The Role of Calcineurin Inhibitor Therapy in Treatment of Primary Focal Segmental Glomerulosclerosis

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July 2015

A THESIS SUBMITTED TO MCGILL UNIVERSITY IN PARTIAL FULFILLMENT
OF THE DEGREE OF

MASTER OF SCIENCE
In
EPIDEMIOLOGY

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ABSTRACT

Background: Primary focal segmental glomerulosclerosis (FSGS) is the most common cause of nephrotic syndrome in adults. Glucocorticoids have been studied as treatment of primary FSGS in retrospective studies. Although some studies evaluated the role of calcineurin inhibitors (CNIs) in a steroid-resistant primary FSGS population, their role as early therapy remains poorly established.

Objectives: To evaluate the efficacy of CNIs in treatment of primary FSGS.

Methods: Two studies were performed. The first was a systematic review to describe the efficacy of CNIs compared to placebo, supportive therapy and other immunosuppressive agents. The second study used registry data and time-dependent Cox models to compare time to end-stage kidney disease (ESKD) between different immunosuppressive therapies while controlling for potential confounders, including those influencing choice of therapy and renal survival.

Results: Study 1**:** Six randomized controlled trials and 2 cohort studies were reviewed. All but one suggested CNIs may be beneficial in steroid-resistant primary FSGS compared to placebo or supportive therapy. No prospective trial studied the efficacy of calcineurin inhibitors in first-line treatment of FSGS. Study 2: In adjusted Cox regression, immunosuppressive therapy with glucocorticoids and/or CNIs was associated with a better renal survival [hazard ratio 0.49 (95% confidence interval 0.28, 0.86)] than no therapy. Although not statistically significant, CNIs ± glucocorticoids were associated with a lower likelihood of ESKD [hazard ratio 0.42 (95% confidence interval 0.15, 1.18)] than glucocorticoids alone.

Conclusions: Our results suggest that immunosuppressive therapy with CNIs and/or glucocorticoids is associated with better renal survival compared with supportive therapy alone in patients with FSGS. Whether early use of CNIs alone or in combination with glucocorticoids are superior to glucocorticoids alone in attaining remission and preventing ESKD, remains to be established.

Keywords: focal segmental glomerulosclerosis; calcineurin inhibitors; renal outcomes

ABRÉGÉ

Contexte : L'hyalinose focale et segmentaire (HFS) primaire est la cause la plus commune de syndrome néphrotique chez l'adulte. Les glucocorticoïdes ont été étudiés comme traitement de première ligne dans l'HFS primaire dans des études rétrospectives. Malgré le fait que des études ont évalué le rôle des inhibiteurs de la calcineurine (ICN) dans une population d'HFS primaire résistante aux stéroïdes, leur rôle comme thérapie au début de l'évolution de la maladie reste mal établi.

Objectifs : Évaluer l'efficacité des ICN dans le traitement de l'HFS.

Méthode : Deux études ont été conduites. La première était une revue systématique pour décrire l'efficacité des ICN par rapport à un agent placebo, à un traitement non-immunologique, ainsi qu'à d'autres agents immunosuppresseurs. Dans la deuxième étude, nous avons utilisé les données d'un registre et des modèles de régression de Cox afin de comparer le temps à l'insuffisance rénale terminale (IRT) entre différents traitements immunosuppresseurs et ce, en ajustant pour des facteurs confondants potentiels, incluant ceux connus pour influencer le choix du traitement et l'issue rénale.

Résultats : Étude 1 : Six essais cliniques randomisés et 2 études de cohorte ont été revus. Tous les articles sauf un suggèrent un effet bénéfique des ICN dans l'HFS primaire résistante à la corticothérapie. Aucune étude prospective n'a évalué l'efficacité des ICN dans le traitement de première intention de l'HFS. Étude 2 : Dans la régression de Cox ajustée, l'immunosuppression avec des

glucocorticoïdes avec ou sans ICN est associée à une meilleure survie rénale [rapport risque 0.49 (intervalle de confiance à 95% 0.28, 0.86)] qu'une absence d'immunothérapie. Quoique qu'il ne soit pas statistiquement significatif, les ICN ± glucocorticoïdes sont associés à un risque moindre d'IRT [rapport risque 0.42 (intervalle de confiance à 95% 0.15, 1.18)] que les glucocorticoïdes seuls.

Conclusions : Nos résultats suggèrent qu'un traitement immunosuppresseur composé d'un ICN avec glucocorticoïdes, ou de glucocorticoïdes seuls est associé à une meilleure survie rénale qu'une thérapie de support seule chez les patients atteints d'HFS. La supériorité des ICN avec ou sans glucocorticoïdes vis-à-vis les glucocorticoïdes seuls pour induire une rémission ou prévenir l'IRT reste indéterminée.

Mots clés : hyalinose focale et segmentaire; inhibiteurs de la calcineurine; issues rénales

Acknowledgments:

I offer my sincere gratefulness to both of my thesis supervisors Dr. Bethany Foster and Dr. Patrick Nachman for all the help and advices they gave me while elaborating this dissertation. I especially thank Dr. Nachman for making me a better nephrologist and a rigorous clinical scientist, and Dr. Foster for her continuous feedback and constant commitment towards perfection. I would like to acknowledge Dr. Ronald Falk, Dr. Vimal Derebail, Caroline Poulton, Dr. Julie McGregor, Susan Hogan and all my colleagues from the UNC Kidney Center for their support during those two wonderful years. A special thank is owed to Dr. Vincent Pichette for his extraordinary mentorship. I thank the faculty, staff and my colleagues at McGill University for stimulating me to work in this field. I also offer my gratitude to my parents for their unconditional support throughout my years of education. Finally, I gratefully thank my dear wife Gabrielle for her love, patience and continual support.

Preface and Contributions of Authors:

This thesis in total constitutes research completed under the supervision of Dr. Patrick Nachman and Dr. Bethany Foster and is presented in four main chapters. Chapter One is an introduction to the major issues related to research in focal segmental glomerulosclerosis. Chapter Two includes a comprehensive literature review presented as a systematic review on the efficacy of calcineurin inhibitors in the treatment of focal segmental glomerulosclerosis. Chapter Three presents original work from a cohort study that analyzed the association between exposure to immunosuppressive therapy and renal survival among patients with primary focal segmental glomerulosclerosis. This chapter has resulted in a manuscript that will be submitted to a major nephrology journal. Chapter Four presents the strengths and limitations of my dissertation including recommendations and future research plans.

Author contributions for Chapter 1: Louis-Philippe Laurin (LPL), with assistance from Bethany J. Foster (BJF) and Patrick H. Nachman (PHN), authored chapter 1.

Author contributions for Chapter 2: LPL and BJF contributed to the conception and design of the study, analysis and interpretation of data, and drafting the article; PHN contributed to the analysis and interpretation of the data and revising the manuscript for important intellectual content.

Author contribution for Chapter 3: LPL, BJF, PHN and Ronald J. Falk were involved in the development of study concept and design. Acquisition of the data was done by LPL and Caroline J. Poulton. Review of the histology slides was performed by Adil M. Gasim and J. Charles Jennette. Statistical analysis was primarily performed by LPL. Drafting of the manuscript was done by LPL, with assistance from BJF and PHN.

Author contributions for Chapter 4: LPL, with assistance from BJF and PHN, authored chapter 4.

Ethics Approval: The clinical data used in this study were obtained as part of the Glomerular Disease Collaborative Network registries. This study was reviewed by the University of North Carolina Biomedical Institutional Review Board in accordance with U.S. federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40CFR 26 (EPA), where applicable.

Introduction:

Nephrotic syndrome, a condition characterized by proteinuria (protein in the urine) in excess of 3.5 g/day, along with hypoalbuminemia (<35 g/l), edema, hyperlipidemia and lipiduria (Mace and Chugh 2014), results from a number of diseases affecting the filtering units of the kidney (glomeruli). Proteinuria is thought to be the driving force behind the nephrotic syndrome, because other features of the syndrome happen when proteinuria reaches the so-called nephrotic threshold (3.5 g/day).

Focal segmental glomerulosclerosis (FSGS) is the most common cause of nephrotic syndrome in adults, representing 40% of cases (Korbet 2012), and the second most common cause in children. FSGS represents a histological finding rather than a pathophysiological process. It is characterized by a segmental obliteration of glomerular capillaries by the extracellular matrix. Entrapment of plasma proteins as hyalinosis frequently accompanies the sclerosis (D'Agati, Kaskel et al. 2011). There are several causes of FSGS. Primary FSGS may be the result of mutations in specific podocyte genes (Pollak 2002) coding for structural components of the glomerulus, or may be idiopathic. The primary idiopathic form of FSGS has been attributed to an as yet unidentified circulating “permeability factor”, but the specific cause remains unknown (D'Agati, Kaskel et al. 2011). Major secondary causes of FSGS include human immunodeficiency virus type 1, and drug exposures (e.g. heroin, pamidronate, lithium). FSGS can also be an adaptive response to glomerular hypertension, as seen in conditions with reduced renal mass (D'Agati, Fogo et al. 2004).

Five histologic subtypes of FSGS have been recognized: collapsing, cellular, tip, perihilar and not-otherwise-specified (NOS) (D'Agati 2003). These variants differ in their demographic and epidemiologic characteristics, clinical presentation, clinical course and prognosis (Chun, Korbet et al. 2004, Thomas, Franceschini et al. 2006). As such, it is plausible that FSGS may represent a group of diseases of different etiologies, rather than a single uniform disease entity.

The natural course of disease progression in patients with FSGS varies with the severity of proteinuria. Proteinuria is believed to cause further renal damage, extending the glomerular injury to the tubulointerstitial compartment by protein overload and complement activation (Abbate, Zoja et al. 2006). A minority of patients (<15%) with non-nephrotic proteinuria (<3.5 g/day) progress to end-stage kidney disease over 5 to 10 years, whereas ≥50% patients with nephrotic-range proteinuria develop end-stage kidney disease (ESKD) by 10 years (Velosa, Holley et al. 1983). Besides severity of proteinuria, other factors have been associated with disease progression: severity of renal dysfunction at onset, significant (>20%) interstitial fibrosis on renal biopsy, and non-response to therapy (absence in reduction in proteinuria) (Korbet 2012). Given the association between the severity of proteinuria and progression to ESKD, and the long disease duration, a reduction in proteinuria has been considered an important outcome measure to determine efficacy of treatment in FSGS. The remission in proteinuria has been categorized as either complete (<0.3 g/day) or partial (<3.5 g/day). The change in estimated glomerular filtration rate over time

(i.e. slope) has also been used as outcome measure of treatment efficacy in FSGS. However, the most important outcome is renal survival, defined as time to development of renal failure requiring treatment with dialysis or transplantation.

Although the pathophysiology of primary FSGS remains poorly understood, it is believed to be an immunologically mediated disease. As such, glucocorticoids have been historically the mainstay first line-therapy (Pei, Cattran et al. 1987, Banfi, Moriggi et al. 1991, Rydel, Korbet et al. 1995, Cattran and Rao 1998). Calcineurin inhibitors (CNIs) are drugs that specifically and competitively inhibit calcineurin, a calcium and calmodulin dependent phosphatase; this ultimately reduces transcriptional activation of various cytokines, thus reducing lymphocyte proliferation (Wiederrecht, Lam et al. 1993). CNI agents (tacrolimus and cyclosporine) have been studied in FSGS patients not attaining proteinuria remission after being exposed to a variable course (usually >4 weeks) of glucocorticoids. With an aggressive immunosuppressive approach, a substantial proportion of patients with nephrotic syndrome may attain a significant reduction in proteinuria in the subnephrotic range with preservation of renal function (Korbet, Schwartz et al. 1994). This remission in proteinuria, either partial or complete, has been associated with a better renal survival (Troyanov, Wall et al. 2005).

Current literature defining the role of CNI therapy in a steroid naïve primary FSGS population is scant. Patients with comorbidities such as diabetes, glaucoma and obesity may experience side effects from glucocorticoids. As such,

defining a role for an alternative agent is important for patient care. The goal of this dissertation is to establish the efficacy of immunosuppressive therapy on renal survival in patients with primary FSGS, and more specifically to examine the association between CNIs and renal survival.

Two studies were performed. The first was a systematic review to describe the efficacy of CNIs compared to placebo, supportive therapy and other immunosuppressive agents in terms of remission of proteinuria and renal survival. In the second study, we used registry data and time-dependent Cox models to compare time to ESKD between different early choice immunosuppressive therapies while controlling for potential confounders, including those influencing choice of therapy and renal outcome.

Preface to Chapter 1:

Chapter 1 provides an overview of the different issues found in the current literature on FSGS with respect to risk factors, treatment, outcome measures and generalizability. It describes the major obstacles that research in FSGS has faced over the past few decades.

This chapter lays the groundwork for the subsequent chapters.

Chapter 1: Background

Nephrotic syndrome is characterized by significant proteinuria (>3.5 g/day), edema, hypoalbuminemia and hypercholesterolemia. Persistent nephrotic syndrome related to FSGS is associated with progression to renal failure (Cameron, Turner et al. 1978), and its complications significantly hamper quality of life (e.g. thromboembolic disease, infections) (Gipson, Trachtman et al. 2011). If left untreated, nephrotic syndrome related to primary FSGS will progress to ESKD in 6 to 9 years in 50% of patients (Cattran and Rao 1998). The association between proteinuria and renal function decline has been described as less pronounced in women than men (Cattran, Reich et al. 2008). Of note, the prevalence of FSGS is increasing among adult African Americans, accounting for 80% of lesions seen on renal biopsy (Korbet 2012). Risk alleles G1 and G2 for the apolipoprotein L1 (APOL1) gene, found in 55-65% of African Americans, have been associated with a higher risk of FSGS, and progression to ESKD (Pollak, Genovese et al. 2012, Genovese, Friedman et al. 2013).

Few studies have examined the effect of treatment on progression to renal failure, recognized as the ideal “hard outcome”, due to the slow progression of FSGS, and the challenges in conducting very long prospective studies. Most studies considered intermediate outcomes. Proteinuria has been accepted as a surrogate maker for progressive renal damage for decades (Velosa, Holley et al. 1983). The association between treatment and remission in proteinuria (response to therapy) has been established in both retrospective and prospective studies (Rydel, Korbet et al. 1995, Cattran and Rao 1998, Cattran, Appel et al. 1999).

The association between remission in proteinuria and preservation of renal function was demonstrated subsequently, but not definitively validated (Troyanov, Wall et al. 2005). Similarly, the rate of change in estimated glomerular filtration rate was proposed as a surrogate endpoint for end-stage kidney disease, but appears less useful in clinical practice due to the need for longer follow-up to observe a clear trend in renal function decline (Troyanov, Wall et al. 2005).

In 2012, the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline for glomerulonephritis was published in an effort to improve consistency of definitions, and assist with clinical decision-making (KDIGO Glomerulonephritis Work Group 2012). Standard definitions of partial and complete remission were agreed upon. Complete remission was defined as a reduction of proteinuria to <0.3g/day or <300 mg/g (<30 mg/mmol) urine creatinine in the setting of normal serum creatinine and serum albumin >35 g/l. Partial remission was defined as a reduction of proteinuria to 0.3–3.5 g/day or 300–3500 mg/g (30–350 mg/mmol) urine creatinine with stable serum creatinine (change in creatinine <25%) or reduction of proteinuria to 0.3–3.5 g/day or 300–3500 mg/g (30–350 mg/mmol) urine creatinine **and** a decrease of 50% from baseline, with stable serum creatinine (change in creatinine <25%). On the other hand, the rate of change in estimated glomerular filtration rate is usually measured as the slope of creatinine clearance in the glomerular disease literature.

A lack of consistency across FSGS studies with respect to studied populations has also made interpretation of prior studies challenging. Important disease characteristics were not uniformly defined across FSGS trials. Indeed, the exact duration of high dose glucocorticoid therapy needed to declare a patient steroid-resistant has been a matter of debate. The KDIGO guideline defined glucocorticoid resistance as a persistence of proteinuria despite prednisone 1 mg/kg/d or 2 mg/kg every other day for >4 months (16 weeks). This definition was based solely on retrospective cohort analysis, and was not applied upon uniformly. The level of proteinuria needed to be included into a trial, and benefit from therapy, has also been inconsistent. For example, in a recent large scale FSGS clinical trial (Gipson, Trachtman et al. 2011), defined steroid resistance and persistent proteinuria as persistence of proteinuria >1 g/g after only one month of therapy whereas previous reports used 2 months in patients with nephrotic-range proteinuria (Cattran, Appel et al. 1999). Low baseline estimated glomerular filtration rate (eGFR) of various degrees (<30 mL/min/1.73m^2 to <60 mL/min/1.73m^2) was used as exclusion criterion in most primary FSGS studies; thus, the impact of immunosuppressive therapy in patients with substantial renal dysfunction at baseline has not been well described.

Prognostic factors in FSGS:

A number of prognostic factors have been identified in primary FSGS. Besides persistent nephrotic-range proteinuria (>3.5 g/day), the level of kidney function at baseline and the severity of tubulointerstitial injury have been

recognized as important predictors of progression to ESKD (Chitalia, Wells et al. 1999). Female sex has also been associated with better renal survival than male sex (Cattran, Reich et al. 2008).

In the last decade, several retrospective studies provided important insights on the prognostic value of histologic variants in predicting the rate of progression to ESKD (Schwartz, Evans et al. 1999, Chun, Korbet et al. 2004, Thomas, Franceschini et al. 2006). For example, collapsing FSGS variant is associated with a higher frequency of ESKD whereas tip lesion responds well to glucocorticoid therapy. Only one prospective study (D'Agati, Alster et al. 2013) examined the clinical outcomes associated with FSGS variant. This study, in which a well-defined immunosuppression protocol was used in steroid-resistant patients, confirmed the poor renal survival associated with collapsing FSGS, and the more favourable renal survival associated with tip lesion. Nevertheless, the influence of FSGS variant on renal outcome in patients treated with a first-line agent other than glucocorticoids remains undefined.

Outcomes in FSGS studies:

FSGS is characterized by increasing proteinuria, which can progress to a clinically apparent disease with nephrotic range proteinuria. This proteinuria is often accompanied by a slow decline in renal function. Even among patients who have achieved remission of proteinuria with immunosuppressive therapy, relapses are common (Troyanov, Wall et al. 2005). Thus, prolonged follow-up is needed to evaluate clinically relevant outcomes such as death or ESKD.

Although reduction in proteinuria (complete and partial remission) has been proposed as an intermediate outcome for ESKD (Troyanov, Wall et al. 2005), complete and partial remissions have not been validated as endpoints acceptable to regulatory agencies (such as the U.S Food and Drug Administration) in evaluating therapies for FSGS. Furthermore, the definition of remission in proteinuria was not consistent prior to the publication of the KDIGO guideline. Many prior studies did not consider level of renal function in the assessment of remission of proteinuria. The new KDIGO definition of proteinuria remission is an attempt to ensure that reduction in proteinuria reflects a true decrease in disease activity rather than a reduction in glomerular filtration rate.

Hence, although several potential surrogate endpoints for ESKD have been suggested, their usefulness in predicting ESKD has not been definitively established. This is important because existing clinical trials and observational studies used intermediate or surrogate endpoints (remission of proteinuria) rather than renal survival as the primary outcome. A better understanding of the pathophysiology behind FSGS would help establish the most clinically relevant surrogate endpoints. However, studies examining renal survival are also needed.

Treatment of FSGS:

Current evidence regarding best therapy for FSGS is based on observational studies and a few small clinical trials. The main agents used to treat FSGS include glucocorticoids and CNIs. A variety of other agents have also been considered.

Glucocorticoids are considered the first-line therapy in primary FSGS (Pei, Cattran et al. 1987, Banfi, Moriggi et al. 1991, Rydel, Korbet et al. 1995, Cattran and Rao 1998). Evidence supporting treatment with glucocorticoids comes from small, uncontrolled, retrospective studies that examined response to therapy in terms of remission in proteinuria. Analyses were mostly descriptive, with minimal adjustment for potential cofounders due to inadequate statistical power. Although progression to ESKD was also examined in some studies (Banfi, Moriggi et al. 1991, Cattran and Rao 1998), very few patients reached this endpoint during the course of the study, limiting the ability to draw conclusions. No randomized placebo controlled trial has been conducted to definitively establish the value of glucocorticoids in preventing ESKD in patients with FSGS. Nevertheless, initiation of immunosuppressive therapy is recommended in patients with idiopathic FSGS and clinical features of nephrotic syndrome, based on the association between persistent nephrotic-range proteinuria (>3.5 g/day) and subsequent kidney failure (Cameron, Turner et al. 1978).

CNIs have been studied subsequently as therapy for steroid-resistant patients. Their superiority in inducing a reduction in proteinuria was initially demonstrated compared to placebo or supportive therapy (Ponticelli, Rizzoni et al. 1993, Lieberman and Tejani 1996, Cattran, Appel et al. 1999). CNI have not been not confirmed superior to mycophenolate mofetil (MMF) combined with dexamethasone (Gipson, Trachtman et al. 2011). Although CNIs appear to be effective in reducing proteinuria in steroid-resistant patients, the relapse rate is high following discontinuation. The influence of CNI therapy on long-term renal

survival remains unknown. CNIs have been considered as therapy for steroid-naïve patients with relative contraindications or expected intolerance to high-dose glucocorticoids based on their efficacy in steroid-resistant patient. However, no studies clearly demonstrated that including CNIs in the initial therapy of FSGS is associated with improved renal survival.

Other therapies, such as fresolimumab or sparsentan (dual angiotensin receptor and endothelin receptor blocker), are currently under investigation.

Given the paucity of data on important aspects inherent to the treatment of primary FSGS, research recommendations have been formulated by the leaders in the field (KDIGO Glomerulonephritis Work Group 2012). These include the need for a randomized controlled trial on the timing of glucocorticoid therapy as first-line treatment, a randomized controlled trial on the comparative efficacy of CNIs, alkylating agents and MMF in steroid-resistant FSGS, and validation studies on the pathological classification of FSGS with respect to outcome and response to therapy.

Preface to Chapter 2:

Chapter 2 provides a systematic review of the literature on therapy with CNIs with the aim of defining the impact of such therapy on renal survival in patients with primary FSGS. Results from this literature review served as justification for the cohort study presented in Chapter 3.

Chapter 2: Literature Search: A Systematic Review of the Efficacy of Calcineurin Inhibitors in Primary Focal Segmental Glomerulosclerosis

Calcineurin Inhibitors in the Treatment of Primary Focal Segmental Glomerulosclerosis: a Systematic Review of the Literature

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ABSTRACT:

Background: Primary focal segmental glomerulosclerosis (FSGS) is the most common cause of nephrotic syndrome in adults. Glucocorticoids have been evaluated in the treatment of primary FSGS in numerous retrospective studies. Evidence suggesting a role for including calcineurin inhibitors (CNIs) in early therapy remains limited. The aim of this study was to systematically review the literature examining the efficacy of CNIs in the treatment of primary FSGS both as first-line therapy and as an adjunctive agent in steroid-resistant patients, with respect to remission in proteinuria and renal survival.

Methods: We performed a systematic review of studies evaluating the clinical efficacy of CNIs in primary FSGS. PubMed and EMBASE were searched from inception to August 2014 for prospective controlled trials, and case-control and cohort studies. Evidence emerging from these studies was reviewed and a meta-analysis was performed.

Results: After systematically applying our inclusion criteria, a total of 152 titles and abstracts were identified. Six randomized controlled trials and 2 cohort studies were reviewed. Three randomized controlled trials compared CNIs to placebo or supportive therapy. Three studies compared CNIs to another immunosuppressive agent. All prospective trials were conducted in patients with primary FSGS deemed steroid-resistant.

Conclusions: The efficacy of CNIs has been evaluated in steroid-resistant primary FSGS patients. There is no evidence supporting their role as first-line therapy. Further studies are needed to determine this role.

Keywords: focal segmental glomerulosclerosis; calcineurin inhibitors; renal outcomes

INTRODUCTION:

Idiopathic FSGS is one of the most common causes of the nephrotic syndrome in adults, with an increased incidence over the last three decades, especially in African Americans.[1] FSGS lesions have been reported in up to 35% of patients who have undergone a kidney biopsy for nephrotic syndrome.[2] Primary FSGS represents 2.3% of patients with ESKD in North America.[3]

Patients with FSGS may benefit from immunosuppressive therapy. Glucocorticoids were evaluated several decades ago in primary FSGS in observational studies; the evidence supporting glucocorticoid therapy in FSGS is based on small observational cohorts of patients (n<100) with inadequate statistical power for adjustment for potential confounders. CNIs have been studied more recently in primary FSGS. Evidence supporting their use in the initial therapy for FSGS is scarce. CNIs have been prospectively evaluated mainly in a steroid-resistant FSGS population.

The purpose of this systematic review was to summarize the existing evidence regarding the effectiveness of CNIs for the treatment of primary FSGS, both as first-line therapy and as an adjunctive agent in steroid-resistant patients. The search strategy focused initially on the most relevant renal outcome, renal failure; however, articles describing the effectiveness of CNIs in achieving remission in proteinuria were also reviewed.

METHODS:

Data sources and searches:

EMBASE and PubMed were electronically searched from their inception dates (EMBASE from 1974 and PubMed from 1966) to August 2014. We initially searched each database for 'glomerulosclerosis, focal segmental' AND ('cyclosporine' OR 'tacrolimus') AND ('renal insufficiency' OR 'kidney failure, chronic'). These terms were selected using the PICO (Population Intervention/exposure Comparison Outcome) strategy. We then broadened the search strategy by searching for 'glomerulosclerosis, focal segmental' AND ('cyclosporine' OR 'tacrolimus') due to a low number of potentially relevant citations identified in PubMed. The Cochrane Library was also searched for the presence of systematic reviews on primary FSGS, and results were compared to our searches. We also reviewed related articles and bibliographies of relevant articles.

Study selection:

Inclusion and exclusion criteria: Studies that described use of CNIs in patients with primary FSGS, including randomized and/or controlled trials, case-control studies, and cohort studies were included. Case reports or case series were excluded due to absence of control or comparison group. Editorials, clinical guidelines, commentary, letters to the editor, and meeting reports were also excluded. We restricted results to human studies published in English. A single individual (LPL) performed the literature searches and the study selection.

Comparison groups had to include any of the following: a control group receiving supportive treatment (i.e. no immunosuppression), placebo or no treatment group, or a group receiving another agent of interest (e.g. if the intervention was a CNIs, the comparison might be MMF). The outcome of interest for an article retained to be reviewed was clinical efficacy of the immunomodulatory treatment; this could be measured or defined in several ways: mortality, renal survival (or time to ESKD), proteinuria remission rate (partial and complete), and renal function (eGFR).

Randomized controlled trials were reviewed and their methodology critically appraised using the Cochrane Collaboration's tool for assessing risk of bias.[5] This risk of bias tool comprises six categories of bias: selection bias (random sequence allocation/concealment of allocation), performance bias (blinding of clinicians and participants), detection bias (blinding of participants and outcome assessors), attrition bias (intention-to-treat analysis), reporting bias (selective outcome reporting), and other bias. All categories focus on the internal validity of the study. External validity (generalizability) and precision (free of random error) were separately assessed. Retrospective studies were reviewed and their methodology critically appraised using the Newcastle Ottawa quality assessment scale.[6] This tool was developed to assess the quality of nonrandomised studies in meta-analyses (case-control and cohort studies). It focuses on three domains to assess study internal validity: the selection of the study groups, the comparability of the groups and ascertainment of exposure/outcome. External validity and precision were also separately assessed in retrospective studies.

Meta-analysis:

A meta-analysis was performed for studies analyzing the efficacy of CNIs (with or without low dose prednisone) versus placebo or supportive therapy. For each study, we estimated the risk ratio comparing CNI treatment to controls. The primary outcome was presence of partial or complete remission at six months of active therapy in order to use data of all studies (results at 1 year were not available for all studies). We then performed a meta-analysis to pool relative risks of remission across all three studies. Of note, the definition of complete remission was very similar in all studies whereas definitions of partial remission had variability between studies, but implied a reduction in proteinuria. We used a random effects model which accounts for random error and inter-study variability to estimate the pooled effect measures with 95% confidence intervals. We calculated the Higgin's I-squared statistic that provided a percentage of variance between studies that is attributable to heterogeneity (i.e. not to chance).

RESULTS:

Search results:

Our literature search with appropriate filters yielded 152 citations. We excluded 136 citations because quick review of title/abstract did not meet our inclusion criteria, or satisfied one of our exclusion criteria. Two articles were further excluded because they were unable to be retrieved by our librarian (Figure 1). A total of 14 articles were reviewed in detail. Of these, 6 were

excluded because they did not meet inclusion criteria on closer examination and 8 articles were reviewed for quality assessment and included in this systematic review.

Study characteristics:

Table 1 summarizes the characteristics of all included studies. There were 6 randomized controlled trials and 2 retrospective cohort studies. Studies were of varying sizes, ranging from 28 to 138 patients. All studies included patients with biopsy-proven FSGS, but two also included patients with minimal-change disease. Most studies included patients with any degree of proteinuria; only 2 studies used the more stringent entry criterion of nephrotic syndrome, which includes hypoalbuminemia, hyperlipidemia and presence of edema. Most studies excluded patients with substantial renal insufficiency (eGFR <45 mL/min/1.73m^2); only one retrospective study included patients with any eGFR at baseline. The studies varied considerably in the demographics of the patients included, especially with respect to age; 1 study included exclusively children and 3 studies exclusively adults. Similarly, the definition of steroid resistance for inclusion in the clinical studies varied from a minimum of 2 to 12 weeks of treatment. The most frequent outcome examined was reduction in proteinuria (complete or partial remission). Table 1 summarizes the various definitions used for complete and partial remission. Complete remission was defined in a fairly similar way across studies whereas there was significant variability in the definitions of partial remission. Our quality assessment focused on internal validity (using the Cochrane Collaboration's tool for assessing risk of bias and the Newcastle

Ottawa quality assessment scale), external validity and precision, and is summarized in Tables 2, 3 and 4.

Findings:

Calcineurin inhibitors vs. control

Three randomized controlled trials compared cyclosporine to placebo (with or without low-dose prednisone) or supportive treatment in a steroid-resistant population[7-9]. All three studies showed a higher proportion of patients treated with CNIs achieving partial or complete remission than the comparison group. However, these studies were of relatively short duration (26 to 200 weeks); as such, change in serum creatinine was analysed as a secondary outcome.

Ponticelli et al.[9] conducted an open randomized trial comparing cyclosporine without glucocorticoids to supportive treatment. The study was not limited to patients with biopsy-proven FSGS. There were 14 patients with biopsy-proven FSGS in each arm, and an additional 8 patients in the cyclosporine group and 5 in the supportive treatment group with minimal change disease on biopsy. A 'rescue treatment' with glucocorticoids was allowed in the supportive treatment group if patients experienced "rapidly progressive renal failure or very severe nephrotic syndrome" (although neither were clearly defined). During the first year of active treatment, the cyclosporine group had a significantly higher proportion of patients in remission (36% complete; 27% partial) compared to the control group (16% partial). Among patients with biopsy-proven FSGS, 8 in the cyclosporine group achieved remission within the first year (3 complete; 5 partial); the proportion in the supportive care group who achieved remission was

not reported. The randomized treatment allocation helped minimize the risk of bias. However, the sample was small and the groups were not balanced on some important potential confounders. For example, a greater proportion of patients in the cyclosporine group (36%) than in the supportive care group (26%) had minimal-change disease on biopsy, conceivably biasing towards greater response in the cyclosporine group. In addition, 46% in the cyclosporine group were children compared with 37% in the supportive care group. The lack of blinding also opened the possibility of biased outcome ascertainment. Furthermore, the generalizability of study results to an exclusively adult or pediatric primary FSGS population is questionable given the heterogeneity of the study population. Another significant limitation is the exclusion of patients with eGFR <80 mL/min/1.73m^2 (children) or <60 mL/min/1.73m^2 (adults) at baseline.

In a randomized trial including patients aged 6 months to 21 years old, Lieberman et al.[8] compared cyclosporine without glucocorticoids (n=16) to placebo (n=15). Patients were treated with study drug, without concomitant prednisone. Among those who completed 6 months of active treatment, all patients in the cyclosporine group achieved remission (33% complete; 67% partial) compared with only 17% in the placebo group (100% partial) by 6 months. However, the definitions of partial and complete remission were unclear. Complete remission was defined as a decline in proteinuria to the normal range, with no mention of a requirement for stability in renal function. Partial remission was defined as a ‘reduction in proteinuria’, where the level of proteinuria still remained in the ‘supranormal’ range, with no mention of the magnitude of the

reduction or a threshold goal in proteinuria. In addition, sequence generation and allocation concealment were not clearly explained, but randomized groups were well balanced. Generalizability was limited by the inclusion of patients with 'supranormal' proteinuria, rather than those meeting the criteria for nephrotic syndrome.

The randomized controlled trial by Cattran et al.[7] is considered a landmark study. Both groups were treated with low-dose prednisone. At 26 weeks of active treatment, the proportion of subjects reaching complete or partial remission was significantly higher in the cyclosporine arm (12% complete; 57% partial) than in the placebo arm (4% partial). By week 78 (48 weeks after discontinuation of therapy), relapse in proteinuria occurred in 60% of those who attained a remission. By week 104 (74 weeks after discontinuation of therapy), 8% of patients were in partial remission in the placebo group compared with 32% in remission in the cyclosporine group (4% complete; 28% partial). Strengths of this study include a design minimizing bias and confounding, and a larger number of patients than in previous studies (n=49). Although randomization procedures were appropriate, the placebo group had a higher proportion of males (74 vs. 65%) and African Americans (14 vs. 4%), and heavier proteinuria at presentation (8.7 vs. 6.9 g/day) than the cyclosporine group. The generalizability of this study is somewhat limited by the relatively small proportion of African Americans, and the explicit exclusion of patients with collapsing variant.

All 3 randomized controlled trials comparing cyclosporine to placebo or supportive treatment (with or without glucocorticoids) pointed towards a better

chance of partial or complete remission with calcineurin inhibitors after 6-12 months of active therapy. As illustrated in Figure 2, the pooled relative 'risk' of proteinuria remission associated with cyclosporine was 7.0 (95% confidence interval 2.9-16.8) compared with placebo/supportive therapy. There was very low heterogeneity among these studies with an I-squared of 0%.

Calcineurin inhibitors vs. mycophenolate mofetil

Only one randomized controlled trial compared the efficacy of MMF and dexamethasone pulses (n=66) to cyclosporine (n=72) in steroid-resistant primary FSGS.[10] Both groups received low-dose prednisone for six months. The primary outcome was remission of proteinuria, which was classified into one of six categories (Figure 3). At week 52, on active treatment, the odds of at least partial remission were lower for the MMF/dexamethasone group, but the difference did not reach statistical significance. Among those who achieved at least partial remission at week 52, 33% in the cyclosporine group relapsed at week 78 (26 weeks after discontinuation of therapy) compared to 18% in the MMF/dexamethasone group. Better preservation of eGFR was seen in the MMF/dexamethasone arm. This randomized controlled trial was generally well designed. However, treatment group was not blinded, opening the possibility of bias in outcome ascertainment. The study groups were well balanced at baseline. Generalizability was limited by the inclusion of patients with mild proteinuria (24% had proteinuria <3 g/day [urinary protein-to-creatinine ratio <2g/g]), and the fact that patients with no remission after only 4 weeks of

treatment with high-dose glucocorticoids were qualified as glucocorticoid resistant.

Calcineurin inhibitors vs. alkylating agents

Two randomized controlled trials compared use of an alkylating agent to calcineurin inhibitors in steroid-resistant idiopathic FSGS. In both studies, there was no difference between treatment groups in the proportion of patients who achieved remission in proteinuria.

The first study was characterized by a complex treatment regimen, including several different immunosuppressives in each study arm.[11] The cyclosporine group (n=34) was initially treated with salicylic acid and prednisolone, and if no remission occurred, switched to cyclosporine alone. The chlorambucil group (n=23) was initially treated with prednisolone alone, and if no remission occurred, chlorambucil was added. Then, if no remission occurred, patients were finally treated with cyclosporine alone (n=10). The proportion of patients achieving remission within 48 months of follow-up was almost identical in each study arm (62% in the cyclosporine group vs. 65% in the chlorambucil group). Specifically, for the cyclosporine-treated, complete remission occurred in 23% and partial remission occurred in 38% of patients, with a mean duration of administration of 23±16.5 months. Among the chlorambucil-treated over 6 to 12 weeks, complete remission occurred in 17% and partial remission occurred in 48% of patients within 48 months of follow-up. Renal survival was 83% for both groups after four years of follow-up. Although the study arms were well balanced at baseline, this study had several important limitations. First, random sequence

generation was inadequate, allocation concealment was undefined, and there was no blinding. Furthermore, the complexity of the treatment regimen, and the fact that patients from both groups may have received cyclosporine, make the superiority of one drug over the other difficult to ascertain. Also, the definition of remission did not include a magnitude of reduction in proteinuria over time (e.g. 50% as described in the 2012 KDIGO guideline).

A small study from China compared proteinuria remission among patients with steroid-resistance or steroid-dependence randomized to IV cyclophosphamide (n=18) or tacrolimus (n=15).[12] Both groups were treated with glucocorticoids, and patients with no response after six months were randomized. After 12 months of therapy, there was no significant difference in remission between the two groups: 66.7% in the cyclophosphamide group (50% complete; 16.7% partial) and 73.3% in the tacrolimus group (40% complete; 33.3% partial). The 12-month relapse rate was similar between the two groups (27.8% for cyclophosphamide-treated vs. 26.7% for tacrolimus-treated). Patients treated with cyclophosphamide had poorer renal function at baseline. One patient was withdrawn from the study due to ESKD (cyclophosphamide-treated). The study had several limitations. Sequence generation and allocation concealment were not clearly defined. This study was small and was conducted in a single center in China, which hamper its generalizability to other countries.

Miscellaneous cohort studies including calcineurin inhibitors

A retrospective cohort study by Goumenos et al.[13] compared renal outcomes associated with immunosuppressive therapy (prednisone alone,

prednisone and azathioprine, or prednisone and cyclosporine) with those associated with supportive care. A higher proportion of patients treated with immunosuppressives than supportive care achieved complete or partial remission in proteinuria within the first year of follow-up. The mean duration of therapy was 20±6 months. Treated patients also showed better renal survival using the endpoint of 50% increase in serum creatinine over 5 years of follow-up. However, this study did not adjust for factors influencing decision to treat or choice of treatment such as baseline proteinuria. The multivariate analysis only included presence of glomerulosclerosis at initial kidney biopsy and baseline serum creatinine. Moreover, patients treated with immunosuppression presented with a significantly lower serum albumin than those treated with supportive care (28 vs. 34 g/l), which biased towards an underestimation of treatment effect. This study was characterized by a heterogeneous treatment group, with a small number of patients treated with each immunosuppressive agent. Only 7 patients were treated with prednisolone and cyclosporine. Therefore, treatment with prednisolone alone could not be compared to treatment with cyclosporine and prednisolone due to a lack of statistical power.

A study by Ehrich et al.[14] was conducted to examine the potential benefit of adding IV methylprednisolone to cyclosporine and prednisolone in the treatment of steroid-resistant FSGS in 52 children. Compared with those untreated with IV methylprednisolone, significantly more patients treated with IV methylprednisolone (84 vs. 64%) had cumulative sustained remission (complete or partial). This study had some weaknesses. The untreated cohort was selected

from a different patient population (different time period) than the IV methylprednisolone group, and there was no control for important confounding factors (e.g. baseline proteinuria or estimated glomerular filtration) in the analysis. The generalizability of this study to adults is also limited because the study population was primarily pediatric.

DISCUSSION:

The bulk of existing evidence suggests that CNIs in combination with glucocorticoids may increase the likelihood of complete or partial remission of proteinuria among individuals with idiopathic FSGS. Among steroid-resistant patients, only cyclosporine has been evaluated in prospective trials with comparison against supportive therapy or placebo (with or without low-dose glucocorticoids). Cyclosporine appears effective in inducing remission, but is associated with high relapse rates following discontinuation. Two studies comparing CNIs to supportive therapy/placebo had adequate internal validity with a well-executed randomized design, and established CNIs as effective in achieving remission in proteinuria.[7,9]

Subsequent prospective studies compared CNIs to other active agents. The evidence supporting alkylating agents in steroid-resistant FSGS is not convincing, and CNIs appear to be more effective than MMF.

Only one retrospective study addressed the efficacy of CNIs as a first-line treatment for FSGS.[13] This study was small, and there was no direct comparison with other immunosuppressive agents. In contrast, MMF has been evaluated as a steroid-sparing agent in first-line therapy of primary FSGS. A small prospective

study (n=33)[15] compared MMF (1 g twice daily for 6 months) combined with low-dose glucocorticoids to high-dose glucocorticoids alone for 3 to 6 months. Outcomes were similar for both groups, with 70% of patients in remission in the MMF group compared to 69% in the high-dose glucocorticoid group.

The clinical trials summarized in this review were of relatively short duration, and included fairly small numbers of patients. As a result, they were not able to assess the impact of immunosuppressive therapy on renal or patient survival. Hard endpoints such as ESKD and mortality are infrequent, and require many years of follow-up. Furthermore, the relatively small number of included studies and their heterogeneity with respect to treatment protocols, and possible publication bias, limit conclusions drawn from this systematic review.

This systematic review is distinguished from the KDIGO guideline by its inclusion of more recent studies, and a systematic critical appraisal of the internal validity of each study using recognized tools (Cochrane Collaboration's tool for assessing risk of bias and Newcastle Ottawa quality assessment scale). This study also includes a meta-analysis of the evidence comparing cyclosporine to supportive therapy and placebo (with and without glucocorticoids). This provides a weighted effect estimate of the association between cyclosporine therapy and remission in proteinuria. However, it was not possible to perform other analyses for subgroups of interest (e.g. children) due to paucity of data and heterogeneity in treatment regimens.

In conclusion, further research is needed to assess effectiveness of CNIs as first-line therapy in primary FSGS. A large randomized trial would be challenging

with potential issues in recruitment and retention because glucocorticoids have been used as first-line treatment for decades. Good quality observational studies would be particularly suitable to measure the effect of calcineurin inhibitors on renal survival in steroid-naïve FSGS population.

Figure 1. Literature search and article selection

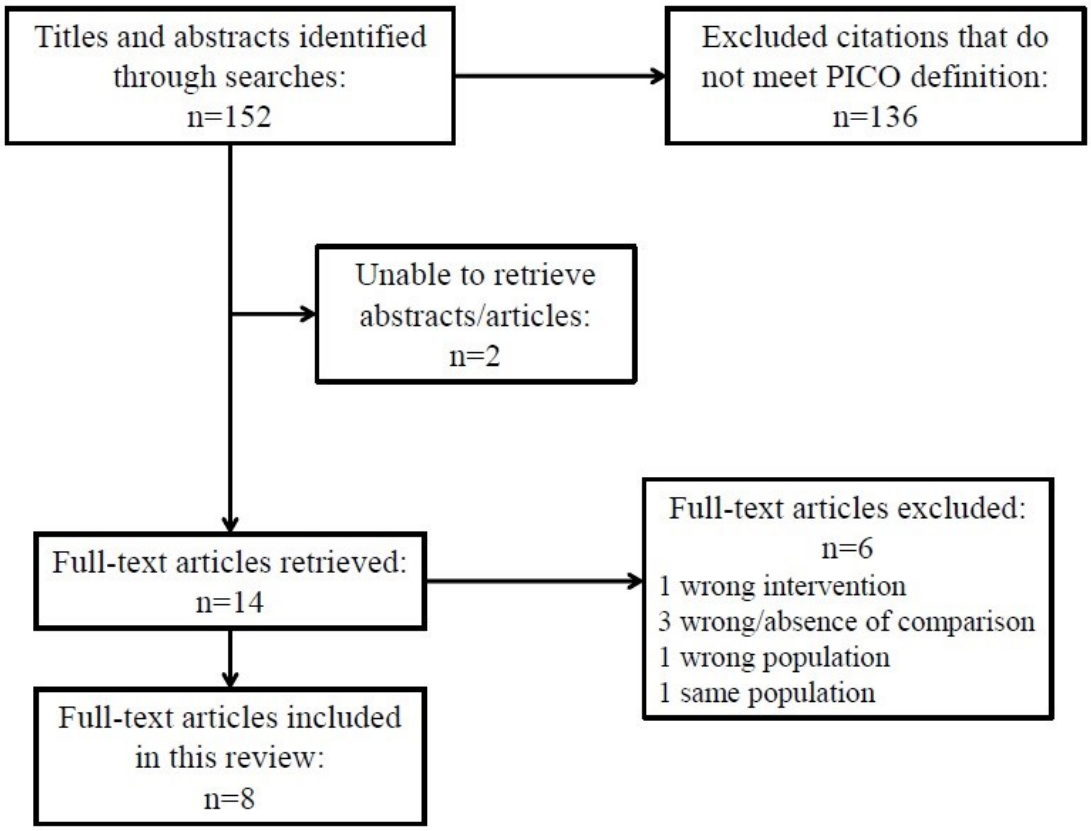

Figure 2. Meta-analysis of remission with calcineurin inhibitors in steroid-resistant FSGS

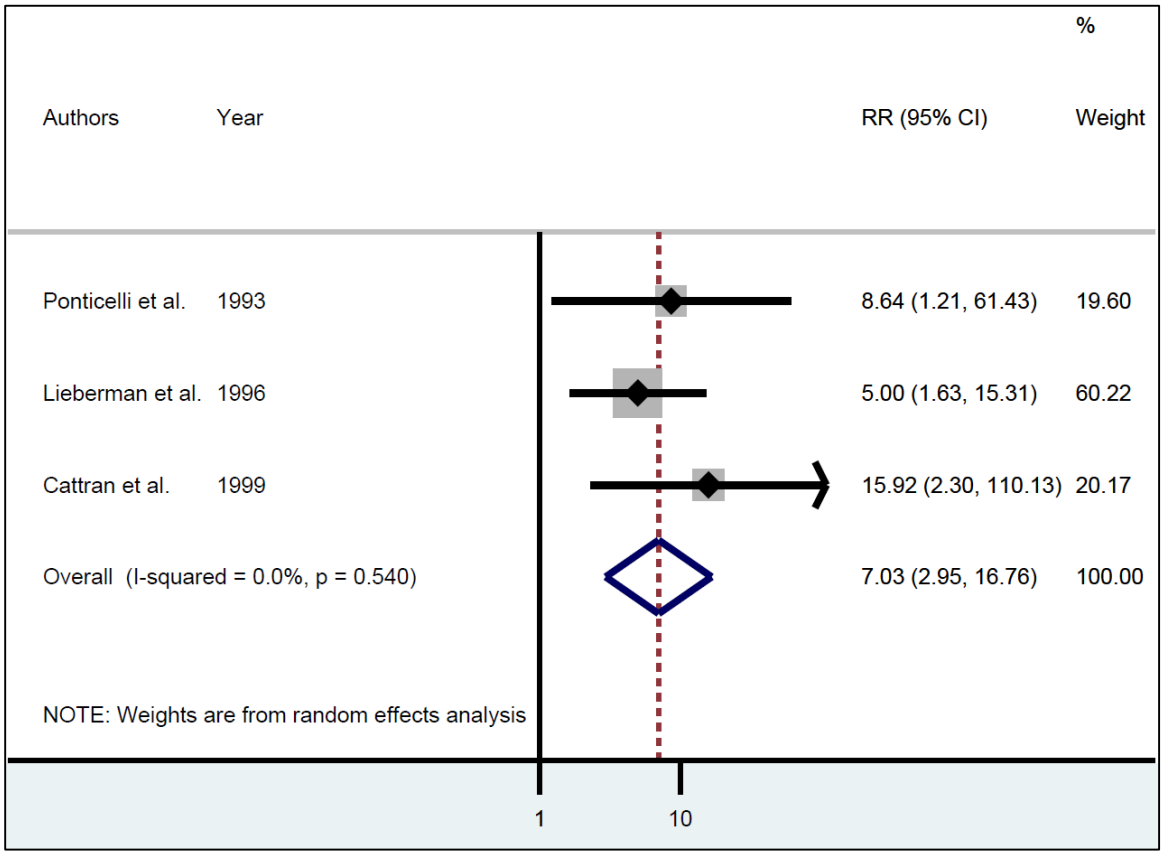

Figure 3. Definitions of the six categories of proteinuria remission from the FSGS Clinical Trial (reproduced from Gipson et al. Kidney International 2011). Category 1: patients who achieved a complete remission by week 26 that was sustained to week 52; Category 2: patients who achieved a partial remission at week 26 and then a complete remission at week 52; Category 3: patients who achieved a partial remission by week 26 that was sustained to week 52; Category 4: patients who achieved a partial remission at week 26 and then had recurrence of proteinuria before week 52; Category 5: patients who achieved a partial remission before week 26 and then had a recurrence of proteinuria before week 26; Category 6: patients who never had a Up/c reduction of >50% and an absolute value below 2 g/g.

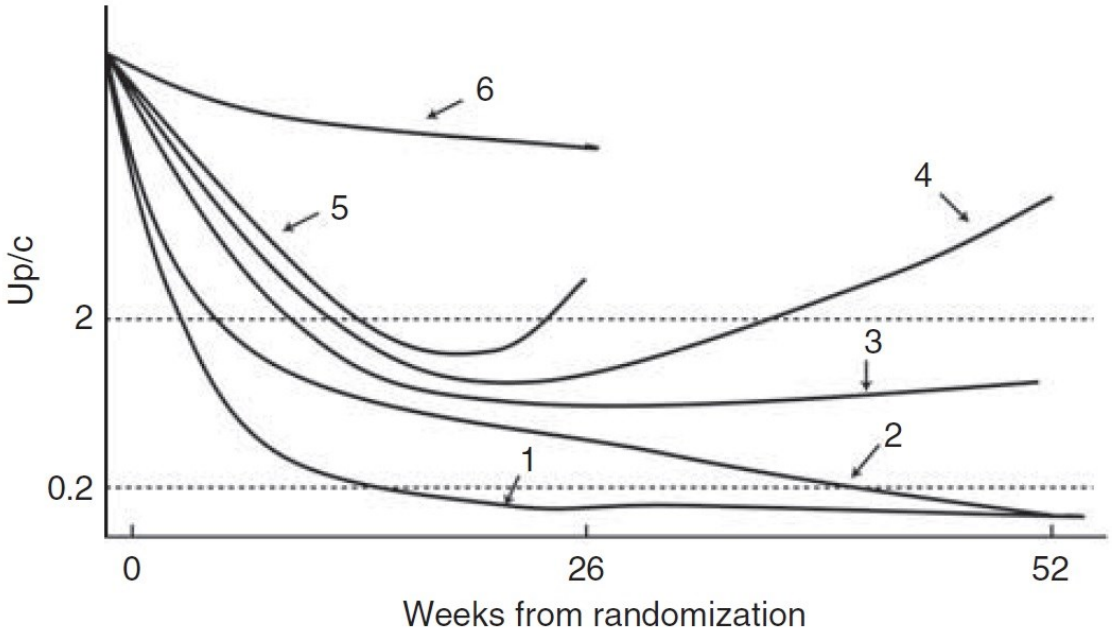

Table 1. Characteristics of reviewed studies

Authors, year	Study design	Participants	Treatment group	Control Group	Outcomes	Follow up duration
Randomized controlled trials in steroid-resistant FSGS						
Ponticelli et al., 1993	Open randomized trial	Steroid resistance: 6 weeks of prednisone 1 mg/kg/d (or 60 mg/m^2/d for children) Inclusion criteria: Biopsy-proven FSGS or MCD, nephrotic syndrome (proteinuria >40 mg/m^2/h or >3.5g/d with variable degree of edema), CrCl >80 mL/min/1.73m^2 in children and >60 mL/min/1.73m^2 in adults Country: Italy Age: 2-65 years Number: Treatment group (22), control group (19)	Adults: Cyclosporine 5 mg/kg/d in 2 divided doses Children: 6 mg/kg/d in 2 divided doses RAAS blockade	Supportive treatment with RAAS blockade. Rescue therapy with glucocorticoids was allowed.	Partial remission: Proteinuria <40 mg/m^2/h or < 3.5g/d for 3 days Complete remission: Proteinuria <4 mg/m^2/h or <0.2g/d for 3 days	Cyclosporine-treated: 18 months (3 to 24) Controls: 24 months (12 to 24)
Lieberman et al., 1996	Placebo-controlled, double-blind, RCT	Steroid resistance: 4 weeks of prednisone at 60 mg/m^2/d Inclusion criteria: Biopsy-proven FSGS, proteinuria >4 mg/m^2/h or Up/c >0.18 in >2 y.o. and >0.49 in <2 y.o., GFR >40 mL/min/1.73m^2 Country: USA Age: 6 months to 21 years Number: Treatment group (15), control group (15)	Cyclosporine 0.03 mL/kg (3.0 mg/kg) in 2 divided doses	Placebo in 2 divided doses	Partial remission: decrease in proteinuria, but supranormal range Complete remission: normal range proteinuria	6 months
Cattran et al., 1999	Placebo-controlled, single blind, RCT	Steroid resistance: 8 weeks of prednisone at ≥1 mg/kg/d Inclusion criteria: Biopsy-proven FSGS, proteinuria ≥ 3.5g/d or ≥ 50 mg/kg, CrCl ≥ 42 mL/min/1.73m^2, BP ≤ 135/90	Cyclosporine 3.5 mg/kg in 2 divided doses and low-dose prednisone at 0.15 mg/kg/d	Placebo in 2 divided doses and prednisone at 0.15 mg/kg/d (maximum daily dose of 15 mg)	Partial remission: 50% reduction of initial proteinuria and ≤3.5 g/d with stable kidney function. Complete remission: proteinuria ≤0.3 g/d +	200 weeks

		mmHg, dietary protein intake ≤ 0.8 g/kg Country: Canada/USA Age: 18-70 years Number: Treatment group (26), control group (23)	(maximum daily dose 15 mg) RAAS blockade	RAAS blockade	stable kidney function. ESKD: CrCl < 12 mL/min, start of dialysis or transplantation or study closure.	
Heering et al., 2004	Open RCT	Steroid resistance: 2-6 weeks of high dose prednisone (1.5 mg/kg/d). Inclusion criteria: Biopsy-proven FSGS, proteinuria > 3.5g/d and serum creatinine <177 µmol/L. Country: Germany Age: 18-79 years Number: Treatment group (23), control group (34)	Prednisolone 1.5 mg/kg/d for 2-6 weeks. If no remission, prednisolone 1.5 mg/kg/d and chlorambucil 0.1-0.4 mg/kg/d. If no remission, cyclosporine 5 mg/kg/d RAAS blockade at the discretion of the physician	Prednisolone 1.5 mg/kg/d and AAS 500mg/d for 6 weeks. If no remission, cyclosporine 5 mg/kg/d. RAAS blockade at the discretion of the physician	Partial remission: proteinuria <3.5 g/d Complete remission: <0.2 g/d	48 months
Gipson et al., 2011	Open RCT	Steroid resistance: 4 weeks of high-dose glucocorticoids Inclusion criteria: Biopsy-proven FSGS, estimated GFR ≥40 mL/min/1.73 m^2, Up/c >1 g/g Country: USA Age: 2-40 Number: Treatment group (66), control group (72)	MMF 25-36 mg/kg/d in 2 divided doses (maximum daily dose 2 g/d) and IV dexamethasone (0.9 mg/kg) for 46 doses + Prednisone 0.3 mg/kg per dose (maximum 15mg) every other day for the first 6 months RAAS blockade	Cyclosporine 5-6 mg/kg in 2 divided doses (maximum daily dose 250 mg) + Prednisone 0.3 mg/kg per dose (maximum 15mg) every other day for the first 6 months RAAS blockade	Six-level categorical assessment of proteinuria remission during the first 52 weeks after randomization	78 weeks

Ren et al., 2013	Open RCT	Steroid resistance: 12 weeks of prednisone (1 mg/kg/d) or relapse on a tapering dose of prednisone Inclusion criteria: Biopsy-proven FSGS, estimated GFR ≥30 mL/min/1.73m^2, nephrotic syndrome or a tendency to develop full nephrotic syndrome, blood pressure ≤135/85 mmHg, no exposure to immunosuppression 3 months prior to randomization Country: China Age: 18-75 years Number: Treatment group (18), control group (15)	IV Cyclophosphamide 0.5-0.75 g/m^2/month and prednisone 0.8 mg/d RAAS blockade	Tacrolimus 0.1 mg/kg/d and prednisone 0.5 mg/kg/d RAAS blockade	Partial remission: decrease of 50% in proteinuria and stable eGFR Complete remission: Proteinuria <0.4 g/d and stable eGFR	12 months
Cohort studies						
Goumenous et al., 2006	Retrospective cohort study	Steroid resistance: None Inclusion criteria: Biopsy-proven FSGS, <65 years, proteinuria >1 g/d, serum creatinine <2.5 mg/dL Country: UK and Greece Age: <65 years Number: Treatment group (25), control group (26)	Prednisone 1 mg/kg or prednisone 1 mg/kg and azathioprine 2 mg/kg or prednisone 0.5 mg/kg and cyclosporine 3 mg/kg RAAS blockade	Supportive treatment with RAAS blockade	Renal failure: -50% increase of baseline serum creatinine -Doubling of baseline serum creatinine -End-stage renal disease Partial remission: proteinuria between 0.3 and 3 g/d. Complete remission: <0.3g/d Remission in proteinuria was only assessed in patients with urinary protein > 3g/d.	5 years
Ehrich et al., 2007	Retrospective cohort study	Steroid resistance: 4 weeks of prednisone 60mg/m^2/d followed by 40mg/m^2 on alternate day for 4 weeks	IV methylprednisolone 300-1000 mg/d and	Cyclosporine 150 mg/m^2/d and prednisolone 40 mg/m^2/d	Complete remission: proteinuria <166 mg/1.73m^2/d for 3 days and serum albumin >35 g/L	5±3.6 years

		Inclusion criteria: Biopsy-proven FSGS for the idiopathic FSGS subgroup, nephrotic syndrome (proteinuria >40 mg/m^2 body surface/h or 1.66 g/1.73 m^2/day, serum albumin >25 g/l, hypercholesterolemia [according to age], edema) Country: Germany Age: 2-20 years Number: Treatment group (25), control group (27)	cyclosporine 150 mg/m^2/d and prednisolone 40 mg/m^2/d		Partial remission: proteinuria between 166 mg/1.73m^2/d and 2 g/1.73m^2/d, and serum albumin >25 g/L	

Table 2. Visual assessment of internal validity of randomized controlled trials

Studies	Sequence generation	Allocation concealment	Blinding	Incomplete outcome	Selective outcome reporting	Other sources of bias
Ponticelli et al.						
Lieberman et al.						
Cattran et al.						
Heering et al.						
Gipson et al.						
Ren et al.						

Low risk of bias
High risk of bias
Unclear

Table 3a. Quality assessment of randomized controlled trials (internal validity)

Authors, year	Internal validity					
	Sequence generation	*Allocation concealment*	*Blinding of participants, personnel and outcome assessors*	*Incomplete outcome data*	*Selective outcome reporting*	*Other sources of bias*
Ponticelli et al., 1993	Low risk of bias; the investigators referred to a random number table	Low risk of bias; the investigators used sequentially numbered, opaque, sealed envelopes	High risk of bias; no blinding, and likely that the outcome might be influenced by the lack of blinding. Rescue therapy was permitted in the supportive group.	Low risk of bias; only 2 patients in the CSA group dropped out before study end (1 due to infection; 1 due to lack of effect). Unlikely to have a clinically relevant impact on the intervention effect estimate.	Low risk of bias; all pre-specified outcomes have been reported in a pre-specified way using the intention-to-treat principle	Low risk of bias; no extreme baseline imbalance between groups, and well-defined outcome with objective criteria.
Lieberman et al., 1996	Low risk of bias; the investigators referred to sequence generator using a computer random number generator	Uncertain risk of bias; the method of concealment is not described	Low risk of bias; blinding of participants and key study personnel (patients and pediatric nephrologists)	Low risk of bias; 2 patients in each group were withdrawn because of noncompliance with the study protocol	Low risk of bias; pre-specified outcomes have been reported in a pre-specified way	High risk of bias; potential risk of information bias due unclear definition of partial remission in proteinuria, and per-protocol analysis
Cattran et al., 1999	Low risk of bias; the investigators referred to a random number table	Low risk of bias; central allocation was used	Low risk of bias; blinding of patients and outcome assessors, but no blinding of clinicians. Clinicians were aware of group allocation because of safety reasons.	Low risk of bias; all patients were followed at least 26 weeks	Low risk of bias; the pre-specified outcomes have been reported in the pre-specified way	Low risk of bias; baseline characteristics between groups were balanced
Heering et al., 2004	High risk of bias; sequence generated by date of birth	Uncertain risk of bias; insufficient information on method of concealment	High risk of bias; no blinding	Low risk of bias; data were missing in only one patient in chlorambucil group	Low risk of bias; the pre-specified outcomes have been reported in the pre-specified way	Although not statistically significant, there was a larger proportion of males in the

						non-chlorambucil group. Definition of partial remission did not include a magnitude of reduction in proteinuria over time.
Gipson et al., 2011	Low risk of bias; use of randomly permuted block of random size	Low risk of bias; central allocation by the Data Coordinating Center, and study investigators blinded to these randomization schedules	Low risk of bias; outcome measurements are not likely to be influenced by the lack of blinding	Low risk of bias; there were few missing visits in each group, well-balanced between the two groups. Two patients missed all three outcome assessment visits, but did not receive study drug following randomization.	Low risk of bias; the pre-specified outcomes have been reported in the pre-specified way. A multilevel ordinal categorical outcomes were used instead of simpler dichotomous classifications for remission in order to increase statistical power and to allow patients to switch to alternative therapy if remission was not achieved.	Low risk of bias
Ren et al., 2013	Uncertain risk of bias; no mention on how sequence generation was performed	Uncertain risk of bias; no mention of the method of concealment of allocation	Low risk of bias; no blinding but outcome measurement is not likely to be influenced by lack of blinding	Low risk of bias; 27 patients out of 33 completed the protocol. 3 patients in each group had missing data.	Low risk of bias; the pre-specified outcomes have been reported in the pre-specified way	Low risk of bias; well-balanced groups at baseline

Table 3b. Quality assessment of randomized controlled trials (external validity)

Authors, year	Effect estimate	Effect estimate precision	External validity
Ponticelli et al., 1993	During the first year, 13 patients out of 22 (59%) reached partial or complete remission compared to 3 out of 19 in the supportive treatment group (16%) RRR: 72% ARR: 43%	Highly statistically significant with p<0.001	A large proportion of patients included in this study showed minimal change disease on renal biopsy. Exclusion of patients with baseline eGFR <60 mL/min/1.73m^2 is also restrictive. Thus, it would be difficult to generalize results to a primary FSGS population.
Lieberman et al., 1996	At six months, 12 patients out of 12 (100%) reached partial or complete remission compared to 2 out of 12 (17%) in the placebo group RRR: 83% ARR: 83%	Magnitude of significance was not reported	Duration of high-dose steroid therapy was only 4 weeks. Patients with only supranormal proteinuria (and not nephrotic syndrome) were included in the study. Results only generalizable to a pediatric population.
Cattran et al., 1999	At week 26 of active treatment, remission in proteinuria occurred in 69% of the CSA group (18/26 patients; 12% complete and 57% partial) compared with a 4% partial remission (1/23 patient) rate in the placebo group RRR: 94% ARR: 65%	Highly statistically significant with p<0.001	A large proportion of patients included were Caucasian. Systematic exclusion of collapsing variant.
Heering et al., 2004	At 48 months, 21 patients out of 34 (62%) reached remission in the non-chlorambucil group compared to 15 patients out of 23 (65%) in the chlorambucil group. RRR: 5% ARR: 3%	Magnitude of significance was not reported	Complex protocol using AAS; both groups may have been exposed to cyclosporine. Included patients with proteinuria >3.5g/d. Other features of the nephrotic syndrome not used as inclusion criteria.
Gipson et al., 2011	The odds of a least a partial remission at week 52 were lower for MMF/DEX than for CSA (OR 0.59; 95% CI 0.30-1.18) but did not reach statistical significance.	Precise estimates with narrow confidence interval	Heterogeneous population with a large proportion of children; these results are therefore not easily generalizable to an adult population. Inclusion of patients with mild range proteinuria (24% had proteinuria <3g/d). Steroid resistance defined as 4 weeks of high dose steroids.

Ren et al., 2013	At 12 months, 12 patients out of 18 (67%) reach remission in the CTX group compared to 11 out of 15 (73%) in the TAC group RRR: 8% ARR:6%	Non-significant estimate	Single center study in China which makes result more or less applicable in North America with a Caucasian population.

Table 4a. Quality assessment of cohort studies

Authors, year	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Outcome not present at start of study	Comparability of cohorts on the basis of the design or analysis
Goumenos et al., 2006	Somewhat representative of the average FSGS in the community	Drawn from the same community as the exposed cohort	By secure record	Yes	Analysis controls for different parameters related to clinical outcome, including baseline serum creatinine. Sample too small to compare efficacy between azathioprine and cyclosporine.
Ehrich et al., 2007	Truly representative of the average steroid-resistant nephrotic syndrome with pediatric FSGS in the community	Drawn from a different source, which consists of patients previously exposed to immunosuppressive therapy in a different era	By secure record	Yes	No control by design or analysis for important potential confounders

Table 4b. Quality assessment of cohort studies (continued)

Authors, year	Assessment of outcome	Duration of follow up	Adequacy of follow up	Treatment effect estimate	Treatment effect estimate precision	External validity
Goumenos et al., 2006	By record linkage	5 years, adequate	Complete follow up for all subjects	At 5 years, 15 treated patients (75%) had remission in proteinuria compared to 4 (31%) in the untreated group	No information on confidence interval	Heterogeneous population with respect to treatment regimen.
Ehrich et al., 2007	By record linkage	Approximately 5 years, which is adequate	Complete follow up for all subjects	Patients in IV methylprednisolone group had higher cumulative sustained remission (84%) than patients without added methylprednisolone (64%)	No confidence interval given, but significant p value (0.02)	Patients aged from 2 to 20 years, therefore limited generalizability to adults. Single center study.

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Preface to Chapter 3:

This chapter presents a study describing the factors influencing initiation and choice of immunosuppressive therapy in primary FSGS. This chapter also investigates the role of CNI therapy in improving renal survival of patients with primary FSGS. A large cohort of patients with primary FSGS was used to perform survival analyses considering the different types of first choice immunosuppressive agents (high-dose glucocorticoids or CNIs with or without glucocorticoids) as the exposure of interest.

Chapter 3: The Role of Calcineurin Inhibitor Therapy in Treatment of Primary Focal Segmental Glomerulosclerosis.

ORIGINAL ARTICLE

The Role of Calcineurin Inhibitor Therapy in Treatment of Primary Focal Segmental Glomerulosclerosis

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ABSTRACT:

Background: There is no clear evidence supporting the role of calcineurin inhibitors (CNIs) as early treatment in focal segmental glomerulosclerosis (FSGS). We sought to determine the patient and disease characteristics associated with choice of therapy early in disease course, and to estimate the association between choice of therapy and end-stage kidney disease (ESKD) in primary idiopathic FSGS.

Methods: We studied an inception cohort of patients diagnosed with primary FSGS by kidney biopsy between 1980 and 2012. Factors influencing choice of therapy were identified using logistic regression. We used time-dependent Cox models to compare time to ESKD between different therapies.

Results: A total of 458 patients were studied (173 treated with glucocorticoids alone; 90 with CNIs ± glucocorticoids; 12 with other agents; 183 with no immunosuppressives). Tip lesion variant, absence of severe renal dysfunction (eGFR ≥30 mL/min/1.73m^2) and hypoalbuminemia were associated with a higher likelihood of exposure to any immunosuppressive therapy. Only tip lesion was associated with choice of glucocorticoids over CNIs. In adjusted Cox regression, immunosuppressive therapy with glucocorticoids and/or CNIs was associated with better renal survival than no immunosuppression [hazard ratio 0.49 (95% confidence interval 0.28, 0.86)]. Although not statistically significant, CNIs ± glucocorticoids were associated with a lower likelihood of ESKD compared to glucocorticoids alone [hazard ratio 0.42 (95% confidence interval 0.15, 1.18)].

Conclusions: There may be a role for CNIs as part of the early immunosuppressive regimen in primary FSGS, but their superiority over glucocorticoids alone remains undetermined.

Key words: focal segmental glomerulosclerosis, calcineurin inhibitors, glucocorticoids, end-stage renal disease

INTRODUCTION:

In adults, idiopathic FSGS represents the most common cause of primary nephrotic syndrome and the most common cause of ESKD related to glomerular disease.[1,2] FSGS encompasses different histologic variants, which differ in their epidemiology, clinical course and response to therapy.[3]

Establishment of effective evidence-based therapies for FSGS has been hampered by lack of access to large patient populations. Glucocorticoids have historically been used as a first-line therapy in FSGS based on retrospective or uncontrolled prospective cohort studies;[4-7] however, no randomized controlled trials were performed to provide direct evidence of their efficacy in preserving renal function. Furthermore, FSGS variant was not taken into consideration in the choice of therapy in these studies.[8] The major randomized controlled trials in FSGS evaluated response to therapy (remission in proteinuria) among steroid-resistant FSGS patients.[9,10] The role of CNIs (tacrolimus or cyclosporine) has never been evaluated in primary FSGS with respect to renal survival, nor were they compared to high dose glucocorticoids in a head-to-head randomized controlled trial.

In this study, we sought to determine the patient and disease characteristics associated with choice of therapy, and to estimate the association between choice of therapy and renal outcome (ESKD) in primary idiopathic FSGS. We hypothesized that the use of CNI therapy is associated with a decrease in the likelihood of ESKD after controlling for other factors affecting renal survival.

METHODS:

Study design and population:

All patients in the Glomerular Disease Collaborative Network (GDCN) with biopsy-proven FSGS who were diagnosed between 1980 and 2012 were considered for this inception cohort study. Patients with a known secondary cause of FSGS, such as human immunodeficiency virus, hepatitis B and C, intravenous drug use, sickle cell disease, single kidney, reflux nephropathy, other glomerulonephritis or transplant recipients were excluded. Perihilar variant FSGS, common in secondary forms of FSGS and believed to be an 'adaptative response to nephron loss or glomerular hypertension'[11], was excluded. All biopsy specimens had a minimum of five glomeruli assessed by light microscopy. FSGS variant was determined using the biopsy report, except for collapsing cases. All available histological biopsy slides from patients with a previous diagnosis of collapsing FSGS were re-verified independently by two nephropathologists (A.M.G. and J.C.J.) who were blinded to the clinical course, treatment and outcome of patients. Patients with any level of proteinuria were included in the study in order to examine the full spectrum of FSGS. All subjects provided written, informed consent for participation in GDCN studies. This study was approved by the University of North Carolina's Institutional Review Board, in agreement with the Declaration of Helsinki.

Clinical data and definitions:

Clinical and laboratory variables were extracted from medical records from the time of renal biopsy to the last available follow up visit and/or initiation of

renal replacement therapy. Data were collected on age, sex, race, FSGS variant, and the following baseline variables: serum creatinine and eGFR, serum albumin, proteinuria, serum cholesterol, body mass index, presence of edema, systolic and diastolic blood pressure, and smoking status. Serum creatinine and eGFR at last follow up were also collected. Presence of arterial hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. For children, arterial hypertension was defined as systolic and/or diastolic blood pressure ≥95th percentile for age, sex, and height.[12] Quantification of proteinuria was based either on spot urinary protein-to-creatinine ratio (Up/c) or 24-hour urine collection as reported in the medical record. Information was also collected on immunosuppressive and antihypertensive therapy. Our primary outcome was ESKD (eGFR< 15 ml/min/1.73m^2, dialysis or transplantation). Estimated GFR was calculated using the Modification of Diet in Renal Disease (MDRD) study group formula[13] for adults and the Schwartz formula for children.[14]

Immunomodulatory therapy was classified into three groups: glucocorticoids alone, CNIs with or without glucocorticoids, and other immunosuppressive agents (e.g. azathioprine, mycophenolate mofetil, rituximab). First choice therapy was defined as the first immunosuppressive treatment started after initial kidney biopsy, regardless of the duration between biopsy and beginning of immunosuppression; any CNI therapy started within 3 months of glucocorticoid initiation was considered as a first choice therapy. Some patients were therefore treated with CNIs and glucocorticoids simultaneously, with the latter given at any

dosage. Patients who were given glucocorticoids alone at a dose ≥30 mg/day (or 0.5 mg/kg for children) were considered treated with glucocorticoids. CNIs at any dosage was considered as treatment with CNIs. Length of CNI therapy was defined as the interval during which the drug was prescribed, regardless of dosage. Length of glucocorticoid therapy was defined as the interval during which the drug was prescribed at high dose (1 mg/kg or ≥30 mg/day). Any treatment with angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or selective aldosterone blocker was defined as renin-angiotensin-aldosterone system (RAAS) inhibition.

Statistical analyses:

Normally distributed continuous variables were summarized as mean ± standard deviation. Variables that were not normally distributed were summarized as median (interquartile range).

Identifying factors associated with choice of therapy

In order to identify potentially important confounders in the association between choice of immunomodulatory therapy and time to ESKD, we first investigated factors associated with a decision to treat with an immunomodulatory agent at all (treated vs. untreated). Patients who were prescribed high dose glucocorticoids (1 mg/kg or ≥30 mg/day), CNIs with or without glucocorticoids at any dosage, or any other immunomodulatory agent were considered treated, regardless of duration of therapy. We used logistic regression to identify factors associated with being prescribed any immunosuppressive therapy (vs. none).

Among those prescribed any immunosuppressive therapy, we fit another logistic regression model to identify variables associated with having been prescribed CNIs (with or without glucocorticoids) compared with high-dose glucocorticoids alone. The 12 patients treated with a variety of other immunomodulatory agents were excluded from this analysis.

Modeling the association between the clinical choice to initiate therapy and ESKD

Cox proportional hazards models were constructed to assess the association between clinical choice to initiate therapy and time to ESKD, adjusted for potential confounders. Time zero was biopsy time. Because we sought to estimate the association between early clinical choice to initiate immunosuppressive therapy and ESKD in an immunosuppression 'naïve' population, dichotomous exposure (not time-dependent) was initially considered in the Cox models. However, there were two problems with this approach. First, there was a violation of the proportional hazards assumption. Second, this approach may lead to immortal time bias, in which observation time prior to initiation of therapy is included in the survival time.[15] Therefore, we used time-dependent Cox models in which the primary exposure (glucocorticoids alone; CNIs ± glucocorticoids) was allowed to change over time; this strategy avoids immortal time bias.[15]

Initially, all variables included in our dataset were considered for inclusion in the models. We excluded those (baseline cholesterol, body mass index, smoking status) with a high proportion of missing values (>20%). The number of

covariates to be included in each regression model was then determined based on an adequate statistical power. We did not face any limitations in the number of covariates that could be included in the logistic regression models. However, given the number of patients on immunosuppressive therapy and the number of ESKD endpoints, we allowed a maximum of seven covariates in the final Cox regression model.

A variety of different variable selection approaches were used to select the covariates included in the Cox models. First, variables identified as important in the logistic regression models were included. We used both a backward and forward stepwise approach, and a change-in-estimate strategy[16] to identify important covariates. A directed acyclic graph was conceived to assess potential confounders of the relationship between choice of immunosuppressive therapy and ESKD (Figure 1).[17] Use of directed acyclic graph is a clear way to illustrate causal relations between variables of interest, and allows complexity compared to algebraic demonstrations. Thus, univariate models or factors deemed to influence the outcome in prior studies were considered when drawing the directed acyclic graph. The proportional hazards assumption was tested using goodness-of-fit testing (Schoenfeld residuals), log-log plots and observed versus expected plots. Only the variable black race violated the proportional hazards assumption; thus, we used a stratified Cox regression on race to address this issue. The stratified Cox model allows the form of the underlying hazard function to vary across levels of the stratification variable. Thus, a single hazard ratio for race is not obtained because the effect estimate for the stratified variable varies

with time (it does not satisfy proportional hazards assumption).[18] RAAS inhibition was not included in our models given that a high proportion of patients on immunosuppression were exposed (>80%), and that controlling for this variable was unnecessary based on our directed acyclic graph.

We used both the Kaplan-Meier survival method and Cox adjusted survival curves to visually evaluate the relationship between treatment with immunosuppressive therapy and our primary endpoint (ESKD). Unadjusted Kaplan-Meier curves were visually similar to adjusted survival curves when examining treatment with any immunosuppression versus none. Statistical tests comparing Kaplan-Meier curves for the different immunosuppression therapies were not created because clinical choice to initiate therapy was treated as a time-dependant variable; immunosuppression status could change over time for each individual. Adjusted survival curves were used to illustrate the association between choice of immunosuppressive agent and renal survival.[19] Adjusted survival curves, stratified on eGFR at baseline (<30 mL/min/1.73m^2 and ≥30 mL/min/1.73m^2), were generated using the following pattern of covariates: mean values for age and baseline serum albumin among patients treated with glucocorticoids alone or CNIs ± glucocorticoids, male sex and NOS variant. Race was not included due to proportional hazards violation (absence of effect estimate).

Managing missing data

There were no missing values for age, sex, FSGS variant or for the primary outcome, ESKD. Baseline serum cholesterol, body mass index and smoking

status could not be included as covariates because data were missing in >20% of cases. Missing values for race, baseline eGFR, baseline 24-hour proteinuria, baseline serum albumin, edema and presence of hypertension at baseline were imputed using an iterative Markov chain Monte Carlo multiple imputation technique (twenty imputations).[16] The primary exposure (immunosuppressive therapy; CNIs treatment ± glucocorticoids) was not imputed in any analysis.

A substantial proportion of patients had proteinuria assessment with Up/c only. The missing 24 hour urine data were handled in two ways. First, we imputed missing values as described above. Second, missing 24 hour urine values were filled in using Up/c values (once with a 1:1 and again with a 1:1.5 conversion factor).[20] We fit Cox regression models using both methods and both conversion factors and compared the hazard ratio estimates.

Results for logistic regression models were expressed as odds ratios with 95% confidence interval (CI), and for Cox proportional hazards models as hazard ratios with 95% CI. Statistical analyses were performed using Stata 13 (StataCorp LP, USA). All authors had access to the primary data; L.-P.L. performed the statistical analysis.

RESULTS:

Patient characteristics:

Of the 458 patients with biopsy-proven FSGS, 183 received no immunosuppressive therapy, 173 received glucocorticoids alone, 90 were treated with CNIs with or without glucocorticoids, and 12 with other immunomodulatory agents (Table 1). Documentation of immunosuppressive therapy was inadequate

for 38 patients (Figure 2). Patients treated with any immunosuppression were younger (median age 36 years, interquartile range [IQR] 18-55 years) than patients not treated with immunosuppression (median age 48 years, IQR 32-63 years); those treated with CNIs (median age 25 years, IQR 13-47 years) were younger than those treated with glucocorticoids alone (median age 41 years, IQR 25-58 years). There was a small proportion of patients with tip lesion among those unexposed to immunosuppression (5.5%) compared to those exposed (19.6%). Among patients treated with immunosuppressive therapy, a higher proportion of those treated with CNIs had collapsing FSGS (23.3 %) compared with those treated with glucocorticoids alone (11.0%); a smaller proportion of patients treated with CNIs had tip lesion (5.6%) compared with those treated with glucocorticoids alone (27.2%). Patients who did not receive immunosuppressive therapy had lower median baseline eGFR (43.8 mL/min/1.73m^2, IQR 27.2-69.9 mL/min/1.73m^2) compared to those who received immunosuppressive therapy (62.8 mL/min/1.73m^2, IQR 41.7-85.7 mL/min/1.73m^2).

Immunosuppression:

Median time to initiation of high-dose glucocorticoids following biopsy was 0.3 months (IQR 0.03-0.8 months) and median time to initiation of CNI therapy following biopsy was 0.7 months (IQR 0.1-3.0 months). Median duration of high-dose glucocorticoid therapy was 3.0 months (IQR 1.5-5.9 months). Among those treated with CNIs, 75% started CNIs within 3 months after kidney biopsy (Figure 3), and only 28 (31.1%) patients were given CNIs alone. CNI treatment had a median duration of 19.6 months (IQR 6.5-34.8 months).

Factors associated with exposure to immunosuppressive therapy:

The logistic regression model compared the 275 patients (60.0%) who were exposed to immunosuppressive therapy to the 183 who were not (40.0%). Only 12 patients were treated with adjunctive agents other than CNI (e.g. azathioprine, mycophenolate mofetil, rituximab, etc.). The factors significantly associated with treatment with immunosuppressive therapy are summarized in Table 2. For each 10-year increment in patient age, there were significantly lower odds of receiving immunosuppression (odds ratio [OR] 0.82, 95% confidence interval [CI] 0.74-1; p=0.01). Patients with a tip lesion on kidney biopsy had 3.00 times the odds of being treated with immunosuppressives compared with those with the NOS FSGS variant (95% CI 1.23-7.32; p=0.02). The odds of being treated were lower in patients with eGFR <30 mL/min/1.73m^2 at baseline than in those with an eGFR ≥30 mL/min/1.73m^2 (OR 0.53, 95% CI 0.29-0.99; p=0.05). Finally, for each 1 g/dL lower baseline serum albumin, the likelihood of being treated with immunosuppressive therapy was higher (OR 2.22; 95% CI 1.59-3.13; p<0.001).

Factors associated with choice of immunosuppressive therapy:

On univariate analysis, several characteristics distinguished patients treated with glucocorticoids alone from those whose treatment included CNIs (Table 3). Patients treated with CNI were younger than those treated with glucocorticoids alone (OR 0.82 for each 10 year increment in age, 95% CI 0.74-0.90; p<0.001). African Americans were more likely to be treated with CNIs (OR 1.77, 95% CI 1.06-2.97; p=0.03). The odds of being treated with CNIs (vs. glucocorticoids alone) were lower in patients with tip lesion FSGS (OR 0.18, 95% CI 0.07-0.47;

p=0.001) than in those with FSGS NOS. A lower baseline serum albumin was associated with higher odds of being treated with CNIs (OR 1.39, 95% CI 1.03-1.85 per 1 g/L lower albumin; p=0.03). Presence of hypertension at presentation was associated with a lower likelihood of receiving CNIs (OR 0.53, 95% CI 0.30-0.94; p=0.03).

The multivariable logistic regression model identified only tip lesion FSGS as a significant correlate of the choice of immunosuppressive therapy. Compared to patients with FSGS NOS, those with tip lesion had significantly lower odds of being treated with CNIs (OR 0.17, 95% CI 0.05-0.53; p=0.002).

Clinical choice to initiate immunosuppressive therapy and time to ESKD:

Any immunosuppression vs. none: Exposure to any immunosuppressive therapy (vs. none) was associated with better renal survival, in both unadjusted and adjusted Cox survival models (Table 4). The crude cumulative probabilities of being ESKD-free at 1, 2 and 5 years were 87.8% (125 at risk), 78.2% (90 at risk) and 60.0% (26 at risk) for patients not treated with immunosuppressive therapy; and 94.4% (204 at risk), 87.9% (154 at risk) and 67.4% (59 at risk) for those treated with any immunosuppressive therapy (Figure 4). Compared with no treatment, treatment with any immunosuppression was associated with significantly lower hazards of ESKD (hazard ratio [HR] 0.49, 95% CI 0.28-0.86; p=0.01).

CNIs vs. glucocorticoids alone: Adjusted survival curves suggested a slightly better long-term renal survival associated with treatment including CNIs than with glucocorticoids alone (Figure 5). Compared with those treated with

glucocorticoids alone, the adjusted risk of ESKD was lower among those treated with CNIs (HR 0.42, 95% CI 0.15-1.18; p=0.1) (Table 5), although this difference was not statistically significant. An additional Cox regression model, including proteinuria at baseline in lieu of baseline hypoalbuminemia as a covariate, showed a similar non-statistically significant lower risk of ESKD associated with CNIs compared with glucocorticoids alone (HR 0.50, 95% CI 0.18-1.44; p=0.2).

There were several other factors found to be significantly independently associated with renal survival among patients treated with immunosuppressive therapy. Hypoalbuminemia at baseline was consistently identified as a correlate of renal outcome. For each 1 mg/dL increment in baseline serum albumin, there was a significantly lower likelihood of ESKD (HR 0.55, 95% CI 0.39-0.78; p=0.001). Proteinuria at baseline was also associated with renal outcome. For each 1 g/day increment in baseline proteinuria, there was a higher likelihood of ESKD (HR 1.06, 95% CI 1.02-1.11; p=0.009). Severe renal dysfunction at baseline (eGFR <30 mL/min/1.73 m^2) was strongly associated with poorer renal survival (HR 2.61, 95% CI 1.37-4.96; p=0.003). Compared with a histological diagnosis of FSGS NOS, tip lesion FSGS was associated with a significantly lower risk of ESKD (HR 0.14, 95% CI 0.04-0.47; p=0.002), whereas there was no significant difference in outcome associated with collapsing FSGS (HR 0.98, 95% CI 0.48-2.00; p=1.0).

Sensitivity analysis for proteinuria at baseline:

We constructed additional multivariable Cox regression models in which missing 24-hour proteinuria data were filled in using Up/c (1:1 and 1:1.5

conversion factors), rather than imputed. The adjusted HR (time to ESKD) obtained from these models for treatment with immunosuppressive therapy were of similar magnitude and significance (HR 0.47, 95% CI 0.27-0.84, p=0.01 for 1:1 conversion; HR 0.46, 95% CI 0.26-0.83, p=0.009 for 1:1.5 conversion) to those estimated when missing 24-hour proteinuria values were imputed (HR 0.49, 95% CI 0.28-0.86; p=0.01).

DISCUSSION:

Idiopathic FSGS is a heterogeneous entity for which evidence of effective immunosuppressive therapy is limited. Current recommendations on first-line treatment are based on retrospective studies conducted over the last three decades using a variety of glucocorticoid regimens.[21] However, patients may experience adverse effects from long-term glucocorticoid therapy, particularly when used in the setting of comorbidities such as diabetes and obesity. There are no randomized controlled trials to support the use of CNIs as first-line immunosuppressive therapy in primary FSGS. However, CNIs have been recommended as an alternative first-line agent based on the evidence emerging from studies in steroid-resistant FSGS.[22] This study describes the role of including CNIs (with or without glucocorticoids) early in the treatment of primary FSGS on renal survival.

Our study identifies the patient and disease characteristics associated with choice of treatment. We show that initiation of immunosuppressive therapy appears to be associated with FSGS variant, baseline renal function and severity of nephrotic syndrome, as indicated by the degree of hypoalbuminemia. Only tip

lesion on renal biopsy was identified as an independent factor favouring choice of glucocorticoids alone over CNI therapy. Our results support a role for immunosuppressive therapy with glucocorticoids and/or CNIs to positively influence renal survival. Our findings suggest that early introduction of CNI therapy (with or without glucocorticoids) may be associated with a lower likelihood of ESKD compared to treatment with glucocorticoids alone, after controlling for variables known to influence renal outcome, although the association did not reach statistical significance (HR 0.42, 95% CI 0.15-1.18; p=0.1). We provide evidence supporting the use of CNIs in combination with glucocorticoids as a choice of early treatment of patients with FSGS who are not "steroid resistant".

Since the 1999 landmark study by Cattran et al.[9], which reported an increased likelihood of remission of proteinuria in steroid-resistant patients treated with CNIs, clinicians may have been more likely to prescribe CNIs as a first choice treatment. Indeed, more than 10% of patients on immunosuppression in our cohort were exposed to CNIs alone. Although several patient and disease characteristics appeared to influence selection of the immunomodulatory agent on univariate analyses, we only found a significant independent association between tip lesion FSGS and glucocorticoid therapy alone. Tip lesion FSGS has been described to share common clinical features with minimal change disease, and to respond promptly to high dose glucocorticoids.[23] Interestingly, collapsing FSGS variant, which has been previously associated with a higher proportion of

patients with renal failure, was not associated with an increased use of CNIs, an agent usually reserved for more resistant cases.[24]

Our study supports a potential role for including CNIs early in the treatment of primary FSGS, but is not able to prove superiority of CNIs with or without glucocorticoids over glucocorticoids alone. Combinations of immunosuppressive agents were frequently used and few patients were exposed to CNIs 'alone' (n=28). Because the majority of patients receiving CNIs were also treated with glucocorticoids, our ability to distinguish the benefits from CNIs alone from the combination of CNIs and high dose glucocorticoids is limited. There were insufficient numbers of patients treated with CNIs alone to provide adequate power for a model examining the association between treatment with CNIs alone and time to ESKD.

Our findings are consistent with previously reported clinical findings. Baseline level of proteinuria was previously demonstrated to have prognostic significance.[25,26] We showed that baseline proteinuria was also predictive of renal survival among patients treated with immunosuppressive therapy. We were not able to examine the impact of remission in proteinuria on renal outcome due to incomplete documentation of proteinuria at fixed intervals. As expected and previously shown, severe renal dysfunction at baseline (eGFR less than 30 mL/min/1.73m^2).[27,28] was predictive of poorer renal survival.

Severe hypoalbuminemia has been shown to be associated with complications of the nephrotic syndrome, such as thromboembolism.[29-31] However, the value of hypoalbuminemia as a predictor of renal outcome in

primary FSGS is controversial. Our results suggest that baseline serum albumin is significantly associated with renal survival, and that this association is stronger than that of proteinuria with renal survival. It was not possible to include both baseline albuminemia and proteinuria in the same Cox regression model when comparing CNIs (with or without glucocorticoids) to glucocorticoids alone due to the small number of renal failure events, resulting in power limitations. Moreover, we could not address the question of the impact of remission from severe hypoalbuminemia on renal survival with the present study design.

Our study has some limitations inherent to its retrospective nature. These include a lack of uniform treatment protocol and systematic laboratory tests at fixed intervals, and variable follow up intervals. As a result, some variables of interest had a significant proportion of missing values, impeding their use in statistical models with multiple imputation. This could lead to residual confounding. For example, presence of diabetes or high body mass index could influence choice of immunosuppressive therapy by favouring steroid-sparing agents, and modify disease trajectory by adding a superimposed glomerular injury. For the balance of the variables, missing values were handled using multiple imputation, which provides less biased estimates than complete case analysis.[16] This was of particular concern with proteinuria quantification for which our sensitivity analysis supported use of imputed 24-hr baseline proteinuria. Our survival model also has limitations. Some patients might have been misclassified as having received glucocorticoids alone when in fact they have been exposed to

CNI later due to non-response. This might have biased results towards the null hypothesis, ascribing response to therapy to glucocorticoids and not CNIs.

We considered using propensity score methods to account for confounding by variables associated with choice of treatment.[32] However, creation of a propensity score matched cohort would have required the exclusion of numerous subjects from our analysis for those not paired, which would have considerably reduced already limited statistical power.

CONCLUSIONS:

This study demonstrates a benefit of early immunosuppression including glucocorticoids and/or CNIs with respect to renal survival, after adjusting for potential confounders. Despite the fact that patients treated with immunosuppressives tended to have evidence of more severe nephrotic syndrome than those who were untreated, we demonstrated significantly better renal survival in patients treated with immunosuppressive therapy compared with none. Among treated patients, those whose treatment included CNIs showed a lower risk of ESKD, although this association was not statistically significant. We also identified presence of tip lesion on renal biopsy as a predictor of better renal outcome, and more severe hypoalbuminemia at baseline as a predictor of poorer renal survival. However, the impact of both FSGS variant and hypoalbuminemia on renal survival await validation in prospective studies. Our findings support a potential role for including CNIs early in the treatment of FSGS (with glucocorticoids). Future prospective trials are needed to confirm the benefit of these therapies in FSGS.

Figure 1. Directed acyclic graph of potential confounders. Solid black arrow indicates direct causal effect. Solid blue arrow indicates probable direct causal effect. Red dotted line indicates new back-door path created by adjustment on a collider (common effect). Variables in square boxes refer to the adjustment set.

S = {age, sex, FSGS variant, eGFR, proteinuria, Salb}

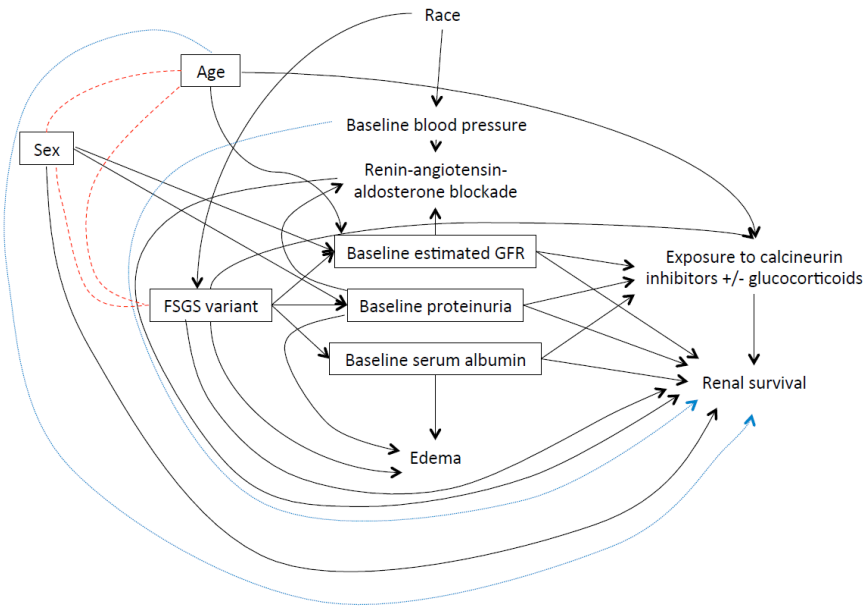

Figure 2. Study flow diagram

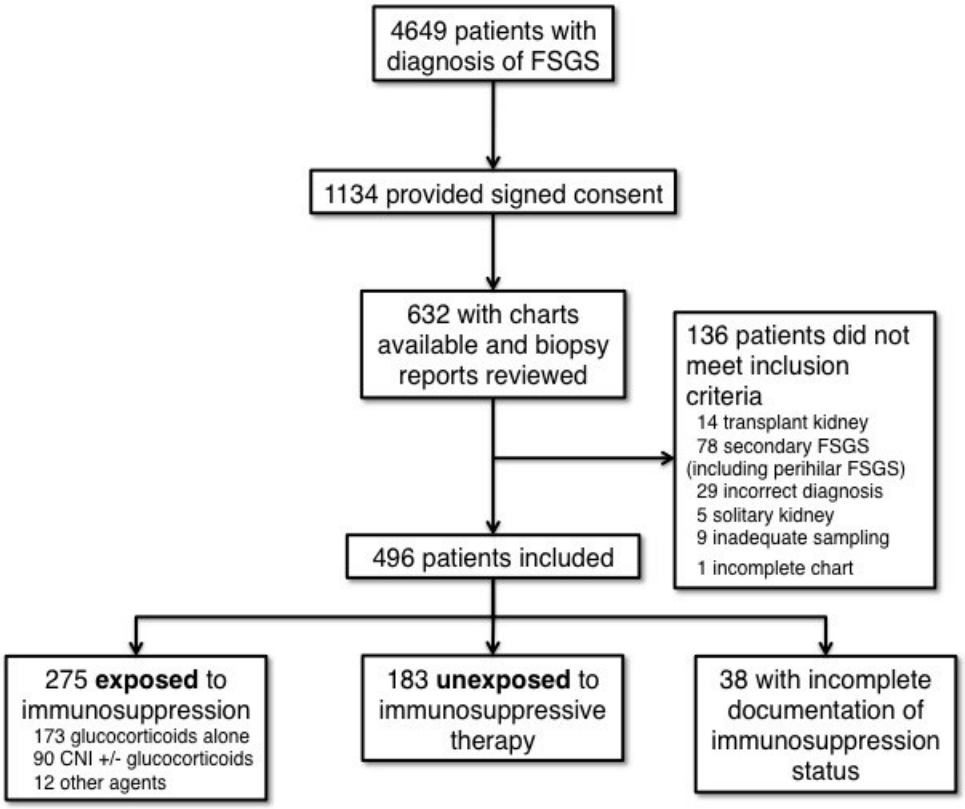

Figure 3. Probability density function of time length between renal biopsy and immunosuppression therapy

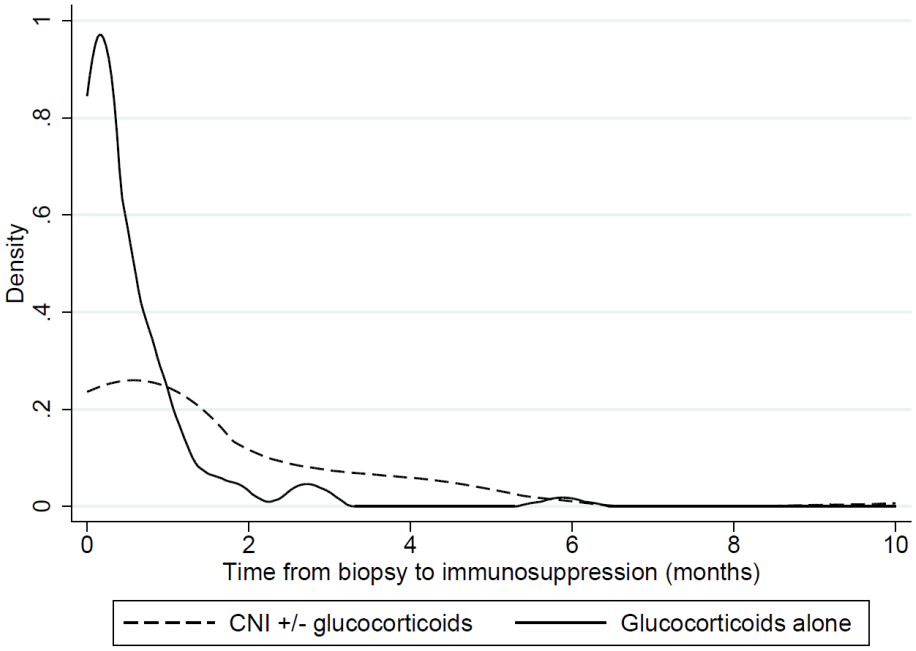

Figure 4. Kaplan-Meier curves by immunosuppression status (p=0.01)

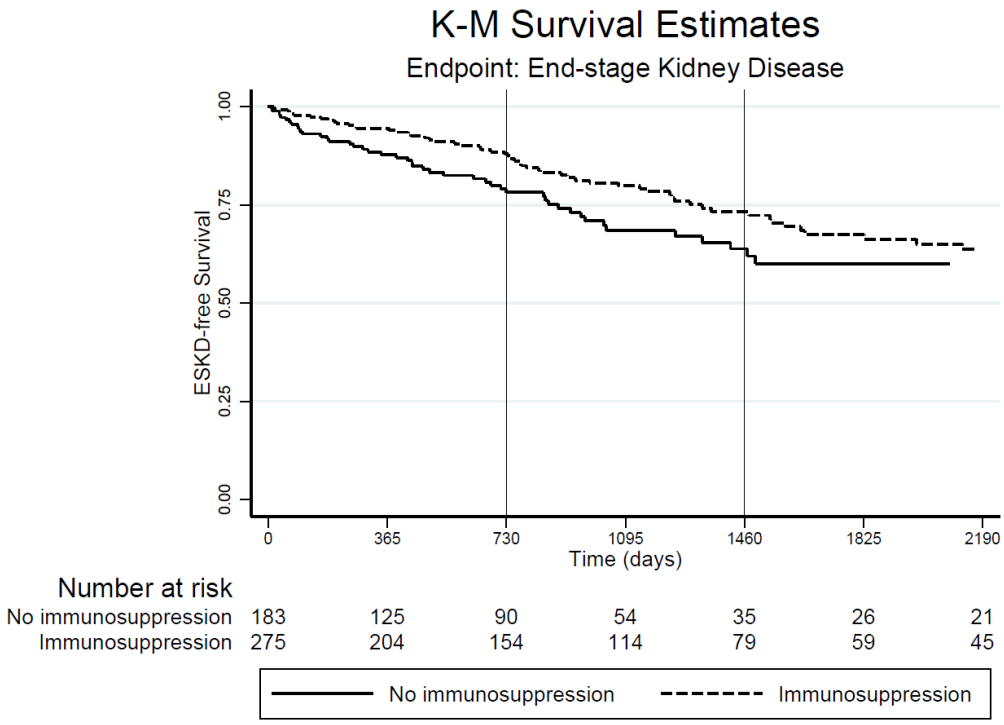

Figure 5. Predicted ESKD-free Nelson-Aalen curves: these curves are predicted for the adjusted Cox time-dependent model (p=0.1); outcomes for the glucocorticoids alone and CNIs ± glucocorticoids groups are computed at the mean (age, male sex, NOS variant, baseline serum albumin eGFR); ESKD, end-stage kidney disease; CNIs, calcineurin inhibitors; NOS, not-otherwise-specified; eGFR, estimated glomerular filtration rate.

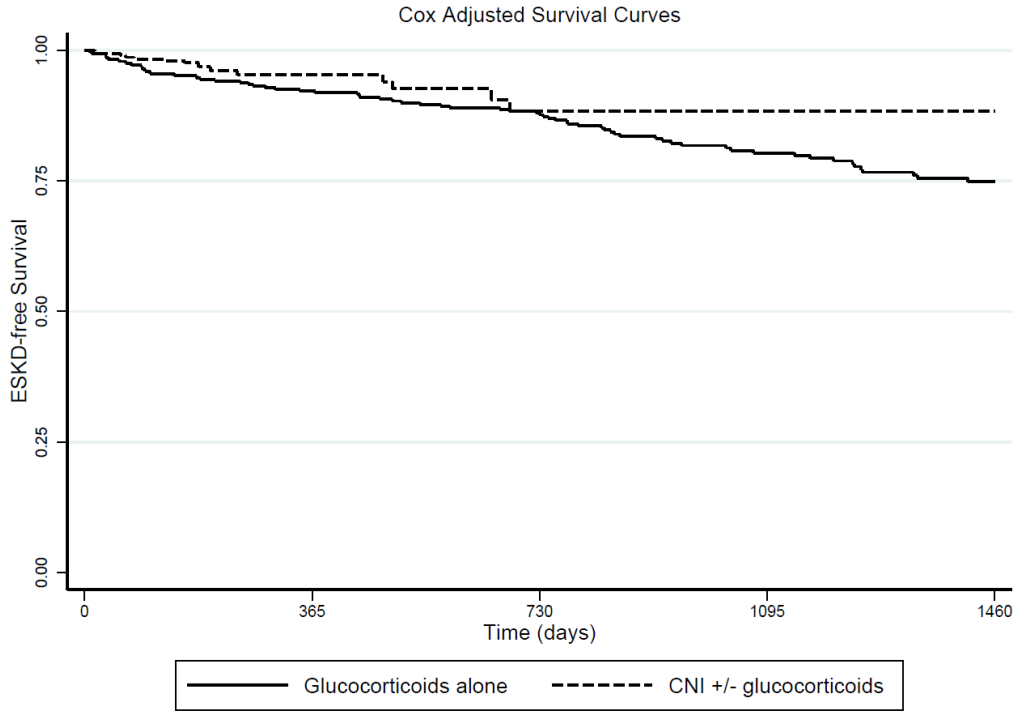

Table 1. Baseline characteristics by immunosuppression status

	No immunosuppression n=183	Immunosuppression* n=275		
		Any immunosuppression n=275	Glucocorticoids alone n=173	Calcineurin inhibitors +/- glucocorticoids n=90
Age at biopsy (years)	48 (32-63)	36 (18-55)	41 (25-58)	25 (13-47)
Female sex (%)	48.1	49.1	45.7	55.6
Black race (%)	45.1	45.9	41.5	55.6
Missing (%)	5.5	3.3	5.2	0
FSGS variant (%)				
NOS	83.6	65.1	61.9	71.1
Tip	5.5	19.6	27.2	5.6
Collapsing	10.9	15.3	11.0	23.3
eGFR at biopsy (mL/min/1.73m^2)	43.8 (27.2-69.9)	62.8 (41.7-85.7)	61.9 (40.5-83.9)	65.5 (46.5-88.2)
Missing (%)	1.6	2.5	1.2	3.3
24-hour proteinuria at biopsy (g/d)	3.8 (2.4-6.6)	6.0 (3.5-12.0)	6.4 (3.8-12.0)	5.5 (2.9-13.0)
Missing (%)	20.2	31.6	19.1	52.2
Serum albumin at biopsy (mg/dL)	3.5 (3-4)	2.4 (1.7-3.3)	2.5 (1.9-3.5)	2.1 (1.5-2.9)
Missing (%)	19.7	16.4	15.6	16.7
Hypertension at baseline (%)	67.3	64.7	68.4	55.4
Missing (%)	11.5	18.6	10.4	27.8
Edema at baseline (%)	45.5	68.3	68.9	70.6
Missing (%)	21.9	11.6	12.7	5.6

Continuous variables expressed as median (interquartile range). FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified; eGFR, estimated glomerular filtration rate.

*12 patients were exposed to a variety of “other” immunosuppressive agents

Table 2. Factors associated with treatment with immunosuppressive therapy

	Univariate		**Multivariate**	
Variables	OR	95% CI	OR	95% CI
Age at biopsy (per 10 year higher)	**0.82**	0.74-0.90	0.82	0.74-1.00
Male sex	0.96	0.66-1.40	1.28	0.77-2.13
Black race	1.02	0.70-1.50	0.87	0.50-1.50
FSGS variant				
NOS	1.00		1	
Tip	**4.62**	2.27-9.37	**3.00**	1.23-7.32
Collapsing	**1.79**	1.01-3.19	1.19	0.53-2.68
Baseline eGFR <30 mL/min	**0.42**	0.26-0.67	**0.53**	0.29-0.99
Baseline proteinuria ≥3.5 g/d	**2.03**	1.32-3.13	1.04	0.56-1.93
Serum albumin at biopsy (per g/dL lower)	**2.44**	1.92-3.03	**2.22**	1.59-3.13
Edema at baseline	**2.59**	1.69-3.96	1.42	0.81-2.49
Hypertension at baseline	0.82	0.54-1.25	0.92	0.52-1.64

OR, odds ratio; CI, confidence interval; FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified; eGFR, estimated glomerular filtration rate.

Table 3. Factors associated with treatment including calcineurin inhibitors (vs. glucocorticoids alone)

	Univariate		**Multivariate**	
Variables	OR	95% CI	OR	95% CI
Age at biopsy (per 10 year higher)	**0.74**	0.66-0.90	0.82	0.74-1.00
Male sex	0.67	0.40-1.12	1.05	0.55-2.04
Black race	**1.77**	1.06-2.97	1.14	0.59-2.19
Variant				
NOS	1.00		1	
Tip	**0.18**	0.07-0.47	**0.17**	0.05-0.53
Collapsing	1.85	0.92-3.70	1.73	0.74-4.02
Baseline eGFR <30 mL/min	0.86	0.40-1.84	1.28	0.51-3.24
Baseline proteinuria ≥3.5 g/d	0.69	0.35-1.36	0.58	0.25-1.36
Serum albumin at biopsy (per g/dL lower)	**1.39**	1.03-1.85	1.43	0.95-2.17
Edema at baseline	1.08	0.61-1.94	1.25	0.59-2.65
Hypertension at baseline	**0.53**	0.30-0.94	0.66	0.34-1.28

OR, odds ratio; CI, confidence interval; FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified; eGFR, estimated glomerular filtration rate.

Table 4. Factors associated with end-stage kidney disease by immunosuppression status

Variables	Hazard ratio	95% confidence interval
Unadjusted		
Immunosuppression None CNIs +/- glucocorticoids OR glucocorticoids alone	 1.00 **0.50**	 0.29-0.87
Adjusted*		
Immunosuppression None CNIs +/- glucocorticoids OR glucocorticoids alone	 1 **0.49**	 0.28-0.86
Age (per year increase)	1.00	0.99-1.01
Male sex	1.15	0.76-1.73
FSGS variant NOS Tip Collapsing	 1.00 **0.21** 1.71	 0.09-0.48 0.99-2.95
Baseline eGFR <30 mL/min/m^2	**4.28**	2.81-6.48
Baseline proteinuria ≥3.5 g/d	1.25	0.68-2.29
Serum albumin at biopsy (per g/dL higher)	**0.69**	0.53-0.90
Hypertension at baseline	1.33	0.81-2.20

CNIs, calcineurin inhibitors; FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified; eGFR, estimated glomerular filtration rate.
*Cox regression adjusted for: immunosuppression, age, sex, Black race, FSGS variant, and baseline eGFR, proteinuria, serum albumin and hypertension.

Table 5. Factors associated with end-stage kidney disease among treated patients

Variables	Hazard ratio	95% confidence interval
Unadjusted		
Immunosuppressive therapy		
Glucocorticoids	1.00	
CNIs +/- glucocorticoids	0.60	0.21-1.70
Adjusted*		
Immunosuppressive therapy		
Glucocorticoids	1.00	
CNIs +/- glucocorticoids	0.42	0.15-1.18
Age (per year increase)	1.00	0.99-1.02
Male sex	1.00	0.56-1.76
FSGS variant		
NOS	1.00	
Tip	**0.14**	0.04-0.47
Collapsing	0.98	0.48-2.00
Baseline eGFR <30 mL/min/m^2	**2.61**	1.37-4.96
Serum albumin at biopsy (per g/dL higher)	**0.55**	0.39-0.78

CNIs, calcineurin inhibitors; FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified; eGFR, estimated glomerular filtration rate.
*Cox regression adjusted for: immunosuppression, age, sex, Black race, FSGS variant, and baseline eGFR and serum albumin; Baseline hypertension was not predictive of renal survival by univariate analysis.

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Preface to Chapter 4:

This chapter presents a summary of the previous chapters, describes study strengths and limitations, and formulates recommendations based on the evidence available in the FSGS literature. Finally, this chapter proposes future directions for research in primary FSGS.

Chapter 4: Summary, strengths, limitations, recommendations and future plans

Summary:

My thesis work included both a systematic review of studies examining the efficacy of CNIs in the treatment of primary FSGS, and a retrospective cohort study aimed at determining the association between immunosuppressive therapy for FSGS and renal outcome. With the systematic review, we found well-designed studies supporting the efficacy of CNIs in inducing remission in proteinuria among patients with steroid-resistant FSGS. The only study that evaluated CNIs in a steroid-naïve population was retrospective, and underpowered to demonstrate its superiority over glucocorticoids or supportive therapy. Our results suggest an association between immunosuppression with glucocorticoids and/or CNIs and better renal survival, but we were not able to demonstrate a statistically significant difference in outcome between those whose treatment included early introduction of CNIs and those treated with glucocorticoids alone.

Strengths:

Systematic review

There are several strengths to our systematic review. We performed searches in the main medical databases. We used MeSH terms in our PubMed search to optimize our results. A meta-analysis was performed using remission outcomes of patients on active treatment for 6 months. We used well-recognized

quality assessment tools to appreciate the internal validity our each study in our systematic review.

Original article

Our retrospective inception cohort included a large number of patients both treated and untreated with immunosuppressive therapies. The Glomerular Disease Collaborative Network is unique in size and demographics, with a significant proportion of African Americans. The racial composition is an important reason why the Glomerular Disease Collaborative Network results may not be easily comparable to other large databases. Indeed, the only other database of similar size in FSGS is the Toronto database, which has a completely different demographic composition. This large cohort allowed us to control for several confounders of the association between immunosuppressive therapies and ESKD. In addition, the long duration of follow-up available in the database allowed us to consider the important outcome of renal survival, which is not the primary outcome in published FSGS prospective trials. Multiple imputation techniques were used to address the pitfalls inherent to missing data.

Limitations:

Systematic review

We recognize limitations to our systematic review. The search terms we used to conduct our literature review may not have captured all relevant studies. Nevertheless, we obtained comparable results to what was found with other systematic reviews published in the last decade.

A significant proportion of patients had missing information on comorbidities that may have influenced choice of therapy. This may have resulted in residual confounding. Diabetes could reasonably constitute one of those unmeasured confounders by its close association with FSGS. There is a hypothesized link between obesity and FSGS, and the obese population is more prone to diabetes. However, presence of diabetic nephropathy was excluded by renal biopsy as our cohort was based on biopsy-proven FSGS.

A direct comparison with previous reports in terms of remission rates was not possible using our database: information on urinary protein excretion was not available at regular intervals for the majority of patients. However, the goal of our retrospective study was to analyze the influence of first chosen therapy on a well-recognized outcome (renal survival) in primary FSGS.

Our Cox regression models using a time-dependent variable for treatment had some limitations due to the relapsing-remitting pattern of the disease, and the multiple therapeutic strategies used to treat patients. Immunosuppressive therapy classification was based on strictly defined choices of treatment. Some patients classified as having received glucocorticoids alone might have been exposed to CNIs later due to non-response. This misclassification might have biased towards the null hypothesis, ascribing response to therapy to glucocorticoids and not CNIs. Retrospective data has inherent limitations: documentation of start/end dates of immunosuppressive therapy is sometimes unclear, which impedes exact determination of time on therapy.

Recommendations:

Until evidence is available from a large prospective cohort study to evaluate disease trajectories under different immunosuppressive agents, our retrospective study suggests that immunosuppressive therapy with glucocorticoids and/or CNIs positively influences renal survival.

Future plan:

Future research in FSGS and other glomerular diseases will depend upon establishment of a large cohort of patients. A NIH-funded multi-center consortium is currently taking place across USA, Canada and Italy to recruit a large and ethnically diverse cohort of glomerular disease patients, and follow them prospectively. This study will allow researchers to address unanswered questions pertaining to treatment of FSGS.

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