic features, clinical presentation and serologic findings are insufficient to accurately predict the glomerular lesion.
6. The significance of lupus podocytopathy needs to be investigated in a different study.

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Appendix 1: Data Collection sheet

Hospital

Age:

Gender: M/F

Repeat biopsy: Yes/No

Clinical presentation: Nephrotic syndrome

Nephritic syndrome

Isolated hematuria

Acute renal failure

Chronic renal failure

Hypertension

SLE

Other

ANA serology: Pos/Neg/Not stated

Titre:

Pattern:

Anti double-stranded DNA: Pos/Neg

Urea:

Creatinine:

HIV status: Pos/Neg/Unknown

Biopsy findings:

Lupus class: I/II/III/IV/V/IV, combined III+V/IV+V

Vascular lesions: Atherosclerosis: Yes/No

Lupus vasculopathy: Yes/No

Necrotising vasculitis: Yes/No

Thrombotic Microangiopathy: Yes/No

Tubulo-interstitial findings: Acute interstitial nephritis; Mild / Moderate / Severe

Chronic interstitial nephritis; Mild / Moderate / Severe

Activity score:

Chronicity score:

Appendix 2: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Pulane Nkagisang Mosiane

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190538

NAME: (Principal Investigator)	Dr Pulane Nkagisang Mosiane
DEPARTMENT:	Anatomical Pathology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Biopsy proven lupus nephritis: A 5-year clinico-pathologic insight from a single centre
DATE CONSIDERED:	31/05/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Tanvier Omar
APPROVED BY:	Dr CB Penny, ~~Chairperson~~, HREC (Medical)
DATE OF APPROVAL:	31/05/2019

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 the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. 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 **May** and will therefore be due in the month of **May** 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

Appendix 3: Clinical and immunologic criteria used in SLICC classification system

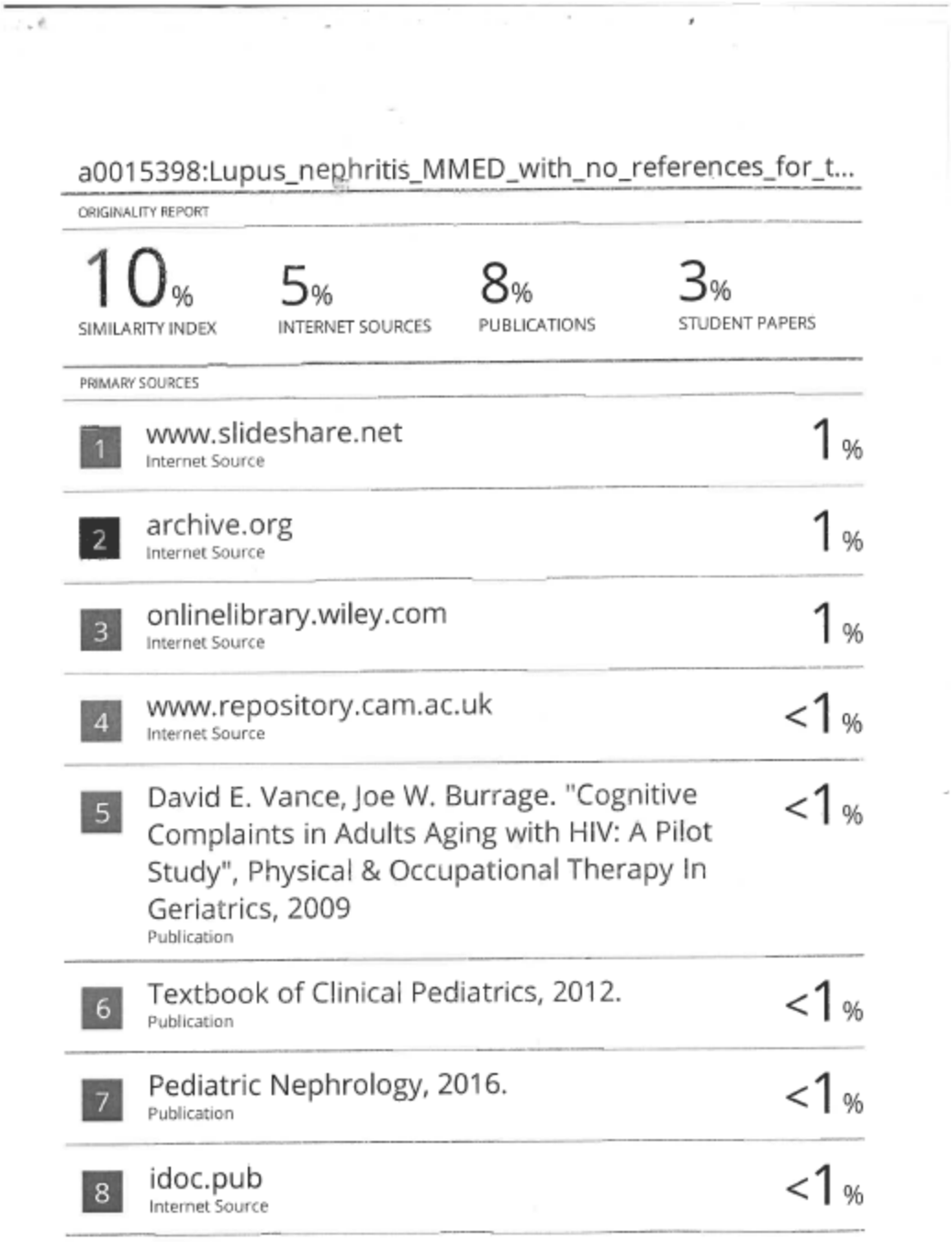
Table 3. Clinical and immunologic criteria used in the SLICC classification system*

Clinical criteria
1. Acute cutaneous lupus, including:
   Lupus malar rash (do not count if malar discoid)
   Bullous lupus
   Toxic epidermal necrolysis variant of SLE
   Maculopapular lupus rash
   Photosensitive lupus rash
   in the absence of dermatomyositis
   OR subacute cutaneous lupus (nonindurated psoriaform and/or annular polycyclic lesions that resolve without scarring, although occasionally with postinflammatory dyspigmentation or telangiectasias)
2. Chronic cutaneous lupus, including:
   Classic discoid rash
   Localized (above the neck)
   Generalized (above and below the neck)
   Hypertrophic (verrucous) lupus
   Lupus panniculitis (profundus)
   Mucosal lupus
   Lupus erythematosus tumidus
   Chilblains lupus
   Discoid lupus/lichen planus overlap
3. Oral ulcers
   Palate
   Buccal
   Tongue
   OR nasal ulcers
   in the absence of other causes, such as vasculitis, Behçet's disease, infection (herpesvirus), inflammatory bowel disease, reactive arthritis, and acidic foods
4. Nonscarring alopecia (diffuse thinning or hair fragility with visible broken hairs)
   in the absence of other causes such as alopecia areata, drugs, iron deficiency, and androgenic alopecia
5. Synovitis involving 2 or more joints, characterized by swelling or effusion
   OR tenderness in 2 or more joints and at least 30 minutes of morning stiffness
6. Serositis
   Typical pleurisy for more than 1 day
   OR pleural effusions
   OR pleural rub
   Typical pericardial pain (pain with recumbency improved by sitting forward) for more than 1 day
   OR pericardial effusion
   OR pericardial rub
   OR pericarditis by electrocardiography
   in the absence of other causes, such as infection, uremia, and Dressler's pericarditis
7. Renal
   Urine protein–to-creatinine ratio (or 24-hour urine protein) representing 500 mg protein/24 hours
   OR red blood cell casts
8. Neurologic
   Seizures
   Psychosis
   Mononeuritis multiplex
   in the absence of other known causes such as primary vasculitis
   Myelitis
   Peripheral or cranial neuropathy
   in the absence of other known causes such as primary vasculitis, infection, and diabetes mellitus
   Acute confusional state
   in the absence of other causes, including toxic/metabolic, uremia, drugs
9. Hemolytic anemia
10. Leukopenia ($<4,000/mm^3$ at least once)
    in the absence of other known causes such as Felty's syndrome, drugs, and portal hypertension
    OR
    Lymphopenia ($<1,000/mm^3$ at least once)
    in the absence of other known causes such as corticosteroids, drugs, and infection
11. Thrombocytopenia ($<100,000/mm^3$) at least once
    in the absence of other known causes such as drugs, portal hypertension, and thrombotic thrombocytopenic purpura

Immunologic criteria
1. ANA level above laboratory reference range
2. Anti-dsDNA antibody level above laboratory reference range (or >2-fold the reference range if tested by ELISA)
3. Anti-Sm: presence of antibody to Sm nuclear antigen
4. Antiphospholipid antibody positivity as determined by any of the following:
   Positive test result for lupus anticoagulant
   False-positive test result for rapid plasma reagin
   Medium- or high-titer anticardiolipin antibody level (IgA, IgG, or IgM)
   Positive test result for anti-β_2-glycoprotein I (IgA, IgG, or IgM)
5. Low complement
   Low C3
   Low C4
   Low CH50
6. Direct Coombs' test *in the absence of hemolytic anemia*

* Criteria are cumulative and need not be present concurrently. SLICC = Systemic Lupus International Collaborating Clinics; SLE = systemic lupus erythematosus; ANA = antinuclear antibody; anti-dsDNA = anti–double-stranded DNA; ELISA = enzyme-linked immunosorbent assay.

Appendix 4: Turnitin report



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