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ORIGINAL ARTICLE

Combined immunosuppressive treatment (CIST) in lupus nephritis: a multicenter, randomized controlled study

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Abstract

Objectives The standard strategy for treating lupus nephritis comprises glucocorticoids together with either intravenous cyclophosphamide or oral mycophenolate mofetil, but the low remission rate is still a challenge in practice. This study was aimed to seek higher remission rate of lupus nephritis using a combined strategy.

Method A 24-week trial was conducted in 17 rheumatology or nephrology centers in China. A total of 191 lupus nephritis patients were randomized to follow a combined immunosuppressive treatment (CIST) with intravenous cyclophosphamide, an oral immunosuppressive agent, namely mycophenolate mofetil, azathioprine or leflunomide, and hydroxychloroquine ($n = 95$), or receive intravenous cyclophosphamide alone ($n = 96$) for 24 weeks. Glucocorticoid was given to both groups. The primary end point was a complete remission with a most stringent standard as proteinuria < 150 mg per 24 h, normal urinary sediment, serum albumin, and renal function at 24 weeks. The secondary end point was treatment failure at 24 weeks.

Results At week 24, both the rate of complete remission (39.5%) and total response (87.2%) was higher in the combined group, compared with CYC group (20.8% and 68.8%, $p < 0.05$). The cumulative probability of complete remission was also higher in

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the combined group ($p = 0.013$). In addition, the combined treatment was superior to routine CYC with less treatment failure (12.8% vs.31.2%, $p < 0.001$). No difference was found between the incidences of severe adverse events in the two arms: 3.2% (3/95 combined group) vs.4.2% (4/96 CYC group).

Conclusion Treatment with a combined immunosuppressive agent is superior to routine CYC only therapy in lupus nephritis.

Keywords Immunosuppressive agents · Lupus nephritis · Remission inducing · Systemic lupus erythematosus

Introduction

Lupus nephritis (LN) is a severe, potentially life-threatening disease, which is often challenging clinically. The standard strategy for treating LN comprises glucocorticoids together with either intravenous (IV) cyclophosphamide (CYC) or oral mycophenolate mofetil (MMF) [1, 2]. However, most patients with LN do not achieve a satisfactory clinical response or renal remission with such therapy. Data from high-quality, prospective-controlled studies show that the complete remission rates induced by CYC and MMF were merely 5.8–25.6% and 8.1–26% in 6 months, respectively [3–6]. Other immunosuppressive agents had been used to treat lupus nephritis, including azathioprine (AZA) [7, 8], cyclosporine [9], tacrolimus [10, 11], and leflunomide (LEF) [12, 13], but the remission rate at 6 months was still low or similar as reported previously. Several studies reported the efficacy of the new biological agents, rituximab and belimumab. However, their use is limited due to unconfirmed efficacy in the majority of patients. Therefore, there is an increasing demand for induction therapy with a higher complete remission and affordable cost in LN patients.

There is little data on the efficacy and safety of combined immunosuppressive drugs in LN. Liu [6] and Bao [14] et al. reported that multi-target therapy, which consists of MMF and a calcineurin inhibitor, tacrolimus, was superior to the traditional CYC therapy in inducing remission in LN. However, the use of the calcineurin inhibitor is limited to selected cases with preserved renal function due to nephrotoxicity of the drug. Here, we conducted a large-scale, multi-centered trial comparing an affordable triple strategy that combined CYC with another immunosuppressive agent plus hydroxychloroquine (HCQ) to CYC only as a remission-inducing treatment for LN.

Materials and methods

This trial was approved by Peking University People's Hospital ethics committee and was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice in 17 rheumatology or nephrology centers of medical institutions in China. All patients provided written informed consent. The study was registered at www.chictr.org.cn (number ChiCTR-TRC-09000587).

Study designs

We conducted a multicenter, open-labeled, parallel-group, 1:1 randomized, controlled efficacy trial from December 2009 through September 2013. Participants were randomized to receive IV CYC or combined strategy and were followed for 24 weeks after randomization.

Patients who met the inclusion criteria were assigned randomly using a sealed opaque envelope containing computer-generated random allocations in a 1:1 ratio to one of the two treatment groups. The statistician who generated the randomization sequence was not involved in the trial.

Patients assigned to the IV CYC group (the CYC group) received 12 CYC pulses at a dose of 0.25–0.5 g/m^2 of body surface area at 2-week intervals within 6 months. Patients assigned to the combined treatment group (the combined group) received CYC at the same dose as the CYC group, combined with HCQ (400 mg/day) and another immunosuppressive agent: MMF (0.75–1.00 g/day), AZA (starting at 1 mg/kg/day and increasing to a target dose of 2 mg/kg/day if tolerated in 2 weeks), or LEF (starting at 10 mg/day increasing to target dose of 20 mg/day if tolerated in 2 weeks). The oral immunosuppressive agent was chosen with consideration of the white blood cell count, hepatic function, and economic situation. The oral immunosuppressive agent started 2 weeks after the first CYC injection and continued at least until week 24.

All patients received oral glucocorticoid therapy at an initial dosage of 1 mg prednisone equivalent/kg/day for 2–4 weeks. The use of intravenous methylprednisolone pulses (three daily pulses of 500–1000 mg IV methylprednisolone) was allowed in critically ill patients (renal impairment or severe extra-renal disease), followed by oral glucocorticoid. After 2 to 4 weeks, the glucocorticoid dosage was tapered gradually to prednisone 10 mg equivalent/day, which was maintained until week 24.

All patients in both arms were treated initially with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers as concomitant medications, excluding those had contraindications.

Patients

Patients were eligible if they met the following inclusion criteria: diagnosis of systemic lupus erythematosus (SLE)

according to the 1997 revised American College of Rheumatology criteria for the classification of SLE [15], age between 18 to 65 years, proteinuria (> 1500 mg of protein in a 24-h urine specimen) with or without hematuria (> 5 red cells per high-power field) and the presence of cellular casts, and a SLE disease activity index (SLEDAI) more than 5. In addition, renal biopsy-proven lupus glomerulonephritis within 6 months before randomization was preferred. We excluded patients who had the following conditions: creatinine clearance < 30 mL/min or serum creatinine on repeated testing > 3.0 mg/dL (265.2 μmol/L), neuropsychiatric lupus erythematosus, pregnancy or lactation, history of malignancy within 5 years, severe infection, or other severe coexisting conditions involving the liver (concurrent increase of aspartate transaminase or alanine aminotransferase to more than double the normal level, or HBsAg positive), eye (visual field defect, mono-ocular function, or cataract), or heart (decompensated heart failure or severe hypertension). Patients treated with CYC within 3 months were also excluded.

Efficacy assessment

The primary end point was complete remission at 24 weeks. The secondary end point was treatment failure at 24 weeks. Complete remission was defined as urinary protein excretion < 0.15 g/day, with normal urinary sediment (RBC < 5/HP, WBC < 5/HP), serum albumin concentration, and renal function. Partial remission was defined as a decrease in the urinary protein excretion rate by more than 50%, with a serum albumin level ≥ 30 g/L and stable renal function. Renal flare was defined as an increase of 50% or more in the baseline serum creatinine level in 1 month, or reoccurrence of nephritic syndrome, or ≥ 1.5 g of protein per 24 h excluding other causes of proteinuria. Treatment failure was defined as death, ESRD, doubling of the serum creatinine level, renal flare, or failure to reach complete or partial remission at 24 weeks. Discontinuation of the study drug or withdrawal from the study due to toxic effects or any other reason was also considered a treatment failure.

Patients were evaluated at baseline and fortnightly during the study. Blood samples were collected at study visits for complete blood cell count and liver function tests; measurements of levels of creatinine, albumin, complements, and anti-double-stranded DNA antibodies. Twenty-four-hour urine sample was collected for the measurement of urine protein. The activity of SLE was evaluated by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).

Maintenance or salvage treatment

After 6 months, patients who achieved CR or PR were given reduced doses of IV CYC (0.25–0.5 g/m^2 of body surface area per month) for another 6 months and then shifted into oral immunosuppressive agents, which continued to at least until 24 months after study inclusion. Those in the combined group kept using the oral immunosuppressive agent, which were added in the induction treatment, as maintenance treatment throughout the study period. Those in CYC group where given either AZA at 1–2 mg/kg/d or MMF at 1–2 g/d after CYC withdrawal.

Patients who had a treatment failure or renal flares would retreat as decided by the attending physicians.

Statistical analysis

The primary end point (complete remission) was used for the power calculation. We estimated a complete remission rate of 20% at 6 months in the CYC group. The clinically meaningful difference was defined as a 15% complete remission rate in the combined group. To detect such a difference, 91 patients needed to be randomized to each arm to obtain a power of 80% and an α level of 0.05.

The primary efficacy analysis was a per protocol (PP) analysis that included all patients who completed this 24-week trial. The safety population comprised all patients who received at least one dose of immunosuppressive agent in the combined or CYC group. Survival curves were derived using Kaplan–Meier methods to estimate the time to complete remission. Between-group differences in the survival curves were assessed using the log-rank test. The efficacy of treatment was estimated using the hazard ratios and their 95% confidence intervals, obtained from a univariate Cox proportional hazard model. Serial data were compared within and between groups by repeated-measures analysis of variance (ANOVA). Unpaired t tests, Mann–Whitney U tests and Wilcoxon's signed–rank tests were used to compare continuous variables between groups. Chi-square tests and Fisher's exact tests were used to compare categorical variables as appropriate. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) ver. 17.0.

Results

Participant characteristics

In total, 191 patients were randomized into the trial: 95 to the combined group and 96 to the CYC group (Fig. 1). The baseline characteristics are detailed in Table 1, and were similar between the two groups. As indicated in Figs. 1, 163 out of 191patients (85.3%) completed the 24-weeks trial: 87 (90.6%) in the combined group and 77 (80.2%) in the CYC group. Twenty-eight patients dropped out of the trial (9 in the combined group and 19 in the CYC group) due to loss of follow-up ($n = 17$), withdrawn by a physician ($n = 4$), or severe adverse events ($n = 7$).

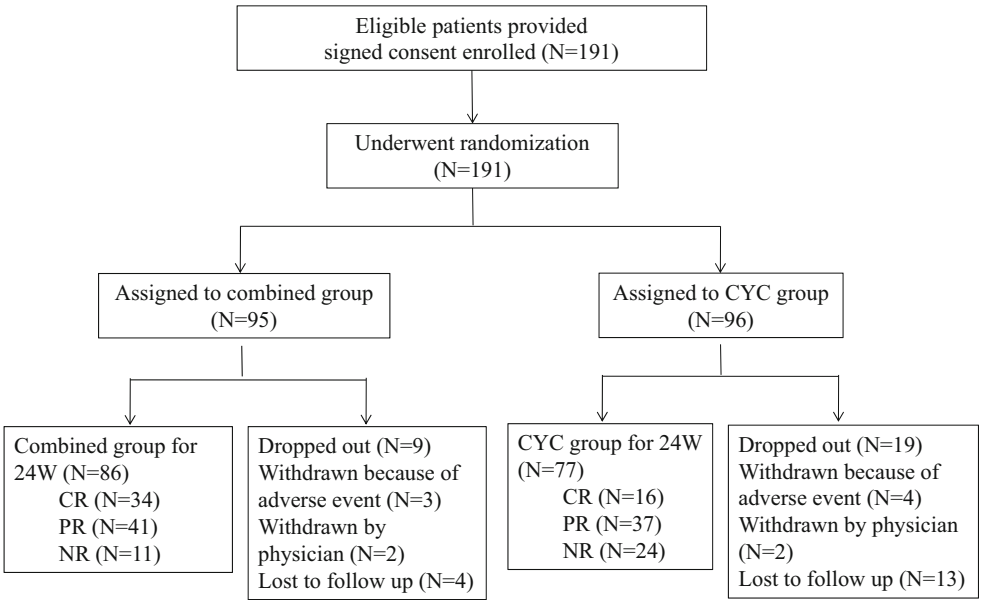


Fig. 1 Enrollment of patients, treatment assignments, and outcomes. Combined group: a combination of intravenous CYC, and another immunosuppressive agent, namely mycophenolate mofetil, azathioprine, or leflunomide, with hydroxychloroquine. CYC group: 12 intravenous CYC pulses at a dose of 0.25–0.5 g/m^2 of body surface area at 2-week intervals within 6 months. CYC, cyclophosphamide; CR, complete remission; PR, partial remission; TF, treatment failure

Dosing and exposure

The starting prednisone doses were similar between the two treatment groups (mean ± SD, 51 ± 15 mg/day in the combined group vs.51 ± 14 mg/day in the CYC group, $p = 0.99$). The cumulative CYC dose per patient after 6 months was 4.5 ± 1.6 g in the combined group and 4.8 ± 1.3 g in the CYC group ($p = 0.06$). In the combined group, 30 patients were prescribed MMF, 9 patients AZA, and 57 patients LEF. The mean (± SD) daily doses of MMF, AZA, and LEF were 1.00 ± 2.78 g, 66.7 ± 25.0 mg, and 12.3 ± 4.2 mg, respectively.

Efficacy

In the PP analysis, at week 24, the rate of complete remission (34/86, 39.5%) was higher in the combined group compared with the CYC group (16/77, 20.8%, $p = 0.011$), yielding an absolute treatment difference of 18.7% (Fig. 2). Within the combined group, the complete remission rates among the patients receiving MMF, AZA, and LEF were 20, 33.3, and 43.9%, respectively. The combined treatment was also significantly superior to CYC in terms of the cumulative probability of achieving complete remission (Fig. 3, hazard ratio = 2.130, 95% confidence interval 1.175–3.858, $p = 0.013$).

Intent-to-treat (ITT) analysis showed similar results: complete remission rate 35.8% (34/95) in combined group vs. 16.7% (16/96) in CYC group, difference 19.1% ($p = 0.003$).

The overall renal response rate was also significantly higher in the combined group than in the CYC group. In the PP analysis, the rate was 87.2% (75/86) in the combined group vs. 68.8% (53/77) in the CYC group ($p < 0.001$, Fig. 2). The total response rates of patients treated with MMF, AZA, and LEF in the combined group were 63.3, 66.6, and 89.8%, respectively. In the ITT analysis, the response rate was 78.9% (75/95) in the combined group vs. 55.2% (53/96) in the CYC group ($p < 0.001$). In addition, the combined treatment was superior to routine CYC with less treatment failure both in the PP analysis (12.8% vs.31.2%, $p < 0.001$, Fig. 2) and the ITT analysis (21.1% vs. 44.8%, $p < 0.001$). Treatment failure occurred in 23.3% of the patients prescribed MMF, 11.1% of those with AZA, and 5.3% of those prescribed LEF.

The levels of serum creatinine, proteinuria, serum albumin, complement C3 and C4, and SLEDAI score improved significantly in both groups during the 24 weeks of follow-up (all $p < 0.05$). No difference was noted between patients in the combined and CYC groups for any of the parameters examined ($p > 0.05$).

After the 24-week trial, further follow-up data was available for 96 out of 128 patients who responded to the initial induction treatment, 59/75 from combine group and 37/53 from CYC group. The median (IQR) follow-up time was 13.5 (9, 18) months. During the extended follow-up, 3/59 (5.1%) patients from the combined group and 4/37 (10.8%) patients from CYC group experienced a renal flare. However, the difference was not statistically significant.

Safety evaluation

As shown in Table 2, there was no difference in severe adverse events between the two groups: 3/95 (combined group) vs. 4/96 (CYC group). The total incidence of adverse events did not differ between the combined (30.5%, 29/95) and CYC (22.9%, 22/96) groups. Infections were observed in both groups, with a rate of 9.5% (9/95) in the combined group and 12.5% (12/96) in the CYC group. The rate of serious infections was similar in both groups: 2/95 (combined group)

Table 1 Baseline demographic and disease characteristics*

Characteristic	Combined group ($n = 95$)	CYC group ($n = 96$)	p value†
Age (years)	33.9 ± 10.8	34.7 ± 10.8	0.646
Female sex—no. (%)	85 (89.5)	89 (93.7)	0.296
Duration (months)	45.7 ± 50.0	3n9.0 ± 51.6	0.366
Renal pathology—no. (%)§	33 (34.7)	29 (30.2)	0.504
II	5 (5.3)	4 (4.2)	0.747
III	4 (4.2)	2 (2.1)	0.444
IV	12 (12.6)	15 (15.6)	0.553
V	4 (4.2)	4 (4.2)	1.000
Mixed membranoproliferative	8 (8.4)	4 (4.2)	0.250
Serum creatinine (μmol/L)	75.2 ± 30.4	72.9 ± 37.3	0.647
Urine protein (gram per 24 h)	4.3 ± 3.0	3.9 ± 2.8	0.374
Serum albumin (g/L)	29.4 ± 7.3	28.4 ± 6.2	0.330
Serum C3 (g/L)△	0.50 ± 0.25	0.48 ± 0.25	0.493
Serum C4 (g/L)△	0.11 ± 0.07	0.10 ± 0.07	0.246
SLEDAI	15.1 ± 5.8	14.5 ± 6.4	0.541

Results given as the means ±*SD*. *CYC*, cyclophosphamide; *SLEDAI*, Systemic Lupus Erythematosus Disease Activity Index

† Between-group p values were calculated by analysis of covariance with adjustment for baseline values. Unpaired t tests, Mann–Whitney U tests, chi-square tests, and Fisher's exact tests were used as appropriate

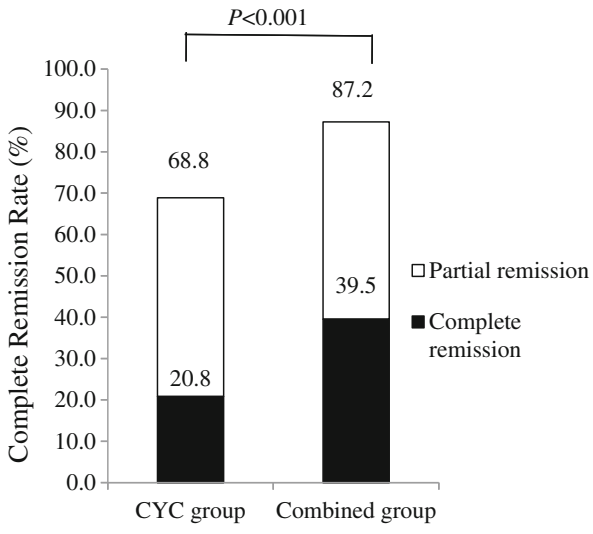
§ According to World Health Organization class [16]. Percentages might not total to 100 because of rounding

△ As the normal ranges for serum complement C3 and C4 assayed at the study sites varied, values were corrected to a single normal reference range for each (for C3, 0.83–0.201 g/L, and for C4, 0.16–0.47 g/L)

vs. 2/96 (CYC group). It seems that leukopenia and herpes zoster infections were more common in the combined group and more infection in general in the CYC group, but there was no difference between the two groups statistically. Three patients died in this trial, two in the combined group due to severe pulmonary infections at weeks 10 and 16, and one in the CYC group due to influenza A at day 6.

Discussion

It has been demonstrated that there was a significant correlation between complete remission and favored long-term outcome in LN [17, 18]. However, based on published data, only 5.8–26% of LN patients achieve complete remission after 6 months of well-recognized treatment with glucocorticoids and either



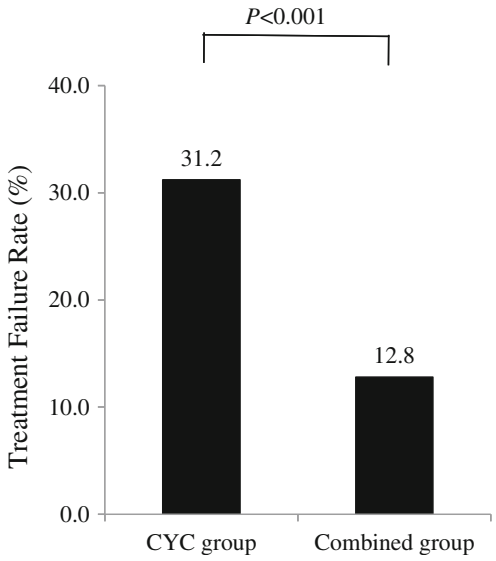


Fig. 2 Rates of complete remission and treatment failure in the combined and CYC groups at week 24. Complete remission was achieved in 34 (39.5%) patients in the combined group, compared with 16 (20.8%) patients in the CYC group ($p = 0.011$), while treatment failure occurred in 20 (12.8%) patients in the combined group, compared with 43 (31.2%) patients in the CYC group ($p < 0.001$)

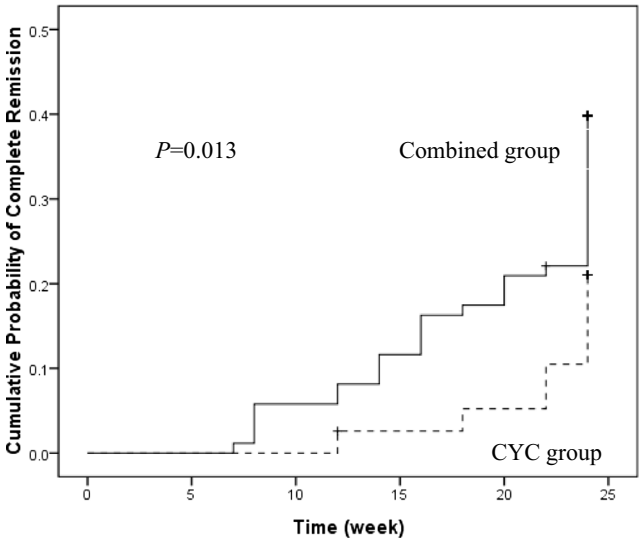


Fig. 3 Kaplan–Meier analysis of the cumulative probability of complete renal remission. The per-protocol population comprised 163 patients, of whom 86 followed combine treatment and 77 were given intravenous cyclophosphamide only. The hazard ratio for complete remission in the combined group with the CYC group was 2.130 (95% confidence interval 1.175–3.858, $p = 0.013$)

MMF or IV CYC [3–6]. It has been shown for decades that CYC, MMF, AZA, and LEF are efficacious in treating proliferative LN. However, there was little study so far to compare the efficacy of combined treatment with CYC alone in LN.

In the present study, we have shown that combined treatment was superior to CYC only in the treatment of patients with active LN. The complete remission rate was dramatically increased from 20.8% in the CYC group to 39.5% in the combined group in 6 months. The benefits of the combined treatment regimen were also supported by a much lower treatment failure rate. Notably, the complete remission rate of the combined group was improved markedly with traditional immunosuppressive agents, not by using costly biological treatments. Recently, Rathi et al. [19] conducted a head-to-head comparison of IV CYC (six biweekly pulses of a fixed 500-mg dose) vs. MMF (1.5–3 g per day). After 6 months, half of the patients in both groups achieved complete response, defined by a proteinuria ≤ 0.5 g/d, return to normal serum creatinine, and inactive urine sediment. The complete remission rate was higher than our study. However, complete remission was defined much more strictly in our study, which is proteinuria ≤ 0.15 g/d, almost a normal standard. This should explain the difference of complete remission rates between the two studies.

Adverse events were similar in both groups. Patients under combined treatment did not experience more adverse events. Moreover, the incidence of severe adverse events leading to death, hospitalization, or withdrawal from the study did not differ between the two arms. It seems that more infection in the CYC group and more leukopenia in the combined group were observed, but there was no difference statistically.

Hydroxychloroquine was administered only to the combined group since we believed it would benefit lupus nephritis patients as part of the combined treatment. It was not used in the CYC group because the effect of HCQ in LN was not confirmed in 2009 when our study was designed and initiated.

MMF, AZA, and LEF were selected as the additional immunosuppressive agent to combine with IV CYC in our study because their efficacies in LN were proved in previous studies

Table 2 Patients reporting adverse events per treatment arm

	Combined group		CYC Group		*p* value
	(*n* = 95)	Percent (%)	(*n* = 96)	Percent (%)	
Leukopenia*	13	13.7	6	6.3	0.086
Anemia†	1	1.1	ND¶	ND¶	1.000
Pancytopenia§	ND¶	ND¶	1	1.0	1.000
Infections	9	9.5	12	12.5	0.504
Herpes zoster	4	4.2	1	1.0	0.358
Upper respiratory infection	2	2.1	5	5.2	0.450
Pneumonia	3	3.2	3	3.1	1.000
Diarrhea	ND¶	ND¶	1	1.0	1.000
Urinary tract infection	ND¶	ND¶	2	2.1	0.497
ALT/AST rise△	4	4.2	2	2.1	0.669
Headache	2	2.1	1	1.0	0.993

*Leukopenia was defined as a white blood cell count below 3×10^9 /L.

† Anemia was defined as a hemoglobin level below the lower limit of the normal range

§ Pancytopenia was defined as a white blood cell count, hemoglobin level and platelet count all below the lower limits of the normal ranges

△ ALT/AST, alanine transaminase/aspartate transaminase. An ALT/AST rise was defined as an alanine transaminase or aspartate transaminase level above 1.5 × ULN (the upper limit of the normal range)

¶ ND denotes not detected

[3–5, 7, 8, 12, 13]. On the other hand, cyclosporine and tacrolimus were not selected despite that they were also effective in LN in concern of their potential nephrotoxicity [9–11]. The low doses of additional immunosuppressive agents were chosen to avoid excessive immunosuppression, as well as overlapping adverse effects such as liver dysfunction and hematocytopenia. The reason we chose three immunosuppressive agents to be added to CYC and HCQ instead of only MMF was that according to previous reports, an increased incident of severe infection was detected in Asian populations compared with other races at the same MMF doses [5]. Since AZA or LEF were showed by several studies to have a similar effect with MMF [3, 5, 12, 20], the following existing protocol investigators were allowed to have other choices when they were concerned about the adverse effect of MMF such as serious infection. Since the patients in the combined group are not randomized to receive the oral immunosuppressive agent, this study was not powered to draw conclusions about subsets of patients using different immunosuppressive agents as a combination component.

The open-label design was chosen for this study, but the potential bias was minimized by randomizing the patients and selecting a primary end point with the use of objective laboratory measures.

One limitation of the study was that not all of the patients underwent renal biopsies and no repeated renal biopsies were performed, which limits the ability to draw a conclusion based on pathological classification. However, the randomization of patients in the two arms should mitigate potential bias produced. As shown in Table 1, the histopathological features of the two treatment groups did not differ at baseline. This situation also resembled clinical practice in the real world. In parts of the developing areas where a renal biopsy may not be readily available due to limitations to expertise or economic reasons, serological activity combined with an active urinary sediment and proteinuria and/or renal functional impairment of varying severity is taken to indicate active severe LN.

Nine patients with class II LN (mesangial nephropathy) were enrolled in this study. Although class II LN is often considered as a benign variant of LN, studies have shown that 18–52% of patients with class II LN required a second biopsy and presented histological transformation, which was the most frequent cause of an unfavorable renal outcome [21–23]. A study by Yang et al. [24] discovered that in class II LN patients, persistent proteinuria more than 0.5 g/d was a risk factor for the end-stage renal disease. There is not much evidence regarding the treatment class II LN patient should receive. European League Against Rheumatism group suggests corticosteroid and immunosuppressive agent for class II LN with persistent proteinuria > 1 g/d, especially in presence of glomerular hematuria [2]. In the present study, all of the 9 patients with class II LN had proteinuria > 1.5 g/d. Seven patients had glomerular hematuria, and 5 of them had extrarenal manifestations (3 with immune thrombocytopenic purpura, 2 with hemolytic anemia). The SLEDAI of the 9 patients was 18.0 ± 4.3, indicating high activity of systemic lupus erythematosus [25].

Long-term clinical trials using CYC have shown that the proportion of responders in patients with proliferative LN continued to increase [8, 26], implying that our 6-month follow-up might underestimate the rate of remission. There was no difference in the relapse rates between the two groups at 6 months, while previous studies using IV CYC found a relapse rate of up to 45% of the patients with LN despite a complete clinical response to induction therapy [27, 28]. A prolonged follow-up during maintenance treatment is needed to explore the impact of the combined treatment on long-term prognosis further, such as renal flare, ESRD, cardiovascular complications, and death.

We conclude that combined immunosuppressive treatment is a safe regime and superior to routine CYC only therapy in inducing remission in patients with LN.

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Compliance with ethical standards

Disclosures None.

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