Chapter . Minimal Change Disease.

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Abstract:

Minimal Change Disease (MDC) is the most common type of nephrotic syndrome in childhood. Currently, advancements in podocyte biology suggest that podocyte may play a pathogenic role in the development of proteinuria in this disease. In the majority of children, MCD presents a relapsing pattern that may persist to adulthood. Steroids are considered the drug of choice to control proteinuria. Recently, new therapeutic modalities including ACTH and rituximab have been tried with variable results. There is a need for multicenter, randomized, controlled studies to define the efficacy of these drugs in MCD.

Keywords: Minimal change disease, nephrotic syndrome, proteinuria, podocyte, CD80, nephrin, cytokine, prednisone.

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Abbreviations: In order of appearance in the text:

MCD- Minimal Change Disease.

Ig- Immunoglobulin.

IL- Interleukin.

CD80 – cluster of differentiation 80

CTLA-4- Cytotoxic T-lymphocyte-associated protein 4.

IFN- Interferon.

INS- Idiopathic nephrotic syndrome.

TNF- Tumor necrosis factor.

VEGF- Vascular endothelial growth factor.

PBMC- Peripheral mononuclear cells.

GBM- Glomerular basement membrane.

PDGF- Platelet-derived growth factor.

TG- Transgenic.

HSP- Heparan-sulfate proteoglycans.

Angptl-4- Angiopoietin-like 4.

FP- Foot processes.

LPS- Polysaccharide.

SCID- Severe combined immunodeficient.

Poly:IC- Polyinosinic:polycytidylic acid.

TLR- Toll-like receptor.

kDa- kilodalton.

PA- puromycin aminoglycoside.

PHN- Passive Heymann nephritis.

ManNAc- Acetylated N-acetylmannosamine.

pI- Isoelectric point.

FSGS- Focal segmental glomerulosclerosis.

CCT- Cortical collecting tubule.

ENaC- Epithelial sodium channel.

ISKDC- International Study of Kidney Disease of Children.

BUN- Blood urea nitrogen.

mg- milligrams.

g- Grams.

URI- Upper respiratory infection.

mEq- Milliequivalents.

Kg- kilograms.

IV- Intravenous.

APN- Arbeitsgemeinschaft für Pädiatrische Nephrologie.

FR- Frequent relapsing.

KDIGO- Kidney Disease: Improving Global Outcomes.

RCT- Randomized controlled trial.

SD- Steroid-dependent.

FR- Frequent relapsing

CNI- Calcineurin inhibitor.

MMF- Mycophenolate mofetil.

Vs- Versus.

ACTH- Adrenocorticotrophic hormone.

FDA- Food and Drug Administration.

MC1R- Mineralcorticoid-1 receptor.

SMPDL-3b- Sphingomyelin phosphodiesterase acid-like 3 b protein.

ASMase- Acid-sphingomyelinase.

ISKDC -

1. Introduction.

Minimal Change Disease (MCD) refers to a type of nephrotic syndrome characterized by the presence of podocyte foot process fusion on electron microscopy and by the absence of major structural glomerular changes and immune deposits on light microscopy and immunofluorescence respectively (1). The terms lipoid nephrosis, minimal lesion, nil disease, have been used as synonyms for MCD.

Among the different types of idiopathic nephrotic syndrome, MCD displays a higher rate of remission to steroids and the best long-term outcome despite the relapsing course of the disease observed in most of MCD patients (2).

2. Epidemiology.

MCD is the most common type of nephrotic syndrome in children and accounts for about 97% of all cases with nephrotic syndrome under 4 years of age. After that, the frequency decreases steadily reaching about 50% of all cases between 8 and 16 years of age (3). The incidence of MCD in < 16 years has been estimated at 2-7 cases/100,000 and the prevalence at 16/100,000. MCD is more predominant in boys (2:1) during childhood but presents with a similar gender distribution among adolescents (4). In adults, MCD represents only the 20% of cases of nephrotic syndrome (5).

3. Etiology.

In the majority of patients, especially in children, MCD is a primary glomerular disease. Secondary causes are shown in Table 1 (6). Relapses in patients with MCD have been reported to follow exposure to inhaled allergens, foods, insect stings, and vaccinations. It is clear that some

patients with MCD may present with nephrotic syndrome after an allergen exposure, and many patients with MCD have increased serum Immunoglobulin (Ig) E levels. Although allergens occasionally have been implicated in triggering nephrotic syndrome in patients with MCD, evidence that blocking the specific allergic agent may prevent relapse is weak. This suggests that the atopic response is associated with the immune abnormality in patients with MCD, rather than having a causal role (6).

A strong clinical association has been established between MCD and Hodgkin's disease. MCD represents the most common form of nephrotic syndrome in Hodgkin's disease (7). However, the incidence of MCD remains very low in patients with Hodgkin's disease (0.4%) (8, 9). Overexpression of C-mip, an 86-kDa protein recruited to lipid rafts, has been found in Reed-Sternberg cells and podocytes from those patients with Hodgkin's disease who developed MCD (10). C-mip has been suggested to interfere with nephrin phosphorylation by blocking the tyrosine kinase Fyn interaction with nephrin, leading to cytoskeleton rearrangement and proteinuria. Although the observation is tantalizing, it remains to be determined whether C-mip displays a casual role in MCD in Hodgkin's disease or may represent an epiphenomenon in the context of podocyte injury.

A study on polymorphisms of interleukin-4 (IL-4) related genes found a lower frequency of the T allele in MCD patients than controls. IL-4 has been associated to atopy. However, the above study did not include any patient with atopy in the control group whereas 7 out of 57 MCD patients presented with asthma or atopy (11). Thus, the clinical significance of IL-4 polymorphism is unclear. The prevalence of a CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) polymorphism (+49GG genotype), associated with decreased expression of CTLA-4, is significantly increased in MCD patients compared to normal controls (12). Impaired CTLA-4

production may play a role in proteinuria as CTLA-4 is the natural inhibitor of CD80, a key molecule in the development of proteinuria in MCD.

MCD is rarely seen in more than one member of a single family. In such cases, MCD may present with a pattern of steroid dependence or resistant, which altogether may suggest an underlying mutation in any of the gene encoding for slit diaphragm proteins.

4. Pathogenesis.

MCD has been assumed to be result of a dysregulation of the immune system. Shalhoub, in 1974, postulated MCD to be a T-cell disorder by which a circulating cytokine (s) released by T cells leads to increase permeability of glomerular basement membrane (GBM) to plasma protein and proteinuria (13). This hypothesis was founded on some clinical and pathological observations: 1) absence of electron-dense deposits, immunoglobulins and complements in MCD patients' glomeruli, 2) remission of proteinuria induced by steroids and cyclophosphamide or spontaneous remission induced by measles; thought to be mediated by suppression of cell mediated immunity, 3) association of MCD with Hodgkin's disease. This hypothesis led to investigators to search for the pathogenic circulating cytokine by using different approaches (see below).

4.1. Circulating Cytokines in MCD.

Studies of cytokines in serum from INS patients have often showed variable results. Thus, serum levels of IL-2, IL-4 and interferon (IFN) -γ have been reported to be high, low or unchanged in patients with idiopathic nephrotic syndrome (INS) during relapse compared to those in remission (14-16). While some authors have reported increased serum levels of IL-2 receptor, IL-5, IL-10, TNF (tumor necrosis factor) -α, VEGF (vascular endothelial growth factor) in INS patients during relapse, others did not find differences in these interleukins among those patients

in relapse compared to those in remission (17-20). The discrepancy among studies may be explained by the lack of kidney biopsy in many patients, therefore including patients without MCD, lack of standardization of assays among authors or the concomitant use of immunosuppressive therapy. An alternative explanation is that the above mentioned cytokines do not play a role in MCD.

Of all cytokines, IL-8 and IL-13 have been suggested to play a major role in proteinuria in MCD. We (21) and others (22) have shown that IL-8 is increased in serum in MCD patients during relapse when levels are compared to those patients in remission. These patients also present with increased IL-8 mRNA on unstimulated peripheral blood mononuclear cells (PBMC) during relapse. In addition, IL-8 infusion into rats resulted in increased ^{35}sulfate (isotope) uptake by the glomerular basement membrane (GBM), decreased in glycosaminoglycans and albuminuria. Finally, the addition of anti-IL8 antibody abolished the changes associated with IL-8 infusion in the rat. These findings suggest a causal link between circulating IL-8 and proteinuria caused by an augmented glycosaminoglycans catabolism (23). In contrast, infusion of other cytokines, such as VEGF, TNF-α or platelet-derived growth factor (PDGF), into rats did not result in proteinuria. Overall, there is strong clinical and experimental evidence supporting a role of IL-8 in proteinuria in MCD through its effect on GBM glycosaminoglycans.

A role of circulating IL-13 in in the pathogenesis of proteinuria has been suggestive by studies in an IL-13 transgenic (TG) rat model reported by Lai (24). These TG rats developed increased levels of serum IL-13 followed by glomerular expression of CD80 (a costimulatory surface protein), podocyte effacement and proteinuria. Of interest, in this animal model, serum IL-13 did not correlate with proteinuria. The clinical significance of the TG model of IL-13 is challenged by the lack of consistent serum IL-13 levels in MCD. Some investigators have reported

increased IL-13 levels in serum of INS patients (16, 18). However, in some of these studies, serum levels of IL-13 were higher in INS patients during remission than during relapse, questioning the presumed pathogenic role of circulating IL-13. It is important to emphasize the often concurrence of atopy or asthma in patients with INS. The increased IL-13 serum levels could be related to the underlying immune process (atopy or allergy) or upper respiratory infection rather than being the cause of proteinuria in MCD. Furthermore, we (25) and others (26) found no detectable IL13 levels in serum or unstimulated PBMC from MCD patients during relapse.

4.2. Mechanism (s) of proteinuria.

4.2.1. Role of capillary wall anionic charges.

MCD is characterized by an increased glomerular permeability to plasma proteins, mostly albumin. This has been historically attributed to a defective glomerular charge-selective barrier caused by the loss of fixed negative charges in the capillary wall (27). This was based on the following observations: 1) the permeability, estimated by fractional clearance studies, of anionic dextran sulfate was lower than those dextran molecules with neutral charge despite sharing a similar size and conformation. In contrast, permeability was enhanced by using cationic dextran molecules (28), 2) Diminished uptake of colloidal iron, a polycation, by glomeruli from MCD patients in comparison to controls, suggesting loss of glomerular polyanion (29).

4.2.2. Glomerular capillary wall and anionic charges.

The glomerular capillary wall is composed of three layers (see Figure 7.1):

a) Endothelium: Endothelial cells form a thin, fenestrated layer that lines the inner portion of the capillary lumen. This layer is covered by polyanionic proteins that provide negative charge to its

surface. The endothelium does not appear to play a key role in MCD since its large fenestrations (70-100 nm diameters) may allow the passage of large proteins in normal circumstances (30).

b) Glomerular basement membrane: It is composed by a central dense layer or lamina densa which is surrounded by 2 electro-lucent layers named lamina rara interna and externa when in contact with endothelial or epithelial cells respectively (31). The major components of the GBM are type IV collagen and heparin-sulfate proteoglycans (HSP) including laminin, perlecan and agrin. HSP provides anionic charges to the GBM, which has been considered the defective charge-selective barrier in MCD for years. Indeed, a decreased number of anion sites and heparin sulfate molecules have been found in the GBM of MCD patients (32). Angiopoietin-like 4 (Angptl-4, see below) and IL-8 have been suggested as mediator of negative charges in the GBM. As previously mentioned, IL-8 augments proteoglycans catabolism in GBM leading to a reduction of anionic charges and mild proteinuria.

However, the causative link between the reduction of anionic sites in GBM and development of proteinuria has been challenged by recent studies. Two knock-out mice models, characterized by the absence of agrin and perlecan and the reduction of heparin sulfate respectively, showed no proteinuria or mild proteinuria despite a significant reduction in GBM´s anionic charges (33). In a different transgenic model, the overexpression of heparanase resulted in a reduction of anionic sites in GBM but mice did not develop proteinuria (34).

c) Podocytes: Podocytes are highly differentiated visceral epithelial cells that are anchored to GBM by integrins and are covered by a surface coat rich in sialoglycoproteins (podocalyxin) on its apical side and slit diaphragm. The role of integrins and podocalyxin in MCD remain uncertain. Thus, podocytes remain attached to the GBM in nephrotic states questioning a role of integrins in

MCD. Expression of podocalyxin has been found reduced in MCD by some authors (35), but not by others (36), arguing against a presumed causative role of podocalyxin in MCD.

4.2.3. MCD as a podocyte disease.

Recent advances in the molecular composition of podocytes and slit diaphragm have suggested a shift in the pathogenesis of proteinuria in MCD from the concept of MCD as immune systemic disease to MCD as a podocyte disease (37).

The current hypothesis is that, in MCD, podocyte reacts to different stimuli such as viral particles, allergens, and/or cytokines leading to structural changes at the level of the slit diaphragm causing proteinuria (38).

Podocytes can be divided into three structural and functional segments: cell body, major processes and foot processes (FP). Neighboring podocyte foot processes interdigitate forming filtration slits of ~30–40 nm width, that are bridged by a thin zipper-like pattern membrane denominated slit diaphragm. Nephrin, the most abundant protein of the slit diaphragm, plays a key role in formation and maintenance of slit diaphragm by providing structural support and by regulating signaling pathways linking the slit diaphragm to the actin cytoskeleton.

There is recent evidence supporting CD80, a transmembrane protein expressed by human podocytes as the key molecule leading to foot processes effacement and proteinuria. CD80 overexpression by podocytes and proteinuria was observed in LPS (lipopolysaccharide)-injected mice. However, no proteinuria was observed in CD80 knock-out mice after LPS injection. These observations were independent of T cells as SCID (severe combined immunodeficiency) mice exposed to LPS also developed foot processes effacement and proteinuria (39). The same pattern

of increased CD80 podocyte expression and proteinuria have been observed in the Poly:IC (Polyinosinic:polycytidylic acid) and IL-13 animal models (24, 40).

LPS and Poly:IC stimulate podocyte CD80 expression by activation of podocyte toll-like receptors (TLR) 4 and 3 respectively (41). This is of great relevance as relapse in MCD patients is often triggered by viral respiratory infections and circulating viral particles are known to stimulate TLR-3. Up to 10% of healthy children developed proteinuria during febrile illnesses. In this cohort of patients, we have found increased urinary CD80 excretion compared to afebrile healthy children (unpublished observations). These findings would support the role of circulating microbial products, rather than interleukins, as the trigger of CD80 podocyte expression and proteinuria in MCD patients. In addition, we have found increased podocyte CD80 expression by cultured podocytes after stimulation with sera from MCD patients in relapse when compared to those in remission (25). No such increased CD80 podocyte expression was observed when podocytes were stimulated with supernatants from cultured PBMC from MCD patients in relapse suggesting the presence of a circulating factor in the serum of these patients rather than a factor released by PBMC.

The above experimental findings suggesting a key role of podocyte CD80 as mediator of proteinuria in MCD are supported by growing clinical evidence. We (42-45) and others (46) have consistently found, in different cohorts of patients, an increased urinary CD80 excretion in MCD patients during relapse compared to those MCD in remission, control subjects, or FSGS patients with massive proteinuria. Urinary CD80 excretion normalized in MCD patients once remission is achieved. The source of the urinary CD80 in MCD patients is likely the podocyte based on the following facts: 1) although serum level of soluble CD80 is decreased in patients with MCD during relapse compared to MCD in remission suggesting increased urinary losses, urinary CD80 in MCD

patients has a molecular weight of 53 kilodaltons (kDa), consistent with it being the cell-membrane associated CD80, in contrast to soluble CD80 (23 kDa) that is present in the circulation (42, 43). 2) CD80 podocyte expression in MCD patients in relapse is increased when compared to expression in MCD patients in remission (43).

We have hypothesized that MCD patients in relapse present a persistent CD80 podocyte expression due to an impaired production of podocyte CTLA-4, the natural inhibitor of CD80, suggesting that MCD is the result of an autocrine podocyte dysregulation (45). We have found that urinary CTLA-4 excretion is increased in MCD patients during relapse compared to those in remission. However, urinary CTLA-4 levels did not correlated with urinary CD80, suggesting a suboptimal CTLA-4 response to the increased podocyte CD80 expression observed in MCD. More important, a polymorphism in the CTLA-4 gene, associated to reduced CTLA-4 production, has been reported to be more frequent in MCD patients than in control subjects (12). Finally, administration of CTLA4-IgG1 to a MCD patient with high urinary CD80 excretion and undetectable urinary CTLA-4, resulted in plummeting of urinary CD80 at 24 hours followed by resolution of proteinuria during 9 days (47). These findings provide strong clinical evidence of the causative link between podocyte CD80 expression and proteinuria in MCD, as suggested by experimental studies.

The mechanism (s) by which CD80 induces cytoskeleton changes and proteinuria remains unresolved. We have found increased CD80 expression and decreased phosphorylated nephrin expression in podocytes exposed to sera from MCD in relapse (poster communication, ASN 2014). However, nephrin phosphorylation was preserved when human podocytes were pre-incubated with CTLA-4, prior to be cultured with sera from MCD patients. This finding suggests that podocyte CD80 may reduce nephrin phosphorylation, likely by disrupting binding of the tyrosine kinases

Fyn and/or Nck binding to nephrin (Figure 7.1). In addition, we and others have found reduced glomerular phosphorylated nephrin by immunohistochemistry in MCD patients (48).

Human Angptl-4, a 45-65 kDa glycoprotein has been proposed as a mediator of proteinuria in MCD by Chugh et al (49). They found glomerular expression of Angptl-4 increased in several animal models of proteinuria including the LPS, puromycin aminoglycoside (PA), Buffalo Mna rats and passive Heymann nephritis (PHN).

LPS-injected wild-type mice developed higher albuminuria than that observed in Angptl-4 knock-out mice (Reference). A transgenic rat model that overexpressed Angptl-4 in podocytes developed by these authors had marked loss of GBM heparan sulfate proteoglycans associated with foot processes effacement and albuminuria at 3 months of age compared to control rats. Proteinuria was reduced by treating tap water with acetylated N-acetylmannosamine (ManNAc), a sialic acid precursor, by shifting Angptl-4 from a high to a neutral isoelectric point (pI). Based on these observations, the authors proposed that in MCD, podocytes secrete Angptl-4 (mostly with pI>8) that migrates to the GBM and endothelium resulting in reduced GBM anionic charges and, therefore, proteinuria (49). In a subsequent study, the same group reported increased plasma levels of Angptl-4 in animal models of proteinuria and in patients with nephrotic syndrome secondary to MCD, focal segmental glomerulosclerosis (FSGS), non-HIV collapsing nephropathy and membranous nephropathy (50).

Data on Angptl-4 in MCD patients during relapse are scarce and only reported by the same group. Angptl-4 oligomers (220 kDa) with pI <8 were detected in urine in 4 MCD patients. One of these patients also had urinary Angptl-4 molecules (55-70 kDa) with a pI>8. In addition, Angptl-4 oligomers (100-160 kDa) with pI<8 and Angtpl-4 molecules (55-70 kDa) with pI> 8 were also detected in blood of few MCD patients (49). In addition, Angptl-4 was mildly expressed, by

immunofluorescence, in kidney tissue from 5 MCD patients in relapse compared to control. Angptl-4 colocalized with podocyte, GBM and endothelial cell markers.

In contrast we have found that urinary Angptl-4 excretion does not correlate with proteinuria in a large cohort of patients with MCD, FSGS and membranous nephropathy. Higher urinary Angptl-4 levels were found in few MCD patients during relapse compared to control subjects (unpublished observations). In contrast to that suggested by Chugh , the source of Angptl-4 detected in urine in MCD is unlikely to be from the podocyte because : 1) we found no or minimal Angptl-4 staining by immunofluorescence in kidney tissue from our MCD patients in relapse, 2) no increased Angptl4 expression was observed in human podocytes cultured with sera from MCD patients in relapse compared to those exposed to sera from MCD patients in remission, 3) contrary to a previous study, Angptl-4 is lower in serum from MCD patients in relapse compared to those in remission. More importantly, Angptl-4 detected in urine from our MCD patients in relapse had a pI of 5.4, in contrast to the high pI reported by others, challenging the significance of the role of charges or pI in the development of proteinuria.

Overall, our findings from a large and well-defined cohort of MCD patients do not support a role of podocyte Angptl-4 in proteinuria in MCD. It seems that urinary Angptl-4 in these patients is the result of a defective glomerular filtration barrier to plasma proteins.

5. Pathological features.

MCD is a glomerular disease. On **light microscopy** the glomeruli show no or "minimal" abnormalities. Some specimens may depict slight increase in mesangial matrix and cellularity. Visceral epithelial cells look normal. The presence of hypertrophied or proliferation of podocytes as well as capsular adhesion are not consistent with MCD.

Globally sclerotic glomeruli in contrast with glomeruli showing segmental sclerosis are also consistent with a diagnosis of MCD only if they represent a small number (<5%) of total glomeruli and are not associated with surrounding tubular atrophy and/or interstitial fibrosis (51).

Although natural senescence of glomeruli may be observed, their clinicopathological interpretation is difficult since there are no well-defined standards for the number of glomeruli affected in normal subjects with age, especially for those under the age of 20 years (52).

Tubules and interstitium show no specific lesions. However, a few cases may present mild focal tubular atrophy, mild segmental interstitial fibrosis, or inflammation. Fat and hyaline droplets may be found in the proximal tubule.

By **immunofluorescence microscopy**, the glomeruli are usually negative for immunoglobulins and complement components. If present they are low in intensity, confined to the mesangium, and not associated with electron dense deposits. A few specimens may have diffuse mesangial Ig M staining which have led to some authors to define it as a different glomerular disease (IgM nephropathy). However, the current consensus regards the presence of IgM in these patients' glomeruli as inconsequential (3). Similarly, predominant C1q deposits have been occasionally described in mesangium from patients with idiopathic nephrotic syndrome. The significance of these C1q deposits, in the absence of immune complex disease such as systemic lupus erythematosus is not clear. The prognosis appears to be associated to the lesions observed on light microscopy, MCD or FSGS, rather than to the intensity of C1q staining.

On **electron microscopy**, the only consistent glomerular finding in MCD is the effacement of the foot processes. The degree of effacement is related to the activity of the disease. The effacement of the foot processes is not pathognomonic of MCD and can be seen in other conditions

presenting with massive proteinuria. The podocytes are firmly attached to the GBM while the slit pore density is decreased resulting in a decreased in the filtration surface area between podocytes (53). The GBM is usually normal and no electron dense deposits are observed.

6. Clinical manifestations.

The main clinical manifestation of MCD is edema. In MCD, edema is pitting, of sudden onset and gravitational (periorbital in the morning and more evident on legs/ankles in the evening). MCD patients may present with massive anasarca that may lead to pleural and/or pericardial effusion resulting in respiratory distress and bowel edema causing diarrhea. There is a strong correlation between the severity of the edema and serum levels of albumin.

MCD patients may develop abdominal pain and emesis as the result of bowel ischemia due to severe hypovolemia. In addition, hypovolemia may lead to orthostatic hypotension or renin-mediated hypertension. These symptoms are alleviated by expanding the intravascular compartment by intravenous albumin infusion. The presence of severe anasarca and/or hypovolemia is a risk factor for acute pancreatitis, renal thrombosis and spontaneous bacterial peritonitis. The presence of macroscopic hematuria argues against MCD as underlying glomerular disease or may be the result of renal vein thrombosis.

The mechanism (s) of edema formation in MCD remains to be defined. It has been proposed that the massive proteinuria leads to hypoalbuminemia and decreased intravascular oncotic pressure (54). As a result, fluid shifts from the intravascular to the interstitial compartment leading to edema and intravascular depletion. The hypovolemia results in renal hypoperfusion which activates the renin-angiotensin-aldosterone axis and sympathetic nervous system. Indeed in MCD patients there is evidence of hypovolemia as well as increased renin and aldosterone serum

levels. The elevated circulating levels of renin, aldosterone and norepinephrine in turn increase the proximal and distal tubular reabsorption of sodium.

There are several arguments that argues against this hypothesis: 1) analbuminemic rats do not develop edema or sodium retention (55), 2) natriuresis and loss of edema that take place during remission occur when patients are still hypoalbuminemic (56), 3) no diuresis is observed after expansion of intravascular compartment with albumin unless a diuretic is given, 4) bilateral adrenalectomy or antialdosterone agents do not reverse sodium retention in MCD patients (57).

The current evidence suggests increased sodium reabsorption in the distal tubule as the primary mechanism leading to edema. Increased reabsorption is triggered by massive proteinuria. This hypothesis is supported by findings in puromycin aminoglycoside rat model of nephrotic syndrome. Ichikawa et al. (58) selectively infused puromycin in one kidney and measured urinary sodium concentration in renal tubules from both rat kidneys by micropuncture technique. A similar amount of sodium was found at the end of distal convoluted tubule in both rat kidneys. However, the final sodium concentration in urine was three times lower in the kidney exposed to PA compared to control kidney, suggesting sodium reabsorption in the cortical collecting tubule (CCT). The latter has been attributed to an increased number of open epithelial sodium channels (ENaC) in the CCT, likely due to the removal of the ENaC gamma inhibitory domain by urinary plasmin (59). Other mechanisms for sodium retention may be also operating since if edema is solely due to ENaC activation, one might have expected a substantial diuretic response with amiloride, a competitive inhibitor of ENaC. However, no such diuretic response to amiloride is observed in MCD during relapse. In addition, sustained activation of ENaC as observed in Liddle syndrome is associated to sodium retention but no edema formation.

7. Laboratory tests.

7.1 Urinalysis.

Nephrotic range proteinuria has been defined in different ways. Thus, proteinuria greater than 50 mg/kg in a 24 hour urine collection in children or greater than 3.5 g/24 hours in adolescents and adults, is considered nephrotic range proteinuria. In children, the International Study of Kidney Disease of Children (ISKDC) defined nephrotic range proteinuria as proteinuria greater than 40 mg/hour/m^2 or 200 mg protein/mmol urine creatinine. ISKDC definition is based on urine collections obtained overnight and not in a 24 hour period. Proteinuria in MCD is highly selective, with a predominance of albumin compared to immunoglobulins or lower molecular weight proteins.

Hyaline casts and fat bodies are often observed in the urine sediment of MCD patients. These bodies are formed by precipitation of albumin and lipoproteins, respectively, with Tamm-Horsfall mucoprotein secreted by renal tubule cells.

Microscopic hematuria may be observed in 20-30% of patients, most of who present with signs of hypovolemia. Thus, it has been thought that hematuria may be consequence of renal ischemia as hematuria resolves once remission is achieved.

7.2. Hypoalbuminemia.

Hypoalbuminemia is the hallmark of nephrotic syndrome. The main mechanism leading to low serum albumin levels is the increased glomerular filtration permeability to plasma proteins. In addition, it is controversial if there is also an increased catabolism of filtered albumin by proximal

tubule cells (60). The current evidence suggests that albumin is reabsorbed intact at that level. The liver production of albumin is increased in MCD patients and gastrointestinal losses are minimal.

7.3. Hyperlipidemia.

Hyperlipidemia is a common feature of patients with nephrotic syndrome. It is the result of an increase hepatic synthesis of cholesterol and triglycerides and a decreased catabolism of lipoproteins. The latter is due to increased urinary losses of albumin which leads to an excess of fatty acids in plasma that will inhibit lipoprotein lipase in fatty tissues and serum. In addition, urinary losses of lecithin cholesterol acyltransferase lead to a reduction of chylomicrons and VDLD clearance.

7.4. Hematology.

Elevated hematocrit is often observed in MCD patients during relapse as many of them present with signs of hypovolemia. Those patients with persistent nephrotic syndrome may develop normocytic-normochromic anemia. This is not due as initially thought to transferring iron losses resulting in iron deficiency because iron stores are normal in most of MCD patients and anemia is not hypochromic. The anemia in MCD has been shown to be due to erythropoietin deficiency (Reference).

7.5. Electrolytes.

Serum electrolytes are usually within the normal range. Hyponatremia is occasionally observed in MCD. It may represent dilutional hyponatremia or pseudohyponatremia, which is found in those patients presenting with severe hypercholesterolemia. In these cases, a normal serum osmolality

will make the diagnosis of pseudohyponatremia. Diuretic therapy may also result in hyponatremia and together with fluid restriction may lead to hypernatremia due to free water deficit.

7.6. Calcium and vitamin D.

Total calcium is consistently decreased in serum of MCD patients during relapse as result of hypoproteinemia. However, ionized calcium is usually within normal range. Urinary losses of vitamin D metabolites binding proteins result in lower serum concentration of these metabolites. However, symptomatic hypocalcaemia or bone disease (except for that associated to the prolonged use of steroids) is rarely seen in MCD patients. Thus, there is no indication to use calcium or vitamin D supplement routinely in MCD patients.

7.7. Complement and IgG levels.

Some patients with MCD lose immunoglobulin in their urine and may have hypogammaglobulinemia. A loss of certain complement proteins such as factor B and properdin may also occur (61). As a consequence, subjects with MCD are at increased risk for bacterial infections, especially from encapsulated organisms. Interestingly, serum C3 levels are elevated in MCD (61).

7.8. Serum creatinine and blood urea nitrogen.

Serum creatinine and blood urea nitrogen (BUN) are usually within normal range in MCD patients. However, mild increased in both serum markers may be found in those MCD patients who present with marked hypovolemia during relapse. In those cases, restoration of normovolemia after albumin infusion will result in normalization of serum creatinine and BUN.

8. Natural course of the disease.

The natural course of MCD before steroid therapy was available cannot be defined because the absence of histological diagnosis in all cases with nephrotic syndrome. There have been only two groups that have compared the long-term outcome of biopsy-proven MCD patients treated with and without prednisone. The study by Black et al. (62) in adults included a total of 31 patients. The remission rate at 4-year follow-up was similar among patients treated with and without steroids. About 50% of patients in the control group underwent spontaneous remission at 15 months follow-up with 20% having persistent proteinuria after 4 years.

Coggins (63) compared the long-term outcome of adults with MCD treated with an alternate regimen of prednisone (average 125 milligrams (mg)/day for 2 months) compared to a placebo group. In agreement to the study published by Black, the percentage of patients achieving complete remission was not statistically different among the 2 groups (93% vs 64%, prednisone vs placebo respectively) at 77 months follow-up.

Similarly, Coggins observed that 6 of 14 MCD patients receiving prednisone (125 mg on alternate days for 2 months) underwent complete or partial remission at 3 months compared to none of 14 patients receiving placebo. However, the long-term outcome of patients from these 2 studies is quite interesting. Of those patients not receiving prednisone, 50% underwent spontaneous remission at 15 months (62) and 80% and 64% of patients had proteinuria <1 gram (g)/day at 48 and 55 months respectively(62, 63). In addition, the rate of remission at 4-year follow-up was similar among patients treated with and without steroids.

Spontaneous remission has been described in children with idiopathic nephrotic syndrome (no histological diagnosis) at the onset of the disease (64) (2%) or in subsequent relapses (65) (31%). Wingen et al. (66) studied the course of 15 and 17 patients with frequently relapsing and

steroid dependent idiopathic nephrotic syndrome (see definitions in Table 7.2)(2) for a mean period of 6 years. Most of patients underwent at least 1 spontaneous remission. Moreover, 23% and 10% of relapses spontaneously resolved in frequent relapsing and steroid dependent idiopathic nephrotic syndrome patients respectively within 2 weeks. These findings led to authors to recommend delaying steroid therapy for few days in the absence of progressive clinical signs to minimize steroid exposure.

8.1. MCD and upper respiratory tract infections.

Upper respiratory infections (URI) seem to trigger relapses in up to 80% cases (67). It is currently thought that circulating viral particles may stimulate toll-like receptors (TLRs) on podocytes resulting in podocyte CD80 overexpression which in turn leads to cytoskeleton rearrangement, opening of slit diaphragm and proteinuria (38, 39). Interestingly, viremia may be also present in subclinical upper respiratory infections and asymptomatic patients.

A leaky glomerular filtration barrier has been proposed as an innate defense mechanism in order to accelerate the shedding of circulating viral particles. Not surprisingly, febrile illnesses are not only associated with a full relapse in MCD but also can result in transient proteinuria in otherwise healthy patients. Therefore, in an MCD patient, it is critical to distinguish between transient proteinuria as result of URI from a full relapse in order to minimize steroids exposure.

MCD patients may develop proteinuria during or shortly after an URI. The rate of relapse during URI was reduced from 48% to 18% when patients received a 7-day course of daily prednisolone at the onset of upper respiratory symptoms (68).

9. Treatment.

9.1. Symptomatic therapy.

9.1.1. Diet. Protein intake requirements in MCD patients are 100% of the recommended daily allowance according to age-predicted stature. High-protein diets result in worsening proteinuria and hypoalbuminemia. In contrast, low-protein diet reduces proteinuria and hypoalbuminemia but leads to malnutrition. Fluids restriction is not advised during the acute phase of edema formation since fluids will aggravate the hypovolemia. If intravenous albumin infusion is administered during this stage, fluids need to be restricted to at least 2/3 of daily maintenance. The same guidelines for fluid restriction are recommended in those patients who are hemodynamically stable. A critical step to control edema formation and avoid steroids side effects is sodium restriction, with a maximum sodium intake of 2 milliequivalents (mEq)/kilograms (Kg) /day in children and a 2 g/day sodium chloride diet in adolescents and adults. The sodium restriction is continued until steroids are tapered off.

9.1.2. Physical activity. Reduced physical activity and bed rest may be recommended in those patients who develop severe anasarca or orthostatic hypotension.

9.1.3. Edema. Sodium restriction is the first action needed in order to reduce edema. Patients with severe anasarca may benefit of intravenous infusion of albumin 25% (1 g/kg of dry weight) followed by diuretics. Albumin infusion will increase the intravascular oncotic pressure shifting fluid from the interstitium to the vascular compartment. Thus, a rise in blood pressure is often observed during albumin infusion enhancing renal perfusion. Interestingly, no massive diuresis is observed despite of fluid shifting unless diuretic therapy is used. Albumin is typically infused over a period of 2 to 6 hours according to the patient's hemodynamic status - 2 hours if the patient is

hypotensive, 4 hours if normotensive and 6 hours if initially hypertensive. Close monitoring of vital signs and clinical status is critical to determine the rate of albumin infusion. Blood pressure should be measured at 30 minute to hourly intervals because albumin infusion may lead to severe hypertension and/or respiratory and cardiac failure.

There are different approaches on when to administer diuretic therapy (usually a loop or distal tubule diuretic) during and after albumin infusion. It is our policy to administer one dose of intravenous (IV) furosemide 1 mg/kg immediately after albumin infusion is completed and then every 8 hour for the following 24 hours as the oncotic effect of infused albumin lasts approximately 24 hours.

9.1.4. Hyperlipidemia. Chronic hyperlipidemia is a risk factor for cardiovascular disease. However, hyperlipidemia resolves upon resolution of proteinuria, which is usually achieved in MCD patients. Therefore no statin therapy is routinely recommended in MCD patients.

9.1.5. Infections. Infections represent one of the most worrisome complications in nephrotic syndrome and they were the main cause of death prior to antibiotic therapy. The increased urinary losses of IgG and complement factors lead to an opsonization defect which puts nephrotic syndrome patients at high risk for Streptococcal pneumonia infections. Prophylaxis with oral penicillin may be indicated during relapse despite the increasing pneumococcal resistance to this antibiotic.

9.1.6. Immunizations. The use of immunosuppressive therapy enhances the risk for viral infection such as varicella. Thus, varicella and pneumococcal vaccination are critical in MCD patients. It is advised to administer these immunizations after 3 months off immunosuppression therapy in order to maximize the immunological response. The efficacy of the pneumococcal vaccine is based on

the bacterial opsonization by anti-pneumococcal immunoglobulin. Low levels of this immunoglobulin are found during relapse of nephrotic syndrome, which may explain why pneumococcal peritonitis still occurs in immunized patients during relapse.

9.2. Treatment of MCD.

9.2.1. Control of proteinuria

The use of steroids in MCD was initially empirical and resulted in increased diuresis in nephrotic patients. Subsequently, their use was justified on the concept that MCD was a T cell disease in which T cells released a cytokine (s) leading to increased glomerular permeability to proteins (Shalhoub's hypothesis) (13). However, the validity of this hypothesis remains uncertain 40 years later given the lack of evidence supporting a role of circulating cytokines in the pathogenesis of proteinuria in MCD.

Molecular discoveries made in the last 20 years have provided a better understanding of the glomerular filtration barrier and the role of podocytes in proteinuria (69). Thus, the concept of MCD has shifted from what was initially thought to be a systemic disease to the current concept of podocyte disease (37). One of the strongest arguments supporting a role of cytokines in MCD was the fact that steroids and cyclosporine induce remission of proteinuria in most patients. However, it has been shown that both steroids and cyclosporine exert a direct effect on podocytes through different mechanisms (48, 70). A decreased in nephrin phosphorylation has been found in kidney tissue from MCD patients in relapse and from rats treated with puromycin aminoglycoside (48). It has been suggested that dysregulation of nephrin phosphorylation, likely caused by podocyte CD80 (unpublished observations) or C-mip overexpression (10), leads to a sequence of events resulting in podocyte foot processes effacement. Interestingly, steroids enhance nephrin

phosphorylation in cultured human podocytes (71). On the other hand, cyclosporine blocks the calcineurin-mediated de-phosphorylation of synaptopodin, which is critical to maintain integrity of the actin cytoskeleton (70). Therefore, both steroids and cyclosporine, aside from their known immunomodulating properties, may act at the level of the podocyte preserving its molecular structure.

Corticosteroids are the cornerstone therapy to induce remission of proteinuria in patients with MCD, leading to remission in up to 95% and 80-90% of children and adults respectively. Although steroid-sparing agents are often used in selected MCD patients, data on their use as first line therapies are scarce. An 8-week course with cyclophosphamide result in resolution of proteinuria in more than 70% of patients. Cyclosporine given to MCD patients induced remission in all patients in a period of 7 to 23 days (72). In addition, the use of cyclophosphamide is limited given its well-known dose-related gonadal toxicity. Likewise, prolonged courses of cyclosporine may result in irreversible renal damage. Altogether, the use of steroid-sparing agents as first line therapy in MCD should be reserved for selective cases such as those diabetes mellitus.

Steroid therapy in MCD includes 2 different stages: 1) Induction phase, in which steroids are administered on a daily basis to induce remission, followed by 2) tapering phase, in which steroids are switched from daily to alternate regimen and tapered down.

9.2.2.1. Induction therapy.

In 1981, the ISKDC published the outcome of 471 children with nephrotic syndrome (363 patients with MCD) treated with steroids (2). The ISKDC arbitrarily defined a treatment protocol for the induction phase of prednisone 60mg/day/m^2 -roughly equivalent to 2 mg/kg- (maximum of 80 mg/day) given in 3 divided doses for four weeks. In this study, most of the MCD patients

(93.1%) underwent remission after an 8-week prednisone course. In fact, 94% of the patients labeled as responders underwent remission by the 4th week of with an average time to remission of 13.3 days.

In the ISKDC study, prednisone was administered in divided doses. In 1989, the Atlanta group demonstrated that prednisone given at 2 mg/kg as single-morning daily dose resulted in complete remission in all 17 patients with MCD in a mean time of 9.6 days (73). This study led to many nephrologist to administer prednisone in a single rather than divided doses.

Data on the treatment of initial episode of nephrotic syndrome in adults are scarce. As mentioned above, the studies by Black and Coggins showed that patients treated with prednisone went into remission more rapidly than those receiving placebo The rate of response to prednisone in adults is less than that observed in children. Complete remission is seen in 80% of adults with MCD, with only 50% occurring within the first month of therapy and with 10-25% patients requiring 6 to 12 weeks of therapy.

The length of induction therapy for the initial episode of nephrotic syndrome has not been defined. It has varied from 4 (2) to 8 weeks (74) (regardless of when remission is induced) to a 3 (75) to 10 (76) day course upon resolution of proteinuria.

In a subsequent study, the Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) compared the outcome of children with initial episode of nephrotic syndrome treated with the ISKDC protocol (60 mg/m²/day for 4 weeks followed by 40 mg/m²/day for 4 weeks) [“short therapy”] versus a 60 mg/m²/day for 6 weeks followed by 40 mg/m²/day for 6 weeks regimen [“extended therapy”] (77). The cumulative rate of patients with sustained remission after 2 years was significantly higher in the extended therapy group compared to the short regimen without

increasing the risk for severe steroid side effects. In addition, a frequent relapsing (FR) pattern was observed more often in those patients under the short compared to the extended prednisone regimen (57% and 29% respectively). Although tantalizing, these results were not confirmed by the Southwest Pediatric Nephrology Study Group (78). In their study, the relapse rate was not statistically different between patients receiving the "short" (average of 28 days) versus "extended" (average of 42 days) induction therapy with prednisone.

In our practice, we complete induction therapy with daily prednisone 2 mg/kg/day for 10 days after resolution of proteinuria, a course based on the length of time it takes for serum albumin to normalize. In our experience, this regimen leads to similar rate and timing of remission that than reported by the ISKDC.

The Japan Cooperative Study Group of Kidney Disease in Children compared two regimens of prednisolone (79). For group A, patients received prednisolone 60 mg/m²/day for 6 weeks compared to those in group B who received the same dose but for a 4-week duration. At the end of the induction period, both groups had a similar timing of remission (median of 10 days in both groups) and rates of response (34/35 and 36/38 patients respectively). Based on this study, a longer induction did not result in a faster or higher rate of remission

The addition of cyclosporine to full dose of prednisone in the induction phase did not increase the remission rate nor shorten the timing of remission.

In children, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines 2012 (80)recommended the induction treatment of the initial episode of nephrotic syndrome in children with daily oral prednisone or prednisolone at 60 mg/m²/day or 2 mg/kg/day (maximum 60 mg/day) for 4-6 weeks. In adults, the recommendation is a single dose of prednisone at 1 mg/kg (maximum

of 80 mg/day) or alternate-day single dose 2 mg/kg (maximum of 120 mg) until complete remission (minimum of 4 weeks to a maximum of 16 weeks).

In summary, there are several guidelines for MCD treatment but as shown by the Canadian-American, and Italian pediatric nephrologists' surveys (78, 81), the guidelines are not followed**.**

9.2.2.2. Tapering therapy

The rationale to taper prednisone is to prevent early relapses and to reduce the risk of side effects such as pseudotumor cerebrii or adrenal insufficiency. During the tapering phase, it is widely accepted that prednisone should be administered on alternate instead of intermittent days (3 consecutive days in a week) (82). However, there is no agreement on the duration of the tapering phase or the dose of prednisone. Thus, the tapering varies from a 4-week period followed by abrupt discontinuation as proposed by the ISCKD to a slow tapering that ranged from 6 weeks up to 7 months proposed by the Japanese group.

This variability was highlighted by the Southwest Pediatric Nephrology Study in centers in the USA and Canada. This retrospective study compared a "standard" versus an "extended" prednisone tapering which ranged from 4-12 weeks for the former and 6-14 weeks for the latter group. Despite the different length of induction and tapering phase, the number of patients suffering relapse was not significantly higher in the standard compared to the extended group (78). Bagga et al (74) showed that an extended prednisolone regimen to 4 months delayed the time to first relapse but there was no statistically difference in the percentage of patients with relapse at 1 year (72.7% extended vs. 91.3 % standard group). The Japan Cooperative Study Group of Kidney Disease in Children reported a longer sustained remission and fewer cases of frequent relapsing cases in children treated with a 28-week prednisolone course compared to those receiving a 12-

week course (79). The relevance of different tapering regimens in these 2 studies (4 weeks vs. 8 weeks respectively) is limited as the induction regimens also differed among groups in each study (4 week vs 8 week respectively).

In 2015, a Cochrane systematic review concluded, based on 3 well-designed randomized controlled trial (RCT), that 6-month prednisone course does not reduce the risk of relapse compared with a 2 or 3-month course in children aged 1-17 years at presentation (83). In our practice, we complete a 6 to 8-week tapering phase starting 10 days upon resolution of proteinuria.

The 2012 KDIGO guidelines for children with idiopathic nephrotic syndrome recommended oral prednisone at 40mg/m² or 1.5 mg/kg on alternate days and continued for 2-5 months with tapering of the dose. The recommendation is more vague for adults, suggesting a slow tapering over a total period of up to 6 months (80).

9.2.2. Natural course of nephrotic syndrome after steroid treatment.

MCD is a relapsing disease in the majority of patients. In 1980, Engle et al. (84) reported a case of late recurrence of nephrotic syndrome in a MCD patient who had been in remission for 19 years after childhood. Subsequently, numerous authors have shown that children with MCD often suffer relapses during adulthood (from 5.5 % to 42%) (85, 86) . A higher number of relapses per patient per year or a frequent relapsing pattern during childhood were risk factors for relapse during adulthood (85).

The ISKDC provided arbitrary definitions regarding the relapse pattern after a 4-week induction phase followed by a 4-week tapering phase (see table 2)**.** However, these concepts have been widely applied by practitioners despite using different induction and/or tapering regimens. Distinction on the pattern of relapse is often misinterpreted making difficult any comparison of

different therapy regimens among studies. One may question whether the significance of a steroid dependent (SD) pattern is similar among different centers given the variability of the tapering schedules. For instance, extended tapering regimens tend to use a very low dose of prednisone towards the end of the tapering period.. Thus, a patient may be labeled as steroid dependent because of a relapse on a much lower dose of steroid as compared to another patient considered steroid dependent according to ISKDC definitions. In summary, longer tapering schedules will lead to a higher number of patients with a "steroid dependent" pattern.

In addition, the original definition of steroid dependence does not take into consideration the role of URI as trigger of nephrotic syndrome. Therefore, it is critical to differentiate the presence of proteinuria associated with viral illness from a full-blown relapse before labeling a patient as steroid-dependent.

This variability is of critical relevance when comparing long-term outcomes of patients labeled as frequent relapser or steroid-dependent to medications including newer drugs such as rituximab and ACTH.

The ISKDC reported the long-term outcome of 363 MCD patients. 93% of patients achieved remission within 4 weeks. Of these patients, 36% had no further relapse and 18% and 39% had an infrequent and frequent relapse pattern (see table 2 for definitions). Koskimies et al. (87) reported a higher rate of frequent relapse pattern (53 %) among children with idiopathic nephrotic syndrome who responded to an initial course of prednisone (78 of 94 children had confirmed MCD). Of these patients, 64% became free of relapse after 3 years. 22% and 24% of patients had infrequent or no relapses after the initial episode of nephrotic syndrome.

9.2.3. Steroid therapy for nephrotic syndrome in relapse.

As for treatment of the initial episode, there is no consensus as to the use of prednisone in the induction and tapering phases. The ISKDC recommended prednisone at 60 mg/m²/day until response (maximum of 4 weeks) followed by prednisone 40 mg/m²/day in 3 consecutive days in a week for a total of 4 weeks (2). Similarly, the APN (77) and Japanese group (79) treated relapses with prednisone 60 mg/m²/day until resolution of proteinuria for 3 days, followed by prednisone 40 mg/m²/48 hours for 4 weeks. Bagga et al. (74) suggested prednisolone 2 mg/kg for 2 weeks and then 1.5 mg/kg on alternate days for 4 weeks. As mentioned previously for treatment of an initial episode of MCD, controlled studies have shown that regimens based on a prolonged course or higher cumulative dose of prednisone do not alter the pattern of subsequent relapses.

Resolution of proteinuria is followed by massive diuresis and resolution of edema despite the presence of hypoalbuminemia, which usually normalizes within 7-10 days after remission. In contrast, it may take months for hyperlipidemia to normalize.

9.2.4. Therapy for frequently relapsing and steroid dependent nephrotic syndrome in MCD.

As shown in Table7. 2, according to the ISKDC, frequent relapsing (FR) is defined by the presence of 2 or more episodes of relapse within 6 months while off steroid therapy for 2 weeks, while steroid dependency (SD) is defined as relapse during the tapering phase or 2 weeks after cessation of steroid therapy.

Historically, studies have included patients without differentiating between frequent relapsing and steroid dependent pattern among these patients. Several drugs have been used to spare the use of prednisone to avoid prolonged exposure to steroids as side effects of high dose

steroids for a prolonged period include cataracts, hypertension, stature growth impairment and obesity.

Chlorambucil (0.15 mg/kg/day for 56 days) or cyclophosphamide (2 mg/kg/day for 56 days) used in combination with low dose prednisone have been shown to induce sustained remission (72% patients at 30 months after cytotoxic drug) in MCD patients with multiple relapses (88). The ISKDC found fewer episodes of relapse (48% vs 88% at 22 months) among those patients with multiple relapses treated with a 42-day course of cyclophosphamide (5 mg/kg./day until induction of cytopenia followed by 1-3 mg/kg/day along with prednisone 10 mg/m²/day for 10 days) **c**ompared to those on an intermittent dose of prednisone (40 mg/m²/3 out of 7 days for 6 months) (89). It is suggested that cyclophosphamide should not be started until the patient has achieved complete remission with prednisone to avoid hemorrhagic cystitis.

Chlorambucil and cyclophosphamide also have serious side effects such neutropenia, hemorrhagic cystitis, late malignancy and gonadal toxicity. Therefore, it is recommended not to exceed a cumulative dose of 168 and 8 mg/kg respectively for these two medications. Second courses of these medications should not be administered.

Prolonged use of cyclophosphamide up to 12 weeks in MCD patients presenting multiple relapses has led to controversial results. The APN reported a higher rate of remission after 2-year follow-up in patients receiving cyclophosphamide for 12 weeks compared to those treated for 8 weeks (67% vs 22% respectively) (90). No such difference was observed by Ueda et al. (91) when compared a 12-week vs 8-week cyclophosphamide regimens in MCD patients after 5 years (24% vs 25% respectively). No benefit was observed when cyclophosphamide was administered

intravenously at 500mg/m² in a monthly base for 6 months. The use of chlorambucil did not add any benefit to the results observed with cyclophosphamide..

Treatment with cyclosporine results in a similar rate of remission compared to cyclophosphamide and chlorambucil in MCD patients with multiple relapses. However, its long-term efficacy is hampered by the onset of relapse shortly after calcineurin inhibitor (CNI) withdrawal (92). CNI may be also considered as second agent in MCD patients who relapse frequently. Cyclosporine is recommended at an initial dose of 5 mg/kg/day, and needs to be adjusted to maintain trough serum levels between 100-150 ng/ml. The length of therapy varies from 12 to 24 months. Mild to moderate cyclosporine-associated nephrotoxicity has been reported in up to 1/3 of MCD patients treated with cyclosporine for more than 3 years (93). Tacrolimus appears to have similar efficacy than cyclosporine but has the benefit of less side effects..

Less data are available on the use of mycophenolate mofetil (MMF) in MCD patients with multiple relapses. Gellermann (94) designed a randomized, open-label, cross-over study to compare the efficacy of MMF vs. cyclosporine in these patients administered during remission (as defined by ISKDC). Patients treated with cyclosporine had a longer free period of relapse during the first year. Only 15% of patients relapsed during cyclosporine therapy compared to 36% patients during MMF therapy. However, this difference was not statistically significant. In another randomized control trial (95), 12 children with biopsy proven MCD and multiple relapses were assigned to received MMF or cyclosporine for 1 year. Patients on MMF group had a higher risk for relapse though the difference did not reach statistical significance likely due to the small sample size.

As previously mentioned, the above studies included patients with multiple relapses but they do not differentiate between patients with steroid dependency or those consider to be frequent relapsers. In 1978, we suggested that these patients have a different response to cyclophosphamide (76). We observed that patients with the frequent relapsing pattern responded very well with a 70 % remission rate after 2 years, while those with steroid dependency did poorly with only 38 % of patients in remission after 3 months of completing therapy. Validating the evaluation of these patients separately, when we combined these two type of patients, the relapse rate was similar to previous studies. These findings were confirmed by APN study in 1983 using the same therapeutic schedule (88). Therefore, we do not recommend the use of either cyclophosphamide or chlorambucil in the steroid dependent MCD patient.

The term steroid dependent nephrotic syndrome needs also to be strictly defined. Relapse while on prednisone should be the sine qua non of the term. As mentioned above, the interpretation of studies comparing outcomes and response to therapy is hampered by the use of prednisone tapering of variable length and dose. Thus, the minimal amount of prednisone to consider a patient as SD may vary from patient to patient but should not be less than 10 mg/day or 20 mg every other day. One alternative approach for the Steroid Dependent patient is to continue with steroids at the lowest dose that keep patient in remission, given on alternate days. A dose of prednisone less than 0.5 mg/kg/day should not interfere with statural growth.

9.2.5. **Other medications in MCD.**

9.2.6.1. Mizoribine. One RCT including 197 children with frequent relapse showed no different rate of relapse among treatment and placebo groups (96). Thus, mizoribine is currently not recommended in nephrotic syndrome.

9.2.6.2. Azathioprine. The ISKDC in a controlled, randomized study concluded that azathioprine has no effect in the relapse rate of children with steroid-sensitive nephrotic syndrome (97).

9.2.6.3. Levamisole. Levamisole anthelminthic agent that has been shown to reduce the risk of relapse in patient with frequent relapsing and steroid dependent NS in single center randomized controlled trials (98, 99). Despite these favorable results and an overall safe profile, the use of levamisole is hampered by the lack of a large-multicenter RCT confirming its efficacy and its unavailability in many countries including USA.

9.2.6.4. Adrenocorticotrophic hormone (ACTH). The use of ACTH was first reported in nephrotic syndrome in 1950 (100). It was found that most of the patients treated with ACTH experienced massive diuresis with about half of them having clinical remission for at least 3 months. For years, ACTH was the first line agent to treat nephrotic syndrome and was approved the Food and Drug Administration (FDA) as therapy for nephrotic syndrome, alongside with steroids and cyclophosphamide. However, the introduction of prednisone for MCD treatment with its benefits of low cost, similar efficacy, and ease of administration halted the use of ACTH in MCD.

The majority of recent data on ACTH in nephrotic syndrome focus on patients with membranous nephropathy. Furthermore, in the passive Heyman nephritis model, ACTH seems to exert its antiproteinuric effect via podocyte MC1R receptor (101).

There is very limited data on ACTH in MCD. In a retrospective case series (102), one MCD patient, previously treated with steroids, mycophenolate and calcineurin inhibitors; received 80 subcutaneous units of ACTH gel twice weekly during 4 months without observing any reduction in proteinuria. In a prospective open-label study (103), two MCD patients resistant to

other immunosuppressive therapy (steroids, mycophenolate, tacrolimus and rituximab in 1 patient) were treated with ACTH for 24 weeks. One patient remained nephrotic at the end of the trial and the other MCD patient underwent partial remission during ACTH trial but relapsed shortly after completion of ACTH therapy. Thus, there is no clinical evidence to support the use of ACTH in MCD at this time. There is an ongoing randomized controlled trial to assess the efficacy and safety of ACTH versus placebo in children with frequent relapsing and steroid dependent syndrome

9.2.6.5. Rituximab. A large number of observational studies have been published in the last decade on the use of rituximab in nephrotic syndrome induced by a variety of glomerulopathies including MCD. Only recently, however, have a few randomized controlled studies been published.

Despite a decade of clinical experience with rituximab in MCD, the mechanism (s) by which it may induce remission is unclear. Since the putative circulating factor likely to cause proteinuria in MCD has been assumed to be a cytokine released by abnormal T cells and rituximab acts on B cells, it has been suggested that B cells have a pathogenic regulatory role on T cells in MCD. This pathogenic role could be "reversed" by rituximab. However, the evidence to support a role of B cell on T cells in MCD is lacking (104). More recently, rituximab was found to bind sphingomyelin phosphodiesterase acid-like 3 b protein (SMPDL-3b) expressed in podocytes, suggesting that rituximab may play a direct, rather than immune-mediated, role on podocytes. SMPDL-3b expression is decreased in kidney tissue from FSGS patients who had recurrence of proteinuria as well as in cultured podocytes treated with sera from FSGS patients with recurrence (105). It has been hypothesized that decreased expression of SMPDL-3b may lead to decreased acid-

sphingomyelinase (ASMase) activity in the raft microdomains which in turn could contribute to actin cytoskeleton remodeling by a mechanism(s) yet to be determined. The above experimental data supports that rituximab may target the podocyte in FSGS but its role in MCD has not been established.

Since 2011, four randomized controlled trials (3 of which came from the same group) were designed to determine the efficacy and safety of rituximab in nephrotic syndrome. Magnasco et al. (106) found that rituximab failed to reduce proteinuria at 3 months in patients with steroid resistant nephrotic syndrome compared to those not receiving rituximab. Only 7 MCD of 31 patients were included in this study. Ravani et al. (107) suggested that rituximab and lower doses of prednisone and calcineurin inhibitors were non-inferior to standard doses of steroids in patients with steroid-dependent nephrotic syndrome. The authors reported that patients in the rituximab group had less proteinuria and a fewer number of relapses at 3 months. However, the relevance of this study to support the use of rituximab in MCD is limited by several facts: 1) it is unclear if the power analysis is based on a non-inferiority-based model as sample size for the 4 groups included in the study are not specified, 2) only 6/27 and 13/27 patients had biopsy-proven MCD in the control and intervention group (rituximab) respectively, 3) patients with high dose steroid dependency (0.7 mg/kg/day) were excluded, 4) it is unclear how many of the patient were in relapse or remission at the time of randomization, 5) data interpretation after 3-month follow-up is limited since many of patients from the control group were switched to the treatment group at 3 months, 6) 75% of patients in the rituximab group relapsed at 1-year follow-up.

The same authors (108) recently reported a non-inferiority randomized controlled trial including children with steroid-dependent nephrotic syndrome in remission. The intervention group received one dose of rituximab at randomization, followed by steroid tapering after 1 month,

similarly to the control group. The primary outcome of the study was the degree of proteinuria within 3 months after randomization. Proteinuria was lower at 3 months in the rituximab group although it did not reach statistical significance. Interestingly, only 34% of patients in the rituximab group relapsed at 1 year follow-up in contrast with the 75 % previously reported by the same group. Of interest, patients on this study received a single dose of rituximab and had steroid dependency at higher dose prednisone (>0.7 mg/kg/day) compared to the already cited previous study in which patients received 1 or 2 doses of rituximab and had a lower degree of steroid dependency.

Finally, Iijima et al. (109) found that patients with "complicated" SD or FR nephrotic syndrome receiving weekly rituximab for 4 weeks after remission had been induced had a significantly longer relapse-free period and fewer relapses compared to those patients receiving placebo (median time to first relapse 267 versus 101 days and 1.54 versus 4.17 relapses/person/year respectively). Treatment failure defined as relapse by 13 weeks after randomization, or steroid resistance between weeks 1 and 53 or FR/SD pattern between weeks 13 and 53, was more frequent in control patients (20/23) compared to those receiving rituximab (10/20). No relapses were reported in the rituximab group during the period of B-cell depletion. The authors concluded that rituximab should be considered as an effective treatment for children with complicated FR/SD nephrotic syndrome during remission. However, such a conclusion may be premature based on the results of this study: 1) Based on authors' definition for treatment failure, 50% of patients in rituximab group failed therapy; 2) most/all patients relapsed in both groups (17/20 vs. 23/23, rituximab vs. control respectively) by the end of the study; 3) the patient population in this study was heterogeneous in terms of being frequent relapses or steroid dependent and in terms of their immunosuppressive regimens and patients included in this study did not

follow the same prednisone tapering used by ISCKD. In addition, the authors presented the long-term outcome of all patients as a group but did not differentiate among those patients presenting with a frequent relapsing versus steroid dependent pattern at the beginning of study. Thus, it is unclear whether FR and SD patients have a similar response to rituximab. In this study, more than 70% of the included patients had steroid side effects such as hypertension, short stature, diabetes, glaucoma, cataract, obesity and osteoporosis. Patients in the rituximab group received a significantly lower cumulative prednisone dose during 1-year follow-up compared to those in the control group. However, no statistically difference in height-for-age Z score and blood pressure were found among both groups of patients at the end of the study.

In summary, the current evidence supporting a role of rituximab in the treatment of MCD is not conclusive. Randomized controlled trials with a larger and well defined cohort of patients and long-term follow-up (>1 year) are needed to assess the efficacy and safety of rituximab before its widespread use in MCD.

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Table 7.1. Secondary causes of Minimal Change Disease.

Drugs.

- Antimicrobials: ampicillin, cefixime, rifampicin
- NSAIDS: ibuprofen, naproxen, zomepirac, indomethacin, fenoprofen, piroxicam, diclofenac
- Lithium
- Probenecid
- Penicillamine

Neoplasms

- Hodgkin's lymphoma
- Non-Hodgkin's lymphoma
- Thymoma

Infections

- Syphilis
- Tuberculosis
- Mycoplasma

Atopy

- Not proven cause-effect: Pollen, dairy product, bee sting, poison oak/ivy

Table 7.2. Clinical definitions in Idiopathic Nephrotic Syndrome.

- Remission: Three consecutive days of trace or negative proteinuria on dipstick or rate of urinary excretion of protein < 4 mg/h/m².
- Initial responder: Attainment of complete remission within initial 8-week of corticosteroid therapy (4 week for KDIGO 2012).
- Relapse: Three consecutive days of ≥3+ proteinuria on dipstick or reappearance of proteinuria ≥ 40 mg/h/m².
- Steroid resistance: failure to achieve remission after an 8-week course of corticoid therapy.
- Infrequent relapse: One relapse within 6 months of initial response, or one to three relapses in any 12-month period.
- Frequently relapse: Two or more relapses within 6 months of initial response, or four or more relapses in any 12-month period.
- Steroid dependence: Two consecutive relapses during corticoid therapy, or within 14 days of ceasing therapy.

Figure 7.1: Molecular anatomy of the podocyte foot process (FP) and actin cytoskeleton in healthy state (left) and in Minimal Change Disease (MCD) during relapse (right). In MCD, microbial products and/or interleukins (ILs) target toll-like receptors (TLR) promoting CD80 and C-mip expression which in turn reduce nephrin phosphorylation by binding Nck and/or Fyn proteins. This may result in a dysregulation of the downstream pathway that links nephrin with cytoskeleton resulting in rearrangement of the actin cytoskeleton.

SMPDL-3b- Sphingomyelin phosphodiesterase acid-like 3 b protein; ASMase- Acid-sphingomyelinase; ARP 2/3- actin related protein 2/3 complex; WASP- Wiskott-Aldrich syndrome protein; CD2AP- CD2-Associated Protein, P- phosphorylated site; DG- dsytroglycans.