NEW DEVELOPMENTS IN THE THERAPY OF CHILDHOOD NEPHRORTIC SYNDROME

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PhD Thesis

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Abstracts:

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TABLE OF CONTENTS

SUMMARY

INTRODUCTION: Eighty to ninety percent of children with steroid-sensitive nephrotic syndrome (SSNS) have relapses. About half relapse frequently and are at risk of the adverse effects of corticosteroids. Non-corticosteroid immunosuppressive agents are used to prolong periods of remission; however these agents have significant potential adverse effects. Currently there is no consensus as to the most appropriate second line agent in steroid–unresponsive nephrotic syndrome (NS) in children.

OBJECTIVES: Our objective is to evaluate the benefits and harms of some non-corticosteroid immunosuppressive agents (i.e. levamisole, cyclophosphamide (CP), cyclosporine A (CSA)) in frequently relapsing (FRNS), steroid-dependent (SDNS) and steroid-resistant NS (SRNS) in children.

PATIENTS AND METHODS: A retrospective study was made on the medical records of 34 children (21 boys, 13 girls) with FRNS (15/34), SDNS (13/34), or SRNS (6/34), whom levamisole was introduced (2 mg/kg per day) after 4 weeks of daily corticosteroid treatment. A second retrospective study was made on the medical records of 37 children, where two groups of patients were identified. Group 1 consisted of 22 children (15 boys, 7 girls) who received CP first as a second-line immunosuppressive drug because of their nephrotic syndrome. Group 2 consisted of 15 children (10 boys, 5 girls) who received second-line treatment with CSA.

CONCLUSIONS: Our findings suggest that levamisole significantly reduces the relapse rate and the cumulative steroid dose in children with FRNS and SDNS and is also a beneficial and safe therapy for SRNS patients. Levamisole may have a place in preventing relapse even after courses of akylating agents, and it may also have some benefit for the treatment of SRNS patients. CP and CSA are effective second-line therapies following steroid monotherapy in idiopathic NS patients, but the relapse rate is lower and the relapse-free period is significantly longer in our CP-treated group. A good remission rate still can be achieved after 5 years following the initial CP and CSA therapy, and the incidence of side effects is low. An important message of our study is that most children who have a difficult course of nephrotic syndrome after an initial remission induced by steroids do well 7-8 years

after presentation. On the other hand, our study is finally not able to give a definitive answer to the question as to which is the better treatment in the examined patient population. This retrospective study works with non equal distribution of focal segmental glomerulosclerosis (FSGS) in the different treatment groups so the question can not be answered due to statistical restrictions. In addition, no accompanying genetic studies were performed, which means that about 10% of our patients are expected to have a genetic disorder.

Further research is still needed to elucidate the disorder's molecular pathogenesis, identify new prognostic indicators, and to develop better approaches to treatment.

ABBREVIATIONS

BAPN, British Association for Paediatric Nephrology

BP, blood pressure

Chl, chlorambucil

Clcreat, endogenous creatinine clearance (ml/min per 1.73 m^2)

CSA, cyclosporine-A

CP, cyclophosphamide

eGFR, estimated glomerular filtration rate

F, female

IgM NP, immunglobulinM nephropathy

IL, interleukin

INS, idiopathic nephrotic syndrome

ISKDC, International Study of Kidney Diseases in Children

FRNS, frequently relapsing nephrotic syndrome

FSGS, focal segmental glomerulosclerosis

MCNS, minimal change nephrotic syndrome

MMF, mycophenolate mofetil

MP, methylprednisolone

M, male

NF-AT, nuclear factor of activated T-cells

NKF K/DOQI CKD, National Kidney Foundation Kidney Disease Quality Outcomes
Initiative stages for chronic kidney disease

NS, nephrotic syndrome

RRT, renal replacement therapy

SD, standard deviation

SDNS, steroid dependent nephrotic syndrome

SRNS, steroid resistant nephrotic syndrome

SSNS, steroid sensitive nephrotic syndrome

UP, urinary protein

1. INTRODUCTION

Idiopathic nephrotic syndrome (NS) is the most frequent glomerular disease in children and is mainly due to minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS) [1−4]. NS generally has a favourable long-term prognosis, about 90 % of affected children exhibit an excellent glucocorticoid responsiveness, but most of them suffer at least one relapse [5]. Steroid-responsiveness is of greater prognostic use than renal histology [6].

According to the literature, about 40 % of the steroid-sensitive (SSNS) cases are frequently relapsing (FRNS) and they commonly become steroid-dependent (SDNS) [7]. Steroid resistance (SRNS) develops in 10 % of children and many of these exhibit FSGS [8].

Treatment of the these NS patients is still challenging because they often require long-term, high-dose immunosuppression (i.e. use of steroids), with a greater prevalence of side effects and complications (poor growth, cushingoid obesity, adrenal suppression, hypertension, osteoporosis, cataracts, and psychological disturbances) [5, 9−11]. The therapeutic aims are to induce complete remission, reduce the rate of relapses, the cumulative dose of corticosteroids, mortality, therapeutic side effects, and the incidence of serious complications [12]. In the past two decades, alkylating agents such as cyclophosphamide (CP) and chlorambucil (Chl), the immunomodulatory drug levamisole, calcineurin inhibitors such as cyclosporine A (CSA) and recently tacrolimus (TAC) and mycophenolate mofetil (MMF), the inhibitor of purine biosynthesis, have been used as the main steroid-sparing agents [13−15]. The effectiveness of cytotoxic therapy depends on the histology of the lesion, they vary in potency and side effects, and there has been no consensus as to which should be used as the first choice of second-line drug [16− 18].

CP has been used in nephrology since the 1960s, whereas CSA and levamisole were added to the armamentarium of therapies in 1987, and a consensus guideline for the treatment of NS was published by the British Association of Paediatric Nephrology (BAPN) in 1994 [19].

Levamisole was originally developed as an antihelminthic drug with a non-specific immunomodulatory effect. It has been hypothesized to normalize deficient cell-mediated immunity [10]. It enhances T-cell responses by stimulating T-cell activation and

proliferation; it potentiates monocyte and macrophage functions, including phagocytosis and chemotaxis, and increases neutrophil mobility, adherence and chemotaxis [20]. This mechanism is important, because SSNS has long been thought to be due to lymphocyte-derived circulating factors leading to podocyte injury with subsequent proteinuria. New studies support this mechanism and implicate the role of T helper 2 cytokine inteleukin (IL) -13. In addition a genetic mutation in familial NS has been reported in a child, who responded to corticosteroid therapy and preliminary trial data on MMF supporting its efficacy as a steroid-sparing agent. Among the different drugs used for their steroid-sparing effect in FRNS, levamisole is the least toxic, and the least expensive. However, it is neither approved for this indication nor widely used in Europe, there are new clinical trial data supporting the efficacy of levamisole in SSNS [21].

The alkylating agent CP is widely used in SDNS and FRNS in children, either orally (2-3 mg/kg per day for 8-12 weeks) or in intravenous form (500-750 mg/m^2 per month for 6 months) [22]. CP has been shown to prevent progressive scarring within the kidney, preserve renal function, induce remission, and to reduce the risk of end-stage renal failure, but it also causes lymphopenia, decreases immunoglobulin secretion, suppresses some of the T-cell functions and enhances the immune response by inhibiting suppressor T-cells.

The toxicity of CP (2-3 mg/kg per day) [23– 25] and chlorambucil (0.2 mg/kg per day for 8-12 weeks) [26, 27] is generally mild and reversible, but it includes bone marrow depression, hemorrhagic cystitis, hair loss, infertility seizures, and rarely, oncogenesis [26–28]. In SDNS and FRNS there is no significant difference between CP and Chl therapeutic results.

CSA, a lipophilic decapeptide, is well recognized to be effective in the treatment of children with SDNS or SRNS. CSA is thought to bind to the cytosolic protein cyclophilin (immunophilin) of immunocompetent lymphocytes, especially T-lymphocytes. This complex of CSA and cyclophilin inhibits calcineurin, which is responsible for activating the transcription of interleukin-2 in T-cells. Activation of the T-cell receptor normally increases intracellular calcium, which acts via calmodulin to activate calcineurin. Calcineurin then dephosphorylates the transcription factor NF-AT (nuclear factor of activated T-cells), which moves to the nucleus of the T-cell and increases the activity of genes coding for IL-2 and related cytokines. CSA prevents the dephosphorlyation of NF-AT by binding to cyclophilin. It also inhibits lymphokine production and IL release and, therefore, leads to a reduced

function of effector T-cells. Several mechanisms have been postulated to explain the CSA-induced reduction in proteinuria in NS. The main mechanisms have been: changes in the properties of the glomerular barrier, resulting in an increased charge and size selectivity [30]; reduction in glomerular plasma flow or ultrafiltration pressure, which reduces proteinuria on a haemodynamic basis [30]; and inhibits the expression of IL-2 receptor at transcriptional level [31].

CSA can be used in teenage boys to avoid gonad toxicity of CP or in children with steroid toxicity. Response rates of 50-100% have been reported in the literature when twice-daily dosing of 5 to 32 mg/kg per day is applied (the usual dose is 5-6 mg/kg per day), blood levels of 70-500 ng/ml being achieved [29]. The efficacy and toxicity of CSA correlate with its serum concentration. Although there is no standard protocol, the initial CSA treatment normally lasts for 1-2 years [6].

The most common side effects of CSA are nephrotoxicity, a transient increase in serum creatinine concentration, a decreased glomerular filtration rate, gingival hyperplasia, hypertrichosis and gastric discomfort [9, 12, 17, 32].

Because of the relatively low prevalence of FRNS, SDNS and SRNS, it is difficult to establish the precise stage at which a steroid-sparing agent should be prescribed to control the disease and minimize steroid toxicity.

2. THE AIMS OF THE STUDY

The aims of our first study were

    1. to investigate the effects of levamisole on the number of relapses in FRNS, SDNS, and SRNS patients in childhood.

    2. to investigate the effects of levamisole on the cumulative steroid dose in FRNS, SDNS, and SRNS paediatric patients.

    3. to investigate the side effects of the levamisole treatment.

The aim of the second study was

    4. to compare the effect and

    5. to compare the long-term outcome of CP and CSA therapy in those idiopathic NS (INS) patients who were originally steroid sensitive but after several relapses became SRNS or SDNS.

3. PATIENTS AND METHODS

3.1. Levamisole study

A retrospective study was made on the medical records of 34 children (21 boys, 13 girls) with FRNS (15/34), SDNS (13/34), or SRNS (6/34) who were admitted to our Department of Paediatrics between January 1998 and January 2003. Their ages at the time of diagnosis ranged between 1.5 and 15 years (median 4.5 years).

Inclusion criteria were age at onset >1 year and <16 years, initial steroid sensitivity, FRNS or SDNS. Exclusion criteria were NS secondary to other systemic diseases or syndromes, and renal histology at onset or subsequently consistent with membranoproliferative or membranous nephropathy.

The first 34 patients who were admitted to our department for FRNS, SDNS, or SRNS and were treated with levamisole were included in the study. Renal biopsy was performed in 23 children and showed MCNS in 13, IgM nephropathy (IgM NP) in 7, and FSGS in 3. Renal biopsy was not undertaken at the initial presentation in 11 steroid-sensitive children in the absence of risk factors indicative of histology other than minimal change disease. However, renal biopsy was performed prior to the introduction of cytotoxic therapy in children with FRNS or SDNS, or in those who developed steroid resistance. In the SRNS patients, levamisole was introduced following cytotoxic therapy when a new relapse developed. Prednisolone was restarted because we wanted to investigate whether these patients would become steroid sensitive with a combined administration with levamisole.

There were 19 patients that received other immunosuppressive therapy before levamisole [9 received CP (2-2.5 mg/kg per day for 8-12 weeks), 10 received Chl (0.2 mg/kg per day for 8 weeks)].

Levamisole was introduced (2 mg/kg per day) after 4 weeks of daily corticosteroid treatment. Following another 4 weeks of alternate-day prednisolone, the dose of steroid was gradually tapered by 10 mg/week. The duration of levamisole treatment was 17±7 months (mean±SD; range: 5-36 months). Levamisole was discontinued for 6 months when leucopenia (white blood cell count $\leq 3\times10^9$/l) occurred and was then restarted.

12

The level of proteinuria at the time of diagnosis was 4.13±2.51 g/day (mean±SD). Before the start of levamisole treatment, the proteinuria level was 2.17±1.34 g/day (mean±SD), the endogenous creatinine clearance was 108.0±47.7 ml per 1.73 m^2 (mean±SD), and the relapse rate was 4.41/year (mean). The cumulative steroid dose before the introduction of levamisole was 7,564.4±3,497.1 mg/year (mean±SD).

3.2. CP versus CSA study

A retrospective study was made of the medical records of 37 children (25 boys, 12 girls) with idiopathic NS (INS) who were admitted to the Department of Paediatrics between 1989 and 2000 (follow-up time 5-13 years, median 7.1 years). At the start of their disease, all were steroid sensitive, but following several relapses, they became steroid dependent or steroid resistant.

Two groups of patients were identified. Group 1 consisted of 22 children (15 boys, 7 girls) age range 2-14 (mean±SD: 7.4±3.6 years) who received CP first as a second-line immunosuppressive drug because of their NS. Group 2 consisted of 15 children (10 boys, 5 girls) who received second-line treatment with CSA. Their ages at the time of the diagnosis lay in the interval 5-16 (mean±SD: 11. 7±4.4) years.

Inclusion criteria were age at onset > 1 year and ≤ 18 years, SDNS or SRNS.

Renal biopsy was performed in all cases, and it was indicated because we wanted to see the histology before the change of steroid therapy to another immunosuppressant. Biopsy revealed MCNS in 19 and FSGS in three patients in group 1. In group 2, FSGS was found in seven and MCNS in eight children.

CP was introduced at 2-2.5 mg/kg per day orally for 8-12 weeks. Mean duration of CP treatment was 2.5±0.5 months (2-3 months). CP therapy was associated with 1 mg/kg oral prednisolone every other day.

The mean proteinuria level at the time of diagnosis was 5.0±3.5 g/day (mean±SD), which became <0.5 g/day during the initial steroid therapy. Before the start of CP treatment, proteinuria level was 3.9±2.9 g/day (mean±SD), endogenous creatinine clearance was

100.4±50 ml/min per 1.73 m^2 (mean±SD), and the relapse rate was 3.4±2.8/year (mean±SD). The cumulative steroid dose before CP introduction was 6,941±2,891.2 mg/year (mean±SD).

CSA was introduced at 3-5 mg/kg per day. Mean CSA treatment duration was 28±15 (7-60) months. CSA serum drug level was monitored monthly; target concentration levels were 100-200 ng/ml at trough and 800-1000 ng/ml at peak. CSA therapy was associated with 1 mg/kg prednisolone every other day.

Proteinuria level at the time of diagnosis was 4.5±2.3 g/day (mean±SD) and became <0.5 g/day at the end of the first steroid course. Before the start of CSA treatment, the mean proteinuria level was 3.9±2.3 g/day (mean±SD), the endogenous creatinine clearance was 86.6±27.3 ml/min per 1.73 m^2 (mean±SD), and the relapse rate was 3.7±3.1/year (mean±SD). The cumulative steroid dose before CSA introduction was 7,656.4±3,517.2 mg/year (mean±SD).

3.3. Definitions

FRNS was defined as two or more relapses within the first 6 months of the initial episode or four or more relapses during any 12-month period.

SDNS was defined as at least two relapses during alternate-day steroid treatment or within 14 days after stopping steroid therapy.

SRNS was defined as no improvement in proteinuria after 1 month of prednisolone therapy of 60 mg/m^2 per day.

Relapse was defined as proteinuria ≥ 1.0 g/day (40 mg/m^2) for 3 consecutive days.

Complete remission was defined as a reduction in urinary protein excretion to ≤ 0.1 g/day (≤ 4 mg/m^2) for 3 consecutive days.

Partial remission was defined as decreased urinary protein by ≥ 50 % from the baseline on initial presentation.

3.4. Corticosteroid protocol

The initial corticosteroid protocol at the start of the disease was prednisolone 60 mg/m^2 daily in two divided doses for 4 weeks, followed by 40 mg/m^2 in a single dose every other day for 4 weeks, with the dose then being tapered by 10 mg/week according to the recommendations of the International Study of Kidney Disease in Children (ISKDC) protocol. Relapses were treated by the same method in all patients. All patients were steroid sensitive at the first course of treatment, but they became FRNS, SDNS or SRNS later on. They received more than one steroid course; the median number was four.

3.5. Clinical assessment

Patients were checked monthly. At each visit, besides the clinical assessment, the following investigations were performed in every case: urinanalysis, 24-h urinary protein / urinary protein:creatinine ratio, endogenous creatinine clearance (based upon 24 hours urine collection), complete blood count, serum creatinine determination, liver function tests were measured. In the second study (CP versus CSA study) estimated glomerular filtration rate (eGFR) was calculated according to the Swartz formula: $GFR = k \ x \ height[cm]/serum \ creatinine[mg/dL]$ where $k=0.55$, serum lipid profile and CSA blood concentration determinations were also performed. Genetic analysis was not performed.

At the start of the study, all the patients had normal liver function tests and a normal blood count in the levamisole group. The endogenous creatinine clearance was initially abnormal in 7/22 patients in the CP and in 5/15 patients in the CSA group.

3.6. Statistical analysis

The clinical data on the patients are reported as median, mean ± standard deviations (SD). Statistical analyses included the Student's t-tests for the comparison of parametric data. The level of statistical significance was taken as ∗p<0.05.

4. RESULTS

4.1. Results of the levamisole study

Of the 34 patients, 28 had SSNS and 13 of these became steroid dependent. Of the 34, 6 developed SRNS before starting levamisole treatment. The patients showed signs of steroid toxicity, including growth retardation, obesity, hypertension, or osteoporosis. Data on patients in the FRNS, SDNS, and SRNS groups are shown in Tables 1, 2, and 3, respectively.

The duration of levamisole treatment was 5-36 (median 17) months. There were 29 patients that received levamisole for at least 18 months, 5 received it for a shorter period because of leucopenia. Of the 29 children, 23 received levamisole for 18 months; 6 had longer therapy because of relapses. The level of proteinuria was 4.13 ± 2.51 g/day at the time of diagnosis and 2.17 ± 1.34 g/day before the start of levamisole treatment.

During therapy the level of proteinuria fell significantly to 0.128 ± 0.213 g/day ($p<0.0001$) and remained low after the cessation of the levamisole adjuvant therapy (0.134 ± 0.301 g/day).

The relapse rate was 4.41/year before levamisole treatment and 0.41/year during levamisole therapy ($p<0.0001$). No relapse occurred in 23 of the 34 patients during this therapy, while 10 children had one, and only one child had two relapses per year. Following the levamisole treatment the relapse rate during the 24-month follow-up was 0.22/year; 28 of the 34 children remained in complete remission and only six of them relapsed. The changes in the level of proteinuria and the cumulative steroid dose are shown in Table 4.

In 23 of the 34 children, steroid administration could be stopped and they were in remission on levamisole alone. Of the 34, 11 still needed prednisolone treatment because of relapses and in one the treatment was supplemented with CSA.

There were 2 of 15 of the FRNS patients, 6 of 13 of the SDNS patients, and 3 of 6 of the SRNS patients that suffered a relapse during the levamisole therapy. The corresponding rates were 3 of 15 in the FRNS group, 2 of 13 in the SDNS group, and 1 of 6 in the SRNS group two years after cessation of levamisole therapy (Table 5).

The connections between the histopathology and the therapeutic response during the treatment and the follow-up are shown in Table 6.

Table 1 Patients with frequently relapsing nephrotic syndrome (FRNS)

Patient no.	Sex	Age (years)	Biopsy	Immunosuppression other than prednisolone before levamisole treatment	Proteinuria (g/day)			Cumulative steroid dose (mg)	
					At diagnosis	At start of levamisole therapy	After levamisole therapy	Before start of levamisole therapy	During levamisole therapy
1	M	5	MCNS	CP	2.3	0.98	0	9240	0
2	M	3	-	-	2.3	1.8	0.54	4930	0
3	M	2	-	Chl	4.1	2.7	0	1850	0
4	F	4.5	MCNS	-	3.2	2.7	0.01	7390	3080
5	F	3.5	MCNS	-	2.1	1.1	0	11080	0
6	M	3	-	CP	4.8	2.4	0.612	7390	0
7	F	6	IgM NP	CP	1.5	1.8	0.1	11090	0
8	F	4	-	-	2.1	0.98	0	8320	3080
9	F	5	-	CP	5.0	1.5	0.17	3080	0
10	M	1.8	-	-	1.5	0.9	0	3400	1680
11	F	2.5	-	-	2.1	0.7	0	15400	0
12	F	5	MCNS	Chl	8.7	3.5	0	12320	0
13	M	4.5	MCNS	Chl	3.7	2.3	0	7530	4920
14	M	4.5	-	-	1.7	0.98	0.05	6460	2060
15	F	5	-	-	1.4	1.1	0.016	6080	4920

M male; *F* female; *MCNS* minimal change nephrotic syndrome; *IgM NP* IgM nephropathy; *CP* cyclophosphamide; *Chl* Chlorambucil.

Table 2 Patients with steroid-dependent nephrotic syndrome (SDNS)

Patient no.	Sex	Age (years)	Biopsy	Immunosuppression other than prednisolone before levamisole treatment	Proteinuria (g/day)			Cumulative steroid dose (mg)	
					At diagnosis	At start of levamisole therapy	After levamisole therapy	Before start of levamisole therapy	During levamisole therapy
1	M	6	MCNS	MP, CP	6.0	2.5	0.54	12320	0
2	M	5	-	-	2.9	1.2	0.1	9240	3080
3	M	15	FSGS	-	5.2	2.4	0.36	13860	5470
4	F	7	MCNS	Chl	6.0	2.6	0.01	6160	0
5	M	1.5	-	-	5.4	3.2	0	3330	1100
6	M	14	MCNS	CP	11.16	2.6	0	6160	1680
7	M	4	MCNS	Chl	5.1	2.4	0.1	3080	0
8	F	6	MCNS	Chl	8.8	6.1	0.02	6160	0
9	M	4	IgM NP	MP	2.5	2.5	0.4	6160	3080
10	M	4.5	IgM NP	CP	4.0	1.15	0.8	6160	2800
11	M	5	FSGS	-	10.3	5.8	0.08	6510	2050
12	M	5	IgM NP	MP	3.7	4.4	0.2	8360	2050
13	M	5	MCNS	-	4.93	2.4	0	5600	0

M male; *F* female; *MCNS* minimal change nephrotic syndrome; *MP* methylprednisolone; *CP* cyclophosphamide; *FSGS* focal segmental glomerulosclerosis; *Chl* chlorambucil; *IgM NP* IgM nephropathy.

Table 3 Patients with steroid-resistant nephrotic syndrome (SRNS)

Patient no.	Sex	Age (years)	Biopsy[a]	Immunosuppression other than prednisolone before levamisole treatment	Proteinuria (g/day)			Cumulative steroid dose (mg)	
					At diagnosis	At start of levamisole therapy	After levamisole therapy	Before start of levamisole therapy	During levamisole therapy
1	F	1.5	MCNS	Chl	5	3.3	0.02	2870	0
2	F	1.5	IgM NP	CP	2.5	0.4	0.01	6600	0
3	M	2	IgM NP	MP, CP	2	0.5	0.024	5540	3900
4	M	15	FSGS	Chl	2.5	1.5	0.15	11370	2050
5	M	3	MCNS	Chl	2.4	1.8	0.32	14690	3080
6	M	5.5	IgM NP	Chl	3.4	1.7	0.18	7390	0

M male; *F* female; *Chl* Chlorambucil; *MP* methylprednisolone; *CP* cyclophosphamide *MCNS* minimal change nephrotic syndrome; *IgM NP* IgM nephropathy; *FSGS* focal segmental glomerulosclerosis.

[a] Patients had a history of MCNS (in patient 4 FSGS). MCNS was combined with IgM NP in patients 2, 3, and 6.

Table 4 The distribution and data of patients with nephrotic syndrome

	FRNS (n=15)	SDNS (n=13)	SRNS (n=6)
Sex (male/female)	9/6	10/3	6/0
Age at diagnosis (years; mean±SD)	3.95±1.25	6.3±3.86	4.75±5.24
Age at start of levamisole therapy (years)	6.67±3.18	9.37±4.1	6.2±5.6

Proteinuria (g/day)
  at diagnosis
  at start of levamisole therapy
  after levamisole therapy
	
	3.1±1.95
	1.69±0.84
	0.09±0.2[**]
		
		5.84±2.68
		3.02±1.53
		0.2±0.25[**]
			
			2.96±1.09
			1.53±1.05
			0.11±0.12[*]

Cumulative steroid dose (mg)
  at start of levamisole therapy
  after levamisole therapy
	
	7704.7±3707.9
	1316.0±1860.7[**]
		
		7165.5±3115.2
		1639.2±1689.1[**]
			
			8086.5±4256.5
			1505.0±1749.7[**]

FRNS frequently relapsing nephrotic syndrome; *SDNS* steroid dependent nephrotic syndrome;
SRNS steroid resistant nephrotic syndrome.
[*] $P<0.01$, [**] $P<0.0001$ vs. start of levamisole therapy; data are given in mean±SD.

Table 5 Relapses during levamisole therapy and during the follow-up period

	Number of relapsed patients during levamisole therapy	Number of relapsed patients 24 month after levamisole therapy
FRNS (n=15)	2	3
SDNS (n=13)	6	2
SRNS (n=6)	3	1

FRNS frequently relapsing nephrotic syndrome; *SDNS* steroid dependent nephrotic syndrome; *SRNS* steroid resistant nephrotic syndrome.

Table 6 Histopathology and therapeutic response

Histology	During levamisole therapy			24 month after levamisole therapy (number of relapses)
	No relapse	1 relapse	2 relapses	
	in 12 month			
MCNS (n=13)	8	4	1	1
IgM NP (n=7)	3	4	-	1
FSGS (n=3)	2	1	-	1
Unknown histology (n=11)	10	1	-	3

MCNS minimal change nephrotic syndrome; *IgM NP* IgM nephropathy; *FSGS* focal segmental glomerulosclerosis.

The endogenous creatinine clearance did not change significantly during the follow-up period, being 108.2 ± 45 ml/min per 1.73 m^2 before the start of levamisole therapy and 108.0 ± 47.7 ml/min per 1.73 m^2 after its completion. Significant changes were not observed in serum electrolyte and liver function parameters.

During the follow-up period, 5 of our patients developed reversible neutropenia (white blood cell count $<3\times10^9$/l). After normalization of the white blood cell count, the levamisole treatment was restarted. None of our patients developed gastrointestinal, cutaneous, or other side effects [20, 33−37]. Side effects of glucocorticoid therapy disappeared during the observation period in all patients.

4.2. Results of the CP treated group

Table 7 and 8 present data on the treatment of patients according to histology and clinical course of the disease. Twenty-two INS patients received CP for 2.5 ± 0.5 months in the first course as a second immunosuppressive drug after a remission with steroids was achieved. The average time from the onset of NS to the start of CP therapy was 2.5 ± 2.0 years. Fifteen of the 22 patients also received methylprednisolone (MP) pulse therapy before administration of CP. Fifteen children had SDNS and seven had SRNS. Nineteen had MCNS and three had FSGS. Proteinuria decreased from 3.9 ± 2.9 to 0.5 ± 1.4 g/day (p<0.01) and remained at 0.9 ± 1.7 g/day, 5 years later. One patient with FSGS, who had heavy proteinuria (12.8 g/day) at the start of CP, still had 6.2 g/day at the end of the 5-year follow-up. There were three patients with MCNS who developed increasing proteinuria, 1.8, 5.2 and 3.0 g/day, respectively, by this time. Nine patients had a reduced creatinine clearance and were ranged into National Kidney Foundation Kidney Disease Quality Outcomes Initiative [38] stages for chronic kidney disease (NKF K/DOQI CKD) stage III-V at the end of CP therapy; one normalized by the time of the 5-year follow-up. Eight of 22 patients had an elevated systolic blood pressure (≥120 mmHg) at the end of the 5-year follow-up period.

Table 7 Statistical data of the cyclophosphamide and cyclosporine-A treated patients according to the clinical course (SDNS or SRNS)

	Group I (CP)						Group II (CSA)					
Clinical classification	SDNS (n=15)			SRNS (n=7)			SDNS (n=8)			SRNS (n=7)		
Age at onset (year)												
- mean±SD	7.7±3.8			6.4±2.9			9.5±4.7			14.3±2.4		
- median (range)	9 (2-14)			7.7 (2.2-12)			8 (4.5-16)			15 (10-16)		
Gender: male/female	12/3			3/4			7/1			¾		
Histology: MCNS/FSGS	12/3			7/0			4/4			4/3		
Duration of treatment (month) (mean±SD)	2.5±0.5			2.4±0.5			20.2±8.5			36.7±17.2		
Urinary protein:creatinine ratio	Before	2 years after	5 years after	before	2 years after	5 years after	Before	2 years after	5 years after	before	2 years after	5 years after
	Therapy											
- < 0.2 mg/mg	1	10	10	0	5	3	7	6	4	7	3	4
- 0.2-2.0 mg/mg	3	3	3	2	2	3	1	2	3	0	3	3
- > 2.0 mg/mg	10	1	1	6	1	2	0	0	1	0	1	0
Urinary protein (g/24 h; mean±SD)	4.1±3.3	0.4±0.8*	0.7±1.6*	3.3±2.3	0.3±0.6*	1.3±2*	4.4±2.6	1.3±3.3*	0.9±2*	3.5±2.1	1.4±2.1*	0.3±0.2*
eGFR (ml/min per 1.73 m^2), (NKF K/DOQI CKD stages)												
- > 90 (stage I)	4	5	7	1	1	4	2	0	5	0	2	3
- 60-89 (stage II)	5	4	3	5	3	0	4	4	1	3	2	3
- 30-59 (stage III)	4	3	3	0	2	1	1	3	1	2	2	0
- 15-29 (stage IV)	1	3	1	0	1	1	1	0	0	0	1	1
- < 15 (stage V)	1	0	1	1	0	1	0	1	1	1	0	0
BP (mmHg; mean±SD)	systolic	diastolic		systolic	diastolic		systolic	Diastolic		systolic	Diastolic	
- before th.	108.3±12.3	72±7		106.4±12.5	67.1±7.6		115.6±9.2	77.5±8.5		119.3±6	77.1±9	
- 1 year after th.	111.2±12.3	71.3±8.5		107.1±10.7	67.9±6.3		117.5±12.8	76.8±7.0		120.7±7.9	78.6±7.5	
- 2 years after th.	113± 15.3	72.2±10.9		104.3±12.4	68.6±10.7		115±17.7	72.5±10		119.2±16.9	75±10.4	
- 5 years after th.	113.3±11.4	74.3±8.0		122.1±13.5	80±12.6		121.9±11.6	78.7±6.4		122.1±12.2	79.3±10.2	
Relapse free period(month, mean±SD)	29.9±21.5			30.3±23.3			28.1±22.4			24±12.7		
Relapse rate/year 5 years after therapy (mean±SD)	0±0.3*			0.1±0.13*			0.8±1.0*			0.5±0.7*		

CP cyclophosphamide; *CSA* cyclosporine-A; *SDNS* steroid dependent nephrotic syndrome; *SRNS* steroid resistant nephrotic syndrome; *MCNS* minimal change nephrotic syndrome; *FSGS* focal segmental glomerulosclerosis; *BP* blood pressure; *eGFR* estimated glomerular filtration rate (according to the Schwarz formule); *NKF K/DOQI CKD stages* National Kidney Foundation K/DOQI stages for chronic kidney disease. *P<0.001 vs. before the start of CP or CSA therapy.

Table 8 Data of cyclophosphamide and cyclosporine-A treatment in idiopathic nephrotic syndrome according to the histology

		Before starting therapy			At the end of therapy			Two years after treatment			Five years after treatment		
		UP g/day	Cl creat ml/min per 1.73m^2	Relapse rate/year	UP g/day	Cl creat ml/min per 1.73m^2	Relapse rate/year	UP g/day	Cl creat ml/min per 1.73m^2	Relapse rate/year	UP g/day	Cl creat ml/min per 1.73m^2	Relapse rate/year
CP (n=22)	MCNS (n=19)	3.4±2.3	104±51.8		0.2±0.4[*]	88.4±28.2		0.2±0.4[*]	83.9±29		0.7±1.3[*]	108.2±45	
	FSGS (n=3)	6.6±5.4	77.5±34.3		2.5±3.5[*]	70.3±37.6		1.6±1.1[*]	75.6±29.5		2.1±3.4[*]	86.1±66.4	
		3.9±2.9	100.4±50	3.4±2.8	0.5±1.4[*]	85.9±29.3	0.5±0.6[*]	0.4±0.7[*]	82.7±28.5	0.2±0.1[*]	0.9±1.7[*]	105.3±47.7	0.1±0.2[*]
CSA (n=15)	MCNS (n=8)	3.5±2.3	83.8±27		0.7±1.1[*]	71±30.9		0.8±1.7[*]	86.9±22.6		0.2±0.2[*]	80.7±52.5	
	FSGS (n=7)	4.5±2.4	88.6±29.2		1±1.7[*]	95.3±37.2		2.1±3.6[*]	80.2±26.7		1±2.2[*]	122.9±18.5	
		3.9±2.3	86.6±27.3	3.7±3.1	0.9±1.4	83.9±35.4	0.9±0.5[*]	1.5±2.8	83.4±24.2	0.9±0.5[*]	0.7±1.6	109.8±35.5	0.6±0.8[*]

CP cyclophosphamide; *CSA* cyclosporine-A; *UP* urinary protein (g/day); *MCNS* minimal change nephrotic syndrome; *FSGS* focal segmental glomerular sclerosis; *Cl creat* endogenous creatinine clearance (ml/min per 1.73 m^2).

Data are presented in mean±SD. [*]P<0.001 vs. before the start of CP or CSA therapy.

The relapse-free period was 30±21.5 months (29.9±21.5 months in SDNS and 30.3±23.3 months in SRNS patients). The relapse rates decreased significantly from 3.4±2.8 to 0.5±0.6/year during the first course of CP therapy and remained at this level (0.1±0.2/year) during the 5-year follow-up (p<0.001). At the end of follow-up, five patients were in remission (<0.4 g/day proteinuria, ≥80ml/min per 1.73 m^2 creatinine clearance) with immunosuppressive therapy.

In relation to CP therapy, 3/22 children had nausea, 2/22 patients had reversible hair loss, 1/22 had leucopenia and 1/22 alopecia.

4.3. Results of the CSA treated group

Table 7 and 8 presents treatment data on the patients according to histology and clinical course of the disease. Fifteen patients received CSA for an average of 28±15 months in the first course as a second immunosuppressive drug after a remission with steroids had been achieved. Twelve of the 15 patients also received MP pulse therapy before administration of CSA. Time from diagnosis to the start of CSA treatment was 3.5±3 years. Eight children had SDNS and seven SRNS. Seven of them had FSGS and eight MCNS. Proteinuria decreased significantly (at the start of CSA treatment, it was 3.9±2.3 g/day, whereas at the end of CSA treatment, it was 0.9±1.4 g/day, p<0.01, 5 years later, it was 0.7±1.6 g/day). Creatinine clearance and eGFR did not change significantly during the 2-year follow-up but increased at the end of the study. Two patients with FSGS and one with MCNS did not respond to this CSA course: proteinuria was 3.5; 2.5 and 4.4 g/day, respectively. Proteinuria increased further in an FSGS case, to 6.1 g/day 5 years after the first CSA period, whereas in the other FSGS and the MCNS patient, a further course of CSA treatment resulted in a decrease in proteinuria to 0.4 and 0.5 g/day, respectively. After 5-years of follow-up, eGFR decreased <15 ml/min per 1.73m^2 in two FSGS patients. In these patients, marked hypertension had also developed.

The relapse-free period was 26.2±18 months (28.1±22.4 months in SDNS and 24±12.7 months in SRNS patients). Relapse rates significantly decreased, from 3.7±3.1 to 0.9±0.5/year during the first CSA course and remained at this lower level (0.6±0.8/year)

during the follow-up (p<0.001). At the end of follow-up in the CSA group, four patients were in remission (<0.4 g/day proteinuria, ≥80ml/min per 1.73 m^2 creatinine clearance) with immunosuppressant therapy.

During CSA treatment 3/15 patients exhibited hirsutism, 2/15 tremor, 2/15 gingival hyperplasia, and 1/15 had nausea and appetite loss.

Table 9 represents the further therapy after the examined period. In the CP group, CSA was introduced when CP results were not sufficient (6/22). Four of 22 patients received prednisolone/MMF therapy as immunosuppression after CP. In the CSA group 5/15 patients received more than one CSA course and 4/15 needed other immunosuppressive therapy. One patient in each group needed renal replacement therapy at the end of the 5-year follow-up.

Figure 1 shows the clinical course according to the histology of the patients in remission or in relapse during the follow-up period.

Table 9 Further immunosupressive treatment after first CP and CSA course

	Number of patients		Number of patients
Only 1 course of CP	10	Only 1 course of CSA	5
CP after CP	0	CSA after CSA	5
Prednisolone and CSA after CP	2	Prednisolone and CSA after CSA	1
CSA after CP	4	CP after CSA	1
Prednisolone after CP	2	Prednisolone after CSA	1
MMF after CP	1	MMF after CSA	1
MMF and Prednisolone after CP	1	MMF and Prednisolone after CSA	0
HD/CAPD and CSA after CP	1	HD after CSA	1

CP cyclophosphamide; *CSA* cyclosporine-A; *MMF* mycophenolate mofetil; *HD* hemodialysis; *CAPD* continous ambulatory peritoneal dialysis.

Figure 1 Clinical course of CP and CSA treated patients after five years follow-up
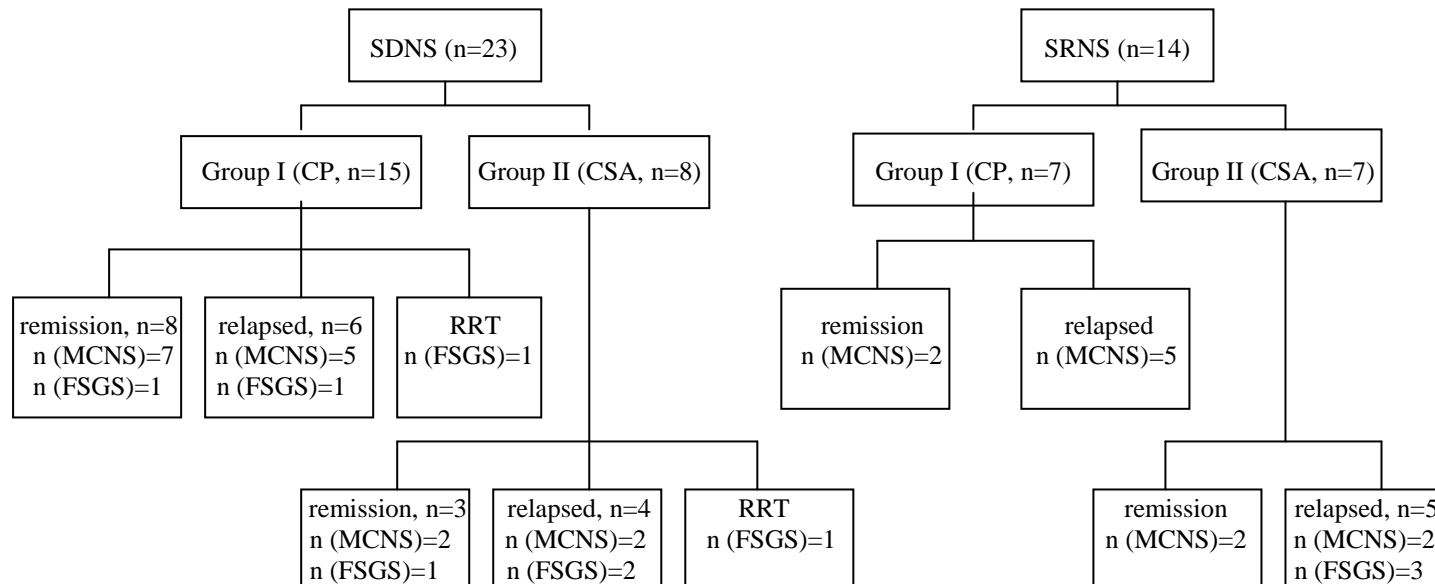
CP cyclophosphamide; CSA cyclosporine-A; SDNS steroid dependent nephrotic syndrome; SRNS steroid resistant nephrotic syndrome; RRT renal replacement therapy; MCNS minimal change nephrotic syndrome; FSGS focal segmental glomerular sclerosis.

5. DISCUSSION

5.1. Discussion of the levamisole study

One of the most difficult tasks in paediatric nephrology is the care of INS patients with multiple relapses, and the situation is even more difficult in SDNS and SRNS patients. These patients are candidates for treatment with steroid-sparing agents [15, 25, 19, 39].

Attempts to maintain remission and to prevent relapses are associated with certain risks. The immunomodulatory agent levamisole has been used as an adjunctive therapy in such patients [40–47] as a good alternative to major immunosuppressives [48]. This treatment leads to decreases in the number of relapses and the amount of prednisolone required. Niaudet et al. treated 30 SDNS patients with levamisole for a mean duration of 9.9 months. Almost half of the patients had no relapses, despite the achievement of a significant reduction in the dose of prednisolone [41].

The British Association for Paediatric Nephrology (BAPN) reported prolonged remission in patients with SDNS given alternate-day levamisole for 16 weeks [45]. However, relapses occurred in the majority of patients after the treatment was stopped. Neuhaus at al. likewise observed that levamisole at a dose of 2.5 mg/kg on alternate days for 6-18 months was effective in inducing remission in more than half of the patients [15]. Bagga et al. suggested that long-term levamisole therapy with low-dose prednisolone can lead to a significant reduction in the relapse rate in SDNS patients [13]. In a prospective trial, Donia et al. treated 20 SDNS patients with levamisole for 6 months and followed them for a further 6 months. At the end of the 6-month treatment period, 10 patients were maintaining remission on levamisole alone. At the end of the 12-month study period, 5 patients (25 %) were still in remission [47]. Ksiazek and Krynski reported a higher proportion (45.5 %) of patients who were able to withdraw from steroids and maintain remission for more than 6 months after levamisole therapy, perhaps because they did not stop levamisole throughout the study [49]. Tenbrock et al. similarly reported a 10-month duration of remission; they may have studied FRNS patients [50]. Alsaran et al. concluded that levamisole is a good alternative to CP as a second-line agent for FRNS patients [23]. Abeyagunawardena et al. reported a retrospective

comparative study in which levamisole was prescribed as a first steroid-sparing agent for 65 children; disease control was achieved in 30%. They concluded that levamisole is an attractive steroid-sparing agent [18]. Boyer et al examined both short- and long-term efficacy of levamisole (i.e. relapse frequency, cumulative annual steroid burden, somatic indices) in 10 children with SDNS. They gave levamisole for 12 months, and followed the patients for three years (from one year prior levamisole treatment to the end of the 12th month after cessation of the drug). They concluded that levamisole is an effective short- and long-term steroid sparing agent [51].

We used approximately double the dose that was used in the BAPN study, because they reported a number of relapses after the treatment was stopped. Therefore we decided to use a higher dose. In our study, no relapse occurred in 23 of the 34 patients (FRNS, SDNS or SRNS) during levamisole treatment, i.e. 67.6% remained in remission, while 10 patients had one and one had two relapses in 12 months. In the 24-month follow-up period after discontinuation of levamisole, 28 children remained in total remission and only 6 had relapses, i.e. a remission rate of 82.3%. There were two relapses among the 15 FRNS patients, six among the 13 SDNS children, and three among the 6 SRNS patients. Although the number of our patients is small, the proportion of relapse-free patients during levamisole treatment is 50% or higher in all three groups. These results are better than the previously reported studies. At the same time one should be aware that this is a retrospective, non-randomized study and there was no control group.

The small number of patients involved in the study does not allow a reliable statistical analysis of the histological findings and the therapeutic effects during levamisole therapy. Renal biopsy was performed in only 23 of 34 patients. It is therefore difficult to draw any conclusion based on the total population when the histopathology was known in only about 75%. Nevertheless, our MCNS patients and those children with unknown histology (indicating a clinical presentation that corresponds to MCNS) exhibited a better response to levamisole than those with IgM NP and FSGS (Table 6). Genetic analysis of the patients was not available in our centre at the time of the study.

The cumulative steroid dose was significantly lower following the introduction of levamisole treatment (i.e. 1468±1766 mg/year). The positive effect of reducing the steroid dosage may be secondary to the previously given alkylating agents. To investigate this, a

prospective study is necessary either with the elimination of alkylating agents or with a separate group of patients treated with alkylating agents only versus alkylating agents followed by levamisole.

The occurrence of side effects during levamisole treatment was rare. We found reversible neutropenia in five patients, but no other side effects.

Some studies report, that other drugs may also have an important role in the treatment of the examined population. High-dose mizoribine [52] or MMF [53] also appears to be useful in maintaining remission and have a steroid-sparing effect in SDNS and SRNS patients. They might be also successfully used in the CSA-dependent FRNS cases [52, 53].

5.2. Discussion of the CP versus CSA study

Our retrospective study documents that the long-term results of CP treatment in childhood SDNS and SRNS are at least as effective as those for CSA.

5.2.1. Clinical course of nephrotic syndrome

In our study, CP treatment duration was 2.5±0.5 months, and the relapse-free period was 30.05±21.5 months (in 5/22 patients ≥60 months). Eighteen of the 22 patients (82%) had a relapse-free period of more than a year following CP therapy without other immunosuppressive treatment. Altogether, 10/22 patients (48%) did not need further immunosuppressive treatment until the end of the fifth year. Of the CP-treated patients, 45.5% were in complete and 36.3% in partial remission after the 5-year follow-up (81.8% altogether). Further immunosuppressive therapy was needed in 12/22 patients because of relapses.

In our CSA-treated group, CSA treatment duration was 28±15 months (7-60 months). The relapse-free period following the start of CSA therapy was 26.2±18 months (in 2/15 patients it was ≥60 months). Of the CSA-treated patients, 46.6 % were in complete and 46.6% in partial remission (altogether 93.2%) at the end of the 5-year follow-up, and 33%

(5/15 patients) did not need therapy. Since many patients relapsed, their treatment was resumed or changed (see table 9). Nevertheless, 9/15 patients still needed maintenance immunosuppressant therapy from the beginning until the end of the 5-year-long follow-up.

At the end of the fifth year, 10/15 patients were in remission (66.6%) in the CSA group and 15/22 (66.2%) in the CP group. The difference between the relapse-free period and the relapse rates after the first CP or CSA period was significant after the 2-year and 5-year follow-up period, with much rarer relapses following CP therapy ($p<0.05$). When estimating our results, we found that partial remission is not equal complete remission and might finally not turn out to be beneficial for the patients.

5.2.2. Renal function, proteinuria and hypertension

Compared with the CSA group, a significant decrease in creatinine clearance was seen in patients who were treated with CP first after steroids, at the end of CP treatment, and 2 years after the CP therapy, but this change was reversible and disappeared after 5 years. Endogenous creatinine clearance and eGFR also showed a significant increase in the CSA-treated group after the 5 years of follow-up. In the CP-treated group, a significantly higher proportion of patients had <0.4 g/day proteinuria (16/22 patients, 72.7%) than those in CSA therapy (9/15 patients, 60%) at the end of the 5-year-long follow-up. At this time, blood pressure was significantly lower after CP than following CSA therapy.

5.2.3. Renal histology

FSGS is an indicator for poor prognosis: 6/10 patients relapsed, 2/10 patients developed end-stage renal failure and needed renal replacement therapy (RRT), and only 1/10 patient was in remission at the end of the fifth year with FSGS histology. In FSGS, the likelihood of remission is much lower than in MCNS which should make a mixture of these two groups obsolete. The low number of the FSGS patients in the CP-treated group (3/25) compared to the 7/15 patients in the CSA group makes it difficult to determine the statistical

significance of our results. Furthermore, we cannot be sure about the homogeneity of these two patient groups due to the lack of genetic analysis reviling the exact source of their symptoms. Although our patients had documented steroid sensitivity at the onset, controversially the histology and the refractory course ending in end-stage renal disease in some patients suggests that some of them might have had a resistant or very poor response to steroids initially.

5.2.4. Adverse events

The types of side-effects during CP and CSA therapy were different. CP therapy applied for less than 3 months was not associated with more side-effects than CSA treatment. Any complications in the two treatment groups proved to be reversible, though none of our patients received more than one course of CP therapy. There were no patients who needed hospitalization related to severe infections during the administration of oral CP or CSA.

The known long-term side-effects of CSA should be taken into account in the treatment decision. Its use for more than 1 year can result in chronic nephrotoxicity in 17-60% of patients [29] (i.e. CSA-associated arteriolopathy, tubulointerstitial lesions and focal glomerular lesions [54]). Other side-effects include hypertension, gingival hyperplasia, and hirsutism. Lijima et al. reported that CSA treatment duration and duration of heavy proteinuria during CSA treatment were independent risk factors for the development of CSA-induced tubulointerstitial lesions in children with MCNS who had been treated with long-term moderate-dose CSA [55]. Also, when using CP, the physician should also take into consideration the potential risk of infertility, cardiac disease and late cancer [56].

5.2.5. The possible use of cyclophosphamide or cyclosporine-A for treatment

Although a correct evaluation of the relative effectiveness of CP and CSA has not been possible because of the lack of a head-to-head randomised trial, studies in which alkylating agents and CSA have been compared have led to the conclusion that a course of

cytotoxic drug leads to a higher rate of cumulative sustained remission compared with CSA [57, 58]. Pena et al. achieved high remission rates (73.3%) in histologically proven MCNS and FSGS SRNS patients using MP pulses and alkylating agents (CP, Chl) together. They also concluded that initial steroid resistance is a poor prognostic factor compared with late-onset steroid resistance [59]. Our patients were steroid sensitive at the beginning, which may explain our better results.

The efficiency of CP and Chl in patients with SDNS, SRNS, and FRNS is defined by remission duration. The effect of cytotoxic drug therapy in SSNS depends on several factors, such as the underlying glomerular disorder, e.g. MCNS, mild mesangial proliferation, IgM NP [55, 60−62], FSGS, steroid sensitivity, FRNS or SDNS course of NS [63], type of cytotoxic drug [64], drug dose [56, 65], treatment duration [66], and concomitant drug therapy [60].

The reported remission rates after cytotoxic therapy vary from 0% after 12 months to about 30% after 5 years [67, 68]. In approximately 10% of the patients, however, NS may relapse during the cytotoxic treatment. The applied CP dose varies from 105 to 588 mg/kg body weight cumulatively [69−71]. Higher dosages are associated with a higher chance of relapse–free intervals, although the risk of side-effects increases. According to the meta-analysis of cytotoxic treatment in FRNS children published by Latta et al., the remission lasted for a maximum of 57 months after the first course of cytotoxic therapy, and the overall relapse-free survival after 4 years is <50%. On average, studies on FRNS result in remission rates of 72% after 2 years and 36% after 5 years; rates for SDNS are 40% and 24%, respectively [33]. Second courses of cytotoxic therapy are reported to increase the rate of long-lasting remissions [72−74]. We gave a CP dose of 112-168 mg/kg cumulatively in one course, and we did not apply a second CP course in any of the NS patients. Our better results may partially be explained by the fact that our patients had SSNS at the start of the disease.

In the study by Cattran et al., 49 SRNS due to FSGS patients were treated with CSA + low-dose prednisolone and compared with low-dose prednisolone + placebo and followed for 200 weeks. They found 70% of the treatment group vs. 4% of the placebo group had a partial or complete remission by 26 weeks. Relapse occurred in 40% of patients by week 52 and in 60% by week 78, but the remainder stayed in remission to the end of 200 weeks. There was a

decrease of 50% in the baseline creatinine clearance in 25% of the treated group compared with 52% of the controls [75].

Ehrich et al. examined 86 children with SRNS due to FSGS and MCNS in a retrospective, non-randomized study, and they concluded that prolonged and intensified treatment with combined prednisolone + CSA therapy including intravenous methylprednisolone pulses resulted in higher rate of remission when compared to CSA monotherapy or other immunosuppressive therapies. Because of a lack of efficacy and the risk of severe side effects of cytotoxic drugs, including gonadal toxicity, their experience does not indicate that children should be treated for steroid-resistant MCNS with CP [76].

Planck et al. conducted a controlled multicentre randomized open label trial to test the efficacy and safety of CSA (n=15) versus CP (n=17) pulses in the initial therapy of children with newly diagnosed primary SRNS and histologically proven MCNS, FSGS or mesangial hypercellularity. At week 12, nine of the 15 (60%) CSA patients showed at least partial remission. In contrast, three of the 17 (17%) CP patients responded (p < 0.05). After 24 weeks, complete remission was reached by two of the 15 (13%) CSA and one of the 17 (5%) CP patients (p = n.s.). Partial remission was achieved by seven of the 15 (46%) CSA and two of the 15 (11%) CP patients (p<0.05). They concluded that CSA is more effective than CP in inducing at least partial remission in SRNS in children [77]. Our better results can be explained by the fact that our patients were steroid sensitive at the onset of their disease.

Based on the data of the above mentioned trial, Hodson et al. concluded that although there was no difference in the number of patients achieving complete remission, those patients receiving CSA treatment were significantly more likely to achieve partial remission than those receiving intravenous CP. They also suggest that CSA rather than intravenous CP should be used as first line therapy for children with SRNS [78].

In the Cochrane Review, 26 trials involving 1173 children were identified. CP and Chl significantly reduced the relapse risk at 6-12 months compared with prednisolone alone. In the single Chl vs. CP trial, no difference in relapse risk was observed at 2 years. There was no difference at one year between intravenous and oral CP. CSA was as effective as CP and Chl, and levamisole was more effective than steroids alone, but the effects were not sustained, once CSA treatment was stopped. There was no difference in the risk for relapse between MMF and CSA. Mizoribine and azathioprine were no more effective than placebo

or prednisone alone in maintaining remission. The reviewers concluded that 8-week-long courses of CP or Chl and prolonged courses of CSA and levamisole reduce the risk of relapse in children with relapsing SSNS compared with corticosteroids alone [5]. Meanwhile the choice of agent depends on the physician and patient preferences related to therapy duration and the type and frequency of complications [5].

Kemper et al. reported that SDNS can recur in patients despite CSA maintenance therapy [79], and despite its efficacy, the majority of patients relapse if CSA is stopped [54, 80−82].

The role of the recently used drugs, such as tacrolimus, rituximab or the most hopeful MMF was not examined in this study. Other studies report that the replacement of CSA with the other calcineurin inhibitor tacrolimus did not lead to better management of severe SDNS [83, 84].

6. CONCLUSIONS

There is no uniformed consensus as to the treatment of steroid–unresponsive NS. Advances in pathogenesis, genetics and biomarkers or surrogate markers might be useful for the diagnosis and identification of patients with steroid-unresponsive NS, severity of disease, progression and response to therapy.

Our findings suggest that

1.   levamisole significantly reduces the relapse rate and the cumulative steroid dose in children with FRNS and SDNS and is also a beneficial and safe therapy for SRNS patients.

2.   Levamisole may have a place in preventing relapse even after courses of akylating agents, and it may also have some benefit for the treatment of SRNS patients.

The confirmation of the favourable effects of levamisole on the reduction of the frequency of relapses and on sparing steroids in an adequately powered, double-blind, placebo-controlled, randomized, multicenter clinical trial will promote consensus on the place of levamisole in the treatment of FRNS in childhood.

3.   In the long term, both CP and CSA are effective second-line therapies following steroid monotherapy in INS patients, but

4.   the relapse rate is lower and the relapse-free period significantly longer in the CP-treated group.

5.   An important message of our study is that most children who have a difficult course of NS after an initial remission induced by steroids do well 7-8 years after presentation.

6.   A good remission rate can be achieved after 5 years following the initial CP and CSA therapy, and the incidence of side effects is low.

On the other hand, our study is not able to give a final an answer to the question as to which is the better treatment in the examined patient population due to its retrospective nature with unequal distribution of FSGS in the different treatment groups. In addition, no supplementary genetic studies were performed; therefore about 10% of our patients are expected to have a genetic origin behind of their symptoms.

Further research is needed to elucidate the disorder's molecular pathogenesis, identify new prognostic indicators and to develop better approaches to treatment.

ACKNOWLEDGEMENTS

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PUBLICATIONS

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