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# Defining diagnostic trajectories in patients with podocytopathies

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## ABSTRACT

Podocytopathies are glomerular disorders in which podocyte injury drives proteinuria and progressive kidney disease. They encompass a broad spectrum of aetiologies, resulting in pathological pictures of minimal-changes, focal segmental glomerulosclerosis, diffuse mesangial sclerosis or collapsing glomerulopathy. Despite improvement in classifying podocytopathies as a distinct group of disorders, the histological definition fails to capture the relevant biological heterogeneity underlying each case, manifesting as extensive variability in disease progression and response to therapies. Increasing evidence suggests that podocytopathies can result from a single causative factor or a combination of multiple genetic and/or environmental risk factors with different relative contributions, identifying complex physiopathological mechanisms. Consequently, the diagnosis can still be challenging. In recent years, significant advances in genetic, microscopy and biological techniques revolutionized our understanding of the molecular mechanisms underlying podocytopathies, pushing nephrologists to integrate innovative information with more conventional data obtained from kidney biopsy in the diagnostic workflow. In this review, we will summarize current approaches in the diagnosis of podocytopathies, focusing on strategies aimed at elucidating the aetiology underlying the histological picture. We will provide several examples of an integrative view of traditional concepts and new data in patients with suspected podocytopathies, along with a perspective on how a reclassification could help to improve not only diagnostic pathways and therapeutic strategies, but also the management of disease recurrence after kidney transplantation. In the future, the advantages of precision medicine will probably allow diagnostic trajectories to be increasingly focused, maximizing therapeutic results and long-term prognosis.

*Keywords:* FSGS, minimal change disease, nephrotic syndrome, podocytes, proteomics

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## INTRODUCTION

Kidney biopsy has represented the reference standard for the classification and the diagnosis of glomerular disorders for a long time. Notwithstanding this, in recent years a growing amount of attention has been paid to reframing the role of histological findings at kidney biopsy, which must be considered patterns of tissue damage rather than diseases themselves, and that should be linked to specific pathomechanisms [1, 2]. This approach has the advantage of helping to precisely identify therapeutic targets and to define prognosis, thus tailoring clinical management. We distinguish three main categories of glomerular diseases: (i) immune-mediated glomerulopathies that encompass distinct histopathological patterns [e.g. immunoglobulin A (IgA) nephropathy, membranous nephropathy (MN), C3 glomerulopathy, etc.], all showing immune deposits on kidney biopsy; (ii) systemic diseases with glomerular involvement [e.g. lupus nephritis (LN), monoclonal gammopathies, metabolic storage diseases, etc.], with a wide spectrum of histologic findings; and (iii) podocytopathies, with a typical pathological picture of diffuse mesangial sclerosis (DMS), minimal-changes (MC), focal segmental glomerulosclerosis (FSGS) or collapsing glomerulopathy (CG) [2]. Podocytopathies have been increasingly recognized as a group of glomerular disorders in which direct or indirect podocyte injury drives proteinuria and progressive kidney disease [2]. Following a huge technological development, many steps forward have been taken in unravelling the causes and the pathogenesis of this group of disorders, pushing nephrologists to face the challenge of integrating different types of information and advanced diagnostic tools in the management of patients suspected to suffer from podocytopathies.

In this manuscript, we will review the current understanding of the main pathophysiologic mechanisms responsible for podocytopathies together with their clinical presentation, diagnostic toolkit and lines of treatment available, providing food for thought for recent, cutting-edge advances in each of these topics.

## WHAT ARE PODOCYTOPATHIES?

Advances in microscopy, genetics and molecular studies have provided significant improvement in the characterization of the glomerular filtration barrier (GFB), raising podocytes as the culprit cells in maintaining structural and functional integrity [2, 3]. Podocytes' adaptive response to stimuli preserves glomerular filtration, prevents the loss of cells and molecules in the urine and promotes to some extent regeneration [4, 5]. Moving forward the traditional anatomopathological classification (i.e. identifying histologic lesions with specific disease entities) triggered off the concept that podocyte patterns of damage could represent the end result of diverse glomerular *noxae* with different mechanisms, prognosis and potentially specific therapeutic targets [1, 2]. Indeed, podocytopathies encompass a broad spectrum of aetiologies, including genetic defects, permeability factor/s, immunologic dysfunction, vascular endothelial growth factor (VEGF) inhibition, infectious agents, drugs, malignancies and maladaptive responses, with different prevalence across infancy, adolescence and adulthood [2, 6] (Fig. 1). To complicate the picture further, growing evidence suggests the possibility of the presence of podocytopathy in the context of other immune-mediated diseases (e.g. lupus podocytopathy, 'podocytopathic features' in the context of IgA nephropathy), with implications for management and prognosis [7, 8].

For a detailed classification of the aetiology and pathomechanisms of podocytopathies, we refer to a recent review on this topic [2].

Increasing evidence supports the hypothesis that podocyte injury can result from a single causative agent or a combination of multiple factors acting with complex mechanisms, prompting multifactoriality as relevant in the pathophysiology of these disorders (Fig. 1). Indeed, multiple, low or medium effect-size genetic, environmental and/or lifestyle-related insults to the GFB can represent additional risk factors finally favouring podocyte injury [2] (Fig. 1). The identification of *APOL1* polymorphic variants (namely G1 and G2 risk alleles) represented a revolution in understanding the racial difference in susceptibility to HIV infection-associated nephropathy, FSGS or 'hypertensive nondiabetic kidney disease' in patients of Black ancestry [9, 10]. In this view, HIV infection probably represents the 'second hit' required to develop glomerular injury and kidney disease in genetically predisposed subjects [11]. Low birth weight (LBW), prematurity, gestational and fetal distress, previous episodes of acute kidney injury (AKI) and nephrotoxins exposure, obesity, diabetes, high-salt diet and ageing represent other examples of risk factors (Fig. 1) [2, 12]. Of note, whatever the cause and the combination of risk factors acting in determining the onset of podocyte injury and loss, the resulting reduced number of functioning nephrons causes the workload to be spread over the remaining nephrons in an attempt to compensate for the metabolic and functional needs of the body [12, 13]. Although representing a functional adaptation, hyperfiltration of the remnant units establishes a vicious circle leading to gradual sclerosis of the entire pool of nephrons and chronic kidney disease (CKD) progression [12, 13].

## CLINICAL MANIFESTATIONS AND INITIAL DIAGNOSTIC WORK-UP

Irrespective of the primary insult, podocyte damage, detachment and loss increase the permeability of GFB, causing proteins to be filtered in the urine. According to this, the clinical hallmark of podocytopathies is proteinuria, ranging from subnephrotic to nephrotic range [14]. When associated with oedema, hypoalbuminaemia and hyperlipidaemia, nephrotic-range proteinuria outlines the complete picture of nephrotic syndrome (NS) [14]. Of note, the severity and variety of clinical features accompanying proteinuria are usually related to the entity and the rate of protein loss, with differences between adults and children (Fig. 2) [2, 15, 16]. In the former, proteinuria usually has a gradual onset, causing protein levels to drop either in plasma or on the extracellular side, preventing the shift of fluids to the interstitium. This makes the development of clinically relevant oedema subtle and mostly dependent on sodium retention [2]. Consequently, proteinuria can be frequently detected incidentally in the adult population. Conversely, in children marked hypoalbuminaemia causes a fluid shift from plasma to the relatively hyperoncotic interstitium, resulting in generalized pitting oedema [15–17]. Of note, these dichotomic presenting features are not exclusive to specific age groups [15, 16]. AKI and microscopic haematuria are not typical features of podocytopathies, and their presence is of no help in either confirming or excluding this hypothesis [18, 19].

Virtually any glomerular disease presenting with proteinuria may resemble a podocytopathy. For this reason, a stepwise approach is fundamental in order to establish a precise diagnosis (podocytopathies versus other glomerular diseases), its aetiology and to guide therapy (Fig. 2). As an initial clinical assessment, detailed medical history is essential: some risk factors

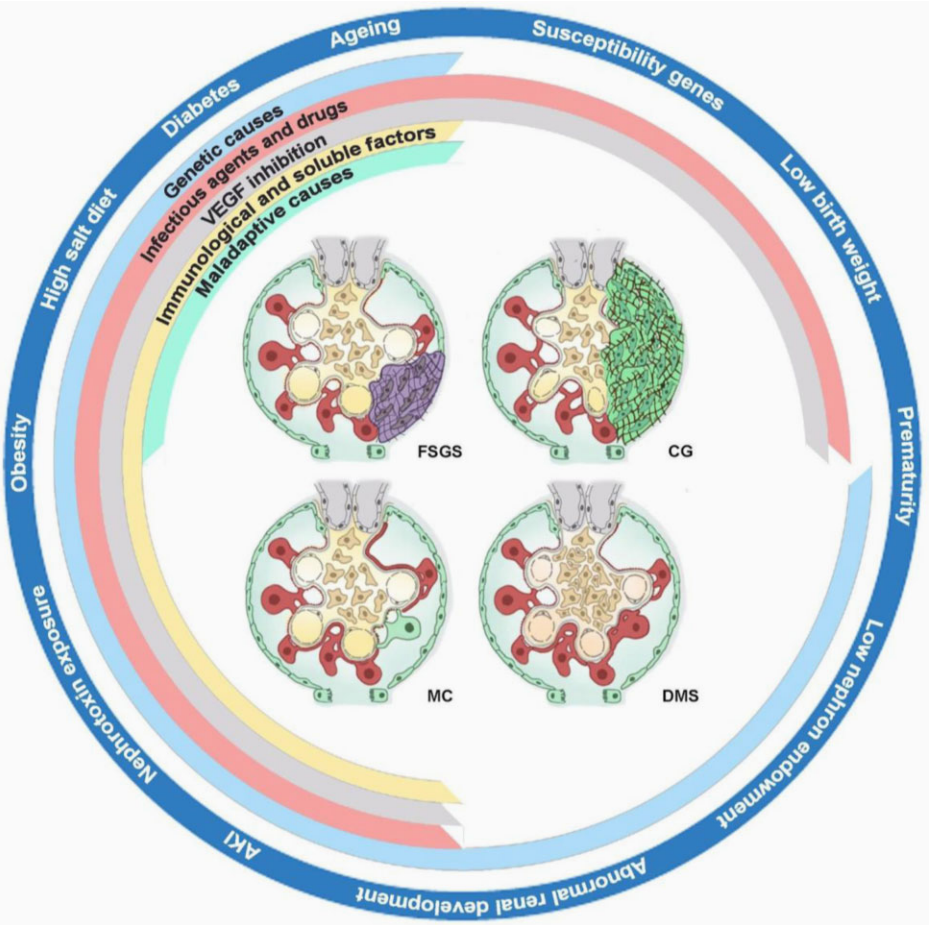


**FIGURE 1:** The spectrum of podocytopathies. Podocytopathies encompass a broad spectrum of aetiologies (inner semicircles) and risk factors (outer blue circle). While the latter are widely associated with podocytopathies, different aetiologic agents are more strictly reported behind the histologic patterns of MC, FSGS, DMS and CG. Genetic causes, light blue semicircle; infectious agents and drugs, red semicircle; VEGF inhibition, grey semicircle; immunological and soluble factors, yellow semicircle; maladaptive, cyan semicircle. Podocytopathies can result from a single causative factor (e.g. monogenic form, single lifespan risk factor) or a combination of multiple genetic and/or environmental risk factors with different relative contributions, identifying compound pathological mechanisms.

or possible causes have different epidemiology according to the patient's age, ethnicity or gender, and can be simultaneously detected in the same patient (Fig. 2). This process is critical in unravelling the aetiology of the disease, irrespective of clinical presentation, the severity of proteinuria and age at onset (Fig. 2). Given the high prevalence of genetic forms in infancy and childhood, the presence of extra-renal signs potentially suggestive of syndromic disorders, as well as a positive familial history of kidney diseases, should be carefully evaluated by deep phenotyping and genetic counselling, since genetic forms are likely to be unresponsive to immunosuppressive therapy [18]. Of note, recent evidence suggests that this is not exclusive to the paediatric population [19]. All additional clinical information (e.g. drug exposure, history of previous or concomitant infections, systemic symptoms, etc.) should be recorded at the time of the first evaluation. Successively, first-line laboratory investigations mainly aimed at excluding alternative diagnostic hypotheses should be ordered (Fig. 2, Table 1) [14, 20]. Kidney ultrasound scanning (US) is usually performed in adults, while it represents a second-tier examination in children [14, 20].

If the initial diagnostic work-up still points towards the hypothesis of a podocytopathy as the cause of proteinuria, then the clinical management differs according to the patient's age and severity of proteinuria (Fig. 2). Patients with sub-nephrotic proteinuria should receive antiproteinuric therapy irrespective of their age at onset. Moreover, if a clear aetiology of podocyte injury is shown by the diagnostic work-up, then patients should be treated accordingly (Fig. 2). While in adults, kidney biopsy is anyway considered in order to confirm the diagnosis and to assess prognostic markers of disease progression, in children it is usually reserved for patients showing disease progression, lack of response to treatment or additional findings (Fig. 2) [2]. Kidney biopsy is mandatory in adults presenting with NS, either to exclude other types of glomerulopathies or to assess the degree of chronicity in podocytopathies (Fig. 2). When kidney biopsy confirms the diagnosis of podocytopathy and blood tests do not point to a specific aetiology, a steroid challenge is suggested [14]. A lack of response defines steroid-resistant NS (SRNS), necessitating the assessment of genetic causes [14]. Conversely, in children kidney biopsy can be initially avoided in favour of a course of steroids when blood pressure, complement levels and kidney function are normal and the suspicion of a podocytopathy is strong [14, 21]. Approximately 80%–90% of patients will experience complete remission within 4 weeks of

**Table 1. Clinical work-up in patients with proteinuria**

| Aetiologic clues | Supporting the diagnosis of podocytopathies | Supporting alternative diagnosis | Ref. |
|---|---|---|---|
| Drugs | | | |
| Lithium, NSAIDs, rifampicin, cephalosporins, IFN therapies, mTOR and calcineurin inhibitors, anthracyclines, bisphosphonates, anabolic steroids, etc. | These drugs may be associated with MC and/or FSGS. In MC, proteinuria generally resolves within weeks if therapy is discontinued. This relationship is less strong in FSGS. | Kidney injury related to these drugs is not limited to podocytopathies. AKI, together with signs of TIN, suggests alternative mechanisms of nephrotoxicity. Specific assessment should be performed. | [22] [23] [24] |
| Penicillamine, gold salts, elemental mercury, anti-TNF therapy, tiopronin, etc. | Not reported or unclear association. | Exposure to these drugs, in the presence of nephrotic proteinuria, may underlie secondary MN. | |
| Infections | | | |
| HIV, CMV, parvovirus B19, EBV, SARS-CoV-2 | Listed viruses are usually associated with FSGS or CG. In the presence of CG, proteinuria is usually more severe than FSGS, and kidney dysfunction is easily traceable. Once the diagnosis is established, treatment should be aimed at resolving the underlying infection. | AKI without proteinuria, TIN and, less commonly, acquired tubulopathies, suggest different mechanisms of infections-mediated kidney injury. Nephrotoxicity caused by anti-infective drugs can make the diagnosis harder. | [24] [25] [26] [27] [28] [29] |
| HCV, HBV | These viruses are usually not associated with podocytopathies. | HCV- and HBV-associated nephropathies include MPGN with or without cryoglobulins, MN and PAN. | |
| VEGF inhibition | | | |
| Pregnancy | The presence of proteinuria, with or without full-blown clinical signs of preeclampsia, suggests a podocytopathy. | Although preeclampsia is the most common cause of AKI in pregnancy, other causes must be ruled out, especially in the absence of proteinuria. | [30] [31] [32] |
| Treatment with VEGF inhibitors | Proteinuria is usually associated with MC or FSGS. | AKI, TMA, MN, nephritic syndrome and proliferative glomerulonephritis have been uncommonly reported. | |
| Autoimmune diseases | | | |
| SLE | In the majority of cases, proteinuria does not support the diagnosis of a podocytopathy in patients with SLE. Lupus podocytopathy, usually presenting with isolated NS in a patient with LES, represents an exception. | Proteinuria in patients with SLE is usually associated with specific histologic patterns (i.e. LN). Additional clinical findings are common (e.g. active sediment). | [33] [34] [35] [36] [37] |
| ANCA vasculitis, Sjögren's syndrome, PAN, other vasculitides | Podocytopathies are not a feature of these diseases. | Even though proteinuria can be a manifestation of these diseases, it is usually subnephrotic and underlies rapidly progressive glomerulonephritis (vasculitis) or a TIN (Sjögren's). | |
| Malignancies | | | |
| Haematologic malignancies (leukemias, lymphomas) | MC and FSGS can be incidentally associated with leukemias and lymphomas. | Tubular dysfunction, AKI and/or cancer drug nephrotoxicity are far more common. MM can anyway present with NS. Suggest the association with MN. | [38] [39] |
| Solid tumours | Rarely associated. | | |
| Maladaptive | | | |
| Congenital (e.g. unilateral renal agenesis, oligomeganephronia, kidney hypodysplasia, etc.) or acquired (e.g. surgical reduction of renal mass, kidney transplant, etc.) conditions | These conditions are associated with FSGS when a biopsy is performed. Proteinuria is usually sub-nephrotic, slowly progressing. Occasionally, NS can be the clinical presentation of these conditions. | Usually associated with hypertension, kidney dysfunction and other clinical signs. | [40] [41] [42] |
| Genetic | | | [43] |
| Extra-renal involvement | Strongly support the diagnosis of podocytopathies, especially in patients with NS. MC, FSGS and DMS represent the typical patterns on kidney biopsy. | Not exclusive to genetic podocytopathies. | |
| Familial history of kidney disease | Strongly support the diagnosis of podocytopathies, especially in patients with NS. MC, FSGS and DMS represent the typical patterns on kidney biopsy. | Not exclusive to genetic podocytopathies. | |
| Resistance to treatment | Strongly support the diagnosis of podocytopathies, especially in patients with NS. MC, FSGS and DMS represent the typical patterns on kidney biopsy. | Other causes of resistance to treatment (e.g. lack of compliance, advanced kidney damage) should be considered in the differential diagnosis. | |

NSAIDs, non-steroidal anti-inflammatory drugs; IFN, interferon; mTOR, mammalian target of rapamycin; TNF, tumour necrosis factor; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HBV, hepatitis B virus; CMV, citomegalovirus; EBV, Ebstein–Barr virus; ANCAs, anti-neutrophil cytoplasmic autoantibodies; PAN, polyarteritis nodosa; TMA, thrombotic microangiopathy; TIN, tubulo-interstitial nephritis; MM, multiple myeloma; MPGN, membrano-prolipherative glomerulonephritis; SLE, systemic lupus erythematosus.

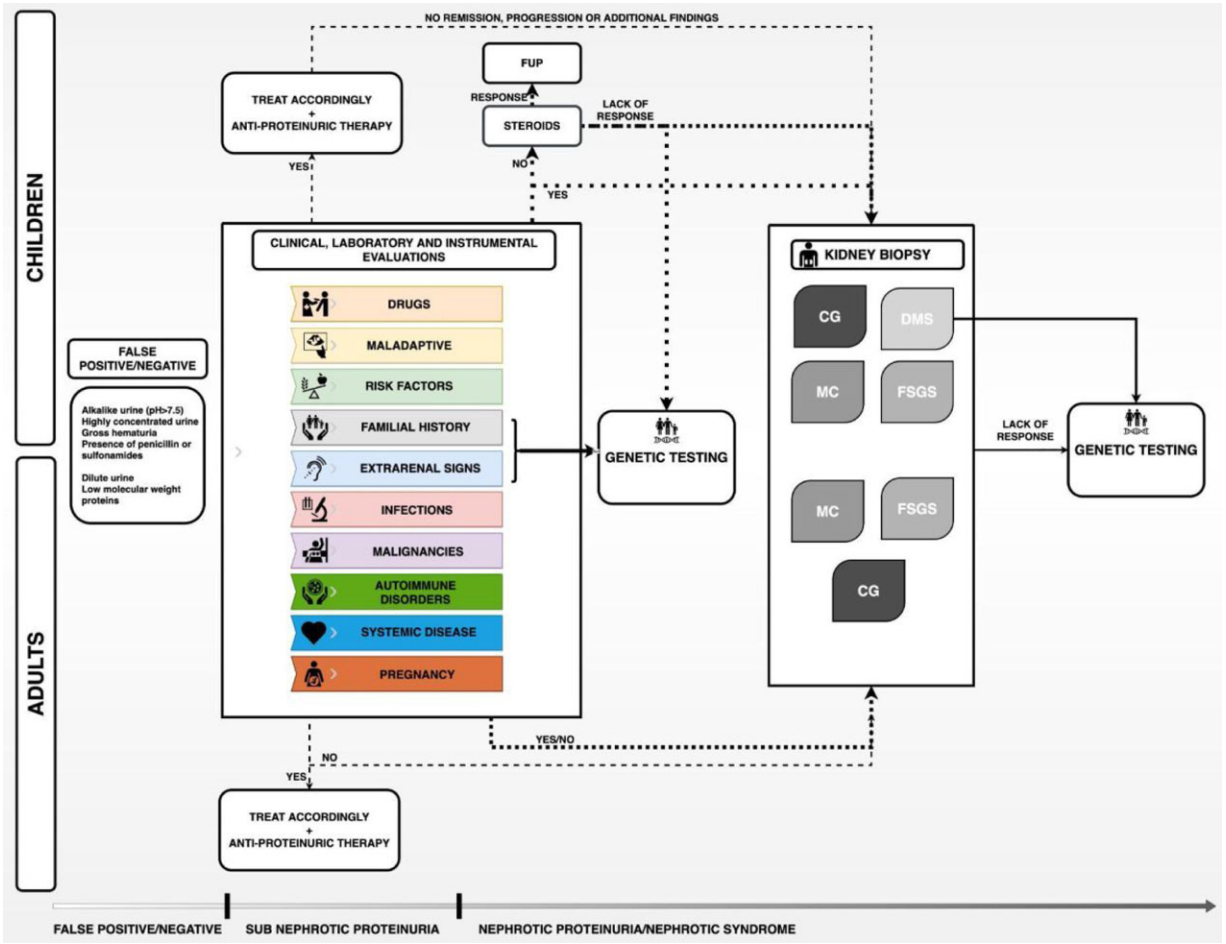


**FIGURE 2:** Approach to patients with suspected podocytopathies. Schematic representation of the clinical approach to patients with proteinuria, according to age (children above, adults below) and severity of proteinuria (from left to right). After exclusion of false positive/false negative proteinuria, a proper assessment should include the assessment of specific causes of podocytopathies. Alternative (i.e. other glomerulopathies) diagnoses should be taken into account at this stage (see Table 1). FUP, follow-up.

therapy, defining steroid-sensitive nephrotic syndrome (SSNS). SRNS imposes genetic testing, kidney biopsy and gradual steroid tapering [14].

## INNOVATIONS IN THE DIAGNOSIS OF PODOCYTOPATHIES

The lack of serum or urinary biomarkers with specific features (i.e. safety, non-invasivity, ease-to-measure, accuracy, consistency between different ethnic groups and genders, etc.) has recently opened up a research quest in the field of podocytopathies. Many hopes to develop innovative diagnostic tools lie in new high-throughput technologies (i.e. 'omics') [44]. Proteomic studies in patients with podocytopathies proposed alpha-1 antitrypsin, transferrin, histatin-3, 39S ribosomal protein L17 and calretinin as new potential urinary markers [45]. Recently, measuring non-coding RNA (e.g. micro-RNA) and single-cell RNA sequencing (scRNAseq) in serum and urine were proposed as innovative non-invasive diagnostic essays [46, 47]. If confirmed in larger cohorts, then these studies can provide other possible sources of knowledge, fostering the capability to differentiate underlying aetiologies and possibly to detect new specific therapeutic targets [48]. In the future, these essays could serve as 'liquid biopsies', potentially preventing invasive investigations as well as bringing forward integration with clinical information and kidney biopsy findings.

## THE ROLE OF KIDNEY BIOPSY

Podocytopathies are associated with MC, DMS, FSGS and CG findings at kidney biopsy. They all share the absence of immune deposits by immunofluorescence and electron microscopy (EM) and can be collectively viewed as a spectrum of glomerular patterns in which progression from normality to global glomerulosclerosis is related to the amount of podocyte loss and the type of parietal epithelial cells (PECs) response. Several lines of evidence support the podocyte depletion hypothesis, showing the histological consequences of precise degrees of podocyte loss, from mesangial expansion to focal and ultimately global glomerulosclerosis [49, 50]. On the other hand, the amount of podocyte replacement driven by PECs that act as podocyte progenitors, together with the efficient or inadequate differentiation into podocytes, is the other determinant of a successful repair strategy, resulting in maintaining normal glomerular

appearance or in the development of glomerular hyperplastic lesions in the Bowman space [51].

MC and FSGS represent a continuum of the same progressive disease [52, 53]. In MC, glomerular appearance by light microscopy is normal and podocyte injury detectable by EM as widespread foot processes effacement, without glomerular basement membrane (GBM) denudation [54]. In the setting of chronic *noxae* not extinguishing over time (e.g. genetic mutations, maladaptive conditions, etc.) [4, 55, 56], and/or an inadequate capacity of PECs to replace lost podocytes usually due to inefficient differentiation [51], podocyte loss exceeds 20% and segmental denudation of GBM initiates the injury cascade leading to scar formation [49]. Glomerulosclerosis can be limited in settings of high regenerative potential as in DMS, a pattern of injury found in children younger than 5 years old with NS progressing to end-stage kidney disease (ESKD) [57]. This particular age span is characterized by a high capacity to generate new podocytes from PECs to support glomerular size growth [4, 58]. In this setting, severe podocyte loss, mainly driven by major genetic alterations, is associated with diffuse accumulation of extracellular matrix protein in the mesangium and signs of a massive podocyte turnover, highlighted by a halo of hypertrophic podocytes surrounding capillary loops [59]. A fast and massive podocyte loss constitutes a particular setting of abrupt capillary collapse, as in CG. This scenario triggers a rapid and catastrophic response of PECs acting as podocyte progenitors in order to replace lost cells. Despite retaining the ability to proliferate, differentiation of PECs into mature podocytes is prevented, thus resulting in the formation of pseudocrescent, a non-inflammatory lesion constituted by a crowd of hyperplastic epithelial cells filling the urinary space [13, 60]. This lesion is encountered in conditions of direct, massive podocyte injury (e.g. drug exposure, viral infections, etc.) [2].

Interestingly, detection of podocyte injury is limited with standard techniques of renal pathology. Principal limitations are due to the focal nature of the injury, which reduces the sensibility of sampling [52], and the scarce evidence of early stages of podocyte damage. Bearing this in mind, the approach to kidney biopsy in podocytopathies needs technical facilities capable of providing not only structural and ultrastructural analysis but also the possibility of *ad hoc* supplementary essays integration and close collaboration with specialists of different fields to include the histologic pattern of injury in a comprehensive evaluation of patient's data.

## INNOVATIONS IN RENAL PATHOLOGY

As patient stratification based only on histological classification has proven to provide little help in defining patient prognosis, therapeutic targets and response to treatment, new approaches are trying to overcome the limits of standard renal pathology and assess risk features of disease progression. An example is represented by the application of super-resolution microscopy (SRM) techniques on tissue sections obtained from the diagnostic routine. SRM has the advantage of allowing 3D visualization of the slit diaphragm, giving direct evidence of structural changes or podocyte loss [61, 62]. In addition, these techniques have the potential to provide panoramic views of the entire tissue samples, thus enabling a more reliable evaluation of prognostic relevant lesions. Apart from morphology, kidney biopsy offers direct access to kidney tissue for high-throughput analysis (including scRNAseq) [63], improving the characterization of mechanisms involved in the pathological processes [64]. The combination of these techniques with digital pathology and bioinformatic analysis offers new perspectives to capture the complex structural changes in kidney biopsies and molecular heterogeneity of these diseases. This integration has the potential to cluster into clinically and biologically relevant subgroups, thus uncovering histologic parameters associated with clinical outcomes and molecular signatures not included in current classification systems [65].

As non-immune-mediated nephropathies, podocytopathies do not show significant immune-complexes deposition by immunofluorescence or EM and occasional low-intensity positive staining for IgM and C3 are considered macromolecular trapping rather than specific deposition. Nevertheless, the recent detection of anti-nephrin autoantibodies in adults and children with biopsy-proven MC provides support for an autoimmune aetiology in a subset of patients [66]. Interestingly, these patients showed podocyte-associated punctate IgG staining at immunofluorescence, together with an increased serum titer of anti-nephrin autoantibodies, suggesting a pathogenic role for a long-searched circulating factor [66]. Considering the paradigm shift induced by the identification of several autoantigens in MN [67], these findings may lay the groundwork to distinguish a subset of patients susceptible to specific treatments.

## PRINCIPLES OF TREATMENT

As for all glomerular disorders, the ideal goal of treatment in podocytopathies is complete remission of the disease (i.e. proteinuria disappearance and normalization of kidney function) [14, 21] that prevents CKD progression and ensures favourable long-term kidney and global outcomes [68–70]. Treatment must target the cause of podocyte injury whenever possible, claiming an aetiologic classification of podocytopathies as fundamental. Unfortunately, in most cases, the primary cause is putative or unknown. This has long led to non-specific therapeutic approaches based on steroids and immunosuppressive drugs (e.g. calcineurin inhibitors, mycophenolate mofetil, rituximab, etc.) as the cornerstones of treatment. Steroids are straightforwardly recommended in routine clinical practice as first-line therapeutic agents, especially in MC and FSGS [14]. Their effectiveness is claimed as a proof-of-concept of the pathogenic role of the circulating factor/s, although experimental evidence of their exact mechanisms of action is still lacking. Of note, resistance to steroids defines the subsequent disease management (Fig. 2) and has prognostic value [68–70]. However, the lack of a consistent definition of steroid resistance represents an important source of bias [1]. Immunosuppressive drugs and plasmapheresis conceptually follow the line traced by steroids in the treatment of podocytopathies. In addition, they can be used as steroid-sparing drugs when repeated steroid cycles are needed or to avoid adverse effects, or as second-line therapies in the case of steroid resistance when a genetic cause is not confirmed or still pending [14].

Besides favouring lexical chaos (i.e. using primary or idiopathic FSGS/MC as synonyms of circulating factor/s-related disease), this approach prevented clinical trials from being correctly designed to address the unmet medical need of providing patients with targeted, effective, non-toxic and tailored (i.e. personalized) therapies. Consequently, in the last few decades, there has been a paucity of novel treatments in the field of podocytopathies [1].

For all these reasons, focusing on slowing the progression towards ESKD does represent the primary goal of the treatment in the majority of patients, especially when interventions aimed at targeting the specific cause are limited or inefficient,

or CKD is already established. Until recently, renin-angiotensin system inhibitors (RASi) have represented the main, if not only, therapeutic options for patients with podocytopathies, together with supportive care (e.g. low-sodium diet, weight control, avoidance of additional insults, etc.). RASi act on the podocyte shear stress driven by hyperfiltration by reducing the transglomerular hydraulic pressure gradient, irrespective of the primary cause of the disease. Very recently, sodium-glucose cotransporter 2 inhibitors (SGLT2i) emerged as extraordinarily promising tools in the nephrologist's armamentarium [71]. Although the exact mechanisms of action are still under investigation, experimental and clinical evidence suggest that SGLT2i are effective in reducing glomerular hyperfiltration and in ensuring nephroprotection in the long run in different types of nephropathies, including FSGS [72]. A dedicated trial is ongoing (EMPA-KIDNEY trial) [73, 74]. Following this principle, the endothelin type A antagonists sparsentan and atrasentan recently proved to be effective in preventing disruption of the actin cytoskeleton in experimental FSGS [75]. Phase II and III trials enrolling FSGS patients are currently ongoing [76, 77].

## INNOVATIONS IN THERAPEUTIC STRATEGIES

Increasing efforts to define the aetiological basis of clinical phenotypes will likely improve the potential success of clinical trials. Starting from the observation that a putative circulating factor could bind to the podocytes glycocalyx, galactose was tested in nephrotic patients because of its potential competitive binding action [78]. The GBM and podocytes themselves are considered as novel potential targets for molecules acting on mitochondrial function and actin–myosin contractile structure [79]. In this regard, a role in stabilizing the podocyte actin cytoskeleton has been proposed also for steroids, CNIs, ACTH and rituximab [80, 81]. New strategies targeting the short transient receptor potential channels [82], the soluble FMS-like tyrosine kinase 1 [83], soluble urokinase plasminogen activator surface receptor [84] and substrate intermediates for coenzyme Q10 are under investigation [85]. Some efforts have also been directed to blocking the progression of scarring and fibrosis following podocyte damage in patients affected by FSGS targeting the inhibition of C-C chemokine receptor type 2 [86, 87], the nuclear factor-$\kappa$B transcription [88] and Slit-2 [89]. Finally, genetic discoveries will play a major role towards personalized medicine, thus tailoring therapies with the best chance of response in carefully selected patients. Novel experimental molecules under investigation include the inhibitors of apolipoprotein L1 (APOL1), such as VX-147, that are currently under investigation in a phase II trial in adults with FSGS and *APOL1* high-risk genotypes [90]. However, the results of these studies still need confirmation to be translated into clinical practice.

## GENETICS OF PODOCYTOPATHIES

Solid evidence about the role of genetic abnormalities in causing podocytopathies is currently available, in both children and in adults. Although the first insights into the pathogenic role of genetic defects date to the end of the 1990s [91], next-generation sequencing (NGS) technologies revolutionized our understanding of the genetic makeup of podocytopathies. Nowadays, we are able to assign a causal relationship between genetic variants and phenotype (namely SRNS, nephrotic-range proteinuria, pathologic patterns of FSGS, MC or DMS) to >50 genes [3, 92, 43]. Historically, the majority of them were classified as 'podocyte genes' since they encode proteins with critical roles in maintaining the integrity of the culprit cells of the GFB [93–96]. The corresponding diseases are referred to as monogenic podocytopathies, with precise indications for treatment and medical management [2, 97, 98]. These conditions encompass a wide spectrum of isolated as well as syndromic kidney phenotypes, all respecting classical Mendelian patterns of inheritance as listed in the Online Mendelian Inheritance in Man (OMIM) database [99]. Syndromic disorders result from the expression of causative genes in extrakidney organs, such as the inner ear, eyes or central nervous system, and are usually recognized with appropriate clinical diagnostic work-up [43]. Overall, monogenic podocytopathies account for ~30% of cases undergoing genetic testing [95, 100]. Interestingly, the frequency is comparable in familial and sporadic cases, and remained stable over time even after the spreading of NGS in diagnostics [43, 95]. As a matter of fact, until recently, the majority of patients presenting with clinical features of podocytopathies finally turned out to be negative to standard genetic investigations, namely NGS targeted sequencing for podocyte genes. An unacceptable proportion of these patients anyway progressed to ESKD, requiring an improvement in understanding of the pathophysiology of these diseases. Indeed, increasing the number of genes tested did not improve the diagnostic rate even with the wide use of whole-exome sequencing (WES) [92, 101]. As in other disorders, a forward approach to genetic investigations risks limiting diagnostic efficacy in podocytopathies. Increasing evidence suggests that phenocopies can represent a proportion of patients presenting with clinical features of podocytopathies as high as that represented by genetically proven monogenic podocytopathies [97, 98]. Indeed, a strictly phenotype-centered *in silico* filtration of variants identified by massive sequencing would miss variants associated with syndromic disorders in patients presenting with either apparently isolated kidney involvement or no familial history [97]. Strategies for the identification of these cases currently rest on a comprehensive genetic screening with WES (i.e. genetic testing including all genes responsible for CKD) and efforts to detect overlooked signs of the disease in the patients or in family members (i.e. reverse phenotyping) in order to confidentially confirm the genetic diagnosis [98, 101–103]. Interestingly, a huge amount of data support mutations in *COL4A* genes as the most frequent cause of familial and sporadic FSGS without overt signs of extra-renal involvement [104, 105]. The demonstration of possible overlapping phenotypes induced by *COL4A* and podocyte genes suggests a conceptual revision of disease boundaries and ontology. As in many other disorders, although few genes are responsible for the majority of cases, singletons are frequently reported as involved in a significant fraction of the remaining cases [98, 106, 107]. Since there is no possibility to *a priori* exclude mutation in one out of the >250 genes, WES applies as the first-choice approach for genetic testing in patients with podocytopathies. Interestingly, recent evidence supports cost-effectiveness of this strategy [108].

## INNOVATIONS IN GENETICS OF PODOCYTOPATHIES

Despite the huge amount of information that it can eventually provide, WES has some limitations that must be taken into account in interpreting the results of genetic testing. Increasing evidence suggests that genetic variants in intronic and regulatory regions of DNA can be relevant in determining phenotypic consequences, mainly affecting splicing and gene expression. Whole-genome sequencing (WGS) could be of help in overcoming this obstacle. Preliminary results including rare kidney

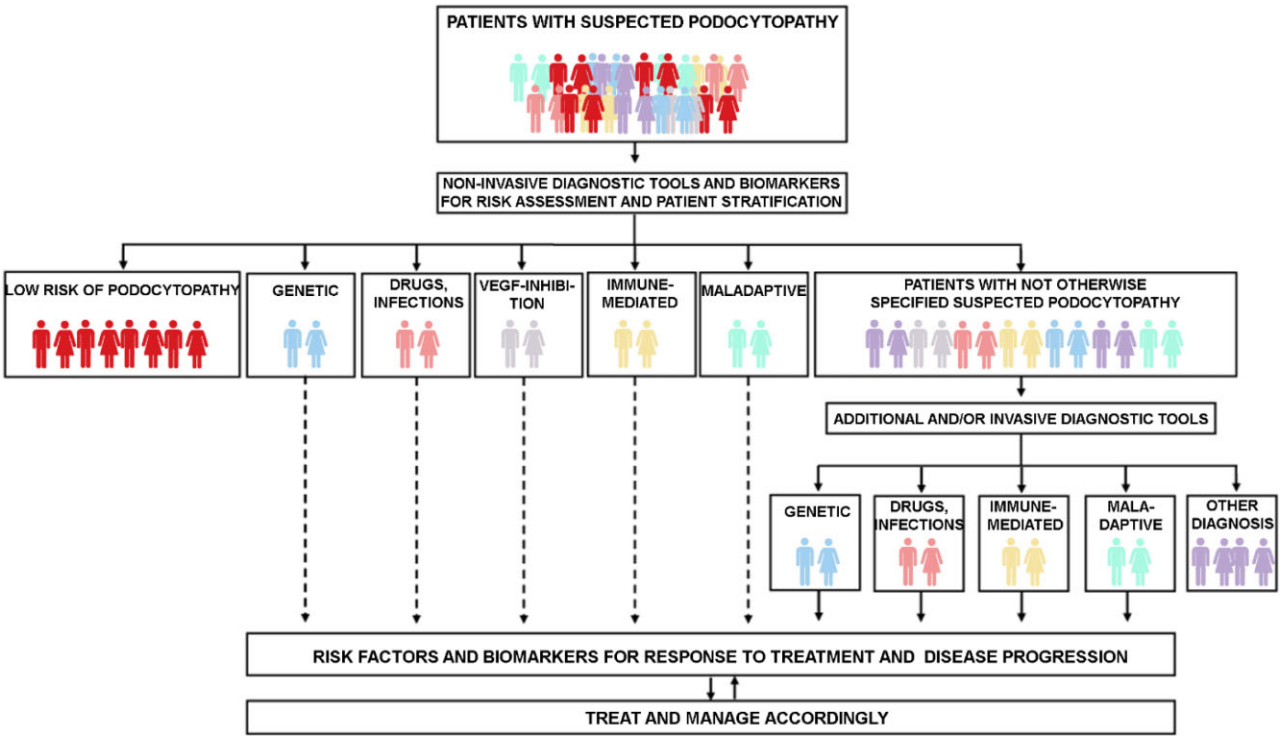


**FIGURE 3:** An ideal approach to patients with suspected podocytopathy. Patients presenting with proteinuria are suspected to suffer from podocytopathies. Ideally, non-invasive diagnostic tools and biomarkers should help in stratifying patients by distinguishing those at low risk of podocytopathy from those at high risk of this group of disorders. Among them, diagnostic first-line tools should address the medical need to identify the underlying cause and pathomechanisms driving podocyte injury. In case of inconclusive or unclear results, additional diagnostic tools, including invasive ones, should be taken into account in the clinical work-up. This additional step should result in the complete classification of disease-causing drivers of podocytopathies and the exclusion of alternative, clinically overlapping, diagnoses. Irrespective of the step when the aetiologic diagnosis is reached, the final step of the ideal clinical management is the assessment of risk factors and biomarkers predictive of response to treatment and disease prognosis, which could eventually result in tailoring treatment by balancing benefits with potential risks and side effects.

diseases have already been published [106]. Although still in its infancy, the clinical utility of WGS could improve the diagnostic efficacy of genetic investigations in the field of podocytopathies. Other technical issues (i.e. analysis of uncovered regions, copy number variations) should also be assessed in a case-by-case setting.

As for many other diseases, we currently classify the results of genetic testing according to strict pathogenicity criteria based on a classical Mendelian concept of genotype–phenotype correlation [109]. However, the genetic make-up of podocytopathies is probably more complex than previously thought. High-frequency and low effect-size variants in podocyte genes (e.g. G1/G2 risk alleles in *APOL1*) already proved relevant in contributing to disease onset and progression when coupled with additional 'hits'. Interestingly, in an experimental model, the equivalent of the human p.R229Q polymorphism in *NPHS2* was associated with the development of proteinuria and ultrastructural glomerular alterations only in ageing mice or when coupled with exposure to nephrotic agents, suggesting a complex pathogenic mechanism [110]. Of note, the second hit could be either genetic or environmental, suggesting a complex pathophysiology of podocyte damage.

## PODOCYTOPATHIES IN KIDNEY TRANSPLANT

Post-transplant recurrence of glomerular diseases represents a relevant contributor to graft failure, together with acute and chronic allograft rejection [111, 112]. Most of the literature about disease recurrence in podocytopathies refers to FSGS. Of note, this term is used as a synonym for disease entity in the majority of studies. Bearing in mind this limitation, the evidence of FSGS relapse immediately after kidney transplantation improving with plasma exchange or immunoadsorption [112, 113], the report of a case of relapsing FSGS resolving after explantation and retransplantation in another recipient with no history of FSGS [114] and experimental models of glomerular lesions induction after treatment with serum from patients with relapsing FSGS [115] strongly support the role of the immune system and/or permeability factor/s in the pathogenesis of a subset of cases of FSGS. The risk of FSGS recurrence is as high as 60% in the first graft and up to 100% in the second, with a risk of graft loss of 40%–60% [111, 112]. Risk factors include recurrence in a previous graft, age at starting kidney replacement therapy >12 years, White and Asian recipients and rapid course to ESKD (<3 years). Notably, the strongest predictor of FSGS recurrence is initial steroid sensitivity [111, 116]. Conversely, protective factors include age at starting kidney replacement therapy <6 years, African–American recipients, genetic and syndromic NS [111].

Disease recurrence after kidney transplant affects three main areas of interest in the field of podocytopathies: (i) clinical, since the course of disease after kidney transplantation correlates to its specific underlying pathogenetic mechanisms; (ii) aetiological, since the occurrence of disease relapse claims against

**Table 2. Summary of innovations and advances in podocytopathies pathophysiology, diagnosis, management and treatment**

| Fields of innovation | Cutting-edge features and relevant issues |
|---|---|
| Improving podocytopathy classification and diagnosis | - High-throughput technologies, like proteomics, may provide innovative diagnostic tools.<br>- Non-coding RNA (e.g. micro-RNA) and single-cell RNA sequencing in serum and urine can represent innovative non-invasive diagnostic essays.<br>- SRM could provide the advantage of allowing 3D visualization of the slit diaphragm, giving direct evidence of structural changes or podocyte loss and a panoramic view otherwise not available with the current techniques. |
| Understanding the complex genetic background of podocytopathies and interplay with environment | - Genetic variants in intronic and regulatory regions of DNA can be relevant in determining phenotypic consequences.<br>- High-frequency and low effect-size variants in podocyte genes (e.g. G1/G2 risk alleles in APOL1) can contribute to disease onset and progression when coupled with additional 'hits', with the second hit being either genetic or environmental. |
| Coupling new pathophysiology discoveries with the development of new treatments | - The GBM and podocytes themselves are considered as novel potential targets for molecules acting on mitochondrial function and actin–myosin contractile structure. New strategies targeting the short transient receptor potential channels, the soluble FMS-like tyrosine kinase 1, soluble urokinase plasminogen activator surface receptor and substrate intermediates for coenzyme Q10 are under investigation.<br>- Other efforts have been directed to blocking the progression of scarring and fibrosis following podocyte damage in patients affected by FSGS targeting the inhibition of C-C chemokine receptor type 2, the nuclear factor-$\kappa$B transcription and Slit-2.<br>- Genetic discoveries will probably play a major role towards personalized medicine, thus tailoring therapies with the best chance of response in carefully selected patients. |
| Tackling disease recurrence in transplant recipients | - Genetic testing before transplantation may be a potential strategy to maximize patient characterization by detecting genetic causes of the disease.<br>- Serum anti-nephrin autoantibodies in patients with podocytopathy recurrence after kidney transplantation may serve as non-invasive diagnostic marker for the pathogenesis of disease relapse.<br>- Serum levels of anti-CD40 have been associated with FSGS, with good accuracy to predict post-transplant recurrence.<br>- Plasma of relapsing patients induces the expression of specific genes (e.g. IL-1beta gene) in cultured podocytes, a potential diagnostic tool to distinguish podocytopathy relapse from other diseases.<br>- The use of SRM to kidney allograft biopsies, together with the implementation of laboratory protocols for the differential diagnosis of disease recurrence to other causes of graft failure (e.g. quantification of cell-free DNA), may serve as additional differential diagnostic tools. |

some causes of disease, while supporting others; (iii) predictive, since quantifying the risk of disease recurrence is pivotal for the post-transplant management of patients and for properly designing clinical trials for therapeutic strategies. Podocytopathies caused by specific aetiologies (e.g. maladaptive, drugs, etc.) do not recur after kidney transplantation, since the leading cause no longer exists. Genetic diseases are supposed not to recur after a transplant. The reported risk of 4%–8% [117] likely depends on an expired attribute of pathogenicity to genetic variants (e.g. in *NPHS2*). Therefore, reports of disease recurrence in genetic podocytopathies should be reassessed according to the current classification system of genetic variants. The label 'primary FSGS' (also known as idiopathic FSGS or circulating factor disease) refers to non-genetic FSGS, whose pathogenic mechanism is thought to be an immune system dysregulation and/or a permeability factor [112, 116, 118]. In this view, disease recurrence after kidney transplantation can be considered as an *ex-post* clue element for the aetiological classification of the primary disorder. However, precision medicine has the goal of being proactive, preventing and predicting instead of merely observing [119]. This target is particularly relevant for kidney transplant medicine due to the shortage of organs together with the increasing use of marginal donors and the clinical complexity of transplant recipients, making the need to precisely assess the clinical risk profile of patients affected by podocytopathies undeniable. Despite its relevance, many factors still prevent this issue from being properly assessed: the correct diagnosis of disease recurrence in clinical studies; the high frequency of patients with CKD of unknown origin undergoing kidney transplantation, hampering the possibility to clearly distinguish disease recurrence or *de novo* disease; the extreme variability in the time of disease recurrence, supporting the contribution of different mechanisms; the availability of pre-transplant graft biopsy and different policies for protocol biopsies [120]. Of note, the diagnosis of podocytopathy recurrence in kidney allograft still relies on pathologic findings at kidney biopsy. As in the native kidney, histologic lesions should be considered as patterns of disease instead of disease-claiming clues. Indeed, pathologic lesions belonging to the spectrum of podocytopathies can be caused by injurious mechanisms other than disease recurrence (e.g., long-term use of CNIs, episodes of AKI, compensatory hypertrophy, etc.), especially at increasing time from graft surgery. Consequently, defining a risk-stratifying strategy for patients, together with advances in the accuracy of diagnostic tools, is

**Table 3. Comprehensive classification of podocytopathies according to clinical history, laboratory investigations, histologic features on kidney biopsy, response to treatment, genetic findings and post-transplant behaviour**

| | Drug-induced podocytopathy | Infection-related podocytopathy | Pregnancy-associated podocytopathy | Lupus podocytopathy | Malignancies-associated podocytopathy | Maladaptive podocytopathy | Genetic podocytopathy | Presumed permeability factor-related podocytopathy |
|---|---|---|---|---|---|---|---|---|
| Clinical history | Onset after drug administration. Proteinuria generally resolves if therapy is discontinued. | Recent or concomitant infections (more commonly viral infections). | Onset of proteinuria (or NS) during pregnancy. Full-blown preeclampsia strongly suggests this diagnosis. | Uncommon manifestation of SLE. The clinical hallmark is NS. | Onset temporarily related to associated neoplasias. | Clinical (e.g. LBW, prematurity, nephrectomy, etc.) or US evidence of loss-nephron mass. | Evidence of familial history of kidney disease, extra-renal involvement, resistance to steroid or immunosuppressants. | Absence of any identifiable aetiologic factor, previous clinically evident episodes. |
| Laboratory findings | Sub-nephrotic proteinuria is more common than nephrotic proteinuria. | Nephrotic-range proteinuria with/without NS. AKI is a common feature. | Nephrotic-range proteinuria, NS. | NS in the absence of active sediment. | Sub-nephrotic proteinuria, nephrotic-range proteinuria with/without NS. | Sub-nephrotic proteinuria; nephrotic proteinuria; NS is uncommon. | Nephrotic-range proteinuria, NS. | NS with SD and FR features. |
| Pathological findings | MC, FSGS, CG Acute or chronic interstitial nephritis are frequent. Tubuloreticular inclusions may be present at EM. | MC, FSGS, CG Tubular microcystic dilation, tubular cell mitosis, apoptosis and interstitial fibrosis may be present, together with tubuloreticular inclusions at EM. | MC, FSGS, CG Podocyte hypertrophy and vacuolation are detected without prominent endotheliosis; occasional non-specific immunoglobulin or complement trapping; with negative fibrinogen staining. | MC, FSGS Mesangial cells and matrix increased; inconstant mesangial immune deposits. Mesangial ED deposits, occasional sub-epithelial or sub-endothelial ED deposits and tubuloreticular inclusions at EM. | MC, FSGS Acute TIN and even massive inflammatory infiltrate may be present. | FSGS | MC, FSGS, DMS GBM alterations may be present at EM. | MC, FSGS Inconstant immune deposit of IgM and/or C3 may be present. |
| Response to treatment | Resolution or improvement of the clinical picture after drug withdrawal. | Resolution or improvement of the clinical picture with specific therapy or infection eradication. | Resolution or improvement of the clinical picture after delivery. | Complete remission with steroid isolated therapy. | Resolution or improvement of the clinical picture with treatment of underlying neoplasia. | Improvement with antiproteinuric therapy (e.g. RASi). Additional benefits from functional imbalance control (e.g. body weight control). | Lack of response to steroids and immunosuppressive therapies. | Complete remission with steroids and immunosuppressive therapies. |
| Genetics | | *APOL1* G1/G2 risk alleles can be found. | | | | Genetic variants in the CAKUT genes support the diagnosis. | Genetic variants in podocyte or phenocopy genes. | |
| Kidney transplantation | Do not usually recur | Do not usually recur | Do not usually recur | Do not usually recur | Do not usually recur | Do not usually recur | Do not usually recur | Recurrence after kidney transplant |

SD, steroid-dependant; FR, frequently-relapsing; CAKUT, congenital anomalies of the kidney and urinary tract; ED, electron-dense.

worth the effort in order to personalize the clinical approach to patients affected by podocytopathies undergoing kidney transplantation.

## INNOVATIONS IN TACKLING DISEASE RECURRENCE

The correct classification of a disease affects not only the possibility to develop new therapies but also the clinical management of transplant recipients. In the field of podocytopathies, this strongly impacts the exact quantification of the risk of disease recurrence and the burden on the overall graft survival. Many studies have explored the association of demographic or clinical variables with the risk of disease recurrence [121]. According to this goal, performing genetic testing before transplantation in otherwise unexplained cases has been suggested as a potential strategy to maximize patient characterization by detecting genetic causes of the disease [112]. Despite the need to be confirmed in additional studies, serum anti-nephrin autoantibodies in patients with podocytopathy recurrence after kidney transplantation may represent a non-invasive diagnostic marker of immune system mechanisms responsible for the pathogenesis of disease relapse [66]. Serum levels of anti-CD40 have been associated with FSGS, with good accuracy to predict post-transplant recurrence [122]. A phase II clinical trial with the CD-40 antagonist bleselumab (blocking T cell co-stimulation to antigen presenting cells, including B cells) enrolling kidney transplant recipients is currently underway [123]. Moreover, it has been observed that the plasma of relapsing patients induces the expression of specific genes (e.g. IL-1beta gene) in cultured podocytes. This could possibly be used as a diagnostic tool to distinguish podocytopathy relapse from other diseases [6, 124]. Extending the use of SRM to kidney allograft biopsies, together with the implementation of innovative tools for the differential diagnosis of disease recurrence to other causes of graft failure (e.g. quantification of cell-free DNA) [125] would represent additional strategies for personalization of kidney transplant medicine, with relevant consequences also for the development of appropriate therapeutic strategies for disease recurrence. All the innovations and avdances presented in this review are summarized in Table 2.

## CONCLUSIONS

Podocytopathies represent a fascinating challenge for precision medicine. In recent years, the efforts of nephrology research have prompted a revision of the classification of podocytopathies based on pathophysiological mechanisms responsible for damage to the GFB. Awareness of the need to integrate information on the pathogenic mechanisms of podocyte injury allowed disease classification to go beyond histology (Table 3) [1, 2]. Indeed, kidney biopsy provides a 'snapshot' of pathological mechanisms active at that time in the organ but frequently misses the cause of the disease. Failure in detecting any contributing component to the pathogenesis of podocyte injury potentially exposes patients to unnecessary or off-target treatments. Cutting-edge genetic, microscopy and high-throughput biological techniques allow diagnostic trajectories to be increasingly focused, pushing nephrologists to get out of the framework of podocytopathies as pure histological entities, in favour of a new paradigm aimed at treating the specific disease of the patients (Table 3). Podocytopathies share an unmatched experimental advantage: translational studies aimed at improving podocyte knowledge could potentially provide a solid foundation for the discovery of disease biomarkers, improving the identification of molecular and cellular mechanisms acting in the disease process and integrating them with the detection of the underlying risk factors that could decompensate nephrons beyond the threshold of kidney reserve. Ideally, precision medicine should provide tools for evaluating an individual's risk of developing podocytopathies even before the clinical onset of the disease, identifying disease-causing mechanisms, providing an accurate diagnosis, estimating prognosis, predicting response to treatment and the risk of disease recurrence, thus tailoring clinical management and therapeutic strategies (Fig. 3). Even if still far from being satisfactorily accomplished, this goal should be pursued in any patient affected by podocytopathies to the aim of optimizing available therapeutic strategies and maximizing long-term prognosis.

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The authors declare no conflict of interest.

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