STEROID RESISTANT NEPHROTIC SYNDROME IN CHILDREN – A CONTINUUM OF CHALLENGES

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Abstract: Nephrotic syndrome represents one of the most frequent glomerular diseases among the pediatric population and while most of the children respond to steroid treatment, almost 20% of them are steroid-resistant. In this report, we present a case study of a 2 years old male with Steroid Resistant Nephrotic Syndrome (SRNS), complicated with spontaneous bacterial peritonitis and sepsis. We underline diagnostic and treatment challenges associated with this pathological entity, as patients with SRNS are at risk of developing chronic kidney disease (CKD), immunosuppressive-related complications as well as acute events such as thromboembolism. This field requires further in-depth studies, as the underlying immunological and genetic mechanisms are largely heterogenous, but we emphasize the multiple clinical-biological aspects that SRNS can present in a single patient, representing a real challenge in pediatric practice.

Keywords: Nephrotic Syndrome; Minimal Change Disease; Steroid Resistant; Pediatrics

Introduction: Nephrotic syndrome (NS) is a glomerular disease characterized by massive proteinuria, hypoalbuminemia, edema and hyperlipidemia, although the last two may not be present in all patients. Definition of NS as by the newest (2022) guideline issued by International Pediatric Nephrology Association (IPNA) (1) is presented in table 1. In pediatric population, NS has an average incidence of 4.7 per 100.000 children, of which an estimate of 12.4% accounts for steroid resistant disease (2). A male to female predominance of 2:1 has been noted (3) with evidence of increased intra-familial incidence when compared to general population (4). Depending on the presence or absence of any systemic disease, NS can be regarded as secondary or idiopathic, the latter representing the most frequent glomerular disease in children (3).

First-line treatment in idiopathic NS consists of corticotherapy, with the majority of patients showing complete remission of proteinuria within 4-6 weeks of daily prednisone/prednisolone, therefore exhibiting steroid-sensitive NS (SSNS). Still, during follow-up, most of them will develop at least one relapse while a certain proportion will experience either cortico-dependence, either steroid resistant nephrotic syndrome (SRNS), defined as absence of remission after a first 4 to 6 weeks course of daily 60 mg/m^2 prednisone. These situations pose difficult diagnostic and therapeutic approaches. Firstly, persistence of massive proteinuria and hypoalbuminemia predispose to a greater risk of developing complications such as acute kidney injury through hypovolemia, thrombosis of renal veins or spontaneous bacterial peritonitis. Secondly, patients that require recurrent

high-dose prednisone, prednisolone or methylprednisolone pulse-therapy for induction of remission are at risk of developing glucocorticoid's deleterious effects such as growth suppression, osteoporosis, glaucoma or cataracts. Thirdly, SRNS can imply a monogenic cause that might not benefit at all from immunosuppressive therapy. In this report, we present the case of a patient diagnosed with SRNS who required multiple induction courses and complex supportive treatment, with evolutive aspects poorly correlated with histological findings.

Case study: Patient M.M., a 2 years old male who was determined to have NS from the age of 12 months, was admitted in our clinic for persistence of edema and oligo-anuria despite multiple courses of glucocorticoids. He is the children of a consanguineous couple with no other medical history apart from NS, for which multiple secondary causes have been ruled out: negative serology for Epstein-Barr virus, cytomegalic virus, Hepatitis B and C, Human Immunodeficiency Virus and syphilis, normal complement and double stranded DNA antibodies levels. Until admission has been receiving multiple courses of both methylprednisolone pulse therapy as well as oral prednisone, with intermittent clinical remission but no complete biological resolution. In the context of his 4th relapse, he was referred to our clinic for further specialized management. The clinical examination revealed: oligo-anuria (< 1 ml/kg/h), palpebral edema and ascites (fig. 1 and 2), hypertension 135/85 mmHg, heart rate 110/minute, respiratory rate 22/minute, with no hearts murmurs or pulmonary crackles. Biological data, as shown in table 2, underlines severe proteinuria with hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia and thrombocytosis. Supportive measures including daily albumin infusion in association with furosemide and dipyridamole were initiated. Taking into consideration his history of lack of response to glucocorticoids alone, monthly Cyclophosphamide 0.5 g/m^2 was initiated alongside with a 3-day course methylprednisolone pulse-therapy 1g/m^2, followed by an alternative regimen of prednisone (60mg/m^2 per two days) and adjuvants (protein-pump inhibitor, vitamin D, potassium chloride). Despite these interventions, no clinical or biological improvement was noted, as also shown in table 2. Facing a SRNS, renal biopsy was performed showing minimal change disease (fig. 3) with positive IgM mesangial infiltration (fig. 4) and fibrinogen deposits on glomerular membrane (fig. 5). In evolution, week 4 of hospitalization, he presented fever with minimum to moderate inflammatory syndrome (C-Reactive Protein 10 mg/L). Spontaneous bacterial peritonitis was suspected, so paracentesis was performed accordingly. The results revealed a transudate, but the peritoneal fluid culture came positive for *Escherichia coli Extended-Spectrum Beta-Lactamase.* Positive hemoculture followed shortly. Treatment with Piperacillin-Tazobactam was initiated according to the antibiogram while also continuing high-dose prednisone for induction of remission, as edema and oliguria persisted in spite of daily albumin infusion and furosemide administration. Thrombosis of renal veins was excluded by Computed Tomography angiography.

Discussion: It is thought that minimal change disease (MCD) NS develops as a disorder of T cells, which release certain cytokine(s) affecting glomerular foot processes, with a consecutive decrease in the synthesis of polyanions. Consequently, the normal negative charge barrier is altered, allowing for albumin leakage. However, the identity of such a single circulating permeability factor remains uncertain and a more sophisticated multifactorial model is arising as a pathophysiological explanation, which

includes numerous candidates as serum circulating factors (SCFs) such as hemopexin, APOL1, anti-CD40 or Angtp14, as well as the concept of a vicious circle of "proteinuria triggering proteinuria" (5). Moreover, some of these SCFs have been associated both with MCD and focal segmental glomerulosclerosis (FSGS). This adds to the debate of whether MCD and FSGS are actually a continuum of the same pathological process. In fact, when a renal biopsy is performed for children with NS, the most common findings consist of MCD showing either normal aspect in optic microscopy (with podocyte foot process effacement in electron microscopy) either mild mesangial proliferation with IgM deposition and less commonly, focal-segmental glomerulosclerosis (FSGC) (6). Histopathology with minimal glomerular damage and mild mesangioproliferative glomerulonephritis has a benign clinical and pathological course without major tubulointerstitial lesions. Instead, focal segmental glomerulosclerosis and diffuse mesangioproliferative glomerulonephritis associate significant tubulointerstitial alterations and an increased risk of developing chronic kidney disease (7).

Regarding IgM deposition, this is not a classical finding in MCD as immunofluorescence is usually negative, but studies argue that IgM nephropathy generally associates with poor steroid response (8,9). Our case aligns with this view, even though in a cohort of 68 children with SRNS, of which 9 with MCD, 26 with mesangio-proliferative glomerulonephritis, 6 membranous glomerulonephritis and 6 FSGS, no statistical correlation between clinical outcomes and histological subtypes were found (10). However, it is certain that MCD, associated or not with IgM deposits, does not always corelate with good clinico-biological outcomes. Even though concomitant infection such as peritonitis and sepsis might be linked to poor response to steroids, the most useful tools for work-up in such a patient are electron-microscopy and genetic testing. Approximately 30% of children with SRNS may exhibit a monogenic cause of their disease, such as mutations in NPHS1 (coding for nephrin), NPHS2 (coding for podocin) or in other more than 30 genes associated with SRNS (11). It is important to note that the possibility of identifying a monogenic cause of SRNS increases with decreasing age and increases to 50 percent in children who are from a consanguineous family (12) - this representing another argument for genetic testing for our patient.

Microscopic analysis of a renal biopsy represents another important way of investigating nephrotic syndromes with onset at pediatric age that have an inadequate response to treatment or develop corticosteroid resistance during evolution (13). Ultrastructural analysis methods include light microscopy, immunofluorescence and electron microscopy. Out of all ultrastructural analysis methods - light microscopy, immunofluorescence and electron microscopy - the latter brings the greatest contribution to both diagnostic process and therapeutical decisions, a fact demonstrated in multiple studies (14,15,16,17). Ben-Bassat *et al.* (18) studied a group of 23 patients with nephrotic syndrome who showed minimal lesions on light microscopy. However, after electron microscopy 17% of cases represented early stages of membranous nephropathy, 4.4% GSFS and 4.4% unclassified glomerulonephritis. At the same time, a recent study evaluating 855 renal biopsies by Zhang *et al.* (19), of which 324 in pediatric patients with nephrotic syndrome, found that in 88.6% of cases the additional information obtained by electron microscopy was crucial for the diagnosis (23.3% of cases represented forms of GSFS).

Treatment possibilities in SRNS include calcineurin inhibitors such as Cyclosporine

and Tacrolimus, alkylating agents such as Cyclophosphamide or the inosine monophosphate dehydrogenase inhibitor Mycophenolate, all of which present serious potential harmful effects, such as medullar aplasia, secondary neoplasms or even posterior reversible encephalopathy syndrome (20).

Otherwise, acute complications may occur in children with NS due to metabolic consciousness of proteinuria, such as hypovolemia (acute renal lesions), infection (edema fluid acts as a culture environment) an increase of ten times thromboembolism venous in comparison then heaven are compared in comparison to the general population (21). In this pandemic period generated by Covid 19, in a pediatric patient, the risk of developing venous thromboembolism is strongly associated with the existence and evolution of underlying pathological conditions. Multisystem inflammatory syndrome in children (MIS-C), which frequently resembles other established diseases, such as thrombotic microangiopathy (22) in a patient with nephrotic syndrome, such as our patient. Acute renal lesions (AKI), in the context of nephrotic syndrome (NS), is a serious and alarming clinical problem (23). Renal angina indications are a construction that aims at a better prediction of AKI by assigning the values of the risk point and signs of injury to the child with acute renal dysfunction (24). In the differential diagnosis of AKI associated with acute tubulointerstitial nephritis or acute / chronic glomerulonephritis can discuss also the hemorrhagic fever with renal syndrome (HFRS) (25). One of the first illnesses to rule out when facing a child with fever, thrombocytopenia, AKI, and acute hepatitis is leptospirosis, as it has a very similar clinical picture with HFRS. In terms of long-term results, it seems that 50 percent of SRN patients will progress to the final stage (26), while SRNs is actually the second most common cause of chronic kidney disease (CKD) in children (27), along with early/ incipient nephropathy in diabetes, due to hyperfiltration lesions (as in nephrotic syndrome where the urinary albumin/ creatinine ratio is the main biomarker) (28). Among all CKD etiologies in children, it is important to mention that NS is associated with the highest risk of thromboembolic events (29), therefore, part of CKD mortality and morbidity can actually be related to these situations. Chronic morbidity affects the quality of life of these patients undergoing long-term hospitalizations, polypragmasia, recurrent interventions. And as survival is not sufficient, identifying medical, psychological, educational and social problems is a necessity to improve the quality of life of these patients (30).

Conclusions: With its optimal treatment remaining still controversial, SRNS is a pathological entity still under research. Our understanding of it would probably benefit a lot out of increased genetic testing and access to electron microscopy. These techniques can also improve clinical practice, as children with monogenic SRNS should not receive immunosuppressive therapy. Systemic infections in patients with idiopathic NS relapses contribute to poor response and extend the treatment duration required to obtain clinical and biological remission.

AUTHOR CONTRIBUTIONS

All authors have read and agreed to the published version of the manuscript. RAB, II, AMLB, ALC, GS, LSG, MAM contributed equally with TIL to this article.

Table 1. Definition of Nephrotic Syndrome as by the newest International Pediatric Nephrology Association (1)

Definition	Nephrotic proteinuria + either hypoalbuminemia (when available) or edema
Nephrotic range proteinuria in children	• proteinuria > 40 mg/h/m^2 - or • ≥ 1000 mg/m^2/day - or • urinary protein creatinine ratio (UPCR) ≥ 200 mg/mmol (2 mg/mg)
Hypoalbuminemia	• < 30 g/L

Table 2. Biological findings at onset, at 14 days and 21 days respectively

Biological findings	*Values at onset*	*Values at 14 days**	*Values at 21 days*	*Normal range*
CBC				
Leukocytes	10.85	12.79	12.47	6.0 – 17.0 *10^3/mm^3
Neutrophils	8.33			1.5 – 8.5 *10^3/mm^3
Lymphocytes	2.3	4.91	5.15	3 – 9.5 *10^3/mm^3
Hemoglobin	9.9		5.5	11.0 - 14.0 g/dL
Thrombocytes	804	6.0	9.0	150-400 *10^3/mm^3
		9.6	950	
		927		
Biochemistry				
Total proteins	33.6	46.18	36.73	60-80 g/L
Albuminemia	6.9			32 – 45 g/L
Total Cholesterol	540	n/a	n/a	100-200 mg/dl
Triglycerides	426	407	603	40-150 mg/dl
Urea	49	803	549	10-40 mg/dl
Creatinine	0.05	33	58	0.38 – 0.54 mg/dl
Uric Acid	6.48	0.2	0.1	2.0 – 5.5 mg/dl
APTT	21.9	8	1	23-34 sec
d-dimers	1232	3.7	6.3	0-250 ng/ml
Alkaline reserve	21	19.4	6	20-28
		671	n/a	
		28	154	
			0	
			22	
Inflammatory markers				

CRP	2.26	0.2	1.2	0 – 5 mg/L
ESR	106	1	3	0-12 mm/1h
Fibrinogen	820	2	n/a	180-400 mg/dl
Urine		n/a	n/a	
24-hour proteinuria	936 mg/kg/24h	n/a	n/a/	

Fig 1 - Palpebral edema. Collection of Nephrology Department, St. Mary's Clinical Emergency Hospital for Children, Iasi

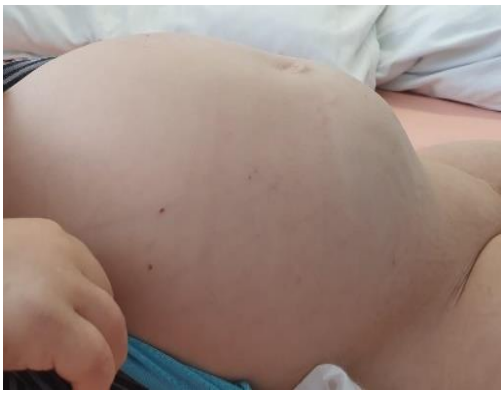

Fig 2 – Enlarged abdomen due to ascites. Collection of Nephrology Department, St. Mary's Clinical Emergency Hospital for Children, Iasi

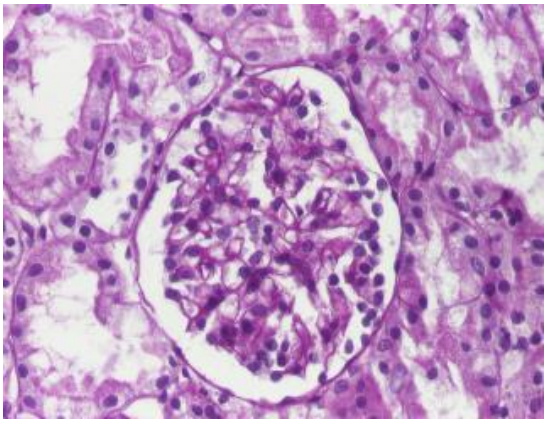

Fig. 3 - Periodic Acid Schiff Coloration on Optic Microscopy x 200 showing normal

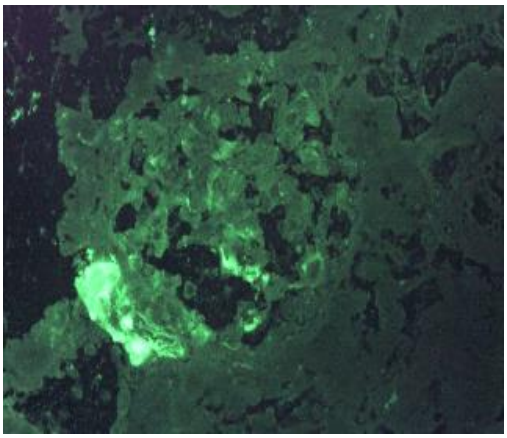

Fig. 4 - Immunofluorescence x 200 showing fine mesangial IgM deposits

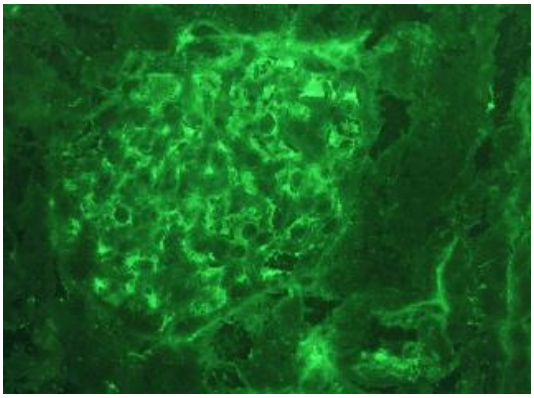

Fig. 5 - Immunofluorescence x 200 showing Fibrinogen deposits

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