Focal Segmental Glomerulosclerosis: 
Pieces of the Puzzle

Een wetenschappelijke proeve op het gebied van de medische wetenschappen

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Chapter 1

General introduction and outline of the thesis

Adapted from Minerva Urol Nefrol 2005;57:211-236
Chapter 1

Focal segmental glomerulosclerosis (FSGS) is a disease entity defined by findings on kidney biopsy. The classical lesion of focal segmental glomerulosclerosis (FSGS), as first described by Rich in 1957, is characterized by the presence of a scarring lesion in a portion (segment) of some (focal), but not all glomeruli.\textsuperscript{1,2} The scar comprises increased mesangial matrix with collapsed glomerular capillaries, an adhesion between the tuft and Bowman’s capsule, and hyaline deposits. The glomerular scar can be accompanied by features such as mesangial hypercellularity and foam cells. Progressive lesions are characterized by periglomerular and tubulo-interstitial fibrosis. In immunofluorescence deposits of IgM and C3 are often found in the sclerotic areas, a result of non-specific trapping of these proteins and not evidence of an immunologic process. It is important to realize that FSGS is a histologic diagnosis and not a single disease entity.\textsuperscript{3} FSGS can be idiopathic (primary; unknown cause) or secondary (with underlying cause; \textit{table 1}).

\textbf{Etiology and pathogenesis of FSGS}

\textbf{Idiopathic FSGS}

For most patients with FSGS the pathogenesis is largely unknown. However, there is strong evidence that idiopathic FSGS may be the result of a circulating factor or factors that alter the permeability of glomeruli.\textsuperscript{4,6} The best evidence supporting the presence of a circulating permeability factor comes from recurrent FSGS after renal transplantation. Proteinuria may develop within a few days after transplantation, and plasma exchange instituted early in the course of recurrent disease removes the putative factor and results in a remission of proteinuria.\textsuperscript{7} In addition, rats infused with serum from patients with recurrent FSGS develop proteinuria.\textsuperscript{8,9} More recent data paint a more complicated picture, suggesting that the increased permeability may be due to the absence or the loss of an inhibitor for the permeability factor.\textsuperscript{10} Studies using isolated perfused glomeruli show that addition of normal serum abrogates the permeability activity of FSGS serum.\textsuperscript{11}

Damage to the podocyte plays an important role in the development of FSGS (as discussed below). In addition, animal models resembling idiopathic FSGS and studies in humans suggest that injury to the parietal cells (PECs) also contributes to the development of idiopathic FSGS.\textsuperscript{12,13} In these studies proliferating cells in Bowman’s capsule were derived from the parietal epithelium. It is hypothesized that the PECs start to proliferate secondary to injury of the podocyte aimed at replacing damaged or injured podocytes.\textsuperscript{14}
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<tr>
<td>1. Familial</td>
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<tr>
<td>A. Mutations in α-actinin 4</td>
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<td>B. Mutations in nephrin</td>
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<tr>
<td>C. Mutations in podocin</td>
</tr>
<tr>
<td>D. Mutations in WT-1</td>
</tr>
<tr>
<td>E. Mutations in CD2-associated protein</td>
</tr>
<tr>
<td>F. Mutations in TRPC 6</td>
</tr>
<tr>
<td>G. Mitochondrial cytopathies</td>
</tr>
<tr>
<td>2. Virus associated</td>
</tr>
<tr>
<td>A. HIV-associated nephropathy</td>
</tr>
<tr>
<td>B. Parvovirus B19</td>
</tr>
<tr>
<td>3. Medication</td>
</tr>
<tr>
<td>A. Heroin-nephropathy</td>
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<tr>
<td>B. Interferon-α</td>
</tr>
<tr>
<td>C. Lithium</td>
</tr>
<tr>
<td>D. Pamidronate / Alendronate</td>
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<td>4. Adaptive structural-functional responses likely mediated by glomerular hypertrophy or hyperfiltration</td>
</tr>
<tr>
<td>4.1 Reduced renal mass</td>
</tr>
<tr>
<td>A. Oligomeganephronia</td>
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<tr>
<td>B. Unilateral renal agenesis</td>
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<tr>
<td>C. renal dysplasia</td>
</tr>
<tr>
<td>D. Cortical necrosis</td>
</tr>
<tr>
<td>E. Reflux nephropathy</td>
</tr>
<tr>
<td>F. Surgical renal ablation</td>
</tr>
<tr>
<td>G. Chronic allograft nephropathy</td>
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<tr>
<td>H. Any advanced renal disease with reduction in functioning nephrons</td>
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<tr>
<td>4.2 Initially normal renal mass</td>
</tr>
<tr>
<td>A. Diabetes mellitus</td>
</tr>
<tr>
<td>B. Hypertension</td>
</tr>
<tr>
<td>C. Obesity</td>
</tr>
<tr>
<td>D. Cyanotic congenital heart disease</td>
</tr>
<tr>
<td>E. Sickle cell anemia</td>
</tr>
<tr>
<td>5. Malignancy (lymphoma)</td>
</tr>
<tr>
<td>Nonspecific pattern of FSGS caused by renal scarring in glomerular disease</td>
</tr>
<tr>
<td>A. Focal proliferative glomerulonephritis (IgA nephropathy, lupus nephritis, pauci-immune focal necrotizing and crescentic glomerulonephritis)</td>
</tr>
<tr>
<td>B. Hereditary nephritis (Alport syndrome)</td>
</tr>
<tr>
<td>C. Membranous glomerulopathy</td>
</tr>
<tr>
<td>D. Thrombotic microangiopathy</td>
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</tbody>
</table>
Secondary FSGS

With our increase in knowledge, the number of secondary causes has steadily grown (table 1). Secondary forms of FSGS were generally considered to result from maladaptive responses that occurred due to the loss of functioning nephrons, hyperfiltration or increased glomerular pressure.

Based on studies in experimental animal models such as the remnant kidney model in the rat, Kriz et al. demonstrated that loss of podocytes was pivotal in FSGS resulting from these maladaptive responses. Of note, all animal models used, had evidence of glomerular hyperfiltration, hypertrophy or increased glomerular pressure. Based on these studies, Kriz proposed that the following sequence of events results in FSGS (figure 1): which starts with injury to the podocyte. The damaged podocytes detach from the glomerular basement membrane (GBM), ultimately followed by loss of the entire cell into Bowman’s space.

Because podocytes are incapable of regenerative replication, loss of podocytes cannot be replaced, which leads to areas of “bare” GBM. Next, parietal epithelial cells covering Bowman’s capsule attach to the bare GBM, leading to the formation of an adhesion between the capillary tuft and Bowman’s capsule. At the site of the adhesion a gap in the parietal epithelium comes into existence.

**Figure 1. Schematic to show the development of focal segmental glomerulosclerosis secondary to maladaptive responses.** (A) Normal glomerulus with vascular and urinary poles. Podocytes are shown in blue, parietal epithelial cells in red. The GBM is shown in black, the parietal basement membrane in yellow, tubular epithelia are shown in white. (B) A dilated and podocyte-denuded capillary is attached to Bowman's capsule. The attachment is accomplished by the affixation of parietal cells to the naked GBM. Thereby a gap in the parietal epithelium has come into existence, permitting filtration/exudation towards the cortical interstitium (arrow). (C) The adhesion has spread to neighboring capillaries resulting in either the collapse or in hyalinosis (shown in a dark grey pattern) of the involved capillaries. Podocytes at the flanks of the adhesion degenerate. The parietal epithelium may either appose those podocytes (arrowhead) or attach directly to the GBM at the flanks of the adhesion. Fluid leakage from perfused capillaries inside the adhesion has created a paraglomerular space (shown in yellow) that contains the sclerotic tuft remnants (that is, collapsed or hyalinized GBM formations). Towards the cortical interstitium this paraglomerular space has become separated by a layer of sheet-like fibroblast processes (shown in green). (D) Via the vascular pole the sclerotic process has reached a further lobule. A small "intact" tuft remnant protrudes into the urinary space still covered by the parietal epithelium. The sclerotic tuft remnants are located outside the parietal epithelium in the paraglomerular space that is separated from the cortical interstitium by a complete layer of cortical fibroblasts. Even in those late stages of injury perfused capillaries are regularly found within the sclerotic regions, probably accounting for the further expansion of the paraglomerular space that may extend onto the proximal tubule. In even later stages fibroblasts will invade the sclerotic area, resulting in fibrous organization. Reprinted with permission.
General introduction and outline of the thesis
Chapter 1

If the attached capillary is still functioning, fluid leaks into the gap in the parietal epithelium (misdirected filtration), leading to the formation of a fluid-rich periglomerular space. Then fibroblasts are stimulated which results in periglomerular and tubulointerstitial fibrosis. The continuing misdirected filtration and podocyte loss results in a further expansion of the adhesion. Inside an adhesion eventually capillaries collapse or become occluded either by deposition of hyaline material or microthrombosis.

In recent years the list of secondary causes has steadily grown and now also includes FSGS not related to glomerular hyperfiltration (table 1). FSGS may result from direct injury to the podocytes due to viral infections (HIV) or the use of drugs (pamidronate/alendronate).17,18

**Familial FSGS and FSGS secondary to sporadic mutations**

The podocyte also plays an important role in familial forms of FSGS. The podocyte is a terminally differentiated cell that contains a contractile system composed of actin, myosin II, α-actinin-4, talin and vinculin (figure 2).19 The actin filament bundles form arches between foot processes of the same podocyte.20 Foot processes are anchored to the underlying GBM via α3/β1 integrin complexes and dystroglycans.21,22 Neighboring foot processes are connected by cell-cell junctions, the glomerular slit diaphragm, which constitutes the main size selectivity filter barrier in the kidney.23,24 Important slit diaphragm proteins are nephrin, an adhesion and signaling protein that bridges the slit between foot processes; podocin, which targets nephrin into lipid raft microdomains and facilitates nephrin signaling and CD2AP which interacts with nephrin and podocin.25-30 A recent discovered slit protein is TRPC6, a non-selective cation channel.31 TRPC6 colocalizes to the slit diaphragm with nephrin, podocin, and CD2AP; in addition, alterations in TRPC6 calcium currents appear to be central to the ability of the podocyte to regulate intracellular and cytoskeletal behaviour.32 During recent years, several mutations in genes encoding for these structural proteins in the podocyte and slit diaphragm have been identified in familial forms of FSGS.

Mutations in nephrin cause a severe type of nephrotic syndrome in newborns (Finnish type congenital nephrotic syndrome).33 These infants are born prematurely, edema is present at birth or appears within a few days. End-stage renal disease develops most often within 3-8 years. Podocin mutations were first described in children with familial steroid-resistant nephrotic syndrome.34 These patients show rapid progression to ESRD. Podocin mutations have also been reported in a few familial cases of FSGS with adolescent or adult onset.35
Figure 2. Glomerular filtration barrier. Two podocyte foot processes bridged by the slit membrane, the glomerular basement membrane (GBM) and the porous capillary endothelium are shown. The GBM is composed mainly of collagen IV (α3, α4 and α5), laminin 11 (α5, β2 and γ1 chains) and the heparan sulphate proteoglycan agrin.

The slit membrane is a porous proteinaceous membrane composed of (as far as is known) nephrin, Neph1, 2 and 3, P-cadherin and FAT1. β1α3 integrin dimers specifically connect the TVP complex (talin, paxillin and vinculin) to laminin 11; the α and β dystroglycans connect utrophin (U) to agrin.

The slit membrane proteins are joined to the cytoskeleton by various adaptor proteins, including podocin, zona occludens protein 1 (ZO-1; Z), CD2-associated protein (CD) and catenins (Caten). TRPC6 associates with podocin (and nephrin; not shown) at the slit membrane. Among the many surface receptors, only the angiotensin II (ANG II) type 1 receptor (AT1) is shown.

Additional abbreviations: Cas, p130Cas; Ez, ezrin; FAK, focal adhesion kinase; ILK, integrin-linked kinase; M, myosin; N, NHERF2 (Na⁺–H⁺ exchanger regulatory factor); NSCC, non-selective cation channel; S, synaptopodin. Reprinted with permission.

The mutations are different from those observed in children, suggesting that podocin retains some function in these patients. In some families the affected patients are compound heterozygous for the R229Q variant. This variant was shown to have a decreased binding to nephrin. Mutations in α-actinin-4 and TRPC 6 have been described in patients with late onset FSGS. Mutations in α-actinin-4 have been identified in several families with an
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autosomal dominant form of FSGS. These patients tend to present in the teenage years or later. FSGS caused by α-actinin-4 mutations runs a more slowly progressive course compared to FSGS caused by mutations in nephrin and podocin. A mutation in TRPC 6, a nonselective calcium channel expressed on podocytes, has recently been identified as a cause of autosomal dominant FSGS. The exact mechanism resulting in FSGS is not known.

Proteinuria usually develops between the age of 20 and 50 years and the duration from onset to ESRD averages 10 years. A special form of inheritable FSGS is caused by podocytic mitochondrial DNA mutations. The mutation has been associated with the MELAS syndrome and more recently with maternally inherited hearing loss, diabetes mellitus, cardiomyopathy and progressive ophthalmoplegia. However, FSGS can be the only clinical feature.

Sporadic (non-familial) mutations in adults with FSGS are rare, with a reported prevalence of 1.5-5%. He et al. screened 78 adult patients with non-familial FSGS (15 steroid-sensitive and 63 steroid-resistant) for known mutations in the podocin gene. Compound heterozygous mutations were detected in only one patient with steroid-sensitive FSGS, no homozygous mutations were found. These results are consistent with the findings of Caridi et al, who found only 3 heterozygous mutations in a cohort of 64 patients with steroid-resistant nephrotic syndrome. The study by He et al. also identified 8 patients with heterozygous R229Q. The allele frequency of this variant did not differ from normal controls. The significance of the heterozygous R229Q variant as a disease causing mutation in FSGS is currently unknown. Sporadic mutations in the gene encoding α-actinin-4 are also rare. Aucella et al. found no α-actinin-4 gene mutations in 33 adults with non-familial FSGS.

Pathological classification of FSGS

Over the years, the pathologic description of FSGS has evolved, and in addition to the classical form other variants have been described. Recently a group of pathologists proposed a new pathological classification for FSGS based on an assessment of glomerular light microscopic features (Columbia classification; table 2; figures 3-7). This classification presumes exclusion of FSGS caused by glomerular scarring in the course of other idiopathic glomerular diseases (table 1). The classification defines five main light microscopic variants: FSGS not otherwise specified (NOS), tip variant, perihilar variant, cellular variant, and collapsing variant. It is important to notice that the presence of sclerosis is no longer
Figure 3. FSGS NOS. Segmental obliteration of capillary lumina by accumulation of matrix and hyalinosis. Features of collapse, glomerular tip lesion or endocapillary hypercellularity are not seen.

Figure 4. Tip variant. Adhesion between glomerular tuft and Bowman’s capsule at the tubular pole adjacent to the origin of the proximal tubule. No collapse of the capillary tuft.

Figure 5. Perihilar FSGS. A lesion of segmental sclerosis and hyalinosis located at the glomerular vascular pole. Features of collapse, glomerular tip lesion or endocapillary hypercellularity are not present.

Figure 6. Collapsing FSGS. Global collapse of the capillary tuft, with hypertrophy and hyperplasia of glomerular epithelial cells.

Figure 7. Segmental endocapillary hypercellularity. Features of collapse or glomerular tip lesion are not present.
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Obligatory for the diagnosis of FSGS, since in particular in the tip-variant and the collapsing variant, sclerosis is often absent.

Although the different variants may reflect different diseases (with different causes and differences in pathogenesis), this is not proven. The different variants may be just a reflection of a different stage of FSGS, dependent on the activity and time of onset of the disease.

Some pathological variants are more likely to occur in relation to certain causes. The perihilar variant of FSGS is associated with hyperfiltration, whereas HIV typically results in collapsing FSGS. However, there is a clear overlap, and patients with idiopathic FSGS may present with either variant. Studies that have assessed the clinical relevance of the histologic variants are few and conflicting.

Table 2. Morphological variants of FSGS

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Characteristics</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS variant</td>
<td>At least one glomerulus with segmental increase in matrix obliterating the capillary lumen.</td>
<td>Exclude perihilar, cellular, tip and collapsing variant</td>
</tr>
<tr>
<td></td>
<td>There may be segmental glomerular basement membrane collapse without overlying podocyte hyperplasia</td>
<td></td>
</tr>
<tr>
<td>Perihilar variant</td>
<td>At least one glomerulus with perihilar hyalinosis, with or or without sclerosis</td>
<td>Exclude cellular, tip and collapsing variant</td>
</tr>
<tr>
<td></td>
<td>&gt; 50% of glomeruli with segmental lesions must have perihilar sclerosis and/or hyalinosis</td>
<td></td>
</tr>
<tr>
<td>Cellular variant</td>
<td>At least one glomerulus with segmental endocapillary hypercellularity occluding lumina, with or without foam cells and karyorrhexis</td>
<td>Exclude tip and collapsing variant</td>
</tr>
<tr>
<td>Tip variant</td>
<td>At least one segmental lesion involving the tip domain (outer 25% of tuft next to origin of proximal tubule)</td>
<td>Exclude collapsing variant</td>
</tr>
<tr>
<td></td>
<td>The tubular pole must be identified in the defining lesion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The tip lesion may be cellular or sclerosing</td>
<td></td>
</tr>
<tr>
<td>Collapsing variant</td>
<td>At least one glomerulus with segmental or global collapse and overlying podocyte hyperplasia</td>
<td>None</td>
</tr>
</tbody>
</table>

Adapted from D’Agati et al.15
Clinical presentation and prognosis

The data on the clinical characteristics and natural history of patients with FSGS are biased by variable inclusion criteria used in the reported studies. Patients with secondary FSGS due to hyperfiltration injury will not be biopsied and are often not included in these studies. Search for etiological causes has been limited especially in the older studies. In most studies patients with idiopathic FSGS will predominate.

Clinical presentation

FSGS can occur at any age. In adults the mean age at onset varies between 40-50 years. The number of affected males and females is roughly similar with a male to female ratio of 1:1. Patients with idiopathic FSGS and patients with FSGS secondary to infections, medication or genetic mutations typically present with a nephrotic syndrome. By comparison, patients with FSGS secondary to adaptive structural-functional responses (hyperfiltration associated FSGS) usually present with a more indolent course without hypoalbuminemia or edema, even when proteinuria exceeds 3-4 g/day. In addition to proteinuria, microscopic hematuria and hypertension are common presenting features of both idiopathic and secondary FSGS.

Prognosis

Both the amount of proteinuria and the level of plasma creatinine are of prognostic significance. Patients with a serum creatinine concentration below 115 μmol/l at presentation have a better renal survival rate than those with higher serum concentrations. Prognosis is excellent in patients with non-nephrotic proteinuria, whereas patients with a proteinuria > 3 g/day have a 50% chance of reaching ESRD within 5-10 years. Renal survival is even worse if proteinuria exceeds 10 g/day, with the majority of patients reaching ESRD within 5 years after presentation. In contrast, attainment of a partial or complete remission of proteinuria (either spontaneous or after steroid treatment) heralds a favorable prognosis, with respectively less than 15% and 2% of such patients developing ESRD.
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Recurrent FSGS after renal transplantation

FSGS can recur after renal transplantation. The reported recurrence rate varies between 20-50%. Recurrence rate is especially high in patients younger than 20 years of age, in patients with a rapid clinical course of their original disease with development of ESRD within 3 years and also in patients with evidence of mesangial proliferation in their native kidney. It is very likely, that the majority of patients with recurrent disease have idiopathic FSGS. Analysis of our own data showed that FSGS mainly recurred in patients with idiopathic FSGS defined by strict clinical criteria. These patients had a recurrence rate of 61%. We did not observe a recurrence in patients with secondary FSGS, except for one patient (7%) with a family history of FSGS. Although one might expect that patients with genetic mutations would not develop recurrent FSGS in the renal transplant, multiple reports have described recurrent disease in patients with mutations in nephrin and podocin. Recurrence of proteinuria has been described in 20% of children with a congenital nephrotic syndrome (nephrin mutation). The recurrence seems to be mediated by the development of anti-nephrin antibodies. In the absence of nephrin, the fetus does not develop tolerance against the protein. Following renal transplantation, the patient develops an immune response to nephrin, expressed on the renal graft.

Recurrent disease has also been reported in approximately 8% of patients with homozygous or compound heterozygous podocin mutations. The recurrence of proteinuria in these patients was initially attributed to other mechanisms than circulating permeability factors. As observed in patients with the congenital nephrotic syndrome, the development of autoantibodies against the unmutated protein of the transplanted kidney seemed a convincing alternative explanation. However, so far, no antipodocin antibodies were detected in affected patients. A study by Carraro et al. suggests that the permeability factor may also play a role in patients with podocin mutations. Using an in vitro assay, these authors demonstrated the presence of the permeability factor in two patients with a heterozygous podocin mutations. These patients also responded to treatment with plasma exchange, which strengthens the hypothesis that possibly both genetic and endogenous factors are involved in the recurrence of FSGS. Without treatment prognosis is poor in patients with a recurrence of FSGS after renal transplantation, and approximately half of the patients will loose their graft.
within 5 years. Treatment with plasma exchange can induce a remission of proteinuria, however long-term effects of plasma exchange are less well known.

Outline of the Thesis

Despite our current knowledge, FSGS remains an enigmatic disease. The aim of this thesis was to gain more insight into FSGS, and to better define risk factors and outcome. This knowledge should allow better treatment of individual patients.

The first two chapters are dedicated to clinical parameters that can predict response to therapy and remission in idiopathic FSGS. In chapter 2 we studied the clinical course of idiopathic FSGS in initially untreated patients. In this group of patients we have tried to define clinical parameters that allow to predict a spontaneous remission and renal prognosis. Important parameters were serum albumin and selectivity of proteinuria. Previous data suggest that fractional excretion of IgG (FE IgG) may predict remission of proteinuria and could also be helpful in the identification of patient at risk for development of ESRD. In chapter 3 we evaluated the usefulness of urinary proteins to predict remission rate, response to therapy and renal outcome of patients with idiopathic FSGS.

The next two chapters examine the prognostic value of FSGS lesions. A new histologic classification of FSGS has been described that distinguishes 5 mutually exclusive FSGS variants on light microscopy. Recognition of these variants may have prognostic value. In chapter 4 we have studied the characteristics and renal outcome of FSGS variants in an adult Dutch population and evaluated the predictive value of the FSGS variants in comparison to other known risk factors for renal failure.

FSGS lesions can also develop secondary to other glomerular diseases. In membranous nephropathy, the existence of FSGS lesions has been associated with a worse renal prognosis. In chapter 5, we have evaluated the prognostic significance of FSGS lesions in idiopathic membranous nephropathy. In addition, we performed a meta-analysis of studies that also evaluated FSGS as prognostic marker in idiopathic membranous nephropathy.

Differentiating between idiopathic and secondary FSGS is important as misdiagnosis can lead to inappropriate treatment with steroids and cytotoxic agents that are not effective in secondary forms of FSGS. However, in daily practice distinguishing between idiopathic and secondary FSGS can be difficult. We have performed a morphometric study of podocyte foot
processes in patients with idiopathic FSGS and FSGS secondary to maladaptive responses. The degree of foot process effacement, estimated by foot process width, in these patients was compared to foot process effacement in minimal change nephrotic syndrome and normal kidneys. We tried to define a cut-off value for foot process width that could predict idiopathic or secondary FSGS with a high sensitivity and specificity (chapter 6).

Recurrence of FSGS after renal transplantation is associated with poor graft survival. Plasma exchange can reduce proteinuria and can even induce a remission. However, limited information is available on the long-term prognosis after treatment with plasma exchange. In chapter 7 we describe the follow-up of patients treated with plasma exchange after recurrence of FSGS in the renal graft. These patients were compared with a group of historical controls, consisting of patients with recurrent FSGS not treated with plasma exchange. Although many patients can be successfully treated with plasma exchange, several patients require prolonged or repeated courses of plasma exchange because of persistent recurrences. Prednisone may be essential in patients with plasma exchange dependent FSGS. The role of prednisone in plasma exchange dependent FSGS is discussed in chapter 8. Rituximab, a chimeric murine/human antibody against the CD20 antigen expressed on immature and mature B-cells may be another option in plasma exchange dependent FSGS. In children, this treatment induced a remission of proteinuria. In chapter 9 we describe the effect of rituximab in a patient with plasma exchange dependent FSGS after a second renal transplantation.

Based on the studies discussed in this thesis and a review of the literature, we provide guidelines on diagnosis and treatment of focal segmental glomerulosclerosis in chapter 10.
General introduction and outline of the thesis

Reference list


Chapter 2

Idiopathic focal segmental glomerulosclerosis: a favourable prognosis in untreated patients?

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Neth J Med 2005;63:393-398
Abstract

Background: Patients with focal segmental glomerulosclerosis (FSGS) are considered to have a poor prognosis and spontaneous remissions are seldom reported. However, FSGS is not a single disease entity. Our aim was to describe the clinical course in initially untreated patients with recently diagnosed idiopathic FSGS.

Methods: This was a retrospective study of patients with a diagnosis of FSGS by histology, who fulfilled the following criteria: proteinuria >3.5 g/day, normal renal function, duration of proteinuria or hypertension of less than one year, normal-sized kidneys, no underlying renal disease, and a negative family history. Renal biopsies were reviewed without knowledge of the clinical course.

Results: Twenty patients (13 male, 7 female) fulfilled the study criteria. Median age was 49.3 (range 21.8 to 73.0) years, serum creatinine 90 ± 20 µmol/l, proteinuria 10.0 ± 5.5 g/day and serum albumin 24 ± 6 g/l. After a median follow-up of 9.4 (2.1-18.6) years, 13 patients (65%) were in remission of proteinuria. Renal function deterioration occurred in seven patients, and prompted treatment in four of them. The ten-year death-censored renal survival was 89%. Renal function deterioration and remission rate could be predicted by selectivity index, serum albumin at three months after renal biopsy and the percentage of glomeruli with segmental sclerosis.

Conclusion: Focal glomerulosclerosis is not a single disease. Case definition using strict clinical criteria identifies a subgroup of patients with idiopathic FSGS who have a good prognosis. In the majority of these patients immunosuppressive therapy is not warranted.
Introduction

In recent years focal segmental glomerulosclerosis (FSGS) has become an increasingly important cause of the nephrotic syndrome in adults.\textsuperscript{1,2} In general, prognosis is considered to be poor in patients with FSGS and a nephrotic syndrome with up to 50% of patients proceeding to end-stage renal disease (ESRD) within five years.\textsuperscript{3,4} Spontaneous remissions are reported to be rare, occurring in less than 6% of untreated patients.\textsuperscript{4-7} Retrospective studies have suggested that complete remissions may develop in up to 60% of patients after prolonged treatment with immunosuppressive drugs.\textsuperscript{6,8} Therefore, a trial of steroid therapy is recommended in all nephrotic patients with primary FSGS.\textsuperscript{3} Attainment of a complete remission heralds a favourable prognosis with less than 15% of such patients developing ESRD. However, the literature data on prognosis and outcome of patients with FSGS must be interpreted with caution since FSGS is a descriptive, histological diagnosis and not a single disease entity.\textsuperscript{9,10}

Secondary forms of FSGS occur frequently, and may result from nephron loss (hypoplastic kidney, reflux nephropathy), other underlying renal diseases, longstanding hypertension, or obesity. If no underlying causes are identified, FSGS is classified as primary, idiopathic FSGS. In recent years it has become evident that in some of these patients the disease may be caused by mutations in podocyte proteins such as α-actinin-4 and podocin.\textsuperscript{11,12} Discrepancies between studies may thus be explained by differences in the distribution of the various types of FSGS in the studied populations. For example, in a recent study that described the natural history of patients with FSGS, the mean serum albumin of the patients was 37 g/l, which strongly suggests that patients with secondary forms of FSGS were included.\textsuperscript{13}

We retrospectively studied the outcome in a subgroup of patients with idiopathic FSGS. To avoid bias caused by including patients with other types of FSGS as far as possible, we applied a set of strictly defined clinical exclusion criteria. Because it was our policy to withhold immunosuppressive treatment in the majority of patients with FSGS without renal function deterioration, we were able to determine the outcome of FSGS in a cohort of untreated patients.
Chapter 2

Materials and methods

We identified all patients diagnosed with FSGS between 1980 and 1996 from the pathology registry at the Radboud University Nijmegen Medical Centre and affiliated hospitals. Since the aim of this study was to assess the clinical course in adult patients with recent-onset idiopathic FSGS, we included adult patients (≥18 years at biopsy) with a proteinuria ≥3.5 g/day and a serum creatinine ≤135 µmol/l in the study. To avoid bias by including patients with hereditary or secondary forms of FSGS or patients with longstanding disease, the following exclusion criteria were used: evidence of hypoplastic kidney, renal agenesis, prior nephrectomy, underlying renal disease, obesity (BMI >32 kg/m2), longstanding proteinuria (>12 months) or hypertension (>12 months), family history of renal disease, underlying malignancy, intravenous drug abuse, evidence of human immunodeficiency virus (HIV) infection or renal insufficiency (serum creatinine >135 µmol/l). During the study period most nephrologists in the participating hospitals considered FSGS to be prednisone resistant, based on the available data that short-term prednisone therapy was mostly unsuccessful. More intensive immunosuppressive therapy was used in patients with evidence of renal function deterioration (increase in serum creatinine >50%).

All renal biopsies of the patients who were included in our study were reviewed by two renal pathologists (KA and ES), who were unaware of patient outcome. A diagnosis of FSGS was made using the criteria recently described by D’Agati et al.14 Based on these criteria we discerned five light microscopic patterns of FSGS: FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant and collapsing variant.

For quantitative analysis all available glomerular cross-sections were numbered. Twenty-five glomerular profiles were selected at random by one of us and these profiles were evaluated by the renal pathologist (KA) for the presence of sclerosis, synechia, hyalinosis, collapse of the glomerular tuft, podocyte hypertrophy and foam cells.

Definitions

End-stage renal disease (ESRD) was defined as a serum creatinine of >450 µmol/l or the need for renal replacement therapy. Nephrotic syndrome was defined as a proteinuria of >3 g/day together with a serum albumin of <30 g/l and massive proteinuria was defined as proteinuria >10 g/day. Patients with a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg were considered hypertensive. A complete remission was defined
Prognosis in untreated patients with idiopathic FSGS

as proteinuria <0.2 g/day with a stable serum creatinine <135 µmol/l and a partial remission was defined as proteinuria between 0.2 g/day and 2.0 g/day with a stable serum creatinine <135 µmol/l. A relapse was defined as a proteinuria >3.0 g/day after prior reduction of the proteinuria to less than 2.0 g/day. Protein selectivity index was calculated as the clearance of IgG divided by the clearance of transferrin. Proteinuria was considered selective if the selectivity index was below 0.2.

Statistical analysis
Values are given as means ± SD or median (range) when appropriate. For comparison between groups, unpaired T-test or Mann-Whitney U test were used for continuous data and Fisher’s exact test was used for categorical data. Cumulative renal survival and cumulative remission rates were calculated with Kaplan-Meier survival curves. A p-value of 0.05 was considered to be the level of statistical significance.

Results
In the period 1980 to 1996 a diagnosis of FSGS was made in 104 patients. Based on strict clinical criteria, 37 of these 104 patients were considered to have recent-onset idiopathic FSGS (figure 1). On revision of the renal biopsies a diagnosis of FSGS was confirmed in 28 patients. FSGS lesions were not present in the biopsies of nine patients. The renal biopsies of these patients showed early lesions consisting of hyalinosis and/or foam cells and/or collapse of the glomerular tuft and/or glomerular epithelial cell hypertrophy and/or mesangial hypercellularity. Twenty patients were initially not treated, and they form the basis of this study. Thirteen out of these 20 patients received an angiotensin-converting enzyme inhibitor (ACEi), which was standard therapy for patients with proteinuria after 1988. Patient characteristics at biopsy of the 20 patients are presented in table 1. For comparison we have also provided the characteristics of the patients (n=8) who were treated with prednisone within a few months after the renal biopsy. Twenty-seven patients were Caucasian and one patient was Indonesian. A nephrotic syndrome was present in all patients, except for one patient who had a serum albumin of 43 g/l at biopsy. Proteinuria >10 g/day was present in ten patients. The findings on renal biopsy are presented in table 2. Most patients were classified as tip variant; only three patients had FSGS-NOS. All patients studied had evidence of diffuse foot process effacement.
Figure 1. Flow chart of selection of patients with idiopathic focal glomerulosclerosis.

104 patients diagnosed as having FSGS

24 patients excluded
- duration of proteinuria > 1 yr n = 18
- proteinuria < 3.5 g/day n = 2
- hypertension > 1 yr n = 4

80 patients

30 patients with secondary FSGS
- hypoplasia/chronic pyelonephritis n = 12
- glomerulonephritis n = 7
- malignancy n = 4
- familial FSGS n = 3
- other n = 4

50 patients with primary FSGS

13 patients with renal insufficiency

9 patients without FSGS after revision of the renal biopsy

28 patients with primary FSGS fulfilling the inclusion criteria
### Table 1. Patient characteristics at biopsy.

<table>
<thead>
<tr>
<th></th>
<th>Initially untreated (n=20)</th>
<th>Treated (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>13/7</td>
<td>5/3</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>49.3 (21.8-73.0)</td>
<td>42.4 (19.5-67.4)</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>24 ± 6</td>
<td>18 ± 5 *</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>90 ± 20</td>
<td>81 ± 17</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min; n=22)</td>
<td>101 ± 34</td>
<td>123 ± 35</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l; n=23)</td>
<td>11.3 ± 2.3</td>
<td>11.6 ± 2.9</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>10.0 ± 5.5</td>
<td>9.8 ± 3.1</td>
</tr>
<tr>
<td>Proteinuria &gt; 10 g/day (n)</td>
<td>7 (35%)</td>
<td>3 (38%)</td>
</tr>
<tr>
<td>Selectivity index (n=23)</td>
<td>0.21 ± 0.09</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>16 (80%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>155 ± 16</td>
<td>146 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>91 ± 9</td>
<td>82 ± 8 *</td>
</tr>
<tr>
<td>Interval presentation-biopsy (months)</td>
<td>2.1 (0.7-10.6)</td>
<td>4.5 (0.7-7.6)</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>9.4 (2.1-18.6)</td>
<td>9.2 (4.0-11.2)</td>
</tr>
</tbody>
</table>

Values are given as means (±SD) or median (range). \*p< 0.05 for comparison between treated and untreated patients.

### Table 2. Findings in renal biopsies of treated and untreated patients.

<table>
<thead>
<tr>
<th></th>
<th>Initially untreated (n=20)</th>
<th>Treated (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glomeruli per biopsy</td>
<td>26 (4-87)</td>
<td>13 (4-62)</td>
</tr>
</tbody>
</table>

**Light microscopy**

- Number of patients with NOS variant: 3 (2)
- Number of patients with tip variant: 13 (6)
- Number of patients with collapsing variant: 2 (0)
- Number of patients with perihilar variant: 2 (0)

**Fluorescence microscopy (n=26)**

- Number of patients with segmental: 17 (6)
- Number of patients without depositions: 2 (1)

\*n = number of patients; \*fluorescence microscopy was not available in two patients.
Outcome of untreated patients

As mentioned above, 20 patients were initially left untreated. Fourteen of these 20 patients developed a spontaneous partial remission. The nephrotic syndrome persisted in six patients. Time between biopsy and partial remission varied between 1.4 and 36.7 months, with a median of 9.9 months.

Cumulative incidence of partial remission at one, three and five years after biopsy was 40%, 73% and 73% respectively. Two patients with a partial response have relapsed, one patient had a sustained partial remission, whereas 11 patients developed a persistent complete remission of proteinuria. Serum creatinine increased more than 50% in all six patients with a persistent nephrotic syndrome and in one patient who relapsed after a partial remission. Four of these seven patients were treated with immunosuppressive drugs, either prednisone monotherapy (n=1) or prednisone combined with cyclophosphamide (n=3). Patients received prednisone at a dose ≥60 mg/day for an average of 2.5 ± 0.9 months; Thereafter the prednisone dose was progressively tapered. Cyclophosphamide was given in a dose of 150 mg/day for three months. Treatment resulted in a partial remission in one patient, and nephrotic range proteinuria persisted in two patients; however, renal function improved in both patients and remained stable until the end of follow-up, and one patient progressed to ESRD. In the three patients with declining renal function who did not receive immunosuppressive drugs, one patient progressed to ESRD, one patient had persistent nephrotic proteinuria and one patient had persistent non-nephrotic proteinuria. Renal function deteriorated progressively in both patients with persistent proteinuria. Treatment with an ACEi was not different between patients who achieved a complete remission (7 out of 11) and patients with deterioration in renal function (4 out of 7).

Table 3. Clinical status at the end of follow-up in treated and untreated patients

<table>
<thead>
<tr>
<th></th>
<th>Initially untreated (n=20)</th>
<th>Treated (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission</td>
<td>11 (55%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Partial remission</td>
<td>2 (10%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Persistent proteinuria</td>
<td>4 (20%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>ESRD*</td>
<td>2 (10%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Deceased</td>
<td>1 (5%)</td>
<td>3 (37.5%)</td>
</tr>
</tbody>
</table>

* End stage renal disease
Clinical status at the end of follow-up is given in table 3. One patient (5%) with persistent proteinuria died due to a myocardial infarction, 2.3 years after diagnosis. Renal survival was 100, 84 and 84% at one, five and ten years after biopsy, respectively. Renal survival censored for death at one, five and ten years after biopsy was 100%, 89% and 89%. For comparison, clinical status at the end of follow-up is also presented for the treated patients (table 3).

In this group of initially untreated patients we tried to define the characteristics that would allow us to predict prognosis. The following parameters were evaluated: serum albumin, proteinuria and protein selectivity index (all measured at the time of biopsy), serum albumin, proteinuria and a combination of increased levels of proteinuria or decreased concentrations of serum albumin (all measured at three months after biopsy), histological variants of FSGS and the percentage of glomerular profiles with sclerosis, synechia or hyalinosis on renal biopsy. A higher percentage (>25%) of sclerosis in the glomeruli, nonselective proteinuria at the time of biopsy and decreased concentrations of serum albumin at three months after renal biopsy were associated with a higher rate of renal failure (table 4).

**Table 4. Predictors of deterioration of renal function in initially untreated patients.**

<table>
<thead>
<tr>
<th>Deterioration of renal function</th>
<th>p-value&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selectivity index&lt;sup&gt;‡&lt;/sup&gt;</strong></td>
<td></td>
</tr>
<tr>
<td>≤ 0.2</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 0.2</td>
<td>7</td>
</tr>
<tr>
<td><strong>Serum albumin at three months</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 g/l</td>
<td>4</td>
</tr>
<tr>
<td>≥ 20 g/l</td>
<td>3</td>
</tr>
<tr>
<td><strong>Sclerosis</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 25% of glomerular profiles</td>
<td>2</td>
</tr>
<tr>
<td>≥ 25% of glomerular profiles</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>†</sup> Deterioration of renal function was defined as an increase in serum creatinine of > 50%.

<sup>‡</sup> P-values were calculated by Fisher’s Exact Test.

<sup>‡</sup> The selectivity index of three patients was not available.
**Discussion**

We aimed to study the outcome in a well-defined subgroup of patients with a nephrotic syndrome due to idiopathic FSGS. Therefore, we selected patients with FSGS who fulfilled strict criteria i.e. normal-sized kidneys, absence of underlying glomerular disorders, negative family history of renal disease, no longstanding proteinuria or hypertension, no morbid obesity and normal renal function. We observed that more than half of the untreated patients showed a spontaneous partial or complete remission of proteinuria, clearly different from previous studies in patients with FSGS that report spontaneous remission rates of 6 to 7%.\(^{4,6,16}\) We feel that our study underlines that FSGS is not a single disease entity, but rather a common phenotypic expression (on histology) of various diseases that differ in pathogenesis, prognosis and response to therapy.\(^{10}\) This is supported by the fact that literature data on the prognosis and outcome of treatment in patients with FSGS are quite diverse. In contrast to our study, many studies that report low remission rates have included patients with decreased renal function at presentation or at the time of renal biopsy,\(^{4,6,17}\) patients with non-nephrotic proteinuria,\(^{16,18}\) and patients with normal serum albumin.\(^{13}\) Furthermore, many studies do not mention the specific criteria that were used to select patients.

In recent years it has become evident that FSGS can result from mutations in podocytic proteins such as nephrin, α-actinin-4 and podocin.\(^{11,12}\) Mutations may not only be present in patients with familial forms of FSGS but also in some patients with sporadic forms of FSGS.\(^{19,20}\) In our patients DNA typing was not performed. However, because of our exclusion criteria (family history of renal disease, age <18 years, longstanding proteinuria) it is unlikely that patients with mutations were included. Although our study is retrospective in nature, we tried to avoid potential bias as far as possible. First, we have included all patients derived from the pathology registry. In addition, the composition of the study population was based on predefined clinical criteria without knowledge of the outcome. Finally, all renal biopsies were revised and only patients with typical lesions of FSGS were included. In the period between 1980 and 1996 not all physicians adhered to the same treatment strategy. Thus eight patients were initially treated. Nevertheless, if untreated and treated patients are grouped together, still 12 out of 28 patients with a normal renal function have a persistent spontaneous remission of proteinuria.
Four of the initially untreated patients received immunosuppressive therapy after deterioration of the renal function. In some of these patients treatment was successful, and renal function improved in three out of four patients. Results might have been even better if we had used immunosuppressive therapy for a longer period of time, as has been suggested by recent studies.\textsuperscript{4,8,21} If spontaneous remissions occur frequently, it is important to be able to predict outcome at an early stage to allow tailor-made treatment. In our patients, nonselective proteinuria, a higher percentage of sclerotic lesions on renal biopsy and low levels of serum albumin at three months after renal biopsy predicted prognosis. Because of the small number of patients in our study we could not define the most predictive variable.

Recent studies suggest that the occurrence of the ‘tip lesion’ variant of FSGS identifies a group of patients with a better prognosis.\textsuperscript{22,23} Due to the small number of patients with FSGS variants other than the tip lesion we were unable to determine whether the histological variant of FSGS can predict the occurrence of a spontaneous remission. However, in agreement with our findings, these studies have also demonstrated that a higher percentage of sclerotic lesions predicts a worse prognosis.\textsuperscript{22,23} In a recent study Bazzi et al. concluded that the fractional excretion of IgG (FE IgG) predicted prognosis in patients with FSGS.\textsuperscript{15} These authors measured FE IgG in 29 patients with FSGS and normal renal function of whom 27 were treated. FE IgG could predict remission in 91\% and progression to ESRD in 71\% of patients, respectively. This confirms our findings that qualitative markers of proteinuria such as FE IgG and selectivity index are important predictive variables.

In conclusion, clinical criteria allow us to identify a group of patients with recent-onset idiopathic FSGS that have a favourable prognosis. Many of these patients will develop a spontaneous remission of proteinuria. In these patients immunosuppressive therapy is not warranted unless renal function deteriorates. Future studies should allow identification of laboratory parameters and histological criteria that predict outcome with high sensitivity and specificity.
Reference List


Chapter 3

Fractional excretion of high and low molecular weight proteins and outcome in primary focal segmental glomerulosclerosis.

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Abstract

**Aims:** Predicting prognosis in patients with a nephrotic syndrome due to primary FSGS remains difficult. Recently, it was suggested that the fractional excretion (FE) of IgG (threshold 0.14%) predicts remission, progression to renal failure and response to therapy in FSGS. In the present study, we evaluated the usefulness of FE IgG to guide treatment of patients with primary FSGS in clinical practice.

**Methods:** From 1995 onward, FE of IgG was measured in 32 adult patients with biopsy-proven primary FSGS. In addition, we quantified 24-hour proteinuria, selectivity index (SI) and FE of albumin, IgG, transferrin and β2-microglobulin (β2m). We evaluated outcome in patients with FE IgG above and below 0.14%. Receiver-operating curves were used to determine the best cut-off values for other urinary proteins in predicting remission, response to therapy and renal survival.

**Results:** Mean age was 45 ± 17 years, serum creatinine 128 ± 58 μmol/l, proteinuria 10.3 ± 4.7 g/day and serum albumin 18 ± 7 g/l. Twenty-three patients received immunosuppressive therapy (9 prednisone and 14 prednisone and cyclophosphamide). After a median follow-up of 58.3 (4.9–127.6) months, 17 patients were in remission (10 complete, 7 partial), 6 patients still had a nephrotic syndrome, renal failure developed in 6 patients, and 3 patients had died. Remission rate was similar in patients with FE IgG less or greater than 0.14%. More patients with FE IgG ≥ 0.14% had received immunosuppressive therapy. Additional analysis revealed that the predictive value of FE of albumin, transferrin and β2m was low. In untreated patients, FE β2m < 1 predicted a better renal survival.

**Conclusions:** Our data indicate that a FE IgG ≥ 0.14% is not invariably associated with a poor outcome in patients with primary FSGS. Therefore, high FE IgG should not lead to therapeutic nihilism. Low FE β2m predicted a good prognosis without immunosuppressive therapy.
Introduction

Primary, idiopathic focal segmental glomerulosclerosis (FSGS) is an important cause of the nephrotic syndrome in adults, accounting for 15–35% of cases. Since the first description of FSGS by Rich, many authors have emphasized the poor outcome in adult patients with FSGS, characterized by unresponsiveness to therapy and progression to end-stage renal disease (ESRD). However, it is now clear that up to 60% of patients can achieve a remission of proteinuria with aggressive corticosteroid therapy. Patients who attain a partial or complete remission have a favorable prognosis, with, respectively, less than 15 and 2% progressing to ESRD. Treatment of patients with FSGS could be optimized if we could predict the likelihood of attaining a remission in an individual patient.

In patients with membranous nephropathy, we and others have studied the predictive value of different urinary proteins. Specifically these studies have evaluated markers of glomerular size selectivity (IgG and protein selectivity index) and markers of tubulointerstitial injury (the low-molecular weight proteins β2-microglobulin and α1-microglobulin). Both urinary IgG excretion and urinary excretion of low-molecular weight proteins accurately predicted disease progression. Recently, Bazzi et al. reported that in patients with primary FSGS, fractional excretion (FE) of IgG predicted remission rate, progression to ESRD and response to therapy. A cut-off value of 0.14% proved most discriminative with a sensitivity of 87.5% and a specificity of 100%. Specifically, remission rate was 0% in patients with a FE IgG ≥ 0.14% despite treatment with steroids and cyclophosphamide. If confirmed, use of this parameter could help in guiding the decision to refrain from cytotoxic medication in these patients.

In the present study, we analyzed the predictive value of a threshold of 0.14% for FE IgG for remission, response to therapy and renal survival in patients with idiopathic FSGS. In addition, we assessed the predictive value of other urinary proteins.

Subjects and methods

In our centre, patients with proteinuria are evaluated using a standard protocol. In all these patients, standardized urine and blood measurements are performed as summarized below. For the present study, we included adult patients (> 18 years at biopsy) with a nephrotic syndrome due to idiopathic FSGS, evaluated from 1995 onward. The diagnosis of idiopathic
FSGS was established by the presence of a nephrotic syndrome with out evidence for another primary glomerular disease in the renal biopsy or a disease associated with secondary FSGS.\textsuperscript{14}

The renal biopsy specimens were reviewed by a renal pathologist without knowledge of the clinical outcome and categorized according to the criteria described by D’Agati \textit{et al.}\textsuperscript{15} Only patients with definite pathologic findings of FSGS on light microscopy and either negative immunofluorescence or only segmental IgM and/or C3 were considered for the study.

\textbf{Standardized measurement of urinary proteins}

Patients came to the ward after an overnight fast. On the evening before the patients had taken 4,000 mg of sodium bicarbonate to ensure a urinary pH above 6.0, which is mandatory for the measurement of urinary $\beta_2$-microglobulin. Upon arrival, up to 500 ml tap water is given to enforce diuresis. The patients remain supine during 2 hours except for voiding. Timed urine samples are collected, and in the middle of the collection period, a blood sample is drawn. In addition, two 24-hour samples are collected for assessment of daily excretion of total protein and creatinine.

\textbf{Laboratory measurement}

Creatinine, cholesterol, albumin, transferrin, IgG, $\beta_2$-microglobulin ($\beta_2$m) were measured in blood samples. In the timed urine samples we measured creatinine, albumin, transferrin, IgG and $\beta_2$m. Measurement of creatinine was done with the modified Jaffe technique on a Hitachi 747 autoanalyzer (Roche, Almere, The Netherlands). The concentration of albumin, transferrin and IgG in serum and urine were measured by immunonephelometry on a BNII nephelometer (Behring, Marburg, Germany) using antibodies whose specificity was checked by Ouchterlony double immunodiffusion and immunoelectrophoresis (Dako, Glastrop, Denmark). Urinary and serum $\beta_2$m were measured by ELISA.\textsuperscript{15}

\textbf{Calculations}

The amounts of albumin, transferrin, IgG and $\beta_2$m in the timed urine samples are expressed as fractional excretion, using the following formula: (urinary protein/serum protein) $\times$ (serum creatinine/urinary creatinine) $\times$ 100. The selectivity index (SI) was calculated as the clearance of IgG divided by the clearance of transferrin.
Definitions
Renal failure was defined as a sustained increase of the serum creatinine of > 50% from baseline measurement or progression to end-stage renal disease (ESRD). ESRD was defined as a serum creatinine of more than 450 μmol/l or the need for renal replacement therapy or kidney transplantation. Nephrotic syndrome was defined as proteinuria of ≥ 3 g/day in association with serum albumin of ≤ 30 g/l. A complete remission was defined as proteinuria < 0.3 g/day with a stable serum creatinine (< 50% increase from baseline) and a partial remission was defined as proteinuria between 0.3 g/day and 2.0 g/day with a stable serum creatinine. A relapse was defined as a proteinuria > 3 g/day after prior reduction of the proteinuria to less than 2.0 g/day.

Statistics
Values are given as means ± SD or median (range) when appropriate. For comparison between 2 groups Mann-Whitney U-test was used for continuous data and Fisher’s exact test was used for categorical data. Differences in continuous data between more than 2 groups were analyzed with use of the Kruskal-Wallis test, with multiple comparison procedures (Dunn’s method) for comparison between groups. A p-value < 0.05 was considered as the level of statistical significance. Receiver operating characteristics (ROC) curves were used to determine the most discriminative threshold for FE albumin, FE transferrin, FE IgG, FE β2m, proteinuria and SI in predicting renal failure or persistence of nephrotic syndrome. The difference in renal survival and remission rate for the selected thresholds was analyzed with Kaplan-Meier statistics. Log-rank test was used for comparison of the Kaplan-Meier curves. To adjust for multiple comparisons, a p-value <0.008 was considered as the level of statistical significance.
Chapter 3

Results

Between 1995 and 2002, a total of 32 patients with idiopathic FSGS who fulfilled the inclusion criteria, were evaluated. Baseline characteristics are given in table 1. The baseline measurement was performed within 16 months from biopsy in 90% of the patients. Nine patients had already received a course of immunosuppressive therapy before baseline measurement. Seven patients were non responsive (prednisone n=2, prednisone and cyclophosphamide n=3, prednisone and cyclosporine n=2) and 2 patients, both treated with prednisone, had relapsed after a partial remission. Baseline measurement was performed without use of immunosuppressive medication. Review of the renal biopsy showed the tip lesion in 22 patients (69%) and FSGS NOS in 10 patients (31%). Thirty patients were treated with an ACE inhibitor. Twenty-three patients received immunosuppressive therapy, 3.2 (range 0.03–91) months after the baseline measurement. Nine patients were treated with prednisone monotherapy for a median of 6.5 (range 3.9–18.3) months. Fourteen patients were treated with prednisone for a median of 6.2 (range 1.3–21.4) months in combination with cyclophosphamide for a median of 3 (range 1.3–3.9) months. Prednisone was given as a single dose of 1 mg/kg/day and subsequently tapered. Cyclophosphamide was administered orally at a daily dose of 1.5–2 mg/kg. The nephrotic syndrome was more severe in patients treated with prednisone or prednisone and cyclophosphamide compared to untreated patients. In addition, patients treated with prednisone and cyclophosphamide had a significantly higher serum creatinine and FE of proteins compared to untreated patients (table 1). Six out of 9 patients (67%) treated with prednisone and 6 out of 14 patients (43%) treated with prednisone and cyclophosphamide attained a remission of proteinuria. In addition, 6 out of 9 untreated patients (67%) developed a spontaneous remission. Two patients, initially responsive to prednisone, experienced a relapse and were successfully treated with prednisone and cyclophosphamide. Two patients responsive to prednisone and cyclophosphamide had 1 or more relapses, all relapses responded to retreatment with prednisone and cyclophosphamide.

At the end of follow-up, 17 patients (53%) were in remission (10 complete and 7 partial), 6 patients still had a nephrotic syndrome (1 patient with a serum creatinine > 135 μmol/l) and 6 patients had developed renal failure. Three patients died during follow-up; Two patients died while nephrotic, due to a myocardial infarction and myocarditis with arrhythmia, 1 patient with a partial remission died due to a myocardial infarction.
Table 1. Patient characteristics at baseline measurement

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Treated patients</th>
<th>Untreated Patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Prednisone</td>
<td>Prednisone + cyclophosphamide</td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>9</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>23/9</td>
<td>8/2</td>
<td>9/4</td>
<td>6/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45 ± 17</td>
<td>47 ± 20</td>
<td>47 ± 17</td>
<td>40 ± 14</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>18 ± 7</td>
<td>16 ± 7</td>
<td>15 ± 5</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>128 ± 58</td>
<td>106 ± 40</td>
<td>161 ± 65</td>
<td>97 ± 31</td>
</tr>
<tr>
<td>Serum creatinine &gt;135 μmol/L</td>
<td>11</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>9.4 ± 3.1</td>
<td>8.3 ± 1.6</td>
<td>11.4 ± 3.4</td>
<td>7.5 ± 2.0</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>10.3 ± 4.7</td>
<td>10.7 ± 3.0</td>
<td>12.4 ± 5.5</td>
<td>6.7 ± 2.9</td>
</tr>
<tr>
<td>Previous immunosuppressive therapy (n)</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>58 (5-128)</td>
<td>37 (7-128)</td>
<td>72 (5-110)</td>
<td>38 (27-104)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission (partial/complete)</td>
<td>17 (10.7)</td>
<td>5 (3/2)</td>
<td>6 (3/3)</td>
<td>6 (4/2)</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Renal failure</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Time urine samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE albumin (%)</td>
<td>0.68 (0.05-4.9)</td>
<td>0.70 (0.12-1.92)</td>
<td>1.22 (0.24-9)</td>
<td>0.25 (0.05-0.42)</td>
</tr>
<tr>
<td>FE IgG (%)</td>
<td>0.28 (0.007-1.9)</td>
<td>0.17 (0.03-0.66)</td>
<td>0.37 (0.02-1.9)</td>
<td>0.05 (0.007-0.08)</td>
</tr>
<tr>
<td>FE β2m (%)</td>
<td>4.6 (0.06-24.2)</td>
<td>1.1 (0.2-10.2)</td>
<td>3.6 (0.15-24.2)</td>
<td>0.49 (0.06-1.55)</td>
</tr>
<tr>
<td>FE transferrin (%)</td>
<td>0.91 (0.05-3.8)</td>
<td>0.73 (0.11-1.91)</td>
<td>1.21 (0.19-3.8)</td>
<td>0.23 (0.05-0.4)</td>
</tr>
<tr>
<td>Selectivity index (%)</td>
<td>0.25 ± 0.1</td>
<td>0.23 ± 0.08</td>
<td>0.31 ± 0.1</td>
<td>0.19 ± 0.05</td>
</tr>
</tbody>
</table>

* p<0.05 untreated vs prednisone; † p<0.01 untreated vs prednisone+cyclophosphamide; ‡ p<0.05 untreated vs prednisone+cyclophosphamide; § p<0.05 prednisone vs prednisone+cyclophosphamide
Predictive value of FE IgG less or greater than 0.14%

We analyzed the predictive value of FE IgG less or greater than 0.14%. Baseline characteristics are given in table 2. Patients with FE IgG ≥ 0.14% had worse baseline characteristics. Serum creatinine was significantly higher in patients with FE IgG ≥ 0.14%. In addition, the nephrotic syndrome was more severe in patients with FE IgG ≥ 0.14% as reflected by a significantly lower serum albumin and a significantly higher proteinuria and serum cholesterol. Significantly more patients with FE IgG ≥ 0.14% received immunosuppressive therapy, 100 versus 40% (table 2, p<0.001).

Table 2. Characteristics of patients with FE IgG less or greater than 0.14%

<table>
<thead>
<tr>
<th></th>
<th>FE IgG &lt; 0.14% (n=15)</th>
<th>FE IgG ≥ 0.14% (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>9/6</td>
<td>14/3</td>
<td>0.24</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40 ± 17</td>
<td>45 ± 17</td>
<td>0.09</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>24 ± 6</td>
<td>18 ± 7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>94 ± 27</td>
<td>128 ± 58</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum creatinine &gt; 135 µmol/l (n)</td>
<td>1 (7%)</td>
<td>10 (59%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>7.8 ± 2.0</td>
<td>9.4 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>7.8 ± 3.6</td>
<td>10.3 ± 4.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>38 (15-123)</td>
<td>58 (5-128)</td>
<td>0.98</td>
</tr>
<tr>
<td>Remission of proteinuria (n)</td>
<td>8 (53%)</td>
<td>10 (59%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Renal failure (n)</td>
<td>4 (27%)</td>
<td>2 (12%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Immunosuppressive medication (n)</td>
<td>6 (40%)</td>
<td>17 (100%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prednisone</td>
<td>4 (26%)</td>
<td>5 (29%)</td>
<td></td>
</tr>
<tr>
<td>Prednisone and cyclophosphamide</td>
<td>2 (13%)</td>
<td>12 (71%)</td>
<td></td>
</tr>
</tbody>
</table>

Remission

Although patients with FE IgG ≥ 0.14% had worse baseline characteristics, remission rate was not significantly different compared to patients with FE IgG < 0.14%, respectively 59% and 53%, (p=NS, table 2). Survival analysis also showed that FE IgG less or greater than 0.14% did not predict a remission of proteinuria (figure 1, p=0.37).
Renal failure
Twenty-seven percent of patients with FE IgG < 0.14% and 12% of patients with FE IgG ≥ 0.14% developed renal failure (p=NS, table 2). Survival analysis showed no significant difference in progression to renal failure between patients with FE IgG less or greater than 0.14% (figure 2, p=0.44).

Figure 1. Remission rate in patients with a FE IgG less or greater than 0.14%

Figure 2. Renal survival in patients with a FE IgG less or greater than 0.14%
Predictive value of FE of proteins, selectivity index and proteinuria

We separately analyzed the predictive value of all measured urinary proteins. Threshold values for FE of proteins (albumin, transferrin, IgG and β2m), SI and 24-hour proteinuria predicting persistent nephrotic syndrome or renal failure were selected using ROC curves (table 3). Values selected showed the best combination of sensitivity and specificity. As reflected by the low area under the curve, the discriminative ability of the threshold values was relatively poor. Survival analysis showed that the selected threshold values for FE of proteins, SI and 24-hour proteinuria did not predict a remission of proteinuria or progression to renal failure with statistical significance. Similarly, the response to prednisone or prednisone and cyclophosphamide could not be predicted by FE of proteins, SI or 24-hour proteinuria. In contrast, in untreated patients, a FE β2m <1 was associated with a significant better renal survival (100% versus 0%, p=0.005). Remission rate was also higher in untreated patients with FE β2m <1, however, this difference did not reach the level of statistical significance (86% versus 0%, p log-rank=0.08). Treatment with immunosuppressive medication resulted in similar remission rates and renal survival for patients with FE β2m less or greater than 1.

We also analyzed all data with out the 9 patients who had al ready received a course of immunosuppressive therapy before baseline measurement. Exclusion of these patients did not change the results (data not shown).

Table 3. Sensitivity and specificity of the most discriminative threshold levels of fractional excretion of proteins, selectivity index and proteinuria in the prediction of renal failure or persistent nephrotic syndrome.

<table>
<thead>
<tr>
<th>Threshold value</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (g/day)</td>
<td>10.5</td>
<td>0.631</td>
<td>71</td>
</tr>
<tr>
<td>Selectivity Index</td>
<td>0.2</td>
<td>0.627</td>
<td>79</td>
</tr>
<tr>
<td>FE β2m (%)</td>
<td>1.0</td>
<td>0.571</td>
<td>79</td>
</tr>
<tr>
<td>FE IgG (%)</td>
<td>0.05</td>
<td>0.480</td>
<td>86</td>
</tr>
<tr>
<td>FE transferrin (%)</td>
<td>0.2</td>
<td>0.431</td>
<td>93</td>
</tr>
<tr>
<td>FE albumin (%)</td>
<td>0.2</td>
<td>0.421</td>
<td>93</td>
</tr>
</tbody>
</table>

AUC=area under the curve; FE=fractional excretion
Fractional excretion of high and low molecular weight proteins in primary FSGS

FE of proteins, SI and 24-hour proteinuria in the different histologic variants

FE of proteins, SI and 24-hour proteinuria were not significantly different between patients with the tip variant and patients with FSGS NOS. Patients with the tip variant had a median FE IgG and FE β2m of 0.19 (range 0.01–1.9) and 1.5 (range 0.06–24.2), compared to 0.08 (range 0.01–0.75) and 0.88 (range 0.1–16.7) for patients with FSGS NOS (p=0.56 and p=0.57 for the differences between the tip variant and FSGS NOS). Selectivity index was 0.26 ± 0.1 in patients with the tip variant and 0.25 ± 0.11 in patients with FSGS NOS (p=0.6).

Discussion

Predicting prognosis in patients with primary FSGS remains difficult. Several clinical and histological parameters such as the degree of proteinuria, serum creatinine and tubulointerstitial damage have been described that are associated with outcome in primary FSGS. However, these parameters lack sensitivity and specificity and do not allow to guide treatment. Thus far, the best prognostic indicator in patients with primary FSGS is attainment of a remission. Therefore, patients with primary FSGS are usually treated with immunosuppressive therapy in an attempt to induce a remission. However, it would be ideal if outcome and response to treatment could be predicted before initiation of immunosuppressive therapy. A recent study by Bazzi et al. in 29 patients with a normal renal function reported that FE IgG less or greater than 0.14% not only predicted remission and progression to ESRD but also responsiveness to immunosuppressive therapy. In fact, a FE IgG above 0.14% was associated with complete unresponsiveness to steroids alone or in combination with cyclophosphamide. In contrast, our study shows that FE IgG ≥ 0.14% is not associated with a worse outcome. Although baseline characteristics were less favorable, remission rate and renal survival were not different from patients with FE IgG less than 0.14%. Of note, patients with FE IgG ≥ 0.14% only attained a remission after immunosuppressive therapy. We, therefore, agree that patients with a FE IgG ≥ 0.14% have more severe disease and are more often in need of immunosuppressive therapy. However, with treatment, prognosis is favorable. We have further analyzed our data and evaluated other threshold values for FE IgG as well as other proteins. As indicated by the rather low AUC of the ROC curves, neither FE IgG, albumin, transferrin and β2m nor 24-hour
proteinuria and SI predicted remission or progression to renal failure with high sensitivity/specificity.

Admittedly, our study was retrospective and, thus, has limitations. Still, the data are sufficient to question the conclusions of Bazzi et al.\textsuperscript{13} We feel that it is not justified to withhold immunosuppressive therapy to patients with FSGS based on FE IgG.

An explanation for the different results in study by Bazzi et al. is not immediately evident.\textsuperscript{13} However, it is likely that the relatively small number of patients with FE IgG \( \geq 0.14\% \) \((n=7)\) included in the study by Bazzi et al. contributed to the different findings. As our study shows, still 41\% of patients with FE IgG \( \geq 0.14\% \) do not attain a remission with immunosuppressive therapy. Therefore, if only a few patients with FE IgG \( \geq 0.14\% \) are included, a response to immunosuppressive medication may be missed. The difference might also be explained by an inadequate course of immunosuppressive medication in some patients in the study by Bazzi et al.\textsuperscript{13} To attain a remission in primary FSGS, immunosuppressive therapy has to be sufficiently long, up to 6–8 months.\textsuperscript{4,5,20} Although at first sight the duration of immunosuppressive therapy in the Italian study appears adequate and not significantly different from our study, at closer look, several patients included in the study were treated between 1974 and 1991. During that period, immunosuppressive courses were often limited to 8 weeks.\textsuperscript{20,21} Especially in patients with a high FE IgG, this period may be too short to induce a remission. This notion is supported by the study of Bazzi et al., showing that to achieve a remission, immunosuppressive therapy has to be continued for a longer period of time as FE IgG becomes higher.\textsuperscript{13}

Alternatively, based on the rapid progress to ESRD it was suggested that patients with a FE IgG \( \geq 0.14\% \) included in the Italian study were unresponsive to immunosuppressive medication due to a sporadic podocytic mutation.\textsuperscript{13,22} Although a possible relationship may exist between immunosuppressive-resistant FSGS due to a podocytic mutation and a high FE IgG, our study shows that this should not lead to the conclusion that all patients with a high FE IgG are therapy resistant.

It is important to emphasize that the favorable prognosis in patients with a higher FE IgG in our study, does not exclude the possibility that FE of proteins can be used to guide treatment as reported by Bazzi et al.\textsuperscript{13} In the present study, the decision regarding the type of immunosuppressive therapy was made by the patient’s physician. Therefore, 14 patients received a combination of prednisone and cyclophosphamide after characterization of
proteinuria. We cannot exclude that the use of an escalating treatment regimen (i.e. first prednisone in case of no response followed by prednisone and cyclophosphamide) would have shown that the response to prednisone or prednisone and cyclophosphamide can be predicted by FE of proteins. FE of proteins may even al low to identify patients who can attain a remission without immunosuppressive therapy. In the present study, we observed a significant better renal survival in untreated patients with a FE β2m <1. These findings are in agreement with a previous study that showed a good renal survival in patients with primary FSGS and a selective proteinuria. Therefore, FE β2m may be a useful marker in selecting the best treatment for patients with primary FSGS. However, the small number of untreated patients in the present study does not allow to draw definitive conclusions, and prospective studies using a stepwise treatment regimen are necessary to establish whether FE of proteins can be used to decide whether or not immunosuppressive treatment should be started and if so which regimen is most effective.

We did not detect a significant difference in FE of proteins between patients with the tip variant and FSGS NOS. However, the number of patients with FSGS NOS was relatively low. Definitive conclusions about differences in FE of proteins between patients with the tip variant and FSGS NOS can only be reached after studies including more patients with different histologic types, preferably also collapsing FSGS and perihilar FSGS.

In conclusion, our study shows that in patients with primary FSGS, a FE IgG ≥ 0.14% is not inevitably associated with a poor renal prognosis or unresponsiveness to treatment and should not lead to therapeutic resignation. Treatment with immunosuppressive therapy results in a high remission rate in patients with FE IgG ≥ 0.14%. Patients with a FE β2m < 1 have a good renal prognosis even without immunosuppressive therapy. Further studies are necessary to determine if FE of proteins can guide treatment.

Reference List


Chapter 4

Pathological Variants of Focal Segmental Glomerulosclerosis in an Adult Dutch Population: Epidemiology and Outcome.

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Nephrol Dial Transplant 2007; in press
Abstract

**Background:** A working group has defined five subtypes of focal segmental glomerulosclerosis (FSGS) based on light microscopic assessment (Columbia classification). Limited information is available on the prognostic and therapeutic implications of this classification in an European population. We conducted a retrospective analysis in 93 adult patients with biopsy-proven FSGS to determine the clinical features and outcome of FSGS variants.

**Methods:** Renal biopsy specimens of adult patients (>16 year) diagnosed with FSGS between 1980 and 2003 were reviewed according to the Columbia classification without the knowledge of clinical outcome. The medical records were reviewed for clinical data. Primary outcomes were remission rate and renal survival.

**Results:** The frequencies of the FSGS variants was: 32% NOS (FSGS not otherwise specified), 37% tip, 26% perihilar and 5% collapsing. Cellular FSGS was not found in the biopsies. The nephrotic syndrome was less frequent in FSGS NOS (57%) and perihilar FSGS (25%) compared to the tip variant (97%). Renal function was significantly better in patients with the tip variant compared to FSGS NOS ($p<0.05$). Glomerular sclerosis and hyalinosis was most severe in patients with perihilar FSGS, intermediate in FSGS NOS and the least severe in patients with the tip variant. Patients with perihilar FSGS were less likely to receive immunosuppressive medication. Renal survival at 5 years was significantly better for patients with the tip variant (78% for tip vs 63 and 55% for FSGS NOS and perihilar FSGS,$p=0.02$). Type of FSGS and serum creatinine concentration were independent predictors of renal survival. Remission rate was higher in patients with the tip variant ($p=0.1$).

**Conclusion:** The collapsing variant was rare in our population. Renal survival and remission rates were higher in patients with the tip variant. Use of the classification scheme for FSGS may be clinically useful.
Introduction

Focal segmental glomerulosclerosis (FSGS) is primarily a histological diagnosis, characterized by the presence of segmental sclerotic lesions involving some but not all glomeruli. Since the first descriptions by Fahr and Rich, several different histological variants of FSGS have been described. A group of renal pathologists redefined these histological variants and proposed a standardized pathological classification system for FSGS based entirely on light microscopic examination. This classification presumes previous exclusion of other primary glomerular diseases associated with FSGS, such as IgA nephropathy, diabetic glomerulosclerosis, membranous nephropathy and Alport’s syndrome. Five histological variants were described: FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant and collapsing variant. The prognostic significance of this classification is still not clear. A study by Chun et al. was unable to detect a significant difference in remission rate among patients with collapsing FSGS, FSGS NOS and the tip variant. In contrast, two other studies reported a lower remission rate and worse renal survival among patients with the collapsing variant compared to the tip variant and FSGS NOS. Patients with the cellular variant had intermediate remission rates. In the reported studies, 32–67% of the patients were Afro-Americans. It is questionable if the conclusions of these studies are applicable to an European population.

In the present study, we analysed the characteristics and renal outcome of FSGS variants in adult Dutch patients and evaluated their predictive value compared to other known risk factors for renal failure.

Patients and methods

From the pathology registry we identified all adult patients (age >16 years at biopsy) diagnosed with FSGS between 1980 and 2003 at the Radboud University Nijmegen Medical Center and affiliated hospitals. The renal biopsy specimens were reviewed by the renal pathologist (E.J.S) without knowledge of the clinical outcome. A minimum of five glomeruli in the light microscopy section was required for inclusion in the study. This number was chosen for a better comparison with previous studies by Chun et al. and Thomas et al. Light microscopic assessment of glomeruli for FSGS lesions was performed in accordance with the Columbia classification system described by D’Agati et al.
Chapter 4 defines five light microscopic patterns of FSGS: FSGS NOS, perihilar variant, cellular variant, tip variant and collapsing variant. Adult patients with one of the above light microscopic variants of FSGS and either negative immunofluorescence or only segmental IgM and/or C3 were considered for the study. Patients with FSGS due to other primary glomerular diseases, such as IgA nephropathy, membranous nephropathy, pauci-immune glomerulonephritis, lupus nephritis or hereditary nephritis were excluded.

Renal biopsies

For light microscopy, pieces of kidneys were fixed in Bouin’s solution overnight at room temperature, dehydrated and embedded in Paraplast. Two micrometer-thick sections were stained with periodic acid Schiff and methenamine silver. For immunofluorescence, kidney fragments were snap frozen in liquid nitrogen, and 2 mm cryostat sections were incubated with fluorescein-labelled antisera directed to human IgG, IgM, IgA, C1q, C3, κ, λ, and fibrinogen. Electron microscopic examination was performed on small pieces of kidneys fixed in glutaraldehyde. Glomerular cross sections were arbitrarily assessed for the extent of sclerosis and hyalinosis, and called normal, mild, moderate or severe.

Clinical data

Medical records were reviewed for clinical data at presentation, at biopsy and at 3 months intervals thereafter. Data collected were: age, sex, weight, blood pressure, level of protein excretion, serum creatinine concentration, serum albumin concentration, serum cholesterol concentration, use of immunosuppressive therapy and antihypertensive medication, initiation of dialysis and death. In addition, the medical records were reviewed for diseases associated with secondary FSGS: morbid obesity, renal dysplasia, solitary kidney, reflux nephropathy, infections (HIV, parvovirus B19), medication (pamidronate, lithium, interferon-a) or intravenous drug abuse, family history of renal disease and sickle cell anaemia.

Definitions

Presentation was defined as the time when proteinuria was first detected. Renal failure was defined as a sustained increase of the serum creatinine concentration of >50% from baseline (renal biopsy), or development of end-stage renal disease (ESRD). ESRD was defined as a serum creatinine concentration of >450 μmol/l or the need for renal replacement therapy or
kidney transplantation. Nephrotic syndrome was defined as proteinuria of $\geq 3$ g/day in association with serum albumin concentration of $\leq 30$ g/l. Patients treated with antihypertensive drugs or with a systolic blood pressure $>140$mmHg or a diastolic blood pressure $>90$mmHg were considered hypertensive. A complete remission (CR) was defined as proteinuria $<0.3$ g/24 h with a stable serum creatinine concentration ($<50\%$ increase from baseline) and a partial remission (PR) was defined as proteinuria between $0.3$ g/24 h and $2.0$ g/24 h with at least $50\%$ reduction in proteinuria from baseline and a stable serum creatinine concentration. A relapse was defined as a proteinuria $>3$ g/24 h after prior reduction of the proteinuria to less than $2.0$ g/24 h.

**Statistical analysis**

Values are given as means $\pm$ SD or median (range) when appropriate. Differences in continuous data were analysed with use of the Kruskal–Wallis test, followed by Dunn’s post hoc rank test in case of more groups. Fisher’s exact test was used for differences in categorical data. Renal survival and remission rates for the different FSGS variants were estimated with Kaplan–Meier statistics. The log-rank test was used for comparison of the Kaplan–Meier curves. The univariable Cox proportional-hazards model was used to select variables associated with renal survival or attainment of a remission. The following variables were tested: serum creatinine concentration, serum albumin concentration, serum cholesterol concentration, proteinuria and mean arterial blood pressure all measured at biopsy, sex, age and type of FSGS. As for strongly skewed variables the high values may have a disproportionate influence on the outcome of the analysis, the log transformation was used to reduce their impact. In order to identify independent predictive parameters, the multivariable proportional hazards model was used in a forward stepwise fashion, with $p<0.05$ for inclusion of variables. Due to the low number of patients, data for the collapsing variant are presented but not included in the statistical comparison among variants.

Throughout, a two-sided $p$-value $<0.05$ was considered as the level of statistical significance. The analysis was performed using SPSS 14.0 for windows (SPSS Inc., Cambridge, MA, USA). Multiple comparison procedures (Dunn’s method) was performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, CA, USA.
Results

A search of the pathology registry identified 116 patients with a diagnosis of FSGS. Twenty-three patients were not included in the study due to missing slides or less than five glomeruli in the renal biopsy. Baseline characteristics of the remaining 93 patients are shown in table 1. The majority of the patients were native Dutch. There were no Afro-American patients. One patient was Indonesian, two patients were Indian and one patient was Turkish. In this cohort, the frequency of the FSGS variants was: 32% NOS, 37% tip, 26% perihilar and 5% collapsing. Notably, the cellular variant was not observed. Renal function was significantly better in patients with the tip variant compared to FSGS NOS (p<0.05). Significantly, more patients with the tip variant presented with a nephrotic syndrome compared to FSGS NOS and perihilar FSGS (p<0.01). The nephrotic syndrome was also more common in patients with FSGS NOS compared to perihilar FSGS (p<0.05). A secondary cause was identified in eight patients. Four patients with FSGS NOS and two patients with perihilar FSGS had a solitary kidney. Reflux nephropathy was present in one patient with perihilar FSGS and one patient with the tip variant was diagnosed with a thymoma. There were no cases of HIV.

Pathology

The median number of glomeruli for evaluation by light microscopy was 12 (range 5–62). The number of glomeruli was significantly higher in patients with the tip variant (16, range 5–62) compared with patients with perihilar FSGS (11, range 5–35; p=0.01). The median number of glomeruli in patients with FSGS NOS was 12 (range 5–60) and 20 (5–28) for patients with collapsing FSGS. Glomerular sclerosis and hyalinosis was most severe in patients with perihilar FSGS, intermediate in FSGS NOS and the least severe in patients with the tip variant (p<0.001, perihilar vs the tip variant and FSGS NOS and p<0.01, tip variant vs FSGS NOS). Electron microscopic examination was available for 51 patients. These patients were equally distributed among the four groups. Significantly more patients with the tip variant (90%) showed diffuse foot process fusion compared with patients with FSGS NOS (50%; p=0.01) and perihilar FSGS (27%; p<0.001). Diffuse foot process fusion was present in 67% of the patients with collapsing FSGS.
### Table 1. Patient characteristics at biopsy

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=93)</th>
<th>NOS (n=30)</th>
<th>Tip (n=34)</th>
<th>Perihilar (n=24)</th>
<th>Collapsing (n=5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>63/30</td>
<td>23/7</td>
<td>24/10</td>
<td>13/11</td>
<td>3/2</td>
<td>0.22</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>49 ± 16</td>
<td>51 ± 17</td>
<td>44 ± 16</td>
<td>50 ± 12</td>
<td>63 ± 18</td>
<td>0.12</td>
</tr>
<tr>
<td>Dutch descent</td>
<td>96%</td>
<td>93%</td>
<td>97%</td>
<td>96%</td>
<td>100%</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum creatinine concentration (μmol/l)</td>
<td>147 ± 99</td>
<td>176 ± 117</td>
<td>119 ± 78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141 ± 88</td>
<td>199 ± 140</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum creatinine concentration &gt; 135 μmol/l</td>
<td>39%</td>
<td>50%</td>
<td>26%</td>
<td>38%</td>
<td>60%</td>
<td>0.19</td>
</tr>
<tr>
<td>Serum albumin concentration (g/L)</td>
<td>27 ± 10</td>
<td>28 ± 11&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20 ± 6&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>37 ± 6</td>
<td>23 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria (g/24 hours)</td>
<td>8 ± 5.3</td>
<td>6.9 ± 4.9</td>
<td>10 ± 5.7&lt;sup&gt;ace&lt;/sup&gt;</td>
<td>5.2 ± 2.6</td>
<td>10.4 ± 6.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>8.9 ± 3.2</td>
<td>7.9 ± 2.8</td>
<td>11.2 ± 2.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>6.7 ± 1.9</td>
<td>9.2 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>63%</td>
<td>57%&lt;sup&gt;d&lt;/sup&gt;</td>
<td>97%&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>25%</td>
<td>80%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>110 ± 14</td>
<td>109 ± 17</td>
<td>110 ± 11</td>
<td>111 ± 12</td>
<td>104 ± 19</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>68%</td>
<td>66%</td>
<td>68%</td>
<td>80%</td>
<td>71%</td>
<td>0.9</td>
</tr>
<tr>
<td>Presentation to biopsy (months)</td>
<td>3.8 (0.1-303)</td>
<td>4.3 (0.1-151)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.3 (0.2-10.6)&lt;sup&gt;bce&lt;/sup&gt;</td>
<td>49.0 (0.7-303)</td>
<td>1.9 (1.5-2.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.05 tip vs NOS; <sup>b</sup>p<0.01 tip vs NOS; <sup>c</sup>p<0.01 tip vs perihilar; <sup>d</sup>p<0.05 NOS vs perihilar; <sup>e</sup>p<0.01 NOS vs perihilar
### Table 2. Treatment and outcome of the FSGS variants.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=93)</th>
<th>NOS (n=30)</th>
<th>Tip (n=34)</th>
<th>Perihilar (n=24)</th>
<th>Collapsing (n=5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEi/ARB</td>
<td>75 (81%)</td>
<td>22 (73%)</td>
<td>26 (76%)</td>
<td>22 (92%)</td>
<td>5 (100%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroids + cytotoxic medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission rate at 5 years</td>
<td>44%</td>
<td>38%</td>
<td>57%</td>
<td>25%</td>
<td>50%</td>
<td>0.1</td>
</tr>
<tr>
<td>After immunosuppressive therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>Without immunosuppressive therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5-year renal survival (%)</td>
<td>66%</td>
<td>63%</td>
<td>78%</td>
<td>55%</td>
<td>30%</td>
<td>0.02</td>
</tr>
<tr>
<td>Follow-up from biopsy (months)</td>
<td>66 (1-273)</td>
<td>59 (12-214)</td>
<td>85 (25-273)</td>
<td>49 (2-92)</td>
<td>50 (1-173)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ESRD=End-stage renal disease; *p<0.05 Tip vs NOS; †p<0.01 Tip vs NOS; ‡p<0.05 Tip vs perihilar; ‡‡p<0.01 Tip vs perihilar; ‡‡‡p<0.01 NOS vs perihilar
Pathologic variants of FSGS

Treatment

Seventy-five patients (81%) were treated with an ACE inhibitor or an angiotensin receptor blocker. A total of 40 patients (43%) received immunosuppressive therapy (table 2). There was a significantly greater use of immunosuppressive medication in patients with the tip variant and FSGS NOS compared with patients with perihilar FSGS ($p<0.001$ and $p=0.018$, respectively). Patients treated with immunosuppressive medication had significantly more proteinuria, a lower serum albumin concentration and a higher serum cholesterol concentration (table 3). The initial course of immunosuppressive therapy consisted of prednisone alone (n=24) or prednisone and cyclophosphamide (n=15). One patient was treated with prednisone and azathioprine due to severe side-effects shortly after initiation of treatment with cyclophosphamide. Patients received high dose prednisone (1 mg/kg/day) for a median of 2.3 months (range 0.4–8.6). The total duration of prednisone treatment was 5.6 months (range 1.3–55.3). Cyclophosphamide was administered orally in a dose of 1.5–2 mg/kg/day for a median of 3 months (range 2–12). The duration of immunosuppressive therapy was not significantly different among the groups.

Table 3. Baseline characteristics of patients treated and not treated with immunosuppressive medication.

<table>
<thead>
<tr>
<th></th>
<th>Treated patients (n=40)</th>
<th>Untreated patients (n=53)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>27/13</td>
<td>36/17</td>
<td>0.99</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>48 ± 17</td>
<td>49 ± 15</td>
<td>0.99</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>146 ± 88</td>
<td>148 ± 109</td>
<td>0.62</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentration (μmol/l)</td>
<td>48%</td>
<td>32%</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>21 ± 9</td>
<td>32 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>concentration (g/L)</td>
<td>10.4 ± 5.8</td>
<td>5.8 ± 3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria (g/24 hours)</td>
<td>9.9 ± 3.3</td>
<td>8.1 ± 2.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>83%</td>
<td>49%</td>
<td>0.001</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>107 ± 14</td>
<td>112 ± 14</td>
<td>0.13</td>
</tr>
<tr>
<td>Nephrotic syndrome (%)</td>
<td>68%</td>
<td>70%</td>
<td>0.83</td>
</tr>
<tr>
<td>Mean arterial blood</td>
<td>13</td>
<td>17</td>
<td>0.01</td>
</tr>
<tr>
<td>pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSGB variant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOS</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Tip</td>
<td>21</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Perihilar</td>
<td>3</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Collapsing</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Remission and renal survival

Remission rate at 5 years was 44% (table 2). Patients with the tip variant had the highest remission rate compared with patients with FSGS NOS and perihilar FSGS ($p=0.2$ and $p=0.04$, respectively); however, the overall difference between the three variants did not reach the level of statistical significance ($p=0.1$; table 2). A spontaneous remission occurred more often in patients with the tip variant compared with patients with FSGS NOS or perihilar FSGS ($p<0.01$; table 2). Two patients with collapsing FSGS also had a spontaneous remission. At the end of follow-up a remission was attained by 12 patients (6 CR/6 PR) with FSGS NOS, 18 patients (15 CR/3 PR) with the tip variant, 5 patients (3 CR/2 PR) with perihilar FSGS and 2 (2 CR) patients with collapsing FSGS. The overall renal survival was 66 and 54% at 5 and 10 years, respectively. Patients with the tip variant had a significantly better renal survival compared with FSGS NOS and perihilar FSGS ($p=0.02$; table 2 and figure 1). Renal survival at 5 and 10 years of patients attaining a remission was 100% for all variants.

Figure 1. Renal survival of patients with FSGS by variant subtype.
Predictors of remission and renal survival

On univariable analysis, a remission was predicted by a lower serum creatinine concentration, a lower serum albumin concentration and a higher serum cholesterol concentration (*table 4*). Type of FSGS, proteinuria at renal biopsy and immunosuppressive therapy did not predict a remission. On multivariable analysis, only serum albumin concentration (*p*=0.03) predicted a remission (*table 4*).

On univariable analysis, renal survival was predicted by a lower serum creatinine concentration, a lower serum albumin concentration, a higher serum cholesterol concentration and type of FSGS (tip variant; *table 5*). Proteinuria at renal biopsy and immunosuppressive therapy did not predict renal survival. On multivariable analysis both serum creatinine concentration (*p*=0.02) and type of FSGS (tip variant; *p*=0.03) predicted renal survival (*table 5*).

*Table 4.* Cox proportional hazard analysis of factors influencing remission rate.

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>FSGS</td>
<td>0.99 (0.97-1.01)</td>
<td>0.20</td>
</tr>
<tr>
<td>Tip</td>
<td>2.72 (1.01-7.33)</td>
<td>0.05</td>
</tr>
<tr>
<td>NOS</td>
<td>1.69 (0.59-4.79)</td>
<td>0.33</td>
</tr>
<tr>
<td>Perihilar**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.00 (0.97-1.03)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex</td>
<td>1.00 (0.98-1.02)</td>
<td>0.67</td>
</tr>
<tr>
<td>Serum creatinine concentration (µmol/l)*</td>
<td>0.96 (0.93-0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum albumin concentration (g/L)</td>
<td>1.12 (1.01-1.23)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum cholesterol concentration (mmol/l)</td>
<td>1.04 (0.98-1.11)</td>
<td>0.16</td>
</tr>
<tr>
<td>Proteinuria (g/24 hours)</td>
<td>1.04 (0.98-1.11)</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>0.99 (0.97-1.02)</td>
<td>0.67</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td>1.03 (0.53-2.01)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Log-transformed values; ** Reference; HRR: Hazard rate ratio
Chapter 4

Table 5. Cox proportional hazard analysis of factors influencing renal survival.

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th></th>
<th>Multivariable analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRR (95% CI)</td>
<td>p-value</td>
<td>HRR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>FSGS Perihilar**</td>
<td>0.26</td>
<td>-</td>
<td>0.29 (0.11-0.74)</td>
<td>0.01</td>
</tr>
<tr>
<td>FSGS Tip</td>
<td>0.28 (0.11-0.70)</td>
<td>0.006</td>
<td>0.43 (0.18-1.04)</td>
<td>0.06</td>
</tr>
<tr>
<td>FSGS NOS</td>
<td>0.64 (0.29-1.4)</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years**)</td>
<td>1.02 (0.99-1.04)</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Male</td>
<td>0.76 (0.38-1.52)</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Female**</td>
<td>0.30 (0.12-0.74)</td>
<td>0.03</td>
<td>0.43 (0.18-1.04)</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>5.99 (1.60-22.4)</td>
<td>0.008</td>
<td>6.30 (1.30-30.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>concentration (µmol/l)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum albumin</td>
<td>1.04 (1.00-1.07)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentration (g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.88 (0.78-0.99)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentration (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria (g/24 hours)</td>
<td>0.95 (0.88-1.03)</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>1.01 (0.99-1.03)</td>
<td>0.35</td>
<td>0.98 (0.48-2.00)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* Log-transformed values; ** Reference; HRR: Hazard rate ratio

Discussion

In the present study, we examined the characteristics and outcome of FSGS variants in an adult West-European population. In our population collapsing FSGS was rare. This is in agreement with other studies showing that 54–91% of patients with collapsing FSGS were Afro-Americans. In a more detailed study of 42 patients with non-HIV collapsing FSGS only five patients were of European descent and three were hepatitis C positive after intravenous drug abuse. Thus, collapsing FSGS is rare in our population, and the small number of patients precludes firm conclusions on prognosis and outcome. We also did not observe the cellular variant. There is some dispute if the cellular variant is a separate entity. Some authors do not discriminate between the cellular and collapsing variant. Furthermore, careful analysis of biopsies with the cellular lesion often results in reclassification into the tip
and the collapsing lesion.\textsuperscript{8} In two studies by Thomas \textit{et al.} and Stokes \textit{et al.}, the prevalence of the cellular variant was only 3–4.5%.\textsuperscript{8,9} In view of these low percentages it is not unexpected that we did not observe the cellular lesion in our study population. The tip variant was the most frequent variant in our study. This finding is somewhat in contrast to literature data reporting a lower prevalence of the tip variant compared to FSGS NOS.\textsuperscript{8,9} Most likely, this reflects the difference in composition of the study populations. In contrast to previous reports, there were no Afro-American patients in our study. Alternatively, this series, like most others, relies on the diagnosis of FSGS given contemporaneously with biopsies, almost always by different pathologists. Therefore, it is likely that not all biopsies were classified as FSGS and may be missed, especially in case of the tip variant, which may have been diagnosed as minimal change disease (MCD). This may also be part of the explanation of differences in outcome between reported series. Patients with the tip variant more often presented with a nephrotic syndrome, had a better renal function and less histological damage compared with patients with FSGS NOS and perihilar FSGS. These findings are in agreement with literature data.\textsuperscript{8,9,11} The perihilar variant is often associated with secondary forms of FSGS, especially due to maladaptive responses that follow loss of functioning nephrons, hyperfiltration or increased glomerular pressure.\textsuperscript{12} Typically, these patients present with nephrotic range proteinuria without a full nephrotic syndrome. Serum albumin concentration usually remains normal even though proteinuria exceeds 3 g/day.\textsuperscript{13} Most patients with perihilar FSGS in our study also presented without a nephrotic syndrome. Nevertheless, an identifiable secondary cause was rare in our patients with perihilar FSGS. Admittedly, this conclusion is not unexpected since in patients with a readily identifiable cause of secondary FSGS, a renal biopsy is not done. Nearly all patients included in the study underwent examination by ultrasound or intravenous pyelogram, which makes it unlikely that secondary causes due to a reduced renal mass, e.g. renal agenesis or dysplasia, were missed. Alternatively, long-standing hypertension, a known cause of secondary FSGS, may have been present in our patients with the perihilar variant. Most patients with perihilar FSGS indeed had a history of hypertension before presentation to our centre or one of the affiliated hospitals. However, due to the retrospective nature of the study, it was not possible to establish the duration and severity of hypertension in these patients. Most patients with perihilar FSGS did not receive immunosuppressive therapy. This is likely due to the low number of patients with a nephrotic...
syndrome. Although data on the use of immunosuppressive therapy in nonnephrotic patients are lacking, currently most authors would advise against immunosuppressive therapy in these patients. In addition, Praga et al. showed that patients with secondary FSGS due to maladaptive responses are usually characterized by a normal serum albumin concentration even though proteinuria is in the nephrotic range. Therefore, most treating physicians did not use immunosuppressive medication in patients with perihilar FSGS. Prognosis of patients with perihilar FSGS is comparable to FSGS NOS. After 10 years renal function remains stable in ~50% of the patients.

The tip variant was associated with the highest remission rate (57%). The remission rate compares very well to previous studies reporting remission rates varying between 53% and 59%. Notably, a large proportion of patients with the tip variant in the present study attained a spontaneous remission. Stokes et al. demonstrated that the presenting features and outcome of the tip variant more closely resembled MCD. Although patients with MCD usually receive immunosuppressive therapy, up to 50% of the patients with MCD can attain a spontaneous remission after a prolonged period. We have published our experience in patients with primary FSGS. Spontaneous remissions occurred relatively frequent in a subgroup of patients that more closely resembled MCD, i.e. a more selective proteinuria and few sclerotic lesions. Howie et al. reported similar results in a group of patients presenting with only tip lesions. Some patients behaved as though they had MCD. Half the patients did not progress even though some may not have been treated or had received similar treatment as those who progressed. Our results and those of Howie suggest that some patients with the tip lesion may not need immunosuppressive therapy to attain a remission. Admittedly, in multivariable analysis the tip variant did not predict attainment of a remission. This is probably related to a relatively low number of patients. A low serum albumin concentration was an independent predictor of a remission. However, inclusion of serum albumin as a variable may not be justified since clinical decisions may have depended on the actual serum albumin concentration. Although the differences in remission rate can be disputed, our study clearly showed a renal survival advantage for patients with the tip variant compared with patients with FSGS NOS and perihilar FSGS. The better renal survival of the tip variant only became apparent after 5 years of follow-up. This explains why Thomas et al. were unable to show a renal survival benefit in patients with the tip variant, in spite of a higher remission rate. In their study follow-up ended after four years. The tip variant was an
Pathologic variants of FSGS

independent predictor of renal survival. Only serum creatinine concentration at biopsy predicted renal survival better. In agreement with a previous study by Chun et al, the overall renal survival in our patients attaining a remission was good irrespective of the type of FSGS. Therefore, the difference in renal survival, especially between the tip variant and FSGS NOS, is most likely explained by a better prognosis in patients with the tip variant who did not attain a remission. Half of these patients did not progress to renal failure compared with only 22% of patients with FSGS NOS. Our results support the concept of Howie, who suggested that the tip variant may be a manifestation of two diseases, i.e. MCD and FSGS. In some patients with MCD, tip lesions may occur, however, these patients do not progress to sclerosis or renal failure. On the other hand, tip lesions may occur early in the course of classical nephrotic FSGS, and these patients can progress to renal failure. The existence of two forms of FSGS may also explain the different results reported in studies by Stokes and Chun. In the study by Stokes et al., of eight patients with the tip variant who did not attain a remission, only one progressed to ESRD. In contrast, Chun et al. reported a poor prognosis in patients with the tip variant not attaining a remission. Patients with the tip variant of FSGS present more often with a nephrotic syndrome, a better renal function and less histological damage. Patients with the tip variant have a better prognosis, and can attain a remission without treatment. Our data demonstrate the clinical usefulness of the FSGS classification.

Reference List


Chapter 5

Focal segmental glomerulosclerosis is not a sufficient predictor of renal outcome in patients with membranous nephropathy.

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Eric Steenbergen² and Jack F. M. Wetzels¹

¹Department of Nephrology and ²Department of Pathology, Radboud University Nijmegen
Medical Centre, The Netherlands

Abstract

**Background:** The course of idiopathic membranous nephropathy (iMN) is variable in untreated patients. Accurate prediction of renal outcome would allow optimal treatment decisions. We demonstrated that urinary \( \beta \)2-microglobulin (\( \beta \)2m) predicted prognosis in iMN with high sensitivity and specificity. It has been suggested that focal segmental glomerulosclerosis (FSGS) is a discriminative parameter with independent prognostic value.

**Methods:** We selected patients with iMN biopsied between 1988 and 2002. Biopsies were analysed for the presence of FSGS, interstitial fibrosis and vascular lesions. Serum creatinine, creatinine clearance, proteinuria and blood pressure were recorded at baseline. Outcome variables included remission of proteinuria, renal death (RD) defined as serum creatinine >135 mmol/l or increase of serum creatinine of >50%, or end-stage renal disease (ESRD). In a subgroup of patients, urinary \( \beta \)2-microglobulin (\( \beta \)2m) was measured.

**Results:** We included 53 patients (33M, 20F). Mean age was 51 years, serum creatinine 99 \( \mu \)mol/l, and proteinuria 7.0 g/10 mmol creatinine. FSGS was present in 22 patients. These patients were characterized by a higher serum creatinine at time of biopsy \((p=0.035)\), more severe interstitial fibrosis \((p=0.001)\) and higher stage of membranous nephropathy \((p=0.001)\). During follow-up 24 patients developed RD, almost equally distributed between patients with and without FSGS. Renal survival was numerically, but not significantly, lower in patients with FSGS. In Cox proportional hazard analysis, only serum creatinine at the time of biopsy was an independent predictor of RD or ESRD \((p<0.001)\). In patients with known urinary \( \beta \)2m, there was no significant correlation with FSGS score \((p=0.174)\).

**Conclusion:** FSGS is not an accurate prognostic marker in iMN. Histological scoring of FSGS is inferior to measurement of urinary proteins in predicting renal outcome in iMN.
Introduction

Idiopathic membranous nephropathy (iMN) is one of the most common causes of the nephrotic syndrome in adults. The course of iMN in untreated patients is variable, as approximately 34–62% develop renal insufficiency and 14–56% will have a spontaneous remission. The identification of parameters that predict prognosis with sufficient accuracy would allow individualized treatment and avoid unnecessary immunosuppressive therapy. Well-known risk factors for deterioration of renal function such as proteinuria, age and sex lack sensitivity or specificity. In previous studies, we have found that urinary β2-microglobulin (β2m) accurately predicts prognosis in patients with iMN. Recent studies have provided evidence that morphological parameters—specifically focal segmental glomerulosclerosis (FSGS)—may be used as prognostic parameter.

In the present study, we have evaluated FSGS as prognostic parameter in patients with iMN. In addition, we performed a meta-analysis of studies analysing the prognostic significance of FSGS in iMN.

Subjects and methods

Patient selection

We included patients with a diagnosis of membranous nephropathy, biopsied between 1988 and 2002 in any of five nephrology units in the Nijmegen region. Only patients with iMN were considered in this study.

Clinical data

From selected patients, their age, sex, serum creatinine, serum cholesterol, serum albumin, proteinuria (expressed per 10 mmol creatinine), creatinine clearance (ECC) calculated according to the Cockcroft Gault formula, systolic and diastolic blood pressure and medication had been recorded at the time of biopsy (table 1), and were frequently measured during follow-up. The mean arterial pressure was calculated as the diastolic blood pressure plus a third of the pulse pressure.

In patients who were seen at the University Hospital, additional standardized measurements were done as described to determine urinary excretion of β2-microglobulin. Renal death was defined as a serum creatinine concentration of >135 μmol/l or an increase of serum creatinine...
of more than 50% over time. Patients who met these criteria were advised to start with immunosuppressive therapy. As described before, start of immunosuppressive therapy, because of severe nephrotic syndrome, was also considered as renal death (RD). 8

End-stage renal disease (ESRD) was defined as an ECC <10 ml/min. A complete remission was defined as proteinuria below 0.2 g/10 mmol creatinine and a partial remission as proteinuria between 0.2 and 2.0 g/10 mmol creatinine, without deterioration of renal function. Follow-up started at the time of biopsy and continued until December 2003 or ended at the time of death or development of ESRD.

Pathology
All biopsy specimens were processed for light microscopy using standardized techniques. For the evaluation and quantitation of FSGS lesions we have used the biopsy slides stained with methenamine silver in which FSGS is best recognized. Biopsies with less than five glomeruli were excluded from the analysis. The presence of FSGS was evaluated by a qualitative and a quantitative analysis. In the qualitative analysis, all available slides were screened for the presence of FSGS and tubulo-interstitial fibrosis. The risk of overlooking FSGS was thereby considered negligible. In the quantitative analysis, glomerular slides were selected at random and evaluated in detail for the extent of FSGS. Focal glomerulosclerosis was defined as a focal lesion with mesangial matrix expansion leading to collapse of the glomerular capillary loops. The extent of sclerosis was scored by counting the number of glomerular quadrants with sclerosis. FSGS was expressed as a percentage by dividing the total score of sclerosis by four times the number of evaluated glomeruli. Globally sclerosed glomeruli were excluded in this analysis, but were included in the calculation of total sclerosis. Total sclerosis was defined as the sum of focal sclerosed glomerular quadrants and globally sclerosed glomeruli x 4 divided by the total number of glomeruli x 4. The interstitium was evaluated for the presence and extent of tubulo-interstitial fibrosis, intimal fibrosis and foam cells. Tubulo-interstitial fibrosis was scored semiquantitatively on a scale of 0–3.

The glomerular deposits were evaluated by electronmicroscopy (EM) and the stage of membranous nephropathy was taken from the corresponding pathology report.
Meta-analysis
We performed a thorough search of the MEDLINE, EMBASE and Cochrane Controlled Trial Register databases. The following keywords were used: ‘membranous nephropathy’, ‘membranous glomerulopathy’, ‘FSGS’, ‘outcome’, ‘remission’. The search was limited to studies published in English. The following types of studies were included: randomized controlled trials, non-randomized prospective trials and retrospective studies. Case reports were not included. Only studies analysing the outcome in patients with iMN according to the presence or absence of FSGS were considered. The primary outcome was remission of proteinuria.

Statistical analysis
Statistical analysis was performed using the SPSS version 11.0 for Windows. For differences between groups, Chi-square test, t-test and the Mann–Whitney U test were applied, if appropriate. Correlations were calculated using Spearman’s correlation test. The Kaplan–Meier univariate analysis was used to select variables associated with progression to RD. Significance was determined by the log–rank test. In order to identify independent predictive parameters, Cox proportional hazard was performed in forward stepwise fashion on variables selected by univariate analysis. For this purpose, nonparametric variables were transformed using log 10 or square root transformation. For all analyses, \( p < 0.05 \) was considered significant. All statistical calculations for the meta-analysis were performed using the computer software Review Manager (RevMan) version 4.2.9 provided by the Cochrane Collaboration. A pooled estimate of the odds ratios with 95% confidence intervals was calculated using the random effects model using the DerSimonian–Laird method. A \( p \)-value <0.05 was considered significant. Heterogeneity was evaluated by using the \( \chi^2 \) test. Given the low power of this test, a \( p \)-value <0.1 was considered significant.

Results
Clinical and pathological findings
We identified 61 eligible patients. In eight patients, the biopsy contained an insufficient number of glomeruli. Thus, we have studied 53 patients (33 M, 20 F) with a mean (SD) age of 51 ± 15 years. Mean follow-up was 62 ± 43 months. Mean creatinine was 99 μmol/l and mean proteinuria 7 g/10 mmol. Baseline characteristics of all patients are given in table 1.
FSGS was present in 22 patients (41.5%). The clinical and pathological findings at baseline of patients without FSGS lesions (n=31, group I) and patients with FSGS lesions (n=22, group II) are listed in table 2. Patients with FSGS had a significantly higher serum creatinine and were numerically older. FSGS was significantly associated with a higher MN stage and more severe tubulo-interstitial lesions (table 2). Vascular changes were also more marked in patients with FSGS, however, the differences were not statistically significant.

For the quantitative analysis of FSGS, we evaluated an average of 336 glomerular profiles per biopsy in group I (min 62, max 830) and 342 glomerular profiles per biopsy in group II (min 63, max 811). A mean of 2.3% of glomerular quadrants was sclerosed in group II (min 0.11, max 13.3%). There were significantly more obsolescent glomeruli in group II (8.1%) than in group I (4.9%), $p=0.034$. Serum creatinine at time of biopsy, ECC and serum cholesterol were significantly correlated with the percentage of FSGS (serum creatinine $r=0.339; p=0.013$; ECC $r=-0.333; p=0.031$; serum cholesterol $r=-0.308; p=0.039$).

Table 1. Clinical data at baseline

| Sex (M/F) | 33 / 20 |
| Age (years) | 50.9 ± 15.1 |
| Serum creatinine (μmol/L) | 98.8 ± 41.5 |
| Serum albumin (g/L) | 24.0 ± 6.1 |
| Serum cholesterol (mmol/L) | 10.4 ± 3.3 |
| ECC (ml/min) | 84.5 ± 31.8 |
| Proteinuria (g/10mmol creatinine) | 7.0 ± 3.2 |
| MAP (mmHg) | 105.0 ± 11.9 |
| Follow-up (months) | 62 ± 43 |

Values are given as means ± SD; S= serum; ECC= creatinine clearance; MAP= mean arterial pressure

Renal outcome

At the time of biopsy, serum creatinine was $>135$ μmol/l in four patients. Another 20 patients reached the defined end point RD during follow-up. The reason for RD was a serum creatinine $>135$ μmol/l in 22 patients, a rise of $>50\%$ of serum creatinine in one patient and the start of immunosuppressive therapy with normal serum creatinine in one patient. The outcome was not different between the groups, with development of RD in 41.9% of patients without and 50% of patients with FSGS (table 3).
Table 2. Clinical and pathological data at baseline

<table>
<thead>
<tr>
<th></th>
<th>FSGS – (n=31)</th>
<th>FSGS + (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>20/11</td>
<td>13/9</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.3 (15.3)</td>
<td>54.4 (14.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>95.4 (41.1)</td>
<td>103.5 (34.4)</td>
<td>0.035</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>23.2 (5.1)</td>
<td>25.0 (7.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>11.2 (3.2)</td>
<td>9.4 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>ECC (ml/min)</td>
<td>90.8 (29.6)</td>
<td>73.1 (33.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Proteinuria (g/10mmol creatinine)</td>
<td>6.8 (3.1)</td>
<td>7.4 (3.3)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>104.0 (11.7)</td>
<td>106.5 (12.2)</td>
<td>NS</td>
</tr>
<tr>
<td>% FSGS</td>
<td>-</td>
<td>2.3</td>
<td>0.001</td>
</tr>
<tr>
<td>MN-stage (%)</td>
<td>32.3</td>
<td>13.6</td>
<td>0.001</td>
</tr>
<tr>
<td>I</td>
<td>48.4</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>12.9</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Interstitial fibrosis (%)</td>
<td>9.7</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>0</td>
<td>51.6</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>43.3</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SD. S= serum; ECC= creatinine clearance; MAP= mean arterial pressure; MN= membranous nephropathy; NS= not significant

Table 3. Clinical outcome

<table>
<thead>
<tr>
<th></th>
<th>FSGS – (n=31)</th>
<th>FSGS + (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal death</td>
<td>13 (41.9%)</td>
<td>11 (50.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>ESRD</td>
<td>3 (9.7%)</td>
<td>3 (13.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Death</td>
<td>1 (3.2%)</td>
<td>2 (9.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td>13 (41.9%)</td>
<td>7 (31.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Remission during FU</td>
<td>21 (67.7%)</td>
<td>11 (50.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Remission end FU</td>
<td>21 (67.7%)</td>
<td>10 (45.5%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

ESRD is end-stage renal disease; FU= follow-up; NS= not significant
Renal survival is depicted in figure 1. Only three patients died, two with FSGS lesions (ns). During follow-up, slightly more patients without FSGS had a complete or partial remission of proteinuria (67.7% vs 50% in group II). Also at the end of follow-up a higher proportion of 20 patients without FSGS (67.7% vs 45.5%) had a complete or partial remission. These differences were not statistically significant. A complete remission at the end of follow-up was attained in 11/31 patients without FSGS and in 4/22 patients with FSGS (ns).

Twenty patients received immunosuppressive treatment during follow-up; 13 patients without FSGS, 7 patients with FSGS (ns).

**Risk factors for RD**

To determine which variables were associated with the development of RD, univariate analysis was performed for all of the clinical variables (table 4). The most discriminate variable was serum creatinine at time of biopsy ($p<0.001$, log-rank test). Other variables associated with progression to RD were ECC ($p<0.001$), diastolic blood pressure ($p<0.001$), mean arterial pressure ($p=0.012$), proteinuria ($p=0.004$) and serum albumin ($p=0.035$). Notably FSGS was not associated with progression to RD ($p=0.46$).
Table 4. Risk factors associated with renal death in the study population

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate (p-value)</th>
<th>Multivariate (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.035</td>
<td>NS</td>
</tr>
<tr>
<td>ECC</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>MAP</td>
<td>0.012</td>
<td>NS</td>
</tr>
<tr>
<td>FSGS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

S=serum; ECC=creatinine clearance; MAP=mean arterial pressure; NS=not significant

Cox proportional hazard analysis was performed using variables selected by univariate analysis. Serum creatinine, at time of biopsy, was the strongest independent predictive factor for progression to RD ($p<0.001$). Diastolic blood pressure also had independent significant prognostic value for progression to RD ($p=0.050$).

Our analysis could have been biased because at the time of biopsy several patients already had decreased renal function. A subgroup analysis was, therefore, performed limited to patients with a serum creatinine at the time of biopsy of $<106$ mmol/l. This subgroup consisted of 39 patients, 25 without and 14 with FSGS. During follow-up, 11 patients developed RD, 7/25 without FSGS and 4/14 with FSGS ($p=ns$). In univariate analysis, serum albumin ($p=0.0061$), diastolic blood pressure ($p=0.007$) and mean arterial pressure ($p=0.023$) were significantly related to RD. Also in this subgroup, FSGS was not related to RD. Multivariate analysis revealed that serum albumin ($p=0.005$) and diastolic blood pressure ($p=0.008$) both had significant prognostic value for progression to RD. In 21 patients, urinary $\beta_2$-microglobulin excretion was determined. FSGS was present in eight of 21 patients. Although urinary $\beta_2$-microglobulin ($u\beta_2m$) was higher in patients with FSGS ($u\beta_2m$: 3865.3 vs 1041 ng/min), the difference was not statistically significant. In a previous study, we have demonstrated that urinary $\beta_2$-microglobulin $>500$ ng/min predicted outcome with high sensitivity and specificity. In the present dataset, we observed development of RD in five out of seven patients with $u\beta_2m >500$ ng/min and in one of 14 patients with $u\beta_2m <500$ ng/min ($p<0.01$). In figure 2, urinary $\beta_2$-microglobulin excretion is plotted against the percentage of FSGS. There was no strong correlation between these variables ($p=0.17$).
Chapter 5

Figure 2. Urinary β2m excretion plotted against percentage FSGS. Urinary β2m is a validated prognostic parameter with a reported threshold value of 500 ng/min. There is no significant correlation.

Meta-analysis of studies analysing the prognostic significance of FSGS in iMN

A total of seven studies, including the present study, fulfilled the inclusion criteria. All studies were retrospective by design. Detailed data of one study were unavailable. An overview of all seven studies is given in table 5. The study by Troyanov et al. did not report the absolute number of patients attaining a remission. Only the remission rate of treated and untreated patients with and without FSGS were reported. However, given that FSGS was present in 25% of patients and the percentage of treated and untreated patients with and without FSGS was not reported to be different, we were able to extrapolate the number of patients with and without FSGS attaining a remission.

The six papers included in the review contained a total of 695 patients with membranous nephropathy (235 with FSGS and 460 without FSGS). The results show a significantly higher remission rate for patients with iMN alone (odds ratio, 2.5; 95% CI, 1.3–4.8) compared to patients with iMN and FSGS lesions (figure 3). However these results must be read with caution as there was considerable heterogeneity between studies. Serum creatinine, proteinuria and blood pressure were higher in patients with FSGS, especially in studies showing a higher remission rate in patients with iMN alone. Of note, the results of the meta-analysis were not altered, if we assumed that a higher percentage of patients with FSGS in the study by Troyanov had been treated.
Patients with FSGS had a numerically higher serum creatinine, and male preponderance. No multivariate analysis

Significantly lower renal survival in patients with FSGS ($p=0.021$).

Patients with FSGS had more proteinuria ($p=0.004$), more hypertension ($p=0.006$), and higher serum creatinine ($p=0.11$). No multivariate analysis

Follow-up data in only 67% of patients. Significantly more patients with FSGS developed renal failure (24% vs 6%). Patients with FSGS had higher serum creatinine at baseline ($p<0.05$). No multivariate analysis

Significantly more patients with FSGS developed renal insufficiency. FSGS was significant in multivariate analysis ($p=0.00004$)

Univariate analysis: more often renal failure in FSGS. Multivariate analysis: age, sex, serum creatinine and tubulo-interstitial lesions were independent risk factors for RD, FSGS not ($p=0.11$)

Presence of FSGS produced a trend toward lower survival ($p=0.13$); disappeared after adjusting for differences in baseline characteristics

Multivariate analysis: FSGS not significant; serum creatinine and diastolic blood pressure independent risk factors for renal death

**Table 5. Summary of studies that investigated the prognostic value of FSGS in iMN**

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>FSGS (+/-)</th>
<th>Remission (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Damme et al.</td>
<td>63</td>
<td>28/35</td>
<td>28/60 $p=0.01$</td>
<td>Patients with FSGS had a numerically higher serum creatinine, and male preponderance. No multivariate analysis</td>
</tr>
<tr>
<td>Wakai and Magi</td>
<td>52</td>
<td>27/25</td>
<td>19/28 $p=0.316$</td>
<td>Significantly lower renal survival in patients with FSGS ($p=0.021$). Patients with FSGS had more proteinuria ($p=0.004$), more hypertension ($p=0.006$), and higher serum creatinine ($p=0.11$). No multivariate analysis</td>
</tr>
<tr>
<td>Lee and Koh</td>
<td>95</td>
<td>41/54</td>
<td>6/24 $p&lt;0.05$</td>
<td>Follow-up data in only 67% of patients. Significantly more patients with FSGS developed renal failure (24% vs 6%). Patients with FSGS had higher serum creatinine at baseline ($p&lt;0.05$). No multivariate analysis</td>
</tr>
<tr>
<td>Dumoulin et al.</td>
<td>72</td>
<td>30/42</td>
<td>3/38 $p=0.002$</td>
<td>Significantly more patients with FSGS developed renal insufficiency. FSGS was significant in multivariate analysis ($p=0.00004$)</td>
</tr>
<tr>
<td>Shiiki et al.</td>
<td>949</td>
<td>62/512</td>
<td>NA</td>
<td>Univariate analysis: more often renal failure in FSGS. Multivariate analysis: age, sex, serum creatinine and tubulo-interstitial lesions were independent risk factors for RD, FSGS not ($p=0.11$)</td>
</tr>
<tr>
<td>Troyanov et al.</td>
<td>389</td>
<td>97/292</td>
<td>Treated: 85/75</td>
<td>Presence of FSGS produced a trend toward lower survival ($p=0.13$); disappeared after adjusting for differences in baseline characteristics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Untreated: 44/68</td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>53</td>
<td>22/31</td>
<td>45/68</td>
<td>Multivariate analysis: FSGS not significant; serum creatinine and diastolic blood pressure independent risk factors for renal death</td>
</tr>
</tbody>
</table>

ESRD= end stage renal disease. Definition of remission differs among studies; stable or improving serum creatinine levels remaining at less than 1.5mg/dl and proteinuria less than 0.5g/d of protein in [12]; remission was not defined in [11]; normal serum creatinine level and proteinuria less than 1.0g/d of protein in [10]; proteinuria less than 0.3g/d of protein in two consecutive samples 1 month apart in [9]; an absence of proteinuria and normalized serum albumin concentration for at least 1 month in [14]; a single proteinuria value $\leq 0.3$g/d in a previously nephrotic patient in [15]; proteinuria remaining below 0.2g/10mmol creatinine in our series.
Figure 3. Meta-analysis of studies evaluating the impact of FSGS on remission rate (random effects model). CI, confidence interval; OR, odds ratio

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>IMN n/N</th>
<th>IMN - FSGS n/N</th>
<th>OR (random) 95% CI</th>
<th>Weight %</th>
<th>OR (random) 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Damme</td>
<td>21/35</td>
<td>8/28</td>
<td></td>
<td>18.50</td>
<td>3.75 [1.30, 10.85]</td>
<td>1990</td>
</tr>
<tr>
<td>Wakai</td>
<td>7/25</td>
<td>5/27</td>
<td></td>
<td>14.80</td>
<td>1.71 [0.46, 6.32]</td>
<td>1992</td>
</tr>
<tr>
<td>Lee</td>
<td>8/33</td>
<td>2/31</td>
<td></td>
<td>11.03</td>
<td>4.64 [0.90, 23.90]</td>
<td>1993</td>
</tr>
<tr>
<td>Dumoulin</td>
<td>16/42</td>
<td>1/30</td>
<td></td>
<td>7.69</td>
<td>17.85 [2.21, 144.07]</td>
<td>2003</td>
</tr>
<tr>
<td>Trojanov</td>
<td>210/292</td>
<td>65/97</td>
<td></td>
<td>30.09</td>
<td>1.26 [0.77, 2.07]</td>
<td>2006</td>
</tr>
<tr>
<td>Present study</td>
<td>21/31</td>
<td>10/22</td>
<td></td>
<td>17.90</td>
<td>2.10 [0.70, 6.30]</td>
<td>2007</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>460</td>
<td>235</td>
<td></td>
<td>100.00</td>
<td>2.50 [1.30, 4.80]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 283 (IMN), 91 (IMN-FSGS)
Test for heterogeneity: $\chi^2 = 10.04, DF = 5 (P = 0.07)$
Test for overall effect: $Z = 2.76 (P = 0.008)$

Favours IMN - FSGS    Favours IMN
Discussion

Patients with iMN would greatly benefit if their prognosis could be predicted with high accuracy. Several studies have defined risk factors for deterioration of renal function such as proteinuria, age and sex. These factors, however, do not have sufficient predictive value for renal outcome to allow firm decisions about start of immunosuppressive therapy. The level and duration of proteinuria in a model introduced by the Toronto Glomerulonephritis Registry proved a better predictor. We recently have validated urinary excretion of β2-microglobulin and IgG as prognostic markers of iMN. Sensitivity and specificity exceeded 85%, and when β2-microglobulin excretion and serum albumin were combined, specificity of these parameters was 100%.

Several studies suggested that the presence of FSGS in the renal biopsy predicted a worse outcome in patients with iMN. In the study of Dumoulin et al., FSGS proved an independent predictor of outcome, apparently outweighing clinical parameters such as creatinine and proteinuria. The latter study prompted the current study. Evidently, FSGS did not predict outcome in our patients with iMN. Our patient cohort consisted of patients with a nephrotic range proteinuria. During follow-up, 45% of patients progressed, which is in agreement with the natural history in nephrotic patients with iMN. Obviously, there were some differences in patient characteristics related to the presence of FSGS. Patients with FSGS were somewhat older and had a significantly higher baseline serum creatinine and more proteinuria. Univariate analysis failed in identifying FSGS as a predictor of RD. Clinical and laboratory parameters proved better predictors and multivariate analysis revealed serum creatinine and diastolic blood pressure at time of biopsy as independent predictive variables.

The conclusions of our analysis could be biased by the fact that some patients already had renal insufficiency at the time of biopsy. Therefore, we separately analysed data of a subgroup of patients with normal renal function at time of biopsy. Again, we failed to identify FSGS as prognostic parameter.

Another bias could have been the inclusion of patients with biopsies containing 5–9 glomeruli. Patients with FSGS may not have been identified correctly. In our study, biopsies of 16 patients contained 5–9 glomeruli. In 11 patients, FSGS was not observed. Five of these
developed RD. Even if we would assume that all these patients had FSGS, the analysis
would not reveal FSGS as an independent prognostic marker.
Unfortunately, we lacked information on urinary β2-microglobulin excretion in many
patients. Urinary β2-microglobulin is an accurate predictor of outcome in patients with iMN.
In a validation study, using predefined threshold values, sensitivity and specificity exceeded
85%. Still, the available data show a weak correlation between percentage of FSGS and
urinary β2-microglobulin excretion (figure 2). This underlines the weakness of FSGS as a
prognostic marker.
Several studies have evaluated FSGS as a predictor of outcome. Our meta-analysis of studies
with available data shows that outcome is worse in patients with iMN and FSGS lesions. The
meta-analysis does indicate that the presence of FSGS identifies high-risk patients. However,
our data suggest that FSGS is not an independent risk factor, in agreement with all other
studies but one. In most studies, patients with FSGS had higher serum creatinine, more
proteinuria and more hypertension. A multivariate analysis was done in one of the older
studies and in two recent studies, which both concluded that the presence of FSGS was not
an independent predictor of outcome.9,14,15 FSGS emerged as an independent prognostic
factor by multivariate analysis only in the study of Dumoulin.9 These authors studied 72
patients with iMN, FSGS being present in 42%. The patients in this study were younger and
had somewhat better renal function. The outcome in these patients was rather grim, with only
24% developing persistent remission and 53% progressing to ESRD after ten years. These
differences in patient characteristics may explain the different results.
Especially, the timing of renal biopsy in relation to the natural outcome of the disease may be
relevant. We propose the following sequence of events in progressive iMN: proteinuria →
tubular injury reflected by increased urinary β2-microglobulin → focal sclerosis → renal
failure. The data of Dumoulin suggest that this model may be valid: some patients without
FSGS at initial biopsy progressed to renal failure. These patients were rebiopsied and in all
of them the new biopsy revealed typical FSGS lesions. Thus, the presence of FSGS may be a
better predictor of outcome in studies where biopsies are taken in a later phase of the disease.
It will be difficult to attain high specificity and sensitivity with histological parameters. Most
studies use only qualitative measures. Furthermore, in routine practice renal biopsies often
contain inadequate amounts of tissue. In our study, eight out of 61 biopsies (13%) contained
less than five glomeruli. Thus, overall accuracy would never reach 90%, even if specificity
FSGS in membranous nephropathy

was 100%. The abovementioned studies, in fact, noted a specificity of 56–59%, clearly too low to be used as a guide for treatment decisions.

In conclusion, FSGS is not an accurate predictor of renal outcome in patients with iMN. Renal biopsy results cannot be used to guide decisions on immunosuppressive therapy.

Reference List


Chapter 6

Podocyte foot process effacement as a differential diagnostic tool in focal segmental glomerulosclerosis

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Submitted
Abstract

Background: Podocyte foot process effacement is characteristic of proteinuric renal diseases. In minimal change nephrotic syndrome (MCNS) foot processes are diffusely effaced whereas the extent of effacement varies in focal segmental glomerulosclerosis (FSGS). Little is known about the pathogenesis of foot process effacement. In the present study we studied the determinants of foot process effacement in FSGS in comparison to MCNS and normal kidneys.

Methods: This is a retrospective study of patients with a histological diagnosis of FSGS. Electron microscopic specimens of 27 patients with FSGS, 15 patients with minimal change nephrotic syndrome (MCNS), and 12 control patients were available for examination. Predefined criteria were used to make a clinical diagnosis of idiopathic or secondary FSGS. Foot process width (FPW) was determined by morphometric methods.

Results: We have studied 27 patients with FSGS. A clinical diagnosis of idiopathic FSGS and FSGS secondary to maladaptive responses was made in 13 and 12 patients, respectively. Two patients with FSGS could not be classified based on clinical criteria. Median FPW was 2551 nm in patients with idiopathic FSGS, 1083 nm in secondary FSGS, and 1725 nm in patients with MCNS as compared to 562 nm in controls. Multivariate analysis revealed that FPW did not correlate with proteinuria or serum albumin levels but was significantly associated as an independent factor with the type of disease (idiopathic FSGS, secondary FSGS, or MCNS). An FPW > 1500 nm differentiated idiopathic from secondary FSGS with a high sensitivity and specificity.

Conclusion: Idiopathic FSGS, MCNS and FSGS secondary to maladaptive responses differ in foot process width, independent of proteinuria. Our results show that quantitative analysis of foot processes may offer a potential tool to distinguish idiopathic FSGS from FSGS secondary to maladaptive responses.
Introduction

Podocyte foot process effacement is an invariable feature of proteinuric glomerular diseases such as focal segmental glomerulosclerosis (FSGS), minimal change disease, IgA nephropathy and diabetic nephropathy.\textsuperscript{1-3} Few studies have assessed foot process effacement in a quantitative way. It was suggested that the degree of foot process effacement might depend on the underlying disease. In patients with minimal change nephrotic syndrome (MCNS), foot process effacement estimated by foot process width, was more extensive than in patients with proteinuric IgA nephropathy.\textsuperscript{2} The degree of foot process effacement appeared to be independent of the level of proteinuria, suggesting that the extent of foot process effacement is determined by the nature of podocyte injury.\textsuperscript{2} Podocyte damage in idiopathic MCNS and idiopathic (primary) FSGS has been linked to a putative glomerular permeability factor, which would suggest that the extent of podocyte damage is similar in the two disorders.\textsuperscript{4-6} Alternatively, the group of Kerjaschki demonstrated that the expression of dystroglycan, an adhesion molecule between the podocyte and the glomerular basement membrane, is significantly lower in minimal change disease compared to FSGS, suggesting that the mechanism of foot process fusion may differ in MCNS and FSGS.\textsuperscript{7}

To our knowledge the degree of foot process fusion in FSGS has never been compared to that in MCNS. We are aware of a limited number of semiquantitative studies on podocyte alterations in patients with idiopathic and secondary forms of FSGS.\textsuperscript{8,9} These studies showed that the mean percentage of the glomerular surface area affected by foot process fusion was less in patients with FSGS secondary to maladaptive structural-functional responses, such as obesity, reflux nephropathy compared to idiopathic FSGS. However, there was a considerable degree of overlap. The semiquantitative method used in these studies in FSGS precludes a direct comparison with the quantitative method used to assess the degree of foot process effacement in patients with MCNS.\textsuperscript{2}

In the present study, we have performed a morphometric analysis of podocyte foot processes in patients diagnosed with idiopathic and secondary forms of FSGS. The degree of foot process effacement in patients with FSGS was compared to the degree of foot process effacement in MCNS and normal kidneys.
Subjects and Methods

Patients and controls

Twenty-seven patients with biopsy proven FSGS were included in the study. Light microscopic assessment of glomeruli for FSGS lesions was performed in accordance with the Columbia classification system described by D’Agati et al. This classification defines five light microscopic patterns of FSGS: FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant and collapsing variant. Adult patients with one of the above light microscopic variants of FSGS and either negative immunofluorescence or only segmental IgM and/or C3 were considered for the study.

For comparison we have used renal biopsy material of patients with MCNS and control patients. Data on foot process width of 12 patients with MCNS and six patients after renal transplantation (used as control) were available from a previous study. We have added three additional patients with MCNS and six control renal tissue, consisting of the apparently unaffected part of kidneys removed because of a malignancy.

Light microscopy and electron microscopy

For light microscopy pieces of kidneys were fixed in Bouin’s solution overnight at room temperature, dehydrated and embedded in paraplast (Amstelstad Amsterdam The Netherlands). Two μm-thick sections were stained with periodic acid Schiff and methenamine silver.

For electron microscopy small pieces of kidneys were fixed in 2.5% glutaraldehyde dissolved in 0.1M sodium cacodylate buffer, pH 7.4 overnight at 4° C and washed in the same buffer. The tissue fragments were postfixed in cacodylate-buffered 1% OsO₄ for 2 h, dehydrated, and embedded in Epon 812 (Merck, Darmstadt, Germany). Ultrathin sections were cut on an ultratome (Leica, Reichert Ultracuts, Wien, Austria), and contrasted with 4% uranyl acetate for 45 min and subsequently with lead citrate for 4 min at room temperature. Sections were examined in a Jeol 1200 EX2 electron microscope (JEOL, Tokyo, Japan).

Measurements of foot processes and glomerular basement membrane

Negatives of electron micrographs (magnification x6000) were scanned at 600 d.p.i. resolution using a scanner (Epson Perfection 1200 Photo, Epson Europe, Amsterdam), resulting in a specimen-level pixel size of ~ 7 x 7 nm². Measurement of the resulting images
was performed using Zeiss KS400 (Carl Zeiss Imaging Systems, Germany). The system was calibrated using the marker bar on the electron micrographs. The magnification data were verified via a grating replica with parallel lines (2160 lines/mm; EMS, Washington, USA). The glomerular basement membrane (GBM) was indicated interactively using a graphic tablet. The image analysis software was used to measure the length of the GBM for each loop. Also, for each loop the number of podocyte foot processes overlying the capillary basement membrane was manually counted. A foot process was defined as any connected epithelial segment butting on the basement membrane, between two neighbouring filtration pores or slits. For each patient, the average foot process width was calculated by dividing the total number of foot processes by the total length of the GBM. A correction factor of $\pi/4$ was used to correct for presumed random variation in the angle of the section relative to the long axis of the podocyte.\textsuperscript{11,12} The measurements were performed without knowledge of the clinical data. As indicated above, we have used data of 18 patients that were previously reported.\textsuperscript{2} To evaluate the influence of inter-observer variation and differences in equipment, we have randomly measured 32 negatives of electron micrographs from these patients. There was a high correlation between the results obtained in both centers ($r=0.97; p<0.001$), although there was a systematic bias, measurements in our center (RUNMC) being 11% (confidence interval 6-16%) lower. FPW measured by our method was 1584 $\pm$ 740 nm fitting very well with the results from the previous study, which measured an FPW of 1432 $\pm$805 nm (p=ns). The difference in FPW was 11% with a 95% confidence interval of 6 to 16%. FPW measured by our method showed a high correlation with the data from the previous study ($r=0.97; p<0.001$).

**Clinical data**

Medical records were reviewed for clinical and laboratory data at renal biopsy. Data collected were: age, gender, race, weight, blood pressure, level of protein excretion, serum creatinine, serum albumin, serum cholesterol, use of immunosuppressive therapy and antihypertensive medication, initiation of dialysis and death. In addition, the medical records were reviewed for diseases associated with secondary FSGS: obesity (BMI > 30 kg/m$^2$), renal atrophy, unilateral renal agenesis, reflux nephropathy, infections (HIV, parvovirus B19), medication (pamidronate, lithium, interferon-$\alpha$) or intravenous drug abuse, family history of renal disease, sickle cell anemia or malignancies.\textsuperscript{10,11}
Definitions

Presentation was defined as the time when proteinuria was first detected. Nephrotic syndrome was defined as proteinuria of ≥ 3 g/day in association with serum albumin concentration of ≤ 30 g/l. Patients treated with antihypertensive drugs or with a systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg were considered hypertensive. A complete remission (CR) was defined as proteinuria < 0.3 g/24 hours with a stable serum creatinine concentration (< 50% increase from baseline) and a partial remission (PR) was defined as proteinuria between 0.3 g/24 hours and 2.0 g/24 hours with at least 50% reduction in proteinuria from baseline and a stable serum creatinine concentration.

Idiopathic FSGS was defined as a serum albumin ≤ 30 g/L in two measurements in the 3 month period before and after renal biopsy, with a normal renal size and anatomy (observed by intravenous urogram or renal ultrasound), a body mass index < 35 kg/m² and no other discernible cause of FSGS. A clinical diagnosis of secondary FSGS was made in patients with either an identifiable cause of FSGS or nephrotic range proteinuria (> 3g/day) with a serum albumin > 35 g/L in two measurements in the 3 month period before and after renal biopsy.

Statistical analysis

Values are given as means ± SD or median (range) when appropriate. Differences in continuous data were analyzed with use of the Wilcoxon summed rank test or Kruskal–Wallis test in case of more than two groups. If the result of the Kruskal–Wallis test was significant (p<0.05), then pairwise comparisons were performed with the Wilcoxon rank-sum test. Fisher's exact test was used for categorical data. Spearman’s rank correlation coefficients were calculated to assess the relation of foot process width to proteinuria, serum albumin and disease type (MCNS, idiopathic or secondary FSGS). Multiple regression analysis was performed in a forward stepwise fashion to determine the relationship between foot process width and variables that were significant in univariate analysis, with p<0.05 for inclusion of variables. As for strongly skewed variables the high values may have a disproportionate influence on the outcome of the analysis, the natural log transformation was used to reduce their impact.

Receiver operating characteristics (ROC) curves were used to determine the most discriminative threshold for foot process width in predicting idiopathic FSGS, MCNS or
FSGS secondary to maladaptive responses. A two-sided p-value < 0.05 was considered as the level of statistical significance. The level of significance was adjusted for multiple comparisons (two-sided test, \( p<0.01 \)). The analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Cambridge, MA, USA).

**Results**

Baseline characteristics of the 27 patients with FSGS are shown in *table 1*. By clinical criteria, idiopathic FSGS was diagnosed in thirteen patients and FSGS secondary to maladaptive responses was diagnosed in 12 patients. A secondary cause was identified in 7 out of these 12 patients: reflux nephropathy (n=2), atrophic kidney (n=2), unilateral agenesis of the kidney (n=1), nephrectomy after hydronephrosis (n=1) and obesity (n=1; BMI of 33.2 kg/m\(^2\)).

**Table 1.** Characteristics of patients with FSGS at biopsy.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=27)</th>
<th>Idiopathic FSGS (n=13)</th>
<th>FSGS secondary to maladaptive responses (n=12)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>18/9</td>
<td>9/4</td>
<td>7/5</td>
<td>0.67</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>47 ± 15</td>
<td>46 ± 15</td>
<td>50 ± 14</td>
<td>0.47</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>174 ± 130</td>
<td>123 ± 97</td>
<td>221 ± 154</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum creatinine &gt; 135 μmol/l</td>
<td>48%</td>
<td>23%</td>
<td>67%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>30 ± 10</td>
<td>21 ± 5</td>
<td>39 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>6.8 ± 3.6</td>
<td>9.3 ± 3.5</td>
<td>4.8 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>9.0 ± 3.1</td>
<td>11.3 ± 2.0</td>
<td>6.4 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>113 ± 14</td>
<td>112 ± 11</td>
<td>113 ± 18</td>
<td>0.83</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>74%</td>
<td>69%</td>
<td>83%</td>
<td>0.65</td>
</tr>
<tr>
<td>Presentation to biopsy (months)</td>
<td>7.3 (0.3-477)</td>
<td>2.1 (0.5-10.6)</td>
<td>93 (0.3-477)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSGS variant</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NOS</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Tip</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Perihilar</td>
<td>11</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Collapsing</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Five patients had nephrotic range proteinuria with a normal serum albumin, compatible with a diagnosis of FSGS secondary to maladaptive responses.\textsuperscript{14} The baseline characteristics of these 5 patients were not different from patients with an identifiable secondary cause (\textit{table 2}). Two patients without an apparent secondary cause had a normal serum albumin and non-nephrotic proteinuria at biopsy. These patients could not be classified based on clinical criteria. Perihilar FSGS was more common in patients diagnosed with secondary FSGS, whereas the tip lesion was seen more often in idiopathic FSGS (\textit{table 1}). As expected proteinuria and serum cholesterol were lower in patients with secondary FSGS. These patients also had a higher serum creatinine concentration compared to idiopathic FSGS.

\begin{table}
\centering
\caption{Baseline characteristics of patients with secondary FSGS due to maladaptive responses with and without an identifiable secondary cause.}
\begin{tabular}{lccc}
\hline
 & FSGS secondary to maladaptive responses (n=12) & & \textit{p}-value \\
 & With identifiable secondary cause (n=7) & Without identifiable secondary cause n=5) & \\
\hline
Sex (M/F) & 4/3 & 3/2 & 0.92 \\
Age at biopsy (years) & 54 ± 14 & 45 ± 15 & 0.34 \\
Serum creatinine (µmol/l) & 262 ± 182 & 162 ± 88 & 0.34 \\
Serum creatinine > 135 µmol/l & 71% & 60% & 0.68 \\
Serum albumin (g/L) & 37 ± 5 & 41 ± 3 & 0.27 \\
Proteinuria (g/day) & 5.0 ± 1.8 & 4.5 ± 0.9 & 0.53 \\
Serum cholesterol & 6.8 ± 1.4 & 5.8 ± 1.3 & 0.28 \\
Mean arterial blood pressure (mmHg) & 120 ± 17 & 104 ± 17 & 0.20 \\
Hypertension (%) & 86% & 80% & 0.79 \\
Presentation to biopsy (months) & 63.7 (0.3-135) & 120 (60.1-477) & 0.27 \\
FSGS variant & & & 0.47 \\
\textit{NOS} & 2 & 0 & \\
\textit{Tip} & 0 & 0 & \\
\textit{Perihilar} & 5 & 5 & \\
\textit{Collapsing} & 0 & 0 & \\
\hline
\end{tabular}
\end{table}
This is probably related to the more indolent course in of FSGS secondary to maladaptive responses. As a consequence a renal biopsy is often not performed until renal function deteriorates. Six patients (46%) with idiopathic FSGS and two patients (14%) with FSGS secondary to maladaptive responses received immunosuppressive therapy with prednisone (n=2) or prednisone and cyclophosphamide/cyclosporine (n=6) after renal biopsy. Remission rate at 5 years was significantly higher in patients with idiopathic FSGS (71%) compared to patients with FSGS secondary to maladaptive responses (9%;p<0.01).Patients with MCNS presented with a mean proteinuria of 9.2 ± 4.1 g/day and a mean serum albumin of 21 ± 5 g/L. A renal biopsy was performed within one month after presentation in 63% of patients with MCNS. The controls had no proteinuria with a mean serum albumin of 39 ± 7 g/L.

Morphometric analysis and determinants of FPW

Median FPW was 1606 nm (range 626-6486 nm) in patients with FSGS irrespective of the underlying cause (idiopathic or secondary) and 1725 nm (range 1216-2685 nm) in MCNS (figure 1;p=ns). The foot process width in normal kidneys was significantly lower compared to FSGS and MCNS with a median foot process width of 562 nm (range 508-827 nm; p<0.001).

Figure 1. Median foot process width with 95% confidence interval in patients with FSGS, minimal change nephrotic syndrome and controls
In patients with FSGS, foot process width correlated with proteinuria ($r=0.58; p=0.002$; figure 2) and with serum albumin ($r=-0.68; p<0.001$). Foot process width also correlated with type of disease (idiopathic or secondary FSGS) ($r=0.82; p<0.001$). Exclusion of the 5 patients without an identifiable secondary cause did not change the association between type of FSGS and foot process width. On multivariate analysis, including all patient groups, type of disease (MCNS, idiopathic or secondary FSGS) remained the most important determinant of foot process width ($p=0.001$).

![Figure 2](image)

**Figure 2.** Proteinuria versus foot process width in the individual patients with idiopathic FSGS (Δ), minimal change nephrotic syndrome (○), FSGS secondary to maladaptive responses (▼) and undetermined type of FSGS (▽).

We further analyzed the differences in foot process width between idiopathic and secondary FSGS in comparison with MCNS (figure 3). Foot process effacement was most severe in idiopathic FSGS and intermediate in MCNS, as reflected by a foot process width of 2551 nm (range 1581-6486 nm) and 1725 nm (range 1216-2685 nm) respectively ($p=0.001$ for idiopathic FSGS vs MCNS). Foot processes were relatively preserved in FSGS secondary to maladaptive responses, with a foot process width of 1083 nm (range 626-1800 nm). Statistical significance was $p<0.001$ for idiopathic vs secondary FSGS and $p=0.001$ for MCNS vs secondary FSGS.
The degree of overlap in foot process width between idiopathic and secondary FSGS was low. Patients with idiopathic FSGS were characterized by a foot process width above 1500 nm (table 3). ROC curve analysis showed that this cutoff value differentiated patients with idiopathic FSGS from secondary FSGS with a high sensitivity (100%) and specificity (83%). The degree of overlap between MCNS and idiopathic or secondary FSGS was high, and foot process width could not accurately differentiate between these diseases.

Table 3. Predicting disease type by foot process width or disease type

<table>
<thead>
<tr>
<th></th>
<th>Primary FSGS (n=13)</th>
<th>FSGS secondary to maladaptive responses (n=12)</th>
<th>MCNS (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot process width</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt; 1500 nm</td>
<td>13</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>&lt; 1500 nm</td>
<td>0</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

p<0.001 for difference between idiopathic FSGS and FSGS secondary to maladaptive responses
p=0.02 for difference between MCNS and FSGS secondary to maladaptive responses
p=0.04 for difference between idiopathic FSGS and MCNS
Discussion

Podocyte foot process effacement is present in most proteinuric diseases, such as MCNS, FSGS, membranous nephropathy, and IgA nephropathy. It is considered to be a stereotypical reaction of podocytes to injury or damage. Although insight in the podocytes is still increasing, the exact mechanism resulting in foot process effacement remains unknown.\textsuperscript{15,16} Our study confirms previous findings suggesting that the degree of foot process effacement is primarily dependent on the nature of the underlying disease and not a consequence of proteinuria.\textsuperscript{2} In multivariate analysis, type of disease was the most important determinant of foot process width. Admittedly, in univariate analysis, proteinuria also correlated with foot process effacement in patients with FSGS. However, this finding probably reflects the fact that foot process effacement and proteinuria are consequences of podocyte injury and not necessarily causally related. It also correlates with more recent insights into podocyte biology, indicating that both proteinuria and morphological alterations in podocytes or slit pores are consequences of podocyte injury.\textsuperscript{15,17,18} In an experimental model of acutely induced proteinuria, we observed widespread effacement of foot processes before the onset of proteinuria.\textsuperscript{18} Also from human studies, there is evidence that proteinuria and podocyte alterations are not causally related.\textsuperscript{19} We have previously described a familial nephropathy, characterized by marked longstanding proteinuria but with normal podocytic foot processes.\textsuperscript{20} These observations strengthen our conclusion that it is the type of disease (i.e. MCNS, idiopathic or secondary FSGS), rather than the amount of proteinuria, that determine foot process effacement.

Over the last years several causes of podocyte injury have been identified that can lead to foot process effacement.\textsuperscript{17} Important causes are interference with structural components of the slit diaphragm complex and its lipid rafts, direct interference with the actin-cytoskeleton and interference with podocyte-GBM interaction.\textsuperscript{21} In addition, at least in some patients with idiopathic FSGS and MCNS there is evidence that podocyte injury is the direct result of a circulating factor.\textsuperscript{4-6} Thus far identification of this factor has been unsuccessful. The difference in foot process effacement in idiopathic FSGS and MCNS in our study suggests that the underlying cause of podocyte injury differs between the two disorders. This is in agreement with the notion that different plasmafactors appear to be involved in idiopathic FSGS and MCNS.\textsuperscript{22} Inclusion of patients with genetic mutations in basement membrane of podocyte proteins (dystroglycans, nephrin, podocin or actinine) may be another explanation.
for the difference in foot process effacement. However, it is unlikely that mutations in any of these proteins contributed to the difference in foot process effacement. Familial forms were excluded after examination of the patient charts. Sporadic genetic mutations are also unlikely, since these mutations are rare in adult patients and if present they are characterized by therapy resistance. Alternatively, the difference may not be associated with the underlying cause, but merely represent a difference in time to biopsy. Patients with MCNS were biopsied earlier after onset of the proteinuria, and foot process effacement may not have reached the maximum extent of foot process effacement. Several animal models also suggest that proteinuria can precede foot process effacement. In mice deficient of laminin $\beta_2$, a component of the GBM, proteinuria develops before widespread foot process effacement. However, since the primary defect in this mouse model resides in the GBM, it may not fully reflect the situation in patients with MCNS. A Japanese group developed an animal model that is similar to MCNS. Injection of mAb 5-1-6, an antigen directed at the extracellular domain of the rat homolog of nephrin, causes massive proteinuria without histologic abnormalities on light microscopy. Even after repeated doses for 25 weeks light microscopy showed minimal glomerular lesions. Eight days after injection of Mab 5-1-6 partial retraction of foot processes was seen on electron microscopic examination. These models show that proteinuria can precede the effacement of foot processes. Thus, if a biopsy is performed shortly after the onset of proteinuria foot process effacement may be less severe.

Foot processes in patients with FSGS secondary to maladaptive responses were more conserved than in idiopathic FSGS and MCNS. In fact, the difference in foot process effacement with idiopathic FSGS was large enough to define a cut-off value for foot process width that can predict idiopathic or secondary FSGS with a high sensitivity and specificity. Our results are in agreement with studies in a specific group of patients with secondary FSGS due to obesity. These latter studies demonstrated that obesity-related FSGS was characterized by relatively mild foot process fusion, indicating that idiopathic and FSGS secondary to maladaptive responses can be distinguished by different morphologic features. Admittedly measurement of foot process width is time consuming and cannot be routinely performed. Clinical parameters such as serum albumin are often sufficient to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses. In a study of 37 patients with nephrotic range proteinuria due to biopsy proven FSGS, Praga et al. showed...
that serum albumin was significantly lower in patients with presumed idiopathic FSGS (serum albumin < 30 g/L) compared to FSGS secondary to maladaptive responses (serum albumin > 35 g/L). However, in patients with a serum albumin between 30 and 35 g/L without an apparent secondary cause the distinction between idiopathic and secondary forms of FSGS often poses a challenge to the nephrologist caring for patients with FSGS. In these patients foot process measurement could be helpful in guiding diagnosis and prevent inappropriate treatment with steroids and cytotoxic agents that are not effective in secondary forms of FSGS. Our study did not include patients with a serum albumin between 30 and 35 g/L without a secondary cause, therefore future studies in this patient group are needed to determine whether morphometric analysis of podocytic foot processes can guide diagnosis and treatment of FSGS.

There are some potential limitations to the study. First, clinical criteria were used to make a diagnosis of idiopathic or secondary FSGS. By using a combination of predefined clinical criteria, we have tried to avoid misdiagnosis. Second, in five patients secondary FSGS was diagnosed without an apparent secondary cause. Nevertheless, we do feel that classification of these cases as FSGS secondary to maladaptive responses is justifiable. Baseline clinical characteristics and prognosis of these five patients were indistinguishable from patients with a secondary cause. Renal biopsies of all five patients showed the perihilar variant, which is known to be associated with FSGS secondary to maladaptive responses. Even more important, the results were not altered after exclusion of these patients from the analysis.

In conclusion, our study demonstrates that foot process width correlates very well with idiopathic FSGS, MCNS and FSGS secondary to maladaptive responses, independent of the degree of proteinuria. Foot processes are more effaced in idiopathic FSGS than in MCNS suggesting different mechanism of podocyte injury. In selected cases measurement of foot process width can be useful to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses.
Reference List

Chapter 7

Plasma exchange improves graft survival in patients with recurrent focal segmental glomerulosclerosis

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Chapter 7

Abstract

Recurrence of primary focal glomerulosclerosis (FSGS) after renal transplantation is associated with poor graft survival. Plasma exchange (PE) can reduce proteinuria and even induce complete remission of proteinuria. It is, however, unknown whether the use of PE therapy improves long-term graft survival. In our center, PE has been used to treat recurrent FSGS after renal transplantation since 1994. Thus far, 13 patients have been treated with PE for recurrent FSGS and followed for up to 77 months after the onset of the recurrence. We reviewed the transplantation data in these patients, and, for comparison, ten patients who underwent transplantation between 1973 and 1991 and were not treated with PE served as historical controls. Recurrence of FSGS occurred within 4 weeks of transplantation in 74% of the patients. PE was started within 14 days of the onset of proteinuria in 85% of the patients. Two patients lost their graft within the first month of transplantation due to untreatable rejection; the remaining 11 patients (85%) achieved complete (n=7) or partial (n=4) remission. Seven patients remained in remission after a short period of treatment with PE (≤ 18 sessions in 2 months), whereas four patients needed prolonged treatment (median of 58 sessions).

The need for prolonged PE was associated with a late (>30 days after transplantation) recurrence of FSGS (p=0.02). A comparison with the historical control group revealed not only a significant reduction in proteinuria, but also significantly better long-term graft survival in the treated group, 85% and 30%, respectively, at 5 years (p=0.02). In conclusion, PE is an effective form of treatment for recurrent FSGS, especially if initiated early. Failure to maintain stable remission after the initial period of PE does not necessarily imply a poor outcome, and sustained remissions can be achieved after prolonged treatment.
**Introduction**

The reported recurrence rate of focal glomerulosclerosis (FSGS) after renal transplantation averages 30%.\(^1\) The risk of recurrence is even higher (50%) in patients younger than 20 years of age, in patients with a rapid clinical course of their original disease and also in patients with evidence of mesangial proliferation in their native kidney biopsy.\(^3\) Recurrent FSGS typically becomes manifest within 3 months of transplantation.\(^6\) Alterations in baseline immunosuppressive regimens have not influenced recurrence rate.\(^8\) Prognosis is poor in patients with a recurrence, and approximately half the patients will lose their graft within 5 years.\(^4\)

Recent studies have suggested that recurrent FSGS is mediated by a circulating protein factor that alters the permeability of glomeruli.\(^3,12\) This finding has led to the use of plasma exchange for the treatment of recurrent FSGS. We and others have reported that plasma exchange treatment effectively reduces proteinuria, especially when plasma exchange is started shortly after onset of proteinuria.\(^2,3,13,14,15,16,17\) Long-term results of plasma exchange are less well known.

Earlier, we published the short-term effect of plasma exchange treatment in seven patients with recurrent FSGS.\(^13\) In this paper we describe the extended follow-up of these patients, who have now been followed for up to 77 months after the onset of the recurrence. In addition, the results of plasma exchange in six more patients are reported. For comparison, we have used a group of historical controls, consisting of patients with recurrent FSGS not treated with plasma exchange.

**Patients and methods**

In the period between 1973 and 2002 a diagnosis of recurrent FSGS was made in 23 adult patients (≥17 years) who received a renal graft at our center. In 19 patients the original disease was biopsy proven FSGS, but in four patients a biopsy was not available pre-transplantation. In these patients recurrent FSGS was considered the most likely diagnosis because heavy proteinuria developed almost immediately after transplantation and the transplant biopsies did not disclose evidence of other glomerular diseases. A clinical diagnosis of recurrent FSGS was made in the case of a rapid onset or firm increase in proteinuria of > 3 g/day. The renal transplant biopsy of patients with recurrent proteinuria
either disclosed glomeruli with evidence of focal and segmental glomerulosclerosis on light microscopy (LM) or normal glomeruli on LM, but evidence of effacement of the epithelial foot processes on electron microscopy.\textsuperscript{13} In three patients with recurrent proteinuria no renal biopsy was done; however, the clinical course was compatible with a recurrence of FSGS, and there was a rapid improvement of proteinuria after the start of plasma exchange.

During the study period we used different regimens of immunosuppressive therapy. From 1973 to 1983, basic immunosuppressive therapy consisted of prednisone (25 mg/day for 1 month tapered to 10 mg/day after 4 months) and azathioprine (3 mg/kg per day). From 1983 to 1985, patients were treated in a randomized study with either cyclosporine A (CyA; starting with 17.5 mg/kg per day and tapering to 5 mg/kg per day at 3 months) and prednisone for the first 3 months, followed by conversion to azathioprine and prednisone thereafter, or azathioprine and prednisone for the whole period.\textsuperscript{18} From 1985 to 1989, all patients received CyA and prednisone during the first 3 months and azathioprine and prednisone thereafter. In the period from 1989 to 1992, patients were treated with CyA (starting with 12 mg/kg per day and tapering to 4 mg/kg per day at 3 months) and prednisone for the first 3 months and thereafter randomized for continued treatment with either azathioprine and prednisone or CyA monotherapy.\textsuperscript{19} From 1992 to 1997, patients were treated with a combination of CyA and prednisone, and since 1997, patients have been treated in a randomized study with mycophenolate mofetil (MMF; 1000 mg b.i.d.), prednisone and high-dose (10 mg/kg per day) or low-dose (6 mg/kg per day) CyA.\textsuperscript{20} Since 2000, patients have been treated with tacrolimus and MMF in combination with either prednisone or daclizumab. Patients who received an HLA-identical living, related-donor kidney were treated with different immunosuppressive regimens. Until 1985 these patients received azathioprine and prednisone. Thereafter, they were treated with CyA (starting with 12 mg/kg per day and tapering to 4 mg/kg per day at 3 months) and prednisone for the first 3 months, followed by conversion to azathioprine and prednisone.

During their hospital stay, patients’ serum creatinine level and urine protein levels were measured daily. After discharge, all patients were followed weekly for the first 4 months, every 2–4 weeks thereafter, and at least every 3 months from 1 year on. At each visit, serum creatinine level and urinary protein concentration were recorded. For the purpose of this study the medical records of the 23 patients with recurrent FSGS were analyzed. The following data were documented for each patient: gender, age at diagnosis of the original
Plasma exchange improves graft survival in recurrent FSGS disease, biopsy result, time from diagnosis to end-stage renal failure, time on dialysis, age at transplantation, donor source, immunosuppressive therapy post-transplantation, time from transplantation to recurrence, proteinuria, serum creatinine, and time from recurrence to the start of plasma exchange therapy. Before 1998, the time of onset of recurrent FSGS was defined as proteinuria exceeding 3.5 g/day. At that time we became aware that plasma exchange should be started as quickly as possible after onset of proteinuria. Therefore, since then, even lower values of proteinuria were considered compatible with recurrent FSGS. Since 1994, all patients with recurrent FSGS have been treated with plasma exchange. Plasma exchange was performed with the CS-3000 Plus Cell Separator (Baxter, Deerfield, Ill., USA). A total of 1.5 plasma volumes were replaced per session with either fresh frozen plasma or 5% albumin. The treatment protocol consisted of daily plasma exchanges for up to 3 days. Thereafter, intensity of plasma exchange treatment was decreased, depending on the clinical response. The initial cycle of plasma exchange treatment consisted of a maximum of ten treatment sessions. In cases of relapse, plasma exchange was re-instituted and the frequency of treatment sessions was slowly reduced, based on the effect on protein excretion. Two patients, recipients of a kidney from a living donor, received pre-emptive treatment with plasma exchange. The pre-emptive treatment regimen consisted of one plasma exchange session on the day before transplantation. Complete remission was defined as proteinuria of less than 0.2 g/day and stable serum creatinine; a partial remission was defined as a decrease in proteinuria of more than 50% to less than 2 g/day.

**Statistical analysis**

The values are given as means ± SD or median (range) when appropriate. Survival probabilities were calculated with the Kaplan-Meier method. Log-rank test was used for comparison of survival curves. For comparison between groups, an unpaired t-test or Mann-Whitney U-test were used. Categorical variables were assessed with use of the X2-test or Fisher’s exact test, as appropriate. A p-value of 0.05 was considered as the level of statistical significance.
Chapter 7

Results

A diagnosis of recurrent FSGS was made in 23 patients. Ten patients, who received transplants before February 1991, were not treated with plasma exchange and served as historical controls. In Table 1 the demographic characteristics of the 13 patients with recurrent FSGS who were treated with plasma exchange are compared with those of the controls. It should be noted that recurrence could not be prevented by any of the immunosuppressive protocols that were used. Four patients in the plasma-exchange group received a graft from a living donor, and three patients had already lost one or more previous renal grafts due to recurrent FSGS.

Table 1. Characteristics of patients with recurrent FSGS treated or not treated with plasma exchange (PE). Values are given as mean ± SD or as median (range) or non-parametric data.

<table>
<thead>
<tr>
<th></th>
<th>Treated with PE (n=13)</th>
<th>Not treated with PE (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>8/5</td>
<td>6/4</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>27 ± 13</td>
<td>24 ± 12</td>
</tr>
<tr>
<td>Time to ESRD (years)</td>
<td>8.4 ± 8.8</td>
<td>7.1 ± 4.9</td>
</tr>
<tr>
<td>Age at transplantation (years)</td>
<td>40 ± 16</td>
<td>33 ± 13</td>
</tr>
<tr>
<td>1st/2nd/3rd/4th transplantation</td>
<td>10/1/1/1</td>
<td>10/0/0/0</td>
</tr>
<tr>
<td>Donor source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadaver</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Living related</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Living unrelated</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Immunosuppressive medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pred/Aza</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pred/CyA</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Pred/CyA/Aza</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pred/MMF/Tacro</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Pred/MMF/CyA</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Acute rejection (number of patients)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Onset recurrence (days)</td>
<td>4 (0-2100)</td>
<td>9 (0-137)</td>
</tr>
<tr>
<td>Proteinuria at onset (g/day)</td>
<td>5.7 ± 3.9</td>
<td>5.8 ± 4.8</td>
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<tr>
<td>Interval recurrence-biopsy (days)</td>
<td>7 (2-93)</td>
<td>28 (0-217)</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>3.4 ± 2.4</td>
<td>3.6 ± 2.6</td>
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<tr>
<td>Allograft loss (n)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Due to recurrence</td>
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<td>0</td>
</tr>
</tbody>
</table>

ESRD = end-stage renal disease; CyA = cyclosporine; Pred = prednisone; Aza = azathioprine; Tacro = tacrolimus. *p<0.001
In the control group, renal biopsy showed characteristic changes of FSGS on LM in eight patients. In addition, there was evidence of acute interstitial rejection in two, and of a chronic vascular rejection in one of these patients. In two patients, LM showed no evidence of FSGS, only changes compatible with acute interstitial rejection or cyclosporine toxicity. In both patients there was evidence of foot process effacement on electron microscopy. No patient in the control group achieved remission of proteinuria. Heavy proteinuria persisted, both in patients treated with azathioprine and in those treated with CyA. The median time between the diagnosis of recurrence and graft failure was 44 months (range 0.3-97 months). Graft failure occurred in all ten control patients.

Recurrent FSGS was the sole cause of graft failure in five patients. Graft failure was due to recurrent FSGS in combination with acute interstitial rejection in four patients and recurrent FSGS in combination with chronic vascular rejection in one patient. Graft survival at 3 and 5 years was 70% and 30%, respectively (figure 1). Graft survival of the cohort of patients without FSGS, aged 15-55 years, who received a first cadaveric renal graft in the same period (1973-February 1991; n=672) was 65% and 59% at 3 and 5 years, respectively (figure 1). Thus, in the period that plasma exchange was not regularly used, graft survival was significantly lower in the patients with recurrent FSGS (p<0.01).

Figure 1. Kaplan Meier probability of graft survival in patients with recurrence of FSGS who either underwent transplantation after 1991 and were treated with plasma exchange (solid line) or underwent transplantation before 1991 (broken line). For comparison, graft survival curves are depicted in grey for the contemporary patient cohort, transplanted before (broken line) and after (solid line) 1991.
Individual data for the patients treated with plasma exchange are given in tables 2 and 3. FSGS recurred early (within 30 days of transplantation) in ten patients and late (more than 30 days after transplantation) in three patients. A renal-graft biopsy was performed in ten patients. No glomerular abnormalities were found by LM in eight patients (nos. 1, 3, 5, 7, 9-12); however, by electron microscopy, effacement of the epithelial foot processes was seen in all of them. There was also evidence of acute interstitial rejection in two of these patients (nos. 1 and 5) and acute vascular rejection in another patient (no. 10). In patient no. 2, whose biopsy showed signs of acute tubular necrosis and rejection, an adhesion was found in one glomerulus, suggestive of FSGS. Patient no. 6, who was biopsied 14 days after onset of the proteinuria, had evidence of focal segmental sclerosis on LM. A renal biopsy was not performed in two patients (nos. 8 and 13) because renal function and proteinuria improved within several days of initiation of plasma exchange.

Finally, in one patient the diagnosis of recurrence was made late after transplantation (no. 4). This patient was treated with cyclosporine monotherapy. She developed mild proteinuria and a decrease in renal function. A renal biopsy revealed vascular changes compatible with cyclosporine toxicity and/or chronic vascular rejection. In the glomeruli there was mesangial matrix and cell proliferation with mild influx of mononuclear leukocytes. There was mild swelling of the epithelial cells. Electron microscopy (EM) showed a focal fusion of the foot processes. Cyclosporine was replaced by azathioprine and prednisone. Shortly thereafter, a tremendous increase in proteinuria occurred, without evidence of renal function deterioration. A clinical diagnosis of recurrent FSGS was made, but a renal biopsy was not done in view of the known abnormalities in the previous biopsy.

Patients with evidence of rejection in the biopsy received anti-rejection treatment consisting of oral prednisone (no. 4), intravenous (i.v.) methylprednisolone (no. 5), i.v. methylprednisolone followed by anti-thymocyte immunoglobulin (no.1), i.v. methylprednisolone followed by oral prednisone (no. 2) or i.v. methylprednisolone followed by monoclonal anti T-cell antibody therapy (no. 10).

Plasma exchange was initiated within 14 days of recurrence of FSGS in 85% of patients (n=11) and in the two other patients at 19 and 50 days after the onset of a recurrence. Two patients (nos. 2 and 5) lost their graft after 0.7 and 1.0 month because of concomitant biopsy proven untreatable rejection.
In all the remaining patients treatment with plasma exchange resulted in a decrease in proteinuria. Seven patients achieved sustained remission of proteinuria, either complete (n=5) or partial (n=2). Four patients suffered a relapse and needed prolonged treatment. However, after a median of 58 sessions (range 43-63 sessions) these four patients also achieved complete (n=2) or partial (n=2) remission.

Subgroup analysis of the 11 patients without graft failure showed that those with a late recurrence (> 30 days after transplantation) were more likely to need prolonged therapy (p=0.02). These patients also tended to be younger (p=0.08). During follow-up, serum creatinine has remained stable in all 11 patients. For patients treated with plasma exchange, graft survival after recurrence of FSGS was as high as 85% at 5 years (Fig. 1). Graft survival was significantly better for patients who underwent plasma exchange than for historical controls (p=0.02). Graft survival of the cohort of patients without FSGS, aged 116-69 years, who received either a renal graft from a living donor or a cadaveric renal graft in the same period (after May 1991; n=1,032) was not significantly different, with 72% at 5 years.
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Time to recurrence (days)</th>
<th>Proteinuria before PE (g/day)</th>
<th>Creatinine before PE (mg/dL)</th>
<th>Interval rec-PE (days)</th>
<th>Proteinuria after PE (g/day)</th>
<th>Creatinine after PE (mg/dL)</th>
<th>Number of PE sessions</th>
<th>Duration of PE (months)</th>
<th>Follow-up after recurrence (months)</th>
<th>Outcome</th>
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<td>73</td>
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<td>50</td>
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<td>45</td>
<td>71</td>
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<td>19</td>
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<td>51</td>
<td>59</td>
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<td>1</td>
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<td>0</td>
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<td>1</td>
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<td>CR</td>
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<td>6.5</td>
<td>1.4</td>
<td>6</td>
<td>0.2</td>
<td>0.9</td>
<td>18</td>
<td>2</td>
<td>9</td>
<td>CR</td>
</tr>
</tbody>
</table>

*Proteinuria measured before second PE sessions; CR=complete remission; PR=partial remission; ESRD=end-stage renal disease; PE=plasmapheresis; Rec=recurrence.
Discussion

Our study clearly demonstrates that patients with recurrent FSGS after transplantation benefit from treatment with plasma exchange. We have extended our previous observations that plasma exchange therapy rapidly decreases proteinuria in the majority of patients. Most importantly, the beneficial effects of plasma exchange are well maintained during longer follow-up, thus improving graft survival rates in our cohort of treated patients when compared with untreated, historical controls. Admittedly, the use of historical controls introduces a bias, since, in recent years, supportive therapy and immunosuppressive treatment have changed. However, most authors would agree that outcome is poor in patients with recurrent FSGS who are not treated with plasma exchange. Reported rates of graft failure in untreated patients vary from 58% to 68% in adults and from 50% to 80% in children, values comparable to the 70% graft failure rate at 5 years in our historical controls. Moreover, we have compared graft survival rates of our patients with recurrent FSGS with graft survival rates of patients who received transplants in the same periods. It is evident that graft survival of patients with recurrent FSGS was significantly less in the period before 1991. This survival disadvantage was completely lost with the introduction of plasma exchange after 1991. This indicates that plasma exchange, and not a different immunosuppressive regimen, caused the difference in graft survival between patients treated with plasma exchange and the historical controls.

Could our results have been biased otherwise? We have included three patients in whom a diagnosis of recurrent FSGS was made on clinical grounds, i.e., the rapid occurrence and incremental rise of proteinuria after transplantation. Some authors have required histological evidence of FSGS in the allograft before starting plasma exchange. However, in early biopsies, glomerular lesions are mostly absent under LM. Moreover, we feel that the clinical course in patients with recurrent FSGS is quite specific. Recently, Kaplan- Pavlovic et al. have also provided evidence that the development of nephrotic range proteinuria early after transplantation is sufficient to allow a diagnosis of recurrent FSGS.

We have included two patients who were pre-emptively treated with plasma exchange therapy. One might argue that the inclusion of these patients may have favored the outcome data. This is unlikely, however, since these patients still developed massive proteinuria after transplantation, which necessitated further plasma exchange therapy. Also, exclusion of these
patients did not alter the results. From the patients’ characteristics, one might even have suspected a worse prognosis in our treated patients, since we have included patients who received a second, third and fourth transplant after having lost a previous graft due to recurrent FSGS. Also, we have allowed living-donor transplantation. This demonstrates the change in our recent policy, being less restrictive in accepting patients with FSGS for transplantation, in view of the potential benefits of plasma exchange therapy.

Overall, 11 patients (85%) achieved sustained remission of proteinuria (54% complete, 31% partial). Admittedly, in four of these patients repeated courses of plasma exchange therapy were needed. Young age and development of a recurrence somewhat later after renal transplantation characterized these patients. Still, it is evident that prolonged treatment is justified in view of the good ultimate outcome. The overall rate of sustained remissions in our study is quite high when compared with the reported remission rates in adult patients, which range from 15% to 67%. A likely explanation for the difference in remission rates is the early initiation of plasma exchange therapy in our patient group, with treatment being started within 14 days in 85% of patients. Larger series also suggest an association between rapid initiation of plasma exchange and remission rates, since all remissions in these studies occurred in patients who were started on plasma exchange early after the onset of recurrent disease.3,14,17,26 In addition, although the onset of a recurrence cannot be prevented by any of the immunosuppressive regimens used, relapse after plasma exchange might be prevented by an immunosuppressive maintenance regimen containing prednisone. We have recently described a patient (no. 12) who was initially managed on steroid-free immunosuppression.27 Proteinuria recurred whenever plasma exchange therapy was reduced. Only after reintroduction of prednisone was sustained remission achieved. A review of the literature data on recurrent FSGS supports the notion that prednisone must be part of the immunosuppressive therapy to achieve a sustained remission of proteinuria after plasma exchange treatment (table 4). In addition to our study, sustained responses in adult patients have also been reported by Artero et al.2 In contrast, in the studies of Dantal et al. most patients suffered a relapse of proteinuria after discontinuation of plasma exchange.3,28 Differences in baseline characteristics and timing of initiation of plasmapheresis treatment cannot explain these differences. However, the maintenance immunosuppressive regimen was clearly different, prednisone being stopped within 60 days of transplantation by Dantal. Matalon et al. also reported a low rate of sustained remissions. In this study, however,
Plasma exchange improves graft survival in recurrent FSGS

plasmapheresis was initiated more than 30 days after recurrence in over half the patients.\textsuperscript{17} This probably contributed to the lower rate of remission. Furthermore, the latter authors do not report the duration or dose of prednisone therapy. Plasmapheresis treatment has been more effective in children, with over 60\% achieving sustained, plasmapheresis-independent, remission of proteinuria (table 4).\textsuperscript{14,15,16,29}

Table 4. Results of plasmapheresis for recurrent FSGS after renal transplantation in adults and children.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients ($n$)</th>
<th>Interval recurrence to plasmapheresis (days)</th>
<th>Sustained remission (%)</th>
<th>Prednisone part of maintenance immunosuppressive therapy?</th>
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<td></td>
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<tr>
<td>Dantal et al.\textsuperscript{28}</td>
<td>8</td>
<td>63 (11-292)</td>
<td>13</td>
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<tr>
<td>Matalon et al.\textsuperscript{17}</td>
<td>13</td>
<td>60 (0-1520)</td>
<td>8</td>
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<tr>
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</table>

\textsuperscript{a}Two children also had an acute rejection and plasmapheresis treatment was delayed for 2 months in another child. NA = not available.

Notably, in children, plasmapheresis treatment has been accompanied by more intensive immunosuppression, either pulse solumedrol, cyclophosphamide or high-dose CyA. Furthermore, in all studies in children, prednisone has been part of maintenance immunosuppression.

In children, there is also limited evidence that recurrent FSGS can be successfully treated with high-dose intravenous CyA, without the need for plasma exchange. A recently published uncontrolled study by Salomon et al. demonstrated that high-dose intravenous CyA could induce persistent remission of proteinuria in 65\% of children with recurrent
FSGS. The role of high-dose intravenous CyA in adults is unknown. Of note, we observed recurrences of FSGS in 15 patients who were treated with i.v. CyA for 3 days, followed by high-dose oral CyA therapy.

In conclusion, plasma exchange therapy improves outcome in patients with recurrent FSGS. For a high success rate to be obtained, plasma exchange therapy must be instituted shortly after onset of the recurrence. If a relapse of proteinuria occurs after the first course of plasma exchange, prolonged treatment is warranted. In view of the improved outcome with plasma exchange, we have adopted a less-restricted policy for transplantation in patients with primary FSGS.

Reference List

Plasma exchange improves graft survival in recurrent FSGS


Chapter 8

Treatment of recurrent focal segmental glomerulosclerosis after renal transplantation: is prednisone essential to maintain a sustained remission?

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Patients with focal glomerulosclerosis (FSGS) are at high risk for developing recurrent disease after renal transplantation.\textsuperscript{1} Recent studies indicate that plasmapheresis effectively reduces proteinuria in most patients with recurrent FSGS.\textsuperscript{2-5} However, in many patients proteinuria recurs after cessation of plasmapheresis treatment.\textsuperscript{3} Thus far, the role of immunosuppressive therapy in preventing or treating recurrent FSGS is unclear. We describe a patient with recurrent FSGS in whom maintenance therapy with prednisone proved essential to achieve a persistent remission of proteinuria. In 1992, a 42-year-old woman presented with biopsy proven FSGS. She developed end-stage renal disease in 1996 and received a cadaveric renal graft in 1999. There was immediate graft function. Six weeks after transplantation, overt proteinuria was noted. At that time, immunosuppressive medication consisted of prednisone (15 mg daily), cyclosporine A (target trough level 200–400 ng/mL), and mycophenolate mofetil (1 g twice daily). A renal biopsy specimen was normal except for complete effacement of the epithelial foot processes on electron microscopy. A diagnosis of recurrent FSGS was made, and treatment with plasmapheresis was started, resulting in a complete disappearance of proteinuria (figure). However, the subsequent course was characterized by recurrences of proteinuria, which responded to intensified plasmapheresis treatment (figure). Plasmapheresis at weekly intervals was needed to maintain a stable remission. In the meantime, the immunosuppressive regimen had been changed. Prednisone treatment was tapered from 3 months after transplantation and ultimately stopped at 11 months because of severe osteoporosis. Furthermore, immunosuppressive maintenance therapy with mycophenolate mofetil (750 mg twice daily) was not tolerated because of severe gastrointestinal side effects. Cyclosporine A caused renal dysfunction and sirolimus persistent ulceration of the mouth. Therefore, 15 months after transplantation, prednisone (10 mg twice daily) was started again in combination with tacrolimus. One month later, plasmapheresis treatment had to be stopped because of an acute thrombosis of the arteriovenous-fistula; an attempt to create a new one failed. Much to our surprise, proteinuria did not recur. To minimize side effects, prednisone was slowly tapered from 18 months after transplantation and finally stopped and replaced by azathioprine at 26 months. Within 1 month, proteinuria returned.
Figure. The time course of proteinuria (○) after renal transplantation. Plasmapheresis sessions are indicated (△) and the period of prednisone treatment is indicated with an open arrow (▲) indicating the end of prednisone treatment and a closed arrowhead (▼) restart of prednisone treatment.

Prednisone in recurrent FSGS
Chapter 8

Reintroduction of prednisone (10 mg twice daily) resulted in an almost immediate decrease of proteinuria (figure). Currently the patient is treated with tacrolimus, azathioprine, and prednisone (5 mg twice daily), with no proteinuria and a stable creatinine of 1.2 mg/dL. It is evident that prednisone treatment cannot prevent the development of recurrent FSGS. In fact, even the introduction of the newer immunosuppressive agents has not influenced the incidence of recurrent FSGS. Although not proven in controlled studies, plasmapheresis therapy effectively induces remission of proteinuria. The literature is somewhat conflicting about the potential to achieve a persistent remission after cessation of plasmapheresis. We believe that the course in our patient strongly suggests that the use of prednisone may be essential in maintaining a sustained remission of proteinuria in patients with plasmapheresis-dependent recurrent FSGS after renal transplantation. Admittedly, prednisone may not be the only beneficiary agent. The potential role of the immunosuppressive regimen in the treatment of recurrent FSGS is further illustrated by the fact that sustained remissions are more often observed in children than in adults: in the former group, plasmapheresis therapy is most often combined with the addition of cyclophosphamide or a large increase in cyclosporine dose.5

Reference list

Chapter 9

Rituximab for plasma exchange dependent recurrent focal segmental glomerulosclerosis after renal transplantation.

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Submitted
Abstract

We describe a 24-year old female patient with recurrent focal segmental glomerulosclerosis (FSGS) after renal transplantation. At the age of 10 years, the patient presented with a nephrotic syndrome due to biopsy proven FSGS. Three years after presentation she progressed to end-stage renal disease. A first renal transplant failed due to recurrent FSGS. Seven years later the patient received a second renal transplant. One week after transplantation, recurrent FSGS was diagnosed. Treatment with plasma exchange (PE) resulted in a complete remission. However, three months later proteinuria recurred, which responded to another course of PE. A third relapse was diagnosed two years later. A renal biopsy confirmed recurrent FSGS. A new remission was attained with PE, however the following 15 months attempts to reduce PE frequency failed and the patient remained dependent on PE. Therefore, it was decided to treat the patient with 4 weekly doses of rituximab i.v. (375 mg/m²). Over the next months PE treatment could be reduced and was ultimately stopped. A relapse was successfully treated with a new infusion of rituximab. At present the patient has attained a partial remission of proteinuria with a stable serum creatinine. In conclusion, B-cell depletion with rituximab may benefit patients with PE dependent recurrent FSGS.
Rituximab in recurrent FSGS after renal transplantation

Introduction

Focal segmental glomerulosclerosis (FSGS) can recur after renal transplantation and is associated with a reduced graft survival. In case of recurrent FSGS, treatment with plasma exchange (PE) results in a remission of proteinuria in up to 85% of patients, especially if started shortly after the onset of recurrence.\textsuperscript{1,2} However, many patients require repeated courses of PE because of frequent relapses.\textsuperscript{1,3} Recently, a 7-year old boy with recurrent FSGS after renal transplantation responded to rituximab, a monoclonal anti-CD20 antibody, which was administered for a transplantation-related lymphoma.\textsuperscript{4} We describe the results of treatment with rituximab in a 24-year-old female patient with recurrent FSGS after renal transplantation.

Case

At the age of 10 years, the patient presented with a nephrotic syndrome due to biopsy proven FSGS. Treatment with prednisone, cyclophosphamide, and cyclosporine was unsuccessful and end-stage renal disease (ESRD) developed within 3 years after presentation. At the age of 13 years the patient received a renal graft, which failed after one year due to recurrent FSGS. Seven years later, she received a second renal graft. Baseline immunosuppressive therapy consisted of prednisone (10 mg), tacrolimus (target through level 15-20 mg/l) and mycophenolate mofetil (MMF 750 mg bid). There was almost immediate graft function. One week after transplantation the patient developed nephrotic range proteinuria. A presumptive diagnosis of recurrent FSGS was made. Treatment with PE was started immediately and resulted in a complete remission. Three months after cessation of PE proteinuria recurred. At that moment the immunosuppressive regimen consisted of prednisone (15 mg) and tacrolimus (target through level 5-10 mg/l). A second course of PE (8 sessions) again resulted in a complete remission. A third relapse occurred 2 years later. To exclude other causes of proteinuria a biopsy of the renal graft was performed, which demonstrated diffuse foot process effacement, without significant lesions on light microscopy and IF, favoring a diagnosis of recurrent FSGS. Although treatment with PE induced a remission of proteinuria, repeated trials of cessation of PE failed (figure). A remission of proteinuria could only be maintained with continuous PE (one session every 7-10 days), even though the patient was...
Figure. Clinical course of proteinuria after third relapse of FSGS and response to plasma exchange (PE) and rituximab.
Rituximab in recurrent FSGS after renal transplantation

treated with a more intensive immunosuppressive regimen consisting of prednisone (10 mg),
tacrolimus (target through level 5-10 mg/l), and MMF (500 mg bid), which was replaced by
azathioprine (2 mg/kg/day) because of gastrointestinal side effects. Because of PE
dependency, the decision was made to start treatment with rituximab. She tolerated the entire
course of 4 weekly infusions (375 mg/m2) without significant side effects, although we
observed a temporary neutropenia. B-cell markers were measured 4 months after completion
of rituximab therapy. Both CD19+ and CD20+ B-cells were undetectable. In the first 4
months after treatment with rituximab three PE sessions were necessary because of
increasing proteinuria. Thereafter, proteinuria gradually decreased without further
interventions. Seven months after treatment with rituximab a partial remission was attained.
Nine months later she experienced a relapse of proteinuria (figure). At that time CD19+ and
CD20+ B-cells were still undetectable. The patient was retreated with a single infusion of
rituximab 1000 mg. Proteinuria gradually decreased, and a partial remission was reached
two months after treatment.

Discussion

Our case report indicates that rituximab may be effective in patients with recurrent FSGS
after renal transplantation. Admittedly, we cannot exclude that PE contributed to the
response after initial therapy. However, the efficacy of rituximab was virtually proven by the
response to rituximab monotherapy after relapse.

Rituximab was used with apparent success in children with recurrent FSGS (table). These
children were treated with rituximab for EBV-related post-transplant lymphoproliferative
disease (PTLD). Coincident with the remission of the PTLD proteinuria also disappeared. In
contrast, two children without PTLD failed to respond to treatment with rituximab for
recurrent FSGS after renal transplantation. Data in adults are also conflicting. Recently,
Hristea and Kamar reported three adult patients with recurrent FSGS after transplantation.
One patient did not respond to rituximab, the other two patients attained a
remission of proteinuria. However, since both patients were simultaneously treated with PE,
it cannot be excluded that PE alone induced the remission. In contrast, Gossmann reported a
patient who was resistant to treatment with PE but responded to rituximab. Recurrent FSGS
has been attributed to a thus far unidentified circulating permeability factor and T-cells have
Table 1. Results of treatment with rituximab in patients with recurrent FSGS after renal transplantation

<table>
<thead>
<tr>
<th>Study</th>
<th>Donor source</th>
<th>Immunosuppression</th>
<th>Age at transplantation</th>
<th>Time to recurrence</th>
<th>Concurrent disease</th>
<th>Plasma exchange</th>
<th>Rituximab dose</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hristea⁷</td>
<td>LRD</td>
<td>Pred; Tac; MMF; Basiliximab</td>
<td>22 yr</td>
<td>2 days</td>
<td>None</td>
<td>Yes, partial response</td>
<td>375 mg/m² once weekly; 2 doses</td>
<td>Complete remission; PE and rituximab were used concomitantly</td>
</tr>
<tr>
<td>Gossmann⁷</td>
<td>Post-mortal</td>
<td>Pred; Tac; MMF; ATG</td>
<td>48 yr</td>
<td>40 days</td>
<td>None</td>
<td>Yes, no response</td>
<td>375 mg/m² once weekly; 6 doses</td>
<td>Complete remission after rituximab</td>
</tr>
<tr>
<td>Kama⁷</td>
<td>Post-mortal</td>
<td>Pred; CsA; MMF; Basiliximab</td>
<td>25 yr</td>
<td>1 day</td>
<td>None</td>
<td>Yes, preemptive, partial response</td>
<td>375 mg/m² once weekly; 2 doses</td>
<td>Complete remission; however, PE and rituximab were used concomitantly; A relapse was also treated with rituximab and PE; proteinuria had already decreased significantly before rituximab treatment</td>
</tr>
<tr>
<td>Nozu⁵</td>
<td>LRD</td>
<td>Pred; Tac; Miozolimine</td>
<td>12 yr</td>
<td>Immediately after transplantation</td>
<td>EBV related PTLD, 5 months after transplantation</td>
<td>No</td>
<td>375 mg/m² once weekly; 4 doses</td>
<td>Initially spontaneous decrease in proteinuria; Recurrence of proteinuria with PTLD, remission after rituximab</td>
</tr>
<tr>
<td>Pescovitz⁶</td>
<td>Post-mortal</td>
<td>Pred; Tac; MMF; Dacluzimab</td>
<td>7 yr</td>
<td>Immediately after transplantation</td>
<td>EBV related PTLD, 5 months after transplantation</td>
<td>Yes, no response</td>
<td>375 mg/m² once weekly; 6 doses</td>
<td>Remission after rituximab</td>
</tr>
<tr>
<td>Marks⁶</td>
<td>Post-mortal</td>
<td>Pred; Tac; Aza</td>
<td>6 yr</td>
<td>“early recurrence”*</td>
<td>CMV infection after PE</td>
<td>Yes, no response</td>
<td>375 mg/m² once weekly; 4 doses</td>
<td>No remission after rituximab</td>
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*exact time to recurrence not available; LRD=living related donor; Aza=azathioprine; MMF=mycophenolate mofetil; Pred=prednisone; Tac=tacrolimus; CMV=cytomegalovirus; EBV=Epstein Barr virus; PTLD=post transplant lymphoproliferative disease; PE=plasma exchange
been implicated as a source of the permeability factor.\textsuperscript{2,10,11} The efficacy of rituximab might suggest that recurrent FSGS is dependent on a B-cell derived antibody or cytokine. However, B-cells are important for T-cell activation, thus rituximab may exert its effect by indirectly affecting T-cell function.\textsuperscript{12,13} In our patient, the first remission was attained more than 5 months after treatment with rituximab. The time to remission is clearly longer compared to previous case reports in recurrent FSGS and minimal change disease, but similar to studies performed in patients with refractory SLE or ANCA associated vasculitis (AAV).\textsuperscript{4,5,7} In these patients, especially those with SLE, attainment of a remission can take up to 8 months after treatment with rituximab.\textsuperscript{14,15} The slower response has been attributed to different kinetics of tissue and peripheral blood B-cells.\textsuperscript{16} Studies involving a murine model for human CD20 expression demonstrated that depletion is slower for tissue B-cells than for peripheral blood B-cells.\textsuperscript{17} In humans with rheumatoid arthritis, treatment with rituximab also resulted in a variable degree of depletion of B-cells in bone marrow.\textsuperscript{18} Slow depletion of tissue B-cells may thus explain the slower response in our patient. Alternatively, the difference in response to rituximab may be associated with different types of plasma cells. It is known that some plasma cells have short life spans, whereas other plasma cells are able to live for extended periods of time continuously secreting antibodies.\textsuperscript{19,20} Rituximab only results in depletion of immature and mature B-cells, but not plasma cells. Thus, depending on the type of plasma cells that are involved (short- or long-lived), time to remission may differ. Our patient relapsed before the return of circulating B-cells. This has also been described in a few patients with SLE and AAV.\textsuperscript{14} This finding also underscores the possible role of tissue B-cells. Stimulation of T-cells by tissue B-cells that return before peripheral blood B-cells are measurable could explain the development of a relapse in the presence of undetectable CD19 positive B-cells.\textsuperscript{14} In conclusion, rituximab appears to offer a new treatment option for patients with recurrent FSGS after renal transplantation. Further studies are necessary to confirm our findings and to elucidate the pathogenetic role of B-cells.
Reference list

Chapter 10

Current guidelines on diagnosis and treatment of focal segmental glomerulosclerosis

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submitted

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Abstract
Focal segmental glomerulosclerosis (FSGS) is one of the most important causes of the nephrotic syndrome in adult patients. FSGS is not a disease entity. The identification of underlying causes of FSGS (secondary FSGS) has increased our insight in the pathogenesis of FSGS. Moreover, differentiating between primary (idiopathic) and secondary forms of FSGS is important to allow appropriate treatment. Recently a new pathological classification of FSGS was proposed, expanding FSGS to include non-sclerotic lesions. In this review we discuss the current diagnostic and therapeutic options in patients with FSGS.

Introduction
In 1957, Rich provided the first detailed pathologic description of focal segmental glomerulosclerosis (FSGS).\textsuperscript{1} It was not until the 1970’s that FSGS emerged as a separate clinico-pathologic entity.\textsuperscript{2} Currently, FSGS is one of the most common patterns of glomerular injury encountered in human renal biopsies.\textsuperscript{3} A recently proposed pathology classification has pointed to the existence of new, non-sclerotic forms of FSGS.\textsuperscript{4} FSGS is usually divided in idiopathic (primary) FSGS or secondary FSGS (table 1). This distinction is important because treatment differs depending on the underlying cause.\textsuperscript{5} For a long period of time idiopathic FSGS in adults has been considered to be prednisone resistant.\textsuperscript{6} The initial results of treatment with corticosteroids were extremely disappointing with complete remission rates below 15% in almost all studies performed before 1980.\textsuperscript{7-14} However over the last 25 years the results of treatment have improved, with complete remission rates exceeding 30-40%.\textsuperscript{15-27} As put forward by Korbet, the improvement in response appears to be associated with longer duration of treatment with corticosteroids.\textsuperscript{28} Based on the experience in children, the earlier studies in adults limited the use of corticosteroids to eight weeks. However, the response of adults to corticosteroids appears to be much slower than in children.\textsuperscript{15;23;29} In this review, we provide an update on the diagnosis and treatment of FSGS.
Table 1. Etiologic classification of FSGS

Idiopathic (primary) FSGS

Secondary FSGS

1. Familial
   A. Mutations in α-actinin 4
   B. Mutations in nephrin
   C. Mutations in podocin
   D. Mutations in WT-1
   E. Mutations in CD2-associated protein
   F. Mutations in TRPC 6
   G. Mitochondrial cytopathies

2. Virus associated
   A. HIV-associated nephropathy
   B. Parvovirus B19

3. Medication
   A. Heroin-nephropathy
   B. Interferon-α
   C. Lithium
   D. Pamidronate / Alendronate

4. Adaptive structural-functional responses likely mediated by glomerular hypertrophy or hyperfiltration
   4.1 Reduced renal mass
      A. Oligomeganephronia
      B. Unilateral renal agenesis
      C. renal dysplasia
      D. Cortical necrosis
      E. Reflux nephropathy
      F. Surgical renal ablation
      G. Chronic allograft nephropathy
      H. Any advanced renal disease with reduction in functioning nephrons
   4.2 Initially normal renal mass
      A. Diabetes mellitus
      B. Hypertension
      C. Obesity
      D. Cyanotic congenital heart disease
      E. Sickle cell anemia

5. Malignancy (lymphoma)

Nonspecific pattern of FSGS caused by renal scarring in glomerular disease

   A. Focal proliferative glomerulonephritis (IgA nephropathy, lupus nephritis, pauci-immune focal necrotizing and crescentic glomerulonephritis)
   B. Hereditary nephritis (Alport syndrome)
   C. Membranous glomerulopathy
   D. Thrombotic microangiopathy

Adapted from D’Agati et al.4
Diagnosis of idiopathic and secondary FSGS

Distinguishing among idiopathic and secondary forms is not merely semantic, but has important therapeutic implications as discussed below. Generally, the distinction can be made from the history (with special attention to secondary causes) and some additional laboratory (serum albumin) and radiologic studies (chest X-ray and kidney ultrasound).

In patients with nephrotic range proteinuria (≥ 3 g/day), serum albumin is an important clinical parameter to distinguish between FSGS secondary to hyperfiltration and other forms of FSGS. In a study of 37 patients with nephrotic range proteinuria due to biopsy proven FSGS, Praga et al. showed that serum albumin was significantly lower in patients with presumed idiopathic FSGS (serum albumin <30 g/L) compared to FSGS secondary to massive obesity, vesicoureteral reflux, or renal mass reduction (serum albumin > 35 g/L). In contrast to hyperfiltration-associated FSGS other forms of secondary FSGS such as viral-associated FSGS, drug-induced FSGS and familial FSGS may also present with a low serum albumin. Especially, the latter form cannot be differentiated from idiopathic FSGS on clinical grounds.

Although clinical data and serum albumin levels thus often allow to differentiate between idiopathic FSGS and FSGS secondary to hyperfiltration, sometimes discussion remains, especially in patients with a serum albumin between 30 g/L and 35 g/L. It has been suggested that these forms could be differentiated by evaluation of foot process effacement in electron microscopy, with mild effacement occurring in secondary FSGS and complete effacement in idiopathic FSGS. In a recent study of podocyte alterations we noted that the mean foot process width was significantly higher in patients with idiopathic FSGS compared to secondary FSGS mediated by adaptive structural-functional responses. The association between foot process width and type of FSGS was independent of proteinuria. Furthermore, the degree of overlap in foot process width between idiopathic and secondary FSGS was low, suggesting that quantitative assessment of foot processes may provide a means for distinguishing between idiopathic and secondary FSGS. Electron microscopy can also help in determining other underlying causes of FSGS. Special attention should be paid to the presence of tubulo-reticular inclusions in endothelial cells (HIV), viral particles, or the presence of abnormal mitochondria in the podocyte (mitochondrial DNA mutation).
A positive family history identifies patients with FSGS based on mutations in podocytic proteins.\textsuperscript{34-36} Obviously, it is important to consider if patients with FSGS need genetic testing to evaluate spontaneous mutations. In contrast to children, spontaneous mutations in adults with FSGS are rare, with a reported prevalence of 1.5-5%.\textsuperscript{37-39} He et al. screened 78 adult patients with non-familial FSGS (15 steroid-sensitive and 63 steroid-resistant) for known mutations in the podocin gene. Compound heterozygous mutations were detected in only one patient with steroid-sensitive FSGS, no homozygous mutations were found. These results are consistent with the findings of Caridi et al, who found only 3 heterozygous mutations in a cohort of 64 patients with steroid-resistant nephrotic syndrome.\textsuperscript{38} The study by He et al. also identified 8 patients with heterozygous R229Q, a podocin polymorphism. The allele frequency of this variant did not differ from normal controls. The significance of the R229Q variant as a disease causing mutation in FSGS is currently unknown.\textsuperscript{40} Similarly, mutations in the gene encoding \(\alpha\)-actinin-4 are also rare. Aucella et al. found no \(\alpha\)-actinin-4 gene mutations in 33 adults with non-familial FSGS.\textsuperscript{39} In view of the low incidence of spontaneous mutations, we do not recommend mutation screening in adult patients with non-familial FSGS.

**Prognosis**

Important clinical features predicting the clinical course of FSGS are the amount of proteinuria and the level of plasma creatinine. Half of the patients presenting with a proteinuria >3 g/day or a serum creatinine over 115 \(\mu\)mol/l progress to end-stage renal disease within 5-10 years.\textsuperscript{16,21,41} Renal survival is even worse if proteinuria exceeds 10 g/day, with ESRD occurring in majority of patients within 5 years after presentation. In contrast, non-nephrotic proteinuria portends a much better prognosis, with a renal survival of >80% after 10 years.\textsuperscript{30,42} Still, the single best predictor of a favorable outcome is attainment of a complete or partial remission of proteinuria. Less than 15% of patients attaining a remission progress to ESRD.\textsuperscript{15,27,43} Most reported remissions were induced by steroid therapy and it is generally suggested that spontaneous remissions occur infrequently in patients with FSGS. However, we have recently shown a high spontaneous remission rate of 60% in patients with idiopathic FSGS who present with a normal renal function. These patients were characterized
by a selective proteinuria (selectivity index <0.2) at presentation and by a serum albumin > 20 g/l and a proteinuria < 8 g/day at three months after renal biopsy.\textsuperscript{44}

Other authors have attempted to determine if prognosis in patients with FSGS can be predicted. Bazzi \textit{et al.} showed that a fractional excretion (FE) of IgG <0.14\%, was associated with a high remission rate after immunosuppressive therapy.\textsuperscript{45} In contrast, patients with a FE of IgG > 0.14\% had a dismal prognosis even with immunosuppressive therapy. Recently, we have also reported on the predictive value of FE IgG in 32 patients with idiopathic FSGS.\textsuperscript{46} Our data do not support the conclusion of Bazzi that FE IgG predicts resistance to immunosuppressive medication. Although a FE IgG >0.14\% was associated with worse baseline characteristics, remission rate was high (59\%) and not different from patients with FE IgG < 0.14\%. Therefore, until more data become available, FE IgG should not be used to guide treatment in patients with FSGS.

Approximately 50\% of patients who develop a remission after prednisone therapy will develop recurrent proteinuria during tapering of the prednisone (steroid dependent) or after stopping treatment (relapse). Patients who do not develop a remission after a minimum of 8-16 weeks of prednisone therapy are called steroid resistant. The latter definition is subject to debate (see below)

proteinuria (selectivity index <0.2). In addition these patients were characterized by a serum albumin > 20 g/l and a proteinuria < 8 g/day at three months after renal biopsy.\textsuperscript{44}

\section*{Treatment}

Obviously, in patients with secondary FSGS therapy should be directed at the underlying disorder or removal of the inciting drug. In patients with severe obesity, weight loss (>10\% of BMI) induces a significant decrease of proteinuria, which is almost similar to the effect of ACE\textsuperscript{i}. \textsuperscript{47} However, maintaining the weight loss is often difficult and many patients relapse.\textsuperscript{48} Small cohort studies suggest that antiretroviral therapy improves renal survival in patients with HIV-associated FSGS.\textsuperscript{49,50} Case reports also provide support for the use of antiretroviral therapy, with recovery of dialysis-dependent renal failure after initiation of antiretroviral therapy.\textsuperscript{51} FSGS associated with hematological conditions such as multiple myeloma and (non) Hodgkin lymphoma’s often responds with a resolution of the proteinuria after successful treatment of the underlying hematological condition.\textsuperscript{52,53} Familial forms of FSGS
are known to be steroid-resistant.\textsuperscript{35,54-56} A possible exception are patients with a non-familial (sporadic) heterozygous podocin mutation.\textsuperscript{57}

It is well known that blood pressure control and reduction of proteinuria significantly slows the progression of renal insufficiency in patients with proteinuric nondiabetic renal disease.\textsuperscript{58-62} Proteinuria should be reduced to $<0.5$ g/day. Target blood pressure are $\leq 130/80$ mmHg in patients with proteinuria $\leq 1$ g/day and $\leq 125/75$ mmHg in patients with proteinuria $>1$ g/day.\textsuperscript{60,63} ACE inhibitors (ACEi) or in case of side effects angiotensin receptor blockers (ARB), are preferred because they are more effective than other antihypertensive agents in slowing the progression of most nondiabetic kidney diseases.\textsuperscript{58,63,64} Despite the recognized beneficial effect of ACEi in patients with chronic kidney disease, little is known about the efficacy of ACEi in patients with idiopathic FSGS. Although ACEi reduce proteinuria in idiopathic FSGS, the few available studies suggest that ACEi rarely induce a complete remission and development of end-stage renal disease is not prevented.\textsuperscript{65,66} However, there is some evidence that ACEi slow the progression to ESRD.\textsuperscript{57} Furthermore, treatment with ACEi also improves the hypoalbuminemia and hyperlipidemia that is associated with idiopathic FSGS.\textsuperscript{68} Therefore treatment with ACEi is recommended for all patients with FSGS. The antiproteinuric effect of ACEi is most prominent in patients who are on a low sodium diet (50-100 mmol/day) or who are treated with diuretics, since relative volume depletion results in greater angiotensin II-dependence of the glomerular microcirculation. Other antihypertensive drugs should be added if the goals for blood pressure and proteinuria are not reached with ACEi, a low sodium diet and diuretics.

An abnormal lipid metabolism usually accompanies a nephrotic syndrome. Most prominent are an increased LDL-cholesterol, hypertriglyceridemia and an increased lipoprotein A (LP(a)).\textsuperscript{69,70} This combination is highly atherogenic.\textsuperscript{71,72} HMG CoA reductase inhibitors (statins) are very effective in lowering total and LDL cholesterol and to a lesser degree triglycerides and LP(a), also in patients with a nephrotic syndrome.\textsuperscript{73-77} Although a cardioprotective effect of statins has never been proven in patients with a nephrotic syndrome, prevention studies in the general population with similar lipid disorders have shown a marked reduction of cardiovascular diseases.\textsuperscript{78,79} Notably, recent studies have also suggested that the use of statins in patients with proteinuria attenuates the deterioration of renal function.\textsuperscript{80,81}
Immunosuppressive therapy should be considered in patients with idiopathic FSGS and a proteinuria $\geq 3$ g/day. Current recommendations for the initial treatment of idiopathic FSGS are almost entirely based on retrospective studies.\(^\text{15}\) Data from these studies show that corticosteroids remain the mainstay of treatment in idiopathic FSGS. Remission rates do not improve if cytotoxic agents are added to the initial treatment of FSGS with corticosteroids.\(^\text{23}\)

To attain a remission both the duration and dose of corticosteroid therapy are important factors. The median time until a remission is attained is 3-4 months, with the majority of patients entering a remission within 6 months.\(^\text{16,18-23,26}\) In addition, studies reporting higher remission rates are also characterized by a longer duration (2-4 months) of high dose treatment.\(^\text{21}\) FSGS is often considered to be steroid-resistant if no remission has occurred after 2-4 months of treatment with high dose prednisone.\(^\text{82}\) However, a significant number of patients do attain a remission four to six months after initiation of treatment with high dose prednisone. It is our experience that patients who eventually will develop a remission show some reduction in proteinuria within the first months of treatment. Therefore, one could argue that patients who respond to treatment with a significant reduction of proteinuria (>50%), should be treated for a total of six months before considering them resistant to corticosteroids.\(^\text{83,84}\)

Few studies have addressed the best therapeutic approach for relapsing or corticosteroid-dependent FSGS. The most important goal is to achieve a stable remission without the need for long-term prednisone. Both cyclophosphamide and cyclosporine appear to be beneficial, inducing a new remission in respectively 78% and 73% of patients.\(^\text{15}\) However, despite similar remission rates, a new relapse is more common after cessation of cyclosporine. In contrast, cyclophosphamide induces more stable remissions.\(^\text{85}\) Cyclophosphamide is usually given in a dose of 2 mg/kg/day for 2-3 months.

Several studies have evaluated the effect of cytotoxic agents and cyclosporine in patients with corticosteroid resistant FSGS. The definition of steroid-resistance was quite variable in these studies, ranging from 4-16 weeks of treatment with prednisone. Alkylating agents do not seem to benefit patients with steroid-resistant FSGS. Retrospective studies in adults report low remission rates for alkylating agents compared to cyclosporine (11% vs 40%).\(^\text{8,13,14,18,22,26,86-92}\) Two prospective trials been conducted in adults, comparing 6-12 months of treatment with cyclosporine to placebo.\(^\text{87,88}\) Remission rate was significantly
higher in patients treated with cyclosporine (60-69%) compared to placebo (4-33%). However, within one year after discontinuation of cyclosporine, 60-80% of the patients had relapsed.

The high relapse rate may decrease with prolonged use of cyclosporine. A study by Meyrier et al. suggests that continuing treatment with cyclosporine for one year in case of a remission followed by a slow tapering of the dose results in a more durable remission. However, this study included a low number of patients with FSGS with relatively mild FSGS lesions on the first renal biopsy. Therefore, the results should be interpreted with caution.

A major concern is the nephrotoxicity of cyclosporine. Continuous use for more than 12 months is associated with a significant increase in tubulointerstitial fibrosis. In most cases serum creatinine does not significantly change despite the aggravation of fibrosis. In addition, cyclosporine may accelerate the progression of FSGS. The number of glomeruli with sclerotic lesions increases significantly during treatment with cyclosporine, even in patients with a partial or complete remission. Cyclosporine nephrotoxicity is associated with higher doses of cyclosporine (> 5.5 mg/kg/day), a higher percentage of glomeruli with FSGS lesions and renal insufficiency prior to treatment. Therefore, cyclosporine dose should not exceed 5.5 mg/kg/day and treatment with cyclosporine should be limited to patients with a creatinine clearance > 60 ml/min/1.73 m²).

Uncontrolled studies have demonstrated an improvement in both renal function and proteinuria for patients with HIV associated FSGS treated with corticosteroids. The data from these studies are conflicting regarding increased risk for serious infections and hospitalization. Nevertheless, a recent guideline for the management of chronic kidney disease in HIV infected patients advised to consider prednisone therapy at 1 mg/kg/day (maximum dose 80 mg/day) for 2 months, followed by 2-4 months taper in patients with HIV associated FSGS whose kidney function deteriorates despite use of antiretroviral therapy. Before considering corticosteroids active infection and active illicit intravenous drug use should be excluded. In patients with obesity, treatment with corticosteroids can even be detrimental, because of a further increase in body weight.
Chapter 10

A new pathological classification of FSGS

Recently D’Agati et al. proposed a consensus classification of FSGS based entirely on light microscopic examination of the renal biopsy. This schema presumes previous exclusion of FSGS conditions caused by glomerular scarring in the course of other primary glomerular diseases. Five light microscopic variants are defined, including FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant, and collapsing variant (table 2).

Clinical presentation and sociodemographic findings differ between the FSGS variants (table 3). Collapsing FSGS has a predilection for Afro-Americans and typically presents with a severe nephrotic syndrome and substantial renal insufficiency. The tip variant has a low proportion of Afro-Americans. The majority of these patients also presents with a severe nephrotic syndrome (>90%). Renal function is usually preserved in patients with the tip variant. Perihilar FSGS has the lowest frequency of nephrotic syndrome.

Table 2. Morphological variants of FSGS

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Characteristics</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS variant</td>
<td>At least one glomerulus with segmental increase in matrix obliterating the capillary lumen. There may be segmental glomerular basement membrane collapse without overlying podocyte hyperplasia</td>
<td>Exclude perihilar, cellular, tip and collapsing variant</td>
</tr>
<tr>
<td>Perihilar variant</td>
<td>At least one glomerulus with perihilar hyalinosis, with or without sclerosis &gt; 50% of glomeruli with segmental lesions must have perihilar sclerosis and/or hyalinosis</td>
<td>Exclude cellular, tip and collapsing variant</td>
</tr>
<tr>
<td>Cellular variant</td>
<td>At least one glomerulus with segmental endocapillary hypercellularity occluding lumina, with or without foam cells and karyorrhexis</td>
<td>Exclude tip and collapsing variant</td>
</tr>
<tr>
<td>Tip variant</td>
<td>At least one segmental lesion involving the tip domain (outer 25% of tuft next to origin of proximal tubule) The tubular pole must be identified in the defining lesion The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck The tip lesion may be cellular or sclerosing</td>
<td>Exclude collapsing variant</td>
</tr>
<tr>
<td>Collapsing variant</td>
<td>At least one glomerulus with segmental or global collapse and overlying podocyte hyperplasia</td>
<td>None</td>
</tr>
</tbody>
</table>

Adapted from D’Agati et al.
Patients with FSGS NOS tend to have clinical parameters intermediate between the tip variant and perihilar FSGS, whereas cellular FSGS had clinical parameters intermediate between the tip variant and collapsing FSGS.

Several retrospective studies have reported on the prognostic utility of the Columbia classification. A recent study by Chun et al. was unable to detect a significant difference in remission rate among patients with collapsing FSGS, FSGS NOS and the tip variant. However, the statistical power of this study to detect a clinically significant difference was low due to the small number of patient with the tip variant. In contrast, two other studies reported a lower remission rate and worse renal survival among patients with the collapsing variant compared to the tip variant and FSGS NOS. Patients with the cellular variant had remission rates between those of patients with the tip variant and collapsing FSGS. A study by our group showed a significant better renal survival in patients with the tip variant compared to FSGS NOS and perihilar FSGS. These studies suggest that the tip variant has a higher remission rate and a better renal survival compared to other FSGS variants. At present classification of FSGS should not influence therapeutic decisions.

### Table 3. Clinical characteristics of FSGS variants as reported in two North American and one West-European study

<table>
<thead>
<tr>
<th></th>
<th>NOS (n=200)</th>
<th>Tip (n=128)</th>
<th>Perihilar (n=76)</th>
<th>Collapsing (n=83)</th>
<th>Cellular (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>55%</td>
<td>57%</td>
<td>55%</td>
<td>47%</td>
<td>61%</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>43 ± 5</td>
<td>48 ± 7</td>
<td>50 ± 6</td>
<td>35 ± 4</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>Afro-American</td>
<td>30%</td>
<td>11%</td>
<td>20%</td>
<td>60%</td>
<td>32%</td>
</tr>
<tr>
<td>Serum creatinine concentration (μmol/l)</td>
<td>177 ± 91</td>
<td>129 ± 46</td>
<td>166 ± 113</td>
<td>317 ± 133</td>
<td>179 ± 53</td>
</tr>
<tr>
<td>Renal insufficiency*</td>
<td>49%</td>
<td>35%</td>
<td>38%</td>
<td>75%</td>
<td>57%</td>
</tr>
<tr>
<td>Serum albumin concentration (g/L)</td>
<td>31 ± 5</td>
<td>22 ± 5</td>
<td>37 ± 6</td>
<td>22 ± 5</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>Proteinuria (g/24 hours)</td>
<td>5.5 ± 2.9</td>
<td>8.9 ± 3.7</td>
<td>4.7 ± 3.1</td>
<td>9.2 ± 2.7</td>
<td>10.9 ± 4.2</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>7.3 ± 2.0</td>
<td>8.2 ± 2.1</td>
<td>6.7 ± 1.8</td>
<td>7.7 ± 1.6</td>
<td>6.3 ± 2</td>
</tr>
<tr>
<td>Nephrotic syndrome (%)</td>
<td>60%</td>
<td>94%</td>
<td>46%</td>
<td>85%</td>
<td>84%</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>108 ± 14</td>
<td>111 ± 14</td>
<td>107 ± 13</td>
<td>106 ± 14</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>69%</td>
<td>62%</td>
<td>80%</td>
<td>68%</td>
<td>70%</td>
</tr>
</tbody>
</table>

* The definition of renal insufficiency varied between studies, either a serum creatinine >106 μmol/l or > 135μmol/l
Chapter 10

Guidelines for diagnosis and treatment of FSGS

The Dutch Federation of Nephrology recently published guidelines for the diagnosis and treatment of patients with FSGS. An outline of the most important diagnostic steps is given in figure 1. Medical history and family history are important to rule out secondary causes of FSGS. We do not advocate routine use of mutational screening. In patients with nephrotic range proteinuria (≥ 3 g/day), serum albumin is an important clinical parameter to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses. If serum albumin is inconclusive, electron microscopy examination can also help to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses. Although for comparative studies the use of the Columbia classification is advised, this should not influence treatment decisions.

Immunosuppressive therapy should be limited to patients with idiopathic FSGS. Since spontaneous remissions occur frequently in patients with idiopathic FSGS, a normal renal function and a selective proteinuria, a wait and see approach should be considered in such patients (figure 1). Otherwise, patients with idiopathic FSGS and a nephrotic syndrome should initially be treated with high-dose prednisone for 4-6 months (table 4). To induce a remission, the initial immunosuppressive therapy should consist of high dose prednisone (1 mg/kg/day, up to 80 mg/day) for 4 months. In the elderly (>65 years) an alternate day regimen (2 mg/kg/day) is also effective with less complications.20

In patients with steroid-dependent or frequently relapsing FSGS, cyclophosphamide 2 mg/kg/day for 2-3 months in combination with prednisone results in more stable remissions. In steroid resistant FSGS, the most effective treatment consists of cyclosporine (target trough levels 125–225 ug/l) for six months. Treatment should be limited to patients with a relatively well-preserved renal function, to prevent nephrotoxicity. In case of a remission cyclosporine treatment should be continued for one year and then slowly tapered off to prevent a relapse. In the absence of a remission cyclosporine should be stopped after six months.
Figure 1. Flowchart of diagnosis and treatment of FSGS

* Patients fulfilling these criteria are eligible for immunosuppressive therapy, although the effect of symptomatic therapy can be awaited for a short period of time.
Table 4. Immunosuppressive therapy in adult patients with primary FSGS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosing scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial treatment</strong></td>
<td></td>
</tr>
<tr>
<td>prednisone*</td>
<td>1 mg/kg/day in patients &lt; 65 yr (up to 80 mg/day) or alternate day</td>
</tr>
<tr>
<td></td>
<td>prednisone 2 mg/kg (up to 150 mg) in the elderly (&gt; 65 years) for 4 months; if a remission is not attained but proteinuria decreases &gt; 50%, continue treatment for a maximum of 6 months</td>
</tr>
<tr>
<td></td>
<td>in case of a complete remission taper prednisone: reduce dose with 10 mg per 2 weeks up to 0.15 mg/kg/day, next taper dose every 2-4 weeks with 2.5 mg</td>
</tr>
<tr>
<td></td>
<td>In steroid-resistant patients taper prednisone over 6 weeks</td>
</tr>
<tr>
<td>cyclophosphamide†‡</td>
<td>2 mg/kg/day in patients &lt; 65 years or 1.5 mg/kg/day in the elderly (&gt; 65 years) for 3 months (round off to 25 mg), in case of young patients who wish to have children limit treatment to 2 months because of fertility risks</td>
</tr>
<tr>
<td>prednisone*</td>
<td>1 mg/kg/day in patients &lt; 65 years (up to 80 mg/day) or alternate day</td>
</tr>
<tr>
<td></td>
<td>prednisone 2 mg/kg (up to 150 mg) in the elderly (&gt; 65 years) for 3 months; in case of a complete remission taper prednisone after 3 months: reduce dose with 10 mg per 2 weeks up to 0.15 mg/kg/day, next taper dose every 2-4 weeks with 2.5 mg</td>
</tr>
<tr>
<td></td>
<td>In steroid-resistant patients taper prednisone over 6 weeks</td>
</tr>
<tr>
<td><strong>Therapy for early relapsing or steroid dependent FSGS</strong></td>
<td></td>
</tr>
<tr>
<td>cyclophosphamide‡</td>
<td>2 mg/kg/day in patients &lt; 65 years or 1.5 mg/kg/day in the elderly (&gt; 65 years) for 3 months (round off to 25 mg), in case of young patients who wish to have children limit treatment to 2 months because of fertility risks</td>
</tr>
<tr>
<td>prednisone*</td>
<td>1 mg/kg/day in patients &lt; 65 years (up to 80 mg/day) or alternate day</td>
</tr>
<tr>
<td></td>
<td>prednisone 2 mg/kg (up to 150 mg) in the elderly (&gt; 65 years) for 3 months; in case of a complete remission taper prednisone after 3 months: reduce dose with 10 mg per 2 weeks up to 0.15 mg/kg/day, next taper dose every 2-4 weeks with 2.5 mg</td>
</tr>
<tr>
<td></td>
<td>In steroid-resistant patients taper prednisone over 6 weeks</td>
</tr>
<tr>
<td><strong>Therapy for late relapse of FSGS</strong></td>
<td></td>
</tr>
<tr>
<td>These patients should receive another course of high dose prednisone as described under initial treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Therapy for steroid-resistant FSGS</strong></td>
<td></td>
</tr>
<tr>
<td>cyclosporine§**</td>
<td>3-5 mg/kg/day: in two divided doses</td>
</tr>
<tr>
<td></td>
<td>in case of a remission continue treatment for 1 year then try to slowly taper</td>
</tr>
<tr>
<td></td>
<td>cyclosporine: reduce cyclosporine dose with 25% every 2 months</td>
</tr>
<tr>
<td></td>
<td>If no remission by 6 months, discontinue cyclosporine treatment &amp;</td>
</tr>
<tr>
<td>prednisone*</td>
<td>0.15 mg/kg/day for 4-6 months then taper off over 4-8 weeks</td>
</tr>
</tbody>
</table>

* prevention of peptic ulcers: histamine H₂-receptor antagonist or proton pump inhibitor
† consider pneumocystis jiroveci pneumonia prophylaxis with cotrimoxazole 1 dd 480 mg
‡ dose adjustment for cyclophosphamide: Leucocytes < 4,0*10⁹/l or platelets < 100*10⁹/l: dose should be halved; Leucocytes < 3,0*10⁹/l or platelets < 75*10⁹/l: discontinue cyclophosphamide. If leucocytes return to normal, restart cyclophosphamide at a dose 50 mg lower than the previous dose
§ dose adjustment cyclosporine: reduce dose by 25-50% if there is: >30% increase in serum creatinine, over the basal values; an increase in serum potassium level to 6 mEq/liter or more; a doubling of the levels of serum transaminases, alkaline phosphatase, bilirubin and/or γGT; an arterial hypertension (>145/90 mmHg) refractory to treatment; if the reduction of the dose did not normalize the abnormal parameters within two weeks, further reduce CsA by 25 to 50%, if abnormalities still persist after two weeks discontinue CsA.
** treatment with cyclosporine should be limited to patients with a creatinine clearance > 60 ml/min

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Guidelines on diagnosis and treatment of FSGS

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Chapter 11

Summary
Focal segmental glomerulosclerosis (FSGS) is an important cause of the nephrotic syndrome in adults. FSGS can be idiopathic (primary) or secondary to a still increasing number of causes. New underlying causes of FSGS have been identified and improved our understanding of the pathogenesis of FSGS. Nevertheless, FSGS remains a puzzling disease, with significant differences in prognosis and response to therapy, even in patients who seem to have a similar form of the disease.

Initially, most authors emphasized the poor outcome in patients with FSGS, characterized by unresponsiveness to therapy and progression to end-stage renal disease (ESRD). However, more recent studies show a better response to immunosuppressive therapy and nowadays most authors have become more optimistic about the efficacy of immunosuppressive medication in idiopathic FSGS. Whether all patients with idiopathic FSGS need immunosuppressive therapy is unclear. Most textbooks still report a low spontaneous remission rate of 5% or less. However, literature data must be interpreted with caution since many studies have included patients with normo-albuminemia, evidence of secondary hyperfiltration-associated FSGS. Furthermore, many studies have included patients with severe renal insufficiency at presentation. Thus, the spontaneous remission rate is probably underestimated.

Therefore, in chapter 2 we have studied the remission rate in 20 initially untreated patients with recently diagnosed idiopathic FSGS and a relatively normal renal function (serum creatinine < 135 μmol/l). These patients were defined by strict criteria to ensure only inclusion of patients with idiopathic FSGS. In this patient group, we observed a high spontaneous remission rate of 60%. In addition, we tried to define prognostic parameters to predict renal outcome. Predictors of renal function deterioration were a nonselective proteinuria (selectivity index >0.2) at renal biopsy and decreased concentrations of serum albumin at three months after renal biopsy. A higher percentage of sclerosis, synechia or hyalinosis on renal biopsy also predicted a worse renal prognosis.

In chapter 3, we assessed the value of urinary parameters in predicting remission rate, renal survival and response to treatment in idiopathic FSGS. In 32 adult patients the following urinary parameters were evaluated: 24-hour proteinuria, fractional excretion (FE) of albumin, IgG, transferrin and β2-microglobulin. A previous study suggested that FE IgG predicts remission rate, progression to ESRD and response to therapy. A cut-off value of 0.14%
proved most discriminative, in fact a FE IgG > 0.14% predicted complete unresponsiveness to immunosuppressive medication. Therefore, we specifically evaluated the usefulness of a cutoff value of 0.14% for FE IgG. In our study remission rate and renal survival of patients with a FE IgG > 0.14% were not different from patients with a FE IgG ≤ 0.14%. The predictive value of FE of albumin, transferrin and β2-m was also low. In untreated patients a FE β2-m < 1% predicted a better renal survival. However, the number of untreated patients was low, and these results should be confirmed in a larger study. Nevertheless, our data show that a FE IgG > 0.14% is not invariably associated with a poor outcome in patients with idiopathic FSGS. Therefore a high FE IgG should not lead to therapeutic nihilism.

A working group of renal pathologists has recently proposed a standardized pathological classification system for FSGS based entirely on light microscopic examination. The prognostic significance of this classification is not clear. In chapter 4 we present the data of a retrospective analysis in 93 adult patients with biopsy-proven FSGS to determine the clinical features and outcome of FSGS variants. The majority of the patients were native Dutch. The frequency of FSGS variants was: 32% NOS, 37% tip, 26% perihilar and 5% collapsing. The cellular variant was not present in our population, this is not surprising given the low prevalence of this variant (3-4.5%). Patients with the tip variant presented more often with a nephrotic syndrome, a better renal function and less histological damage on the renal biopsy. Five-year renal survival was significantly better in patients with the tip variant compared to FSGS NOS and perihilar FSGS (respectively 78%, 63% and 55%). Type of FSGS was an independent predictor of renal survival. Remission rate was also higher in patients with the tip variant, however, the difference with FSGS NOS and perihilar FSGS did not reach the level of statistical significance.

FSGS may also be used as a prognostic marker in other glomerular diseases. Several studies suggested that the presence of FSGS in the renal biopsy of patients with idiopathic membranous nephropathy predicts a worse renal outcome. In chapter 5 we compared renal outcome and remission rate in patients with idiopathic membranous nephropathy with (iMN+FSGS;n=22) and without FSGS lesions (iMN;n=31). There was no difference in renal function deterioration between patients with and without FSGS lesions. Only serum creatinine, but not FSGS was an independent predictor of renal function deterioration or
progression to ESRD. At the end of follow-up a higher proportion of patients with iMN had a complete or partial remission. The difference with patients with iMN+FSGS was not statistically significant. There was no correlation between the extent of FSGS lesions and urinary β2-microglobulin excretion. A meta-analysis of 6 studies analyzing the prognostic significance of FSGS showed a significantly higher remission rate for patients with iMN alone compared to patients with iMN+FSGS. However, these results should be interpreted with some caution, because of the considerable heterogeneity between studies. In comparison to other prognostic factors, such as β 2-microglobulin, histological scoring of FSGS is inferior.

Podocyte foot process effacement is a characteristic feature of proteinuric renal diseases. However, the degree of foot process effacement differs depending on the underlying disease. In minimal change nephrotic syndrome (MCNS) foot processes are diffusely effaced, whereas the degree of foot process effacement appears to vary between idiopathic and FSGS and FSGS secondary to maladaptive responses. In contrast to MCNS, a quantitative assessment of foot processes has never been performed in patients with FSGS. We have assessed the degree of foot process effacement, estimated by foot process width (FPW), in 27 patients with FSGS (chapter 6). The results were compared to foot process width in MCNS and normal kidneys. Based on predefined criteria, a clinical diagnosis of idiopathic FSGS and FSGS secondary to maladaptive responses was made in respectively 13 and 12 patients. Two patients could not be classified based on clinical criteria. Foot process effacement was most severe in idiopathic FSGS, intermediate in MCNS. Foot processes were relatively preserved in FSGS secondary to maladaptive responses. On multivariate analysis, type of disease was the most important determinant of FPW. Our result also showed that FPW could be used to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses.

FSGS can recur after renal transplantation. Depending on the underlying cause, recurrence rate can vary from 0% in patients with secondary FSGS to 60% in patients with idiopathic FSGS. Without treatment these patients will progress to ESRD. In a previous retrospective study, we published the short-term effect of plasma exchange (PE) treatment in patients with recurrent FSGS. However, long-term results of PE are less well known. In chapter 7 we
describe the extended follow-up (3.4 years) after PE in 13 patients with recurrent FSGS after renal transplantation. Ten patients, not treated with plasma exchange, served as historical controls. Baseline characteristics were similar. Out of 13 patients treated with PE, 11 patients (85%) attained a remission. Two patients (15%) lost their graft within the first month after transplantation. Four patients needed prolonged treatment with PE, because of a relapse. After a median of 58 PE sessions these patients attained a sustained remission. These patients were characterized by a late recurrence (> 30 days) after transplantation. In the untreated patients, proteinuria persisted and eventually graft failure occurred in all ten patients. Graft survival at 5-years after transplantation was significantly better for treated patients (85% vs 30%).

Plasma exchange (PE) is an effective treatment for recurrent FSGS after renal transplantation. However, after cessation of PE proteinuria can recur. Often these patients need continuous treatment with PE to maintain a remission. The role of immunosuppressive therapy in preventing or treating recurrent FSGS is unclear. In chapter 8 we describe a 42-year old female patient with PE dependent FSGS after renal transplantation. Proteinuria was first discovered 6 weeks after renal transplantation. Plasma exchange at weekly intervals was necessary to maintain a stable remission. Proteinuria recurred the moment PE frequency was reduced. Because of severe osteoporosis, prednisone was replaced by other immunosuppressive medication. However, severe side effects necessitated reintroduction of prednisone. After cessation of PE, proteinuria did not recur. A second attempt to discontinue prednisone, again resulted in a relapse. Proteinuria disappeared after treatment with prednisone. These observations suggest that prednisone therapy may be essential in maintaining a sustained remission of proteinuria in patients with PE dependent recurrent FSGS after renal transplantation.

Recently, successful use of rituximab, a chimeric monoclonal anti-CD20 antibody, was reported in a child with recurrent FSGS after renal transplantation. This drug may be an alternative therapy for patients with recurrent FSGS, especially in case of plasma exchange (PE) dependency. In chapter 9 we describe successful treatment with rituximab in a 24-year-old female patient with recurrent FSGS after a second renal transplantation. Although treatment with PE induced a remission of proteinuria, repeated trials of cessation failed.
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Because the patient remained PE dependent for more than 15 months, she was treated with 4 weekly infusions of rituximab. After rituximab treatment, PE could be discontinued. Currently the patient is in partial remission.

In chapter 10, we propose a diagnostic and therapeutic strategy in patients with FSGS based on the studies in this thesis and a review of the literature.
Chapter 12

Nederlandse samenvatting
Focale segmentale glomerulosclerose (FSGS) is een belangrijke oorzaak van het nefrotisch syndroom bij volwassenen. Op basis van een etiologische classificatie wordt FSGS onderverdeeld in een idiopathische (primaire) vorm en een groot aantal secundaire vormen. Onze kennis over de pathogenese van FSGS is sterk toegenomen door de identificatie van nieuwe secundaire vormen. Desondanks blijft FSGS een complex ziektebeeld, met grote verschillen in prognose en reactie op behandeling, zelfs bij patiënten die eenzelfde vorm van FSGS hebben.

De eerste ervaringen met het gebruik van afweeronderdrukkende medicijnen bij patiënten met FSGS waren teleurstellend. Mede hierdoor werd lang aangenomen dat patiënten met FSGS een slechte prognose hebben met een grote kans op het ontwikkelen van terminaal nierfalen. Recentere studies laten echter veel betere resultaten zien van behandeling met afweeronderdrukkende medicijnen bij patiënten met idiopathische FSGS. Dit heeft ertoe geleid dat tegenwoordig de meeste patiënten met idiopathische FSGS behandeld worden met afweeronderdrukkende medicijnen. Het is echter de vraag of alle patiënten met idiopathische FSGS zo behandeld moeten worden. De meeste leerboeken geven aan dat slechts bij 5% van de patiënten met idiopathische FSGS, de aandoening spontaan verdwijnt (spontane remissie).

De gegevens waarop deze aanname is gebaseerd moeten echter met enige voorzichtigheid worden geïnterpreteerd, aangezien veel studies patiënten includeerden met een normaal serum albumine, hetgeen kenmerkend is voor secundaire FSGS, een vorm die niet reageert op afweeronderdrukken medicijnen. Daarnaast includeerden veel studies patiënten met een gestoorde nierfunctie. Hierdoor wordt de kans op een spontane remissie waarschijnlijk veel lager geschat dan dat deze daadwerkelijk is.

In hoofdstuk 2 hebben wij derhalve het remissiepercentage geanalyseerd van 20 initieel onbehandelde patiënten met idiopathische FSGS en een relatief normale nierfunctie (serum creatinine <135 μmol/l). Van deze patiënten ontwikkelde de meerderheid (60%) een spontane remissie. Aansluitend hebben wij gekeken naar voorspellende factoren voor progressief nierfalen. Verslechtering van de nierfunctie werd voorspeld door een niet-selectieve proteinurie (selectiviteitsindex > 0,2) ten tijde van de nierbiopsie, een serum albumine >20 g/l op 3 maanden na de nierbiopsie en een hoog percentage sclerose, synechien of hyalinose in het biopt.
In hoofdstuk 3 hebben wij bij 32 patiënten met idiopatische FSGS onderzocht wat de waarde is van het meten van de uitscheiding van eiwitten in de urine voor het voorspellen van een remissie, behoud van nierfunctie en de respons op behandeling. Bij 32 volwassen patiënten zijn de volgende eiwitten gemeten in de urine: totale hoeveelheid eiwit per 24 uur, fractionele uitscheiding (FE) van albumine, IgG, transferrine en β2-microglobuline (β2m). In een eerdere studie werd gesuggereerd dat FE IgG kan worden gebruikt als voorspeller van remissie, progressief nierfalen en respons op behandeling. Een drempelwaarde van 0.14% leek de hoogste sensitiviteit en specificiteit te hebben. Derhalve hebben wij in onze studie specifiek de betrouwbaarheid van een drempelwaarde van 0.14% onderzocht. Wij vonden echter geen verschil in remissiepercentage en progressie naar nierfalen tussen patiënten met een FE IgG meer en minder dan 0.14%. De voorspellende waarde van FE albumine, transferrine en β2m waren ook laag. Bij onbehandelde patiënten bleek een FE β2m < 1% een betere renale overleving te voorspellen. Gezien het kleine aantal onbehandelde patiënten in onze studie, is het wel noodzakelijk om deze resultaten te bevestigen in een grotere studie. Desalniettemin toont onze studie aan dat een FE IgG > 0.14% bij patiënten met een idiopatische vorm van FSGS niet altijd gepaard gaat met een slechte prognose. Immunosupressieve behandeling moet derhalve niet onthouden worden aan patiënten met idiopatische FSGS en een FE IgG > 0.14%.

Recent is tijdens een internationale consensus bijeenkomst een nieuwe histologische classificatie van FSGS ontwikkeld, gebaseerd op lichtmicroscopisch onderzoek. De klinische relevantie van deze classificatie is nog onduidelijk. In hoofdstuk 4 presenteren wij de gegevens van een retrospectieve studie bij 93 volwassen patiënten met FSGS, gericht op de klinische kenmerken en de prognose van de verschillende histologische varianten. De meerderheid van de patiënten in onze studie was van Nederlandse afkomst. FSGS NOS werd bij 32% van de patiënten gediagnosticeerd, de tip variant bij 37%, perihilaire FSGS bij 26% en de collaborerende variant bij 5% van de patiënten. The cellulaire variant werd niet aangetroffen in onze populatie. Dit is niet onverwacht, gezien de lage prevalentie van deze variant (3-4,5%). Patiënten met de tip variant presenteren zich vaker met een nefrotisch syndroom, een betere nierfunctie en minder histologische afwijkingen in het nierbiopt. De 5-jaars overleving was significant beter bij patiënten met de tip variant in vergelijking met patiënten met FSGS NOS en perihilaire FSGS (respectievelijk 78%, 63% en 55%). Het type
FSGS was een onafhankelijk voorspellende factor voor de ontwikkeling van nierinsufficiëntie. Patiënten met de tip variant hadden ook een hoger remissie percentage, hoewel het verschil met patiënten met FSGS NOS en perihilaire FSGS niet statistisch significant was.

De aanwezigheid van FSGS laesies is mogelijk ook van voorspellende waarde bij andere glomerulaire nierziekten. In een aantal studies bij patiënten met idiopathische membraanze glomerulopathie (iMN) werd gesuggereerd dat de aanwezigheid van FSGS in het nierbiopt gepaard ging met een grotere kans op progressief nierfunctieverlies. In hoofdstuk 5 vergeleken wij patiënten met iMN en FSGS laesies in het nierbiopt (iMN+FSGS;n=22) met patiënten zonder FSGS laesies (iMN;n=31) in het nierbiopt. Hierbij is specifiek gekeken naar progressief nierfunctieverlies en remissiepercentage. Het aantal patiënten met progressief nierfalen verschilde niet tussen patiënten met en zonder FSGS laesies. Alleen de serumkreatinine concentratie, maar niet de aanwezigheid van FSGS laesies, was een onafhankelijk voorspellende factor voor het ontstaan van progressief nierfunctieverlies. Aan het eind van de follow-up hadden meer patiënten met iMN een remissie bereikt, het verschil met patiënten met iMN+FSGS was echter niet statistisch significant. Er kon geen verband worden aangetoond tussen de aanwezigheid van FSGS laesies en de uitscheiding van β2-microglobuline in de urine. Een meta-analyse van onze studie en 5 soortgelijke studies bij iMN toonde een significant hoger remissiepercentage bij patiënten met iMN in vergelijking met patiënten met iMN+FSGS. Bij de interpretatie van deze resultaten moet wel rekening worden gehouden met de grote mate van heterogeniteit tussen de verschillende studies. De voorspellende waarde van FSGS laesies in het nierbiopt bij patiënten met iMN is minder goed in vergelijking met andere voorspellende parameters zoals β2-microglobuline.

Versmelting van de voeten van podocyten is kenmerkend voor nierziekten die gepaard gaan met eiwitverlies in de urine. De mate van voetjesversmelting is echter afhankelijk van de onderliggende aandoening. Bij minimal change nephrotic syndrome (MCNS) zijn de voetjes totaal versmolten, terwijl de mate van voetjesversmelting sterk wisselt bij idiopathische FSGS en FSGS secundair aan overbelasting van nefronen. In tegenstelling tot MCNS, is de mate van voetjesversmelting bij FSGS nooit door middel van een kwantitatieve meting geanalyseerd. Wij hebben bij 27 patiënten met FSGS de mate van voetjesversmelting
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bepaald door middel van meting van de breedte van de podocytenvoetjes (PVB) (hoofdstuk 6). De resultaten werden vergeleken met de PVB bij MCNS en normale nieren. Met behulp van vooraf opgestelde klinische criteria werd bij de patiënten met FSGS onderscheid gemaakt tussen idiopathische FSGS (n=13) en FSGS secundair aan overbelasting van nefronen (n=12). Bij 2 patiënten was het niet mogelijk om op grond van klinische criteria onderscheid te maken tussen idiopathische en secundaire FSGS. De mate van voetjesversmelting was het meest prominent bij patiënten met idiopathische FSGS en milder bij patiënten met MCNS. De voetjes waren redelijk normaal bij patiënten met FSGS secundair aan overbelasting van nefronen. De onderliggende aandoening bleek bij multivariate analyse de meest belangrijke voorspeller van de breedte van de podocytenvoetjes. Daarnaast bleek dat de breedte van podocytenvoetjes ook goed gebruikt kan worden om onderscheid te maken tussen patiënten met idiopathische FSGS en FSGS secundair aan overbelasting van nefronen.

FSGS kan na transplantatie terugkomen in de transplantaatnier. Afhankelijk van de onderliggende oorzaak, varieert de recidiefkans van 0% bij patiënten met secundaire FSGS tot 60% bij patiënten met idiopathische FSGS. Zonder adequate behandeling ontwikkelen deze patiënten uiteindelijk terminaal nierfalen. In een eerdere retrospectieve studie bij patiënten met een recidief FSGS na niertransplantatie, hebben wij de korte termijn resultaten laten zien na behandeling met plasmaferese. De resultaten van plasmaferese op de langere termijn zijn echter onduidelijk. In hoofdstuk 7 beschrijven wij een langdurige follow-up (3,4 jaar) na behandeling met plasmaferese bij 13 patiënten met een recidief van FSGS in de transplantaatnier. De gegevens van deze patiënten werden vergeleken met een historische controlegroep van 10 patiënten met een recidief FSGS, die nooit zijn behandeld met plasmaferese. De uitgangskennmerken van de beide groepen waren niet verschillend. Van de 13 met plasmaferese behandelde patiënten bereikten elf patiënten (85%) een remissie van de proteïnurie. Bij twee patiënten leidde het recidief tot volledig transplantaatfalen. Vanwege een recidief na de eerste plasmaferese behandeling, was bij vier patiënten langdurige behandeling met plasmaferese noodzakelijk. Na een mediane behandeling met 58 plasmafereseessenties, bereikten deze patiënten uiteindelijk ook een stabiele remissie. Deze vier patiënten werden gekenmerkt door een laat optredend recidief (> 30 dagen na de niertransplantatie). In de onbehandelde historische controlegroep persisteerde de proteïnurie...
en uiteindelijk ging de functie van het transplantaat bij alle patiënten verloren. De 5-jaars overleving van de transplantaatnier was significant beter bij patiënten behandeld met plasmaferese (85% vs 30%).

Plasmaferese is een effectieve behandeling bij een recidief van FSGS in de transplantaatnier. Echter na staken van de plasmaferese kan een nieuw recidief optreden. Bij deze patiënten is vaak langdurige behandeling met plasmaferese noodzakelijk om een remissie te handhaven. De rol van de immunosuppressieve behandeling in het voorkomen danwel behandelen van het recidief is onduidelijk. In hoofdstuk 8 beschrijven wij een 42 jarige patiënt met plasmaferese-afhankelijke FSGS na niertransplantatie. Het recidief ontstond 6 weken na de niertransplantatie. Wekelijkse behandeling met plasmaferese was noodzakelijk om een stabiele remissie te handhaven. De protéinurie recidiveerde zodra de plasmaferese frequentie werd verlaagd. Vanwege ernstige osteoporose werd de prednison vervangen door andere immunosuppressieve medicatie. Deze medicatie veroorzaakte echter ernstige bijwerkingen, waardoor opnieuw gestart moest worden met prednison. Vervolgens bleek de protéinurie niet terug te komen na staken van de plasmaferese behandeling. Een tweede poging om de prednison te stoppen leidde opnieuw tot een recidief van de protéinurie. Na starten met prednison verdween de protéinurie weer. Deze observaties suggereren dat bij patiënten met een recidief FSGS na niertransplantatie behandeling met prednison essentieel is om een remissie te handhaven.

Recent is bij een kind met een recidief FSGS na niertransplantatie, succesvolle behandeling gerapporteerd met rituximab, een chimerisch monoclonaal antilichaam gericht tegen het CD20 antigeen op B-lymfocyten. Rituximab kan mogelijk als alternatieve behandeling dienen bij patiënten met een recidief FSGS, met name in het geval van plasmaferese afhankelijkheid. In hoofdstuk 9 beschrijven wij succesvolle behandeling met rituximab bij een 24 jarige patiënt met een recidief FSGS na een tweede niertransplantatie. Hoewel behandeling met plasmaferese resulteerde in een remissie van de protéinurie, lukte het niet om de plasmaferese te staken. De patiënt werd behandeld met rituximab, één keer per week gedurende 4 weken, aangezien zij gedurende meer dan 15 maanden afhankelijk bleef van plasmaferese. Na de behandeling met rituximab kon de plasmaferese worden gestaakt. Op dit moment heeft patiënt een partiële remissie.
In hoofdstuk 10 presenteren wij een diagnostische en therapeutische behandelstrategie voor bij patiënten met FSGS, gebaseerd op de studies gepubliceerd in dit proefschrift en een analyse van de literatuurgegevens.
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Dit proefschrift was nooit tot stand gekomen zonder de bijdrage van een groot aantal
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onderzoek naar glomerulaire nierziekten verder kunnen uitbreiden onder andere met behulp
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List of Publications


Curriculum Vitae