

A Randomized Pilot Trial Comparing Cyclosporine and Azathioprine for Maintenance Therapy in Diffuse Lupus Nephritis over Four Years

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There is not agreement about the best maintenance treatment for patients with diffuse lupus nephritis. This multicenter, randomized trial compared the safety and efficacy of cyclosporine and azathioprine. Seventy-five patients with diffuse proliferative lupus were given three intravenous methylprednisolone pulses followed by prednisone and oral cyclophosphamide for a median of 90 d. Subsequently, patients were randomly assigned either to cyclosporine or to azathioprine for 2 yr (core study). Treatment continued for up to 4 yr (follow-up study). The primary outcome measure was the incidence of disease flares. Secondary end points were proteinuria per day, creatinine clearance, and adverse effects. Seven flares occurred in the cyclosporine group, and eight occurred in the azathioprine group. At the end of the core study, mean proteinuria decreased from 2.8 ± 3.57 to 0.4 ± 0.85 g/d ($P < 0.0001$) in the cyclosporine group and from 2.2 ± 1.94 to 0.5 ± 0.78 g/d ($P < 0.0002$) in the azathioprine group. After 4 yr, mean proteinuria was 0.2 ± 0.24 and 0.3 ± 0.33 g/d, respectively. At the core study end and at the follow-up completion, creatinine clearance and BP levels did not change significantly from baseline in either group. Five of 36 patients who were receiving cyclosporine and four of the 33 who were receiving azathioprine stopped the treatment because of adverse effects. For patients with diffuse proliferative lupus nephritis, azathioprine or cyclosporine combined with corticosteroids demonstrated equal efficacy in the prevention of flares.

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Although earlier diagnosis and the refinement of the available therapies have improved the prognosis of lupus nephritis, long-term treatment still remains a major challenge for clinicians. The prolonged administration of corticosteroids is burdened by a number of severe and even life-threatening adverse effects (1). Although a major advance has been obtained with the use of the intermittent administration of intravenous pulsed cyclophosphamide (2), it still leads to a significant risk for ovarian failure and infection (3), and a number of patients develop lupus exacerbations (4,5). Consequently, considerable efforts are being made to find alternative therapeutic approaches. One controlled trial showed that the combination of mycophenolate mofetil and prednisolone is as effective as a regimen of cyclophosphamide and prednisolone followed by azathioprine but is less toxic (6). Another controlled trial showed that short-term therapy with intravenous cyclophosphamide followed by main-

tenance therapy with mycophenolate mofetil or azathioprine seems to be more efficacious and safer than long-term therapy with intravenous cyclophosphamide (7).

Uncontrolled studies have shown good long-term results using methylprednisolone pulses and oral cyclophosphamide for induction and the treatment of renal flares and low-dose prednisone and azathioprine for maintenance (8,9). Clinical benefits obtained by administering cyclosporine in combination with prednisone even in patients whose disease has failed to respond to a previous steroid therapy have been described (10,11), but no controlled trial has been reported fully until now. To compare the effectiveness of cyclosporine and azathioprine in the maintenance therapy of patients with lupus nephritis, we organized a multicenter, prospective, randomized, open, blinded-end point, controlled trial in which patients with active disease initially were treated with an induction therapy and then randomly assigned to low-dose prednisone plus cyclosporine or azathioprine.

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Materials and Methods

Participants

Male and female patients who were aged at least 16 yr and who gave their written informed consent were admitted to the study when they

had lupus diagnosed on the basis of the American College of Rheumatology criteria (12) and biopsy-proven World Health Organization class IV, Vc, or Vd nephritis (13) with a chronicity index of ≤ 4 , according to Austin *et al.* (14). The patients who had a new diagnosis of lupus nephritis or were experiencing a new flare of a previously quiescent disease were enrolled provided that they had active urine sediment (≥ 5 erythrocytes/high-power field), proteinuria >1 g/d in the case of newly diagnosed nephritis or >2 g/d in the case of a new renal flare, and serum creatinine levels of ≤ 4 mg/dl. The exclusion criteria were potential silent nephritis (15), *i.e.*, patients without relevant clinical signs of nephritis, renal diseases unrelated to lupus, treatment with cyclosporine or azathioprine in the 6 mo preceding the screening visit, a cumulative cyclophosphamide dose of >200 mg/kg, any contraindications to the trial drugs, and previous malignancy.

Inclusion and Exclusion Criteria at Randomization

After induction or flare treatment, the patients had to have no major extrarenal signs or symptoms of lupus that required aggressive therapy. Proteinuria had to be >0.5 g/d at least twice in the 2 wk preceding randomization, serum creatinine <132 μmol (≤ 1.5 mg/dl), and creatinine clearance >60 ml/min. Diastolic BP had to be <90 mmHg with a maximum of two antihypertensive drugs, and the oral prednisone dose had to be ≤ 0.5 mg/kg per d.

Objectives and Study Design

Objectives of the study were to evaluate the efficacy of a maintenance treatment that is based on cyclosporine or azathioprine in preventing the disease flares in patients with diffuse proliferative lupus nephritis and to compare the efficacy and the safety of the two treatments.

Patients who met the inclusion criteria were studied for 2 yr (core study). At the end of core study, the patients were invited to continue to be followed up to 4 yr.

The study was conducted in accordance with the Declaration of Helsinki. The study design and treatment protocols were approved by the Ethics Committees of the participating hospitals.

Interventions

Induction/Flare Treatment. One intravenous methylprednisolone pulse was given every 24 h for 3 consecutive days (0.5 g each for patients who weighed ≤ 50 kg; 1 g each for patients who weighed >50 kg), after which oral prednisone was administered at a dose of 1 mg/kg per d for 10 to 15 d and then tapered to 0.7 mg/kg per d for the next 10 to 15 d and then to 0.5 mg/kg per d up to the end of 2 mo. Oral cyclophosphamide also was given at a dose of 1 to 2 mg/kg per d for 3 mo.

Study Treatments. After induction treatment was completed, all patients received oral prednisone, which had to be reduced from 0.5 to 0.2 mg/kg per d by the end of month 6 in the case of normal levels of serum creatinine and proteinuria of <0.5 g/d and in absence of extrarenal symptoms. A further reduction until complete withdrawal could have been attempted at the investigators' discretion.

Cyclosporine (Neoral; Novartis AG, Basel, Switzerland) was administered at an initial dose of 4 mg/kg per d. After the first month (or sooner if trough blood levels exceeded 200 ng/ml), the dose was reduced by 0.5 mg/kg every 2 wk to a maintenance dose of 2.5 to 3.0 mg/kg per d (keeping trough levels between 75 and 200 ng/ml) if proteinuria was <1 g/d. In the case of higher levels of proteinuria, cyclosporine was reduced more slowly. The dose had to be reduced by 25 to 50% if serum creatinine increased to $>30\%$ above baseline levels unassociated with a lupus flare and/or if serum potassium increased to 6 mEq/L or more, liver enzyme levels doubled, or the patient devel-

oped treatment-refractory hypertension. If the abnormal parameter(s) did not return to normal within 2 to 4 wk, cyclosporine therapy had to be stopped.

Azathioprine (Azathioprina Wellcome, GlaxoSmithKline, London, UK) was given at an initial dose of 2 mg/kg per d, with an optional reduction to 1.5 mg/kg per d after 1 mo, if proteinuria was <1 g/d and serum creatinine was stable. The dose had to be halved if the white blood cell count was $<4000/\text{mm}^3$ and stopped for at least 2 wk if it was $<3000/\text{mm}^3$. Persistent or repeated neutropenia (neutrophils $<1000/\text{mm}^3$) required its discontinuation.

Neither cyclosporine nor azathioprine was increased if renal or extrarenal signs of lupus activity occurred. Prednisone was increased at clinical discretion of the clinician in the case of arthralgias and mild fever, according to the severity of symptoms. In no case did the daily dose exceed 25 mg.

Recruitment of Patients

From March 1999 to March 2001, 157 patients who had a diagnosis of systemic lupus erythematosus (SLE) (12) were considered for the study. We excluded 12 patients because their serum creatinine was >4 mg/dl (352 $\mu\text{mol/L}$) and 34 patients because renal biopsy did not show class IV, Vb, or Vc (13) or because the chronicity index was higher than 4 (14). Thirty-two patients were excluded because they were treated with either cyclosporine or azathioprine in the 6 mo preceding the screening. Four patients refused to participate in the study.

Outcomes

The primary outcome measure was the incidence of SLE flares over 2 yr. Secondary outcome measures were the levels of proteinuria as expressed in g/d, serum creatinine (mg/dl), and adverse effects.

Sample Size

The aim of this study initially was to show superiority of maintenance therapy with low-dose cyclosporine plus prednisone *versus* azathioprine plus prednisone on the incidence of renal flares. A sample size of 206 patients were to be enrolled in 24 mo. Because of an enrollment rate much lower than expected (27% of total sample in the first 20 mo), a protocol amendment was issued on which basis the aim of the study was switched from confirmatory to pilot and the target sample size was set to 80 patients. The new sample size was based solely on feasibility considerations. Because there no longer was a confirmatory aim, no new formal hypotheses were drawn and therefore no new power calculations were made.

Randomization

Randomization was stratified by center and was performed centrally. Investigators made telephone calls to the randomization center, where a computer program assigned each patient to one of the two treatment groups. Randomization occurred after verification of the eligibility of the patient for the treatment phase of the trial. The randomization to treatment was made according to a biased coin design (16).

Visit Schedule

During the induction period, the patients underwent frequent laboratory tests and a clinical examination. For the aim of the study, data had to be recorded on days -90 , -60 , and -30 and at baseline (day 0). During the core study, they were examined on days 15, 30, 45, and 60; at the end of months 4 and 6; and then every 3 mo until month 24. Patients who participated in the follow-up study were examined every 3 mo until month 48.

The laboratory tests included the measurement of 24-h protein excretion and serum creatinine, urea, electrolytes, cholesterol, triglyceride, glucose, liver enzymes and bilirubin, total proteins and albumin, and a complete blood count with leukocytes. Anti-dsDNA, C3, and C4 were tested at each scheduled visit. The Systemic Lupus Activity Measure (SLAM) index (17) was recorded on days -90 and -60, at baseline, and then after 12 and 24 mo.

Definitions

A nephritic flare was defined as a rapid increase in serum creatinine of $\geq 30\%$ above baseline associated with an increase in proteinuria, and/or active urine sediment, and/or a reduction in serum C3 and C4, and/or an increase in anti-dsDNA antibody levels; a proteinuric flare was defined as a rapid increase in proteinuria of at least 2 g/d if the previous proteinuria level had been ≤ 3.5 g/d or a doubling if previous proteinuria had been > 3.5 g/d (18). Severe extrarenal flares included skin or visceral vasculitis, neurologic manifestations, thrombocytopenia ($< 50,000/\text{mm}^3$), hemolytic anemia (a decrease in hemoglobin levels of ≥ 5 g/dl together with an increase in serum bilirubin and reticulocytes), lupus pneumonitis, lupus myocarditis, or lupus serositis. The term definitions of specific lupus features are those included in the criteria of the American College of Rheumatology (13).

Active urine sediment was defined as the presence of ≥ 5 erythrocytes/high-power field. The creatinine clearance has been calculated according to Cockcroft and Gault (19).

Statistical Analyses

All enrolled patients were considered for evaluation during the induction period; all randomly assigned and treated patients were considered for the safety evaluation, whereas only patients who had at least one postbaseline evaluation were eligible for the efficacy analyses according to the intention-to-treat principle. All results are given considering all available observations at each visit; no imputation of missing values was done. Given the exploratory nature of the analysis, no correction for multiplicity was made in reporting the results of the analyses of multiple variables. Within-group comparisons were done by means of paired *t* test; between-group comparisons were done by means of unpaired *t* test. Variability estimates to be used in calculation of confidence intervals (CI) were obtained by analysis of covariance using baseline values as covariates.

The results are given as mean values \pm SD unless otherwise specified. All analyses were performed using SAS V8.2 (SAS Institute, Cary, NC). Flares were not censored in the data analyses

Results

Induction Therapy

Seventy-five patients were enrolled in the screening period and received the induction treatment; 57% had a new diagnosis of SLE, and 43% had a renal flare. One woman with a long history of lupus nephritis and repeated flares died of pneumonia a few days after starting induction therapy; another patient developed a perirenal hematoma and fever after biopsy and received a different treatment.

Among the remaining 73 patients (eight men and 65 women; median age 32 yr), the median duration of induction was 90 d. The mean daily dose of cyclophosphamide was 91.5 ± 23.8 mg, and the mean daily dose of oral prednisone was 39.2 ± 11.1 mg.

The mean serum creatinine decreased from 1.0 ± 0.46 to 0.9 ± 0.27 mg/dl at the end of induction ($P = 0.0028$). The mean proteinuria decreased from 3.6 ± 2.5 to 2.4 ± 2.8 g/24 h ($P =$

0.0015). There was an increase of serum C3 (from 57.1 ± 32.2 to 93.0 ± 27.4 mg/dl) and serum C4 levels (from 9.0 ± 6.0 to 21.4 ± 35.1 mg/dl) and a decline of the mean SLAM score (from 21.1 ± 7.7 to 13.7 ± 5.4 ; $P < 0.0001$). Three patients were not randomly assigned because of adverse effects: High liver enzyme levels, severe leukopenia as a result of a wrong dose of cyclophosphamide, and renal failure during the screening period that needed re-treatment. These three patients and a fourth who withdrew informed consent were not randomly assigned.

Demography at Randomization

Of the 69 patients who were randomly assigned, 36 were assigned to cyclosporine and 33 were assigned to azathioprine. The mean age and gender distributions in the two groups were comparable. The initial biopsy showed a fair distribution of World Health Organization classes between the two groups and similar activity and chronicity indices. There were no significant differences in the baseline values of serum creatinine, daily urine protein excretion, or the SLAM index (Table 1).

Numbers Analyzed

All patients but one were followed for at least 1 yr. One patient in the azathioprine arm was converted to mycophenolate mofetil because of inefficacy. Between the first and second years, five patients in the cyclosporine arm and four in the azathioprine arm stopped treatment because of adverse effects (see below). One patient in each group was lost to follow-up. Forty-seven patients (24 in the cyclosporine group and 23 in the azathioprine group) accepted to participate to the follow-up study (Figure 1).

Treatments

Cyclosporine Group. The mean starting dose of cyclosporine was 3.5 ± 0.5 mg/kg per d (range 2.5 to 4.3); at the 24th month, it was 2.7 ± 0.7 mg/kg per d (1.4 to 4.1). The mean dose during core treatment was 3.0 ± 0.4 mg/kg per d (2.0 to 3.9). The mean starting dose of oral prednisone was 24.2 ± 7.3 mg/d (6.3 to 50.0) with variations according to the variable residual activity of the disease; at the 24th month, it was 7.5 ± 4.9 mg/d (2.5 to 25.0; Table 2). The average dose during the core study was 11.9 ± 6.0 mg/d (5.5 to 28.8). Two patients completely stopped corticosteroids.

In the follow-up study, the mean exposure to cyclosporine was 2.3 ± 1.08 mg/kg per d in the third year and 2.1 ± 0.97 mg/kg per d in the fourth year. The mean doses of prednisone were 7.0 ± 8.28 and 6.7 ± 9.8 mg/d, respectively.

Azathioprine Group. The mean starting dose of azathioprine was 1.6 ± 0.49 mg/kg per d (0.79 to 3.03); at the 24th month, it was 1.4 ± 0.37 mg/kg per d (0.70 to 1.89). Mean exposure during the core study was 1.5 ± 0.54 mg/kg per d (0.73 to 3.85). The doses of azathioprine were 1.08 ± 0.37 mg/kg per d at the third year and 0.90 ± 0.35 mg/kg per d at the fourth year. The mean starting dose of oral prednisone was 22.9 ± 8.1 mg/d (5.0 to 40.0); at the 24th month, it was 7.2 ± 5.3 mg/d (2.5 to 25.0; Table 2). The average dose during the core study period was 12.3 ± 5.3 mg/d (5.7 to 28.0). Two patients completely stopped corticosteroids. In patients who continued

Table 1. Main demographic characteristics at randomization^a

	Cyclosporine	Azathioprine
No. of patients	36	33
Age (yr)	31.7 (\pm 9.1)	31.2 (\pm 11.7)
Female gender	33 (91.7%)	29 (87.9%)
Years since first diagnosis	5.4 (\pm 8.0; median 0.4; range 0 to 24.5)	2.3 (\pm 3.6; median 0.1; range 0 to 9)
Serum creatinine (mg/dl)	0.9 (\pm 0.23)	0.9 (\pm 0.29)
Creatinine clearance (ml/min)	92.5 (\pm 21.5)	104.1 (\pm 46.5)
Proteinuria (g)	2.8 (\pm 3.57)	2.2 (\pm 1.94)
Hemoglobin (mg/dl)	12.2 (\pm 1.4)	12.4 (\pm 1.6)
BP (mmHg)		
SBP	125 (\pm 16.4)	129 (\pm 14.2)
DBP	81 (\pm 9.1)	81 (\pm 9.8)
SLAM total score	13.1 (\pm 5.5)	14.2 (\pm 6.1)
Biopsy WHO category (%)		
IV	84	91
Vc or Vd	16	9
Chronicity index	2.5 (\pm 2.6)	2.8 (\pm 3.7)
Activity index (median/interquartile range)	7 (2 to 24)	7 (2 to 20)

^aDBP, diastolic BP; SBP, systolic BP; SLAM, Systemic Lupus Activity Measure; WHO, World Health Organization.

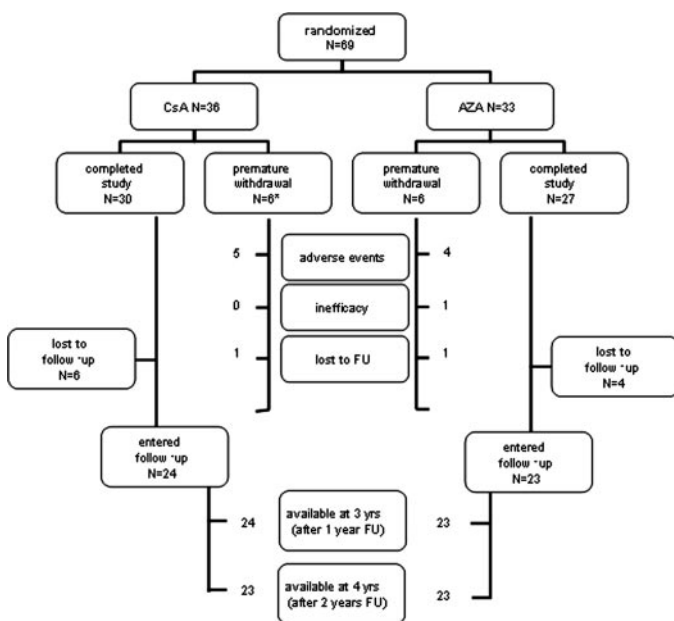


Figure 1. Patients' disposition in the core study (69 patients) and in the follow-up study (47 patients). Twenty-four months' data for two premature withdrawals were made available by the center and were actually used in all analyses according to the intention-to-treat principle.

the study, at 3 yr the mean dose of prednisone was 5.0 ± 4.21 mg/d (0 to 20). At 4 yr, the mean dose was 32.0 ± 132.0 mg/d (median 5 mg/d). The high mean dose was accounted for by the fact that a patient had a flare at the end of the fourth year and was given high-dose methylprednisolone pulses.

All patients but two who were receiving cyclosporine were

given antihypertensive therapy. Of them, seven who were receiving cyclosporine and three who were receiving azathioprine were given angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers.

Efficacy

After randomization, no patient died or entered end-stage renal failure. During an overall exposure of 65.9 patients/yr in cyclosporine group, seven flares of SLE occurred, which represents an incidence density of 10.6 flares/100 patient-years. Two of them developed within the end of the second year, and five developed between the third and the fourth years. Eight flares occurred in the azathioprine group: Three in the first 2 yr and five after the end of the second year. The overall exposure was 59.8 patients/yr, representing an incidence density of 13.4 flares/100 patient-years. There was one nephritic flare per group. After flare treatment, serum creatinine returned to normal in the patient who was receiving cyclosporine, whereas it did not improve in the patient who was receiving azathioprine (from 1.6 to 1.9 mg/dl). There were four proteinuric flares in the cyclosporine arm and six in the azathioprine arm. In one patient per group, proteinuria did not respond to the reinforcement of therapy and remained in a nephrotic range. Two extrarenal flares occurred in the cyclosporine arm and another one in the azathioprine arm. They completely reversed after appropriate therapy. Data from patients who developed relapses have not been censored for the purpose of data analysis.

In the cyclosporine group, mean creatinine clearance decreased from 92.5 ± 21.5 ml/min at baseline to 82.6 ± 20.0 ml/min after 24 mo (NS). At 4 yr, the mean values were 80.0 ± 15 ml/min for the 23 patients who completed the follow-up study, not significantly different from the basal values of the

Table 2. Oral prednisone administration during treatment^a

Variable	Visit/Period	Cyclosporine (n = 36)					Azathioprine (n = 33)				
		No. of Patients	Mean	SD	Minimum	Maximum	No. of Patients	Mean	SD	Minimum	Maximum
Current dose (mg/d) ^b	Baseline	29	24.2	7.3	6.3	50.0	29	22.9	8.1	5.0	40.0
	30 d	34	21.7	8.3	10.0	50.0	33	23.1	7.2	6.3	40.0
	60 d	33	17.2	9.2	6.3	50.0	33	18.9	7.1	6.3	40.0
	6 mo	33	12.9	7.5	2.5	37.5	32	12.6	5.2	6.3	25.0
	12 mo	33	8.8	6.2	2.5	37.5	28	8.9	4.3	2.5	25.0
	24 mo	27	7.5	4.9	2.5	25.0	24	7.2	5.3	2.5	25.0
Cumulative dose (mg)		36	7667	4724	1161	24,623	33	7377	4014	1456	23,255

^aStatistics are based on all patients who take at least one prednisone dose from baseline to month-24 visit. No significant differences were seen at any time point between treatments. The cumulative dosage of prednisone was not significantly different.

^bStatistics are calculated on patients who actually were taking prednisone. Patients who were not taking prednisone (dose = 0) are not considered.

same patients (-6.9 ± 21 ml/min; NS). The corresponding figures in the azathioprine group were 104.1 ± 46.5 and 109.9 ± 43.9 ml/min (NS) at 2 yr. At 4 yr, the mean values were $104. \pm 40.1$ ml/min for patients who completed the follow-up study with a NS reduction when compared with the basal values of the same patients (-5.1 ± 38.7 ml/min; NS; Figure 2). The cyclosporine-azathioprine difference between the 24th month mean changes from baseline was equal to 12.0 ml/min ($P = 0.044$). The comparison between the cyclosporine and azathioprine groups in changes of creatinine clearance at 4 yr was NS. The areas under the curve of serum creatinine in the two groups were not significantly different. The mean reciprocal of serum creatinine also remained almost unchanged during treatment in both groups (data not shown). A control renal biopsy was performed after 2 yr in 14 patients who were assigned to cyclosporine and in 15 patients who were assigned to azathioprine. The activity index decreased from 5.9 ± 3.9 to 1.4 ± 3.2

in the cyclosporine group and from 7.4 ± 4.2 to 0.5 ± 1.3 in the azathioprine group. The chronicity index passed from 2.3 ± 1.5 to 3.7 ± 1.8 in the cyclosporine group and from 1.7 ± 2.0 to 3.1 ± 2.1 in the azathioprine group.

Mean proteinuria levels decreased from 2.8 ± 3.57 to 0.38 ± 0.85 g/d ($P < 0.0001$) in the cyclosporine group at the end of core study and from 2.2 ± 1.94 to 0.53 ± 0.78 g/d ($P = 0.0004$) in the azathioprine group. The reduction occurred earlier in the cyclosporine group (Figure 3). By the 24th month, median proteinuria was 0.15 g/d (range 0.0 to 4.7) in the cyclosporine group and 0.2 g/d (0.0 to 2.9) in the azathioprine group. The cyclosporine-azathioprine difference between the 24th month mean changes from baseline was NS. At the end of 4 yr, the mean proteinuria was 0.23 ± 0.24 g/d in the cyclosporine group and 0.33 ± 0.33 g/d in azathioprine group (NS; Figure 3). When compared with the basal values, in patients who completed the follow-up study, proteinuria decreased by $1.76 \pm$

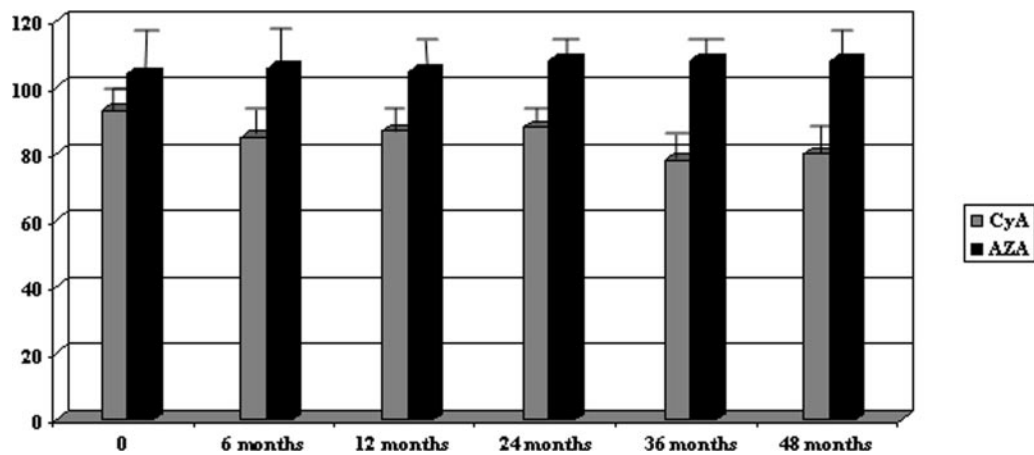


Figure 2. Variations of the mean levels of creatinine clearance, after randomization in patients who were assigned to azathioprine (■) and cyclosporine group (▒). Time 0 refers to the first observation at randomization. No significant differences between groups were observed at any time point. The lines on the top of the columns represent the SEM.

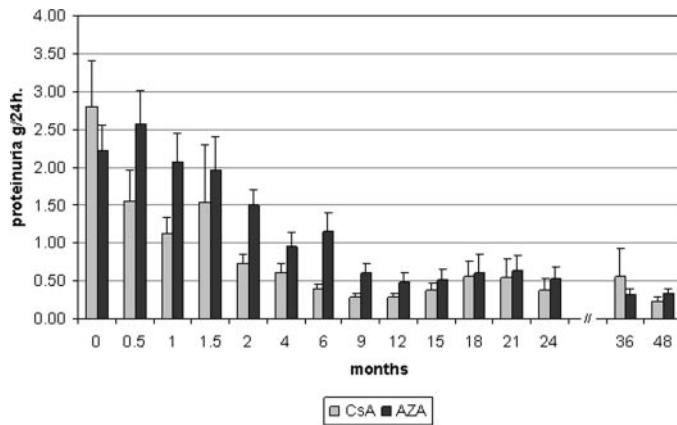


Figure 3. Mean levels of daily urinary protein excretion in azathioprine (■) and cyclosporine group (▨). Time 0 refers to the first observation at randomization. No significant differences between groups were observed at any time point. The lines on the top of the columns represent the SEM.

2.76 g/d ($P = 0.0058$) in the cyclosporine group and 2.11 ± 2.23 g/d ($P = 0.0002$) in the azathioprine group, without differences between groups (NS). At the end of the follow-up study, 15 (41.7%) of the 36 patients who were assigned to cyclosporine and five (15.1%) of the 33 patients who were assigned to azathioprine had undetectable proteinuria levels ($P = 0.045$). Mean systolic and diastolic BP levels tended to remain stable in both groups (Table 3). After 2 yr, the SLAM total score significantly decreased in both groups, from 13.1 ± 5.5 to 8.8 ± 7.2 in the cyclosporine group ($P = 0.002$) and from 14.2 ± 6.1 to 5.6 ± 3.0 in the azathioprine group ($P < 0.0001$).

Safety

During the core study, five patients in the cyclosporine group discontinued treatment: Two because of arterial hypertension, one because of increased BP and serum creatinine and potassium levels, one because of gastrointestinal intolerance, and one because of interstitial pneumonitis. Four patients in the azathioprine group discontinued the treatment because of leukopenia or infection.

A number of other adverse events were encountered during the core study (Table 4). Minor infection and leukopenia were

more frequent in the azathioprine group, whereas gastrointestinal disorders and arthralgias were more frequent in the cyclosporine group. In no case were these side effects severe enough to lead to treatment discontinuation in either group. After the core study was completed, adverse effects occurred less frequently, possibly as an effect of the reduction of immunosuppressive and steroid agents.

Discussion

One aim of the study was to evaluate which of the two regimens offered better protection against renal flares, a factor that was associated strictly with long-term disease outcome (18,20). However, during the 4-yr follow-up, seven flares occurred in the cyclosporine group (10.6 flares/100 patient-years) and eight in the azathioprine group (13.4 flares/100 patient-years). This small number of flares precludes any statistical analysis but underlines the efficacy of both regimens in preventing renal exacerbations. Most renal exacerbations were proteinuric flares that showed a fair outcome even in the long term, when appropriately treated (18). No patient died or entered end-stage renal failure during the 4-yr follow-up.

Mean creatinine clearance levels decreased early during the core trial in patients who were treated with cyclosporine. This variation was expected because of the known vasoconstrictive effect of cyclosporine on renal vessels. The decrease in creatinine clearance occurred soon after the start of the treatment; subsequently, creatinine clearance remained almost unchanged until the end of the core and the follow-up study. In control renal biopsy, in both the cyclosporine and azathioprine groups, there was a reduction in activity index and a correspondent increase in chronicity index. Because protocol biopsy was performed in a minority of patients, no firm conclusions about cyclosporine nephrotoxicity can be drawn.

An important result is the significant reduction in daily proteinuria in both treatment arms in the core study. These data are highly relevant because there now is a bulk of evidence indicating that the remission of proteinuria is a strong predictor of a fair long-term renal outcome not only in patients with primary glomerular diseases (21,22) but also in those with lupus nephritis (23,24).

The renal efficacy of treatment was matched by the attenuation of the general and extrarenal manifestations of the disease.

Table 3. Mean levels of systolic and diastolic BP in cyclosporine and azathioprine groups^a

Month	Cyclosporine		Azathioprine	
	Patients	SBP/DBP (mmHg)	Patients	SBP/DBP (mmHg)
0	36	125/81	33	129/81
6	33	128/84	32	128/80
12	32	127/82	29	123/79
24	30	126/80	27	124/77
36	24	125/78	23	124/77
48	23	120/78	23	124/79

^aMonth 0 refers to the first observation at randomization. No significant differences between groups were observed at any time point.

Table 4. Adverse events recorded during the core study, regardless of event severity^a

	Cyclosporine		Azathioprine	
	<i>n</i> (%)	Incidence Density ^b	<i>n</i> (%)	Incidence Density ^b
No. of patients	36		33	
Leukopenia	4 (11.1)	6.1	10 (30.3)	16.7
Anemia	5 (13.9)	7.6	5 (15.2)	8.4
Hypertension	7 (19.4)	10.6	5 (15.2)	8.4
Hypercholesterolemia	2 (5.6)	3.0	4 (12.1)	6.7
Gum hyperplasia	2 (5.6)	3.0	0	0.0
Hypertrichosis	2 (5.6)	3.0	0	0.0
Diabetes	0	0.0	1 (3.0)	1.7
Hyperkalemia	1 (2.8)	1.5	0	0.0
Hypertensive crisis	1 (2.8)	1.5	0	0.0
Infections	7 (19.4)	10.6	14 (42.4)	23.4
Arthralgias	14 (38.9)	21.2	3 (9.1)	5.0
Gastrointestinal disorders	11 (30.6)	16.7	3 (9.1)	5.0

^aIn no case hospitalization was required. Numbers shown are the counts of patients who reported each adverse event. Patients reporting multiple events are therefore counted for each reported adverse event.

^bNumber of events per 100 patients-years of follow-up.

The mean SLAM scores of lupus clinical activity significantly decreased with both treatments, with somewhat lower scores in the azathioprine group. Five patients per group had to stop cyclosporine or azathioprine during the core study because of adverse effects. The rate and the severity of adverse effects were lower than those observed with other therapies that are used in lupus nephritis, such as corticosteroids (1) and intravenous cyclophosphamide (2–7), and similar to those with mycophenolate mofetil (6,7). One concern about the prolonged administration of cyclosporine is the possible risk for renal toxicity. One patient in the cyclosporine group had to discontinue treatment because of an increase in serum creatinine levels; however, there was no other case of severe renal function impairment. It should be noted that for maintenance treatment, the mean doses of cyclosporine ranged approximately 2 mg/kg per d. At this dosage, a vasoconstrictive effect of cyclosporine still is present, but the risks for nephrotoxicity, arterial hypertension, hyperlipidemia, and other cyclosporine-related adverse effects are very infrequent.

Conclusion

Today the clinician may choose from among several options for treating lupus nephritis. Besides cyclophosphamide and corticosteroids, azathioprine and mycophenolate mofetil have been used with success in recent randomized, clinical trials (6,7). These data confirm an important role for azathioprine in the maintenance treatment of lupus nephritis and also show that cyclosporine can be considered as a further therapeutic option that is particularly useful in patients with high proteinuria. We do not advocate a long-term administration of cyclosporine in all patients; rather, we believe that the possibility of rotating agents with different mechanisms of action and different adverse effects in a long-lasting disease such as diffuse

lupus nephritis may help to increase the therapeutic index of our treatment strategies.

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References

1. Doria A, Vitali C, Tincani A, Balestrieri G, Galeazzi M, Meroni PL, Neri R, Tavoni A, Bombardieri S: International survey on the management of patients with SLE. The results of a questionnaire regarding renal involvement. *Clin Exp Rheumatol* 14[Suppl 16]: S23–S30, 1996
2. Austin HA 3rd, Klippel JH, Balow JE, Le Riche N, Steinberg AD, Plotz PH, Decker JL: Therapy of lupus nephritis. *N Engl J Med* 314: 614–619, 1986
3. Illei GG, Austin HA 3rd, Crane M, Collins L, Gourley MF, Yarboro CH, Vaughan EM, Kuroiwa T, Danning CL, Steinberg AD, Klippel JH, Balow JE, Boumpas DT: Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann Intern Med* 135: 248–257, 2001
4. Illei GG, Takada K, Parkin D, Austin HA, Crane M, Yarboro CH, Vaughan EM, Kuroiwa T, Danning CL, Pando J, Steinberg AD, Gourley MF, Klippel JH, Balow JE, Boumpas DT: Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy. Long-term follow-up of a cohort of 145 patients participating in randomized controlled trials. *Arthritis Rheum* 46: 995–1002, 2002
5. Houssiau FA, Vasconcelos C, D’Cruz D, Sebastiani GD, Garrido ED, Ede R, Danieli MG, Abramovicz D, Blockmans D, Mathieu A, Direskeneli H, Galeazzi M, Gul A, Levy Y, Petera P, Popovic R, Petrovic R, Sinico RA, Cattaneo R, Font J, Depresseux G, Cosyns JP, Cervera R: Immunosuppressive therapy in lupus nephritis. The Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 46: 2121–2131, 2002
6. Chan TM, Li FK, Tang CS, Wong RW, Fang GX, Ji YL, Lau CS, Wong AK, Tong MK, Chan KW, Lai KN: Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N Engl J Med* 343: 1156–1162, 2000
7. Contreras G, Pardo V, Leclercq B, Lenz O, Tozman E, O’Nan P, Roth D: Sequential therapies for proliferative lupus nephritis. *N Engl J Med* 350: 971–980, 2004
8. Cameron JS: Lupus nephritis. *J Am Soc Nephrol* 10: 413–424, 1999
9. Ponticelli C, Moroni G: Lupus nephritis. *J Nephrol* 13: 385–399, 2000
10. Miescher PA, Favre H, Le Moine R, Huang YP: Drug combination therapy of systemic lupus erythematosus. *Springer Semin Immunopathol* 16: 295–311, 1994
11. Hallegua D, Wallace DJ, Metzger AL, Rinaldi RZ, Klinenberg JR: Cyclosporine for lupus membranous nephritis: Experience with ten patients and review of the literature. *Lupus* 9: 241–251, 2000
12. Hochberg MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [Letter]. *Arthritis Rheum* 40: 1725, 1997
13. Churg J, Dernstein J, Glasscock RJ: Lupus nephritis. In: *Classification and Atlas of Glomerular Diseases*, 2nd Ed., edited by Churg J, Tokyo-New York, Igaku Shoin, 1995, pp 151–156
14. Austin HA 3rd, Muenz LR, Joyce KM, Antonovych TA, Kullick ME, Klippel JH, Decker JL, Balow JE: Prognostic factors in lupus nephritis. Contributions of renal histologic data. *Am J Med* 75: 382–391, 1983
15. Leehey DJ, Katz AI, Azaran AH, Aronson AJ, Spargo BH: Silent diffuse lupus nephritis: Long-term follow-up. *Am J Kidney Dis* 2[Suppl]: 188–196, 1982
16. Simon R: Restricted randomization designs in clinical trials. *Biometrics* 35: 503–512, 1979
17. Liang MH, Socher SA, Larson MG, Schur PM: Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. *Arthritis Rheum* 32: 1107–1118, 1989
18. Moroni G, Quaglini S, Maccario M, Banfi G, Ponticelli C: “Nephritic flares” are predictors of a bad long-term renal outcome in lupus nephritis. *Kidney Int* 50: 2047–2053, 1996
19. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
20. Mosca M, Bencivelli W, Neri R, Pasquariello A, Batini V, Puccini R, Tavoni A, Bombardieri S: Renal flares in 91 SLE patients with diffuse proliferative glomerulonephritis. *Kidney Int* 61: 1502–1510, 2002
21. Passerini P, Pasquali S, Cesana B, Zucchelli P, Ponticelli C: Long-term outcome of patients with membranous nephropathy after complete remission of proteinuria. *Nephrol Dial Transplant* 4: 525–529, 1989
22. Korbet SM, Schwartz MM, Lewis EJ: Primary focal segmental glomerulosclerosis. Clinical course and response to therapy. *Am J Kidney Dis* 23: 773–783, 1994
23. Drenkard C, Villa AR, Garcia-Padilla C, Perez-Vazquez EM, Alarcon-Segovia D: Remission of systemic lupus erythematosus. *Medicine* 75: 88–98, 1996
24. Korbet SM, Lewis EJ, Schwartz MM, Reichlin M, Evans J, Rohde RD: Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. *Am J Kidney Dis* 35: 904–914, 2000