PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS: WHY ARE PIECES OF THIS PUZZLE STILL MISSING?

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ABSTRACT

Focal segmental glomerulosclerosis (FSGS) can be classified as primary or secondary. Moreover, many causes of primary FSGS have been identified in recent years. In this regard, genetic circulating permeability factors and the abnormal podocyte expression of co-stimulatory molecules have been reported. However, the classification of this entity remains difficult to understand, mainly due to the fact that it describes a morphologic pattern of scarring. FSGS is a histological pattern shared by almost all the glomerulonephritides that describes a podocyte lesion and not a disease. Therefore, it should be reclassified according to the new pathophysiological findings and the biomarkers encountered in each triggered pathway.

Keywords: Focal segmental glomerulosclerosis (FSGS), soluble factor urokinase type plasminogen activator receptor (suPAR), B7-1, proteinuria.

MORPHOLOGY AND PATHOPHYSIOLOGY

Since the introduction of kidney biopsy in 1951, the urinary anomalies observed either macro or microscopically, plus the quantification of proteinuria and the clinically diagnosed kidney diseases, were consolidated into one main group in internal medicine: glomerular diseases.[1] Nephrosis and nephritis were better distinguished and classified according to optic microscopy observed patterns, to which electron microscopy and immunofluorescence microscopy added more information. Therefore, morphology was employed as a solid base on which clinical and laboratory findings could be explained and sustained in order to establish diagnosis and to eventually prescribe an available treatment. As recently remarked by Sethi et al.,[2] adequate quantification of the percentage of glomeruli affected by sclerosis can only be made by examination of sufficient glomeruli within a specimen, which helps to ensure that the specimen is reasonably representative of the population of glomeruli in the kidney as a whole. Of course, this ideal situation can be far from possible in a clinical setting.

Despite this often overlooked consideration, the morphological patterns of glomerulopathies have been facing new challenges in the last decades. As the pathophysiological pathways of glomerular diseases have been unravelled, the glomerular diseases have become better understood. As a consequence, the dissection of the molecular mechanisms of disease has started to demonstrate that a similar morphological pattern in a biopsy can be shared by different entities based on quite diverse pathophysiological pathways. This important issue obliged the nephrology community to reconsider the classification of many glomerulopathies, in which the pathophysiology would prevail over the morphological patterns. Moreover, the chain of events is facing a new challenge. The identification of appropriate, specific, and commercially available plasmatic or urinary biomarkers that do not only correlate with the histological variants of a certain glomerulopathy, but also inform the therapeutic approaches to be followed.

THE COMPLEX DEFINITION OF PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is even more complex, as it refers to a morphological pattern that can not only be present in its primary variants, but also occur as a consequence of secondary insults and as a common final pattern of glomerular obliteration.

FSGS is a morphological pattern of glomerular injury primarily directed at the podocyte and defined by the presence of sclerosis in parts (segmental) of some (focal) glomeruli, as observed by light microscopy of a renal biopsy.[2] However, the name itself is misleading. Morphometric analysis of complete glomeruli from renal biopsies obtained from patients with FSGS shows that the volume of the sclerotic lesions averages just 12.5% of the entire glomerular volume.[3] Moreover, as remarked by Sethi et al.,[2] renal biopsies with <15 glomeruli cannot exclude FSGS with confidence. This is further complicated by the well-known fact that the inner (deep) juxtamedullary glomeruli are preferentially affected in the early phases of primary FSGS.[4] A biopsy specimen containing only cortical glomeruli may underestimate the frequency of FSGS lesions in the whole kidney. In order to maximise accuracy, the diagnostic set should be comprised of consecutive sections selected from 12-15 routinely cut serial sections[5,6] and should contain a minimum of 8 glomeruli.[3]

Another point frequently overlooked is that the electron microscopy-observed changes are the initial phase of the pathological process; only with time will the characteristic sclerotic lesion develop. This can explain the absence of FSGS lesions in an initial biopsy while a second biopsy, performed months or even years later, clearly demonstrates lesions of FSGS.[7] The 'bottom line' is that the lesion of FSGS observed by light microscopy, which is how FSGS is defined, is not really segmental and is only rarely truly focal in its distribution. While FSGS is not a very common cause of nephrotic syndrome (NS) in the elderly,[8] some of these patients may present with NS and with an FSGS as the only apparent lesion in optical microscopy. Finally, evaluation of non-sclerosed glomeruli by electron microscopy can be helpful in identifying a primary podocytopathy, and can support the use of immunosuppressive therapy in the setting of widespread foot process effacement.

GENETIC CAUSES OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Currently, mutation analysis is expensive and single genes are analysed separately. Therefore, a cost-effective approach requires information on the prevalence of causative mutations in a given population.[9] However, certain concepts must be taken into account when a genetic cause of FSGS is suspected. The genetic causes of FSGS comprise proteins that are mainly expressed in the podocyte or in the slit diaphragm itself and are engaged either in the organisation of the slit diaphragm or the podocyte actin cytoskeleton, thus regulating glomerular membrane permeability and selectivity. FSGS caused by mutations in nephrin (*NPHS1*), podocin (*NPHS2*), CD2-associated protein (*CD2AP*), phospholipase C epsilon-1 (*PLCe1*), and myosin 1e (*MYO1E*) is characterised by an autosomal recessive pattern of inheritance. As a rule, the onset of disease occurs in childhood. In contrast, mutations in *ACTN4, TRPC6,* and *INF29* cause autosomal dominant FSGS. In most patients, onset of disease is in adulthood, and many patients do not develop a full-blown nephrotic syndrome. FSGS can also be caused by mutations in genes that encode proteins that are expressed not only in the podocytes but also, or even more so, in other tissues and cell types. In these syndromic forms of FSGS the extrarenal manifestations are most prominent and often diagnostic, and in some of these diseases FSGS may be the only or the presenting manifestation, thus mimicking isolated FSGS. Well-known examples are mutations in the transcription factor Wilms tumour 1 (*WT1*) and certain mitochondrial mutations.[9]

PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS PATHOPHYSIOLOGY: suPAR

FSGS is a major cause of chronic kidney disease in children and adults.[10-12] It can occur as a primary disorder (called primary acquired FSGS) as a consequence of genetic mutations in podocyte-specific or slit diaphragm proteins (also called primary genetic FSGS), or as a secondary disorder.[13,14] In recent years, much of the progress obtained in unravelling the pathophysiological events in FSGS has been focussed primarily on the identification of genetic mutations of membrane and podocyte slit diaphragm proteins and on immune factors, but the real identity of the

primary acquired variant apparently caused by circulating permeability factors remains elusive. In this regard, the role of these permeability factors in the pathogenesis of proteinuria has also shown progress in recent years, although the results are not entirely convincing and appear to lack specificity for a unique type of glomerular disease, as has been found in other glomerular diseases such as minimal change disease and membranous nephropathy.[15] Lately, the soluble factor urokinase-type plasminogen activator receptor (suPAR) has become one of the most studied permeability factors with a potential pathophysiological involvement in FSGS. It is reported to be responsible for the contraction of podocytes and their eventual detachment from the glomerular basement membrane, which denudes it and causes proteinuria in the majority of primary acquired cases of FSGS.[16]

Abnormally high circulating levels of suPAR have been associated with the pathogenesis of acquired primary FSGS, since approximately two-thirds of patients with acquired FSGS have increased circulating levels of suPAR.[16] suPAR then binds to and activates αvβ3 integrin in podocytes by a lipid-dependent mechanism,[17] leading to alterations in the morphology and dynamics of the metabolism of podocytes and foot process effacement, detachment and podocyturia, finally resulting in proteinuria and the beginning of glomerulosclerosis, nephrotic syndrome, and renal insufficiency.[17,18] According to Li et al.,[19] steroid responsiveness may be related to the levels of suPAR in some primary FSGS cases. The authors propose a suPAR concentration of 3,400 pg/ml to be used as an optimal cut-off value for corticosteroid therapy.[19]

What is the cellular origin of this increased membrane urokinase-type plasminogen activator receptor (uPAR) and circulating suPAR in FSGS? Wei et al.[17] suggest that neutrophils and monocytes may be culprits, but another possibility lies in circulating T cells, since there is an association between T cell activation and systemic proteinuria. In turn, and as mentioned above, not all cases of idiopathic acquired FSGS display increased circulating levels of suPAR. This is another confirmation that histological FSGS is not a disease but a form of kidney damage characterised by common histopathological features and with completely different pathophysiological pathways.[20] In other words, FSGS is a morphological description that is denoting podocyte injury; it is a lesion and not a disease.[2]

With regard to suPAR and FSGS, this concept is not shared by others who question whether elevated levels of suPAR are indeed pathogenic or are merely markers of a split uPAR (CD87) molecule. Moreover, proteinuria does not occur in other clinical settings in which suPAR is elevated.[21-23] An elevated concentration of suPAR is not a specific marker of FSGS as levels can also be high in patients with other glomerulopathies, as well as in patients without glomerular derangement. In addition, elevated suPAR levels are not always encountered in recurrences of FSGS following transplantation.[21-23] Finally, some authors suggest that it is the presence of suPAR in urine that is the real cause of primary acquired FSGS.[20-23]

Various molecules can activate uPAR, including urokinase-type plasminogen activator (uPA), plasminogen, chymotrypsin, various metalloproteinases, and some elastases.[24-27] Studies are generally based on the action of these molecules on uPAR but, as suPAR slightly shares the same molecular structure as uPAR, these proteases are also likely to cleave suPAR fragments. Furthermore, suPAR or uPAR are capable, once activated, of catalysing the conversion of plasminogen to plasmin, which is an important molecule in fibrinolytic processes and in the activation of several matrix metalloproteinases, in the recycling and degradation of the extracellular matrix, in cell activation, migration, contraction, vasculogenesis, and in vitronectin degradation.[28-31] This phenomenon may occur in plasma, on the podocyte surface, or in renal distal tubular cells.[18-32] It is noteworthy that patients with NS present with elevated serum levels of plasminogen and plasmin.[33] In turn, and after being filtered, urinary plasminogen is converted to plasmin by podocyte or distal renal tubular epithelial uPA/uPAR.

At this distal location, plasmin has been reported to function as a regulator of water and sodium absorption, which is a key event in the pathogenesis of oedema, and also as a mediator in calcium tubular transport.[32,34,35] It is known that uPAR is needed to activate the integrin αvβ3 in podocytes, which promotes cell motility and activation of small GTPases that control cell division, such as cdc42 and cdc40. If αvβ3 integrin is activated, the podocyte contracts and proteinuria ensues. However, it is believed that suPAR has inhibitory properties on adhesion and uPAR-dependent migration but not on cell contraction. Thus, it would be able to interact with αvβ3 integrin, vitronectin, or plasmin.[36,37]

PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS PATHOPHYSIOLOGY: B7-1

A new and provocative proposal recently came onto the scene when expression of the B7-1 molecule on podocytes was found to be present in patients with primary FSGS.[38] B7-1 is a 53 kDa membrane-associated protein that, in the glomerulus, is localised exclusively in podocytes, although it can also be found in renal tubules.[39,40] It is better known for its role in the immune system as a co-stimulatory receptor involved in T cell activation.[40] Activation of B7-1 by puromycin in cultured podocytes was found to attenuate expression of nephrin and results in foot process effacement and retraction.[41] The ability of B7-1 to regulate podocytes' filtering capacity is also shown when lipopolysaccharide (LPS) is injected into mice, which results in increased B7-1 expression and proteinuria; proteinuria does not occur in B7-1 knockout mice.[39] Therefore, the immune stimulatory role of B7-1 within the glomerulus would support the idea that it may modulate immune-mediated injury to podocytes.[42]

T cells require two signals to become activated. The first signal comes from the interaction between the antigen-presenting cell (APC) and the T cell receptor via the major histocompatibility complex. This signal alone leads to anergy or tolerance.[43] The second signal is named the co-stimulatory or accessory signal, and is mediated via interactions between CD28 expressed on the surface of T cells and the lymphocyte activation antigens B7-1 or B7-2 (also known as CD86) expressed on the surface of APCs.[44] B7-1 modulates the activity of responding CD4+ and CD8+ T cells by alternatively binding to the surface glycoprotein CD28 co-stimulator, which is constitutively expressed on the surface of naïve and activated T cells, or the cytotoxic T lymphocyte-associated protein 4 (CTLA 4) co-inhibitor, which is inducibly expressed on both CD4+ and CD8+ T cells upon activation. As mentioned above, podocytes do not express the B7-1 molecule on their extracellular membrane in normal conditions. However, various rodent models of glomerular diseases are associated with an upregulation of B7-1 in podocytes.[45-48] In this respect, the podocyte would act as an APC to T cells, which would then activate other T cell populations as well as B cell populations, triggering antibody synthesis and also potentially influencing the synthesis or release of suPAR from leukocytes. These findings and speculations may portend relevant implications for the role the second signal should be playing at the initial steps of the immune response involved in glomerulopathies.

In this regard, there are at least two implications related to the podocyte expression of B7-1. First, the APC role of podocytes in abnormal conditions; second, B7-1+ podocytes have a reduced capacity to attach to the surrounding matrix (the glomerular basement membrane) through β1 integrin.[49,50] Whereas in T cells B7-1 acts by binding to CD28 or CTLA-4 through its extracellular domains, in podocytes the cytoplasmic tail of B7-1 is necessary and sufficient to block β1-integrin activation by competing with talin for β1-integrin binding.[51,52] B7-1+ podocytes change their morphological characteristics and their function, promoting podocyte migration through inactivation of β1 integrin and leading to detachment of their foot processes from the glomerular basement membrane, podocyturia, and proteinuria.[53] This is a result of the interaction between T cells and podocytes through B7-1 and B7-2. The inhibition of β1-integrin activation in podocytes by abatacept could be a potential mechanism that could explain the underlying antiproteinuric action of this drug.[53]

B7-1 can be detected and measured in the urine and may be a potential biomarker of podocyte injury, but, as mentioned above, its origin could be either podocytic or tubular.[39,40] Urinary levels of B7-1 in patients with relapsed minimal-change disease are higher when compared with those in patients with minimal change disease in remission, lupus (with and without proteinuria), other glomerulopathies (FSGS, membranoproliferative glomerulonephritis, immunoglobulin A nephropathy, and membranous nephropathy), and healthy control patients.[40] Data from a second study by the same group showed that urinary B7-1 was increased in patients with minimal change disease in relapse compared with patients with minimal-change disease in remission or those with FSGS.[54] Additionally, the level of urinary B7-1 mRNA was found to be enhanced in patients with glomerular kidney disease compared with that of healthy patients.[55] Promising data describing the utility of urinary B7-1 as a biomarker of

podocytopathy have been reported; however, the fact that B7-1 can also be derived from tubular epithelium reduces confidence in its specificity.[40] Moreover, immunohistochemical detection of B7-1 is technically difficult using paraffin-embedded tissue samples.[56] This highlights the need for the development of improved techniques for routine widespread use.

Recently, Yu et al.[38] administered abatacept in one or two intravenous doses of 10 mg/kg to four patients with recurrent FSGS after kidney transplantation and to one patient with primary FSGS.[40] The patients with recurrent FSGS underwent concurrent plasmapheresis. The conclusions from this study must be taken with utmost caution as only five patients were included. Nonetheless, the results were interesting and encouraging: these patients experienced a remission that lasted 10-48 months. As the therapy was beneficial, B7-1 staining of kidney biopsy samples from patients with glomerular diseases was assessed and podocyte B7-1 expression was observed. In the non-transplant patient with primary FSGS, treatment with abatacept 10 mg/kg on Day 1, 15, and 30, and monthly thereafter, was associated with partial remission and proteinuria decrease at 12 months. Several hypotheses for this response could be proposed. Firstly, abatacept is capable of modulating the immune response by affecting B7-1 and CD28 co-stimulation, which in turn could decrease leukocyte-derived circulating factors, such as suPAR, and consequently protect podocytes from contraction.[20] Secondly, abatacept might bind to podocyte B7-1, thus altering the cellular downstream function of this receptor in relation to the roles of actin and integrin in podocyte contraction.[20,38] Thirdly, plasmapheresis could have removed a circulating factor and this removal induced remission independent of podocyte B7-1 expression and/or abatacept infusion.[20,49] In summary, a small subset of patients with primary FSGS who are B7-1+ may prove to be responsive to abatacept. It cannot be concluded that abatacept is a specific treatment for FSGS.

Finally, abatacept could play a role in podocyte toll-like receptor (TLR) signalling through B7-1 interaction or independent of B7-1. This mechanism could be, for example, via the endogenous calprotectin system composed of TLR4 agonists S100A8/S100A9 and present in monocytes.[57] These proteins have been shown to play critical roles in LPS-induced sepsis, vasculitis, and certain types of glomerulonephritis.[58,59] To my knowledge, this hypothesis has not been explored in this field. Although the podocyte B7-1 pathway seems to play an important role in some glomerular diseases, clinical results suggest that targetting this mechanism needs further study in randomised controlled trials. As commented by Haraldsson,[59] the relevance of distinguishing B7-1+ from B7-1— glomerulopathies could predict the response to abatacept.

THE REASON WHY PRIMARY FSGS IS A DIFFICULT PUZZLE TO COMPLETE

Histopathologic morphological patterns have played a valuable and critical role in the classification and comprehension of glomerulopathies. However, as the dissection of the pathophysiological pathways of glomerular diseases continue to be revealed, the classical morphological classifications must be aligned with the biomarkers involved in the development of glomerular injuries and changed to a molecular-based classification. Immunohistochemistry can add important functional information to the morphological patterns. So long as FSGS is considered a disease, the completion of the puzzle will remain elusive. FSGS is a morphological lesion. Many new pathophysiological advances have taken place in recent years that can justify the splitting of this entity into a more comprehensive classification. Morphology has paved the road for nephrologists to face proteinuria. However, with respect to FSGS, the newly discovered pathophysiological pathways and the different adjunctive biomarkers are showing that new roads are stretching out ahead, which will require a new classification of this erroneously denominated disease. Therefore, more than one puzzle could be made, in which the pieces would be fewer and ought to fit more easily and smoothly.

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