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Primary focal segmental glomerulosclerosis in adults: presentation, prevalence and treatment outcomes in patients at Charlotte Maxeke Johannesburg Academic Hospital

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1. Background: Primary Focal Segmental Glomerulosclerosis

1.1 Introduction

Focal segmental glomerulosclerosis (FSGS) is considered one of the most important causes of end-stage kidney disease in adults with an annual incidence of approximately 7 per 1 million and is also the most common histological finding in Black African patients diagnosed with glomerular disease [1-3]. There is currently very little South African data available regarding the prevalence and response to treatment of FSGS. The aim of this study was to determine the prevalence of FSGS and treatment outcomes in the population of patients seen at Charlotte Maxeke Johannesburg Academic Hospital's renal unit.

1.2 FSGS and Chronic Kidney Disease

The incidence of end-stage kidney disease (ESKD) in Sub-Saharan Africa was estimated to be just fewer than 100 per million of the population in 2006, contributing to a significant and increasing burden of disease [3]. It is estimated that by the year 2030, 70% of patients with ESKD will be from the developing world [3]. In 1994 glomerular disease was reported as the cause of ESKD in 52% of patients in South Africa, however there is still no reliable data regarding the aetiology of ESKD in Africa [4]. FSGS accounts for approximately 40% of patients who present with the nephrotic syndrome and is the most common primary glomerular disease causing ESKD in the United States [5,6]. It is also the most prevalent histologic finding in Black African patients with glomerular disease, with prevalence rates of 15-25% [3].

1.3 Pathogenesis of FSGS

The exact aetiology of primary FSGS is unknown; however, electron microscopy suggests that a common pathway leading to the development of FSGS is podocyte injury. This podocyte injury may arise through a variety of mechanisms; a circulating factor has been suggested to be responsible for some cases of primary FSGS as evidenced by recurrence of FSGS in some patients after transplantation for ESKD secondary to FSGS [7]. Soluble urokinase plasminogen activating receptor (suPAR) has been proposed in some studies to be the circulating factor responsible for the injury to glomerular cells resulting in primary FSGS. The evidence confirming the role of suPAR has, however, been inconclusive [8,9].

Genetic mutations involving the podocin and nephrin genes have also been implicated in the pathogenesis of FSGS in Caucasian populations [7-9]. Interestingly, the incidence of focal segmental glomerulosclerosis is higher and the rate of renal survival is worse among Black patients; genetic studies in large populations have identified risk factors for focal segmental glomerulosclerosis and end-stage renal disease in this group [5]. In particular, mutations to the *MYH9* gene on chromosome 22 (which codes for myosin) have been identified as being responsible for the development of primary FSGS in patients of African descent, although further studies have instead found a greater association between the *APOL1* gene on chromosome 22 and FSGS. The G1 and G2 mutations of the *APOL1* gene have been demonstrated to be prevalent in individuals of African ancestry with FSGS and HIV-associated nephropathy (a form of the collapsing variant of FSGS) [5,10-12].

1.4 Classification of FSGS

FSGS comprises a syndrome of multiple pathological processes characterized by progressive glomerular fibrosis that is diagnosed on renal biopsy [13]. Patients usually present with the nephrotic syndrome or proteinuria but can present with an acute

nephritic syndrome or with urinary abnormalities alone [14]. FSGS can be classified as idiopathic (primary) or secondary (where an underlying cause is present) and can usually be differentiated as such by histological findings on renal biopsy. Characteristically, primary FSGS is associated with a more diffuse pattern of podocyte injury (best appreciated on electron microscopy) and negative or weakly positive staining on immunofluorescence (which in cases of primary FSGS usually only detects either IgM or albumin). In contrast, secondary FSGS demonstrates podocyte injury that is often patchy in distribution. In cases of FSGS secondary to immune complex mediated glomerulonephritis, immunofluorescence staining is often strongly positive for IgG, IgM, IgA, albumin, and complement. Glomerular size may also assist in the differentiation of primary and secondary forms of FSGS as FSGS secondary to glomerular hyperfiltration (for example, obesity, reflux nephropathy, and sickle cell anaemia) is often associated with glomerulomegaly [15,16].

1.5 Secondary FSGS

The damage to the glomerulus in secondary FSGS may result from an initial insult such as glomerular hyperfiltration (from previous scarring or intraglomerular hypertension), direct podocyte toxicity, or other glomerular diseases such as membranous glomerulonephritis [16,17].

Glomerular hyperfiltration may be a consequence of conditions in which there are reduced numbers of functioning nephrons (such as renal agenesis & reflux nephropathy) or as a result of increased strain placed on a normal numbers of nephrons (e.g., obesity, sickle cell disease, hypertension, and obstructive sleep apnoea). This hyperfiltration eventually results in further nephron loss initiating a cascade of events which ultimately manifests as glomerulosclerosis [17,24].

Direct podocyte injury may result from a variety of causative agents including viruses and drugs. Certain viruses are able to damage podocytes either directly or indirectly (through immune-mediated mechanisms); the virus most extensively studied in this regard is the Human Immunodeficiency virus type 1 (HIV-1). HIV-1 causes a form of secondary FSGS commonly known as HIV-associated nephropathy (HIVAN) which has become an important cause of ESKD in individuals of African ethnicity infected with the virus [18-20]. Certain drugs including heroin, bisphosphonates and interferon therapy are capable of causing podocyte toxicity, resulting in FSGS [21-23].

Patients with secondary forms of FSGS tend to manifest more insidiously and may not present with the typical nephrotic syndrome (peripheral oedema, hypoalbuminaemia and hypercholesterolaemia are minimally present or absent in these cases); instead, such patients may present with gradually worsening renal function and sub-nephrotic range proteinuria [5,17.]

The importance of distinguishing primary and secondary forms of FSGS lies in the different approaches to treatment; primary FSGS is generally treated with immunosuppression, whereas the treatment of secondary FSGS lies in addressing the underlying cause [22,23]. Remission rates in idiopathic primary FSGS are highly variable and range from 31-74 % [26].

1.6 Histological Variants of FSGS

Five histologic variants of FSGS exist, namely: cellular, tip, collapsing, perihilar and not otherwise specified (NOS)/classical (Figure 1.6.1). These entities are important to distinguish as they may represent different pathologic processes and thus have differing prognoses and treatments [27]. Overall the prognosis of primary FSGS is poor, with only 40% of all sufferers not progressing to ESKD at 10 years [28.] Thomas et al have shown that a high proportion of African-Americans with FSGS have the

collapsing variant (up to 91% in some studies) with very few (15%) having the tip variant [27]. Historically the collapsing form of FSGS has been found to be the least responsive to treatment with remission rates of 14% leading to poor renal survival (33% renal survival at 3 years) , whereas the tip variant carries the most favourable prognosis with remission rates of 50% and a 3-year renal survival of 73% [26,27].

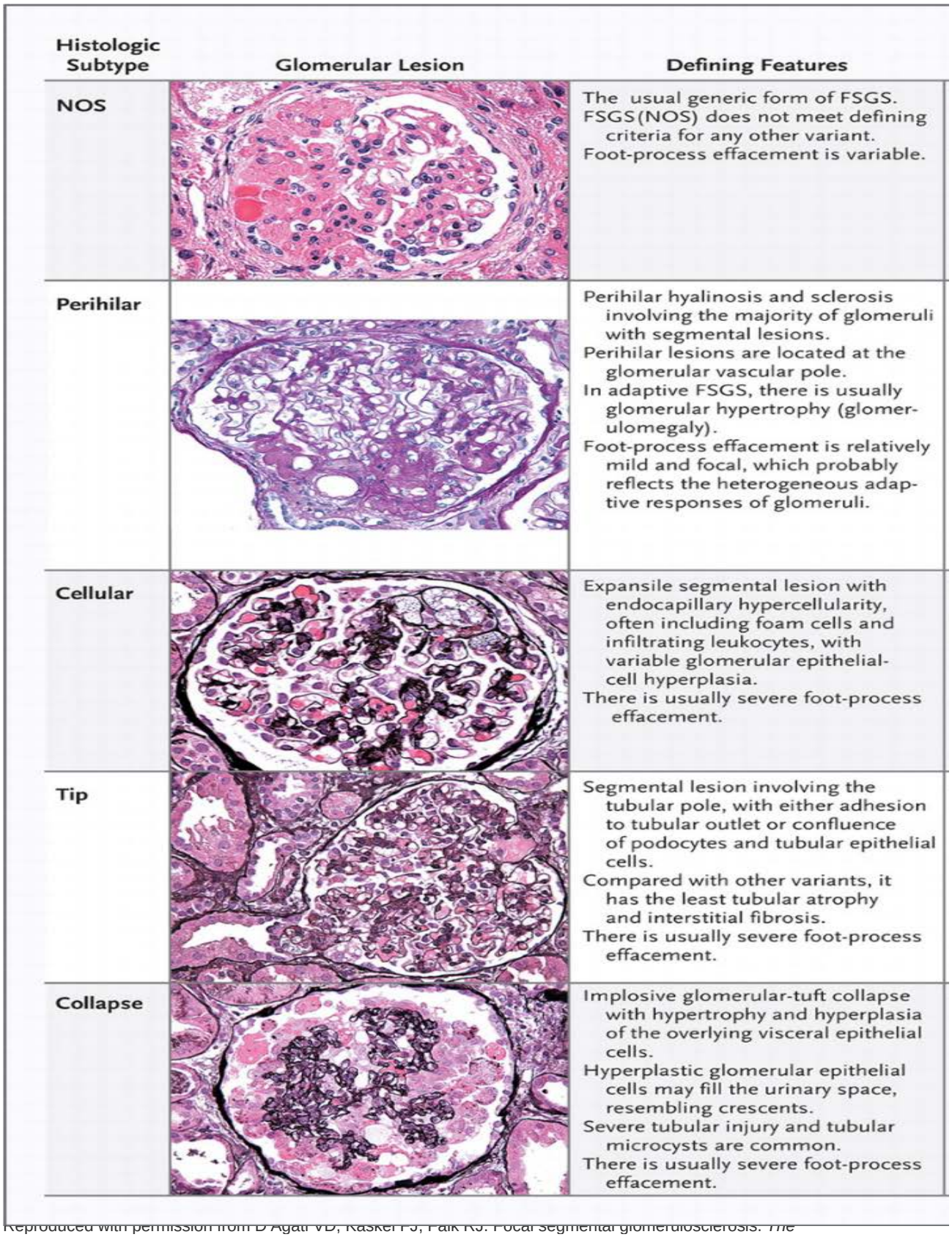
Other factors affecting prognosis adversely include patients who present with the nephrotic syndrome or nephrotic range proteinuria (>3.5g/24 hrs), elevated serum creatinine (1.3 mg/dL or 114 mmol/L) and the degree of tubulointerstitial fibrosis on biopsy [28,29]. The most important predictor of outcome however is response to treatment irrespective of histological variant present on biopsy (Table 1.6.1) [30].

Table 1.6.1 Factors Predicting Poor Prognosis in Primary FSGS [26-30]

Poor Prognostic Factors: FSGS
Histological variant: collapsing
Nephrotic range proteinuria >3.5g/24 hrs
Elevated serum creatinine >114mmol/L
Higher degree of tubulointerstitial fibrosis on biopsy
Poor response to treatment

In 2004 Chun et al reported that, contradictory to previous evidence, 64% of patients with the collapsing variant compared to 78% with the tip variant attained remission. This study also concluded that the 10-year survival for patients who attained remission was excellent irrespective of histological lesion [30]. The discrepancy in treatment outcomes observed in different studies further motivated this research in determining clinical end-points in the cohort of patients at CMJAH with primary FSGS.

Figure 1.6.1 Histological Variants of FSGS



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1.7 Epidemiology of FSGS

International data indicates a geographical variation in the prevalence and epidemiology of FSGS; with a prevalence (of all primary glomerular disease) of 6% in Chinese biopsy registries [31], 10.4% in Italian registries [32], and approximately 12% in Spanish studies [33]. The United States Renal Data System recorded biopsies over a period of 21 years and identified FSGS as the commonest primary glomerular lesion in patients with end-stage kidney disease (2.3% of cases of ESKD) [34]. Rydel et al reported patient characteristics in 81 patients with primary FSGS; 53% of patients were Black, 58% male and the median age of patients at the time of biopsy was 39 years of age [29].

1.8 Clinical Presentation of FSGS

Patients with primary FSGS usually present with acute or sub-acute proteinuria and eventually manifest with features of the nephrotic syndrome (peripheral oedema, hypoalbuminaemia and nephrotic range proteinuria). Patients may also have hypertension and/or haematuria, which occurs in up to 37% of patients in some studies [29]. Nephrotic range proteinuria has been reported to occur in 60-75% of cases at presentation, hypertension in 45-65% & renal dysfunction in 25-50% [29,35].

1.9 Treatment of FSGS

The goal of treatment for primary FSGS is to induce complete or partial remission in order to prevent progression of renal dysfunction and ultimately ESKD. Initial therapy in patients with FSGS includes renin-angiotensin system blockade and sodium restriction [29]. Additional therapy using 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) Reductase inhibitors are often administered to reduce the elevated lipids often associated with the nephrotic syndrome [25]. Patients with nephrotic-range proteinuria should be given a course of high-dose glucocorticoid therapy at 1mg/kg/day or 2mg/kg on alternate days for a period of up to 16 weeks after which

this dose is tapered (dependent on clinical response) over the next 3-6 months. Remission rates vary from 40-80% in different studies [29,30,35.] Patients who are classified as glucocorticoid-resistant i.e. who do not achieve a partial or complete response after 16 weeks of treatment are treated with calcineurin-inhibitors (Cyclosporine or Tacrolimus) in addition to Prednisone for 12 months before tapering the dose. Glucocorticoid and calcineurin-inhibitor therapy have been found to be successful in about 50% of patients [5,35]. Tacrolimus has been less extensively studied than Cyclosporine, however preliminary uncontrolled studies have shown promise in inducing remission in patients who are steroid and Cyclosporine resistant [36]. Randomised controlled trials have found no benefit in using Mycofenolate Mofetil (MMF) over Cyclosporine in steroid-resistant patients, while uncontrolled trials have demonstrated that MMF is a useful drug in inducing remission in Cyclosporine and steroid-resistant patients [26,37,38]. Sirolimus has been shown to be effective in some cases but concerns exist as to its tendency to exacerbate proteinuria thus contributing to nephron damage [39]. Other treatment options that have been investigated include alkylating agents, plasmapheresis and anti B-cell monoclonal antibodies (rituximab), however none of these have been shown to be useful in the treatment of primary FSGS [5,40]. Patients undergoing renal transplantation for ESKD secondary to primary FSGS have a 40% rate of recurrence in the allograft, and plasmapheresis in this clinical setting has been shown to be effective [41].

Current recommendations for treatment of primary FSGS in patients with nephrotic syndrome are glucocorticoid therapy as first-line treatment for 16 weeks. Patients who are found to be steroid resistant / dependent are usually switched to calcineurin inhibition (most commonly Cyclosporine) and steroid therapy for 1 year (Table1.9.1) [42].

Table 1.9.1 Therapy Guidelines & Outcomes (Adapted from the KDIGO Clinical Practice Guidelines for Glomerulonephritis) [42]

Drug	Duration of treatment	Outcome
1st line therapy: • **Prednisolone**	1mg/kg/day OR 2mg/kg alternate days	40-80% remission
Steroid-resistance therapy: • **Cyclosporine Or** • **Tacrolimus And** • **Prednisolone**	1 year-in case of remission 1 year-in case of remission 4-6 months	Upto 50% remission Upto 50% remission
Other Therapies: • **Sirolimus, MMF, alkylating agents**	Insufficient data	Insufficient data

Response to treatment may be partial (defined as a reduction in proteinuria by >50% and to subnephrotic levels <3.5g/24 hrs) or complete (defined as proteinuria <0.3g/24 hrs) [42]. Relapse occurs most commonly within the first 2 months of stopping steroids (around 75%) [38]. Steroid dependence can be defined as relapse of disease on steroid-therapy or the need for ongoing glucocorticoid therapy in order to maintain remission. Steroid resistance refers to the inability to achieve at least partial remission after a duration of at least 12-16 weeks of adequate steroid therapy [7,29,41-48]. Rydel et al reported that 50% of patients with nephrotic range proteinuria due to primary FSGS respond to glucocorticoid therapy within 4 months and all patients respond by 9 months [29]. These findings contrasted with previous studies, which demonstrated a less than 20% response-rate to therapy which resulted in the historical conviction that FSGS was steroid-unresponsive [7,50-54].

1.10 The South African Perspective of FSGS

Conflicting findings in various studies regarding prognostic indicators and treatment outcomes have prompted this review of patients with primary FSGS. Particular emphasis was placed on epidemiological data and responses to therapy in order to

identify prognostic features and compare clinical outcomes to other previously studied population groups.

Of particular interest in this regard is the indigenous Black population with FSGS, which has not been previously investigated. The current data on FSGS available in Black populations is derived from African-American patient cohorts who may be genetically distinct from Sub-Saharan Africans as these patients are historically descended from the Central and West Africans. The *APOL1* mutations associated with kidney disease and FSGS have been investigated in the African-American population, however the penetrance of this gene throughout Sub-Saharan Africa is currently unknown [55].

The prevalence of primary FSGS and its response to therapy in the South African context has not been well studied. Review of the available literature has identified only one study conducted in South Africa describing the clinical course, histological grading and response to treatment of children with FSGS in Kwa-Zulu Natal. The study found an increasing incidence of FSGS in general (from 1.8% to 20% in Indian children and 5% to 28% in Black children over a 25-year period), as well as similarities between Indian and Black children in terms of epidemiological data and response to treatment. It additionally found that Indian children remitted more frequently and Black children relapsed more often [56].

1.11 Rationale for Study

Primary FSGS is an important cause of ESKD, particularly in the Black population, and contributes to an increasing global burden of disease. Therapy for FSGS has demonstrated variable outcomes in different population groups, however these outcomes are additionally influenced by multiple presenting clinical parameters.

There is currently a lack of data regarding the demographics, histological variant and response to treatment of FSGS in the South African population. Country-specific data regarding disease-entities provides the clinician with better insight in terms of identifying and managing their local patient populations optimally. It also provides the health-care community with a broader understating of the nature of clinical disorders and the impact of diseases within the local community. This study was undertaken to better characterise the presentation and outcomes in patients with primary FSGS with the aim of furthering knowledge in the assessment and management of this condition.

1.12 Aim of Study

The aim of this study was to analyse the demographic data and treatment of patients with primary FSGS and to compare the different treatment outcome groups in terms of clinical and demographic data.

1.13 Study Objectives

- To determine the prevalence of FSGS as a proportion of all native kidney biopsies performed at CMJAH from 2001-2010.

- To describe the demographic data, histological lesion, Urine Protein: Creatinine ratio (UPCR), renal function and clinical presentation at the time of biopsy in patients diagnosed with primary FSGS for the period 2001-2010.

- To review the treatment which each patient with biopsy-proven primary FSGS received and their outcomes including the proportion of these patients achieving remission and the proportion with progression of renal dysfunction. Long-term outcomes such as sustained remission, relapse and end-stage kidney disease were also determined. Analysis was made of the nature of

remission (i.e., complete or partial) and the impact thereof on relapse rates and progression to ESKD

- To compare groups of patients by sex, race & histological variant, in terms of presenting features, treatment outcomes and cumulative outcomes (chronic kidney disease and/or death).

2. Methods

2.1 Study Description

This was a single centre retrospective, observational study conducted on all patients who underwent native kidney biopsies at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from 01/01/2001-31/12/2010. This study contains descriptive and comparative elements.

2.2 Study Population

All native kidney biopsies conducted at CMJAH on patients aged 18 years and older between the years 2001-2010 were analysed in order to identify the number of patients with FSGS. CMJAH is a tertiary level academic hospital situated in central Johannesburg and serves a population that is racially and ethnically varied. A total number of 525 biopsies on native kidneys were reviewed for the study period, from which a total of 38 patients diagnosed with primary FSGS were identified for inclusion in this study. Eight out of the total 38 patients had no follow-up data and thus only presenting parameters were documented in this group. Treatment response and patient outcomes were analysed in the remaining patients.

2.3 Inclusion Criteria

- All patients 18 years and older during the period January 2001-December 2010 undergoing native kidney biopsy
- All adult patients with retrospective review of renal biopsy and patient presentation confirming the diagnosis of primary FSGS between January 2001-December 2010
- Minimal duration of follow-up at least 3 months following biopsy

- Patients who were followed up for less than 3 months were analysed in terms of their presenting parameters only

2.4 Exclusion Criteria

- All patients in whom retrospective review of renal biopsy, serological investigations and clinical presentation suggested a secondary cause for FSGS were excluded from further analysis
- All patients undergoing transplant kidney biopsy
- All patients under the age of 18 years were excluded from the study

2.5 Data Extraction

The renal database which records every renal biopsy performed at CMJAH was used to determine the total number of biopsies performed on native kidneys from 2001-2010. Renal biopsy reports were used to identify patients with primary FSGS. Once patients with primary FSGS were identified, the National Health Laboratory Service (NHLS) and patient records were used to evaluate the following:

- Presenting Data: a total of 38 patients were analysed in respect of the following:
    - Demographics: age, sex, race, reason for renal biopsy
    - Laboratory parameters: histological subtype, serum White Cell Count (WCC) ($X10^9$/L), serum Haemoglobin (Hb)(g/dL), spot Urine Protein:Creatinine ratio (uPCR) (g/mmol), Urine White Cell Count (UWCC)/ml, Urine Red Cell Count (URCC)/ml, urine dysmorphic cells (%), Creatinine (micromoles/L), Glomerular Filtration Rate (eGFR) (ml/min/1.73m^2)

- Spot uPCR was utilised to assess degree of proteinuria as this test was used with greater frequency than a 24-hour uPCR.
- Treatment and Outcomes: 30 patients had follow-up data and were analysed as follows:
    - Treatment: glucocorticoid therapy received (yes/no), dosage, duration, follow-up UPCR, second-line therapy, type of second-line therapy, reason for second line therapy
    - Remission and outcomes: remission (yes/no), partial or complete remission, final outcome (if known)

2.6 Definitions

As per the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines on Glomerulonephritis, the following definitions were applied:

- Nephrotic range proteinuria was defined as a spot uPCR >300-350 mg/mmol (uPCR >0.3-0.35 g/mmol)
- Sub-nephrotic proteinuria was defined as a spot uPCR >30 mg/mmol but <300 mg/mmol (uPCR > 0.03g/mmol but <0.3g/mmol)
- eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) 4 variable equation: eGFR=175 X Serum Creatinine$^{-1.154}$ X age$^{-0.203}$ X 1.212 (if patient Black) X 0.742 (if female) [57]
- Complete remission was defined as a reduction of proteinuria to <30 mg/mmol (<0.03g/mmol). Other parameters used in the KDIGO guidelines to further define complete remission such as normal serum creatinine and normal serum albumin were not utilised in this study.
- Partial remission was defined as a reduction of proteinuria to 30–350mg/mmol (0.03-0.35g/mmol)

- Relapse was defined as an increase in protein : creatinine to a level >350 mg/mmol (>0.35g/mmol) urine after remission had been obtained

2.7 Statistical Analysis

All data was collected on Microsoft ® Excel 2013. Data analysis was then performed using Statistica^{TM} Version 9. The Shapiro Wilk W test was used to test for normality of distribution. Because most variables were not normally distributed, medians and interquartile ranges were used to represent the central tendency and measure of statistical dispersion respectively.

Comparative analysis was performed using the Mann Whitney U test for continuous variables and Fisher exact test for categorical variables. Survival analysis was assessed using the Kaplan-Meier estimator. A $p<0.05$ was considered statistically significant.

3. Results

3.1 The Epidemiology and Presenting Features of Primary FSGS

3.1.1 The Epidemiology of FSGS

A total of 525 renal biopsies were performed on native kidneys during the 10-year study period. The prevalence of primary FSGS was determined to be 7.2% with a total number of 38 patients identified for inclusion in this study. The prevalence of FSGS from 2001-2005 was 7.7% and from 2006-2010 was 6.9% of all biopsies. Two patients were excluded from this series due to inadequate data (histological variant was not clear on biopsy report).

3.1.2 The Presenting Features of FSGS

Of the 38 patients included in this series 8 had no follow-up data available for analysis, therefore only presenting variables were analysed in this group. A large proportion (86.8%) of patients were Black (33 out of 38), 3 were White and 2 Indian; there were no patients of mixed race in this study. The ratio of Black to Non-Black patients was 6.6:1. A male preponderance of 65.8% vs 34.2% for females was noted with a male to female ratio of 1.92:1 Patients in this series most commonly presented with nephrotic-range proteinuria (89.5% of cases) with a median uPCR of 0.77 g/mmol (0.77g/mmol); subnephrotic range proteinuria and renal dysfunction occurred much less frequently (2.6% and 7.9% respectively). The most common histological variant was found to be the classical variant with a prevalence of 47.4%, the second most common being the collapsing variant at 21.1% and the least common being the tip variant (7.9%). The perihilar and cellular variants constituted 4 and 5 cases (10.5% and 13.2%) respectively out of the 38 patients (Figure 3.1.1).

One patient in this series was initially diagnosed with pre-eclampsia presenting with proteinuria during the second trimester of pregnancy. This patient later underwent a renal biopsy 12 weeks post-partum, due to ongoing proteinuria, and was subsequently diagnosed with primary FSGS.

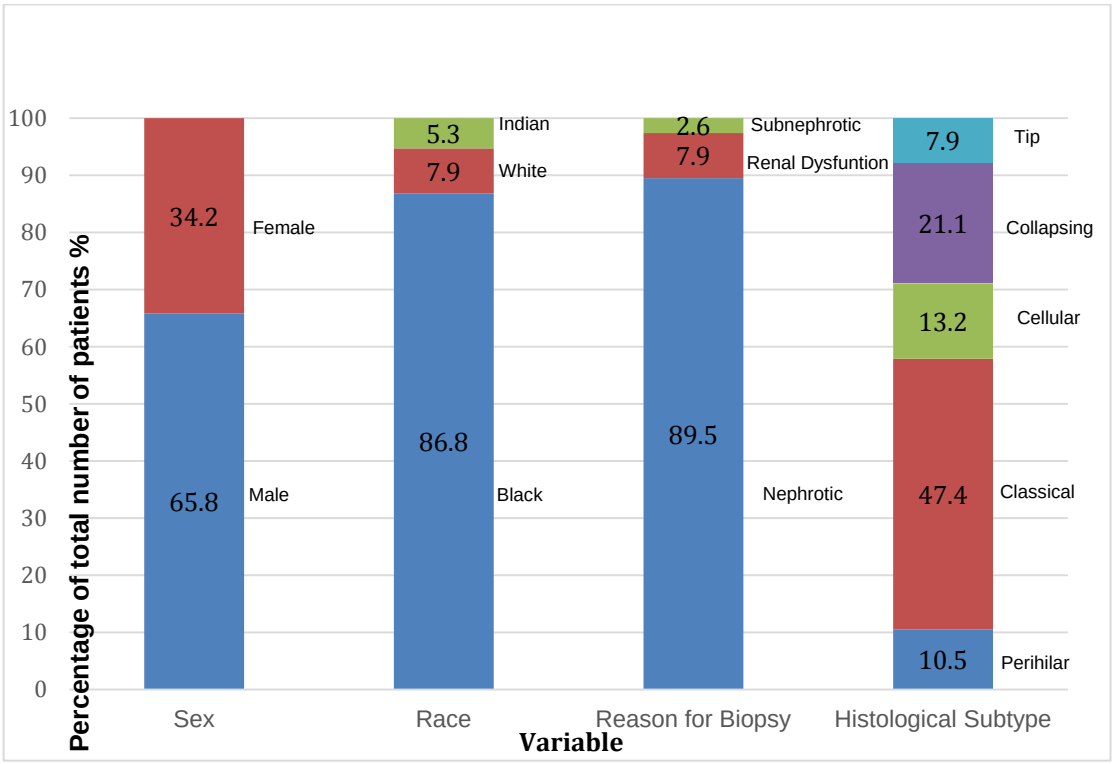
Table 3.1.2.1 Patient Demographics, Indication for Biopsy & Histological Variant of FSGS

Category	Count (N)	Percent of total number of patients (%)
Sex		
• Male	25	65.8
• Female	13	34.2
Race		
• Black	33	86.8
• White	3	7.9
• Indian	2	5.3
Indication for Biopsy		
• Nephrotic	34	89.5
• Renal Dysfunction	3	7.9
• Sub-nephrotic	1	2.6
Histological Subtype		
• Classical	18	47.4
• Collapsing	8	21.1
• Cellular	5	13.2
• Perihilar	4	10.5
• Tip	3	7.9

N = number of patients with data; %Valid obs = Valid N/Total N

Graphic representation of the above descriptive statistics is demonstrated in Figure 3.1.2.1

Figure 3.1.2.1 Bar-graph Representation of Variables by Percentage



The median age at time of presentation for biopsy was 31 years of age (IQR 23-42). The earliest age at which a diagnosis of primary FSGS was made was 17 years and the oldest was 65 years. At presentation, the median serum White Cell Count (WCC) was $6.7X10^9$/L (IQR 5.5-9.8) and Hb 12.3g/dL (IQR 10.8-14). Urine WCC and Urine Red Cell Count (uRCC) were not documented in all patients in this series (19 and 17 cases respectively out of the total 38 patients); amongst those patients in whom urinalysis was performed the median uWCC was 43 000/ml and the median uRCC was 22 000/ml. eGFR was calculated using the MDRD 4-variable equation; the median eGFR was 68.1ml/min/1.73m^2 (IQR 40-111.9) indicating that on average, patients had stage 2 CKD according to the KDOQI guidelines at presentation (Table 3.1.2.2).

Table 3.1.2.2 Presenting Features of FSGS

Variable	Valid N*	% Valid obs.	Median	Range***	IQR**
Age (years)	38	100.0	31	17-65	23-42
WCC: $X10^9$/L	38	100.0	6.7	3.8-23	5.5-9.8
Haemoglobin (g/dL)	38	100.0	12.3	6.8-17	10.8-14
uPCR (g/mmol)	38	100.0	0.77	0.04-1.8	0.57-1.2
Urine WCC/ml	19	50.0	43000	1000-192000	5000-90000
Urine RCC/ml	17	44.7	22000	1000-90000	2000-45000
Creatinine: micromoles/L	38	100.0	108.5	68-676	80-172
Glomerular Filtration Rate: ml/min/1.73m^2	38	100.0	68.1	10.2-149	40-111.9

*Valid N = number of patients with data; %Valid obs = Valid N/Total N *** Range= minimum-maximum
** Interquartile range = Lower quartile – upper quartile
Abbreviations: UPCR- Urine Protein:Creatinine ratio; WCC- White Cell Count; uWCC-Urine White Cell Count; uRCC-Urine Red Cell Count

3.1.3 The Role of Gender in the Presentation of FSGS

Comparative analysis in presenting parameters by gender was undertaken demonstrating no statistically significant difference in age, serum WCC, uPCR and renal function between males and females. The median age at presentation in males was 30 vs 32 years in females (p=0.67), median WCC was the same in both groups with a value of 6.7 $X10^9$/L (p=0.83). The median uPCR was lower in the male group 0.74g/mmol compared to 1.2, however not significantly so (p=0.2). A marginally higher eGFR was noted in males (p=0.54).

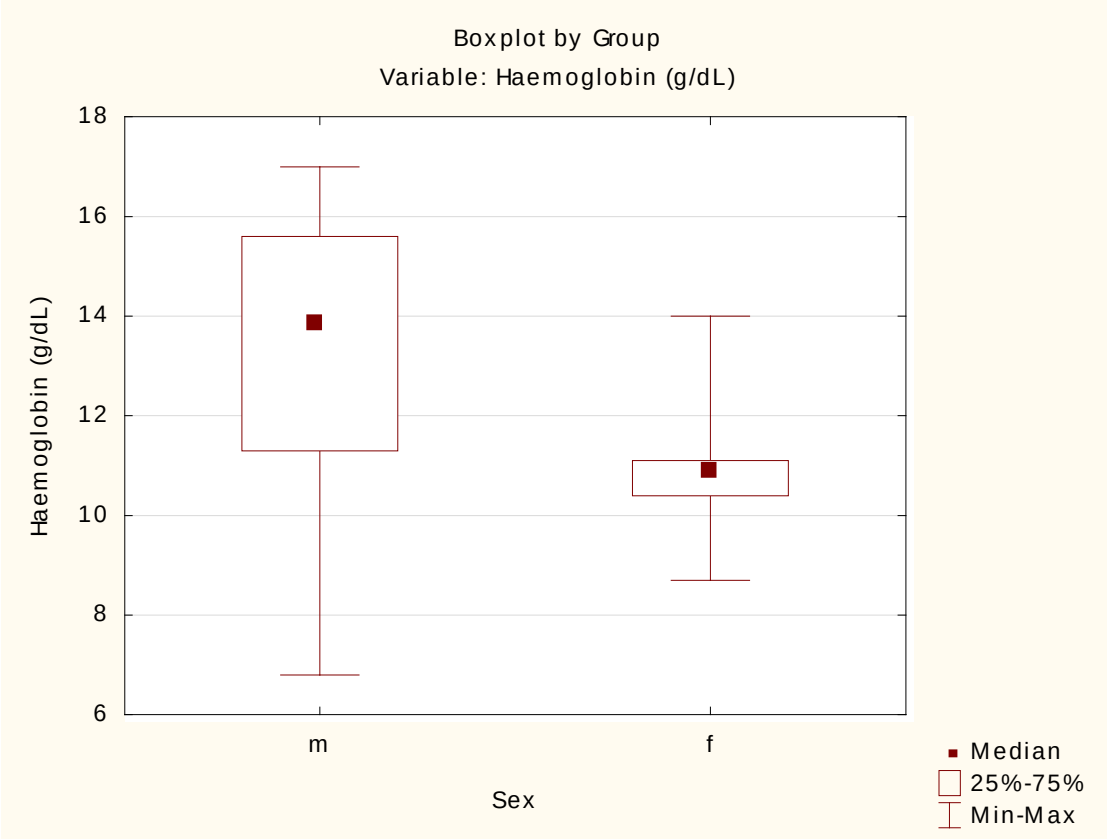
Table 3.1.3.1 Comparison of Males and Females by Presenting Parameters

Variable	Males	Females	p-value
Age	30 (24-42)*	32 (22-42)*	0.67**
WCC $X10^9$/L	6.7 (5.4-9.8)*	6.7 (6.03-8.9)*	0.83**
Hb (g/dL)	13.8 (11.3-15.6)*	10.8 (10.4-11.0)*	0.0014**
uPCR g/mmol	0.74 (0.61-0.98)*	1.2 (0.57-1.6)*	0.2**
UWCC/ml	43 000 (1 900-72 000)*	50 000 (14 500-112 500)*	0.599**
URCC/ml	19 000 (2 000-32 000)*	53 000(11 000-79 000)*	0.22**
Creatinine micromol/L	112 (86-185)*	98 (80-134)*	0.50**
eGFR ml/min/1.73m^2	71.1 (40.1-120)*	68.1 (47-87.9)*	0.54**

*median (Interquartile range) **Mann Whitney U Test
Abbreviations: WCC-White Cell Count; Hb-HAemoglobin; UPCR- Urine Protein:Creatinine Ratio; UWCC- Urine White Cell Count; URCC-Urine Red Cell Count; eGFR- Glomerular Filtration Rate

Haemoglobin was the only presenting variable noted to be significantly different between males and females in this series. The median haemoglobin was noted to be 10.8 g/dL (IQR 10.4-11.0) in females and 13.8 g/dL (IQR 11.3-15.6) in males with a p-value of 0.0014.

Figure 3.1.3.1 Comparison of Haemoglobin in Males and Females

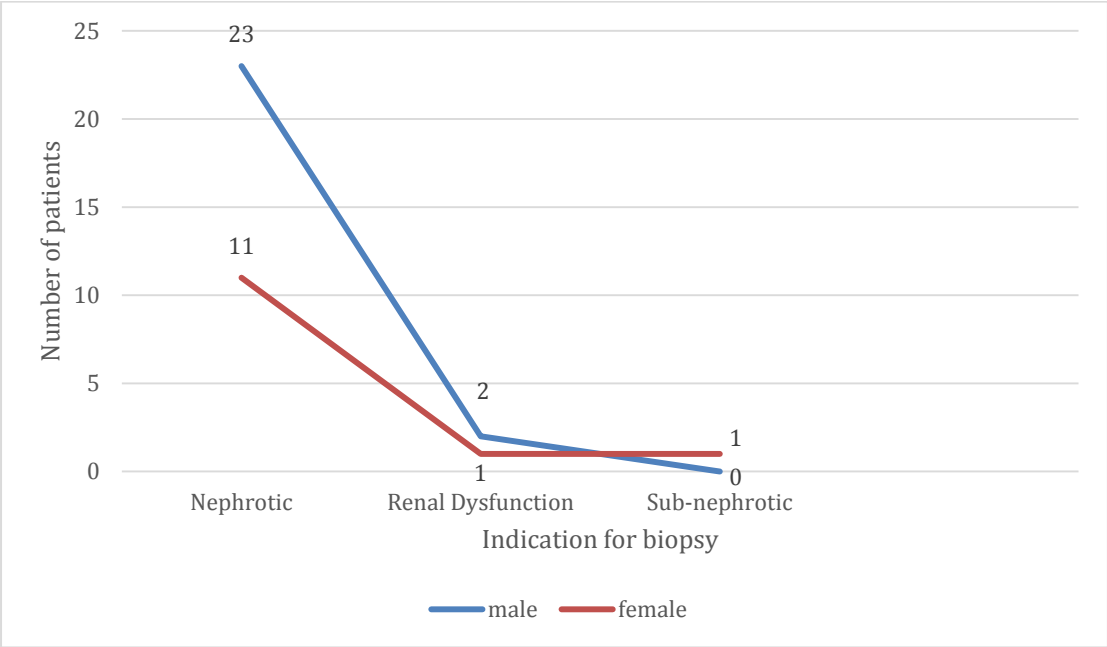


m-males; f-females

A total of 25 males and 13 females were compared in terms of indication for biopsy. There was no significant difference in indication for biopsy when comparing males with females (p=0.37). In both groups of patients, the most prevalent indication for biopsy was nephrotic-range proteinuria. 23 of the total 25 males included in this study presented with nephrotic-range proteinuria; this translates to 92% of all male patients studied. 8% of all males presented with renal dysfunction and no male

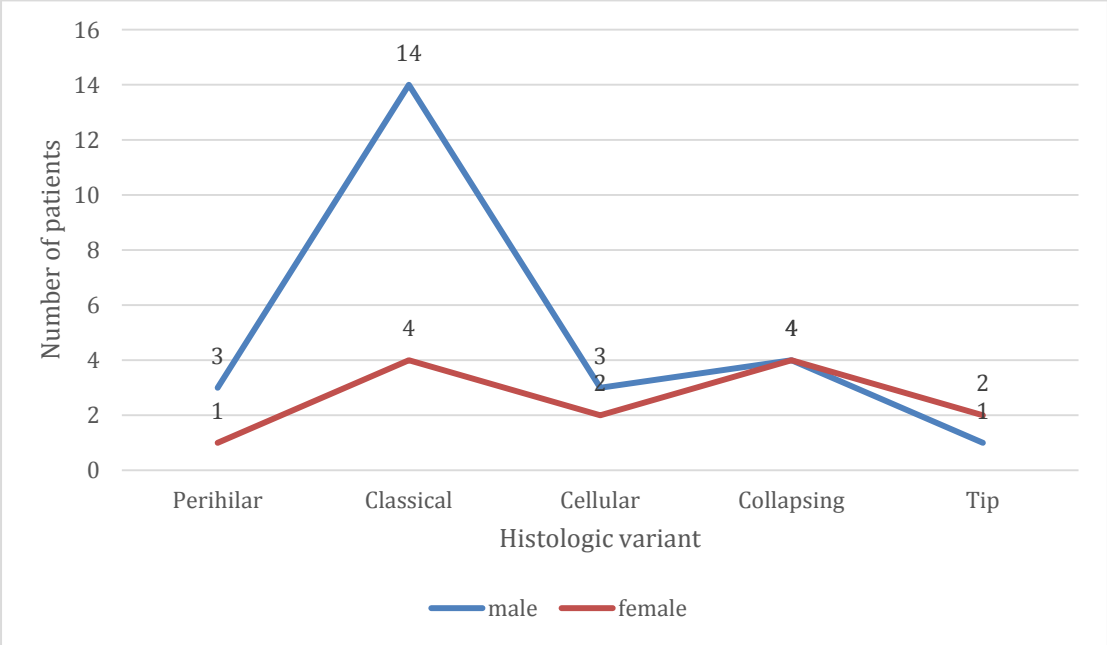
subjects had sub-nephrotic range proteinuria. 11 females (84.6% of total number of female patients) presented with nephrotic-range proteinuria, and 1 each (7.7%) had sub-nephrotic proteinuria and renal dysfunction at initial diagnosis (Fig 3.1.3.2)

Figure 3.1.3.2 Comparison of Males and Females by Indication for Biopsy



There was no significant difference when comparing males to females by prevalence of histological subtype (p=0.45). The most common variant encountered in males was the classical lesion (56% of all histologic variants in males) which occurred in 14 cases, whereas in females the classical and collapsing subtypes occurred with equal frequency (30.8% each) as the most common lesion; a total number of 4 females with the classical and 4 with the collapsing variant were identified. The least common variant in males was the tip variant (4%), whereas the least commonly occurring subtype in females was the perihilar lesion (7.7%). In males, the perihilar lesion accounted for 12%, cellular for 12% and collapsing for 16% of all male cases. The cellular and tip variants each accounted for 15.4% of cases in females (Figure 3.1.3.3)

Figure 3.1.3.3 Comparison of Numbers of Males and Females by Histological Variant



3.1.4 Differences in Presentation of FSGS by Race

A total of 33 Black patients, 3 White and 2 Indian patients were identified. Because of the small number of Non-Black patients in this study, the Non-Black patients were grouped together and compared with the Black patients. There was insufficient data recorded for subjects' uWCC & uRCC to include these variables in the comparison. Laboratory data including WCC, Hb, UPCR and Creatinine were found not to be statistically significantly different when comparing Blacks and Non-Blacks. The median WCC in Black patients was $6.6X10^9$/L vs $8.0X10^9$/L in Non-Blacks (p=0.15). haemoglobin was found to be lower in black subjects (11.6 g/dL) compared with Non-Blacks (14.0) with a p-value of 0.15. uPCR was similar in both groups (0.7 Black vs 0.79 for Non-Blacks, p=0.5). Serum creatinine was slightly lower in Black patients, however not significantly so (p=0.5) (Table 3.1.4.1)

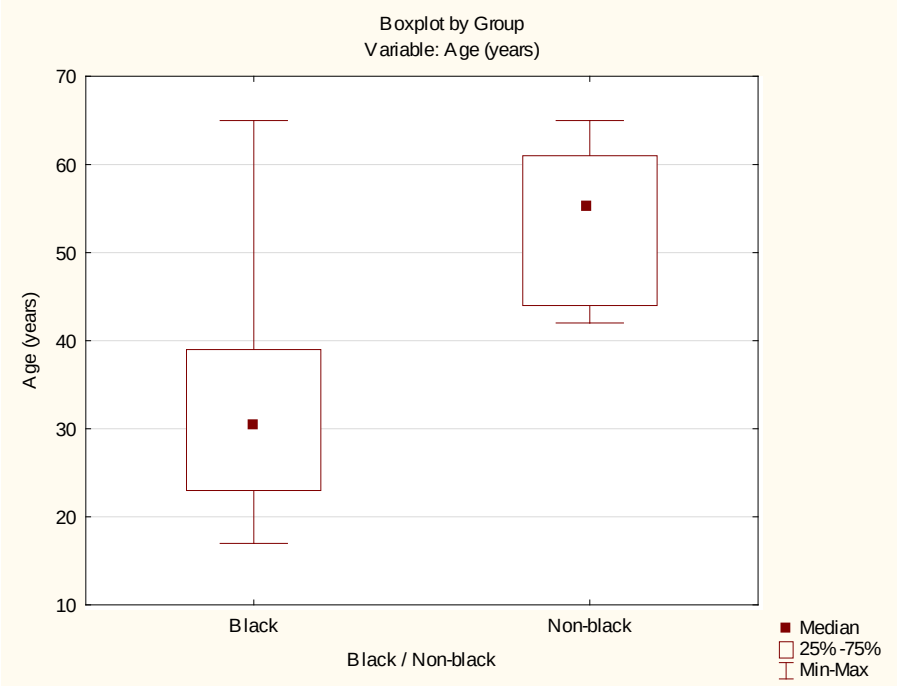
Table 3.1.4.1 Comparison of Presenting Parameters in Black and Non-Black patients

Variable	Black	Non-black	p-value
Age	30 (23-39)*	55 (44-61)*	0.002**
WCC X10^9/L	6.6 (5.4-8.9)*	8.0 (7.9-10.0)*	0.15**
Hb (g/dL)	11.6 (10.5-14.2)*	14.0 (12.7-14.0)*	0.47**
UPCR g/mmol	0.79(0.54-1.3)*	0.7(0.7-0.7)*	0.50**
Creatinine micromol/L	104 (80-162)*	172 (152-203)*	0.50**

*median (Interquartile range) **Mann Whitney U Test
Abbreviations: WCC-White Cell Count; Hb-Haemoglobin; UPCR- Urine Protein:Creatinine ratio;

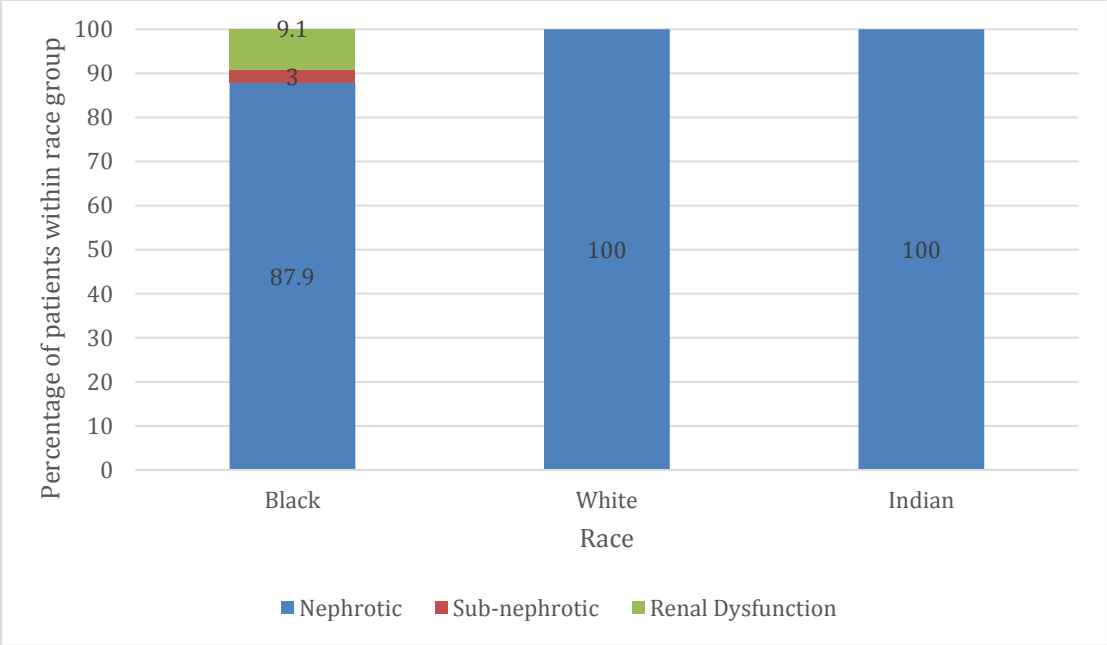
Age at presentation was the found to be significantly different when comparing Black to Non-Black patients. The median age of Black patients was 30 years (IQR 23-39) whereas the median age for patients who were Non-Black was 55 years (IQR 44-61), with a p-value of 0.002 (Fig 3.1.4.1)

Figure 3.1.4.1 Ages of Black and Non-Black patients



The majority of patients in this series across all racial groups presented with proteinuria within the nephrotic range. 29 (87.9%) of the 33 Black patients, 3 (100%) out of 3 Whites and 2 (100%) of the total 2 Indian patients in this study presented with the nephrotic syndrome. No White or Indian patients presented with sub-nephrotic proteinuria or renal dysfunction as the indication for renal biopsy. 3 Blacks (9.1% of all Black patients) presented with renal dysfunction and 1 (3.0% of Black patients) presented with sub-nephrotic proteinuria. There was no significant difference in indication for biopsy across race groups (p=0.95).

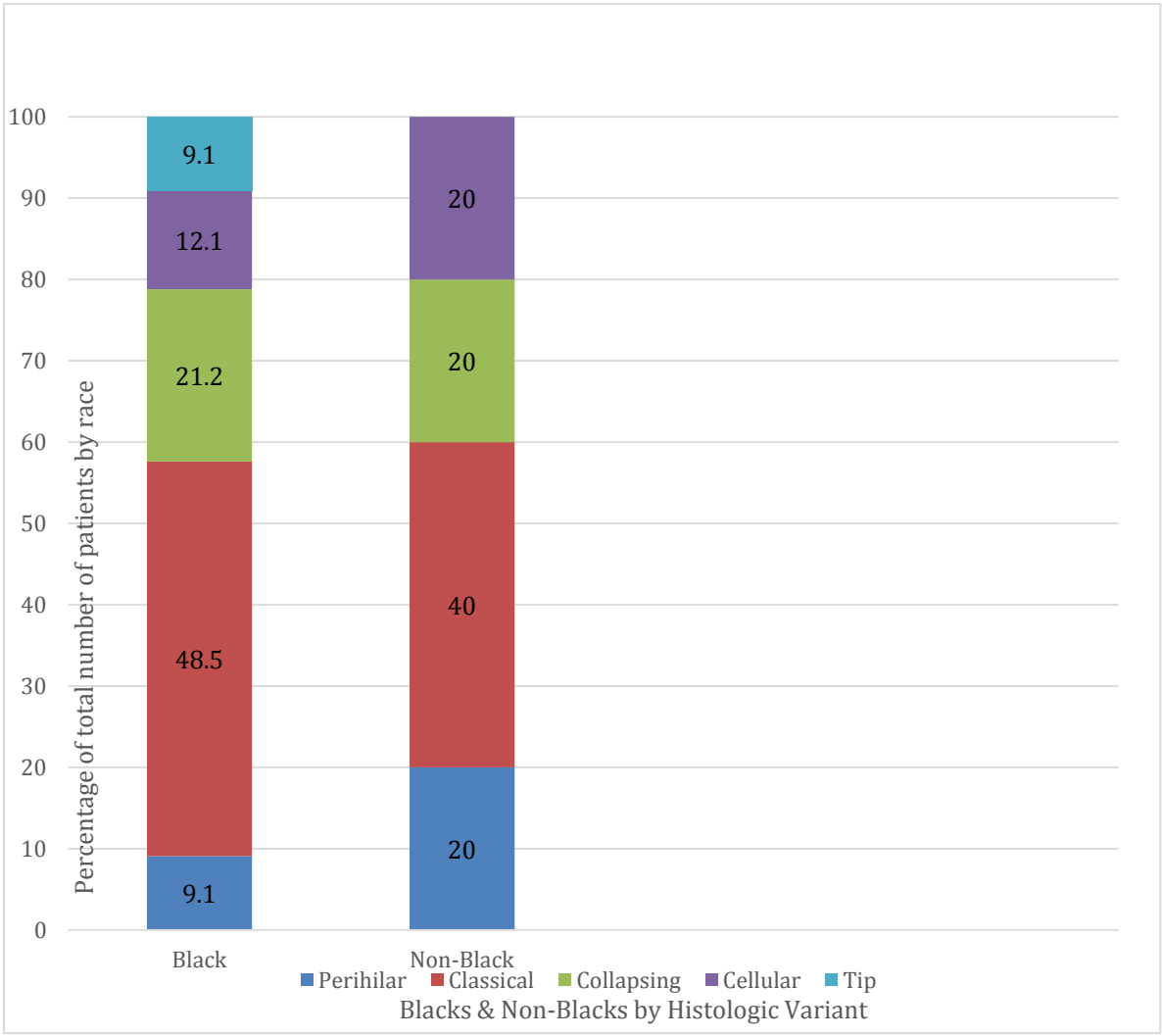
Figure 3.1.4.2 Comparison of Race Groups by Indication for Biopsy



Histological variant was analysed comparing Black with Non-Black patients. The commonest subtype in both groups was found to be the classical/NOS subtype (prevalence 48.5% Blacks and 40% in Non-Blacks). All of the remaining subtypes occurred with equal frequency in the Non-Black group of patients at 20% each (excluding the tip variant which occurred in no Non-Black patients). The second

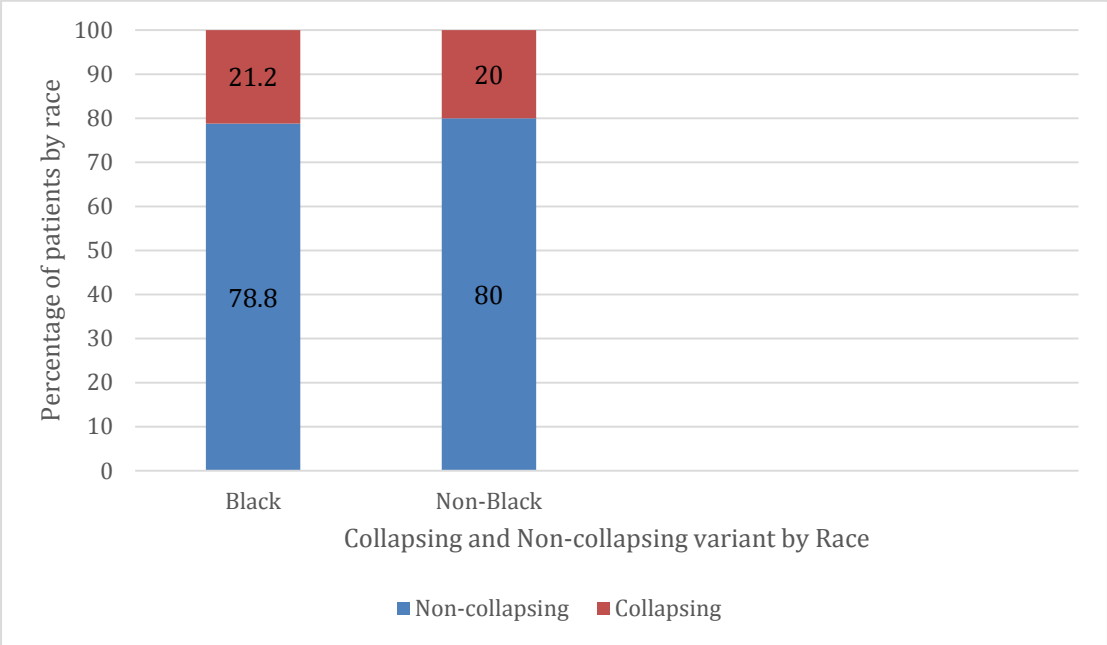
most common lesion in Blacks was the collapsing variant at 21.2%, with the cellular variant occurring in 4 patients (12.1%) and the tip and perihilar subtypes being present in 9.1% each. No significant difference was found when comparing Blacks with Non-Black patients by histological variant (p=0.87).

Figure 3.1.4.3 Comparison of Blacks and Non-Blacks by Histological Variant



Since a significant number of Black patients were found at biopsy to have the collapsing variant of FSGS, sub-analysis was performed comparing Black with Non-Black patients who had this variant.

Figure 3.1.4.4 Comparison of Black and Non-Black patients by Collapsing vs Non-collapsing subtype



7 (21.2%) of the 33 Black patients had the collapsing variant of FSGS, compared with 26 (78.8%) Blacks who had one of the other variants. 20% of Non-Black patients had the collapsing subtype, compared with 80% who did not. The ratio of non-collapsing to collapsing variants in Black patients was 3.7:1 vs 4:1 for Non-Blacks and the ratio of Black: Non-Black patients with the collapsing variant was 7:1 (however this ratio is influenced by the high total number of Black patients in this study) (p=0.95).

In summary, significant findings in this component of the analysis were that Black patients were significantly younger at presentation than their Non-Black counterparts. No other statistically significant differences in presentation by race were observed. The collapsing variant occurred more frequently in Blacks (second most common lesion in Blacks vs equal predominance with all other lesions in Non-Blacks after the Classical variant) compared with Non-Blacks, however not significantly so (p-value 0.95). The commonest subtype across all racial groups was the classical variant and

the most frequent reason for biopsy in both groups was nephrotic-range proteinuria. The younger age at presentation in Black patients and the higher incidence of the collapsing variant in Blacks may indicate a genetic predisposition in this group of patients; however, this observation will need to be confirmed by further studies.

3.1.5 Presentation of the Histological Variants of FSGS

The median ages at presentation for the subtypes of FSGS were 49 years for perihilar, 35 for classical, 26 for cellular, 25 for collapsing and 30 for the tip variant. Serum creatinine at diagnosis was similar across the groups. Median values for serum creatinine in ascending order (in micromol/L) were 90.5 for collapsing, 101 for classical, 106 for cellular, 154 for tip and 167 for the perihilar subtype.

Table 3.1.5.1 Comparison of Presenting Features by Histological Variant

Variable	Perihilar N=4 (10,5%)	Classical N=18 (47,4%)	Cellular N=5 (13,2%)	Collapsing N=8 (21,1%)	Tip N=3 (7,9%)
Age	49(39.5-60.5)*	35 (23-44)*	26 (20-32)*	25 (22-35)*	30 (23-62)*
UPCR g/mmol	1.16 (0.47-1.79)*	0.76(0.68-1.18)*	0.95 0.71-1.2)*	0.72 (0.3-1.0)*	1.01 (0.1-1.2)*
Creatinine micromol/L	167 (116.5-181.5)*	101 (87-185)*	106 (86-112)*	90.5 (75-152)*	154 (111-414)*
eGFR ml/min/1.73m^2	39.8 (37.9-89.9)*	82 (44-113)*	92.5 (47.7-125)*	88.1 (55.1-110.8)*	59.4 (12.1-67.9)*

*median (Interquartile range)
Abbreviations: UPCR- Urine Protein:Creatinine ratio; eGFR- Glomerular Filtration Rate

Given the small numbers in the patient groups with the perihilar (5), tip (3) and cellular (4) variants, statistical comparison for these groups was not undertaken. Certain trends however were observed including that the median age for patients presenting with the perihilar lesion was higher (49 years) than other variants. The median uPCR across the groups ranged from 0.72 for the collapsing group to 1.16

for the perihilar group. The eGFR was similar across most groups, however was noted to be <60ml/min/1.73m^2 in the tip and perihilar group.

Subanalysis of this series was conducted comparing patients with the collapsing variant and those with the classical variant (Table 3.1.5.2)

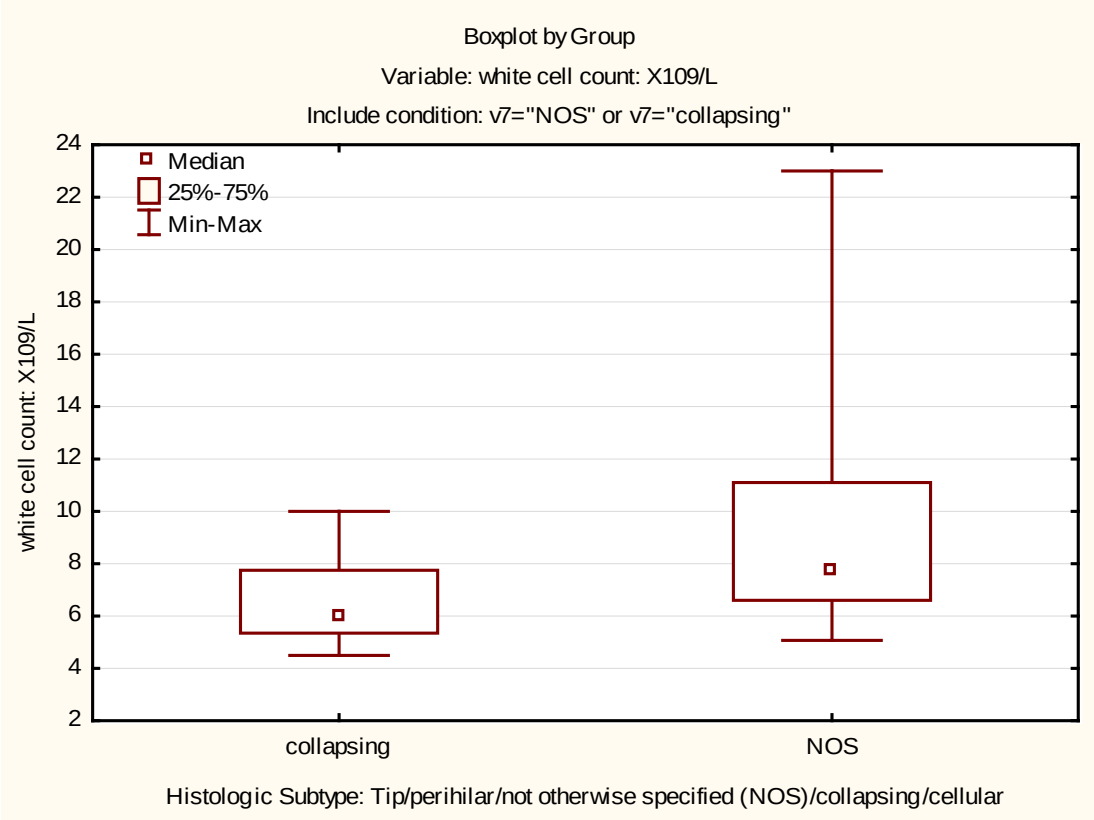
Table 3.1.5.2 Comparison of Collapsing with Classical Variant by Clinical Presentation

Variable	Collapsing N=8 (21,1%)	Classical N=18 (47,4%)	p-value
Age	25 (22-35)*	35 (23-44)*	0.26**
WCC X10^9/L	6.0 (5.4-7.8)*	7.7 (6.6-11.1)*	0.03**
Hb (g/dL)	12.4 (10.7-13.9)*	12.7 (10.8-14.9)*	0.71**
Creatinine micromol/L	90.5 (75-152)*	101 (87-185)*	0.44**
eGFR	88.1 (55.1-110.8)*	82 (44-113)*	0.79**

*median (Interquartile range) **Mann Whitney U Test
Abbreviations: WCC- White Cell Count; Hb- Haemoglobin; eGFR- Glomerular Filtration Rate

The only variable found to be statistically significantly different in this comparison was serum WCC which was found to be lower in those with the collapsing variant than in those with the classical subtype (p=0.03). Age and haemoglobin were not found to be significantly different at presentation when comparing the classical and the collapsing variants of this disease. No significant differences in creatinine or eGFR were noted in the collapsing versus classical subtypes. P-values for age, Hb and renal function are represented in Table 3.1.5.2.

Figure 3.1.5.1 Comparison of White Cell Count in Collapsing and Classical Variant



Abbreviations: NOS- Not Otherwise Specified/Classical

3.2 The Treatment of Primary FSGS

3.2.1 Therapy for Primary FSGS

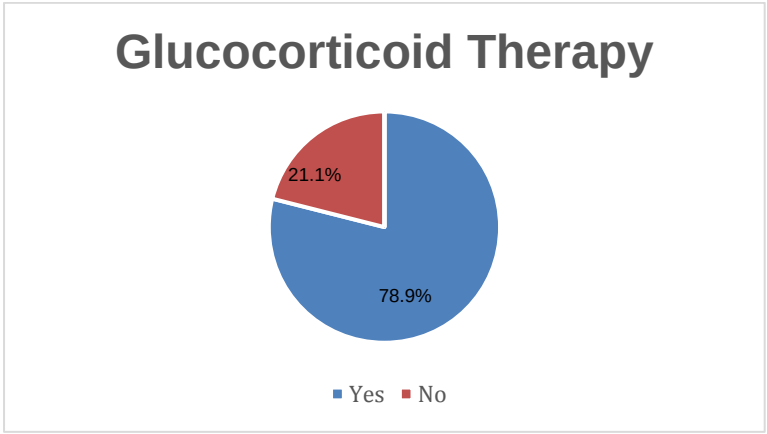
The initial treatment of primary FSGS is glucocorticoid therapy, typically a dose of 1mg/kg/day or 2mg/kg on alternate days. All patients in this series received doses of 1mg/kg/day. 8 of the total 38 patients were lost to follow-up after initial biopsy and therefore treatment data was not available for these cases. (Table 3.2.1.1)

Table 3.2.1.1 Treatment of FSGS

Variable	Count: N	Percent of total number of patients: %
Glucocorticoid Therapy • Yes • Unknown	 30 8	 78.9 21.1
Remission after first-line therapy • Yes • No • Unknown	 28 2 8	 73.7 5.3 21.1
Remission • Complete • Partial • Unknown • No	 6 22 8 2	 15.8 57.9 21.1 5.3
Second-line therapy/Second course Glucocorticoids • Yes • No/unknown	 8 30	 21.1 78.9
Reason for second-line therapy • Relapse • Non-response • Other (overlap FSGS/Membranous GN)	 6 1 1	 15.8 (75) 2.7(12.5) 2.7(12.5)

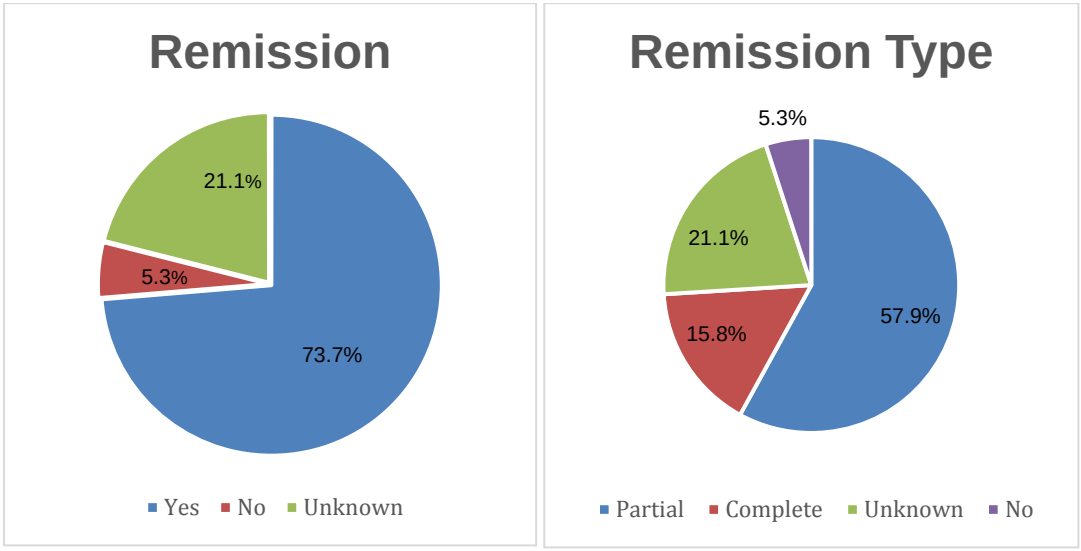
N = number of patients with data; %Valid obs = Valid N/Total N
() - percentage of number receiving second-line therapy
Abbreviations: FSGS- Focal Segmental Glomerulosclerosis; GN- Glomerulonephritis

Figure 3.2.1.1 Therapy in patients with FSGS



28 patients achieved remission after a course of first-line therapy. One patient demised from sepsis 6 weeks after being initiated on glucocorticoid therapy without achieving remission at the time of death, and 1 did not achieve remission on first-line therapy and was changed to second-line therapy after 6 months on glucocorticoid therapy with no response. Most patients achieved partial remission (57.9%); those who achieved complete remission constituted 15.8% of the total number of patients included in this study (partial remission being defined a uPCR of 30-350 mg/mmol) (Figure 3.2.1.2)

Figure 3.2.1.2 Remission Status in FSGS



8 of the 30 patients who received first-line therapy were changed to second-line therapy or were given a second course of steroid therapy Two patients received a second course of steroid therapy after having achieved remission; these patients subsequently defaulted follow-up and relapsed during the default period. 5 patients were given Cyclosporine as second-line therapy (1 for non-response and 4 for relapse after achieving remission). 1 patient was given Cyclophosphamide as

second-line therapy; this patient was initially diagnosed with Primary FSGS, however subsequently this diagnosis was changed to an overlap Membranous glomerulopathy with FSGS.

The median duration of induction glucocorticoid therapy was 16 weeks (IQR 12-20) and median follow-up uPCR was 0.23 g/mmol (IQR 0.08-0.93), confirming that most patients achieved partial remission. The median duration for withdrawal therapy in this series was 17 weeks (IQR 12-101) (Table 3.2.1.2).

Table 3.2.1.2 Duration of Therapy and Follow-up of FSGS

Variable	Valid N	% Valid obs.	Median	IQR
Induction therapy duration (weeks)	30	78.9%	16	12-20
Follow-up UPCR (g/mmol)	30	78.9%	0.23	0.08-0.93
Withdrawal therapy duration (weeks)	21	55.3%	17	12-101
Time to outcome (weeks)	38	100.0000	52	16-104

Valid N = number of patients with data; %Valid obs = Valid N/Total N
Abbreviations: UPCR- Urine Protein:Creatinine ratio

3.2.2 The Role of Presenting Features in Determining Response to Therapy

Patients who achieved partial remission were compared with those who achieved complete remission to determine any differences in presenting parameters which may predict response to treatment. None of the analysed variables at presentation including age, uPCR, creatinine, eGFR and duration of therapy appeared to significantly influence response to therapy. The median age at presentation of those who achieved partial remission was 31 compared to 29 years in those who achieved complete remission (p=0.89). uPCR was similar in both groups (0.88 in the partial group and 0.71 in the complete remission group) with a p-value of 0.24. Likewise, renal function in both groups was also similar, with eGFR in the group who achieved

partial remission 75ml/min/1.73 m^2 compared with 87.6ml/min/1.73m^2 in the complete remission group (p=0.88). The median duration for induction therapy was 14 weeks in the group who attained partial remission and 16 weeks in the group in whom complete remission was induced (p=0.26) (Table 3.2.2.1).

Table 3.2.2.1 Comparison of Remission Status by Presenting Parameters

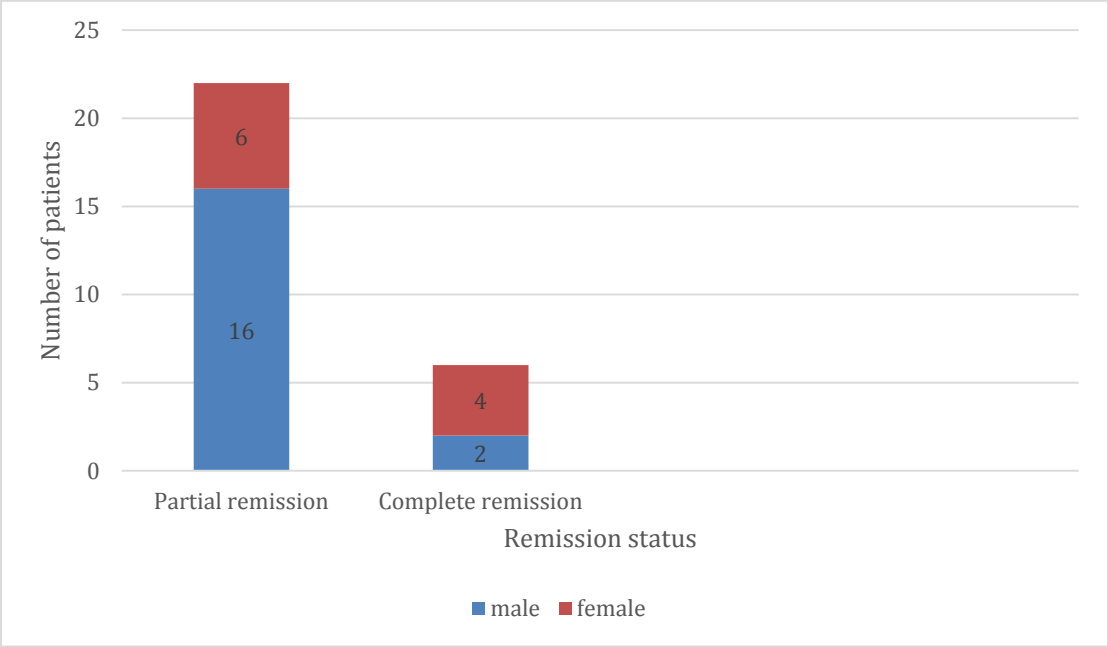
Variable	Partial Remission	Complete Remission	p-value
Age	31 (22-42)*	29 (23-41)*	0.89**
uPCR (g/mmol)	0.88 (0.71-1.4)*	0.71 (0.11-1.2)*	0.24**
Creatinine (micromole/L)	106 (79-160)*	97.5 (87-111)*	0.89**
eGFR (ml/min/1.73m^2)	75 (44-127)*	87.6 (67.9-88.1)*	0.88**
Induction Therapy Duration (weeks)	14 (12-16)*	16 (16-16)*	0.26**

*median (Interquartile range) **Mann Whitney U Test
Abbreviations: uPCR- Urine Protein:Creatinine ratio, eGFR- Glomerular Filtration Rate

3.2.3 The Role of Gender in Determining Treatment Outcomes

Treatment outcomes were compared by sex and were found not to be significantly different (p=0.15) by Fisher-exact test. A total of 16 males (88.9% of all male patients who achieved remission) were identified who achieved partial remission and 2 (11.1%) who achieved complete remission. A ratio of 8:1 was calculated in males for those achieving partial vs complete remission. Six (60%) females achieved partial remission and 4 (40%) attained complete remission, with a ratio of 1.5:1. The ratio of females who went into complete remission was observed to be higher than in males, however this was not statistically significant (Figure 3.2.3.1)

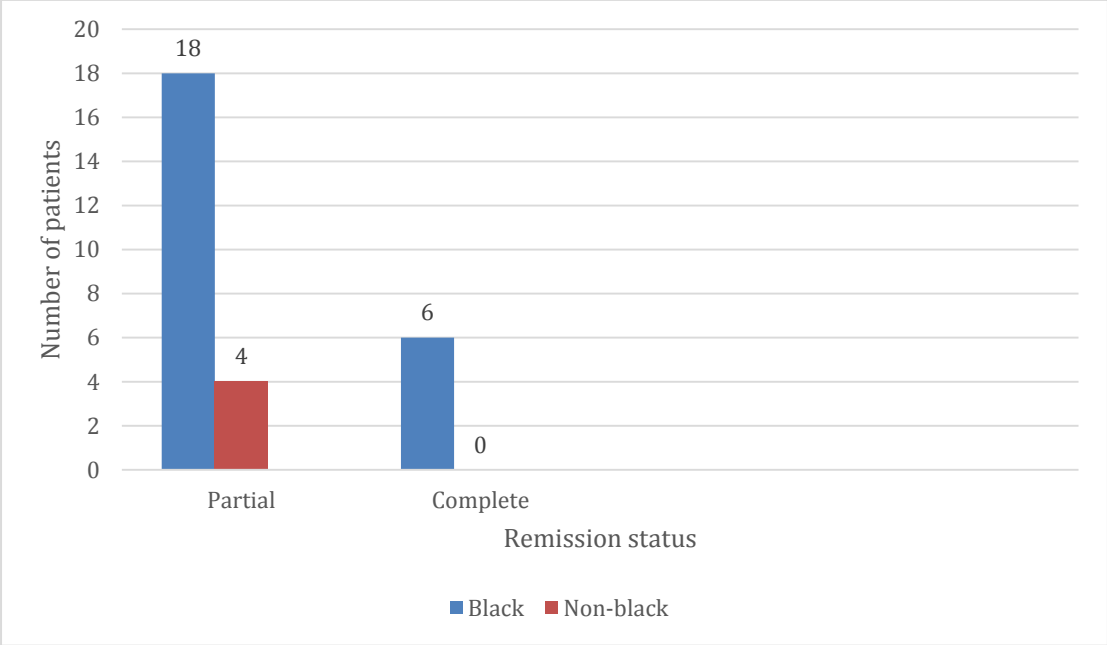
Figure 3.2.3.1 Comparison of Females and Males by Remission Status



3.2.4 Differences in Treatment Outcomes by Race

Treatment outcomes were compared by race; due to the fact that there were very few White and Indian subjects these patients were combined and analysed as Non-Black. Available literature suggests that Black patients' therapeutic response is poorer than their Non-Black counterparts and analysis was undertaken to determine if this is true in the South African population. Partial remission was induced in 18 Black patients (75% of all Black patients that achieved remission) and complete remission in 6 (25%). Amongst the Non-Black patients, 4 (100%) achieved partial remission and none obtained complete remission (Figure 3.2.4.1). Given the small numbers in the Non-Black group, these findings need to be treated with reserve. The difference in treatment outcomes in these groups was not significantly different ($p=0.55$) by Fisher-exact test.

Figure 3.2.4.1 Comparison of Blacks and Non-Black patients by Remission Status

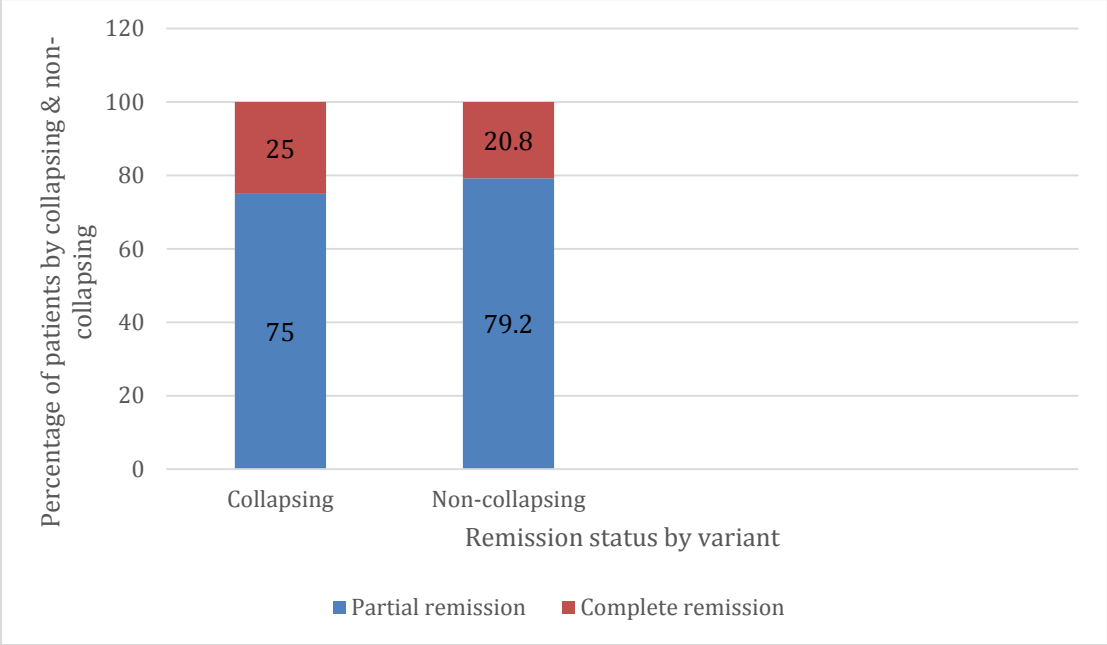


3.2.5 Histological Variants and Response to Therapy

The histological variants were compared to ascertain if any group demonstrated a significantly reduced response to therapy. In the perihilar group, 100% (N=3) achieved partial remission and no cases achieved complete remission. In the classical group 85.7% (N=12) obtained partial remission and 14.3% (N=2) complete remission. Those who had the cellular variant of the disease achieved partial remission in 75% (N=3) and complete remission in 25% (N=1). In the tip variant group, 33% (N=1) went into partial remission and 66.7% (N=2) complete remission. Notably, the tip variant was the only subtype in which more patients achieved complete remission than partial remission. Subanalysis was performed comparing the collapsing group (historically associated with poorer response to therapy) with the other variants as a group to determine any difference in treatment response. No significant difference in response to therapy was noted (p=0.64 by Fisher-exact test),

with 75% (N=3) of patients with the collapsing variant achieving partial and 25% (N=1) achieving complete remission. In the non-collapsing group, 79.2% achieved partial and 20.8% achieved complete remission (figure 3.2.5.1). However, the small sample sizes may have influenced the analysis.

Figure 3.2.5.1 Comparison of Collapsing and Non-Collapsing Variant by Remission Status



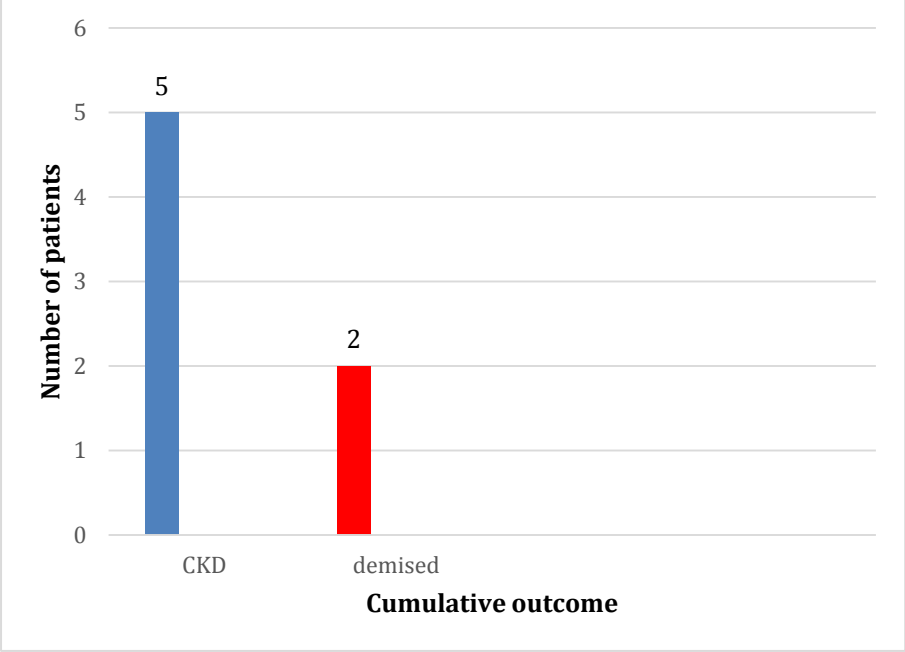
3.3 Long Term Outcomes & Survival of Primary FSGS

3.3.1 Outcomes of Patients with FSGS

Survival analysis was performed comparing those patients with a cumulative outcome of death or development or progression to advanced CKD against those surviving with reasonable renal function. Advanced CKD was defined as Kidney Disease Outcomes Quality Initiative (KDOQI) stages 4 & 5 (GFR <30ml/min/1.73m^2); these stages are considered to be advanced CKD during which symptoms and

complications of uraemic toxin accumulation become increasingly prevalent [58]. A total of 7 patients were identified who fell into the above category, 8 were lost to follow-up after diagnosis, 2 patients had relapsing disease but were lost to follow-up thereafter and therefore long-term outcomes were unknown in these cases. The remaining 21 patients had a favourable outcome. Of the patients reaching poor cumulative outcomes, 2 died within 1 year of diagnosis of Primary FSGS. In both instances, the patients developed sepsis and demised in the ICU setting, which may have been related to immunosupression. Of the patients that developed CKD; 4 had received second-line therapy and the remainder had defaulted follow-up and presented at a later stage with CKD.

Figure 3.3.1.1 Total number of patients who developed CKD or Death



3.3.2 Comparison of Cumulative Outcomes by Clinical Presentation

Patients reaching the cumulative outcome of CKD or death were compared with those who had favourable outcomes by presenting features, in order to determine which (if any) presenting parameters might predict a poorer outcome. The median

age at presentation for those with poor cumulative outcome was 37 years and in those with favourable outcomes this was 30 years (p=0.64). Laboratory findings such as WCC and Hb were not significantly different between the two groups when comparing survival (p=0.68 and p=0.40 respectively). UPCR at presentation was similar in both groups with median values of 0.78 in the poor cumulative outcome group and 0.88 in the favourable group (p=0.88). The median time to outcome in those patients who had a poor outcome was 104 weeks as opposed to 16 weeks for those with a favourable outcome. The p-value for the observation was 0.26 indicating that this finding is not statistically significant; this finding may arise as a result of small numbers in this series.

Table 3.3.2.1 Comparison of Survival Groups by Presenting Features

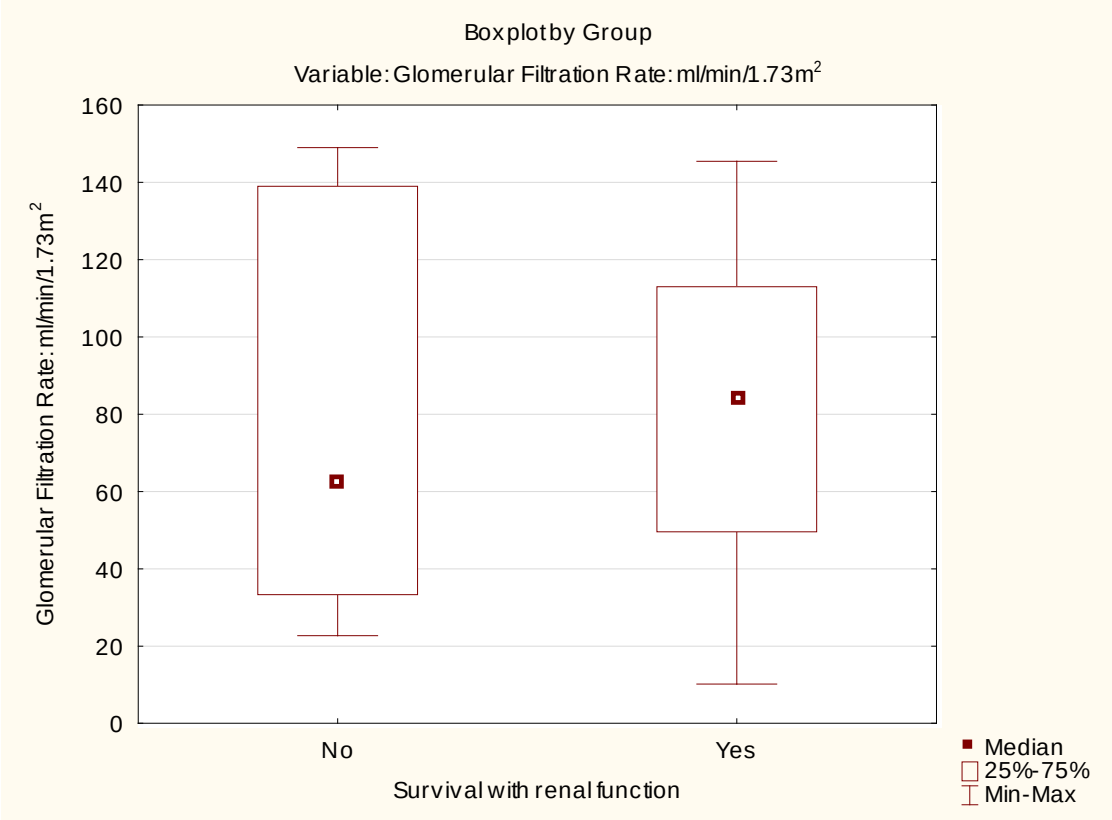
Variable	Cumulative Survival No	Cumulative Survival Yes	p-value
Age (yrs)	37 (22-42)*	30 (23-41)*	0.64**
WCC (X109/L)	7.7(6.03-11)*	7.8(6.6-9.8)*	0.68**
Hb (g/dL)	13.6(10.8-14)*	11.3(10.4-13.9)*	0.40**
uPCR (g/mmol)	0.78 (0.68-1.7)*	0.88 (0.7-1.2)*	0.88**
Creatinine(micromol/L)	112 (73-203)*	101(87-152)*	0.96**
eGFR (ml/min/1.73m^2)	62 (33.3-139)*	84.1(49.6-113)*	0.94**
Time to Outcome (weeks)	104 (52-208)*	16(16-20)*	0.26**

*median (Interquartile range) **Mann Whitney U Test
Abbreviations: WCC- White Cell Count; Hb-Haemoglobin; uPCR- Urine Protein:Creatinine ratio; eGFR- Glomerular Filtration Rate

Renal function was analysed comparing the two survival outcomes groups; the median serum creatinine in the group with poorer outcomes was 112 micromol/L (IQR 73-203) and 101 micromol/L (IQR 87-152) in the group with better outcomes. The p-value for this analysis was 0.94. A trend towards a lower eGFR was noted in

the group with worse outcomes (62 ml/min/1.73m^2) compared with the group who had favourable outcomes (84.1 ml.min/1.73m^2). This observation was not statistically significant, probably due to small sample sizes, however it suggests that patients in the worse outcome group had poorer renal function at the time of diagnosis, and thus were more likely to develop CKD/poorer outcomes (Table 3.3.2.1)

Figure 3.3.2.1 Comparison of Cumulative Outcomes by eGFR

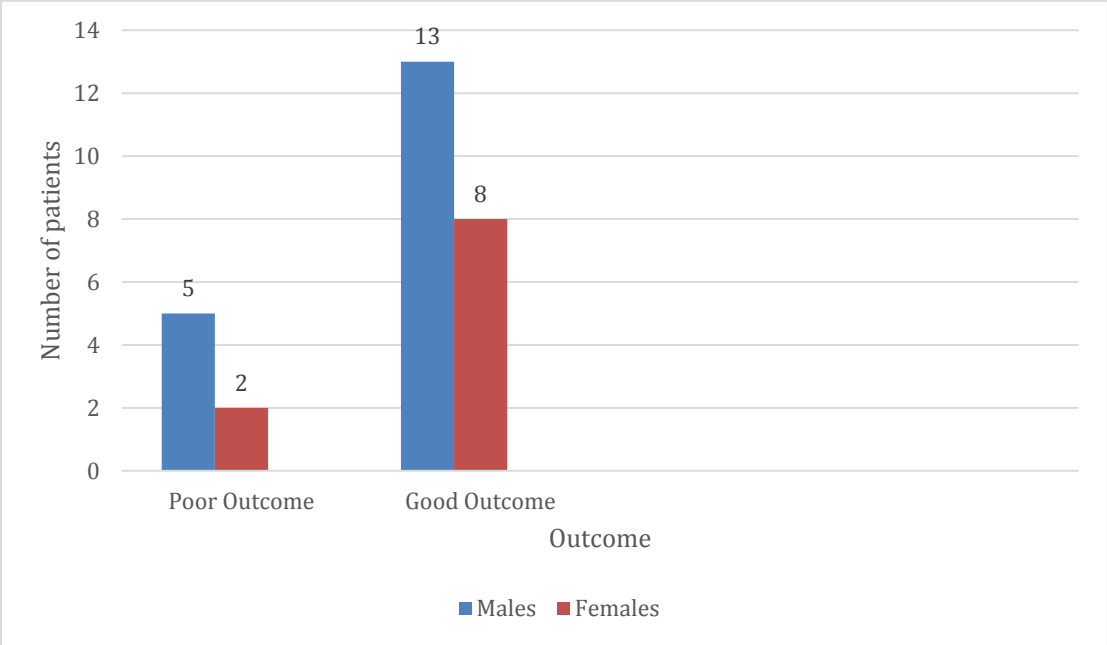


No-Poor Outcome; Yes-Good Outcome

3.3.3 The Role of Gender in Cumulative Survival

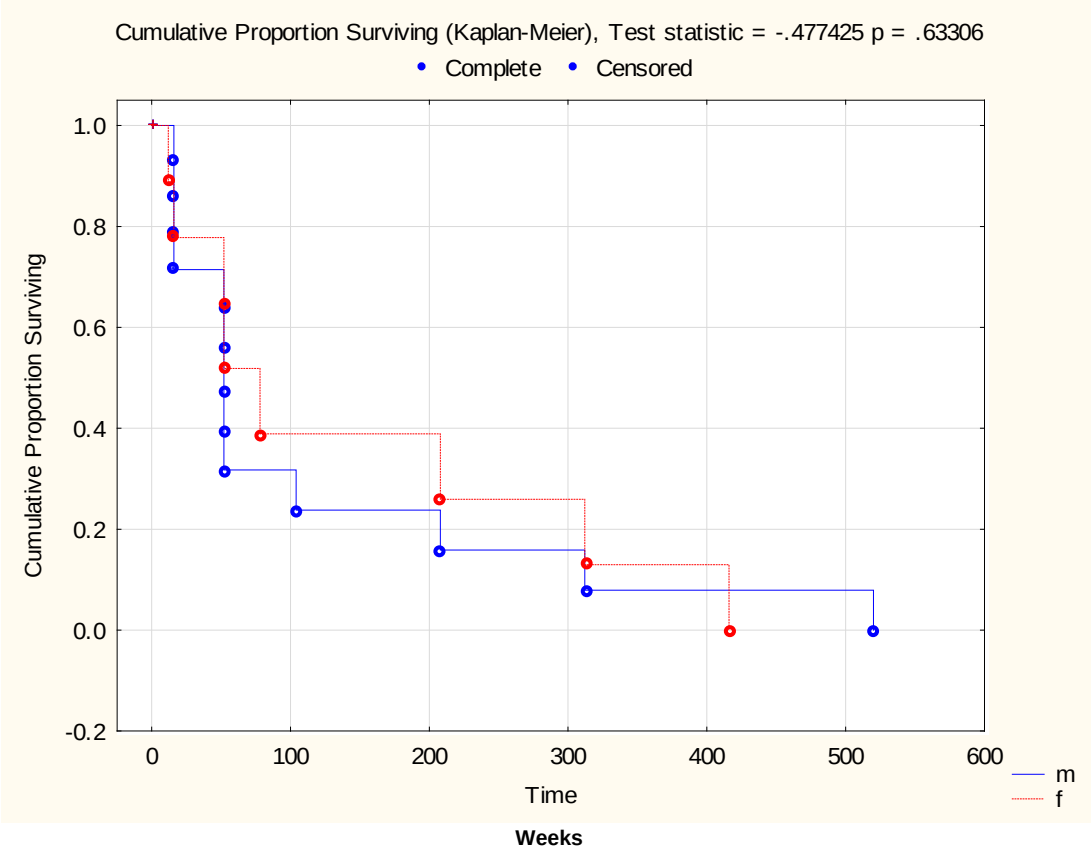
Comparative analysis of survival outcome by gender was made. A total of 5 males (27.8% of all males where outcome was known) had poor cumulative survival whereas 13 (72.2%) had favourable outcomes. Two (20%) females had poor cumulative outcomes and 8 (80%) had good outcomes. The ratio of males with good to poor outcomes was 2.6:1 and the corresponding ratio in females was 4:1. The p-value for this observation was 0.51 by the Fisher-exact test.

Figure 3.3.3.1 Comparison of Cumulative Outcome by Sex



The Kaplan Meier survival estimator demonstrated that the cumulative survival in males and females was much the same (p=0.63)

Figure 3.3.3.2 Kaplan Meier Survival curve for Males & Females

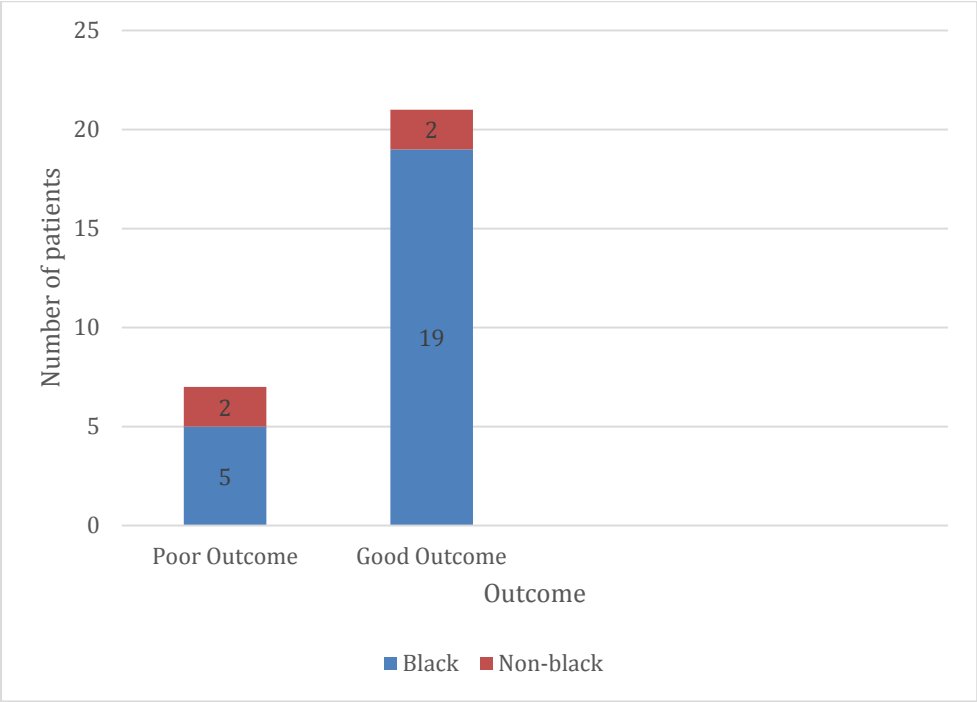


m-Males; f-females

3.3.3 Differences in Survival by Race

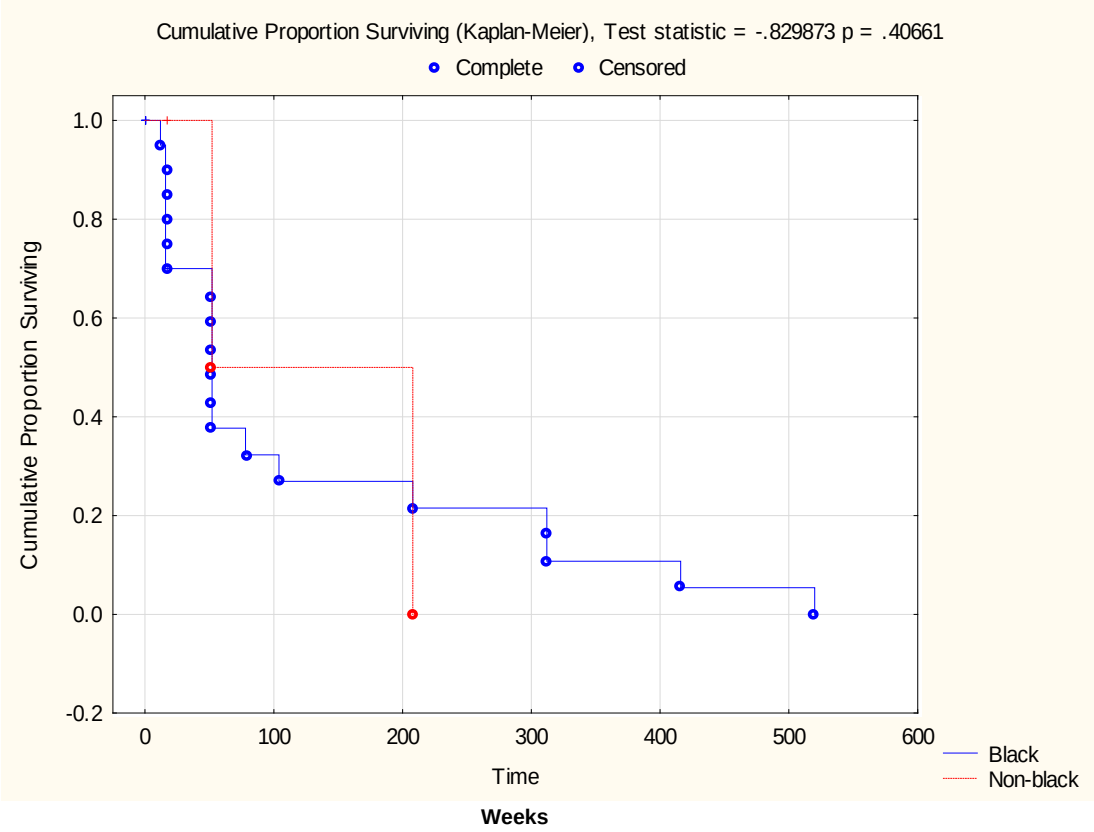
Survival analysis was compared between race groups, and as previously mentioned, Non-Blacks were combined to form one group and compared with Black patients. The small number of Non-Blacks in this series made comparative analysis challenging; nonetheless, 5 Black patients (20.8% of all Blacks with known survival outcomes) were found to have poorer outcomes whereas 79.2% (19) had favourable outcomes. This is somewhat in contrast with the Non-Black patients where equal numbers had poor and favourable outcomes (50%, 2 patients each), however the small sample size makes these findings unreliable. The p-value for this analysis was 0.25 by Fisher-exact test indicating that it was not statistically significant.

Figure 3.3.3.1 Comparison of Survival in Blacks and Non-Blacks



Kaplan Meier survival analysis demonstrated that Non-Black and Black patients had a similar cumulative survival. The P-value for the whole period analysed was 0.4.

Figure 3.3.3.2 Kaplan Meier Survival curve of Blacks and Non-Blacks

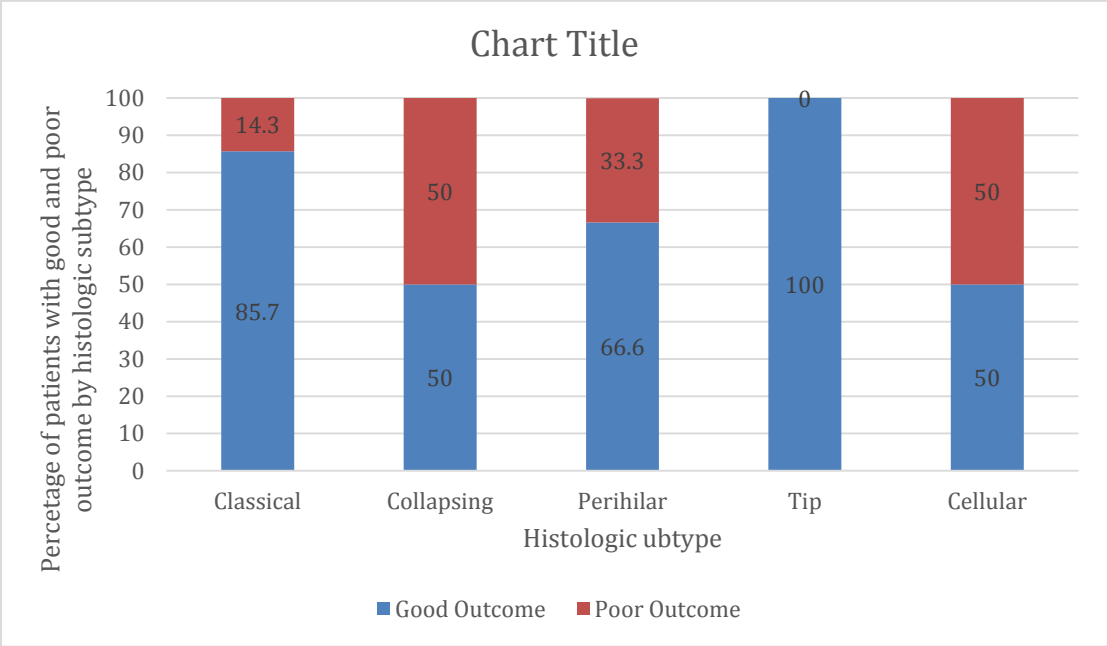


3.3.4 The Role of Histological Variant in Predicting Cumulative Survival

Histological subtypes were compared by outcome demonstrating that no particular variant predisposed patients to a worse outcome (p=0.33). The largest number (47.4%) of subjects had the classical variant of the disease; 14.3% (N=2) with the classical subtype had poor outcomes whereas 85.7% (N=12) had a good outcome. In patients with the perihilar variant, 33.3% (N=1) had poor outcomes and 66.6% (N=2) had good outcomes. 50% of patients with the collapsing variant (N=2) had poor outcomes and the remianing 50% (N=2) had favourable outcomes. Patients with the tip variant appeared to have the best outcomes; 100% (N=3) had favourable outcomes and 0% with poor outcomes. Of those who had the cellular variant; 50% (N=2) had good outcomes and 50% (N=2) poor outcomes. Figure 3.3.4.1 below

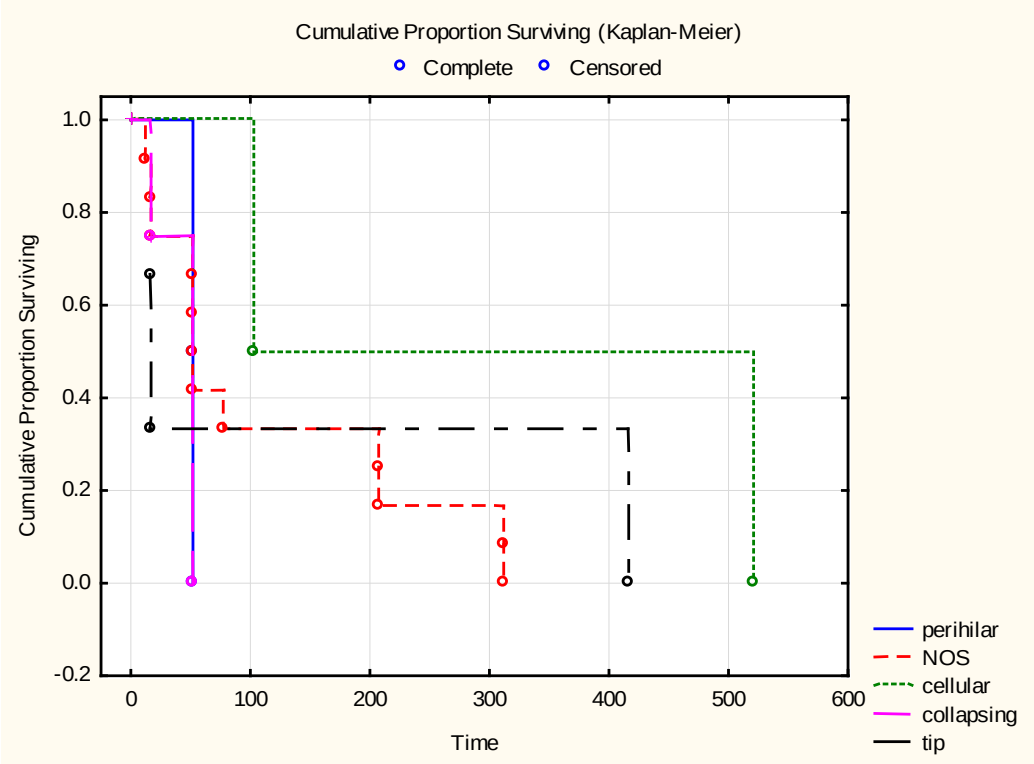
demonstrates the percentage of patients with good and poor outcomes by histological variant of the total number of patients.

Figure 3.3.4.1 Comparison of Survival by Histological Variant



Outcomes were compared between the collapsing (being historically the lesion with the worse prognosis) and non-collapsing variant cohorts to determine if any differences were present. 19 (67.9%) patients in the non-collapsing group showed favourable outcomes and 5 (17.9%) demonstrated poor outcomes compared with 2 (7.14%) each in the collapsing group demonstrating favourable and poor outcomes. The ratio for good to poor outcomes in the non-collapsing group was 3.8:1 vs 1:1 in the collapsing group (p=0.25).

Figure 3.3.4.2 Kaplan Meier Survival curve of Histological Variants



Weeks **Histologic variants:**

Abbreviations:NOS- Not Otherwise Specified/Classical

4. Discussion

4.1 The Epidemiology & Presenting Features of Primary FSGS

4.1.1 The Epidemiology of FSGS

The prevalence of Primary FSGS in this study was found to be 7.2% of all biopsies performed from 2001-2010. This is lower than the findings by Haas et al in the United States documenting the incidence of Primary FSGS in all biopsies performed from 1987-1993 at approximately 12.2%. The prevalence of this disorder is variable worldwide; with prevalence rates of 6% in China, 12% of all cases presenting with the nephrotic syndrome in Spanish studies and 2.3% of all causes of ESKD in the United States (US). FSGS as a percentage of all primary glomerular disorders or as a percentage of cases who presented with the nephrotic syndrome was not assessed in this series, and therefore an accurate comparison of these variables in this study could not be made [51-54,59]. Patients with primary FSGS in the South African developing-world setting possibly present later with more advanced renal dysfunction, precluding biopsy; possibly explaining the lower prevalence of FSGS in this predominantly Black cohort compared with international studies.

The incidence of Primary FSGS is rising in the United States and in parts of Africa. Korbet et al found that the prevalence of FSGS in the US increased from 29% of all primary glomerular disorders presenting with the nephrotic syndrome in 1975-1985 to 38% in 1985-1994. A similar trend was noted in Africa (Democratic Republic of Congo) where FSGS was previously documented to have a frequency of 6%, whereas newer studies have demonstrated rates as high as 41% in patients with primary nephrotic syndrome [60-62]. These findings were not confirmed in this study. The prevalence of FSGS in the first 5 years of this study was 7.7% and subsequently

6.9% in the last 5 years, however the period under review may have been not long enough to assess this variable adequately.

4.1.2 The Presenting Features of FSGS

FSGS was found to be more prevalent in males in this study, with males constituting 65.8% of the study cohort, giving rise to a male-to-female ratio of 1.92:1. This observation is supported by Kitiyakara et al showing that males have a 1.5-2 fold higher risk of FSGS than females [2]. Rydel et al documented a male prevalence of 58% in their study on FSGS in the United States, further confirming that FSGS is indeed a disease that affects males more frequently [29,54]. Female sex hormones may be protective in preventing primary glomerular diseases in women by exerting antioxidant effects in the mesangial microenvironment, and controlling the release of cytokines and growth factors; possibly explaining the consistently lower prevalence in multiple studies in this group [63].

The median age at presentation in patients with primary FSGS in this series was 31 years of age, somewhat lower than the mean age of 40-50 years, as described in the literature [27,29,30]. This finding may be explained by the presence of a susceptibility gene in this predominantly Black cohort of South African patients; this gene (*MYH9/APOL1* gene) has previously been demonstrated in the African-American population.

Analysis of this series demonstrated an overwhelming majority of affected subjects being Black (86.8%) concurring with previous studies which found FSGS to be more common in Black patients; a prevalence of 56% was described by Rydel et al and a 4-fold increased risk of FSGS related ESKD in Blacks was found by Kitiyakara et al [2,29,54]. However, the prevalence of FSGS in Black patients in this particular study is considerably higher than described in previous literature; a possible explanation for

this may be the racial demographics of the South African population which are likely to be mirrored in this series; the 2011 Census found that 79.2% of South Africans are Black [64]. This study was conducted in a public hospital setting serving lower socio-economic groups; which in the context of historical South African injustices tend to be predominantly Black, possibly further explaining the high prevalence of Black patients in this series. The younger age at presentation and the predilection of FSGS for Black patients may allude to the presence of a genetic vulnerability in this group. Mutations in The *APOL1* and *MYH9* genes on chromosome 22 have been identified as a causative factor in the development of FSGS, and these mutations are prevalent in the African-American population (although to date, no studies determining the penetrance of this gene in the Sub-Saharan African population have been conducted) [10-12].

Nephrotic-range proteinuria has been reported to occur at presentation in between 60-75% of patients with primary FSGS [29,31]. Eighty six point eight percent (86.8%) of patients in this study had nephrotic-range proteinuria at initial presentation, confirming that this is the most frequent presenting syndrome in patients with FSGS. Renal dysfunction (7.9%) occurred less commonly, somewhat inconsistent with international reports documenting frequencies of 25-50%; this may however reflect local biopsy practices in which biopsies are not performed on patients with advanced renal dysfunction. An alternative explanation for the lower rates of renal dysfunction in our population may be a possibly more acute-onset of presentation (however symptoms pre-presentation were not described in this study). [29,31]. Renal dysfunction was defined by Rydel et al in their study as a serum creatinine greater than 115micromol/L; the median creatinine at presentation in their cohort was 150micromol/L indicating that most patients had renal dysfunction at presentation. Contrary to Rydel et al's findings, median serum creatinine in this study was 109

micromol/L, indicating that most patients in this study did not manifest with renal dysfunction at presentation.

The most common histological variant in this study was found to be the classical subtype occurring at 47.4%, the second most common being the collapsing variant and the least common variant being the tip subtype. This is somewhat different from that described by Deegens et al in Holland, who reported the tip variant as the most prevalent lesion (37%), classical being the second most common and the collapsing variant being the least common lesion, occurring at 5% [65]. These discrepant findings however need to be interpreted in the context of a study conducted by Thomas et al, who documented very high rates of the collapsing variant in Black patients and a very low prevalence of the tip variant in this same population [27]. Given the extremely high numbers of Black patients in this study, the distribution of the subtypes appears to suggest that the collapsing variant is common in this population and the tip variant occurs very infrequently.

4.1.3 The Role of Gender in the Presentation of FSGS

Differences in presentation by gender were analysed in this study, which found no statistically significant difference in presenting parameters by gender other than haemoglobin. The median haemoglobin (Hb) in males was 13.8 g/dL and 10.8g/dL in females (p=0.001). This is most likely a reflection of the normal difference in Hb by sex, as females usually tend to have lower Hb's due to menstrual losses [66]. There is a dearth of studies comparing difference in presentation between males and females; however a study by Cattran et al described no statistically significant difference in presentation between males and females. Cattran et al however did not analyse all parameters that were described in this study, including differences in Hb by gender [67].

4.1.4 Differences in Presentation of FSGS by Race

Presenting variables were compared between Black and Non-Black subjects in this study to identify any significant differences; the only finding that proved to be of statistical significance was the difference in age at presentation. Black patients presented at a younger age (median age of 30) compared with their Non-Black counterparts (median age of 55), with a p-value of 0.002. The finding that Black patients were significantly younger may suggest that a genetic predisposition *(APOL 1/MYH9* gene) may be present in the South African Black population. The *APOL1* gene has been described as being particularly prevalent in Black patients with the collapsing variant of FSGS and interestingly, the collapsing variant occurred more frequently in Blacks compared with Non-Black patients in this series (although this did not achieve statistical significance) [10-12,29]. This finding has been confirmed in a number of studies that have described a higher prevalence of the collapsing variant in Black participants [27,30] These observations, however, need to be interpreted in the context of the small numbers of Non-Black patients, possibly indicating a sampling bias. Rydel et al compared Black with Non-Black patients and also described no significant difference in presenting parameters by race; they did however find that Black patients were nephrotic at presentation more often than Non-Blacks.

4.1.5 Presentation of the Histological Variants of FSGS

Differences in presenting parameters by histological variant were impractical to compare statistically as the numbers in some groups were too small. Certain trends were however noted including that the median age at presentation in patients with the perihilar lesion was higher than others. The perihilar variant of FSGS commonly occurs as a consequence of an underlying chronic disease, such as obesity and hypertensive nephrosclerosis [16]. These disorders are more frequently encountered in older patients (accounting for the older median age in this group), possibly reflecting an undiagnosed underlying secondary cause for FSGS in the perihilar

group of this study. Another interesting observation was that eGFR was relatively similar between the variants. This may suggest a possible selection bias, as participants who presented with significant renal dysfunction may not have been biopsied due to local biopsy practices and are therefore inadequately represented in this study. Chun et al's study comparing the histological subtypes also demonstrated no significant difference between the variants other than an older age at presentation in those with the tip lesion, and more severe proteinuria in those with the collapsing subtype [16,30]. Subanalysis in this study comparing the collapsing variant with the classical variant found only serum WCC to be significantly lower in the collapsing group. The presence of HIV infection in a patient was an exclusion criterion in this study and therefore is not a possible explanation for the low WCC in the collapsing group. Parvovirus B19 infection is a known secondary cause of collapsing glomerulopathy and is known to cause leucopaenia; patients in the collapsing group may have been incorrectly categorised as primary FSGS, rather than FSGS secondary to Parvovirus infection, as Parvovirus infection was not tested routinely in patients [16]. Alternatively, studies have demonstrated that White Cell Count in healthy Blacks on average are lower than their White counterparts, which may possibly explain the significantly lower WCC in Black patients in this series [68].

4.2 The Treatment of Primary FSGS

4.2.1 Therapy for Primary FSGS

First line therapy for primary FSGS is glucocorticoid therapy as per the KDIGO glomerulonephritis guidelines [38]. Most patients in this cases series received glucocorticoid therapy at the dose recommended in the guidelines (1mg/kg/day) excluding the 8 patients who were lost to follow-up. Patients in this study were therefore managed according to current guidelines.

The median period for induction therapy in the 30 subjects treated was 16 weeks. The recommended duration of therapy is not clear, however a study conducted by Banfi et al demonstrated that prolonged therapy for 16 weeks before weaning was associated with better outcomes in patients with FSGS [46]. This finding was confirmed by Ponticelli et al who described a 61% remission rate in those patients treated for >16 weeks vs 15% in those receiving therapy for <16 weeks [45]. Seventy three point seven percent (73.7%) of the total number of patients analysed in this series achieved remission, this number is higher than that described in previous studies; Rydel et al described a remission rate of 50% in their study over a median period of 100 weeks [29]. A possible explanation for a higher response rate in this study may be the short duration of follow-up for many patients (52 weeks) who may have experienced a subsequent undocumented relapse of disease; some patients were followed-up for as short a period as 16 weeks (IQR 16-104 weeks). Fifty seven point nine percent (57.9%) achieved partial remission and 15.8% complete remission after the induction period of therapy. Rydel et al described slightly different response rates with 5 patients achieving partial remission and 10 patients achieving complete remission [29,41,42].

4.2.2 The Role of Presenting Features in Determining Response to Therapy

Patients who achieved partial remission were compared with those who achieved complete remission, interrogating their presenting parameters to determine any differences which may predict type of remission. None of the presenting variables analysed including age, uPCR or renal function were found to be significantly different in these 2 groups. The median duration of induction therapy was slightly shorter in the group who achieved partial remission compared with the group who achieved complete remission (without statistical significance), supporting previous studies suggesting a longer duration of therapy to achieve remission [42]. Troyanov et al similarly found no significant difference in presenting parameters when comparing

individuals who obtained partial vs complete remission, aside from proteinuria which was significantly lower in the partial remission group [41]. There were too few non-responders to therapy in this case series to analyse treatment outcomes by responders versus non-responders; Rydel et al analysed these 2 groups in their study and similarly observed no significant difference at presentation between the 2 groups except for age at onset of disease was found to be significantly younger at presentation in the group of non-responders [29].

4.2.3 The Role of Gender in Determining Treatment Outcomes

Differences in response to therapy were compared by sex, demonstrating no significant difference when comparing males with females. The lack of a significant difference in response by gender in this study concurs with Troyanov et al; even when comparing responders with those who did not respond to therapy at all (Rydel et al and Cattran et al) no difference between males and females has been documented [29,41,67].

4.2.4 Differences in Treatment Outcomes by Race

Remission status was compared between Black and Non-Black patients in order to determine whether there was a difference in therapeutic response between Black and Non-Black patients with FSGS. This study identified similar response to therapy in patients between these race groups. This finding concurs with that of Troyanov et al [41]. Rydel et al compared non-responders with responders to therapy and also demonstrated no significant difference between Black and Non-Black patients [29,41].

4.2.5 Histological Variants and Response to Therapy

Historically, the collapsing variant has been described as the least responsive to therapy and therefore associated with the poorest outcome. Chun et al have compared response to therapy of the different histological variants and found that

64% of patients with the collapsing variant vs 78% with the tip variant attained remission, contradicting findings documented in previous literature. To gain more clarity on this issue, the collapsing variant was compared with all other subtypes as a group (termed non-collapsing) in this series by treatment outcomes; the findings in this study confirmed Chun et al's results that the collapsing variant does not respond inferiorly to treatment compared with the other variants [30]. One can argue therefore, that epidemiological and clinical parameters at presentation should not deter clinicians from dispensing therapy, as no single variable can predict response to therapy in patients with primary FSGS [26,27,30].

4.3 Long Term Outcomes and Survival of Primary FSGS

4.3.1 Outcomes in Patients with FSGS

Poor long-term outcomes in this study were defined as CKD stage 4 or 5 and death. 7 subjects were identified for inclusion in this group, of which 2 died secondary to sepsis and the remaining 5 went on to develop advanced CKD.

4.3.2 Comparison of Cumulative Outcomes by Clinical Presentation

Historically, factors predicting a poor outcome include the collapsing variant, nephrotic-range proteinuria, renal dysfunction at presentation, degree of tubule-interstitial fibrosis on biopsy and poor response to therapy; however these factors have not consistently been demonstrated to predict a poor outcome [28-30]. Contrary to previously published data on predictors of outcome, Rydel et al found that only serum creatinine at presentation predicted renal survival; all other variables including race, sex, age and degree of proteinuria were not significant. No single presenting parameter was found to significantly affect renal survival in this cohort of patients. A non-significant trend towards a lower eGFR was noted in patients in this study who

had poor long term outcomes, consistent with the findings of Rydel et al [28-30]. A significant follow-up duration is required in order to determine progression to CKD in patients with primary FSGS, as this disease usually progresses in a sub-acute/chronic manner [7]. The median duration of follow-up for those patients with a poor long-term outcome was 104 weeks, whereas the median duration of follow-up in those with a good outcome was 16 weeks; suggesting that those with good outcomes may not yet have had sufficient follow-up to accurately classify outcome.

4.3.3 The Role of Gender in Cumulative Outcome

This study demonstrated no significant difference in renal survival when comparing males with females. A Kaplan Meier survival analysis was performed and no statistically significant difference by sex in cumulative survival was found. This is somewhat different from Cattran et al's findings that females had an overall better outcome than males. A possible explanation for the findings in this study may be the small numbers of females in this study, making adequate statistical analysis challenging [67].

4.3.4 Differences in Survival by Race

Black and Non-Black patients showed no difference in long-term outcomes, concurring with Rydel et al [29]. Kaplan Meier survival analysis demonstrated no difference in cumulative survival when comparing Black with Non-Black patients.

4.3.5 The Role of Histological Variant in Predicting Cumulative Survival

No particular histological subtype significantly predisposed sufferers of FSGS in this study to a worse long-term outcome. Further interrogation of this cohort comparing the collapsing variant to the other subtypes (as a group) demonstrated a trend towards a worse outcome in the collapsing variant group, however this observation was not statistically significant. This finding, despite being not significant, is

consistent with previous literature documenting poorer outcomes in patients with the collapsing variant of FSGS [26-28] .

5. Conclusion

FSGS is an important primary glomerular disorder, especially in the South African Black population. Younger age at onset and the frequency of occurrence of the collapsing variant in Black patients suggests the possibility of the presence of a genetic susceptibility in this group, however this needs to be further characterised in future studies. The decision to administer or withhold therapy should not be dependent on presenting parameters, as these variables poorly predict response to treatment. Prolonged courses of glucocorticoid therapy should be initiated timeously to prevent adverse outcomes and induce remission.

6. Limitations of Study

- Patient records: in certain cases, patient records were not traceable, therefore making analysis of therapy impossible in this group. In other cases, patient records (in particular urine White Cell Count and Red Cell Count) were incomplete thus preventing certain variables from being adequately analysed. Laboratory parameters in most patients however, made it possible to determine presenting features in all subjects.

- Small study group: continuous data in this study was not normally distributed, given the small number of patients that were eligible for inclusion. Statistical analysis was therefore performed using non-parametric testing; however it is not possible to completely eliminate bias given the small cohort of patients.

- Histological reporting: the classification of histologic subtype is dependent on inter-observer variability between pathologists and this may have also affected data and results.

- Retrospective nature of study: inherent to the study type, selection and information bias may have negatively affected the accuracy of certain elements of this study.

7. Future Studies

The true prevalence of Primary FSGS as a proportion of primary glomerular disorders was not analysed in this study. A renal biopsy registry, documenting the prevalence of primary and secondary glomerular disorders, as well as tubulo-interstitial nephropathies and vascular nephropathies would be useful in the South African setting.

Further studies of a similar nature with larger sample sizes and at multiple institutions in South Africa are required to substantiate or refute the findings in this particular study. Given the small group of patients in this case series, many findings were not statistically significant; it would be valuable to determine if observed trends in this study would constitute significant findings in a larger population.

A genetic predisposition in the South African Black population was possible suggested by the findings in this study. Genetic studies in the Black population with Primary FSGS (described in this study) would elucidate the presence or absence of a genetic susceptibility in this particular racial group.

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