“CAROL DAVILA”
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MEDICINE FIELD OF STUDY

Mycophenolate Mofetil- Therapeutic Approach in Idiopathic Nephrotic Syndrome

PHD THESIS SUMMARY

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List of Abbreviations

CP	Cyclophosphamide
CsA	Cyclosporine
GSFS	focal and segmental glomerulosclerosis
KDIGO	*eng* Kidney Disease Improving Global Outcomes
LGM	minimal glomerular damage
MMF	Mycophenolate mofetil
SNCS	corticosteroid-sensitive nephrotic syndrome
SNCD	corticosteroid-dependent nephrotic syndrome
SNCR	corticoid-resistant nephrotic syndrome
RTX	Rituximab

Work Hypothesis and General Objectives

Nephrotic syndrome is the most frequent glomerular disease or podocytopathy encountered at paediatric age. The diagnostic criteria are represented by:

- nephrotic-range proteinuria- defined by urinary protein excretion > 50 mg/kg/day or >40 mg/m2 or according to the KDIGO guide from urine/24h or from the first sample urine protein/creatinine ratio> 2 mg/mg or 3+ on strips.
- Hypoalbuminemia- defined by the value of serum albumin < 3g/dL (30g/L) or by the presence of oedema when we do not have a specified value of serum albumin.
- dyslipidemia (1-4).

The topic approached, Mycophenolate Mofetil (MMF)- therapeutic approach in idiopathic nephrotic syndrome arose from the desire to:

- evaluate different therapeutic alternatives

- study certain particularities of the patient (age, sex, treatment compliance, biological markers) that could represent landmarks in terms of long-term prognosis, disease progression, and anticipation of adverse situations

- to create clinical and paraclinical landmarks, which can indicate an effective therapeutic approach in the short and long term, depending on the chosen treatment.

The PhD thesis study is retrospective, observational, and it included paediatric patients admitted to the Nephrology Department of the Emergency Clinical Hospital for Children “Maria Skłodowska Curie” Bucharest, in the period 2016-2020, diagnosed since 2013.

The clinical and paraclinical data of the patients were extracted from the observation sheets and the electronic database of the Emergency Clinical Hospital for Children “Maria Skłodowska Curie” Bucharest and they concerned:

- the patient’s personal data
- personal and heredo-collateral pathological antecedents
- clinical examination of the patient
- laboratory tests
- the treatment chosen and the evolution
- complications and adverse reactions

We have conducted two studies. The first study aimed at analysing the clinical and paraclinical data by age groups and type of nephrotic syndrome in order to highlight the similarities and differences between patient groups. The second study focused on 2nd-line immunosuppressive treatment with MMF:

- to which groups of patients, it was administered
- if it was a first-line treatment
- if previously administered treatments influenced the evolution under treatment with Mycophenolate mofetil
- what was the evolution of the nephrotic syndrome: how many relapses and at what interval, how much time the remission was maintained
- side effects during treatment period

The patients included in the study were diagnosed with idiopathic nephrotic syndrome and classified as: cortico-sensitive (SNCS), cortico-dependent (SNCD), cortico-resistant (SNCR). The cases of secondary nephrotic syndrome were excluded, the patients being evaluated based on paraclinical data for: systemic lupus erythematosus, vasculitis, viral hepatitis with B or C virus, HIV, and those over 12 years old, based on renal biopsy.

The limitations of this study are the small number of patients who received 2nd-line immunosuppressive therapy and the availability of Mycophenolate mofetil therapy in the past. For the future, this study wants to introduce the treatment with Mycophenolate mofetil in the therapeutic scheme of more patients, the dosage of mycophenolic acid in the blood to correlate the administered dose with the efficiency of the treatment and the comparison of the therapeutic results with the histopathological aspects.

Analysis of the Particularities of the Patients with Nephrotic Syndrome

The study group is represented by 124 patients with idiopathic nephrotic syndrome, aged between 1 and 16 years, diagnosed or evaluated over a period of at least 2 years within the Department of Nephrology of the Emergency Clinical Hospital for Children “Maria Skłodowska Curie” Bucharest. A number of 92 patients (74%) were diagnosed and monitored within the department, and 32 patients (26%) were diagnosed within other paediatric hospitals, but they continued monitoring within the Nephrology Department of the Clinical Hospital of Emergency for Children “Maria Skłodowska Curie”. The patients were

divided based on the age into the following groups: 1-3 years, 4-6 years, 7-9 years, 10-12 years, and over 12 years.

The study aims at analyzing some paraclinical markers according to age group, the type of nephrotic syndrome in order to:

- highlight similarities and differences
- analyze whether they can be included as factors that anticipate the evolution of the disease
- analyze whether there is a cause-effect relationship

The markers evaluated comprise:

- serum creatinine, serum urea
- inflammatory markers: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen
- total serum proteins
- lipid profile: total cholesterol, triglycerides, total lipids
- proteinuria/24h

Results

From the total of 124 patients included in the study, 78 patients were males (63%) and 46 patients were females (37%). The male: female ratio is approximately 1.7:1. Most patients with nephrotic syndrome come from urban areas, 70 cases (70.56%), and 54 cases from rural areas (54.44%). The total distribution of cases according to the county of origin can be seen in Figure 1.

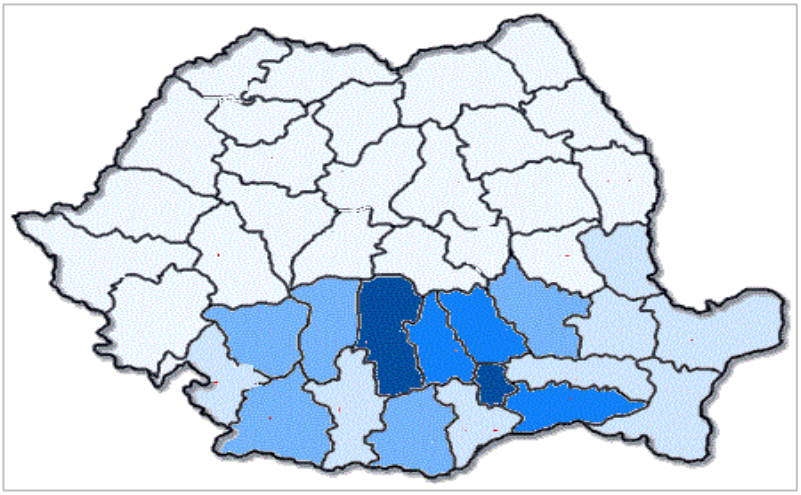

Figure 1. Distribution of cases according to county of origin

Most cases are from Bucharest (30 cases) and Argeș (13 cases), followed by Dâmbovița, Călărași, and Prahova counties. The counties with the fewest cases were: Mehedinți, Tulcea, and Suceava.

According to the type of nephrotic syndrome, most patients were diagnosed with SNCS- 62 cases (50%), SNCD- 37 cases (30%), and SNCR- 24 cases (20%). Aspects highlighted by Figure 2.

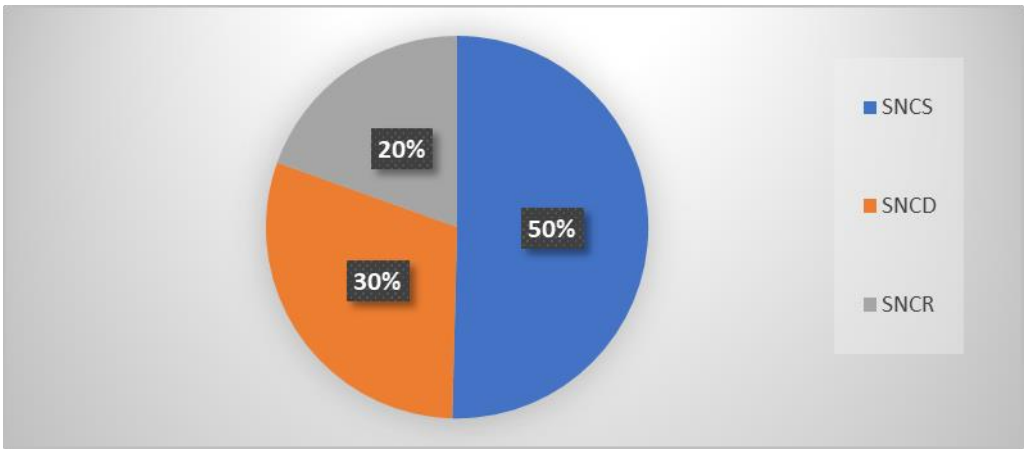

Figure 2. Distribution of cases according to the type of nephrotic syndrome

The distribution of cases according to age group and the type of nephrotic syndrome are highlighted in Figure 3. Most cases of nephrotic syndrome are in the age groups 1-3 years (63 cases) and 4-6 years (39 cases).

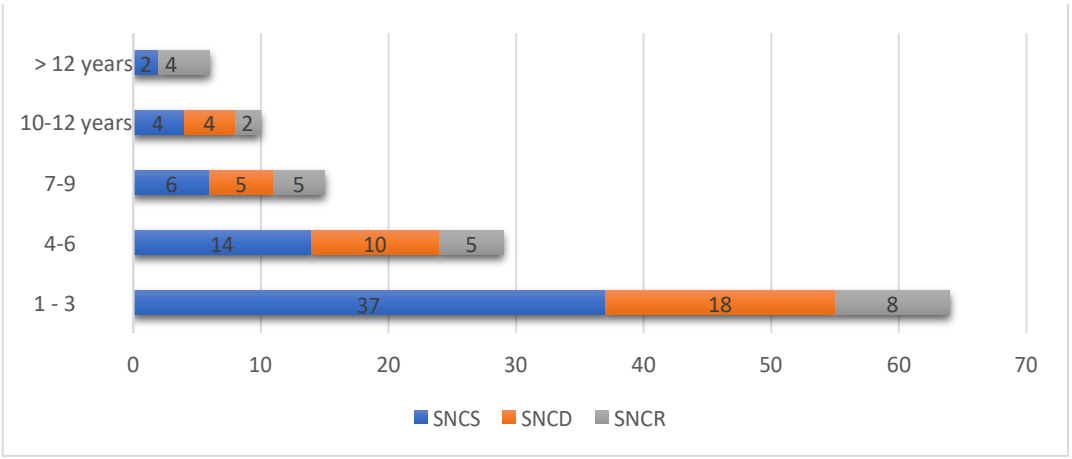

Figure 3. Distribution of cases according to gender, age group and type of nephrotic syndrome

The nephrotic syndrome was mostly diagnosed in patients aged below 6 years, especially in those aged 2 years and 3 years, respectively.

We analyzed the distribution of cases according to gender, age and type of nephrotic syndrome. For the female gender, the average age of the patients with SNCD was 5.61 ± 3.86 years, and for those with SNCR, the average age was 3.77 ± 3.11 years. For the male gender, the average age of the patients with SNCD was 3.3 ± 2.07 years, and for those with SNCR, the average age was 7.66 ± 4.86 years. In SNCS, the average age for the female and male gender was the same, namely that of 4 years - Figure 4.

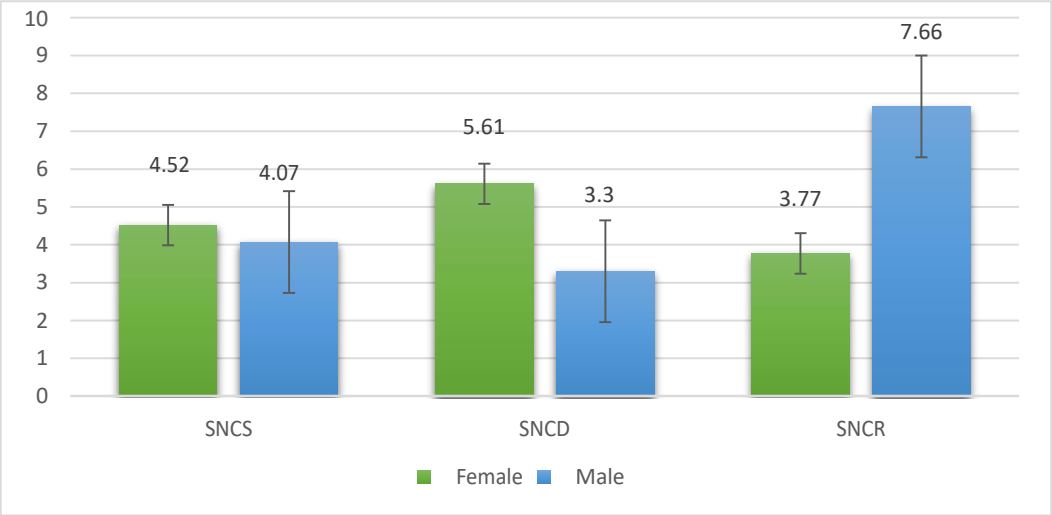

Figure 4. Average age of SNCS, SNCD, SNCR patients by gender.

Age group 1-3 years

The age group 1-3 years has the most cases, a total of 63 cases (50.8%). Most patients are with SNCS - 37 cases (29.38%), 18 cases with SNCD (14.51%), and 8 cases with SNCR (6.45%) - Figure 5. The age with the most cases was 2 and 3 years.

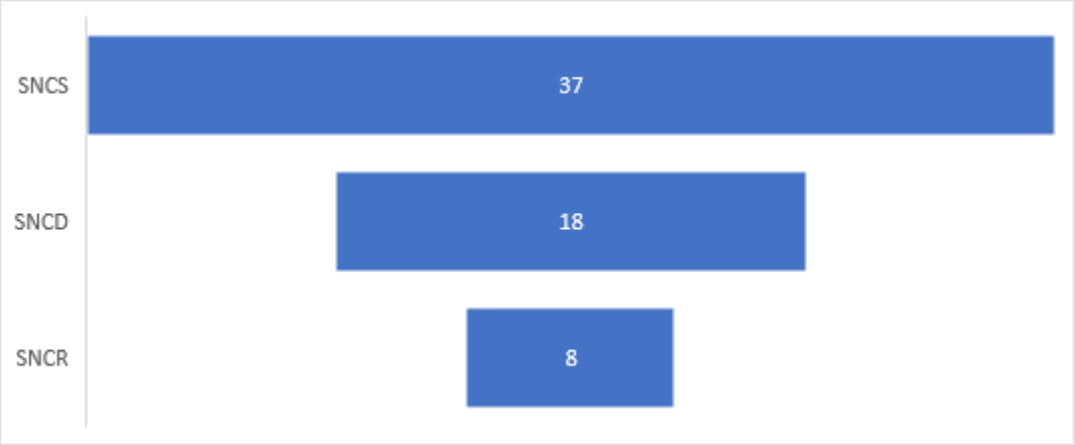

Figure 5. Distribution of cases according to SNCS, SNCD, SNCR

Patients with SNCS, SNCD and SNCR were evaluated based on the paraclinical constants - Table I. At a first evaluation, there are no big differences, the mean value of serum creatinine for SNCS is 0.27 ± 0.10, lower compared to the values for SNCD: 0.35 ± 0.14 or SNCR: 0.32 ± 0.1.

Table I. Mean value ± standard deviation of biological constants in patients with SNCS, SNCD, SNCR

Paraclinical variables	**SNCS**	**SNCD**	**SNCR**
Serum creatinine(mg/dl)	0.27±0.10	0.35±0.14	0.32±0.1
Serum urea (mg/dl)	22.95± 8.23	24.31±9.96	26.48±13.96
Uric acid (mg/dl)	4.1± 1.22	5.09±1.88	4.84±1.18

The comparative statistical analysis of the SNCS - SNCR subgroup highlights statistically significant differences for the value of serum creatinine (p= 0.005, independent student T-test). The mean value of serum creatinine for patients with SNCS is lower compared to that of patients with SNCR - Table II. For the other SNCS-SNCD and SNCD-SNCR subgroups, no statistically significant differences were highlighted (p> 0.05, independent student T-test).

Table II. Comparative analysis of biological constants in patients with SNCS and SNCR

Paraclinical variables	**SNCS**	**SNCR**	**p**
Serum creatinine(mg/dl)	0.27±0.10	0.32±0.1	0.005
Serum urea(mg/dl)	22.95± 8.23	26.48±13.96	0.05

We used linear regression, R= 0.02 to demonstrate a correlation between serum creatinine values in patients with SNCS and those with SNCR - Figure 5. There is a correlation between the 2 groups, which could explain the evolution of some patients with SNCS to SNCR. Performing the creatinine cut-off, we obtained a value of 0.39 mg/dL, the value that could represent a marker for monitoring the evolution of patients with nephrotic syndrome. Patients with this value of serum creatinine may be predisposed to SNCR.

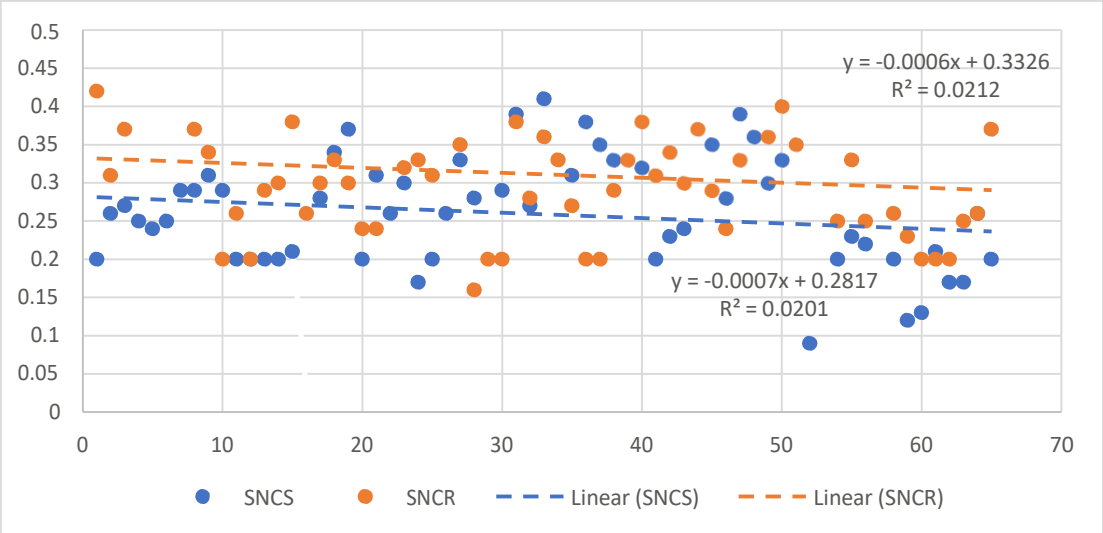

Figure 5. Linear regression of serum creatinine values in SNCS and SNCR patients

Another aspect evaluated in the study was the lipid profile: total cholesterol, triglycerides, total lipids, which is the average of the values of these constants and how these values evolve.

For the Age group 1-3 years, we obtained the following mean values ± the standard deviation:

- SNCS: total cholesterol- 2 4 5 .77±126.79 m g /dL, triglycerides- 154.97±147.62 mg/dL, total lipids- 804.59±401.83 mg/dL
- SNCD: total cholesterol - 290±134.96 mg/dL, triglycerides- 147.65±122.77 mg/dL, total lipids- 884.37±378.31 mg/dL
- SNCR: total cholesterol- 366.44±259.11 mg/dL, triglycerides- 321.77±517.81 mg/dL, total lipids- 1357±1065 mg/dL

There are no statistically significant differences regarding the lipid profile values depending on the type of SNCS, SNCD, and SNCR.

However, there are differences in the case of SNCS and SNCD patients regarding the normalization of the lipid profile values. The tendency towards normalization of the values appears primarily in triglycerides and total lipids. These values normalize within a maximum of 30 days after obtaining remission. For cholesterol values, the evolution is a little different. We analyzed these values in the patients included in the study on the first day of onset or relapse, subsequently after 30 days, 60 days, and 90 days. We analyzed 24 patients with SNCS and 24 patients with SNCD. For an easy correlation of the data, we made a graph with the average value on the first day, after 30 days, 60 days, and 90 days. If in the case of SNCS patients the cholesterol value normalizes on average between 30-60 days, for SNCD patients the duration of normalization of total cholesterol values is on average 40-90 days - Figure 6.

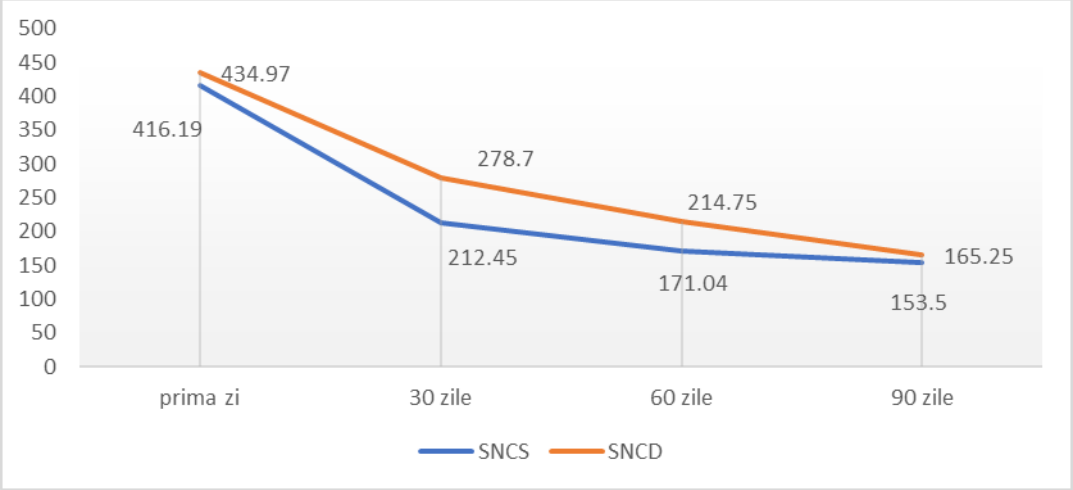

Figure 6. Evolution of mean values of total cholesterol, depending on the type of SNCS or SNCD

We analyzed according to the type of SNCS, SNCD, SNCR the mean value of total serum proteins, ESR and fibrinogen, to highlight whether there are statistically significant differences.

The mean value of total serum proteins was: in SNCS - 5.74 ± 1.21g/dL, in SNCD – 5.59 ± 1.23 g/dL, in SNCR - 5.6 ± 1.18 g/dL.

Inflammatory markers, in the studied group, do not show different values depending on the type of nephrotic syndrome, but their increase is noticed in the context of hypoproteinemia. The ESR had as average value: 30.23 ± 31.52 mm/h in SNCS, 36.17 ± 38.64mm/h in SNCD and 52.06 ± 36.01 mm/h in SNCR. For fibrinogen we obtained the following values: 449.24 ± 230.5 mg/dL in SNCS, 421.68 ± 232.07 mg/dL in SNCD,

621.27 ± 273.79 mg/dL. There is an inversely proportional relationship between ESR and total serum proteins. While total serum proteins are low, ESR increases.

Proteinuria is a marker that can influence the long-term evolution. In order to be able to make a comparison with the other age groups, we calculated the average value and the standard deviation for SNCS, SNCD, SNCR. The results were different. If for SNCS the average value of proteinuria was 1.61±2.37g/24h, for SNCD it was slightly increasing, 2.59±4.08g/24h, and the average value of proteinuria for SNCR patients was 3.24±2.74g/24h, double compared to the value of SNCS patients.

Age group 4-6 years

The age group 4-6 years includes a number of 39 patients and represents 23% of the total lot of 124 children included in the study. The largest share is occupied by patients with SNCS, 14 cases (48%), followed by 10 cases with SNCD (35%) and 5 cases with SNCR (17%).

We evaluated the biological constants for each type of nephrotic syndrome. In patients with SNCS, the mean value ± standard deviation of serum creatinine was 0.41 ± 0.11 mg/dL, of serum urea 26.07 ± 10.48 mg/dL and of uric acid of 4.58 ± 1.19 mg/dL. In SNCD patients, serum creatinine has an average value of 0.32 ± 0.08 mg/dL, serum urea 23.87 ± 6.5 mg/dL and uric acid 4.5 ± 1.16 mg/dL. In the case of SNCR patients, we have the following values: serum creatinine 0.48 ± 0.29 mg/dL, serum urea 32.36 ± 27.49 mg/dL, and uric acid 6.19 ± 1.51 mg/dL

The comparative analysis based on the biological constants for the subgroup of patients with SNCS - SNCD and the SNCS - SNCR group did not reveal statistically significant changes ($p > 0.05$, independent student T-test).

Continuing the analysis of the 4-6 years group, we evaluated lipid profiles: total cholesterol, triglycerides, total lipids related to the type of nephrotic syndrome. For patients with SNCS, the following values were obtained:

- total cholesterol, an average value of 229.38 ± 116.88 mg/dL
- triglycerides, an average value of 117.96 ± 107.39 mg/dL
- total lipids, an average value of 684.44 ± 326.33 mg/dL

For SNCD patients, the following mean values ± standard deviation were highlighted:

- total cholesterol 270 ± 137.92 mg/dL
- triglycerides 127.48 ± 100.3 mg/dL
- total lipids 820.55 ± 367.34 mg/dL

In the case of SNCR patients, the calculated values were:

- total cholesterol 316.66 ± 120.9 mg/dL
- triglycerides 163.66 ± 117.1 mg/dL
- total lipids 953.41 ± 328.85 mg/dL

The comparative analysis between the subgroups SNCS – SNCD, SNCS – SNCR and SNCD – SNCR did not highlight statistically significant changes ($p>0.05$, independent student T-test). An increase in lipid profile values is noticed depending on the type of nephrotic syndrome and age group.

The same characteristics are maintained as in the age group 1-3 years regarding the duration of normalization of lipid profile values.

The values of the inflammatory markers, ESR is maintained in an inversely proportional relationship with the value of the serum proteins, an aspect highlighted in figures 7.

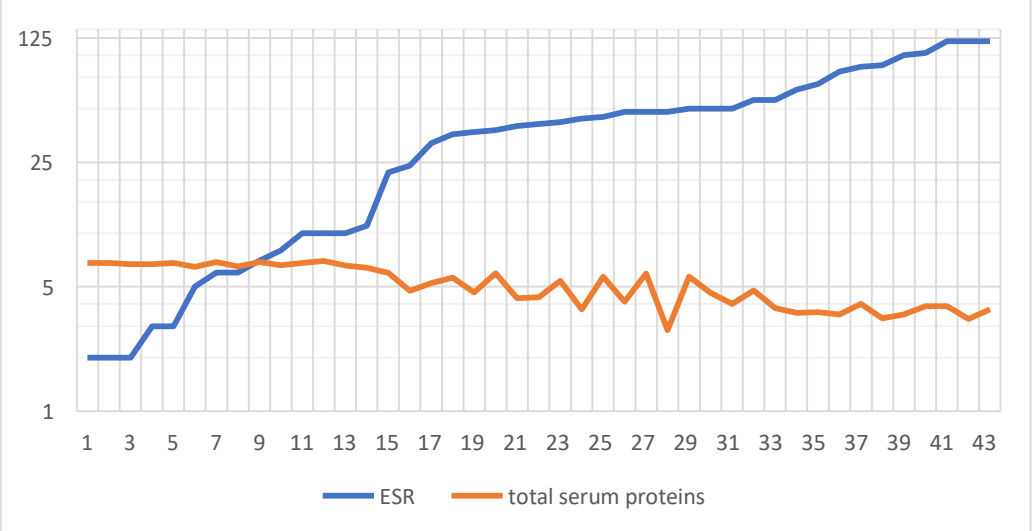

Figure 7. The relationship between ESR and total serum proteins for age group 4-6 years.

The average values of serum total proteins, ESR and fibrinogen, for the age group 4-6 years are shown in table III. There are no statistically significant differences, but an increase in values is noticed, according to the type of nephrotic syndrome.

Table III. The mean of biological variables related to the type of nephrotic syndrome

Biological variables	SNCS	SNCD	SNCR
ESR (mm/h)	30.62 ±28.01	34.85 ± 26.86	44.9 ±24.07
Fibrinogen (mg/dL)	441.15 ±207.01	457.98 ± 263.79	432.38 ±211.51
Total serum proteins (g/dL)	6.1 ± 1.11	5.54 ± 1.23	5.19 ± 1.18

An increase in the average value of proteinuria/24h is noticed. For SNCS, the mean value of proteinuria was 1.68 ± 3.2 g/24h, for SNCD it was slightly increasing 2.1 ± 3.03 g/24h, and the mean value of proteinuria for SNCR patients was 2.37 ± 2.76 g/24h.

Age group 7-9 years

The age group 7-9 years represents 12.9% of the group of 124 patients. In this group, there are 16 patients, approximately with equal weight, distributed as follows: 6 cases of SNCS, 5 cases of SNCD and 5 cases of SNCR. The peculiarity of this group is the age distribution. If for the age of 7 years there are patients with SNCS, SNCD and SNCR, for the age of 8 years there are only 2 patients with SNCR, and for the age of 9 years 2 patients with SNCS and 1 patient with SNCD.

In patients with SNCS, the mean ± standard deviation of serum creatinine is 0.37 ± 0.12 mg/dL, of serum urea 25.01 ± 12.76 mg/dL, and of uric acid of 4.62 ± 1.01 mg/dL.

In patients with SNCD, serum creatinine has an average value of 0.33 ± 0.11 mg/dL, serum urea 24.38 ± 8.03 mg/dL, and uric acid 5.1 ± 0.74 mg/dL. In the case of SNCR patients, we have the following average values: serum creatinine 0.42 ± 0.21 mg/dL, serum urea 32.39 ± 18.46 mg/dL, and uric acid 5.48 ± 1.7 mg/dL. No statistically significant differences were noticed.

The lipid profile analysis: cholesterol, triglycerides, total lipids, for age group 7-9 years offers limited information because of the small number of patients. The average values obtained in this group are indicative considering the limited number of entered values,

secondary to the small number of patients included in the study. The average values are highlighted in figures 8.

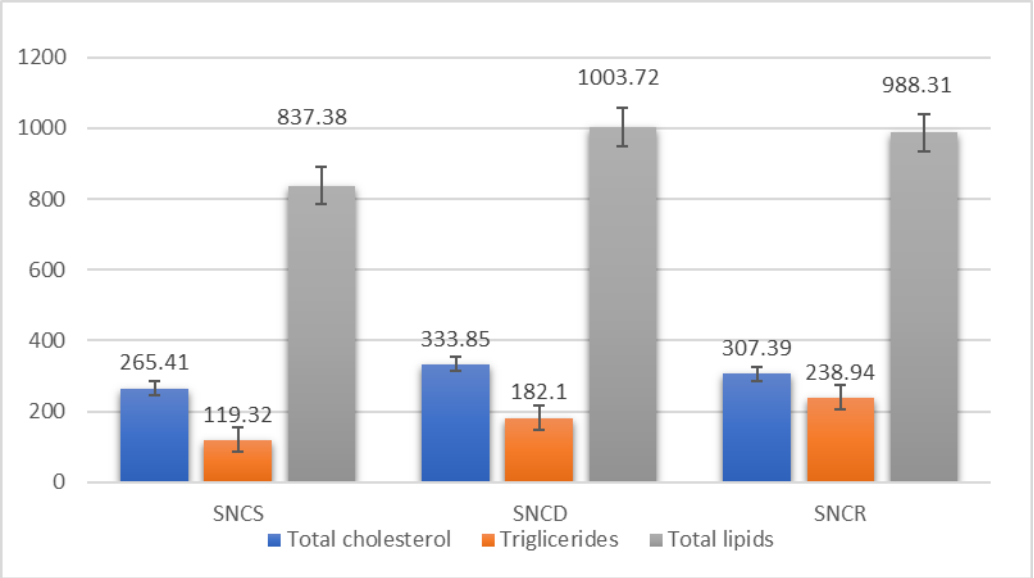

Figure 8. The average of the lipid profile values according to SNCS, SNCD, SNCR

The characteristics of the inflammatory markers described in the previous age groups are also preserved in this category. The mean value for total serum proteins was: in SNCS- 5.41 ± 1.43 g/dL, in SNCD- 4.94 ± 1.33 g/dL and in SNCR- 5.38 ± 1.18 g/dL. For ESR and fibrinogen the mean values are shown in Table IV

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Table IV. The mean of the inflammatory markers for each type of nephrotic syndrome

Inflammatory markers	**SNCS**	**SNCD**	**SNCR**
ESR (mm/h)	42.47 ± 38.44	49.03 ± 33.98	43.84 ± 31.83
Fibrinogen (mg/dL)	425 ± 250.47	497. 04 ± 216.38	414.97 ± 111.8

For SNCS the mean value of proteinuria was 2.49 ± 3.46 g/24h, for SNCD it was slightly increasing 2.88 ± 3.63 g/24h and the mean value of proteinuria for SNCR patients was 3.64 ± 3.32 g/24h.

Age group 10-12 years and over 12 years

The 2 groups represent the lowest percentage of the lot of 124 included patients. Age group 10-12 years consists of 6 cases (4.83%), and age group over 12 years consists of 8 cases (6.45%). The same characteristics as in previous age groups are maintained.

Discussions

We analyzed for each age group the average value for: total cholesterol, triglycerides, total lipids, total serum proteins, proteinuria, ESR and fibrinogen. A slight increase in values is noticed from group to group. These differences may exist because of the different number of patients in each age group, the changes in biological parameters that occur with age. Lipid profiles, especially cholesterol values, correlate with hypoproteinemia, especially with albuminemia. In our study, the value of total serum proteins was used, taking into account the fact that serum albumin represents 35-50% of the value of total serum proteins [2,3]. Podocyte injury determines important losses of serum albumin, which secondarily causes changes in the lipid profile, with the increase in particular of total cholesterol and the LDL-cholesterol fraction, but also the decrease of oncotic pressure, which secondarily has a negative effect on the lipid profile. Higher values of the lipid profile also correspond to higher values of proteinuria [4, 5, 6].

Differences in values, highlighted in the study, depending on the type of nephrotic syndrome: SNCS, SNCD, SNCR and age group, can signal certain characteristics such as:

- the lipid profile is more important in SNCR patients, the values increase according to the age
- the value of serum creatinine is dependent on more variables, being related to muscle mass, age, gender [7].
- ESR depends on the total serum proteins and reflects the inflammatory status existing in the nephrotic syndrome. The persistence of the inflammatory status causes irreversible structural changes, which can evolve into chronic kidney disease [8,9].
- proteinuria appears to be age-dependent, the values being higher with age advance and type of nephrotic syndrome. In SNCR patients, proteinuria values above 3 g/dL are a marker of progression to chronic kidney disease.

Study Limits

We included in the study 124 patients diagnosed with idiopathic nephrotic syndrome. The limits of this study were:

- unequal number of patients for each age group

- the unequal number of cases diagnosed with SNCS, SNCD and SNCR

- the test protocol for samples collected for each patient at each evaluation. This test protocol is dependent on hospital resources at the time of collection.

Mycophenolate Mofetil- Therapeutic Approach compared to other

Immunosuppressants

The basic treatment in nephrotic syndrome, until now, remains corticotherapy. Depending on the evolution of the disease, the toxicity of corticosteroids, the 2nd line immunosuppressant therapy is introduced, taking into account the long-term effects on prognostic and the quality of life. Out of the 124 patients included in the study, 49 cases required 2nd line immunosuppressant therapy.

Depending on the form of nephrotic syndrome, patient compliance, availability of treatment, the most used drugs in the study group were: Cyclophosphamide (CP), Cyclosporine (CsA) and MMF- Figure 9. The therapeutic schemes were different and included one, two or more immunosuppressants of the 2nd line, administered at different intervals.

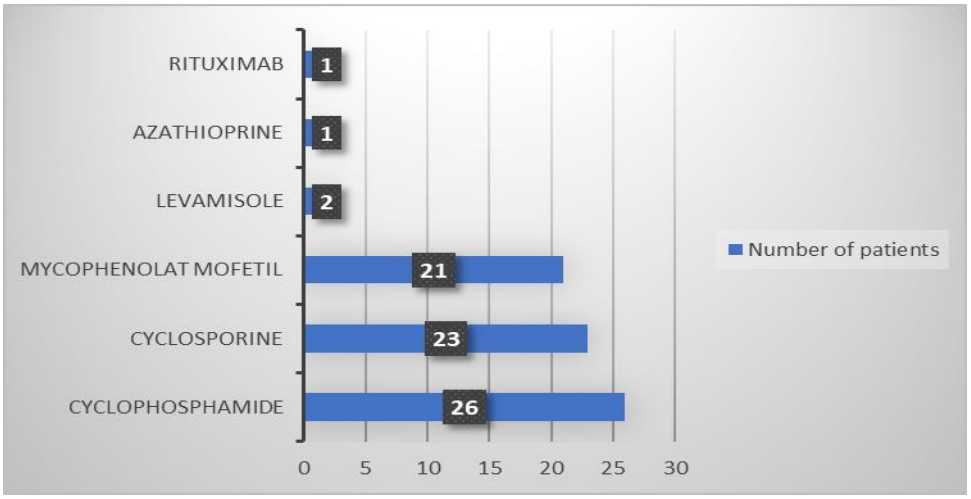

Figure 9. Use of 2nd line immunosuppressant therapy

Mycophenolate Mofetil Monotherapy- Comparative Aspects with Cyclophosphamide and Cyclosporine

MMF was administered as the unique 2nd-line immunosuppressant for seven patients with SNCD and one patient with SNCR-late responder. Among the SNCD patients, two patients were females and five patients were males. The age for this group was included in the range (1 year- 13 years), with an average of 5.7 ± 3.86 years – Figure 10.

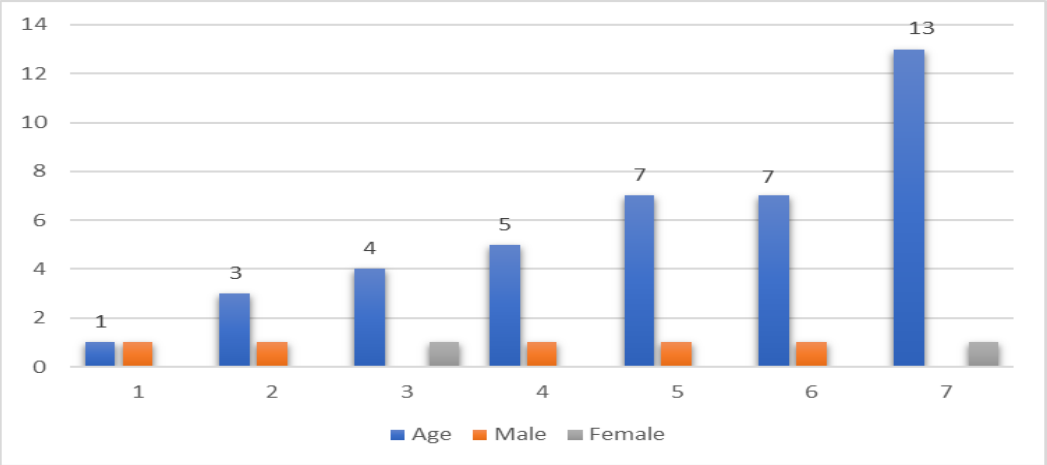

Figure 10. Distribution of patients who received MMF according to age and gender

Patients who received 2nd-line immunosuppressive treatment continued corticosteroid therapy in parallel, with a progressive decrease in the dose of Prednisone. The period of MMF treatment was 2 years, with a dose of 1200mg/m2.

During the administration of MMF, the evolution of the patients was favorable, with a decrease in the number of relapses. Most patients had two relapses during the 2nd-line immunosuppressive treatment. The two patients aged 7 years had three relapses in the first year of MMF treatment. The first patient, aged 7 years, presented relapses at 2 months, 5 months and 8 months after the initiation of MMF. The second patient aged 7 years presented relapses at 3 months, 4 months and 10 months after starting MMF treatment. Patients aged 3 and 4 years did not have relapses during the 2 years of MMF treatment. The 13-year-old patient had two relapses, the first at 2 months and the second at 10 months, and the 5-year-old patient had relapses at 6 months and 8 months after starting MMF.

In general, for the 7 patients, the first relapse was within the first 2-6 months, and the 2nd relapse between 4-10 months after the initiation of treatment - Figure 11.

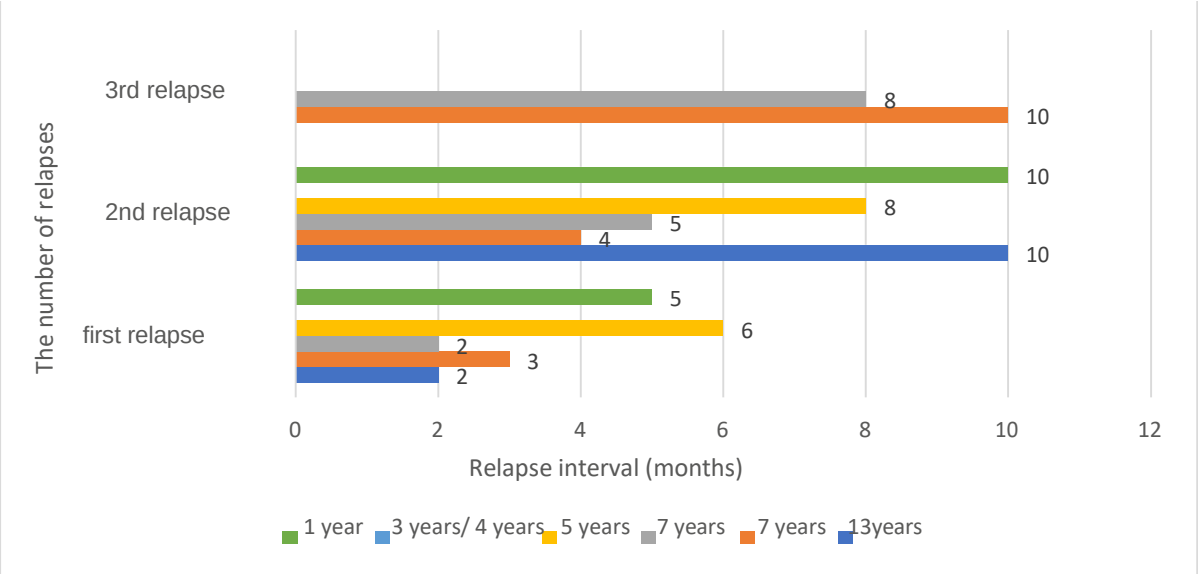

Figure 11. Number of relapses and relapse interval after the initiation of MMF treatment

After the end of the 2 years of treatment with MMF, the remission was maintained for 6 months for three cases, 8 months for 1 case and one year for another three cases - Figure 12.

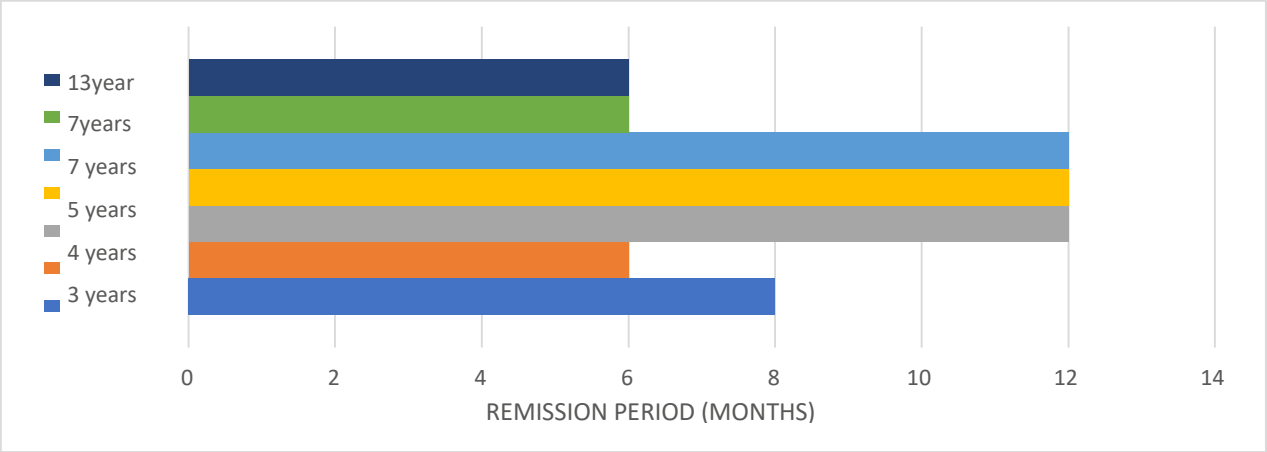

Figure 12. Remission period after the end of MMF treatment

No adverse reactions were documented during MMF treatment. Paraclinically, blood count values, renal function (serum creatinine, serum urea, and uric acid), proteinuria were preserved at normal values, the parameters being evaluated monthly in the first 3 months, then every 2-3 months. The treatment was well tolerated and compliant patients, with favorable results.

MMF, compared to the 2 immunosuppressants, CP and CsA, had good tolerability, favorable results, maximum 3 relapses in the first year of administration, subsequently without relapses; maintenance of remission after the end of therapy being between 6-12 months.

The administration of the medicine at home makes it acceptable to patients, and the clinical and paraclinical evaluation can be performed in an outpatient facility or during 24 hours of hospitalization. From the point of view of adverse reactions, according to the patients' anamnesis, they were absent during administration.

CP proved to be effective during the 6 months of administration, for seven patients with SNCD. No patient had relapses during this period. The disadvantage of this treatment is the administration in the hospital, which presupposes a hospitalization of at least 3 days; the first day being for clinical, paraclinical evaluation and hydration, the second day for treatment administration and the following 24 hours of post-administration monitoring. Adverse reactions were described during administration: nausea, remitted at the end of the course. The unfavorable aspect was registered after the end of the treatment, when patients presented the first relapse in the first 3 months, and the second relapse in the first 6-8 months.

CsA was well tolerated by the patients and was found to be more effective in the five patients with SNCR compared to the three patients with SNCD. If in SNCD patients the first relapse is in the first year of treatment, the second relapse in the second year, the third relapse occurs at least 12 months after the end of therapy. CsA decreased the number of relapses in these patients, taking into account the fact that before therapy, their number was 3-4 relapses in 6 months. In SNCR patients, during CsA treatment, they did not have relapses, but this fact may be related to the positive effect of CsA on the histopathological changes: LGM and membranous glomerulonephritis. No effect in the patient with GSFS, who had five relapses during treatment, most in the second year of administration. It is tolerated by patients and no clinical or paraclinical side effects have been documented during therapy.

Apparently, MMF is effective in SNCD, hence the need to continue the study, to include more patients of different ages in order to analyze these aspects as accurately as possible.

Mycophenolate Mofetil and Cyclophosphamide

In this situation there were 4 cases with SNCD and 3 cases with SNCR. The peculiarity in the case of SNCR patients is that CP was administered during the period when the diagnostic was SNCD, later at different intervals, they became SNCR - late responder (remission was obtained after 6 weeks of daily corticotherapy) and they were under treatment with MMF.

The SNCD patients had an age in the range (2 – 4 years), with an average of 2.75 ± 0.95 years, three male children and one female child - Figure 13. The SNCR patients had the age in the range (6 – 14 years), with an average of 9.33 ± 4.16 years, of which two male patients and one female patient - Figure 14.

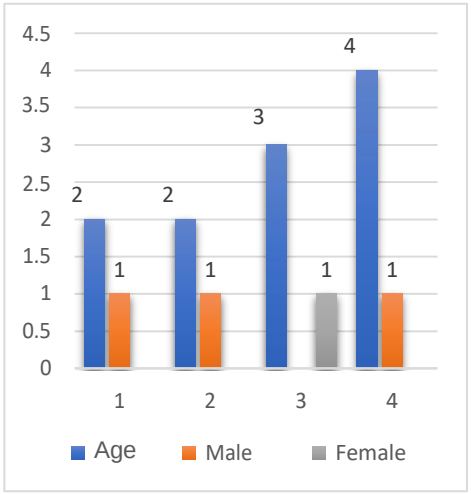

Figure 13. Distribution of patients with SNCD according to age and gender

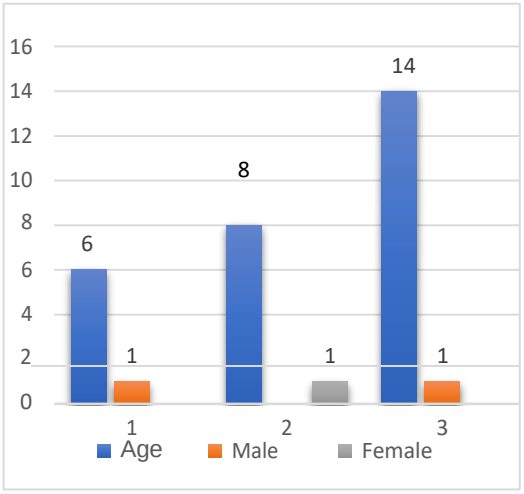

Figure 14. Distribution of patients with SNCR according to age and gender

The first patient with SNCD, aged 2 years, male, initially received CP. Later, after the end of CP treatments, 2 months later, MMF treatment was initiated. During the administration of the CP courses, the patient had repeated relapses: the first 2 months after the start of the CP courses and the following 3 relapses at 1-month intervals. During the treatment with MMF, the patient had a relapse 6 months after the initiation of therapy. Subsequently, the remission was maintained for a period of 18 months, after the end of MMF therapy, without documented adverse effects.

For the second patient, aged 2 years, male, the evolution was different considering that the first administered immunosuppressant was CP, then MMF. During the 6 courses of CP, the patient had a relapse 2 months before the administration of the second CP course, subsequently the remission was maintained for 8 months after the end of the courses and MMF therapy is continued. During MMF administration, the patient had three relapses at intervals of: 4 months, 8 months, and 10 months after the initiation of treatment. After the end of MMF therapy, remission was maintained for 12 months, when the patient had a first relapse, then the second relapse was 24 months after the end of MMF therapy.

In the case of the female patient, aged 3 years, with SNCD, the first therapeutic option was MMF, subsequently CP. During the first year of MMF, the number of relapses was four. The interval at which the patient relapsed was: 2 months, 3 months, 4 months, 7 months, 10 months after starting MMF administration. In the second year of treatment, the patient had a relapse, in the first 2 months, then the remission was maintained for 10 months. After the end of the 2 years of treatment, the patient presented two consecutive relapses and it was decided to initiate therapy with CP, 6 courses. During CP treatment, the evolution was favorable without relapses. After the completion of the 6 CP courses, remission was maintained for 12 months.

The fourth case of SNCD, a 4-year-old male patient, had as first immunosuppressant a 2nd line immunosuppressant, MMF, and later CP. In this case, the first year of treatment was without relapses. The situation for this case changed in the second year of administration, when it presented four relapses within 8 months. The first relapse 3 months after the start of the second year of MMF treatment, then at 4 months, 6 months, and the fourth relapse at 8 months. The treatment with MMF is interrupted 4 months before the end of the 2 years of administration of the drug and it is decided to administer 6 CP courses. The remission is maintained for the duration of the 6 courses and for the duration of 12 months after the end of the courses.

For SNCR patients, one patient evolved to chronic kidney disease stage V-dialytic, being multidrug resistant. For the other 2 patients, the evolution was favorable after the end of MMF treatment, maintaining remission for a period longer than 6 months.

Mycophenolate Mofetil, Cyclophosphamide and Cyclosporine

The therapeutic scheme including MMF, CP, and CsA was documented in five cases with SNCR. The age of the children was in the range (2 – 7 years), with an average of 4 ± 2.12 years, three patients being female and two patients being male - Figure 15.

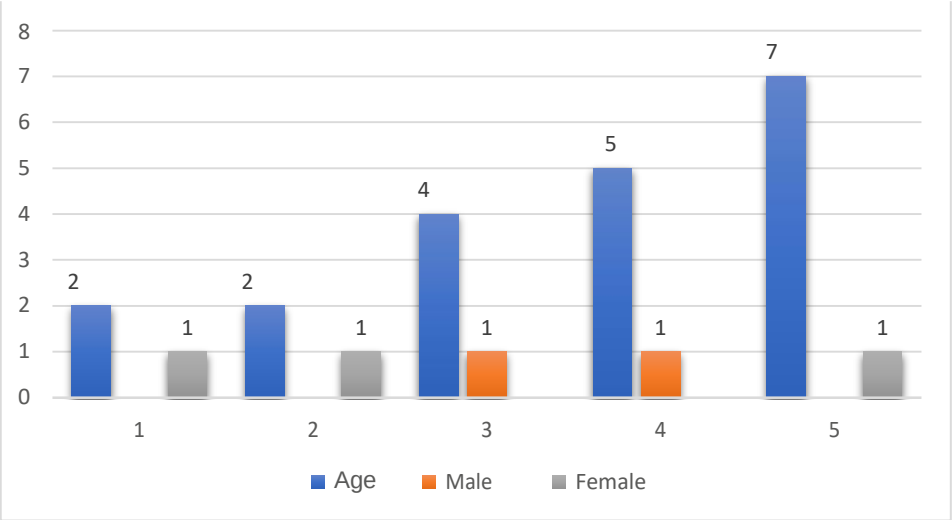

Figure 15. Distribution of patients according to age and gender in SNCR

From the histopathological point of view, the results showed: 4 cases with focal and segmental glomerulosclerosis (GSFS), and 2 cases with minimal glomerular damage (LGM) - Figure 16.

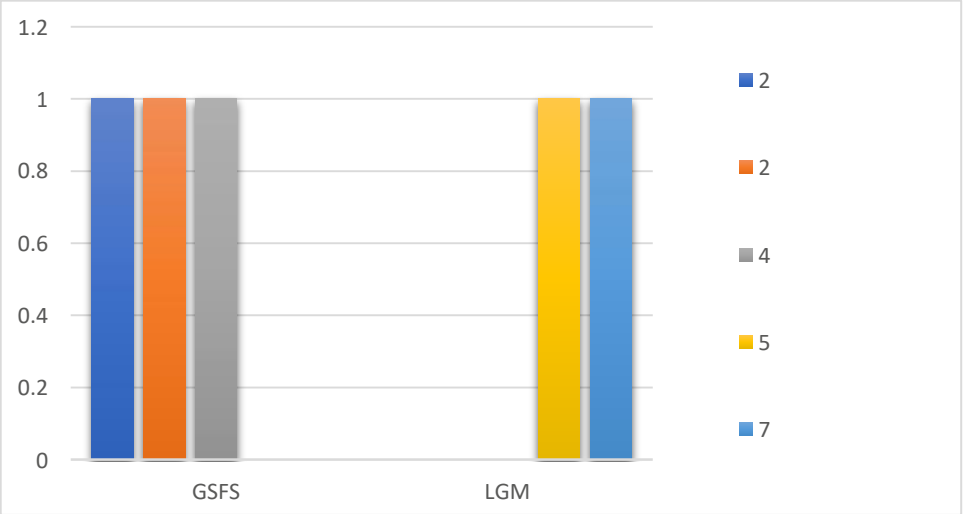

Figure 16. Histopathological appearance according to age

We evaluated the effectiveness of MMF treatment, depending on the result of the renal biopsy and we acknowledged:

- female patients, aged 2 years and 4 years, with GSFS, under treatment with MMF, achieved partial remission 6 months after the initiation of therapy, subsequently another 2nd line immunosuppressant was introduced.

- female patients aged 2 years with GSFS and 7 years with LGM, during the treatment with MMF, did not show a reduction in proteinuria below the value of 1.5g/24h.

- the 5-year-old male patient with LGM achieved remission with Csa, but it was continued with MMF to maintain remission.

Mycophenolate Mofetil, Cyclosporine and Levamisole

The therapeutic scheme comprising Levamisole, CsA and MMF was applied in the case of a 2-year-old male patient with SNCD.

Levamisole was initially introduced, in an alternative regimen, for a duration of 24 months, with a relapse during the scheme. The adverse event was 3 months after the end of treatment, when three consecutive relapses occurred within 6 months. The second option in the therapeutic regimen was CsA, with maintenance of remission throughout the 22 months of treatment. 2 months before the end of the CsA period, when the dose was minimal (25mg), the patient was hospitalized for a relapse. The next relapse was 4 months after the end of CsA treatment. The therapeutic scheme ends with the introduction of MMF, which maintains the remission, until the end of the study, for a period of 6 months.

Mycophenolate Mofetil, Cyclophosphamide and Rituximab

CP, MMF and Rituximab (RTX) were part of the therapeutic scheme of a 9-year-old male patient who evolved from SNCS, initially SNCD, then SNCR and later the progression was to chronic V-dialytic stage kidney disease.

The first MMF therapeutic option was initiated when the patient was diagnosed with SNCD. 3 months after the initiation of therapy, he became cortico-resistant and a renal biopsy was performed, which revealed GSFS.

In evolution, proteinuria values begin to increase from 1.04 to 3.4 g/24h, with persistent edema, resistant to treatment. 4 doses of RTX are administered at an interval of 2 weeks between administrations, without adverse reactions during administration or after administration, with the persistence of proteinuria, values above 3.5 g/24h and continuation of MMF therapy. 4 months after the end of RTX therapy, concurrently with MMF therapy, CP courses are initiated, without improvement of proteinuria or improvement of the effects of nephrotic syndrome. Within one year after the initiation of MMF therapy, the patient is introduced to the chronic hemodialysis program.

Discussions

In the treatment of nephrotic syndrome, MMF as a 2nd line immunosuppressant therapy is still incompletely studied. In the literature, based on the studies carried out so far, it finds its effectiveness mostly in the cases of frequent relapse nephrotic syndrome (SNFR) and SNCD.

In forms of SNCD, MMF maintained remission for at least 6 months with fewer relapses, regardless of whether it was used as first-line treatment or not. Younger age, under 6 years, shows that MMF is more effective in cases of SNCD [10, 11,12].

SNCR cases represent a challenge for the clinician, especially the forms with GFSF, where they are often multidrug resistant. Among the 49 patients who required 2nd line immunosuppressant therapy, five patients with SNCR aged between 2 years and 7 years, three females and two males had in the therapeutic scheme: MMF, CP, and CsA. In all these cases MMF was not used as a first option, being effective in maintaining remission, but not in achieving it.

In SNCR, MMF has a limited use [13]. It is not effective in achieving remission, but it can help maintain remission where other drugs, used for more than 2 years, they can become

nephrotoxic, as is the case with CsA [14, 15]. Csa is effective in cases of SNCR with LGM, membranous nephropathy and in some cases with GSFS, as we also found in the current study, aspects also highlighted in the literature [16,17, 18].

Study Limits

In this study, 49 patients who required 2nd line immunosuppressant therapy were included and only 21 patients received MMF. The limits of this study were:

- the small number of patients who received MMF treatment
- the impossibility of dosing mycophenolic acid, because of the costs, in order to properly evaluate the effectiveness of the treatment and to adjust the doses, if necessary.

Personal Contributions

The present study included a number of 124 pediatric patients admitted to the Nephrology Department of "Maria Skłodowska Curie" Emergency Clinical Hospital for Children Bucharest, between 2016-2020, diagnosed with idiopathic nephrotic syndrome since 2013, of these, 49 cases required 2nd line immunosuppressant therapy.

The objectives of this study were:

- to analyze the evolution of patients with idiopathic nephrotic syndrome in the first year of diagnostic, based on paraclinical markers, to highlight the similarities or differences that could influence the long-term evolution of the disease.

- to evaluate the effectiveness of MMF treatment compared to other immunosuppressants of the 2nd line 2, depending on the type of nephrotic syndrome: SNCD or SNCR.

Patient sheets were analysed in the first 12 months after the onset of the nephrotic syndrome, during and after the end of the 2nd line immunosuppressant therapy.

In the first study, for a more detailed analysis, patients were divided according to age into groups of: 1-3 years, 4-6 years, 7-9 years, 10-12 years, and over 12 years. We evaluated according to each age group and type of nephrotic syndrome different biological markers (serum creatinine, serum urea, lipid profile, inflammatory markers, total serum proteins, proteinuria) that could have an impact on the evolution of the nephrotic syndrome. We highlighted relationships of interdependence and particularities between these paraclinical markers depending on the type of nephrotic syndrome.

The second study analyzed therapeutic schemes including MMF in the treatment of

patients with nephrotic syndrome. We evaluated the effectiveness of the treatment according to the type of SNCD or SNCR. Clinical and paraclinical parameters were monitored during and after the end of MMF treatment. Depending on the type of nephrotic syndrome, MMF was either the first or the second treatment option. We tracked the number of relapses during and after the end of MMF therapy and documented adverse reactions.

Because of the small number of patients enrolled, the study requires continuation to reproduce the results on larger cohorts of patients, to verify whether the results highlighted in the present study are maintained.

Conclusions

- Our study included 124 patients monitored in the Pediatric Nephrology Department of the Emergency Clinical Hospital for Children "Maria Skłodowska Curie" Bucharest.
- From the lot included in the study, there is a predominance of the male gender, being a male: female ratio of 1.7:1.
- Most of the cases monitored in the clinic come from the South-East of the country, an aspect supported by the increased addressability of cases for the capital.
- Most cases of nephrotic syndrome were described in age group 1-3 years, followed by age group 4-6 years.
- An increase in the mean values for biological markers determining nephrotic syndrome (serum creatinine, serum urea, lipid profile, and proteinuria) is noticed with advancing age.
- In the age group 1-3 years, the average value of serum creatinine of SNCS patients was 0.27 ± 0.10 mg/dL, and for SNCR patients it was 0.32 ± 0.1 mg/dL. The comparative statistical analysis of the SNCS-SNCR subgroup highlighted statistically significant differences for the serum creatinine value ($p=0.005$, independent student T-test). Linear regression highlighted a $R= 0.02$, indicating a relationship between the two types of nephrotic syndrome. The cut-off of serum creatinine offered us a value of 0.39 mg/dL, which could represent a marker for possible patients who can evolve from SNCS to SNCR. This relationship has not been described in the other age groups, which is why we have to consider that the serum creatinine value is closely related to the muscle mass, age and gender of the patient.
- Podocyte injury causes important losses of serum albumin, which secondarily causes changes in the lipid profile, with the increase especially of total cholesterol and the LDL-cholesterol fraction, but also the decrease of oncotic pressure, which secondarily has a negative effect on

the lipid profile. High values of the lipid profile correlate with higher values of proteinuria.

- An aspect noticed in patients with SNCS and SNCD, regardless the age group, is the tendency to normalize lipid profile values, primarily triglycerides and total lipids. These values normalize within a maximum of 30 days after obtaining remission.
- The normalization of total cholesterol values is different depending on the type of SNCS or SNCD. For patients with SNCS, the value of total cholesterol normalizes on average between 30-60 days, and for patients with SNCD on average between 60-90 days.
- Within our study, fibrinogen is increased at the onset of nephrotic syndrome or in the time of relapses.
- ESR, frequently used as an infection marker, in nephrotic syndrome, has an inversely proportional relationship with total serum proteins. The more severe the hypoproteinemia, the higher the ESR value, supporting the existence of an important inflammatory process. The highest values of ESR have been described in patients with SNCR, supporting the persistence of an inflammatory status, which in the long term may contribute to irreversible structural changes that may evolve into chronic kidney disease.
- Proteinuria is age-dependent, values being higher with advancing age and type of nephrotic syndrome. In patients with SNCR, proteinuria values above 3 g/24h represent a marker of progression to chronic kidney disease. Urinary loss of proteins causes a decrease in serum proteins, which causes the alteration of the lipid profile, by increasing its values and the presence of an inflammatory status, which in turn maintains urinary protein losses. All these changes are interconnected and any disruption of one causes the decline of the others.
- From the lot of 124 patients included in the study, 49 patients required 2nd line immunosuppressant therapy. MMF was included in the treatment regimen of 21 patients.
- Apparently, MMF is effective in SNCD, especially in patients under 6 years of age, which shows the need to continue the study and include more patients of different ages to analyze these aspects as accurately as possible.
- Patients who received MMF in the therapeutic scheme had an average duration of remission maintenance between 6 and 12 months.
- MMF has limited results in SNCR cases, being used as a secondary option to maintain remission.
- MMF is effective in maintaining long-term remission, especially in patients treated with Calcineurin inhibitors, to minimize the risk of nephrotoxicity.
- During the study, there were no clinically or paraclinically documented changes specific to

the adverse effects of MMF, but because of the small number of patients, the study must be continued to compare the results on a larger lot.

- The advantage of MMF treatment is that it is administered at home, and the patient's clinical and paraclinical evaluation can be performed in an outpatient facility or within 24 hours of hospitalization at most.

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List of Scientific Works Published

ISI or BDI indexed articles

1. Loredana POPA, Mihaela BALGRADEAN, **Anca CROITORU**-Long-Term Study in Children with Steroid-Resistant Nephrotic Syndrome Progressing to End- Stage Renal Disease - *Maedica* a Journal of Clinical Medicine. 2022;17(2): 271- 276.

   https://www.maedica.ro/articles/2022/2/2022_17(20)_No2_pg271-276.pdf

2. **Anca CROITORU,** Mihaela BALGRADEAN- Treatment-Associated Side Effects in Patients with Steroid-Dependent Nephrotic Syndrome- *Maedica* a Journal of Clinical Medicine. 2022;17(2): 285-290.

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