Electron microscopic morphometry of podocyte foot process effacement as a tool to distinguish primary from secondary focal segmental glomerulosclerosis (FSGS)

Dr Nelson da Costa

Student number: DCSNEL001

A dissertation submitted to the Faculty of Health Sciences, University of Cape Town, in partial fulfilment of the requirements for Master of Medicine (MMed) in Anatomical Pathology

Date submitted: 15 March 2024

Supervisor: Dr Brendon Price

Co-supervisors:

Dr Shivani Singh

Dr Bianca Davidson

Dr Nadia Ikumi

Plagiarism Declaration

"This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor."

Name: Dr Nelson da Costa

Student number: DCSNEL001

Signature:

Signed by candidate

Date: 12 March 2024

AFFILIATIONS OF STUDENT AND SUPERVISORS

STUDENT

Dr Nelson da Costa (BSc, MBChB, DA(SA), FC Path(SA) Anat)
Registrar Anatomical Pathology at National Health Laboratory Service, Groote Schuur Hospital
Division of Anatomical Pathology, Department of Pathology, University of Cape Town
Email address: DCSNEL001@wf.uct.ac.za

SUPERVISORS

PRINCIPAL SUPERVISOR

Dr Brendon Price (PhD, MBBCh, FC Path(SA) Anat, MMed (Anat Path))
Specialist Anatomical Pathologist at National Health Laboratory Service, Groote Schuur Hospital
Division of Anatomical Pathology, Department of Pathology, University of Cape Town
Email address: brendon.price@nhls.ac.za / brendon.price@uct.ac.za

CO-SUPERVISOR

Dr Shivani Singh (MBChB, FC Path(SA) Anat, MMed (Anat Path))
Specialist Anatomical Pathologist at National Health Laboratory Service, Red Cross War Memorial Children's Hospital
Division of Anatomical Pathology, Department of Pathology, University of Cape Town
Email address: shivani.singh@nhls.ac.za / shivani.singh@uct.ac.za

CO-SUPERVISOR

Dr Bianca Davidson (MBChB, FCP(SA), MPhil (Int Med), Cert Nephrology (SA) Phys)
Specialist Nephrologist, Division of Nephrology and Hypertension at Groote Schuur Hospital
Nephrology and Hypertension Research Unit, University of Cape Town
Email address: bianca.davidson@uct.ac.za

CO-SUPERVISOR

Dr Nadia Ikumi (BSc, MSc, PhD)

Lecturer - Postgraduate Research

Division of Anatomical Pathology, Department of Pathology, University of Cape Town

Email address: nadia.ikumi@uct.ac.za

DECLARATION PAGE

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

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature:

Signed by candidate

Date: 12 March 2024

ACKNOWLEDGEMENTS

- Dr Nadia Ikumi, who I believe has been sent from above as a miracle worker. Thank you for the time, effort, support, patience, wealth of research knowledge, and moments of laughter.
- Dr Brendon Price, for his much-appreciated contributions as a great supervisor, for taking the time to introduce me to the electron microscope, his continued support, patience, and wealth of research knowledge since 2019.
- Dr Shivani Singh, for her contributions as co-supervisor and continued support since 2019.
- Prof. Komala Pillay, for her advice, wealth of knowledge, words of encouragement, and continued support since 2019.
- Dr Bianca Davidson, for her contributions and advice, particularly from the clinical perspective.
- Dr Erika Jones, for taking the time to teach me how to navigate and use REDCap.
- The Division of Nephrology and Hypertension, Groote Schuur Hospital, for the use of patient files and the opportunity to create an online database for FSGS patients on REDCap.
- Mr Ebrahim Dollie, for his assistance with the electron microscope, including the acquisition of images, his time, support, encouraging words, and numerous moments of laughter, too many to count.
- Miss Courtni Veldsman, for her hard work, effort, and countless hours spent preparing and imaging the cases for electron microscopy.
- Mr Muneeb Adonis, for his assistance with exportation of REDCap data.
- Dr Robert Freercks, for supplying us with treatment response information regarding the patients biopsied and treated at Livingstone Hospital.
- Miss Yanelisa Sentiwe, for assisting with obtaining the patient files from the Division of Nephrology and Hypertension at GSH for our study.

DEDICATIONS

I would like to dedicate this dissertation to my loving grandparents, Carlos, Helena and Vitalina, my generous parents, Victor and Ana, my brother, Ricardo, and my caring husband, Matthew, for without their unwavering support and continuous appraisal I would not be where I am today.

ABSTRACT

Background

Focal segmental glomerulosclerosis (FSGS) is a histological pattern of glomerular injury and one of the most common causes of end-stage renal disease in adult patients. Two major subtypes of FSGS (primary and secondary) have been identified, with differences in clinical presentation, electron microscopy (EM) foot process width (FPW) and effacement (FPE), and treatment options. Primary FSGS commonly presents as nephrotic syndrome (NS), shows diffuse FPE with a FPW >1500nm, and is generally responsive to steroid therapy. Secondary FSGS does not present with NS (non-nephrotic), shows focal FPE and a FPW <1500nm, and does not respond to steroids. Sometimes, it may be clinically very difficult to differentiate between the two. In these scenarios, EM becomes of paramount importance. In this study, we evaluated podocyte FPW and FPE in FSGS patients to investigate whether a significant difference exists between primary and secondary FSGS.

Methods

Cases histologically diagnosed as FSGS in adult native renal biopsies over a 5-year period at Groote Schuur Hospital and Livingstone Hospital were reviewed. Using the 2021 KDIGO guidelines, cases were classified as nephrotic syndrome (NS) or non-nephrotic syndrome (NNS). Using EM and imaging software, podocyte FPWs were calculated and the extent of FPE determined for each case. The results were analysed and correlated with multiple variables.

Results

Of a total sample size of 35, 32 cases of FSGS and 3 controls were reviewed. 23 patients presented with NS while 9 patients did not meet the criteria for NS. The NS group had a calculated median FPW of 3076nm, while the NNS group had a median FPW of 1322nm (p=0.003). 83% of the NS group had diffuse FPE, whereas 78% of the NNS group had focal FPE (p=0.003). Logistic regression revealed a FPW threshold value of 2550nm correlated with an 80% probability of a NS diagnosis. A strong correlation between primary FSGS and oedema (p=0.006), proteinuria (p=0.0005), cholesterol (p=0.003) and albumin (p=0.0002) were found. A strong correlation existed between FPW and proteinuria (p=0.0021), eGFR (p=0.017), and albumin (p=0.012). No differences were identified with regards to age, gender, and HIV status. Unsupervised hierarchical cluster analysis with no *a priori* assumptions identified three clusters, one NNS cluster and two

NS clusters, the latter demonstrating 2 separate populations differing with respect to uPCR, creatinine, and FPW. The significance of this finding will be investigated in follow up studies.

Conclusion

This is the first study, to our knowledge, in the sub-Saharan African setting to use EM to calculate FPW and determine FPE in FSGS patients. The current study results align with those of previously published international studies. In this study, primary FSGS is generally associated with diffuse FPE and FPW>3000nm, whereas secondary FSGS is associated with focal FPE and FPW <1500nm. In clinically difficult scenarios, EM FPW calculation and FPE determination remains an important adjunct to histopathology and clinical parameters in the differentiation of primary from secondary FSGS in the South African population. The significance of making this distinction lies in completely different patient management regimens between the two groups.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANA	Antinuclear antibody
Anti-DNAse B	Antideoxyribonuclease B
Anti-dsDNA	Anti-double stranded deoxyribonucleic acid
APOL1	Apolipoprotein 1
ASOT	Antistreptolysin O
BP	Blood pressure
C3	Complement 3
C4	Complement 4
cANCA	Central antineutrophil cytoplasmic antibody
CNI	Calcineurin inhibitor
DRC	Departmental Research Committee
eGFR	Estimated glomerular filtration rate
eGFR-MDRD	Estimated glomerular filtration rate – Modification of Diet in Renal Disease equation
eGFR-CKD-EPI	Estimated glomerular filtration rate – Chronic Kidney Disease – Epidemiology Collaboration equation
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
ESRD	End-stage renal disease
FPE	Foot process effacement
FPW	Foot process width
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane

HCA	Hierarchical cluster analysis
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIVAN	HIV-associated nephropathy
HREC	Human Research Ethics Committee
IFN	Interferon
IgM	Immunoglobulin M
IQR	Inter-quartile range
KDIGO	Kidney Disease: Improving Global Outcomes
LM	Light microscopy
MCD	Minimal change disease
MMed	Master of Medicine
NHLS	National Health Laboratory Service
NNS	Non-nephrotic syndrome
NOS	Not otherwise specified
NS	Nephrotic syndrome
pANCA	Peripheral antineutrophil cytoplasmic antibody
RAAS	Renin Angiotensin Aldosterone System
RPR	Rapid Plasma Reagin test
SD	Standard deviation
SLE	Systemic lupus erythematosus
SUPAR	Soluble urokinase receptor
Urine PCR / uPCR	Urine protein creatinine ratio

UCT	University of Cape Town
USA	United States of America
VDRL	Venereal Disease Research Laboratory test
VEGF-A	Vascular endothelial growth factor A

LIST OF APPENDICES

1. CHAPTER ONE - INTRODUCTION

1.1. FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is the leading glomerular cause of end-stage renal disease (ESRD) in the United States of America (USA). It is responsible for approximately 40% of adult nephrotic syndrome (NS) and 20% of paediatric NS cases[1]. The prevalence of FSGS is increasing globally[2,3]. The incidence rates are generally higher in men, being 1.5-fold higher than in women[2]. Proteinuria is the cardinal presenting clinical feature of FSGS[4].

FSGS is a morphologic/histologic pattern of injury defined as a "segmental obliteration of glomerular capillaries by extracellular matrix."[3] The lesion of FSGS represents a segmental increase in glomerular matrix with the accumulation of intracapillary lipid-laden macrophages leading to obliteration of the capillary lumina in at least one glomerulus in the entire kidney biopsy[4,5]. The sclerosis is usually accompanied by hyalinosis, accumulation of plasma proteins in the adherent capillary loop[3,5]. FSGS begins within the juxtamedullary nephrons, progressing to involve the outer cortex at a later stage of disease; hence renal biopsy sampling should be adequate to identify the focal diagnostic lesions[3,6]. Regarding adequacy, at least 10 glomeruli, both cortical and juxtamedullary, should be sampled[1]. Of note, immunofluorescence usually shows segmental deposits of IgM antibody and C3 complement within the hyalinosis[3].

Podocytes are terminally differentiated, post-mitotic cells unable to proliferate to compensate for lost cells[4]. After podocyte loss has reached a critical point the glomerular basement membrane (GBM) is exposed, resulting in collagen deposition, maladaptive interactions between the GBM and the parietal epithelial cells, capillary loop collapse, and endothelial cell loss, creating the characteristic segmental obliteration of the glomerular capillary tuft[4,7]. "Focal" denotes a heterogeneous involvement of the glomerular population in the renal cortex (<50% of all glomeruli affected on light microscopy (LM))[4,5]. Sclerotic lesions involve the great majority of the glomeruli, revealing that FSGS is not as focal as the name implies[4]. "Segmental" affects only a portion of the glomerular tuft (<50% of the glomerular tuft affected); this must be distinguished from non-specific focal global glomerulosclerosis (affecting the entire glomerular tuft) observed in aging and hypertensive nephropathy[4,5].

FSGS is a syndrome manifested by glomerular podocyte injury (podocytopathy). Another podocytopathy and part of the differential diagnosis of NS is minimal change disease (MCD)[3]. These podocytopathies are characterised by podocyte foot process effacement (FPE), a change within the shape of the foot processes, associated with narrowing of glomerular filtration slits and development of tight junctions between foot processes[3,8,9]. To date, the ultrastructural changes of podocytes described in NS include FPE, microvillous transformation, podocyte vacuolisation, and podocyte detachment from the GBM[9,10]. FSGS lesions are focal and segmental on LM; however, at the electron microscopic (EM) level, diffuse and global FPE of varying levels can be seen, as compared to MCD in which diffuse FPE is seen[8,11]. Da Silva *et al.* found that patients with FSGS showed positive and significant correlation between proteinuria levels and foot process width (FPW) which was not seen in patients with MCD, indicating that proteinuria exhibits a direct relationship with the extension of FPE in FSGS[12]. In their study, the mean FPW was higher in FSGS than in MCD[12]. Interestingly, Ishizuka *et al.* found that the amount of proteinuria did not correlate with FPE percentage or FPW[13]. Similarly, Deegens *et al.* reported no correlation between FPW and proteinuria or serum albumin levels, suggesting that the extent of FPE is determined by the nature of podocyte injury[11]. In the study by Hommos *et al., a*lthough diffuse FPE was highly associated with NS, proteinuria alone had limited correlation with the degree of FPE[14].

The stereotypical response to podocyte injury involves four steps: 1) reorganisation of the actin cytoskeleton resulting in cell body simplification and FPE; 2) disruption of the glomerular filtration barrier leading to increased permeability to proteins, specifically albumin, which has been shown to injure both podocytes and parietal epithelial cells by enhancing apoptosis and scavenging retinoic acid; 3) podocyte death; and 4) podocyte detachment[5]. The glomerulus can recover with the loss of <20% of podocytes; however, a >20% podocyte loss ultimately leads to FSGS[5].

Although FPE is the conventional parameter to assess on EM regarding the podocytopathies, Royal *et al.* suggested that a combination of a variety of structural changes affecting podocytes, endothelial cells, and GBMs may better characterise the heterogeneity in progression and response to therapy among these patients. These included FPE, condensation of the actin-based cytoskeleton, microvillous transformation, loss of primary processes, podocyte detachment, thickening and thinning of the GBM, GBM abnormal texture, tubuloreticular inclusions, glomerular endothelial cell fenestration, endothelium honeycombing-like appearance, and electron densities/hyaline material. They demonstrated that severe FPE and microvillous transformation were associated with proteinuria

remission, podocyte detachment with lack of remission, and prominent endothelial cell and GBM abnormalities with loss of renal function[9].

Extensive podocyte injury at the ultrastructural level with adequate numbers of normal-appearing glomeruli and non-scarred tubulointerstitium by LM suggests MCD. Individuals with a diagnosis of MCD who are resistant to glucocorticoid therapy or manifest deterioration in renal function may have FSGS that was under sampled on the first biopsy and is shown on a subsequent biopsy[2]. Notably, MCD requires less aggressive immunosuppressive therapy and has an excellent long-term prognosis. Nili *et al.* found that certain EM features present in non-sclerotic glomeruli in kidney biopsies of patients with steroid-resistant NS potentially represent an underlying FSGS rather than MCD. These features include moderate to severe multifocal expansion of mesangial matrix, remodelling and duplication of the GBM involving >10% of capillary walls, and podocyte proliferation[15].

Various constituent cells of the kidney have been reported to be involved in the pathogenesis of FSGS, according to Morita *et al.* These include podocyte injury to segmental sclerosis, phenotypic changes in parietal epithelial cells, and crosstalk between glomerular epithelial and tubular epithelial cells and between glomerular endothelial or mesangial cells and podocytes. Podocytes secrete vascular endothelial growth factor A (VEGF-A) and endothelin-1 to regulate glomerular endothelial cell proliferation and function, thereby maintaining glomerular capillary loop homeostasis. Overexpression of VEGF-A in podocytes caused a collapsing glomerulopathy[16]. Daehn *et al.* found that endothelin-1 release by podocytes mediates mitochondrial oxidative stress and dysfunction in adjacent endothelial cells via paracrine endothelin-1 receptor type A activation. This endothelial dysfunction in turn promotes podocyte apoptosis, leading to podocyte loss, albuminuria, glomerulosclerosis, and renal failure. They suggested that the reciprocal interaction between podocytes and endothelial cells may provide opportunities for therapeutic intervention in FSGS[17]. Similarly, Royal *et al.* proved their hypothesis that the glomerular endothelium participates in the pathophysiology of the podocytopathies[9]. Saga, Sakamoto, Matsusaka, and Nagata found that the glomerular filtrate acts in combination with FPE to provide the mechanical force that expands the subpodocyte space into pseudocysts, resulting in local podocyte detachment, which is accelerated by hyperfiltration[18].

Rosenberg *et al.* has found it useful to cluster FSGS into 6 clinical forms, including 2 common forms (primary FSGS and adaptive FSGS), 3 less common forms (high-penetrance genetic FSGS, viral-mediated FSGS, and medication-associated FSGS), and an apo L1 (APOL1)-associated FSGS variant[2].

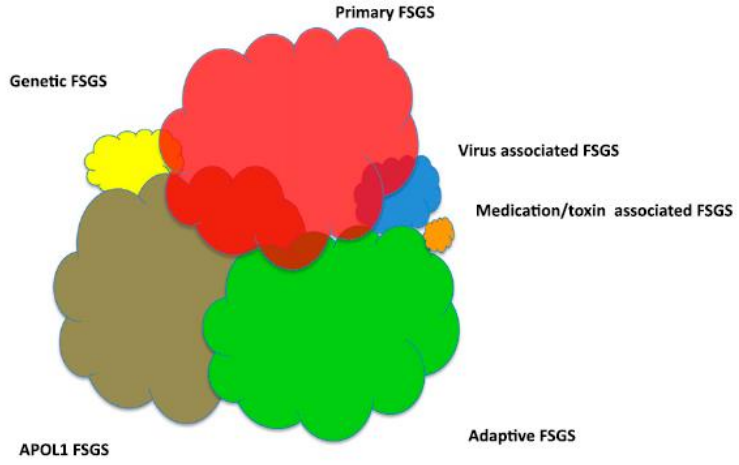


Figure 1: The six forms of clinical FSGS, with the size of each bubble corresponding to the prevalence in a population in the United States[2].

According to the latest Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, FSGS is subdivided into four types as per the diagram below[19,20]:

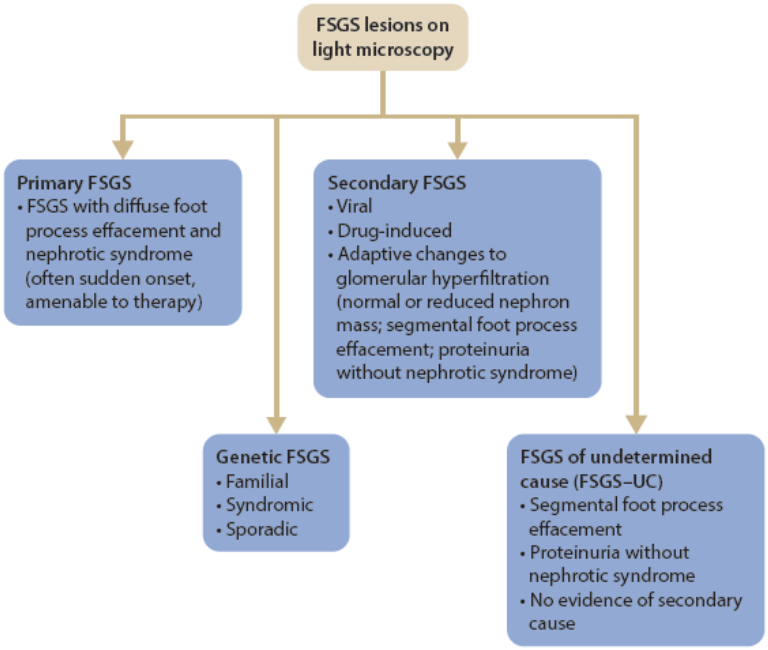


Figure 2: FSGS types according to the KDIGO guidelines[19].

1.1.1. PRIMARY FSGS

A putative circulating permeability factor, toxic to the podocyte, has been proposed to play a role in primary FSGS development[3,21]. It was found that two thirds of patients with primary FSGS have increased levels of SUPAR (soluble urokinase receptor). However, authors of subsequent studies believe that SUPAR is not the putative agent[3].

Patients with primary FSGS typically present with abrupt onset marked proteinuria and severe NS[1,4,21]. Many authors believe that MCD and primary FSGS are the same disease, where FSGS is a more advanced form of MCD[1]. FPE is diffuse and occurs throughout glomeruli in both diseases[7]. Primary FSGS is a diagnosis of exclusion, requiring that secondary causes be ruled out[1,21].

Serum albumin levels are often found to be sufficient to distinguish between primary and secondary FSGS. Deegens *et al.* reported that serum albumin was significantly lower in patients with presumed idiopathic FSGS compared to secondary FSGS and they suggested that in patients with borderline serum albumin levels, foot process measurement could be helpful in guiding diagnosis and treatment[11].

De novo FSGS may occur in the transplanted donor kidney in patients who did not have FSGS as a cause of ESRD in the native kidney. This often occurs >12 months after transplantation and is associated with variable proteinuria, hypertension, and progressive renal allograft dysfunction. Kidney size discrepancies between the graft and the recipient can lead to *de novo* FSGS due to compensatory glomerular hyperfiltration in residual nephrons caused by nephron loss or low nephron number in the allograft[1].

1.1.2. SECONDARY FSGS

Secondary FSGS is caused by numerous different disease processes. These patients present with sub-nephrotic range to nephrotic range proteinuria[1,4,21]. There is usually a slow increase of proteinuria over time and they rarely, if ever, develop NS. Renal insufficiency is therefore less common[1,4]. Sometimes, clinicopathological correlation is missed, resulting in a number of patients with secondary FSGS being mislabelled as primary FSGS and undergoing immunosuppressive therapy. Sethi *et al.* proposed that dividing the FSGS lesion into nephrotic syndrome-associated and non-nephrotic syndrome-associated FSGS as a first approximation, followed by dividing the lesion according to the degree of FPE, will be more clinically relevant for the correct recognition of primary versus secondary FSGS[21].

In secondary FSGS, FPE is primarily limited to sclerotic areas[7]. This form of FSGS results from a mismatch between glomerular load and glomerular capacity in conditions associated with hyperfiltration, glomerular capillary hypertension, and glomerular hypertrophy[4,14].

HIVAN (HIV-associated nephropathy), which occurs in untreated HIV-1 positive patients, is a specific form of viral-induced secondary FSGS[3]. The effect of HIV on podocytes is strongest in individuals with 2 APOL1 risk alleles with an odds ratio of 89 in South Africa. Most individuals with HIVAN have one or two APOL1 risk alleles. APOL1-associated FSGS is a major form of FSGS in countries with individuals of sub-Saharan African descent. The effect is largely recessive, requiring 2 risk alleles, although in certain situations (HIV-positive South Africans), a single copy of a risk allele G1 has a significant association with HIVAN. APOL1 high-risk alleles are strongly associated with collapsing glomerulopathy in several settings: HIVAN, in which 72% have 2 APOL1 high-risk alleles, the use of exogenous interferon (IFN) therapy, and in systemic lupus erythematosus (SLE)[2]. The mechanism of virus associated FSGS involves direct infection of the podocytes, resulting in dysregulation of the cellular phenotype and apoptosis[4]. It is a rapidly progressive disease associated with a poor prognosis[3].

The genetic causes of FSGS, considered as causes of secondary FSGS by some authors and a standalone category by others, may present as sporadic or familial disease, with autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance patterns[4]. The best-known susceptibility genes are the G1 and G2 polymorphisms in the APOL1 gene, which impart a greatly increased risk of adult-onset FSGS, hypertensive nephropathy, and HIVAN in African American patients. Genetic FSGS results from a mutation in genes encoding vital podocyte structural proteins, including those involved in slit diaphragm structure and function (nephrin, podocin, CD2AP, etc.), actin cytoskeleton (α-actinin 4, formin, myosin IIA etc.), or foot process-GBM interaction (LAMB2 and ITGA3)[4,5]. The clinical presentation is variable in each of these disorders[5]. A genetic form of FSGS must be considered when either diffuse or segmental FPE is observed[4].

Direct podocyte toxicity resulting in dysregulation of the cytoskeleton has been described in drug induced FSGS[4].

Secondary to alterations of glomerular epithelial cells	
Viral infections	HIV (established) CMV (probably) Parvovirus B19, EBV, HCV (possibly) Hemophagocytic syndrome (possibly) SARS-COV-2 (with *APOL1* risk genotype)
Drug-induced	Direct-acting antiviral therapy mTOR inhibitors, CNIs Anthracyclines Heroin (adulterants) Lithium Interferon Anabolic steroids NSAIDs
Secondary to adaptive changes with glomerular hypertension	
Reduced nephron number	Reflux nephropathy Renal dysplasia Oligomeganephronia Sickle cell disease Age-related FSGS
Normal nephron number	Obesity-related glomerulopathy Primary glomerular diseases Systemic conditions, e.g., diabetic nephropathy, hypertensive nephrosclerosis

Figure 3: Causes of secondary FSGS[20].

Table 1: Characteristic clinicopathological features of the main types of FSGS[2].

Characteristic Features	Primary FSGS	Adaptive FSGS	APOL1 FSGS	Genetic FSGS	Infection/ Inflammation Associated	Medication-Associated FSGS
Mechanism of Podocyte Injury	Circulating factor, possibly a cytokine	Mismatch between metabolic load and glomerular capacity	APOL1 variant–initiated inflammation	High-penetrance genetic variants (Mendelian or mitochondrial inheritance)	Postulated role of IFN and possible other cytokines	Presumed direct effect on podocytes
History	Acute onset of edema	Reduced renal mass: low birth weight, oligomeganephronia, ureteral reflux, morbid obesity; increased single-nephron GFR: cyanotic congenital heart disease, sickle cell anemia	Family history, may be unremarkable	Family history, may be unremarkable with recessive inheritance genes	HIV, CMV, possible: parvovirus B19, Still disease, natural killer cell leukemia	Bisphosphonate, lithium
Laboratory tests	Many have high-grade proteinuria and nephrotic syndrome	Any level of proteinuria, serum albumin may be normal	Any level of proteinuria	Any level of proteinuria	Any level of proteinuria	Any level of proteinuria
Renal pathology	Widespread foot process effacement	Large glomeruli, perihilar sclerosis variant most typical, partial foot process effacement	May resemble primary or adaptive forms	Variable	Variable	Variable
Treatment and response	May respond to IST	Responds well to RAAS antagonism, often with >50% proteinuria reduction	May respond to therapies used for primary and adaptive forms	High-penetrance genetic mutations: usually does not respond to IST	Treat the virus	Stop the medication
Recurrence after renal transplant	Possible	Unlikely	Possible	Unlikely	Possible if infection/ inflammation persists	Unlikely

CMV, cytomegalovirus; IST, immunosuppressive therapy; RAAS, renin-angiotensin-aldosterone system; CG, collaspsing glomerulopathy. Modified from Kopp (24), with permission.

Table 2: Characteristics of various forms and diseases included in the differential diagnosis of FSGS[1].

Characteristics	Primary FSGS	Secondary FSGS	Genetic FSGS	MCD
Clinical history	Acute onset of nephrotic syndrome without risk factors or previous renal disease history	Risk factors are present, such as obesity, drug consumption, vesicoureteral reflux, renal agenesis or reduced nephron mass or viral infection	Family history of FSGS disease (although frequently there are not familiar records); proteinuria or nephrotic syndrome with onset in early childhood or adolescence	Acute onset of nephrotic syndrome without risk factors or previous renal disease history
Laboratory findings	Nephrotic syndrome: peripheral oedema, hypoalbuminaemia and >3.5 g of proteinuria in 24-h urine; haematuria is common	Non-nephrotic or nephrotic-range proteinuria, without nephrotic syndrome; normal serum albumin levels	Childhood-onset genetic FSGS: usually nephrotic syndrome is present; adolescence or adult-onset genetic FSGS: proteinuria without nephrotic syndrome	More rapid onset of nephrotic syndrome; peripherial oedema, hypoalbuminaemia and >3.5 g of proteinuria in 24-h urine
Pathological findings	LM: segmental areas of sclerosis, partial capillary collapse and hyaline deposits[a]			LM: normal glomeruli
	IF: none or few immune deposits in sclerotic lesions positive to IgM and occasionally to C3		IF: negative	
	EM: usually diffuse (>80%) podocyte FPE	EM: usually segmental (<80%) podocyte FPE	EM: either diffuse or segmental podocyte FPE	EM: diffuse (>80%) podocyte FPE

[a]Depending on the location of the lesions, tip and perihiliar variants are distinguished. Cellular and collapsing variant show their own characteristics. If not a quality biopsy, glomeruli may seem normal.
IF, immunofluorescence.

1.2. FSGS HISTOLOGICAL SUBTYPES

D'Agati *et al.* proposed international recommendations to be used in defining terms and classifying FSGS variants based on histology. There are 5 histologic subtypes of FSGS: perihilar, cellular, tip, collapsing, and not otherwise specified (NOS)[8]. These will be discussed briefly.

1.2.1. PERIHILAR SUBTYPE

The perihilar subtype is defined as segmental lesions that are located at the glomerular vascular pole, present in at least 1 glomerulus. They show hyalinosis and sclerosis involving >50% of glomeruli[3,8]. They are commonly found in secondary FSGS, where the glomeruli are usually enlarged and FPE is usually focal and relatively mild[3,4,6]. These patients present with the highest frequency of hypertension, the lowest frequency of NS, and have a reasonably good prognosis[22].

1.2.2. CELLULAR SUBTYPE

The cellular subtype is the least common form of FSGS. They are defined as segmental lesions having expansile endocapillary hypercellularity involving at least 25% of the tuft, often with foam cells, hyalinosis, infiltrating leukocytes, and karyorrhexis, with variable glomerular epithelial cell hyperplasia[3,6,8]. They are typically found in the peripheral tuft. FPE is usually severe[6].

1.2.3. TIP SUBTYPE

The tip subtype is defined as at least one segmental lesion located at the tubular pole (outer 25% of the tuft next to the origin of the proximal tubule) with either adhesions to the proximal tubular outlet or confluence of podocytes with proximal tubular epithelium[3,8]. They are not characterised by glomerular enlargement[7]. FPE is typically severe and diffuse[6]. This subtype is usually associated with primary FSGS[3,6]. It is more common in older Caucasians, presents with the least impaired renal function, has a greater likelihood of steroid responsivity, and has the best prognosis[3,4,6,8,22].

1.2.4. COLLAPSING SUBTYPE

The collapsing subtype is defined as implosive glomerular tuft collapse with hypertrophy and hyperplasia of the overlying visceral epithelial cells in at least 1 glomerulus[3,8]. Pseudocrescents,

crowding of the urinary space by hypertrophied and hyperplastic podocytes, are diagnostic[3,6]. This is the most aggressive variant of FSGS, affecting younger patients, males, and African Americans more frequently[3,4,6,8,22]. It presents with severe renal insufficiency despite a shorter duration of symptoms and has the worst remission rate and prognosis[3,6,8,16,22]. It may be primary or secondary to viruses (HIV and Parvovirus B19), pamidronate therapy, IFN therapy, and acute vaso-occlusive disease[4,6].

1.2.5. NOS SUBTYPE

The NOS subtype is the most common form of FSGS[3,4]. It is characterised by at least 1 glomerulus showing segmental sclerosis[6,8]. It does not meet the criteria for any other subtype. All other subtypes can evolve into NOS over time[3,8]. FPE is variable but typically diffuse[6]. It is equally distributed among primary and secondary forms[4]. These patients commonly present with hypertension and NS. Very few of these patients undergo complete remission and renal survival is moderate[22].

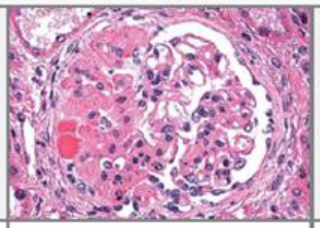
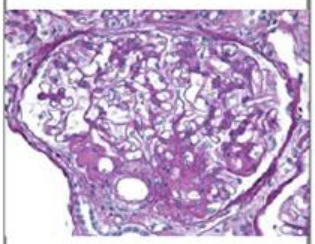
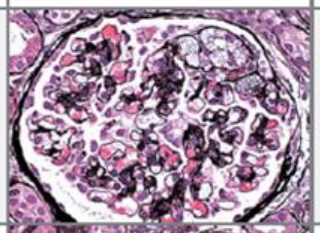
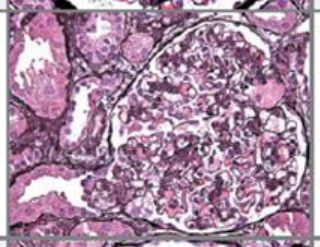
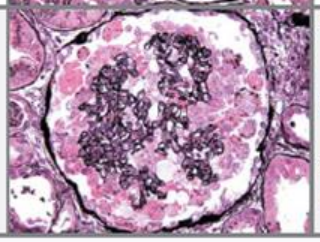
Histologic Subtype	Glomerular Lesion	Defining Features	Associations	Clinical Features
NOS		The usual generic form of FSGS. FSGS(NOS) does not meet defining criteria for any other variant. Foot-process effacement is variable.	Primary or secondary (including genetic forms and other diverse secondary causes). Cross-sectional studies suggest this is the most common subtype. Other variants can evolve into FSGS (NOS) over time.	May present with the nephrotic syndrome or subnephrotic proteinuria.
Perihilar		Perihilar hyalinosis and sclerosis involving the majority of glomeruli with segmental lesions. Perihilar lesions are located at the glomerular vascular pole. In adaptive FSGS, there is usually glomerular hypertrophy (glomerulomegaly). Foot-process effacement is relatively mild and focal, which probably reflects the heterogeneous adaptive responses of glomeruli.	Common in adaptive FSGS associated with obesity, elevated lean body mass, reflux nephropathy, hypertensive nephrosclerosis, sickle cell anemia, and renal agenesis. Predisposition for vascular pole is probably due to normally increased filtration pressures at the proximal afferent end of glomerular capillary bed, which are heightened under conditions of compensatory demand and vasodilatation of the afferent arteriole.	In adaptive FSGS, patients are more likely to present with subnephrotic proteinuria and normal serum albumin levels.
Cellular		Expansile segmental lesion with endocapillary hypercellularity, often including foam cells and infiltrating leukocytes, with variable glomerular epithelial-cell hyperplasia. There is usually severe foot-process effacement.	Usually primary, but also seen in a variety of secondary forms. This is the least common variant. It is thought to represent an early stage in the evolution of sclerotic lesions.	Usually presents with the nephrotic syndrome.
Tip		Segmental lesion involving the tubular pole, with either adhesion to tubular outlet or confluence of podocytes and tubular epithelial cells. Compared with other variants, it has the least tubular atrophy and interstitial fibrosis. There is usually severe foot-process effacement.	Usually primary. Probably mediated by physical stresses on the paratubular segment owing to the convergence of protein-rich filtrate on the tubular pole, causing shear stress and possible prolapse.	Usually presents with abrupt onset of the nephrotic syndrome. More common in white race. Best prognosis, with highest rate of responsivity to glucocorticoids and lowest risk of progression.
Collapse		Implosive glomerular-tuft collapse with hypertrophy and hyperplasia of the overlying visceral epithelial cells. Hyperplastic glomerular epithelial cells may fill the urinary space, resembling crescents. Severe tubular injury and tubular microcysts are common. There is usually severe foot-process effacement.	Primary or secondary to Viruses: HIV-1, parvovirus B19, SV40, EBV, CMV, hemophagocytic syndrome Drugs: pamidronate and interferon Vaso-occlusive disease: atheroemboli, calcineurin inhibitor nephrotoxicity, and chronic allograft nephropathy	Most aggressive variant of primary FSGS with black racial predominance and severe nephrotic syndrome. Worst prognosis, with poor responsivity to glucocorticoids and rapid course to renal failure.

Figure 4: Histologic variants of FSGS[3].

1.3. ELECTRON MICROSCOPIC ULTRASTRUCTURAL ANALYSIS OF FSGS

Podocytes have large cell bodies and numerous foot-like protrusions that extend outwards. These processes interdigitate with the processes of neighbouring podocytes, thereby creating structural support for the glomerular capillaries[3].

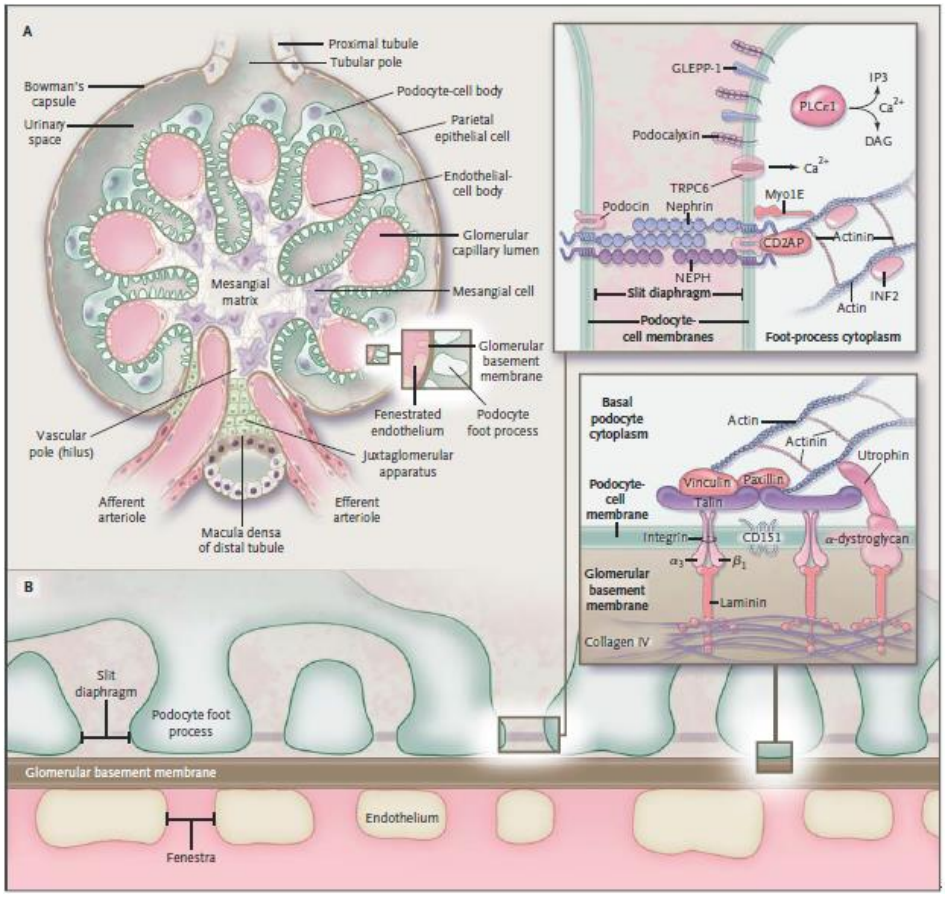


Figure 5: Morphology of the glomerulus and the GBM[3].

Podocyte injury is initially manifested by FPE, which proceeds with retraction, shortening and widening of the foot processes, ultimately resulting in a continuous and flattened cytoplasmic sheet covering the GBM. FPE is potentially reversible and represents a specific, reactive phenotype of the podocyte which may enhance adhesion to the GBM and limit the risk of detachment, at least for some time[4,23].

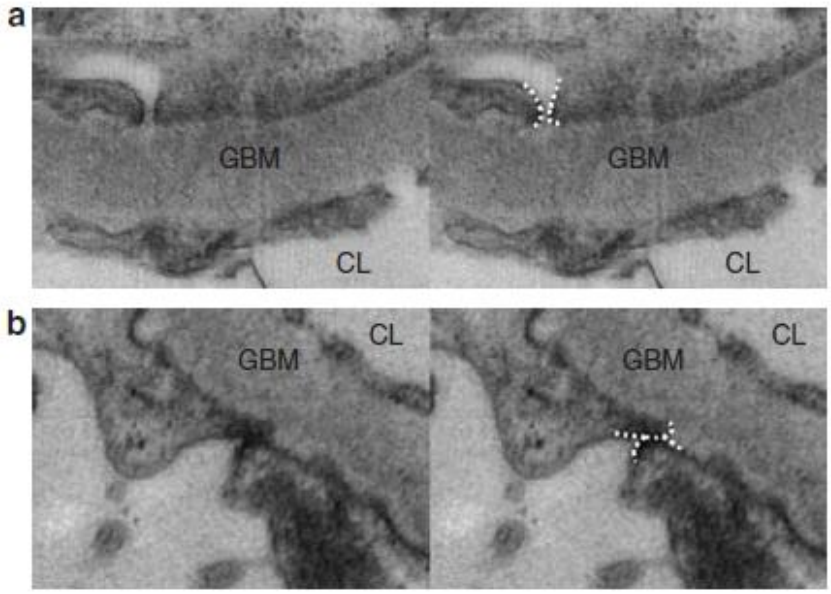


Figure 6: Electron micrographs illustrating the difference between separate foot processes (a, normal) and foot process fusion (b, abnormal). *GBM: glomerular basement membrane; CL: capillary lumen*[11].

Podocyte FPE is seen invariably in glomerular diseases associated with proteinuria, such as FSGS, MCD, IgA nephropathy, and diabetic nephropathy. According to Deegens *et al.*, FPE is more extensive in primary than in secondary FSGS[11]. Podocyte FPE is diffuse (>80% of the analysed surface) and occurs throughout glomeruli in primary FSGS and MCD, while it is segmental (primarily limited to sclerotic areas with <80% involvement) in secondary FSGS[1,7]. It is usually complete overlying the sclerosis, and may vary from mild to severe effacement in the non-sclerotic glomeruli[8]. Cases of collapsing FSGS secondary to HIV, IFN, or pamidronate therapy are characterised by widespread FPE, exceptions to the rule[24].

Ishizuka *et al.* found that all the genetic FSGS patients in their cohort showed segmental FPE up to 38% and FPW <2000nm, while all the primary FSGS patients showed diffuse FPE above 88% and FPW >3000nm. The FPE and FPW of all the patients with secondary FSGS were <38% and <1500nm, respectively[13].

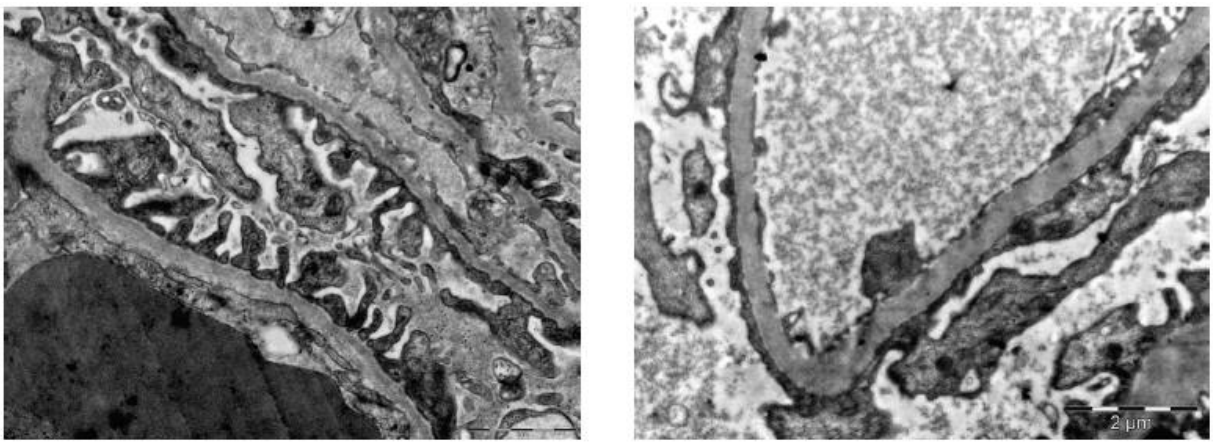


Figure 7: Electron micrographs illustrating well preserved foot processes (left) and complete foot process effacement (right)[25].

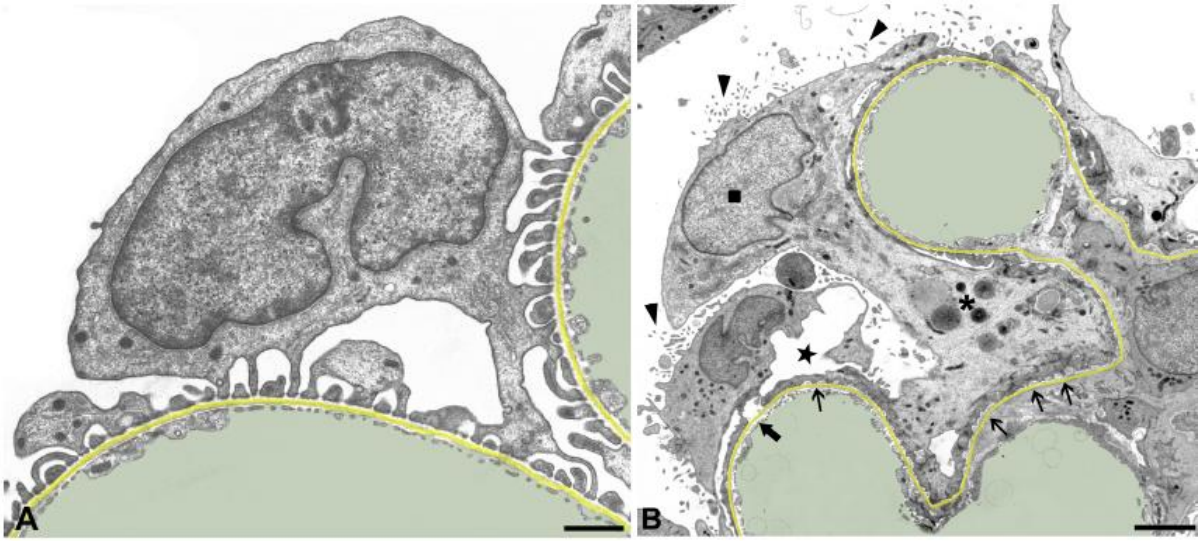


Figure 8: A) demonstrates a healthy podocyte with normal foot processes and B) demonstrates an injured podocyte with widespread FPE and focal complete detachment from the GBM (thick arrow)[23].

Deegens *et al.* found that a FPW >1500nm differentiated primary from secondary FSGS with high sensitivity and specificity, and that the degree of overlap between them was low. Additionally, they reported that the degree of overlap between MCD and the different types of FSGS was high and that FPW could not accurately differentiate between these diseases, suggesting that it is difficult to distinguish MCD from primary FSGS at the ultrastructural level. The degree of FPE between FSGS variants was not significantly different either. Type of disease (primary or secondary FSGS) but not FSGS variant was the only determinant of FPW in patients with FSGS[11].

In the study by De Vriese *et al.,* a FPW >1500nm adequately differentiated primary from secondary FSGS[4].

Taneda *et al.* found that FPW was significantly higher in the FSGS group than that in the MCD and control groups, particularly in the collapsing, tip, and cellular variants. In FSGS, FPW was inversely correlated with disease duration, but not with proteinuria[10].

The Gundersen method is used to calculate the arithmetic mean of the FPW. The following equation sums up the Gundersen method, where π/4 is the correction factor needed to correct for presumed random variation in the angle of section relative to the long axis of the podocyte:

$$FPW = \pi/4 \times \sum \text{GBM length} / \sum \text{foot process}$$[25]

Table 3: Comparison of the different studies found in the literature with important parameters utilised.

Study	Sample size	Time period	Area chosen	Minimum number of measurements needed	Formula used to calculate FPW	Cut-off value for FPE	p value
Kfoury[7]	33	1997-2010	Glomerular capillary loop	At least 5 glomeruli and 20 podocytes per case	Gundersen method	70%	<0.00001
Hommos *et al.*[14]	42	1994-2013	Glomerular capillary loop	2 non-sclerosed glomeruli	Not mentioned	80%	<0.05
Spanu *et al.*[25]	37	1999-2008	Not mentioned	100µm of GBM	Gundersen method	Not mentioned	<0.05
Taneda *et al.*[10]	43	Not mentioned	Glomeruli	8 glomeruli	Gundersen method	Not mentioned	<0.05
Fuiano *et al.*[26]	14	1987-1993	Glomeruli	8 or 9 glomeruli	Not mentioned	Not mentioned	<0.01 (8 glomeruli) or 0.001 (9 glomeruli)
Thomas *et al.*[22]	197	1982-2001	Not mentioned	≥5 glomeruli	Not mentioned	Not mentioned	<0.05
Deegens *et al.*[11]	24	Not mentioned	Glomerular capillary loop	2.6 (primary FSGS) and 3.8 (secondary FSGS) glomeruli	Gundersen method	FPW of 1500nm	<0.05
Sethi *et al.*[21]	41	1999-2012	Glomerular loops	≥8 non-segmentally sclerosed glomeruli	Not mentioned	80%	<0.05

1.4. TREATMENT AND PROGNOSIS OF FSGS

Patients presenting to the Division of Nephrology and Hypertension at Groote Schuur Hospital (GSH) are treated according to the latest KDIGO guidelines (Dr B. Davidson, Specialist Nephrologist, personal communication).

Adults with NS are generally biopsied before treatment is initiated. Patients with primary FSGS usually respond to high-dose oral glucocorticoid therapy as first-line immunosuppressive treatment[3,20,24]. These should be continued until complete remission is achieved, or as tolerated by patients up to a maximum of 16 weeks. Steroid resistance is defined by a lack of an adequate treatment response after 16 weeks of therapy. Those patients who respond to glucocorticoid treatment should receive glucocorticoids for at least 6 months. Adults with contraindications or intolerance to glucocorticoids should receive alternative immunosuppression with calcineurin inhibitors (CNIs). These are administered for a period of at least 18 months[20].

The major concerns of primary FSGS are the dismal renal prognosis in patients with poor response to immunosuppressive therapy, those experiencing relapses, and its recurrence after renal transplantation, where 30-50% develop recurrent disease in the allograft, often leading to renal graft failure[1,3]. This recurrence can occur immediately or months to years after transplantation and clinically presents as primary FSGS with severe NS[1]. It is thought that the permeability factor is responsible for this causation[3]. Risk factors responsible for recurrence include younger age, those who progress to ESRD within 3 years of diagnosis, a history of recurrence in a prior allograft, and patients with higher proteinuria levels pre-transplantation[1]. In these cases, plasmapheresis to remove the permeability factor is beneficial early in the course of recurrence and usually leads to remission[3]. Interestingly, apolipoprotein A-Ib has potential prognostic value, as it can be found in urine before episodes of FSGS recurrence post-transplantation[1].

Patients with a presumed primary cause for FSGS are empirically characterised as steroid responsive, relapsing/steroid-dependent, or steroid resistant based on the time-related reduction in proteinuria level observed after initiating treatment with corticosteroids and its duration after dose reduction or discontinuation. Although, in children, steroid resistance is defined as no response after 4-8 weeks of therapy with high-dose corticosteroids, no consensus exists in adults. A potentially useful biomarker for predicting overall responsiveness to therapy clinically is a modest reduction (>20% from baseline) in proteinuria level after 8 weeks of treatment. Glassock and Fervenza suggested that evaluating the

histological subtypes of FSGS is of little help in phenotyping a patient with presumed primary FSGS and is of no prognostic benefit in adult patients having steroid resistance after 8 weeks of treatment[27].

Patients with secondary FSGS, except those due to genetic forms, receive a conservative approach to therapy, including lifestyle modification in the form of maximal blood pressure (BP) control with the use of angiotensin-receptor blockers, dietary sodium restriction, moderate protein diets, lipid control with the use of a statin, smoking cessation, weight control, and avoidance of nephrotoxic medications[1,3,7,24]. In these patients, this treatment results in a reduction in proteinuria to less than 1g/day, slowing down disease progression depending on the extent of established renal damage[1,3]. Treatment of secondary FSGS therefore includes therapy directed at the cause[3]. Immunosuppression should not be used in adults with FSGS of undetermined cause or in those with secondary FSGS[20]. Unnecessarily administering steroids to these patients would expose them to the side effects generally associated with these medications and suppress their immunity, a potentially hazardous situation in an already immunocompromised population with a high prevalence of HIV and tuberculosis.

Most of the genetic forms of FSGS appear during childhood and are mainly associated with corticosteroid and immunosuppressive therapy resistance[1,4,5]. It may be clinically aggressive with overall progression to ESRD[1]. Genetic screening is recommended in early-onset disease resistant to immunosuppressive therapy, syndromic, and familial disease, but has not been recommended in sporadic adolescence or adult-onset FSGS that does not have clinical evidence of a genetic syndrome. Response to first-line corticosteroid treatment can be taken as an initial confirmation of the diagnosis of primary FSGS and precludes the need for genetic analysis[4]. Approximately 70% of genetic cases of corticosteroid-resistant NS are due to mutations in the nephrin (NPHS-1), Wilms tumour 1 (WT-1), and podocin (NPHS-2) genes. Whole-exome sequencing has found >30 genes related to steroid resistance in NS. A positive genetic test will focus the therapy on an antiproteinuric and symptomatic treatment, avoiding immunosuppressive therapy exposure[1]. Some genetic forms respond to calcineurin inhibitors[3].

Patients with a worse outcome include those of African race, increased rates of proteinuria, and renal insufficiency[3]. Remission rates at 5 years after initiation of immunosuppressive therapy was significantly higher in patients with primary FSGS compared to patients with secondary FSGS[11].

According to the KDIGO guidelines, patients with MCD are recommended to receive high-dose oral glucocorticoids for initial therapy for up to 16 weeks. If a contraindication to glucocorticoids exists, then an immunological agent is recommended[20]. This treatment is the same as for primary FSGS.

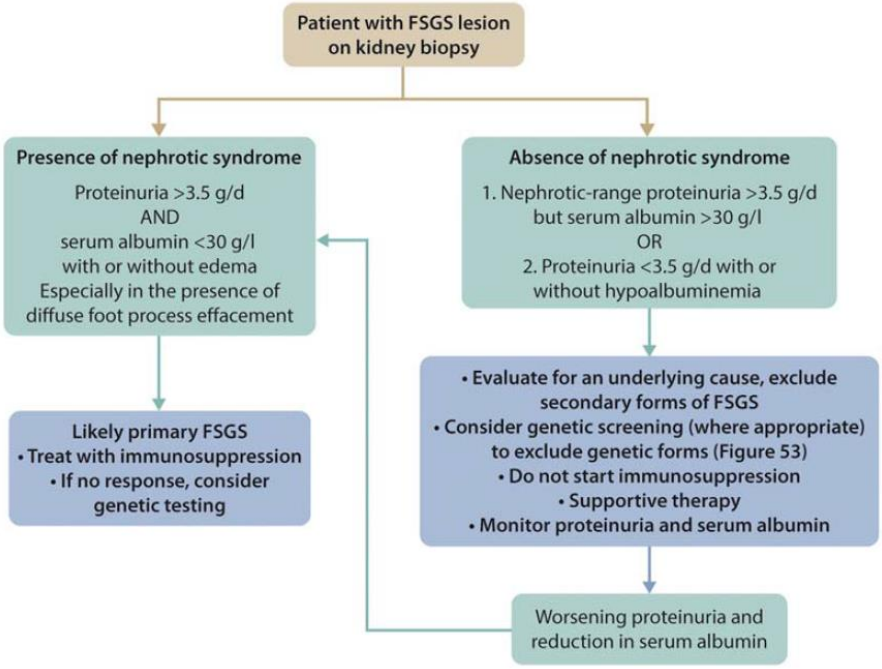


Figure 9: Evaluation of a patient with FSGS on kidney biopsy and no evidence of other glomerular pathology[20].

1.5. PROBLEM STATEMENT

The process to accurately determine whether FSGS is primary or secondary is lengthy, requiring clinical history, serology, LM morphological features, EM measurements, and retrospective review of response to treatment regimens. The importance of determining the type of FSGS lesion present is that it impacts on patient management. More recently, authors have looked at using EM to morphometrically determine whether it can be used as an adjunct to differentiate primary from secondary FSGS. As a result, this additional tool would assist clinicians in distinguishing between the two processes with more confidence, optimising treatment at an earlier time, removing the sequelae associated with unnecessary and potentially dangerous treatment regimens, and saving on healthcare costs.

By proving the notion that EM measurements of podocyte FPW can be used as an important adjunct to diagnose the type of FSGS lesion present, valuable time, medical costs, and potentially dangerous treatment sequelae may be kept to a minimum.

The current study will assess whether FPW measurements in FSGS obtained in patient cohorts in developed countries can be applied to our local population who are genetically distinct and may have different communicable and non-communicable disease profiles. To our knowledge, this is the first study addressing this question.

In patients with frank renal-related NS, diffuse FPE is most likely to occur because of global podocyte damage. However, FPW measurement may be of particular benefit in the following scenarios:

1. Patients with primary podocytopathy who do not yet fulfil the KDIGO criteria for NS and may have features generally associated with secondary FSGS (e.g. obesity, hypertension, etc.)
2. Patients with non-renal pathology that manifest with NS. These may include hypoalbuminaemic states from malnutrition, chronic liver disease, protein losing enteropathies, familial hypercholesterolaemia, etc. In the South African population this poses remarkable difficulties clinically as secondary FSGS patients presenting with NS due to one or more of the above causes are frequently encountered (Dr B. Davidson, Specialist Nephrologist, personal communication).

1.6. HYPOTHESIS

The null hypothesis (H_O) = statistically significant difference in EM FPW measurements does not exist between primary and secondary forms of FSGS in our local cohort.

The alternative hypothesis (H_A) = a statistically significant difference in EM FPW measurements between primary and secondary forms of FSGS exists in our cohort.

1.7. AIMS AND OBJECTIVES OF THE CURRENT STUDY

- To use EM morphometric techniques to measure podocyte FPWs in renal biopsies of adults with a histological diagnosis of FSGS (and MCD) over a five-year period, from 2019 to 2023.
- To analyse and correlate the FPWs with all relevant clinical and laboratory information.
- To obtain a reference range for FPW to differentiate primary from secondary FSGS in our local cohort.
- To perform a retrospective audit of adult patients treated for FSGS over the period stated above.
- To correlate our study findings with those of previously published international studies.
- To create an online database for the adult patients with FSGS treated at the Division of Nephrology and Hypertension at GSH.

2. CHAPTER TWO - MATERIALS AND METHODS

2.1. ETHICS APPROVAL

This study was reviewed and approved by the Departmental Research Committee (DRC). The study was further approved by the Human Research Ethics Committee (HREC).

HREC study number: 832/2020.

2.2. STUDY DESIGN

This was a retrospective cohort study.

2.3. INCLUSION AND EXCLUSION CRITERIA

2.3.1. INCLUSION CRITERIA

- All patients 18 years of age and older with a histological diagnosis of FSGS on an initial renal biopsy performed between 2019 and 2023 by the Division of Nephrology and Hypertension at GSH and Livingstone Hospital in Port Elizabeth.
- All patients 18 years of age and older with a histological diagnosis of MCD on an initial renal biopsy performed between 2019 and 2023 by the Division of Nephrology and Hypertension at GSH and Livingstone Hospital in Port Elizabeth.
- All cases with sufficient tissue (at least one non-sclerosed glomerulus) for EM assessment.

2.3.2. EXCLUSION CRITERIA

- Patients under the age of 18 years with a histological diagnosis of FSGS or MCD.
- Cases whose tissue blocks could not be salvaged for EM image acquisition.
- Cases with no glomeruli on toluidine blue stained slides.
- Cases with inadequate tissue or fat only on toluidine blue stained slides.
- Patients with other primary glomerular diseases leading to diffuse FPE, e.g. diabetes mellitus[10].

- EM performed on ex-wax specimens. In general, ex-wax processing of renal biopsies for EM results in poor preservation of podocyte foot processes, thereby possibly rendering measurements inaccurate.

Control samples included cases diagnosed with only tubulointerstitial pathology (e.g. acute tubular injury). Our study had 3 controls out of the total sample size of 35, 2 of which were biopsies from patients at Livingstone Hospital and 1 at GSH.

2.4. CASE SELECTION

All biopsies performed for any indication by the Division of Nephrology and Hypertension at GSH is recorded on a form and safely kept in a renal biopsy file (organised by year) within the Division itself. The information recorded on these forms includes clinical history, blood results, urine results, the histologic diagnosis, and the treatment provided.

The renal biopsy files for the above stated years were perused. All the cases with a histological diagnosis of FSGS (and MCD) were noted and the information used to populate a Microsoft Excel spreadsheet, the initiation of the database for this study. Some biopsy forms did not contain all the required information. For these specific cases, the relevant clinical blood and urine results and histological diagnoses were retrieved from the National Health Laboratory Service (NHLS) TrakCare Lab Results system and the database subsequently updated.

Initially, the data was captured in an Excel spreadsheet and then transferred to a password protected REDCap database.

Once all the relevant renal biopsy files were perused and the data captured, all the renal biopsy cases signed out by the Division of Anatomical Pathology at GSH during the above-mentioned period were reviewed. This was done to ensure the largest possible sample size in our cohort, to include all the relevant cases referred from Livingstone Hospital, and to decide which cases to use for the controls in our study. The database was then updated.

The inclusion and exclusion criteria were then applied. From an initial sample size of 75 cases, a final sample size of 35 cases (of which 3 were controls) was reached, as outlined below (Figure 10). We first determined whether each case had resin blocks, toluidine blue stained slides, and/or grids to perform

EM. Those cases without blocks, those without glomeruli on toluidine blue stained slides, and those without at least one normal glomerulus on toluidine blue stained slides, were excluded. If a case had no toluidine blue stained slide, the resin block would be cut, and a toluidine blue stained slide made to assess for the presence of at least one normal glomerulus. If adequate, a grid would then be created. Once all the cases had grids, EM images were taken. For the assessment of FPE, only normal appearing glomeruli were assessed ultrastructurally as this allows for distinction of primary from secondary causes.

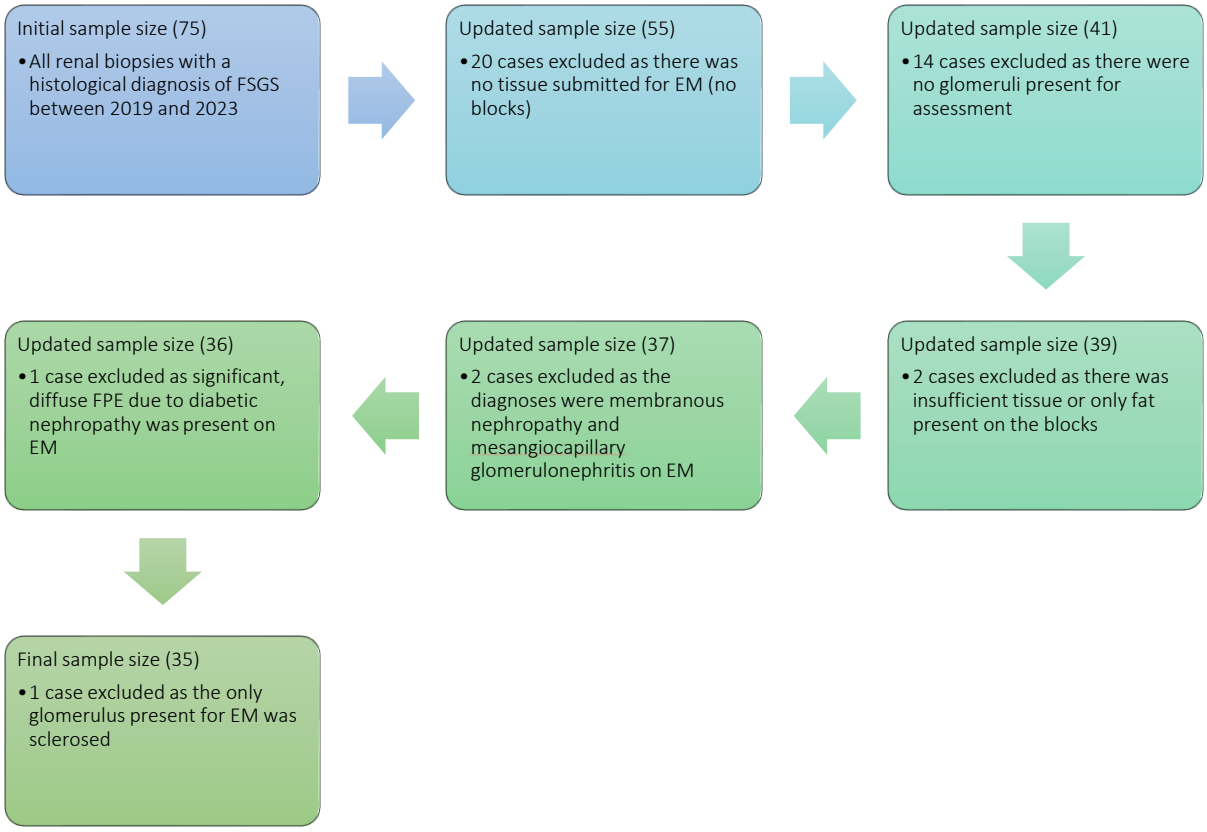


Figure 10: Flow diagram of case selection process.

2.5. COLLECTION OF PATIENT CLINICAL AND LABORATORY DATA

The patient clinical information included on the REDCap online FSGS database for the Division of Nephrology and Hypertension at GSH is listed below:

- Folder number
- Date of birth
- Gender
- Medical history:
    - Hypertension
    - Underlying renal dysfunction and the cause thereof
    - On dialysis
    - Previously diagnosed with an autoimmune disorder
    - Diabetes mellitus
    - Primary dyslipidaemia
    - HIV status
    - Illicit drug use
    - Alcohol use
    - Over the counter analgesic use
    - Smoking
- Clinical examination and side investigation findings:
    - Peripheral oedema
    - Blood pressure
    - Urine dipstick
    - Preliminary clinical diagnosis
    - Indication for biopsy
- Laboratory tests:
    - Urine PCR
    - Cholesterol
    - Albumin
    - HIV ELISA
    - VDRL
    - RPR
    - Hepatitis B surface antigen
    - Hepatitis C virus

- ANA
- Anti-dsDNA
- ASOT
- Anti-DNAse B
- pANCA
- cANCA
- C3
- C4

- Initial biopsy:
  - Date of biopsy
  - Episode number
  - Accession number
  - MRN number
  - Initial histologic diagnosis
- Electron Microscopy:
  - GBM length
  - Number of foot processes
  - Calculated FPW
  - FPE (focal or diffuse)
  - Final pathologic diagnosis
- Treatment and follow-up:
  - Immunosuppressive therapy given?
    - Duration of treatment
    - Response to treatment?
    - Clinical resolution?
  - Lost to follow-up?
  - Defaulted therapy?
  - Oedema at follow-up?
  - BP at follow-up
  - Urine dipstick at follow-up
  - RAAS antagonist given?

2.5.1. DEFINITION OF NS

We used the 2021 KDIGO guidelines definition of NS to categorise all the study cases as nephrotic (NS) or non-nephrotic (NNS). The presence of proteinuria >3.5g/day (uPCR >0.35g/mmol) and serum albumin <30g/L would label a case as nephrotic. If nephrotic-range proteinuria >3.5g/day was present, but the serum albumin was >30g/L the case would be labelled as non-nephrotic. If proteinuria <3.5g/day was present with or without serum albumin <30g/L the case would be labelled as non-nephrotic[20].

2.5.2. eGFR EQUATION CHOICE

Two equations are used for the calculation of the eGFR: the CKD-EPI equation and the MDRD equation. The CKD-EPI equation has been shown to be more accurate than the MDRD equation at an eGFR >60mL/min/1.73m^2 and is preferred for patients with African ancestry (Dr B. Davidson, Specialist Nephrologist, personal communication). It is for this reason that the eGFR CKD-EPI equation results are displayed and discussed.

Dr R. Freercks from Livingstone Hospital supplied the treatment details of the patients treated as primary FSGS/NS. Individual patient files in the Division of Nephrology and Hypertension at GSH were used to obtain the treatment details of the patients treated as primary FSGS/NS. This information included: if a steroid was given; if an immunological agent was added; whether a response was obtained, or steroid resistance prevailed; whether remission was achieved; and whether any relapses occurred at follow-up.

2.5.3. CLASSIFICATION OF CASES

We initially tried to categorise each case as primary FSGS, secondary FSGS or MCD but found it difficult in some cases to make this distinction based on the clinical information available. Some of these difficult cases included those patients presenting with a mixed nephrotic/non-nephrotic picture and those with primary FSGS versus MCD. We then decided that it would be simpler, and the results cleaner, if we divided the cases into those presenting with nephrotic syndrome and those without. Indeed, this was done in the studies by Spanu *et al.* and Sethi *et al.*[21,25]. Patients with NS would inevitably be those with a podocytopathy (primary FSGS and/or MCD) and those without NS would equate to the secondary FSGS group (NNS). Notably, making the distinction between primary FSGS and MCD does not impact

management as they are treated the same. The importance becomes paramount when distinguishing primary FSGS from secondary FSGS (or rather NS from NNS). The analysis commenced after all the cases were categorised according to the above algorithm.

2.6. ULTRSTRUCTURAL MORPHOMETRY BY ELECTRON MICROSCOPY

A JEOL JEM-1011 transmission electron microscope with a currently installed Olympus iTEM imaging platform was used for this study. The details of the imaging software are as follows: Olympus Soft Imaging Solutions GmbH iTEM 5.2 (Build 3554). The Microsoft Windows NT 6.1 (Build 7601) Service Pack 1 is currently installed on the EM computer.

Most cases had an average of 1-2 glomeruli on the grids for EM assessment. Areas in non-sclerosed glomeruli were identified and between 2-5 images taken of the GBM with the adjacent foot processes for each case. Images were taken at 8000x standard magnification across all cases.

At least two different areas in a glomerulus were imaged per case. After an image was obtained, a polyline ruler was used to draw along the lamina densa of an appropriate length of the GBM until approximately 100µm was measured. This value was chosen as the study performed by Spanu *et al.* used a minimum of 100µm of GBM[25]. The EM program calculates the length of the GBM drawn/highlighted (in nm), annotates the images with the polyline drawn, inserts a scale bar for the specified magnification, and allows the image files to be renamed, saved, and stored on the EM computer. Each case was labelled according to the specific biopsy ES number.

The number of foot processes were counted manually per highlighted area of GBM. A foot process was distinguished from the next by a definitive slit/space between them (see Figure 6). This data was captured on a Microsoft Excel spreadsheet. Captured data included: the total length of GBM measured per case; the total number of foot processes counted in the highlighted GBM lengths; and whether FPE was focal or diffuse. A column for the FPW, calculated using the Gunderson method, was added. Thereafter, a definitive statistical analysis of the EM data was performed.

FPE was categorised as focal or diffuse based on the amount of effacement visualised on EM. As per the studies conducted by Jacobs-Cacha *et al.,* Kfoury, and Ishizuka *et al.,* we used a FPE value of 80% or more of the GBM podocyte surface imaged to differentiate diffuse from focal (<80%) effacement[1,7,13].

2.7. STATISTICAL ANALYSIS

2.7.1. CONTINUOUS VARIABLES

These included: age, systolic blood pressure (SBP), diastolic blood pressure (DBP), eGFR, creatinine, uPCR, albumin, cholesterol, and FPW.

2.7.2. CATEGORICAL VARIABLES

These included: gender, NS group, NNS group, hypertension, diabetes mellitus, history of dyslipidaemia, HIV status, oedema, and FPE (focal vs diffuse).

2.7.3. STATISTICAL SOFTWARE AND TESTS OF SIGNIFICANCE

All statistical analyses were performed by Dr N. Ikumi using STATA (version 18.0, Stata Corporation, College Station, Texas, USA) and R (version 4.2.1, R Core Team, Vienna, Austria). The analyses were stratified by two clinical groups, NS and NNS. The proportions described in the study were reported as medians with interquartile ranges instead of variance as the data showed outliers and was not normally distributed. The differences between the groups were compared using Chi-squared/Fisher's exact test and Wilcoxon rank-sum tests. The associations between FPW and all collected continuous data (including uPCR, cholesterol, creatinine, eGFR, and albumin) were assessed using simple linear regression and statistical analysis was performed using the Spearman rank test. A p-value of <0.05 was considered statistically significant.

The results are displayed in different formats. An initial data table expresses the means and medians of the evaluated variables between the different groups with their associated p values (a simple format to view the significance of the data; see Table 4). Additionally, box and whisker plots, graphs, and a bar chart for each of the variables are displayed to view trends in the data. Importantly, the data table (Table 4) shows the results for the variables on their own. The figures (box and whisker plots and graphs) display those variables with relation to FPW.

2.7.4. LOGISTIC REGRESSION

A logistic regression analysis was performed to establish a FPW threshold above which a primary podocytopathy (NS diagnosis) could be predicted. Threshold probabilities of 50% and 80% were used.

We employed the '*glm ()*' function to fit the logistic regression model and the predicted probabilities from the logistic regression model were visualised using the '*ggplot2*' package, both available in R (version 4.2.1).

2.7.5. UNSUPERVISED HIERARCHICAL CLUSTER ANALYSIS (HCA)

An exploratory analysis of all collected continuous data was performed to establish whether clinically relevant groups (NS and NNS) could be grouped in an unsupervised fashion. Based on the agglomerative coefficient, the Ward's minimum variance method was used for the final clustering. We used the *'cluster'* package for the cluster analysis. The '*factorextra*' package was used to visualise the results which were displayed as a dendrogram. Both packages are available in R (version 4.2.1).

We performed the unsupervised HCA to determine whether the identified clusters had any significant differences between them which may explain treatment response and prognosis (specifically, steroid responsiveness and remission). The model included the following parameters: FPW, uPCR, cholesterol, creatinine, and albumin. 28 cases were included in the model. 7 cases were excluded as follows: controls (n=3) and 4 cases for missing values in either uPCR, cholesterol, creatinine, or albumin.

3. CHAPTER THREE - RESULTS

3.1. PATIENT DEMOGRAPHICS, CLINICAL AND LABORATORY VALUES

The collected data is summarised in Table 4. The NS and NNS groups were defined as per the KDIGO guidelines mentioned previously.

Table 4: Demographic, clinical, and laboratory data of NS and NNS cases (statistically significant values highlighted in bold with an asterisk).

		Total (n=32)	Nephrotic (n=23)	Non-nephrotic (n=9)	p value
Age (years)					0.8
	0 – 19	1 (3.1)	1 (4.4)	0 (0)	
	20 – 29	11 (34.4)	9 (39.1)	2 (22.2)	
	30 – 39	11 (34.4)	7 (30.4)	4 (44.4)	
	40 – 49	5 (15.6)	4 (17.4)	1 (11.1)	
	50 – 59	4 (12.5)	2 (8.7)	2 (22.2)	
	Mean (SD)	35.7 (10.7)	34 (10)	39.8 (11.8)	
	Median (IQR)	34 (27 – 44.5)	34 (26 – 43)	37 (31 – 48)	
Gender					0.4
	Female	16 (50.0)	10 (43.5)	6 (66.7)	
	Male	16 (50.0)	13 (56.5)	3 (33.3)	
HIV					0.6
	Negative	25 (78.1)	17 (73.9)	8 (88.9)	
	Positive	7 (21.9)	6 (26.1)	1 (11.1)	
Hypertension					0.7
	Yes	15 (46.9)	10 (43.5)	5 (55.6)	
	No	17 (53.1)	13 (56.5)	4 (44.4)	
History of dyslipidaemia					0.3
	Yes	5 (15.6)	5 (21.7)	0 (0)	
	No	27 (84.4)	18 (78.3)	9 (100)	

SBP (mmHg)					0.5
	Mean (SD)	138.3 (22.9)	135.1 (17.4)	145.8 (32.2)	
	Median (IQR)	135 (120 – 148)	132.5 (120 – 140)	141.5 (120 – 171.5)	
DBP (mmHg)					0.6
	Mean (SD)	85.2 (13.2)	84 (12.2)	88 (15.7)	
	Median (IQR)	82.5 (75 – 94)	80 (75 – 90)	85.5 (75 – 99.5)	
Oedema					**0.006***
	Yes	23 (71.9)	20 (87.0)	3 (33.3)	
	No	9 (28.1)	3 (13.0)	6 (66.7)	
eGFR (ml/min/1.73m^2)					0.4
	Min-Max	4 - 129	4 - 129	12 - 106	
	Mean (SD)	70.6 (46.2)	66.0 (46.2)	83.6 (46.7)	
	Median (IQR)	79 (20 – 122)	65 (19 – 120)	102 (39.5 – 122.5)	
Creatinine (µmol/L)					0.3
	Min-Max	44 – 1071	54 - 1071	44 – 624	
	Mean (SD)	226.7 (255.1)	238.6 (270.3)	196.2 (222.9)	
	Median (IQR)	102.5 (67 – 314.5)	103 (74 – 362)	73 (59 – 229)	
Albumin (g/L)					**0.0002***
	Min-Max	8 - 47	8 – 24	25 - 47	
	Mean (SD)	20.4 (8.9)	16.3 (4.8)	32.4 (6.9)	
	Median (IQR)	18 (14 – 25)	17 (13 – 20)	32.5 (27 – 34)	
uPCR (g/mmol)					**0.0005***
	Min-Max	0.1 – 2.3	0.3 – 2.3	0.1 – 1.0	

	Mean (SD)	0.9 (0.6)	1.1 (0.5)	0.4 (0.3)	
	Median (IQR)	0.9 (0.5 – 1.3)	1.2 (0.7 – 1.3)	0.4 (0.3 – 0.6)	
Cholesterol (mmol/L)					0.003*
	Min-Max	1.7 – 21.1	3.5 – 21.1	1.7 – 8.4	
	Mean (SD)	10.0 (5.7)	12.0 (5.2)	4.5 (1.9)	
	Median (IQR)	9.0 (4.3 – 14.3)	12.9 (8.5 – 14.6)	4.3 (3.8 – 4.8)	
FPW (nm)					0.003*
	Min-Max	406.1 - 10236.16	588.9 - 10236.2	406.1 – 3256.9	
	Mean (SD)	3029.1 (2337.8)	3623.5 (2462.2)	1510.2 (946.6)	
	Median (IQR)	2592.3 (1523 – 3304)	3075.8 (2012.2 – 4297.8)	1321.5 (865.0 – 1680.3)	
FPE					0.003*
	Focal	11 (34.4)	4 (17.4)	7 (77.8)	
	Diffuse	21 (65.6)	19 (82.6)	2 (22.2)	

Statistically significant differences were obtained for oedema, albumin, uPCR, cholesterol, FPW, and FPE.

Non-significant differences were found in age, gender, HIV status, hypertension, history of dyslipidaemia, SBP, DBP, eGFR, and creatinine.

3.2. PARAMETERS WITH STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN NS AND NNS

Box and whisker plots (Figure 11: A-D) were created to show data distribution in parameters that had significant differences between NS and NNS cases.

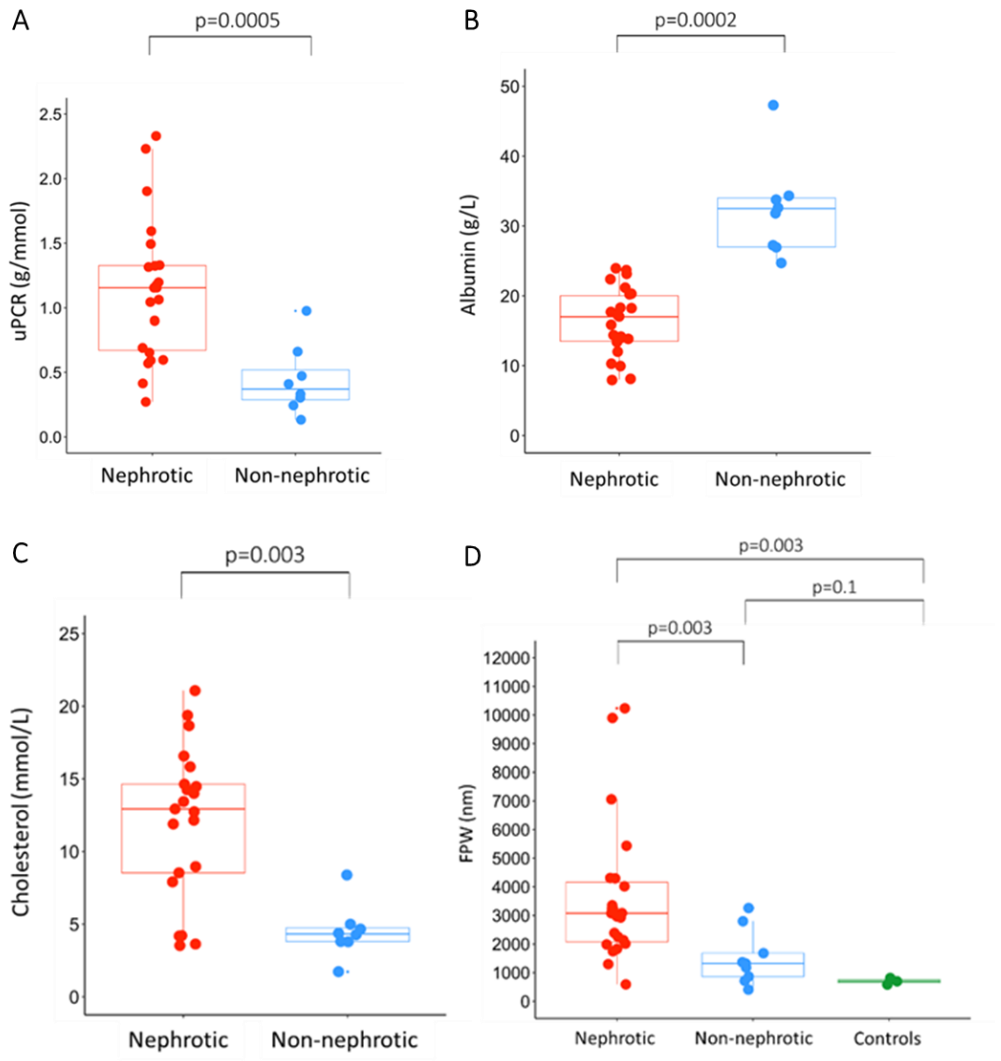


Figure 11: Comparison of NS and NNS groups, bar and whisker plots. A) uPCR; B) Albumin; C) Cholesterol; D) FPW.

Figure 11A shows a wide distribution of uPCR values in the NS group compared to the NNS group, with higher median and IQRs. The maximum uPCR value in the NNS group is lower than the median of the NS group.

Figure 11B demonstrates that the albumin data is more closely distributed in the NS group compared to the NNS group, with notably lower median and IQRs.

Figure 11C (cholesterol) illustrates a similar data distribution to Figure 11A (uPCR).

In Figure 11D, a wide distribution of FPW values in the NS group exists compared to the NNS group, with higher median and IQRs. These results illustrate a similar data distribution to Figure 11A and C, except that in Figure 11D, the maximum FPW value in the NNS group is higher than the NS group median.

3.3. RELATIONSHIP OF FPW WITH OTHER MEASURED PARAMETERS

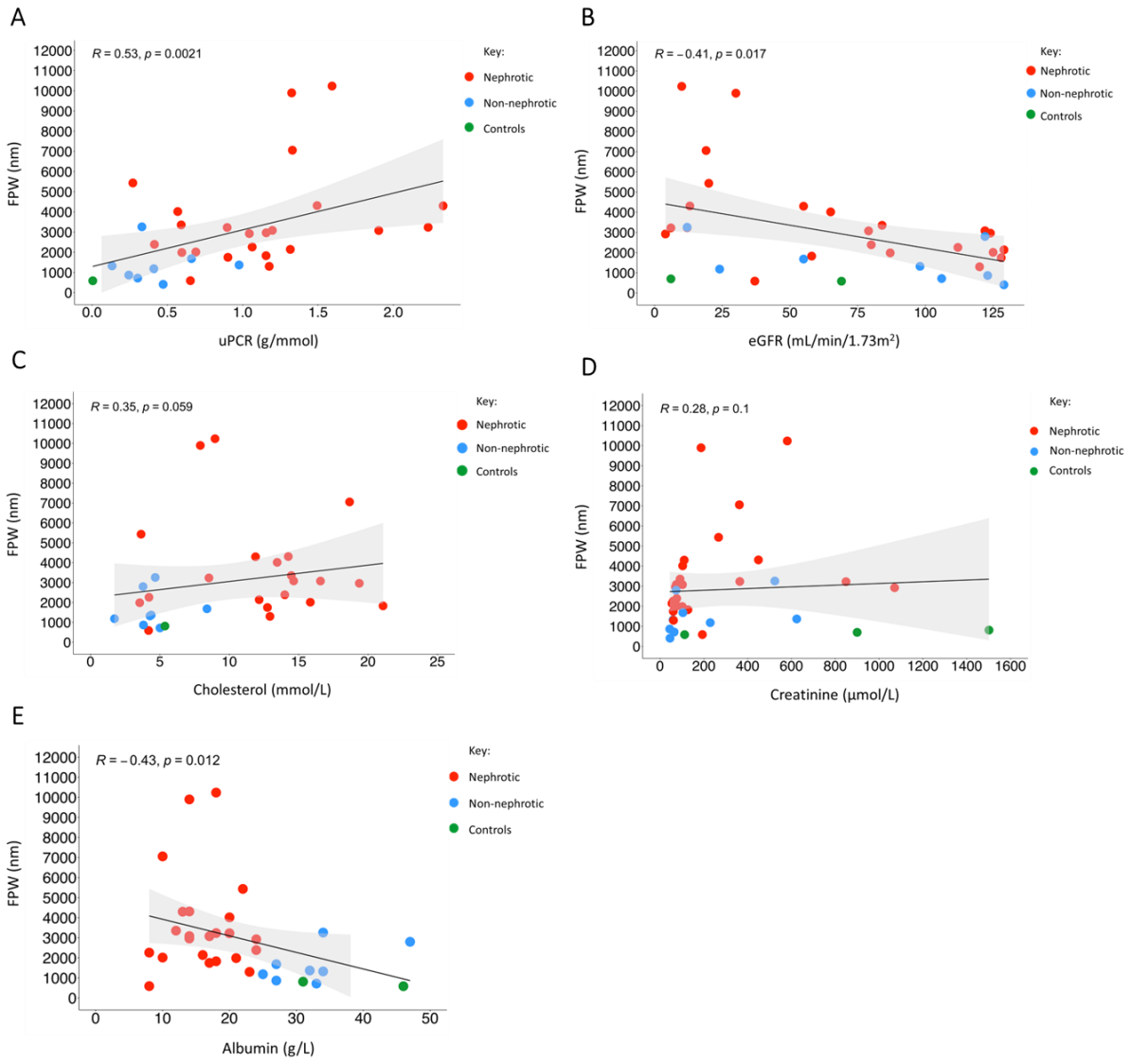


Figure 12: Linear regression scatter plots of FPW against varying parameters for NS, NNS, and controls. A) uPCR; B) eGFR; C) Cholesterol; D) Creatinine; E) Albumin.

Figure 12A shows a mostly linear relationship between the FPW and uPCR up until a uPCR of 1.3g/mmol. Some overlap exists between nephrotic and non-nephrotic cases at uPCR values of 0.3-1g/mmol. At uPCR values >1.3g/mmol, the FPW becomes more variable in distribution.

Figure 12B illustrates the scatter plot of eGFR against FPW with a mostly linear relationship at normal eGFRs. However, the relationship becomes non-linear with more variability in FPW at low eGFR (<50mL/min/1.73m^2).

Figure 12C demonstrates a mostly linear relationship between the cholesterol and the FPW, bar two conspicuous outliers with very high FPWs (discussed later). Interestingly, a few NS cases had normal cholesterol results.

There is no obvious linear relationship between the creatinine and FPW among the two groups, as shown in Figure 12D. Although most patients had normal creatinine results at biopsy with closely grouped FPW scatter points, the FPW values become variable at abnormally high creatinine (>180µmol/L).

Figure 12E illustrates a clear distinction between the NS cases with lower albumin values and the NNS cases with higher, more normal albumin values. A linear relationship exists between the FPW and albumin at values >20g/L, the data becoming more variable at lower values. The trend between FPW and albumin for the NNS cases appears unaffected.

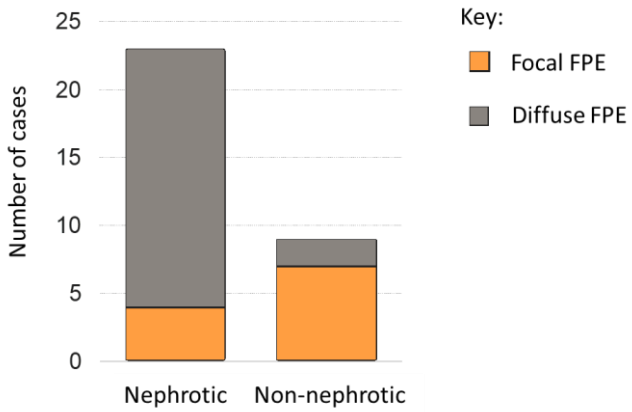


Figure 13: Bar chart displaying the number of cases with focal and diffuse FPE among the two study groups.

Figure 13 illustrates that not all NS cases have diffuse FPE and not all NNS cases have focal FPE. Each discrepant case was reviewed separately and independently, and the discrepancies are discussed later (see Chapter 4: Discussion).

3.4. LOGISTIC REGRESSION

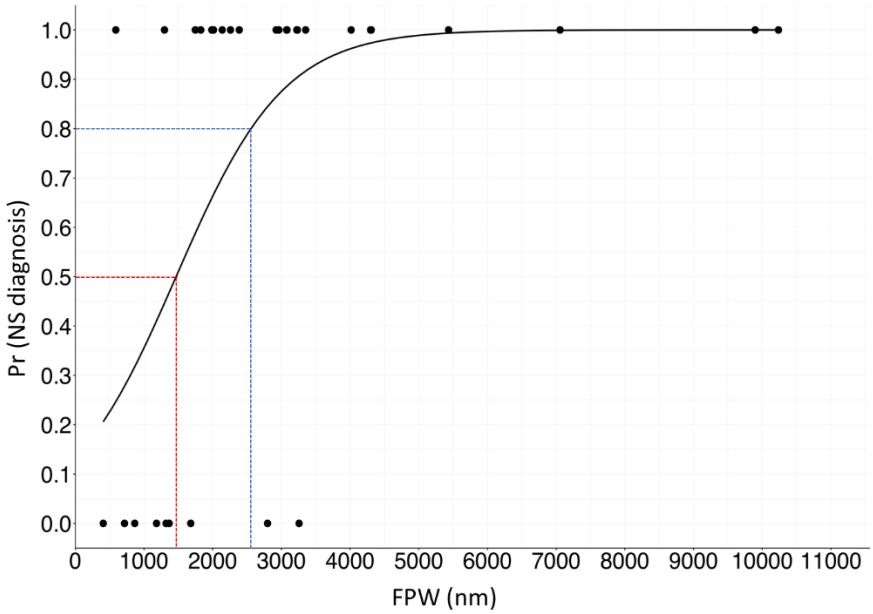


Figure 14: Logistic regression of FPW versus the probability ratio of a definitive NS diagnosis.

We performed a logistic regression of FPW versus the probability of NS to calculate what the threshold value/cutoff in our population is, and to determine whether there is a difference between our and the internationally used threshold of 1500nm used to differentiate primary from secondary FSGS. Figure 14 illustrates that a Pr of 0.5 (50% probability of a NS diagnosis) correlates to a FPW of 1450nm. A Pr of 0.8 (80% probability of a NS diagnosis) correlates to a FPW of 2550nm.

3.5. UNSUPERVISED HCA

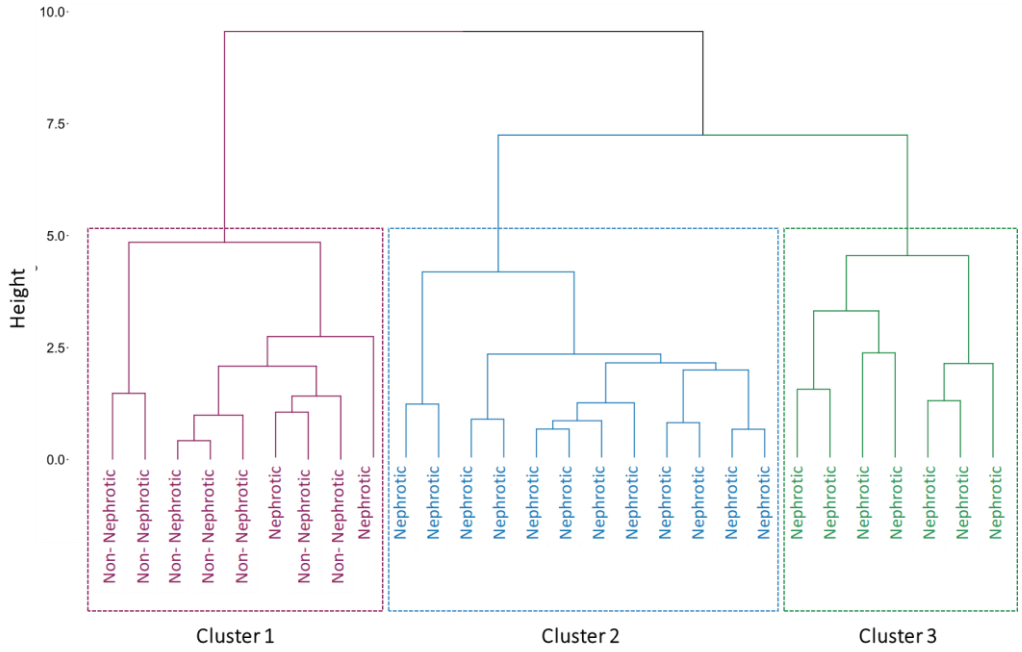


Figure 15: Cluster dendrogram of 28 cases using FPW, uPCR, cholesterol, creatinine, and albumin.

Figure 15 illustrates the cluster dendrogram that resulted from an unsupervised HCA of our data. Three clusters were chosen as it appears that the NS group has two distinct populations (Clusters 2 and 3). Two NS cases clustered with the NNS group (Cluster 1). These differences are discussed in Chapter 4: Discussion.

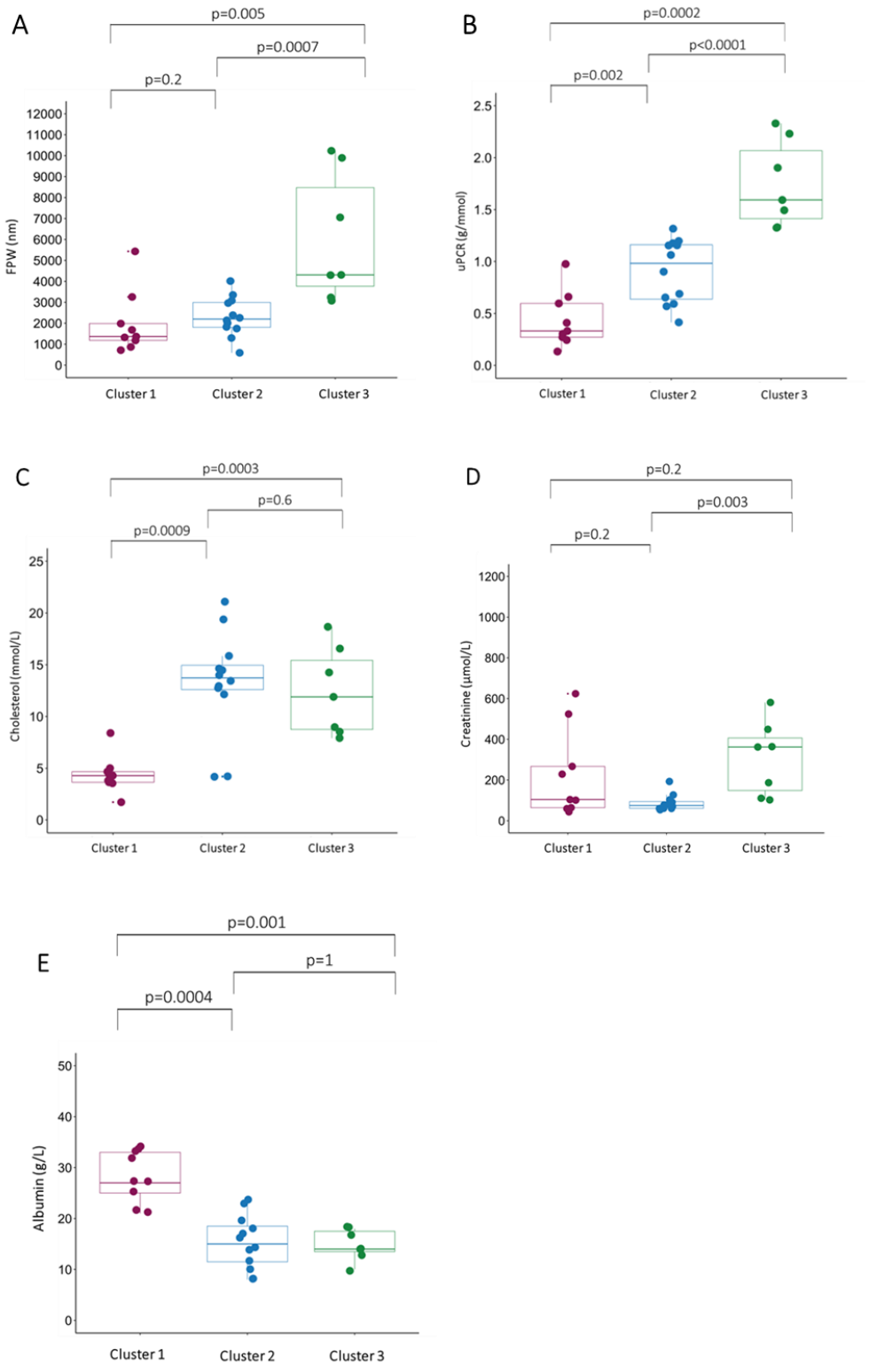


Figure 16: Box and whisker plots of the five variables used in the HCA model between the 3 clusters. A) FPW; B) uPCR; C) Cholesterol; D) Creatinine; E) Albumin.

Figure 16 illustrates the box and whisker plots for the three clusters regarding the different variables analysed in the unsupervised HCA.

Similarities between the clusters include:

1. FPW between Clusters 1 and 2 (Figure 16A).
2. Cholesterol between Clusters 2 and 3 (Figure 16C).
3. Creatinine between Clusters 1 and 2, and Clusters 1 and 3 (Figure 16D).
4. Albumin between Clusters 2 and 3 (Figure 16E).

Differences between the clusters include:

1. FPW between Clusters 1 and 3, and Clusters 2 and 3 (Figure 16A).
2. uPCR between Clusters 1 and 2, Clusters 2 and 3, and Clusters 1 and 3 (Figure 16B).
3. Cholesterol between Clusters 1 and 2, and Clusters 1 and 3 (Figure 16C).
4. Creatinine between Clusters 2 and 3 (Figure 16D).
5. Albumin between Clusters 1 and 2, and Clusters 1 and 3 (Figure 16E).

In summary, Cluster 1 has the lowest FPW, uPCR, and cholesterol, and the highest albumin among all the clusters. Cluster 2 has a FPW and uPCR between Clusters 1 and 3; has a higher cholesterol than Cluster 1; and a lower albumin than Cluster 1. Cluster 3 has the highest FPW and uPCR among all the clusters, with similar cholesterol and albumin to Cluster 2.

4. CHAPTER FOUR - DISCUSSION

4.1. CASE DEMOGRAPHICS, CLINICAL AND LABORATORY PARAMETERS IN NS AND NNS CASES

Previously described differences in NS and NNS from various published sources are summarised in Table 5. The structure of Table 5 is similar to that of the parameters assessed in this study (Table 4; see Chapter 3: Results).

Table 5: Results from previously published FSGS studies.

Variable and Study		FSGS overall	Nephrotic	Non-nephrotic	p value
Sample size					
	Sethi *et al.*[21]	41	18	23	
	Hommos *et al.*[14]	38	11	27	
	Deegens *et al.*[11]	29[a]	17	7	
	Spanu *et al.*[25]	37	17	20	
	Taneda *et al.*[10]	43			
	Kfoury *et al.*[7]	33	17	16	
	Ishizuka *et al.*[13]	20	9	3[b]	
	da Silva *et al.*[12]	22			
Age (years)[c]					
	Sethi *et al.*		46.8	56	
	Hommos *et al.*		53	53	
	Deegens *et al.*		52	54	0.57
	Spanu *et al.*		42	36	0.1
	Taneda *et al.*	38.8			
	Ishizuka *et al.*[d]		4.2	5.4	
	da Silva *et al.*	35.1			
Gender[e]					
	Sethi *et al.*		77.8	70	
	Hommos *et al.*		55	56	

	Deegens *et al.*		70.6	57.1	0.68
	Spanu *et al.*		52.9	40	
	Taneda *et al.*	53.5			
	Kfoury *et al.*	63.6	58.8		
	Ishizuka *et al.*	75	77.8	66.7	
	da Silva *et al.*	59.1			
Oedema					
	Ishizuka *et al.*		100	0	
Diabetes mellitus[f]					
	Hommos *et al.*		0	26	0.08
History of dyslipidaemia[f]					
	Hommos *et al.*		36	48	0.72
Hypertension[f]					
	Hommos *et al.*		73	85	0.39
	Deegens *et al.*		72	86	0.28
	da Silva *et al.*	72.7			
SBP (mmHg)[c]					
	Sethi *et al.*		137	125.4	0.04
	Hommos *et al.*		132	137	0.62
DBP (mmHg)[c]					
	Sethi *et al.*		82.2	73.2	0.04
	Hommos *et al.*		74	79	0.27
eGFR (mL/min/1.73m^2)[c]					

	Taneda *et al.*	53.4			
	da Silva *et al.*	72.9			
Proteinuria (g/day)[c]					
	Sethi *et al.*		18.8	4.0	0.01
	Hommos *et al.*		7.7	3.2	<0.001
	Deegens *et al.*		9.4	5.0	<0.01
	Spanu *et al.*		7.4	1.4	<0.001
	Taneda *et al.*	6.5			
	Ishizuka *et al.*[g]		9.8	1.0	
	da Silva *et al.*	6.3			
Cholesterol (mmol/L)[c]					
	Sethi *et al.*		20.4	11.7	0.001
	Hommos *et al.*		13.5	11.9	0.09
	Deegens *et al.*		11.1	6.8	<0.01
	Spanu *et al.*		19.9	10.9	0.01
Creatinine (µmol/L)[c]					
	Sethi *et al.*		169	146	0.3
	Hommos *et al.*[h]		124	124	0.83
	Deegens *et al.*		112	262	<0.01
	da Silva *et al.*	83.3			
Albumin (g/L)[c]					
	Sethi *et al.*		25	39	<0.0001
	Hommos *et al.*		33	40	<0.001

	Deegens *et al.*		21	37	<0.001
	Spanu *et al.*		21	36	<0.001
	Ishizuka *et al.*[i]		35	61	
	da Silva *et al.*[j]	2.6			
FPW (nm)[c]					
	Deegens *et al.*[h]		3236	1098	<0.001
	Spanu *et al.*		1196	422	<0.001
	Taneda *et al.*[k]	4004			<0.05
	Ishizuka *et al.*[h]		4504	1203	
	da Silva *et al.*	2503			
FPE[c]					
	Sethi *et al.*		96% (diffuse)	48% (segmental)	<0.0001
	Hommos *et al.*[h]		100%	30%	<0.001
	Spanu *et al.*		Diffuse	Segmental	
	Kfoury *et al.*		64.9% diffuse effacement	72.1% focal effacement	<0.0001
	Ishizuka *et al.*		88-100%	0-38%	

[a]5 cases were labeled as "FSGS secondary to maladaptive responses without identifiable secondary cause (possible secondary FSGS)". [b]Non-nephrotic refers to the maladaptive FSGS cohort (excluding the genetic FSGS cohort). [c]Results expressed as means. [d]This study focused on primary versus genetic FSGS. All results for this study are expressed as medians. [e]Results expressed as % male. [f]Results expressed as %. [g]Proteinuria is expressed as uPCR (g/g). [h]Results expressed as medians. [i]Results are for serum total protein and not albumin. [j]Results are expressed as mg/dL. [k]Control group mean FPW=747nm.

This study, although the sample size may be small, can be compared with similar international studies in developed countries because their sample sizes are similar, overall and between NS and NNS groups. The study with the largest sample size comparing primary and secondary FSGS is 41 (Sethi *et al.*)[21]. Note that the study by Taneda *et al.* with a larger sample size (43) compared FSGS overall to MCD[10] (Table 5).

There was no significant difference (p=0.8) regarding patient age and FSGS type in our study (Table 4). This parallels the results of other studies. Most of the patients in our study were younger than those in other studies. Of note, the study by Ishizuka *et al.* focused on primary versus genetic FSGS, hence the very young ages of their cohort (Table 5). The fact that our study had a slightly younger population is probably of no significance, as FSGS is known to have a variable age presentation.

FSGS is generally a male predominant pattern of injury[2]. There was no statistical difference between gender among the two groups in this study (p=0.4). Half of the patients in our study were male and the other half female. Males more often presented with NS (57%); however, of the non-nephrotic patients, 67% were female (Table 4). Our study findings are like those of Spanu *et al.*, where females predominated the NNS group. Our results are in keeping with international ones in that most patients presenting with primary FSGS are male (Table 5).

No statistical difference was found between the two groups regarding HIV status (p=0.6). The NNS group only had one patient with HIV (11%). The NS group had a relatively higher prevalence of HIV (26%; Table 4). This highlights a few noteworthy points:

1. If a patient has HIV, one cannot assume that the FSGS pattern of injury is secondary, e.g. HIVAN.
2. HIV infection does not equate to a direct cause for a podocytopathy. The results imply that other factors are responsible in causation. Indeed, HIV infection can suppress immunity to a degree that allows other factors to directly cause injury.
3. HIVAN is not synonymous with HIV-positive secondary FSGS. The term HIVAN should only be used in the correct context where the clinical and morphological findings are suggestive.

In our study, of the 7 HIV-positive patients, 3 were treated as primary FSGS and 4 as secondary FSGS (of which 2 were labelled as HIVAN).

There was no significant difference between the two groups regarding hypertension in this study (p=0.7). Approximately 47% of our total cohort had hypertension, of which 56% of cases were non-nephrotic and 44% were nephrotic. There was no significant difference between the SBP (p=0.5) and

DBP (p=0.6) of both groups (Table 4). da Silva *et al.* had a much higher prevalence of hypertension in their cohort (73%). The studies by Hommos *et al.* and Deegens *et al.* showed that the NNS group had a relatively higher, although statistically insignificant, percentage of hypertensive cases compared to the NS group (Table 5).

16% of our total cohort had a history of dyslipidaemia. Note that this variable refers to the history of having been diagnosed with hypercholesterolaemia previously and does not include those NS cases who would likely have high cholesterol on subsequent laboratory blood test evaluation. All the cases with a history of dyslipidaemia in our study presented with NS. These results can be explained by the pathophysiology of NS, where hypercholesterolaemia is commonly present. It is interesting to note that there were no dyslipidaemic patients in the NNS group. One would expect an abnormal lipid profile in at least a few cases of secondary FSGS attributable to the metabolic syndrome in the South African population. One cannot deduce compelling conclusions from these results as the p value (p=0.3) was statistically insignificant (Table 4). Hommos *et al.* showed that 36% of NS and 48% of NNS cases presented with dyslipidaemia (Table 5).

A statistically significant difference (p=0.006) was found in this study between the two groups regarding oedema at presentation. 72% of our total cohort presented with oedema, and 87% of the patients with NS had oedema at presentation (Table 4). This is expected as increased proteinuria is associated with a decline in serum albumin and resultant peripheral oedema. Indeed, oedema is not a criterion in the definition of NS according to the 2021 KDIGO guidelines; however, when oedema is present, it may assist in clinically differentiating primary from secondary FSGS[20]. In the study by Ishizuka *et al.*, all their NS patients presented with oedema, whereas the secondary FSGS cases did not have oedema. Having said this, the sample size for their secondary FSGS group was only 3 and thus significant assumptions regarding this cannot be made (Table 5).

One must discuss the 3 non-nephrotic patients who presented with oedema in this study (Table 4). One of the patients was a hypertensive with a BP of 168/94mmHg, a uPCR of 0.410g/mmol, a creatinine of 229µmol/L, an eGFR of 24mL/min/1.73m^2, and an albumin of 25g/L at biopsy. Another of the patients was a hypertensive with a BP of 196/105mmHg, a uPCR of 0.331g/mmol, a creatinine of 524µmol/L, an eGFR of 12mL/min/1.73m^2, and an albumin of 34g/L at biopsy. As can be inferred, both patients had uncontrolled hypertension, abnormally high creatinine, and abnormally low eGFR with evidence of chronic kidney disease. Indeed, oedema can result from the above. However, one must keep in mind non-renal causes for oedema. The third patient was a hypertensive with a BP of 175/114mmHg, a

creatinine of 73µmol/L, an eGFR of 122mL/min/1.73m^2, and an albumin of 47g/L at biopsy. The uncontrolled BP in this case was a likely cause for the oedema. This patient never had a uPCR performed but was treated clinically as primary FSGS with steroids and had a good clinical response with complete remission at follow-up (Dr R. Freercks, Specialist Nephrologist, personal communication).

There was no statistical difference in eGFR between the two groups in this study (p=0.4; Table 4). However, a statistically significant difference in the eGFR was present between the NS and NNS groups when correlated with FPW (p=0.017; Figure 12B). 2 controls had variable eGFR results, with one being normal (69mL/min/1.73m^2) and the other abnormally low (6mL/min/1.73m^2). 1 control did not have an eGFR result. The 23 nephrotic cases had variable eGFR values with 52% having a normal eGFR of >60mL/min/1.73m^2. 8 non-nephrotic cases had variable eGFR values, with 63% having a normal eGFR (Table 4). 1 non-nephrotic case did not have an eGFR result. Taneda *et al.* found a slightly reduced mean eGFR of 53mL/min/1.73m^2 in their FSGS cohort, while da Silva *et al.* found a normal eGFR of 73mL/min/1.73m^2 in theirs (Table 5). Our study showed similar results to da Silva *et al.* with a mean eGFR of 71mL/min/1.73m^2 (Table 4).

The variability in FPW seen at lower eGFR values in Figure 12B may be explained as follows: higher FPW values occur with increasing podocyte damage, which is associated with worsening renal function (a reduction in the eGFR). Caution should be applied in interpreting FPWs in patients with abnormally low eGFRs (chronic kidney disease). In essence, eGFR has a statistically significant correlation with FPW at presentation; however, no significant correlation exists regarding eGFR values alone between the two groups.

There was no statistical significance when comparing creatinine values between the nephrotic and non-nephrotic groups (p=0.3; Table 4). Although not statistically significant, the p value improved to p=0.1 when plotting the data against FPW (Figure 12D). The patients presenting with NS had higher median and mean creatinine values than the NNS group, although the overall medians were within normal reference ranges (Table 4). The 3 controls had variable creatinine results with two patients presenting in chronic kidney disease (112, 900, and 1502µmol/L, respectively). Caution should be applied when interpreting FPW in patients with creatinine >180µmol/L.

Our study findings are like those of others in that no statistical association between creatinine levels and FPW existed (Table 5). Interestingly, Deegens *et al.* found no statistically significant association between FPW and creatinine levels; however, a significant p value of p<0.01 existed between the two

groups when not accounting for FPW[11]. Their NNS group presented with higher creatinine levels (more than double) than the NS group, implying that the former patients were already in chronic kidney disease due to other causes.

A statistically significant difference existed between the albumin values of nephrotic versus non-nephrotic patients when FPW was not accounted for (p=0.0002; Figure 11B). The median albumin was 18g/L with an IQR of 14-25g/L. 63% of the non-nephrotic cases had a normal albumin >30g/L. The remaining 37% had a slightly reduced albumin level, but these values were all ≥25g/L. The 23 nephrotic cases all had low albumin levels below 25g/L. Indeed, the upper limit of albumin in the NS cases was less than the lower limit in the NNS cases (Table 4). 2 controls had normal albumin levels of 31 and 46g/L, respectively. 1 control and 1 non-nephrotic case did not have albumin results.

Our study findings correlate with the albumin criteria used in the 2021 KDIGO definition of NS[20]. When correlated with FPW, statistical significance remained in the albumin levels between the two groups (p=0.012; Figure 12E). The explanation as to why the FPW values become variable at low albumin levels (<20g/L) is due to increased FPW being associated with increased podocyte injury, with subsequent increased albumin leakage across the GBM and proteinuria. Albumin remains a significant factor in categorisation of FSGS patients at the clinical and EM level. One must reiterate that non-renal causes for hypoalbuminaemia (e.g. malnutrition) may play a part in clinical presentation and should be kept in mind. Caution must be taken when interpreting FPW at albumin <20g/L.

Our study results are like those of others in that the NS group had significantly lower albumin levels than the NNS group (Table 5). Interestingly, Deegens *et al.* proved by multivariate analysis that FPW did not correlate with serum albumin levels[11]. In our study, there was a correlation. Note that the value provided by da Silva *et al.* was likely in the incorrect units (2.6mg/dL converts to 0.026g/L; Table 5).

A statistically significant difference existed between the uPCR values of nephrotic versus non-nephrotic patients (p=0.0005; Table 4; Figure 11A). The median uPCR was 0.9g/mmol with an IQR of 0.5-1.3g/mmol. When plotting uPCR against FPW, statistical significance remained (p=0.0021). A higher uPCR value correlated with a higher FPW. This can be explained by the following: an increased uPCR (>1.3g/mmol) implies significant proteinuria which is most likely explained by an increase in injured podocytes with increased FPW (significant effacement).

Only 1 of the 3 controls had a uPCR result (0.005g/mmol). The 23 nephrotic cases had variable uPCR values, but all non-nephrotic cases (n=9) had a uPCR below 1.0g/mmol (Figure 11A). The uPCR on their own could potentially be used to clinically categorise patients as nephrotic or non-nephrotic. However, caution should be applied at higher uPCR (>1.3g/mmol).

Our study findings are like those of others in that statistical significance existed in the level of proteinuria between the two groups. In all the studies, the NS groups had significantly higher proteinuria values than the NNS groups (Table 5). However, a slight difference was noted between the current and other studies. Our study showed a significant correlation between the FPW and proteinuria among the two groups, but Deegens *et al.* performed a multivariate analysis which demonstrated that FPW did not correlate with proteinuria[11]. Similarly, Taneda *et al.* performed a multivariate analysis which illustrated no association between the mean FPW in their FSGS group and proteinuria[10]. Hommos *et al.* mentioned that although diffuse FPE was highly associated with NS, proteinuria alone had limited correlation with the degree of FPE[14]. Interestingly, Spanu *et al.* showed that FPW was correlated to proteinuria when all their patients were analysed together, but a correlation did not exist when the two groups were analysed individually[25]. Of note, developed countries use 24-hour urine collection for proteinuria determination instead of a uPCR, which we use in South Africa. The reason we do not perform the same test is due to our resource-limited setting with financial restraints. 24-Hour urine collection is more sensitive than uPCR for proteinuria assessment. We therefore rely on the trend in serial uPCRs to gauge proteinuria (Dr B. Davidson, Specialist Nephrologist, personal communication).

A statistically significant difference existed between the cholesterol values of nephrotic versus non-nephrotic patients (p=0.003; Table 4; Figure 11C). Only 1 control had a cholesterol result, slightly elevated at 5.36mmol/L (FPW of 811nm). 2 nephrotic cases did not have cholesterol results. 1 non-nephrotic case did not have cholesterol results. 1 non-nephrotic case had a raised cholesterol of 8.39mmol/L. This patient was hypertensive, never flagged as having dyslipidaemia, and treated as secondary FSGS.

The mean and median cholesterol were significantly higher in the NS group (12.0mmol/L and 12.9mmol/L, respectively) compared to the NNS group (4.5 and 4.3mmol/L, respectively; Table 4). Although hypercholesterolaemia is not a requirement for the KDIGO definition of NS, it is strongly associated with it. Regarding the outliers in Figure 12C, one had a FPW of 10236nm with a uPCR of 1.6g/mmol, a creatinine of 581µmol/L, an eGFR of 10mL/min/1.73m^2, and an albumin of 18g/L. The other outlier had a FPW of 9897nm with a uPCR of 1.3g/mmol, a creatinine of 187µmol/L, an eGFR of

30mL/min/1.73m^2, and an albumin of 14g/L. Both these cases had significant proteinuria, hypoalbuminaemia, and creatinine and eGFR values consistent with chronic kidney disease. 4 NS cases had normal cholesterol levels at biopsy. One of these four cases had a comparatively much higher FPW (5433nm). On closer inspection, the major difference between this and the other three cases was a higher creatinine of 267µmol/L and eGFR of 20mL/min/1.73m^2. Unfortunately, statistical significance could not be proven in our study between FPW and cholesterol (p=0.059). Our results suggest that although cholesterol is useful in distinguishing between the two groups clinically, a correlation between cholesterol and FPW was not found (EM is not helpful in this regard).

Other studies found a strong correlation between the cholesterol values among the two groups, except the study by Hommos *et al.* (p=0.09). In all the studies, the NS groups had higher mean cholesterol values than the NNS groups (Table 5).

There was a statistically significant difference between the FPWs of the nephrotic and non-nephrotic groups in our study (p=0.003). A similar difference was noted between the FPWs of the nephrotic and control groups (p=0.003). However, no difference was found between the FPWs of the non-nephrotic cases and the controls (p=0.1; Table 4). The mean and median FPWs in the NS group (3624nm and 3076nm, respectively) were significantly higher (more than double) than the NNS group (1510nm and 1322nm, respectively; Table 4). Figure 12 shows significant variation of the FPWs at high uPCR, low eGFR, and low albumin levels. As mentioned previously, this may be due to severe podocyte injury.

Our study results are like others in that the NS group had a significantly higher FPW than the NNS group (Table 5). Deegens *et al.* proved with multivariate analysis that FPW was significantly associated as an independent factor with the type of disease (idiopathic versus secondary FSGS) and that a FPW >1500nm differentiated idiopathic from secondary FSGS with high sensitivity and specificity (p<0.001)[11].

The logistic regression curve in our study (Figure 14) further highlights the significance of FPW in differentiating primary from secondary FSGS. The 0.5 Pr point on the y-axis correlates with a FPW of 1450nm, the threshold value at which half of all NS cases are identified with accuracy. This threshold value is very close to the 1500nm used in the literature to differentiate primary from secondary FSGS. Our study results mirror those of previously published international studies in developed countries. Having said this, the probability of diagnosing NS is only 50% accurate using this threshold value. It can be suggested that between the Pr 0.5 and 0.8 points a “grey zone” exists where a diagnosis of “possible

NS/primary FSGS" can be made. Above the Pr 0.8 point (FPW of 2550nm) a diagnosis of "probable NS/primary FSGS" can be made with more certainty.

Most of the patients in our study with NS (83%) had diffuse FPE on EM, whereas most (78%) of the non-nephrotic cases had focal FPE (p=0.003; Table 4). Explanations as to why some NS cases had focal FPE include: sampling error; the fact that many times only one non-sclerosed glomerulus was present on EM grids, limiting assessment; and extrarenal causes for hypoalbuminaemia (e.g. malnutrition). One of the NS cases had a FPW of 1298nm, an outlier. Another had a FPW of 1746nm, a value above the median threshold for NNS and below the median threshold for NS.

2 NNS cases had diffuse FPE. A detailed explanation follows regarding this. The first case is an uncontrolled hypertensive (BP=196/105mmHg) with a creatinine of 524µmol/L and an eGFR of 12mL/min/1.73m^2 at biopsy, treated clinically as secondary FSGS. The diffuse FPE is most likely explained by chronic kidney disease secondary to uncontrolled hypertension, where progressive, severe podocyte injury has occurred. The second case is an uncontrolled hypertensive (BP=175/114mmHg) with normal cholesterol, creatinine, eGFR, and albumin at biopsy, treated clinically as primary FSGS (NS), even though he did not meet the KDIGO criteria for NS. There was no uPCR for this patient. He was treated with steroids, responded well, and is in complete remission, currently maintained on low dose prednisone. This scenario highlights the importance of EM in the clinical distinction between primary and secondary FSGS: although he was non-nephrotic according to the KDIGO criteria, the fact that a good clinical response to steroids with complete remission was seen indicates he was correctly managed as primary FSGS initially. EM is useful in this regard in that it showed diffuse FPE with a FPW of 2798nm, confirming the clinical suspicion of primary FSGS.

Our results indicate that diffuse FPE correlates with NS (primary FSGS), whereas focal FPE correlates with NNS (secondary FSGS). These results are like those of other studies (Table 5). Note that the determination of FPE is essentially an estimate of how much of the GBM surface is effaced. Having said this, the estimation may not be an accurate measure to differentiate between primary and secondary FSGS. Rather, a properly calculated FPW lends more accuracy in this regard.

We performed an unsupervised HCA with no *a priori* assumptions regarding "syndrome" to determine how the data clustered and whether any significance could be attributed thereto. Figure 15 illustrates the dendrogram with the three clusters that resulted from this analysis. There was a clear separation between the NS and NNS clusters with the identification of two separate NS populations. Cluster 1 (n=9)

comprised all the non-nephrotic cases and two nephrotic cases. Cluster 2 (n=12) comprised nephrotic cases only. Cluster 3 (n=7) also comprised only nephrotic cases. We can infer the following from the above:

1. Three clusters existed and not two as was expected (NS and NNS).
2. Herein, a significant difference existed between Cluster 2 and Cluster 3, the two nephrotic clusters.
3. Clusters 2 and 3 contain pure nephrotic cases, whereas Cluster 1 contains a mixed population of predominantly non-nephrotic cases with two nephrotic cases.

On closer inspection into an explanation for the first and second points above, Cluster 2 had a statistically lower median FPW, uPCR, and creatinine when compared to Cluster 3. Also, Cluster 2 had a higher median eGFR than Cluster 3 (Figure 16). One can infer that Cluster 3 has more renal dysfunction, podocyte injury, and hence FPE when compared to Cluster 2. This has potential implications in management, specifically response to steroid therapy and remission. This will be investigated in the future. Potentially, this information can assist clinicians in identifying patients (by assessing laboratory results) that will respond more poorly to steroid therapy. Indeed, D'Agati *et al.* stated that patients with a worse prognosis include those with increased rates of proteinuria and renal insufficiency[3] (Cluster 3 in this instance). Interestingly, the cholesterol and albumin values were not significantly different between these clusters, variables that are used daily to help differentiate primary from secondary FSGS clinically.

Regarding the third point above, two NS cases clustered with the NNS group. On further investigation, one of the cases had a FPW of 5433nm, had evidence of chronic kidney disease, and was treated clinically as HIVAN. The other case was diagnosed with MCD at age 12 in 2006. He received steroid therapy and had a good clinical response with complete remission. He continued to have many relapses over the following years while following up at Red Cross War Memorial Children's Hospital. He was lost to follow up but then presented to GSH in March 2022 at 28 years of age with NS. He had a history of hypertension (BP of 144/87mmHg at most recent biopsy) and primary dyslipidaemia (normal cholesterol of 3.54mmol/L at most recent biopsy) on treatment. He was managed as MCD at GSH since his presentation, had a good clinical response to steroids, is currently in complete remission but has continued to have relapses. His calculated FPW was 1986nm. This value may be explained by the fact that in MCD, the FPW is significantly lower than in primary FSGS, as was proven by Taneda *et al.*[10]

The statistical differences between Clusters 1 and 3 were in the uPCR, cholesterol, albumin, and FPW. No difference was found in the creatinine values. Cluster 3 appeared to contain patients with NS that had severe renal dysfunction compared to Cluster 2. One can infer from Figure 16 that laboratory results alone can be used clinically to differentiate NS from NNS (Cluster 3 from Cluster 1), even though EM would find a significantly different FPW between them. In this clinical scenario, EM would not be very useful.

When interpreting the differences between Clusters 1 and 2 a similar approach to the above can be used. Statistical differences were found in uPCR, cholesterol, and albumin. No statistical differences were found in FPW and creatinine values between these clusters. NNS (Cluster 1) can certainly be distinguished from NS (Cluster 2) clinically, using laboratory results, specifically uPCR, cholesterol, and albumin. Cluster 2 appeared to contain patients with NS that did not have overt renal dysfunction (FPW higher but not statistically different from Cluster 1, and a lower uPCR and FPW than Cluster 3; Figure 16). In this clinical scenario, EM would not be very useful.

One may then ask, "Is there a place for EM in the diagnosis of FSGS and if so, in which clinical scenarios?" The answer is yes. We have proven with statistical significance that using clinical parameters, such as the presence of oedema (p=0.006), and laboratory results, such as the uPCR (p=0.0005), cholesterol (p=0.003), and albumin (p=0.0002), clinicians can differentiate primary from secondary FSGS clinically with confidence in most cases without the need for renal biopsy or EM (Table 4). A uPCR >1.0g/mmol, a cholesterol >10mmol/L, and an albumin <25g/L are strongly associated with NS (primary FSGS) (Table 4). However, situations exist where trying to categorise a disease process as primary or secondary becomes clinically very challenging, even when renal biopsy histology is available. It is in these challenging situations where EM becomes an important adjunct in the decision-making process regarding which management algorithm to follow. For example, patients presenting with a mixed nephritic/nephrotic picture, those with a serum albumin at presentation of between 25 and 30g/L, those presenting with a borderline uPCR of 0.35g/mmol, and those without obvious secondary causes on clinical history, may pose difficulty[11]. Indeed, these challenging scenarios commonly occur in our local population with a high prevalence of malnutrition and HIV (Dr B. Davidson, Specialist Nephrologist, personal communication). EM can then assist with distinguishing between primary and secondary FSGS by examining the FPW and FPE of podocytes. Primary FSGS has diffuse FPE (>80% of the GBM effaced) with a median FPW >3000nm. Secondary FSGS has focal FPE (<80% of the GBM effaced) with a median FPW of <1500nm (Table 4; Figure 11D). Clinicians would be able to classify patients and treat accordingly more appropriately. It must be reiterated how important correct classification prior to

treatment initiation is. Steroids unnecessarily given to secondary FSGS patients (many of which are already immunocompromised with malnutrition, HIV, and/or tuberculosis) could have dire consequences. Not administering steroids to primary FSGS patients would delay a therapeutic response with potentially unfavourable outcomes.

4.2. RECOMMENDATIONS

1. Firstly, we would like to discuss situations in which cautious interpretation of FPW be recommended:
    a. Patients with a history of diabetes mellitus. Diabetes is one of the causes of secondary podocyte injury with subsequent diffuse FPE[11], potentially leading to overinterpretation of FPW and erroneous diagnosis as a primary podocytopathy. Indeed, this was the reason one of the cases in our study had to be excluded, as the glomerulosclerosis was so severe.
    b. Patients presenting with a high creatinine result at biopsy (creatinine >180µmol/L). The reasoning behind this and all the recommendations that follow is that, as mentioned before, the FPW becomes variable and inaccurate otherwise.
    c. Patients presenting with a low eGFR result at biopsy (eGFR <50mL/min/1.73m^2).
    d. Patients presenting with high uPCR results (uPCR >1.3g/mmol).
    e. Patients presenting with low albumin levels (albumin <20g/L).
2. We recommend that future studies on this topic use larger sample sizes to increase power and strength.
3. We recommend that future studies perform a comparison of primary FSGS and MCD to investigate whether any significant differences exist between them or if they are indeed part of the same disease process.
4. We recommend a higher threshold value be used to differentiate NS from NNS.
    a. A FPW <1500nm correlates with a NNS diagnosis.
    b. A FPW between 1500 and 2500nm correlates with a “possible NS” diagnosis.
    c. A FPW >2500nm correlates with a “probable NS” diagnosis.
5. We recommend that EM be used as an adjunct in the clinical distinction of the type of FSGS in all suspected cases, although an argument for use in clinically challenging scenarios only has been discussed. The reason for this is that patients in our population frequently present with clinical signs of NS although they have secondary FSGS. Administering steroids to already immunocompromised patients who may present in NS but have secondary FSGS can lead to disastrous results. Confirmation of the type of FSGS with FPW and FPE is justified in all suspected FSGS cases (Dr B. Davidson, Specialist Nephrologist, personal communication).
6. We recommend that a calculated FPW should bear more significance than an estimate of FPE to assist in the differentiation of these patterns of injury.

Study limitations include:

1. The sample size. A sample size of 35 (32 cases and 3 controls) is not large enough to generate powerful conclusions from. Having said this, our sample size was large enough that statistical significance in multiple variables was found. Also, our study sample size was similar to those of internationally published studies (Table 5).
2. Some laboratory results were missing. Not every patient had every laboratory test performed.
3. There were 14 cases with no glomeruli and 2 cases with insufficient tissue or only fat on toluidine blue stained slides for EM assessment, hence these cases were excluded. The sample size could potentially have been considerably larger otherwise.
4. Counting the number of foot processes on EM images can sometimes be difficult due to the quality of the image. Additionally, the slits between foot processes are sometimes not as obvious, especially when processes begin to fuse.
5. The mean FPW calculated would be more accurate if more than one pathologist performed the task of counting foot processes instead of one (the student).

Study strengths include:

1. Our findings mirror many of those previously published internationally. One can therefore infer that the criteria used internationally regarding FPW and FPE can be used in our patient population as well. These findings include:
    a. Most patients presenting with primary FSGS are male.
    b. There is no association between creatinine levels and FPW.
    c. The NS group had significantly higher proteinuria than the NNS group.
    d. The NS group had significantly lower albumin levels than the NNS group.
    e. The NS group had higher mean cholesterol levels than the NNS group.
    f. The NS group had a significantly higher FPW than the NNS group (p=0.003).
    g. The NS group had diffuse FPE on EM, whereas the NNS group had focal FPE (p=0.003).
2. Ours is the first study, to our knowledge, to correlate oedema at presentation with primary and secondary FSGS in adults. 87% of the patients with NS had oedema at presentation (p=0.006).
3. Ours is the first study, to our knowledge, to correlate eGFR with FPW between primary and secondary FSGS. We found a strong negative association in this regard (p=0.017).
4. Our study is the first, to our knowledge, to prove a strong correlation between the FPW and proteinuria among primary and secondary FSGS (p=0.0021).
5. Our study is the first, to our knowledge, to prove a strong association between FPW and serum albumin levels (p=0.012).

6. We performed a logistic regression and found that a FPW threshold value of 2550nm correlated with a probability of diagnosing NS with 80% accuracy, whereas a threshold value of 1450nm (generally accepted internationally) correlated with a Pr of 50%. It is for this reason that we recommend a higher FPW cutoff of 2500nm be used to help differentiate primary from secondary FSGS.
7. Ours is the first study, to our knowledge, to perform an unsupervised HCA of FSGS patients. We found 3 definite clusters (1 NNS cluster and 2 separate NS clusters) with major differences between them. One of the NS clusters comprises patients with significantly more proteinuria, more renal dysfunction, and higher FPWs (more FPE).
8. Ours is the first study, to our knowledge, to compare primary and secondary FSGS in the sub-Saharan African setting.
9. Our study is the first, to our knowledge, to correlate HIV status with FSGS.

What does the future hold?

1. We will apply the current study results in our own Division of Anatomical Pathology going forward. It will be interesting to compare our threshold value of 2500nm to the generally accepted 1500nm and determine whether this impacts pathological diagnosis significantly.
2. We believe our study results are significant enough to be used locally and internationally to assist renal pathologists in distinguishing primary from secondary FSGS with increasing accuracy.
3. We believe the Division of Nephrology and Hypertension at GSH and the Division of Nephrology at Livingstone Hospital will find the results clinically applicable and useful from a management perspective.
4. This research study has constructed a great foundation for future research on this topic in the South African population.
5. An FSGS database has been created for the Division of Nephrology and Hypertension at GSH, which will expand in time.
6. We will expand our investigation to include treatment data. We will retrospectively analyse the response to steroid therapy in the NS group. We will also determine whether differences in steroid responsiveness and overall renal prognosis (survival) exist between the two NS clusters, as we expect.
7. We intend to publish this research soon.

5. CHAPTER FIVE - CONCLUSION

Distinguishing primary from secondary FSGS can be clinically difficult in some situations. It is imperative to categorise the type of FSGS pattern as this has direct impact on patient management. EM FPE has been used as a tool to assist in this regard. Our study is the first, to our knowledge, to investigate this topic in an African setting and to determine whether an association between HIV status and FSGS exists.

In conclusion, our study has proven the alternative hypothesis, which states that a statistically significant difference exists in EM FPW measurements between primary and secondary forms of FSGS. Primary FSGS generally presents with oedema, is significantly associated with diffuse FPE, and has a median FPW>3000nm. Secondary FSGS is significantly associated with focal FPE and has a median FPW<1500nm. We have determined a FPW threshold value of 2500nm to help differentiate primary from secondary FSGS with an 80% probability. We performed an unsupervised HCA and found two discrete NS clusters with major differences in laboratory results and FPW. We suspect that significant differences in treatment response and overall renal survival exist between them. We suggest that EM be used not only in clinically difficult scenarios but for all suspected FSGS cases to assist in the accurate distinction between the types of FSGS so that appropriate and optimal management be applied.

REFERENCES

1 Jacobs-Cacha C, Vergara A, Garcia-Carro C, Agraz I, Toapanta-Gaibor N, Ariceta G, Moreso F, Seron D, Lopez-Hellin J, Soler MJ. Challenges in primary focal segmental glomerulosclerosis diagnosis: from the diagnostic algorithm to novel biomarkers. Clin Kidney J. 2021;14(2):482-491. https://doi.org:10.1093/ckj/sfaa110.

2 Rosenberg AZ, Kopp JB. Focal Segmental Glomerulosclerosis. Clin J Am Soc Nephrol. 2017;12:502-517. https://doi.org:10.2215/CJN.05960616.

3 D'Agati VD, Kaskel FJ, Falk RJ. Focal Segmental Glomerulosclerosis. N Engl J Med. 2011;365:2398-2411. https://doi.org:10.1056/NEJMra1106556.

4 De Vriese AS, Sethi S, Nath KA, Glassock RJ, Fervenza FC. Differentiating Primary, Genetic, and Secondary FSGS in Adults: A Clinicopathologic Approach. J Am Soc Nephrol. 2018;29:759-774. https://doi.org:10.1681/ASN.2017090958.

5 Jefferson JA, Shankland SJ. The pathogenesis of focal segmental glomerulosclerosis. Adv Chronic Kidney Dis. 2014;21(5):408-416. https://doi.org:10.1053/j.ackd.2014.05.009.

6 Stokes MB, D'Agati VD. Morphologic variants of focal segmental glomerulosclerosis and their significance. Adv Chronic Kidney Dis. 2014;21(5):400-407. https://doi.org:10.1053/j.ackd.2014.02.010.

7 Kfoury H. Epithelial cell foot process effacement in podocytes in focal and segmental glomerulosclerosis: a quantitative analysis. Ultrastruct Pathol. 2014;38:303-308. https://doi.org:10.3109/01913123.2014.927405.

8 D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis. 2004;43(2):368-382. https://doi.org:10.1053/j.ajkd.2003.10.024.

9 Royal V, Zee J, Liu Q, Avila-Casado C, Smith AR, Liu G, Mariani LH, Hewitt S, Holzman LB, Gillespie BW, Hodgin JB, Barisoni L. Ultrastructural Characterization of Proteinuric Patients Predicts Clinical Outcomes. J Am Soc Nephrol. 2020;31:841-854. https://doi.org:10.1681/ASN.2019080825.

10 Taneda S, Honda K, Ohno M, Uchida K, Nitta K, Oda H. Podocyte and endothelial injury in focal segmental glomerulosclerosis: an ultrastructural analysis. Virchows Arch. 2015;467:449-458. https://doi.org:10.1007/s00428-015-1821-9.

11 Deegens JK, Dijkman HB, Borm GF, Steenbergen EJ, van den Berg JG, Weening JJ, Wetzels JF. Podocyte foot process effacement as a diagnostic tool in focal segmental glomerulosclerosis. Kidney Int. 2008;74:1568-1576. https://doi.org:10.1038/ki.2008.413.

12 da Silva CA, Monteiro ML, Araujo LS, Urzedo MG, Rocha LB, dos Reis MA, Machado JR. In situ evaluation of podocytes in patients with focal segmental glomerulosclerosis and minimal change disease. PLoS One. 2020;15(11):e0241745. https://doi.org:10.1371/journal.pone.0241745.

13 Ishizuka K, Miura K, Hashimoto T, Kaneko N, Harita Y, Yabuuchi T, Hisano M, Fujinaga S, Omori T, Yamaguchi Y, Hattori M. Degree of foot process effacement in patients with genetic focal segmental glomerulosclerosis: a single-center analysis and review of the literature. Sci Rep. 2021;11:12008. https://doi.org:10.1038/s41598-021-91520-9.

14 Hommos MS, De Vriese AS, Alexander MP, Sethi S, Vaughan L, Zand L, Bharucha K, Lepori N, Rule AD, Fervenza FC. The Incidence of Primary vs Secondary Focal Segmental Glomerulosclerosis: A Clinicopathologic Study. Mayo Clin Proc. 2017;92(12):1772-1781. https://doi.org:10.1016/j.mayocp.2017.09.011.

15 Nili F, Saboori F, Jahanzad I, Mehrazma M. Electron microscopic findings suggestive of focal and segmental glomerulosclerosis in patients with steroid-resistant nephrotic syndrome. Ultrastruct Pathol. 2019;43:6-12. https://doi.org:10.1080/01913123.2019.1584258.

16 Morita M, Mii A, Yasuda F, Arakawa Y, Kashiwagi T, Shimizu A. Diverse Alterations of Glomerular Capillary Networks in Focal Segmental Glomerular Sclerosis. Kidney Int Rep. 2022;7:1229-1240. https://doi.org:10.1016/j.ekir.2022.03.007.

17 Daehn I, Casalena G, Zhang T, Shi S, Fenninger F, Barasch N, Yu L, D'Agati V, Schlondorff D, Kriz W, Haraldsson B, Botttinger EP. Endothelial mitochondrial oxidative stress determines podocyte depletion in segmental glomerulosclerosis. J Clin Invest. 2014;124(4):1608-1621. https://doi.org:10.1172/JCI71195.

18 Saga N, Sakamoto K, Matsusaka T, Nagata M. Glomerular filtrate affects the dynamics of podocyte detachment in a model of diffuse toxic podocytopathy. Kidney Int. 2021;99:1149-1161. https://doi.org:10.1016/j.kint.2020.12.034.

19 Kidney Disease: Improving Global Outcomes Glomerular Diseases Work Group. Takeaways for Clinicians from the KDIGO 2021 Clinical Practice Guideline for the Management of Focal Segmental Glomerulosclerosis. https://kdigo.org/guidelines/gd/.

20 Kidney Disease: Improving Global Outcomes Glomerular Diseases Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int. 2021;100(45):S1-S276. https://doi.org:10.1016/j.kint.2021.05.021.

21 Sethi S, Zand L, Nasr SH, Glassock RJ, Fervenza FC. Focal and segmental glomerulosclerosis: clinical and kidney biopsy correlations. Clin Kidney J. 2014;7:531-537. https://doi.org:10.1093/ckj/sfu100.

22 Thomas DB, Franceschini N, Hogan SL, ten Holder S, Jennette CE, Falk RJ, Jennette JC. Clinical and pathologic characteristics of focal segmental glomerulosclerosis pathologic variants. Kidney Int. 2006;69:920-926. https://doi.org:10.1038/sj.ki.5000160.

23 Kriz W, Shirato I, Nagata M, LeHir M, Lemley KV. The podocyte's response to stress: the enigma of foot process effacement. Am J Physiol Renal Physiol. 2013;304:F333-347. https://doi.org:10.1152/ajprenal.00478.2012.

24 Sethi S, Glassock RJ, Fervenza FC. Focal segmental glomerulosclerosis: towards a better understanding for the practicing nephrologist. Nephrol Dial Transplant. 2015;30:375-384. https://doi.org:10.1093/ndt/gfu035.

25 Spânu S, Craciun C, Spânu C, Gherman-Caprioara M. CORRELATION BETWEEN PODOCYTE FOOT PROCESS EFFACEMENT AND PROTEINURIA IN HUMAN GLOMERULAR DISEASES. Studia Universitatis "Vasile Goldis" Seria Stiintele Vietii (Life Sciences Series). 2011;21(3):463-468.

26 Fuiano G, Comi N, Magri P, Sepe V, Balletta MM, Esposito C, Uccello F, Dal Canton A, Conte G. Serial morphometric analysis of sclerotic lesions in primary "focal" segmental glomerulosclerosis. J Am Soc Nephrol. 1996;7:49-55.

27 Glassock RJ, Fervenza FC. "Presumed" Primary Focal Segmental Glomerulosclerosis: A Novel Nuance for Steroid Therapy. Kidney Int Rep. 2022;7:9-12. https://doi.org:10.1016/j.ekir.2021.11.021.

APPENDICES

APPENDIX 1: CONFIRMATION LETTER OF MMED RESEARCH METHODS COURSE ATTENDANCE

Ms Annemie Stewart
UCT Clinical Research Centre, L51, Old Main Building
Groote Schuur Hospital, Observatory, 7925, Cape Town
☎ 021 406 6498 / 072 408 0459
✉ annemie.stewart@uct.ac.za
W www.crc.uct.ac.za

24 Jan 2024

To Whom it May Concern

Statement confirming MMeds Research Methods

This letter confirms that **Dr Nelson da Costa atte**nded the **Clinical Research Centre's MMeds Research Methods Course on the 5th and 12th May 2022**

If you have any questions or concerns, please do not hesitate to contact me on **021 406 6498** or **072 408 0459**.

Best wishes

Annemie Stewart
Operations Manager
Clinical Research Centre (CRC)
UCT Faculty of Health Sciences

APPENDIX 2: HREC RENEWAL UNTIL 30-12-24

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/12/24
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	21/11/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	20 November 2023		
HREC REF Number	832/2020	Current Ethics Approval was granted until	30 December 2023
Protocol title	Electron microscopic morphometry of podocyte foot process effacement as a tool to distinguish primary from secondary focal and segmental glomerulosclerosis (FSGS)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	X No	
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Brendon Price		

HUMAN RESEARCH ETHICS COMMITTEE
20 NOV 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Department / Office Internal Mail Address	Department of Anatomical Pathology, NHLS. pricebrendon@yahoo.com

1.1 Does this protocol receive US Federal funding?	☐ Yes	X No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	X No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	**X**
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	N/A
Billing Address of Sponsor:	N/A
Vat Number:	N/A

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Contact person	N/A
Telephone number	N/A
Email Address	N/A
2. **Internal Journal Billing:**	
Fund Number:	N/A
Cost Centre Number:	N/A
Account Holder Name:	N/A
Division of Account Holder:	N/A

2. List of documentation for approval

Renewal of ethics approval (HREC832/2020)

3. Protocol status (tick ✓)

☐	Open Enrolment
☐	Closed to enrolment (tick ✓)
X	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	53
Number of participants enrolled, since last HREC Progress report (continuing review)	53
Additional number of participants still required	Unsure

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	N/A

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

6. Cumulative summary of participants

Total number of participants who provided consent	N/A
Number of participants determined to be ineligible (i.e. after screening)	Unsure
Number of participants currently active on the study	53
Number of participants completed study (without events leading to withdrawal)	N/A
Number of participants withdrawn at participants' request (i.e. changed their mind)	N/A
Number of participants withdrawn by PI due to toxicity or adverse events	N/A
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	N/A
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	N/A
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	N/A

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
See attached document

8. Protocol violations and exceptions (tick ✓ all that apply)

X	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

X	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A. This is a retrospective study using formalin fixed paraffin embedded tissue

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	X Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	X Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	X Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	X Not applicable
		DSMB report attached	☐ Yes	☐ No	X Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	X No
If yes, please explain:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
X	Shown no change
If there has been a change, please explain:	
N/A	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.
N/A. This is a retrospective study using formalin fixed paraffin embedded tissue

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes		X No	
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	X No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

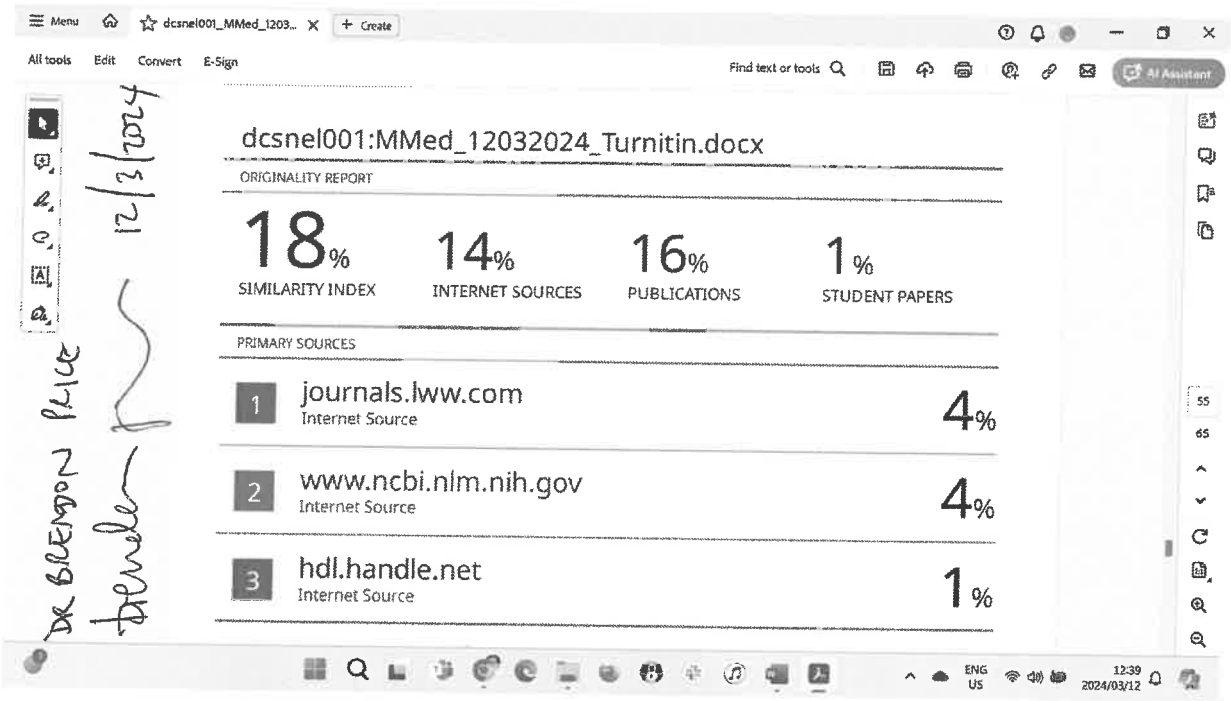
(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	20 November 2023

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

APPENDIX 3: TURNITIN REPORT


Menu
dcsnel001_MMed_1203...
Create
All tools
Edit
Convert
E-Sign
Find text or tools
AI Assistant
dcsnel001:MMed_12032024_Turnitin.docx
ORIGINALITY REPORT
18%
SIMILARITY INDEX
14%
INTERNET SOURCES
16%
PUBLICATIONS
1%
STUDENT PAPERS
PRIMARY SOURCES
1
journals.lww.com
Internet Source
4%
2
www.ncbi.nlm.nih.gov
Internet Source
4%
3
hdl.handle.net
Internet Source
1%
12/3/2024
DR BRENDON PLUG
ENG
US
12:39
2024/03/12