COMBINATION THERAPY WITH PULSE CYCLOPHOSPHAMIDE PLUS CORTICOSTEROIDS IMPROVES RENAL OUTCOME IN PATIENTS WITH LUPUS NEPHRITIS

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ABSTRACT

Background: The prognosis of SLE is influenced by the onset of glomerulone-phritis. Clinical trials in lupus nephritis have demonstrated that cyclophosphamide therapy is the superior regimen in the management of lupus nephritis for preserving renal function.

Objective: The purpose of this study is to define the outcome of renal function with bolus pulses of cyclophosphamide and steroid according to our protocol and also to determine an appropriate pattern of treatment of lupus nephritis.

Methods: In this open-label clinical trial, to evaluate the results, the short-term prognosis and the rate of complications of an immunosuppressive regimen with corticosteroids and cyclophosphamide, twenty-five patients with biopsy-proven lupus nephritis were studied. Treatment was structured in 4 phases: 1) Induction with bolus methylprednisolone and cyclophosphamide. 2) Maintenance with oral prednisolone for 4 weeks and monthly cyclophosphamide pulses for 6 months. 3) Tapering with reduction of prednisolone by 10% each month and continuing cyclophosphamide every other month till one year and for the second year every 3 months. 4) Discontinuation with oral prednisolone slowly tapered to the least effective daily dose and cyclophosphamide discontinued after 2 yr of therapy. We defined primary outcome measures according to these criteria: renal function return to normal limits or become stable, regression of systemic and local inflammatory symptoms, urine protein excretion falling below 0.3 gr/dL or by at least 50%, RBC cast disappearance, C3, C4, Hb, and ESR return to normal limits.

Results: Twenty-three patients with lupus nephritis completed our therapeutic

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protocol. Renal biopsy was performed in 22 cases and indicated type IV in 20 patients (95.2%), and type V in 2 patients. After an average of 4+1.95 months 22 patients achieved remission (95.65%) and only one case remained non-responsive. She became pregnant in her fourth month of therapy. Significant statistical differences were achieved between creatinine, proteinuria, hematuria, leukocyturia, urinary cast, C3, C4, ESR, and Hb before and after therapy ($p<0.05$). Plasma creatinine fell from 1.44+0.95 mg/dL to 0.97+0.78 ($p<0.004$). Proteinuria fell from 1879.78+1854.46 to 408.34+572.92 mg/24h ($p<0.001$). Thirteen episode of relapses were treated again with repeated cycles of cyclophosphamide and all remitted again.

Conclusion: Intensive immunosuppression with steroid and cyclophosphamide provides excellent results with an acceptable rate of complications in the treatment of lupus nephritis.

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INTRODUCTION

Lupus is a complex immunologic disorder with multiple abnormalities in immunoregulation. Target tissue damage is caused by pathogenic autoantibodies, immune complexes and T lymphocytes. Renal involvement occurs in approximately 40% of patients with lupus. In 3-6% of patients, kidney involvement is the first manifestation of lupus and the severity of renal injury determines its prognosis.[1-5]

The WHO classification of lupus nephritis is based on biopsy findings.[6] Proliferative renal involvement like Class IV means life-threatening systemic lupus.

Nowadays we need further advances for treating severe lupus nephritis in order to lower the mortality rate and decrease progression to renal failure or dialysis.

If there is a proliferative component in renal biopsy the most effective therapy is steroid plus cytotoxic agents and cyclophosphamide is the superior drug in this setting.[7-11] In lupus nephritis treatment it is very important to consider the combination of drugs and to define goals of therapy.[12]

Therefore in this study we tried to clearly define inclusion criteria, activity and chronicity index, model and targets of therapy in advance.

The main goals of treatment are control of nephritis to the point of allowing a good quality of life, and non-progression of renal disease associated with a minimal risk of side effects.

PATIENTS AND METHODS

In order to evaluate cyclophosphamide and steroid efficacy in the treatment of lupus nephritis, we studied 25 patients who came to our rheumatology clinic with a diagnosis of lupus with glomerulonephritis according to ACR criteria between 1996 and 2000. Renal biopsy was done for 22 patients. The staging on renal biopsy was performed according to WHO class.[4,13,14] In all renal biopsy specimens the activity and chronicity scores were assessed and reported. We enrolled 24 patients with lupus nephritis in to this open clinical trial study.

Inclusion criteria for this study were the following:

1- Diagnosis of SLE in accordance with ACR criteria.[15]

2- Proliferative glomerulonephritis defined by one of the following:

- Evidence of active proliferative GN in the renal biopsy (WHO Class IV, III, IIb)

-When renal biopsy is not available, WHO class V or GN of undetermined type, with the following clinical or paraclinical findings:

a) The presence of one extrarenal systemic manifestation that needs immunosupressive therapy.

b) Proteinuria > 1gr/24h (or sustained 2 to 4 + proteinuria detected 2 times per year).

c) Progressive renal failure with 30% decrease in creatinine clearance over a 1-year period and/or creatinine >167 µmol/L (1.88 mg/dL) with any pathologic class.

d) The presence of > 5 red cells in HPF of urine sediment at least two times during one year.

e) Presence of RBC, WBC, granular or hyalin cast without active infection.

Exclusion criteria were the presence of any of the following:

WHO class I , II lupus nephritis, end stage renal disease in any WHO class when replacement renal therapy will be indicated or Cr >3 mg/dL for 3 consecu-

tive months, leukopenia (neutrophils $<1.5\times10^9$/L) due to bone marrow suppression, recurrent episodes of bacterial infection, history of cytotoxic drug treatment for more than 2 weeks during the 6 weeks before study entry, IDDM, the presence of only one kidney, and history of pulse therapy with corticosteroids during the 6 weeks before study entry. We evaluated all laboratory and serologic tests (CBC, platelet count, U/A, creatinine, GFR, 24^h proteinuria, C3, C4, Anti-DNA, ANA, ESR) at the beginning of the study and then monthly during the first 6 months, every other month during the first year and every 3 months thereafter. At each study visit, patients were questioned about and examined for adverse events. Each patient had a complete clinical evaluation for detection of other organ involvement besides nephritis and each patient was assesed when an episode of relapse or flare up occurred.

The intervals at which patients were followed were dictated by the activity of lupus and nephritis. All patients but one which was not possible, because of severe thrombocytopenia and recurrent seizures had biopsy specimens. The specimens were processed for optic microscopy and for immunofluorescence studies.

Method of therapy

The treatment consisted of 4 phases:

1) Induction: Methyl-prednisolone 1 gr/m^2 i.v. for 3 days, followed by 0.75-1 gr/m^2 bolus i.v. injection of cyclophosphamide.

2) Maintenance: intravenous cyclophosphamide, given as boluses once a month for 6 consecutive months adjusted with granulocyte count. High divided doses of oral steroid (>1mg/kg/day) for 4 weeks then tapered by 10% decrease in dose monthly.

3) Tapering: i.v. bolus cyclophosphamide once every 2 months for 1 year with the same dose and adjusted with PMN count followed by once every 3 months for another year. Prednisolone was decreased on average 10% every month till it reached a dose of 5 – 10 mg/day p.o.

3) Discontinuation: if our study goal was achieved, the alkylating agent was interrupted and prednisolone further tapered to the lowest possible dose every other day and patients followed up for evidence of relapse. When the patient had a relapse during treatment, if she was in maintenance phase, the dose of steroid increased and if she was in the tapering phase, a new induction was attempted with monthly i.v. bolus cyclophosphamide, the same as maintenance phase. When the tapering was followed by a clinical or laboratory flare, the dose of steroid was increased temporarily.

Goals of therapy or outcome measures

We separated primary from secondary outcome measures to reach a favourable balance between benefits and side-effects.

Primary outcome measures (criteria for partial remission): these criteria were considered essential for our patients:

a) no progression of renal disease (normal or stable renal function)

b) regression of systemic symptoms.

c) decrease of at least 50% in dysmorphic RBC, cellular cast and proteinuria in the absence of doubling of serum creatinine or less than 1gr proteinuria per day.

d) return to within normal limits of stigmata of inflammation (ESR , etc.)

e) return of patient to functional class II in the absence of disabling symptoms and in the presence of an acceptable rate of complications.

Secondary outcome measures (criteria for complete remission):

These targets were useful and important although not at the cost of serious complications.

a) Complete improvement of renal and extrarenal symptoms:

Rise of creatinine less than 30 μmol/L(0.3 mg/dL), less than 300 mg proteinuria per day or only trace proteinuria or less than 50% of initial proteinuria when Cr >150 μmol/l(1.7 mg/dL), avoidance of RBC cast, complete regression of all systemic and vasculitic symptoms.

b) Return to within normal limits of ESR, C3, C4, Hb.

c) Fall of auto-antibody titers.

d) Avoidance of relapses.

e) Avoidance of sterility, AVN and infectious complications.

Criteria for non-responders or progressive disease

When proteinuria fell <50%, hematuria remained and fell <50%, the red cell casts remained in the urine, plasma creatinine rose and stabilized >30 μmol/L above the pretreatment value, and when plasma creatinine became double at the end of the study.

Criteria for relapse

Increase in 2 of the following indices >50% (after reaching the lowest level during therapy), dysmorphic RBC, proteinuria, serum creatinine, cellular cast, doubling of proteinuria if there was nephrotic proteinuria or at least 2 gr/day if basal proteinuria <3.5 gr/day, at least 2 systemic symptoms reappeared.

Refractory to therapy

No renal response in spite of 3 courses of 6 month boluses of cyclophosphamide.

End stage renal disease

When plasma creatinine rose and stabilzed above 450 μmol/L (5 mg/dL) for 3 months.

Disease flare

Appearance of one or more systemic features during the

Table I. The table reports for the individual patients.

Patient No.	Renal Biopsy	Plasma Creatinine mg/dL		Proteinuria g/d		Total follow–up months	Outcomes of treatment				Final status	Cumulative dose of prednisolone and cyclophosphamide during protocol (g)	
		B	E	B	E		Full remission	Partial remission	Relapse	Flare		PDN	CPM
1	IV	0.9	0.6	6.000	0.063	29	—	yes	1	1	remission	10.55	10.20
2	IV	0.8	0.8	1.080	0.100	8	—	yes	—	—	rem	3.15	7.00
3	IV	2	0.8	0.300	0.300	27	—	yes	—	1	rem	11.90	15.00
4	IV	1.2	0.7	1.833	0.150	25	—	yes	2	—	rem	6.00	8.50
5	IV	1.3	0.6	1.450	-	19	Yes	—	—	—	rem	6.37	11.50
6	IV	0.6	0.7	0.280	-	24	—	yes	2	—	rem	7.63	14.50
7	IV	0.9	0.7	0.600	0.300	21	Yes	—	—	1	rem	8.85	13.00
8	IV	1.6	0.8	0.397	0.100	26	—	yes	—	1	rem	12.07	15.00
9	IV	5.2	3.9	6.200	1.100	18	—	yes	1	—	rem	5.73	13.00
10	IV	1.2	0.8	3.700	0.711	8	—	yes	—	1	rem	6.45	8.00
11	IV	1.7	0.9	1.100	-	25	Yes	—	—	1	rem	5.45	14.00
12	IV	1.4	0.8	1.060	-	15	—	yes	1	—	rem	5.92	7.00
13	IV	1.1	0.4	0.200	-	31	—	yes	—	—	rem	7.12	8.00
14	IV	0.7	0.4	2.200	-	16	—	yes	—	1	rem	4.41	10.80
15	IV	1.1	0.9	0.360	0.200	8	Yes	—	—	1	rem	7.36	8.00
16	IV	1	0.7	0.690	-	22	—	yes	1	1	rem	9.79	15.00
17	IV	1	0.6	4.700	0.638	22	—	yes	1	1	rem	10.55	16.00
18	IV	1.8	0.9	1.900	0.100	28	—	yes	1	1	rem	12.97	16.00
19	IV	0.7	2.8	1.000	2.000	14	Non responder				Fail	10.60	11.00
20	V	1.9	1.3	1.850	0.950	28	—	yes	2	—	rem	7.27	15.20
21	V	1.8	0.8	4.800	0.800	18	—	yes	—	1	rem	6.97	13.00
22	——	2.5	0.8	1.400	0.600	25	—	yes	1	—	rem	5.02	6.50
23	IV	0.9	0.7	0.090	-	26	Yes	—	—	—	rem	7.50	8.00

4th phase of therapy, when laboratory inflammatory indices rose or serum complement fell in the tapering phase of steroid, increase <30% in urinary abnormality and plasma creatinine.

All data were analyzed statistically, mean and standard deviations of the mean were computed and the significance of their differences tested by paired or unpaired Student's t–test. All mean values are shown ± 1 SD and P values <0.05 are considered statistically significant.

RESULTS

Twenty-five patients with active lupus nephritis were enrolled in this study. All patients had active disease both histologically and clinically. Two patients didn't complete the course of therapy and discontinued the protocol during 6 months. Our patients' data are reported in Table I. We enrolled 21 females and 2 males with average range of age 23.07 ± 10.56 years in this study.

After an average follow up of 21.00± 6.89 months, plasma creatinine and proteinuria fell significantly. An average duration between onset of disease and renal involvement was 16.21±24.54 months. Average duration between renal involvement and initiation of therapy was 6.94±16.29 months. Average duration of therapy was 21.00 ± 6.89 months. Twenty tissue samples were compatible with WHO class IV (DPGN) and 2 samples were WHO class V (MGN). Average score of disease activity was

Table II. Results of the statistical comparisons among selected variables before and after treatment cycles.

Parameters	Before	After	p value
Proteinuria	1879.78±1854.46	408.34±572.92	.001
Hematuria	20.35±10.40	3.04±6.36	.000
Leukocyturia	19.22±12.27	4.30±7.23	.000
C3	31.10±19.53	79.63±50.81	.001
C4	11.38±5.42	21.45±10.34	.003
Creatinine	1.44±0.95	0.97±0.78	.004
GFR	0.66±0.26	0.88±0.28	.002
ESR	65.04±38.19	23.48±19.90	.000
Hb	9.60±2.36	12.49±3.23	.000

Table III. The table reports the cumulative dose of prednisolone and cyclophosphamide during phases of therapy.

Dose(mg) Phase	Prednisolone	Cyclophosphamide
Phase I	1015.90±511.15	882.60±210.30
Phase II	3144.28±1268.20	5139.13±1620.81
Phase III	3737.21±1649.28	6552.63±4446.86
Phase IV	172.15±103.93	———

9.95 ± 3.40 and chronicity was 1.55 ± 2.4. Average range of diastolic blood pressure was 86.36 ± 17.26 mmHg and systolic blood pressure was 129.77±26.97mmHg. At the beginning of renal disease 91.3% of them had hematuria and leukocyturia and 95.9% had urinary casts. Five patients had proteinuria <500 mg/day (21.73%) and >2 gr/day in 6 cases (26.08 %).

Mean range of creatinine, proteinuria and GFR was 1.44±0.95 mg/dL, 1879.78±1854.46gr/24h, 0.72 ± 0.37cc/min, before treatment respectively. Decrease of C3 and C4 levels were seen in 94.7% and 82.3% at the beginning of therapy. ANA was positive in 83.3% of patients. Renal failure or creatinine >1.9 mg/dL was seen in four patients at the beginning of therapy. Five patients had nephrotic syndrome that didn't correlate with class of pathology and only two of them had membranous GN. Results of the statistical comparisons among selected variables (hematuria, leukocyturia, urinary cast, creatinine, GFR, C3, C4, proteinuria, Hb, ESR) before and after treatment cycles show significant differences ($p<0.05$) with 95% confidence level (Table II).

With regard to criteria for response to therapy 5 patients (21.73%) achieved complete remission, 17 patients out of 23 benefited from therapy by achieving a partial remission (73.91%) and overall 22 out of 23 patients (95.65%) achieved remission (Table I). Most of the patients had recovered within the first five months of therapy (4±1.95). In nephrotic patients there was a significant statistical difference between proteinuria before and after therapy ($p<0.001$). Only one patient had treatment failure and didn't respond to therapy. However this patient became pregnant at the 4th month of therapy. This is the only case of treatment failure in our study. Her pregnancy ended after two months. We didn't have any case refractory to therapy or ESRD or death till the last cycle of protocol. Flare up occurred in 12 out of 23 patients (54.5%) that improved with increasing dose of steroid. Thirteen episodes of relapse were seen in 23 patients. Except one, all episodes of relapse responded with a second remission (88.9%) after repeating the cycle of treatment. At the time of first relapse mean of C4 was 15.50 ± 6.3. Average period of first relapse was 7.75 ± 5.52 months. At the time of remission, proteinuria (71.42%) and hematuria (57.14%) fell sequentially and the first stigmata of relapse was proteinuria in 77.7%. We analyzed the relation between the additive dose of drug and response to therapy and recurrence. There was a direct association between the mean amount of steroids given in the third phase of therapy and time between two relapses ($p<0.01$). There was positive significant correla-

Table IV. Percent of side effects attributable to prednisolone and cyclophosphamide.

Prednisolone & Cyclophosphamide side-effects	No.	Percent
Infections (UTI, subcutaneous abscess, herpes zoster, candidiasis, salmonellosis)	18	41.9%
Leukopenia	5	11.6%
Cushingoid feature	5	11.6%
Hepatitis	2	4.7%
Aseptic necrosis of bone	1	2.3%
Diabetes	1	2.3%
Hemorrhagic cystitis	0	0

tion between steroid and cyclophosphamide dose in the second and third phases of therapy and time between two relapses (r=1.000, $p<0.01$) and positive significant correlation between steroid dose in the third phases of therapy and side effects (r= 0.483, $p< 0027$). Average cumulative dose of steroid and cyclophosphamide during the protocol is shown in Table III.

In this study no significant association was noted between chronicity and activity scores and response to therapy. On the other hand the amount of subendothelial deposits have great importance in predicting time of relapse ($p< 0.001$).

Fifteen out of 23 patients developed side effects during the protocol as mentioned in Table IV. The most frequent side effect was infection (41.9%) which mostly consisted of urinary tract infections and candidiasis. None of the infections were serious and they were all treated with proper therapy. No case of secondary malignancy was reported.

DISCUSSION

Lupus is a recurrent chronic inflammatory disease of connective tissue, which is mostly recognized by involvement of the skin, joints, kidneys and serous membranes. Certain immune complexes including anti-DNA, anti-C1q and anti-nucleosomes combined with an imbalance in apoptosis are capable of binding to renal structures and so causing nephritis.[16,24]

Renal manifestations are seen in about 40% of patients suffering from SLE and in 3-6% of patients renal problems are the first manifestations of disease and the severity of renal dysfunction determines the prognosis. Almost all studies approve bad prognosis if the disease is associated with proliferative renal disease.[25]

Having in mind new therapeutic methods, close association and coordination between the rheumatologist, clinical nephrologist and renal pathologist is the basis for appropriate decision.

The main goal of therapy should be to improve renal function or prevent progressive decrease. In treating lupus nephritis considering specific therapeutic goals and utilizing a logical combination of drugs is of great importance.[12] More studies with the same methodology are needed to reach corresponding conclusions.

In this study we tried to define clearly and carefully the criteria for choosing the patients, the level of chronicity in biopsy, the therapeutic method and especially the suspected goals, in order to clarify the final conclusion on treatment outcome.

According to previous reports, which named the combination of cyclophosphamide and steroids as the most effective treatment especially in patients with systemic disease and active vasculitis, we used cyclophosphamide and prednisolone as the theraputic protocol in four different phases with specified doses.[7-12,29-33]

The aim of the study was to define a form of treatment based on therapeutic goals and to determine an appropriate pattern for planning future studies using more new agents for treatment of lupus nephritis.

We classified treatment into four phases:

1) Induction (pulse therapy with steroids for three days and then bolus cyclophosphamide once)

2) Maintenance, monthly pulses of cyclophosphamide for the first 6 months plus oral prednisolone for 4 weeks.

3) Tapering phase, decreasing prednisolone 10% monthly and continuation of cyclophosphamide pulse every two months for the first year and then every three months until the end of the second year.

4) The fourth phase which consists of discontinuing cyclophosphamide and steadily reducing prednisolone to minimal effective daily doses if favorable response to therapy is seen.

In this study our therapeutic goals can be divided into primary and secondary goals. In the secondary goal the full remission of the patient was considered but for the primary goal partial remission was considered acceptable. It means no progression of renal disease (normal or stable renal function), regression of systemic ex-

trarenal symptoms such as vasculitis, at least 50% reduction in number of dysmorphic RBCs, cellular casts and proteinuria without doubling serum creatinine or excretion of less than 1 gr of protein daily, regression of other inflammatory factors (ESR) and the patient's return to functional class II in the presence of acceptable therapeutic side effects.

The major theory of this study is to determine whether treatment with cyclophosphamide and steroids in the four phasesof the protocol described is effective in complete or partial remission of our patients; whether there is a significant chance of recurrence after treatment with cyclophosphamide and steroids or not? and finally whether there are notable side effects to the protocol implicated?

Of the 23 patients being treated with the therapeutic protocol five achieved full remission (21.70 %) and 17 gained partial remission (73.9%). So with the criteria described, 22 patients (95.6%) were treated all together and only one patient (4.3%) did not respond to therapy. This patient became pregnant in the fourth month of treatment and so the disease did not respond to therapy. There was no reported case of resistance to therapy, ESRD or death till the end of treatment.

The findings concluded the following points:

The response rate to therapy was very valuable and compared favourably with other reports. Most of the patients had recovered within the first five months of therapy (4±1.95). Comparing the lab and biological parameters showed a statistically significant difference ($p<0.05$) before and after treatment. The first laboratory finding detected in the retrieval of the disease was proteinuria (71.42%).

The disease had many recurrences. Only one of which did not respond to therapy (11.1%). Most relapses occurred in the maintenance phase of therapy. The mean amount of C4 showed a reduction in recurrence. The symptoms of recurrence in more than two-thirds of the patients were increased protein in urine at first, reappearing hematuria and finally increased creatinine. It is possible that the reduction in the dose of steroids might be contributed to the recurrence, since almost all reported recurrences happened in the maintenance phase. Thus prescribing higher doses of steroids in this stage or adding a second immunosuppressive drug such as mycophenolate or cyclosporine could be considered.[37,39] Also postponing the early reduction of steroids might be helpful in preventing future relapses. Greater amounts of cyclophosphamide in the third phase decreased the probability of recurrence and increased the time needed for it ($p<0.01$).

In this study there was a direct association between the mean amount of steroids given in the third phase of therapy and time between two relapses. On the other hand a greater mean dose of steroids in the third phase caused a greater rate of side effects. Hence none of the infections were serious and they were all treated with proper therapy so it seems despite the side effects of the drugs the dosage of steroids must not be reduced too quickly.

Analysis of side effects shows no serious effect requiring special therapy or leading to death. 65.2% of patients suffered from side effects, which mostly consisted of infections. Fortunately all cases where controlled with therapy and did not cause serious side effects. There was one case of ANV, which is an unpredictable condition.

The most important factor in the lack of response to treatment proved to be pregnancy. So due to the known adverse effects of pregnancy it is strictly advised that pregnancy be postponed to the time when the patient has either fully recovered or is using the least amount of medication.[34,36]

The first sign of a relapse in more than two-thirds of the patients was an increase in proteinuria. Therefore increased proteinuria can be the most important indication for repeating the biopsy.[40,48]

The rate of response to therapy in this study is similar to those reported in other references.[7,12,26,29,31,32,34] At least 21% of our patients recovered fully. Therefore one can suggest that by stricter regimens and possibly combination therapy gaining full recovery is possible in lupus. In case of recurrence at any stage of therapy the therapeutic protocol should be restarted and the amount of cyclophosphamide adjusted based upon the response to therapy and bone marrow side effects.

Although most relapses were treated with continuation of therapy, yet the following questions are raised: Is using cyclophosphamide with longer intervals for example a single pulse of cyclophosphamide every 3 to 4 months after a full course of treatment useful in preventing relapse? Or will repeating the bolus dose of steroid in longer intervals or adding a second oral drug such as mycofenolate mofetil to the therapeutic protocol improve the results?

In repeated relapses or resistance to therapy adding new drugs such as mycofenolate to cyclophosphamide and assessing response to therapy using the well-defined criteria could be considered.[37,38,49,50]

A similar pattern with strictly defined criteria for parameters such as final goals of treatment, response to therapy, complete or partial recovery and recurrence should be organized in different rheumatology centers.

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