Efficacy of Two Cyclophosphamide Regimens for the Treatment of Lupus Nephritis in Puerto Ricans: Low vs Standard Dose

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Introduction: The clinical outcome and therapeutic response to immunosuppressive agents vary among patients with lupus nephritis of different ethnic populations. Thus, we evaluated the efficacy of two established treatment protocols for lupus nephritis (low-dose versus standard-dose cyclophosphamide) in Puerto Ricans with systemic lupus erythematosus (SLE).

Methods: A retrospective cohort of 49 adult patients with SLE treated with intravenous low or standard-dose cyclophosphamide for clinical or biopsy confirmed lupus nephritis was studied. Demographic parameters, clinical manifestations, autoantibodies and pharmacological treatments were determined prior to cyclophosphamide treatment. Renal parameters, disease activity, damage accrual and corticosteroid use were determined before and after treatment. Cyclophosphamide-associated adverse events were also examined. Univariable and bivariable analyses were used to evaluate group differences.

Results: Thirty-nine SLE patients received the standard-dose treatment and ten patients the low-dose therapy. Prior to cyclophosphamide infusion, demographic parameters, clinical manifestations, autoantibodies profile, disease damage and pharmacologic treatments were similar in both groups. Disease activity was higher in the low-dose group. After cyclophosphamide therapy, significant improvement of renal parameters (increase in the glomerular filtration rate and decrease in hematuria, pyuria, urinary cellular casts, proteinuria and hypertension) were observed only for patients that received the standard-dose therapy. Disease activity and corticosteroids requirement decreased in both groups after treatment. No differences were observed for adverse events associated with cyclophosphamide.

Conclusions: The standard-dose cyclophosphamide therapy appears to be more effective, and similar in terms of drug safety, than the low-dose regime for lupus nephritis in Puerto Ricans with SLE. (*Ethn Dis.* 2010;20[Suppl 1]: S1-116–S1-121)

Key Words: Systemic Lupus Erythematosus, Lupus Nephritis, Cyclophosphamide, Hispanics, Puerto Ricans

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that affects multiple organs and systems.[1] Renal involvement is the most common serious complication, occurring in approximately 50% of patients.[2] Patients with diffuse glomerulonephritis (GN) and severe focal and membranous GN are at higher risk for progressive renal insufficiency.[2] Cyclophosphamide, in combination with glucocorticoids, is used to treat severe lupus nephritis.[3] The standard treatment is the National Institute of Health (NIH) protocol, which consists of intravenous cyclophosphamide [0.5–1 gm/m^2, adjusted to white blood cell (WBC) nadir], given monthly for the first six months then quarterly for at least 12 months.[4] Several alternative treatments have emerged including the Euro Lupus Nephritis Trial protocol which seems to be as effective as the conventional treatment.[5] It comprises six pulses of a low fixed-dose of 500 mg given every two weeks for a cumulative dose of three grams followed by azathioprine as a remission maintenance agent.[5]

Clinical manifestations, treatment response and outcomes of SLE vary among patients of different ethnic populations.[6–8] For example, severe forms of lupus nephritis are more frequent in African Americans and Hispanics from Mexican ancestry compared to Caucasians.[6–8] Previously, we characterized the clinical manifestations, serologic findings and outcomes in Hispanics from Puerto Rico with SLE, and found that these features significantly differ from patients of other ethnic groups.[9,10] Now, we have examined the efficacy of two different cyclophosphamide regimes for lupus nephritis in Puerto Rican patients: the NIH (standard-dose) and the Euro Lupus Nephritis Trial (low-dose) protocols.

Methods

Patient Population

A retrospective cohort of 49 adult patients with SLE treated with intravenous cyclophosphamide for clinical or biopsy confirmed lupus nephritis was studied. Thirty-nine patients received the standard dose cyclophosphamide therapy and 10 patients were treated with the low-dose therapy. Twenty-nine patients in the standard-dose group completed at least 10 months of therapy. All patients in the low-dose group received a cumulative dose of 3 grams. All patients fulfilled the American College of Rheumatology (ACR) classification criteria for SLE.[11] They were followed at the lupus clinics of the University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico from 1990 to 2007. This study was approved by the institutional review board of the University of Puerto Rico Medical Sciences Campus.

Prior to study visit, patients had routine visits at 1–3 months intervals. Additional visits were scheduled as needed per disease activity or complications. At each routine visit a structured questionnaire was completed to gather information about demographic parameters, clinical manifestations, comorbid conditions, disease activity, disease damage, serological features, laboratory tests, pharmacologic treatment, and drug adverse events. A complete lupus autoantibody panel was determined at diagnosis of SLE for each patient.

Variables

Demographic, clinical, serologic and pharmacologic variables were studied. Demographic variables included sex, age at diagnosis of SLE and age at onset of renal disease. The clinical domain included the assessment of SLE manifestations, renal parameters, kidney biopsy findings, comorbid conditions, serologic features, disease activity and disease damage. SLE clinical manifestations were determined as per the ACR classification criteria for SLE.[11] Renal parameters included estimated glomerular filtration rate (GFR), proteinuria (>1 g/24hr), hematuria (>10 red blood cells [RBC] per high power field [HPF] attributed to SLE), pyuria (>5 white blood cell [WBC]/ HPF attributed to SLE) and urinary cellular casts. GFR was determined using the Modification of Diet in Renal Disease (MDRD) equation and was expressed in five categories (≥90, 60–89, 30–59, 15–29 and <15 mL/min) according to the National Kidney Foundation Chronic Kidney Disease classification.[12] Kidney biopsy findings were expressed as defined by the World Health Organization for the classification of lupus nephritis.[13] Comorbid conditions that may influence renal outcome were determined such as arterial hypertension, diabetes, hyperlipidemia and cardiovascular disease (angina pectoris, myocardial infarction, heart failure and/or peripheral artery disease). Disease activity was assessed with the Systemic Lupus Disease Activity Measure (SLAM).[14] Disease damage was determined using the Systemic Lupus International Collaborating Clinics/ ACR Damage Index (SDI).[15]

The following serologic tests were determined at diagnosis of SLE: antinuclear (ANA), anti-double stranded DNA (anti-dsDNA), anti-Smith (anti-Sm), anti-ribonucleoparticle (anti-RNP), anti-Ro, and anti-La antibodies. In addition, serum complements (C3 and C4) were measured at SLE diagnosis.

The use and mean dose of hydroxychloroquine, azathioprine, mycophenolate mofetil, methotrexate and cyclosporin were assessed at baseline, prior to cyclophosphamide therapy. The mean dose of prednisone (or equivalent) was determined before and after cyclophosphamide treatment. Adverse events associated with cyclophosphamide were ascertained; these included nausea, vomiting, diarrhea, hair loss/alopecia, pneumonitis, anemia, leukopenia, thrombocytopenia, hematuria, infections, premature ovarian failure and malignancy.

Statistical Analysis

Statistical analysis was performed by SPSS for Windows software version 12.0 (SPSS Inc., Chicago Il, USA). Demographic parameters, cumulative SLE manifestations, cumulative comorbidities, disease activity, damage accrual, and pharmacological treatments were determined at baseline, defined as the visit before cyclophosphamide treatment. Lupus serologies were measured at SLE diagnosis. Renal parameters, disease activity, damage accrual were determined, and corticosteroid dose were determined at baseline and after cyclophosphamide therapy, defined as the last visit after cyclophosphamide treatment in which all study variables were available for analysis. Cyclophosphamide-associated adverse events were recorded during treatment, except for premature ovarian failure and malignancy that were not ascertained until last patient visit. Differences between study groups were analyzed with the chi-square and Fisher's exact tests. The McNemar test and the Wilcoxon signed ranks test were used to examine differences within the same group before and after treatment. $P \leq .05$ was considered statistically significant.

RESULTS

The baseline demographic parameters, clinical manifestations, serologic features, disease activity and disease damage of lupus nephritis patients who received the low-dose and standard-dose cyclophosphamide therapies are depicted in Table 1. No significant differences were found for sex, age at SLE diagnosis, age at renal disease onset, SLE clinical manifestations, renal parameters, serologic features, comorbidities and damage accrual. The only difference observed was disease activity, which was higher among patients treated with low-dose cyclophosphamide. Kidney biopsy was performed in 29 (59.2%) patients; four patients in the low-dose group and 25 patients in the standard-dose group. No significant differences were found for types of renal pathology (data not shown).

Table 2 shows the pharmacologic treatment for SLE prior to cyclophosphamide. Except for intravenous methylprednisolone pulses that were more common in the standard-dose group (59% vs 20%, $P=.037$) the medication profile was similar for both treatment groups. In addition, no significant differences were observed for the mean dose of prednisone (or equivalent), hydroxychloroquine and azathioprine (data not shown).

The mean (SD) follow-up time between the first cyclophosphamide therapy and last study visit was 2.1 (3.1) and 6.6 (4.2) years for the low-dose and standard-dose groups, respec-

Table 1. Baseline demographic parameters, clinical manifestations, serologic features, disease activity and disease damage

Features	Cyclophosphamide therapy		
	Low-dose (*n*=10)	Standard-dose (*n*=39)	*P* value
Sex, % female	100	89.7	.569
Age at SLE diagnosis, mean (SD) years	29.8 (9.7)	25.3 (9.2)	.186
Age at renal onset, mean (SD) years	30.8 (9.5)	26.6 (9.7)	.226
Renal disease duration prior to cyclophosphamide therapy, mean (SD) years	0.8 (0.8)	2.5 (3.6)	.142
Clinical manifestations, %			
Malar rash*	80.0	84.6	.659
Discoid lupus*	0.0	10.3	.569
Oral ulcers*	30.0	56.4	.171
Arthritis*	60.0	76.9	.422
Pericarditis*	0.0	10.3	.569
Pleuritis*	10.0	15.4	1.000
Psychosis*	0.0	7.7	1.000
Seizures*	0.0	17.9	.319
Anemia (any etiology)	100	94.9	1.000
Hemolytic anemia*	20.0	13.2	.625
Leukopenia*	60.0	61.5	1.000
Lymphopenia*	70.0	89.5	.147
Thrombocytopenia*	30.0	20.5	.673
Renal parameters, %			
Glomerular filtration rate			
≥90 ml/min	60.0	33.3	
60–89 ml/min	30.0	28.2	
30–59 ml/min	10.0	20.5	.447
15–29 ml/min	0.0	10.3	
<15 ml/min	0.0	7.7	
Proteinuria (>1g/24hr)	77.8	88.6	.609
Hematuria (> 10 RBC/ HPF)	20.0	44.7	.074
Pyuria (> 5 WBC/ HPF)	60.0	51.4	.727
Cellular casts	10.0	34.2	.244
Serologic features, %			
Anti-nuclear antibodies	100.0	100.0	1.000
Anti-dsDNA antibodies	90.0	89.5	1.000
Anti-Smith antibodies	55.6	30.4	.240
Anti-Ro antibodies	57.1	35.0	.391
Anti-La antibodies	0.0	25.0	.281
Anti-RNP antibodies	60.0	52.4	1.000
Low C3	87.5	76.3	.669
Low C4	80.0	67.6	.700
Lupus anticoagulant	40.0	13.3	.249
Anti-cardiolipin IgA antibodies	0.0	5.0	1.000
Anti-cardiolipin IgG antibodies	60.0	43.5	.639
Anti-cardiolipin IgM antibodies	20.0	9.5	.468
Comorbidities, %			
Hypertension	10.0	48.4	.061
Diabetes mellitus	0.0	12.8	.569
Hyperlipidemia	55.6	59.0	1.000
Cardiovascular disease	10.0	7.7	1.000
SLAM score, mean (SD)	13.0 (5.6)	9.4 (4.6)	.036
SDI score, mean (SD)	0.1 (0.3)	0.3 (0.7)	.525

* Per American College of Rheumatology classification criteria for systemic lupus erythematosus; SD: standard deviation; RBC: red blood cells; HPF: high power field; WBC: white blood cells; SLAM: Systemic Lupus Activity Measurement; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index

tively. For the standard-dose group the median and mean (SD) number of cyclophosphamide treatments were 10 and 8.9 (2.9), the median and mean (SD) dose were 1,200 mg and 1,198 (363) mg (range 700–2,300 mg), and the mean (SD) duration of treatment was 17.1 (9.3) months. Selected clinical manifestations before and after cyclophosphamide treatment are summarized in Table 3. Patients treated with the standard-dose regime had significant improvement in GFR and had less hematuria, pyuria, urinary cellular casts, proteinuria and hypertension after treatment. However, patients treated with the low-dose did not improve in any of these parameters. Disease activity and corticosteroids requirement decreased in both groups after treatment. However, patients who received the standard-dose therapy accrued more disease damage. In the low-dose group new damage was observed only in renal domain of the SDI, whereas in the standard-dose group new damage was noticed in the ocular, neuropsychiatric, renal, peripheral vascular, musculoskeletal, skin, and diabetes domains.

No differences were found for adverse events attributed to cyclophosphamide treatment except for urinary tract infections, which were more common in patients who received the low-dose therapy (Table 4). Serious infections were uncommon in both groups. Malignancy, premature ovarian failure and pneumonitis were not observed in either group.

DISCUSSION

The Euro Lupus Nephritis Trial investigators examined the efficacy and toxicity of low-dose intravenous cyclophosphamide for lupus nephritis.[5] They found no greater cumulative probability of treatment failure in patients treated with the low-dose regime compared to those who received the standard-dose. This study has important clinical im-

Table 2. Baseline pharmacologic treatment

Features	Cyclophosphamide therapy		
	Low-dose (*n*=10) %	Standard-dose (*n*=39) %	*P* value
Corticosteroids	100	97.4	1.000
IV methylprednisolone pulses	20.0	59.0	.037
Hydroxychloroquine	80.0	41.0	.037
Azathioprine	70.0	38.5	.090
Mycophenolate mofetil	0.0	0.0	—
Methotrexate	0.0	0.0	—
Cyclosporin	0.0	0.0	—

IV=intravenous

plications since the low-dose therapy could have a better safety profile, especially for a long-term outcome since patients are exposed to a lower cumulative dose of cyclophosphamide. Therefore, late adverse events such as malignancy may occur less frequently. The potential benefits of the low-dose therapeutic regimen prompted its use in our population of Puerto Ricans with lupus nephritis. However, it was not as effective as the standard-dose regime since our patients did not have significant changes in GFR, hematuria, pyuria, urinary cellular casts, proteinuria and hypertension.

The contrasting results of our study and the Euro Lupus Nephritis Trial could be attributed to variability in ethnic composition, as renal involvement is more severe in populations of African and Amerindian heritage than Caucasians.[6–8] The participants in the Euro Lupus Nephritis Trial consisted mostly of Caucasians[5]; whereas our study comprised Hispanic patients from Puerto Rico. Puerto Ricans are a population of mixed ethnicity, mainly of African, Caucasian and Amerindian descent.[16] Specifically, the African component predominates in Puerto Ricans with SLE (45%) as determined by admixture studies.[17] Although Puerto Ricans with SLE have a lower frequency of renal involvement, it is possible that renal disease is more severe in those affected, and thus, require a more aggressive immunosuppressive approach. [9]

Another plausible explanation for differences in treatment response could be related to polymorphisms of cytochrome P450, given the variability observed for these alleles among different ethnic groups.[18] It has been reported that the hepatic expression of CYP2C19*2 may be linked to poor metabolism of certain drugs, cyclophosphamide included.[18] Approximately 70–80% of the administered drug is bioactivated by cytochrome P450 enzymes to 4-hydroxycyclophosphamide.[19] The frequency of individuals with poor metabolism in African Americans is 5.4% and some new mutations have been reported for this population.[20] For instance, SLE patients homozygous for CYP2B6*5 or CYP2C19*2 have a higher probability of doubling of serum creatinine levels and developing end stage renal disease.[20] They also have a trend toward lower probability of achieving complete renal response to cyclophosphamide therapy. Therefore, the predominance of certain cytochrome P450 alleles, perhaps surrogate to ethnicity, may contribute to treatment response.

Table 3. Selected clinical variables before and after cyclophosphamide treatment

Feature	Cyclophosphamide therapy					
	Low-dose			Standard-dose		
	Before	After	*P* value	Before	After	*P* value
GFR ≥90 mL/min, %	60.0	36.4		33.3	48.7	
GFR 60–89 mL/min, %	30.0	54.5		28.2	20.5	
GFR 30–59 mL/min, %	10.0	9.1	0.392	20.5	23.1	.032
GRF 15–29 mL/min, %	0.0	0.0		10.3	2.6	
GFR < 15 mL/min, %	0.0	0.0		7.7	5.1	
Hematuria (>10 RBC/HPF), %	20.0	20.0	1.000	44.7	21.1	.001
Pyuria (>5 WBC/HPF),%	60.0	40.0	0.625	51.4	16.2	.002
Cellular casts, %	10.0	10.0	1.000	34.2	10.5	.022
Proteinuria (>1000 mg/24hr), %	77.8	44.4	0.250	86.1	38.9	.001
Hypertension, %	10.0	30.0	0.500	48.4	25.8	.039
SLAM score, mean (SD)	13.0 (5.7)	6.4 (3.2)	0.021	9.3 (4.6)	6.5 (3.7)	.002
SDI score, mean (SD)	0.1 (0.3)	0.6 (1.3)	0.138	0.3 (0.7)	1.0 (1.4)	.006
Prednisone, mean (SD) mg	43.0 (24.2)	15.5 (8.6)	<0.001	42.3 (18.5)	17.3 (12.5)	<.001

GFR: glomerular filtration rate, RBC: red blood cells; HPF: high power field; WBC: white blood cells; SLAM: Systemic Lupus Activity Measurement; SD: standard deviation; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index

Table 4. Adverse events associated to cyclophosphamide treatment

Adverse events	Cyclophosphamide therapy		
	Low-dose (*n*=10) %	Standard-dose (*n*=39) %	*P* value
Gastrointestinal, any type	40.0	63.2	.282
Nausea	30.0	36.8	1.000
Vomiting	30.0	34.2	1.000
Diarrhea	20.0	13.5	.630
Hair loss/alopecia	60.0	41.0	.311
Anemia	30.0	38.5	.726
Leukopenia	40.0	43.6	1.000
Thrombocytopenia	10.0	2.6	.370
Pneumonitis	0.0	0.0	1.000
Hematuria	33.3	15.4	.340
Infections, any type	90.0	69.2	.253
Urinary tract infection	80.0	25.6	.003
Upper respiratory tract infection	10.0	46.2	.066
Skin infections	30.0	25.6	1.000
Sepsis	10.0	0.0	.204
Premature ovarian failure	0.0	0.0	1.000
Malignancy	0.0	0.0	1.000

Our study has some limitations. First, the study group was small, particularly in the low-dose regime group. Nevertheless, since the preliminary analyses disclosed a better efficacy of the standard-dose over the low-dose therapy, no additional patients were treated with the latter therapy. Second, patients were not randomly allocated to particular treatments, which could add bias. Finally, not all patients had renal biopsies to assess the type of lupus nephritis. Therefore, it is possible that more patients in the low-dose treatment had a more severe form of nephritis, and hence, exhibited poorer clinical response. Nonetheless, all patients presented clinically severe forms of lupus nephritis (marked proteinuria and/or rising creatinine) that warranted aggressive immunosuppressive treatment.

In summary, our study shows that Puerto Rican patients with lupus nephritis treated with the standard-dose cyclophosphamide regimen had a better clinical response than those who received the low-dose treatment. Our data highlight the need of pharmacogenetic studies in lupus patients from different ethnic backgrounds in order to provide a more individualized or personalized therapy.

IMPLICATIONS FOR IMPROVING HEALTH DISPARITIES

Health disparities are well-documented in minority populations such as African Americans, Hispanics and Native Americans. These populations tend to be under-represented in clinical trials of new therapies or treatment protocols. Our work shows the need to take into consideration patients from different ethnic backgrounds when a particular treatment is studied.

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