Efficacy and safety of mycophenolate mofetil and tacrolimus combination therapy in patients with lupus nephritis: a nationwide multicentre study

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Abstract

Objective

Recent studies have shown that a combination treatment of mycophenolate mofetil (MMF) and tacrolimus (TAC) may be an option for lupus nephritis (LN) patients that do not adequately respond to initial treatment. We evaluated the efficacy and safety of the combination treatment of MMF and TAC in LN patients with suboptimal response to prior MMF or TAC treatments.

Methods

In this multicentre study, we retrospectively enrolled 62 patients with class III, IV, or V LN who inadequately responded to MMF or TAC treatment. Those patients were then treated with a combination of MMF and TAC for 6 months. The primary outcome was complete remission (CR) at 6 months, and secondary outcomes included overall response and adverse events.

Results

After 6 months of treatment with the drug combination, CR was achieved in 14 of 62 patients (22.6%), and 35 (56.5%) patients responded. A significant reduction in proteinuria and lupus disease activity score was observable after 3 months. After 1 year, the CR rate increased to 36.4% (20 of 55 patients), and the overall response rate (n=38, 69.1%) also increased from 6 months. Twenty-one patients reported 29 adverse events, including severe infection requiring hospitalisation (n=3, 10.3%), infection not requiring hospitalisation (n=2, 6.9%), and herpes zoster (n=4, 13.8%).

Conclusion

Our findings suggest that a combined MMF and TAC treatment, with a favourable adverse-event profile, may be a beneficial option for LN patients with inadequate response to either MMF or TAC treatments.

Key words

combination therapy, complete remission, lupus nephritis, mycophenolate mofetil, tacrolimus

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Introduction

Renal disease is a major organ manifestation of systemic lupus erythematosus (SLE), and up to 60% of SLE patients develop lupus nephritis (LN) (1). Recently, the treatment of LN has improved significantly with the introduction of new immunosuppressive agents into clinical practice (2). However, as many as 45% of LN patients do not respond to immunosuppressive drug treatment (3), and 10–20% of LN patients eventually develop end-stage renal disease (ESRD) (4, 5). Patients with active LN experience a decreased quality of life compared with those without renal disease (6), and their mortality rate is increased by eight-fold compared with the general population (7).

Despite insufficient efficacy and potential toxicities, cyclophosphamide (CYC) in combination with corticosteroids has been the standard of care for many years in the treatment of LN (8). Mycophenolate mofetil (MMF) has recently emerged as an option for LN treatment. Randomised controlled trials (RCTs) have shown that MMF is as effective as CYC, with fewer side effects (9, 10). Nevertheless, one-third of LN patients do not achieve complete or partial remission (PR) with these treatments (9, 10); thus, current induction treatment with CYC or MMF is not ideal due to inefficacy. Many patients who fail to achieve remission with induction therapy eventually progress to renal failure (11-13); therefore, more effective immunosuppressive regimens are needed to manage LN.

Tacrolimus (TAC) is a calcineurin inhibitor (CNI) that has a mechanism of action similar to cyclosporine, and can act as a potent inhibitor of human T cell proliferation. Recent studies have shown that TAC may be a suitable alternative treatment for LN patients with an inadequate response to conventional therapy. Several RCTs and uncontrolled studies have shown that TAC is similar in efficacy to CYC or MMF for the initial treatment of LN (14-16). The combination of TAC therapy with MMF, known as multitarget therapy, has been shown to be more effective than intravenous CYC as an induction therapy for LN (17). The addition of TAC was found to be safe and effective for refractory LN patients who did not achieve renal response with MMF (18, 19). However, there are limited data on the safety and efficacy of the combination treatment of MMF and TAC in patients with LN.

To date, clinical trials using novel biologic agents such as belimumab, abatacept, and rituximab have failed to show superiority over conventional drugs for LN treatment (20-22). While other promising drugs are being developed for the treatment of LN, there is a clear and unmet need within the current options for the management of LN. The aim of this study was to determine the efficacy and safety of the combination therapy of MMF and TAC in patients with LN who did not achieve remission with either MMF or TAC alone.

Patients and methods

Population and study design

In this retrospective observational study, LN patients were enrolled from 11 tertiary academic rheumatologic centres across Republic of Korea between January 2015 and June 2017. Patients with LN who had been treated with a combination therapy of MMF and TAC for at least 6 months were recruited to participate in this study. Inclusion criteria required that all patients were as follows: (1) of either gender and between 15 and 80 years of age; (2) fulfilled the 1997 revised criteria for the classification of SLE (23); (3) had a diagnosis of Class III, IV, or V LN; and (4) had inadequate response to either MMF or TAC monotherapies for 6 months prior to combination treatment of MMF and TAC. Patients with serum creatinine (SCr) >2.0 mg/dl at enrolment were excluded. Patients were also excluded if they had advanced co-morbidity or other diseases associated with kidney dysfunction, including diabetic kidney disease or primary kidney disease.

Renal biopsies were used to confirm LN in all patients and were performed at the time of diagnosis of LN. Renal biopsy specimens were classified according to the International Society of Pathology/Renal Pathology Society

(ISN/RPS) classification (24). Inadequate response to previous regimens was defined as any of the following: (1) failure to improve to <1 g/day, or a urine protein to creatinine ratio (UPCR) <1.0; or (2) 50% of pretreatment baseline values, with or without persistently active urinary sediment (>5 red blood cells (RBCs)/high-power field (HPF) and >5 white blood cells (WBCs)/HPF and no cellular casts).

Patients were followed in 3-month intervals for the assessment of clinical response and adverse events. Of the 62 LN patients, 55 were followed up to 12 months. This study received approval from the institutional review board/ethics committee (IRB/EC) of each medical centre, including Chonnam National University Hospital (CNUN-2015-143), which waived the requirement for informed consent due to the retrospective nature of the study.

Treatment regimens

At the time of enrolment, either MMF or TAC had been added to the treatment regime of LN. As a part of the multitarget treatment, the additional dose of MMF or TAC was based on the clinical judgment of a rheumatologist. MMF or TAC was added at a lower dose and was increased to 2–3 g/day of MMF or 4 mg/day of TAC, according to renal function or clinical presentation of adverse events. Laboratory tests were carried out every 2 to 4 weeks during the dosage step-up period, and every 4 weeks after the target dose was reached. If the SCr level was elevated by more than 30% compared to the baseline or preceding clinic visit, additional MMF or TAC was withheld until SCr levels stabilised. Upon SCr stabilisation, MMF or TAC was added at a lower dose. Dosage was also adjusted if patients experienced adverse effects related to the multitarget treatment.

Assessment of treatment response

The primary endpoint of interest was the incidence of complete remission (CR) after 6 months of multitarget treatment. Secondary endpoints included overall response (CR + PR), progression to ESRD and death, improvement of estimated glomerular filtration rate (eGFR), anti-double-stranded DNA (dsDNA) titre, SLE disease activity index (SLEDAI)-2K score, and adverse events at 6 and 12 months.

CR was defined as a normal eGFR (≥90 ml/min/1.73 m^2) or >25% increase from baseline if the baseline eGFR was abnormal; or as an UPCR <0.5; or as a dipstick test of 0 to trace and inactive urinary sediment (≤5 RBCs/HPF, ≤5 WBCs/HPF, and no cellular casts). PR was defined as having a normal eGFR (≥90 ml/min/1.73 m^2) or >25% increase from baseline if the baseline eGFR was abnormal, and >50% reduction in UPCR, ranging between 0.5 and 2.0. Non-response (NR) was defined as not meeting any remission criteria.

Patient data collection

Baseline characteristics were collected at the time of patient enrolment. Sociodemographic data included age at onset of SLE, age at onset of LN, gender, disease duration of SLE (the time from the diagnosis of SLE until the development of LN), and insurance information. Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg on two or more occasions, and/or patient-reported intake of antihypertensive medications. Diabetes mellitus was defined as having a history of fasting glucose levels ≥140 mg/dl, or the use of insulin or a hypoglycaemic agent.

Renal and extra-renal disease activity associated with SLE was assessed using the SLEDAI-2K score (25). The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (26) was also obtained at enrolment. SLEDAI-2K and SLICC/ACR Damage Index scores were assessed every 3 months during follow-up.

We obtained clinical data, including WBC count, haemoglobin concentration, platelet level, serum albumin level, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, SCr level, and level of 24-h proteinuria (g/day) or UPCR. GFR was calculated according to the Modification of Diet in Renal Disease (MDRD) study equation: Estimated GFR (ml/min/1.73m^2) $= 186 \times [\text{SCr (mg/dl)}]^{-1.154} \times (\text{age})^{-0.203} \times$ (0.742 if female). Parameters for measuring kidney function were obtained every 3 months during follow-up periods. Serological markers, including autoantibodies (such as anti-nuclear [ANA], anti-dsDNA, and anti-Smith) and complements [C3, C4]), were also obtained.

We also investigated patient medication history, including the medications to be used in treatment, as well as previous medications used before the initiation of multitarget treatment, such as hydroxychloroquine and prednisolone. Use of angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) use, which may reduce proteinuria, was also obtained.

Safety assessments included medical histories, clinical presentations, the assessment of laboratory tests, vital signs, spontaneous reporting of adverse events such as gastrointestinal syndrome, new onset hypertension and diabetes mellitus, temporary transaminase rise, SCr elevation, leukopenia, infections such as herpes zoster, viral or bacterial infection, and other manifestations.

Statistical analysis

We performed statistical analyses using SPSS software (v. 18; SPSS Inc., Chicago, IL, USA). Values are expressed as mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. For assessing between-group differences, continuous variables were compared to the Mann-Whitney U-test, and categorical variables were compared to the chi-square test. Within-group comparisons (between baseline and follow-up points) were assessed with the Wilcoxon signed-rank test. *p*-values less than 0.05 were considered to be statistically significant.

Results

Baseline epidemiologic features, clinical presentation, and laboratory findings

A total of 62 ethnically homogenous Korean patients with LN were enrolled in this study and the baseline demographic and clinical characteristics are

Table I. Clinical characteristics of LN patients at study enrolment [a].

	LN (n=62)
Age, years	33.0 ± 10.4
Women (%)	57 (91.9)
Disease duration at onset of LN, months	35.5 ± 62.3
Medical beneficiaries (%)	60 (96.8)
Hypertension (%)	19 (30.6)
Diabetes mellitus requiring treatment (%)	1 (1.6)
SLEDAI-2K score	10.0 ± 11.5
SLICC Damage Index score	0.31 ± 0.59
ISN/RPS histological class (%)	
III	15 (24.2)
III+V	3 (4.8)
IV	25 (40.3)
IV+V	5 (8.1)
Pure V	14 (22.6)
Laboratory findings	
ESR, mm/h	31.0 ± 18.6
CRP, mg/dl	0.75 ± 3.42
Albumin, mg/dl	3.53 ± 2.30
Anti-nuclear antibody positivity (%)	61 (98.4)
Anti-dsDNA, IU/ml	136.1 ± 224.2
Serum creatinine, mg/ml	1.05 ± 1.29
eGFR, ml/min/1.72 m^2	98.1 ± 62.1
Urine protein/creatinine ratio	3.98 ± 3.62
Active urinary sediments (%)	41 (66.2)
Hydroxychloroquine (%)	48 (77.4)
Statin use (%)	29 (46.8)
ACEi or ARB use (%)	40 (64.5)
Previous ineffective regimen (%)	
MMF	50 (80.6)
TAC	12 (19.4)
CYC	26 (41.9)
AZA	17 (27.4)

[a] Unless indicated otherwise, data are presented as mean ± standard deviation.
LN: lupus nephritis; SLEDAI: systemic lupus erythematosus disease activity index; SLICC: systemic lupus international collaborating clinics; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; dsDNA: double-stranded DNA; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MMF: mycophenolate mofetil; TAC: tacrolimus; CYC: cyclophosphamide; AZA: azathioprine.

shown in Table I. The mean age of the patients was 33.0 (SD 10.4) years, and 57 (91.9%) patients were female. The mean disease duration of LN was 35.5 (SD 62.4) months**.** At the time of enrolment, the mean SLEDAI-2K score was 10.0 (SD 11.5) and the mean SLICC score was 0.31 (SD 0.59). For renal biopsy findings, 15 (24.2%) patients exhibited features of class III, 25 (40.3%) patients demonstrated class IV, and 14 (22.6%) patients exhibited pure class V. There were eight mixed proliferative/membranous cases in the ISN/RPS classification, including three III+V cases and five IV+V cases. The mean SCr, eGFR, UPCR, and serum albumin of LN patients at study enrolment were 1.05 mg/ml, 98.1 ml/min/1.72 m^2, 3.98, and 3.53 mg/dl, respectively. ANA positivity was found in 61 (98.4%) patients, and the mean anti-dsDNA titre was 136.1 IU/ml.

Of the 62 LN patients, 25 (40.3%) had been initially treated with intravenous CYC with or without AZA. However, due to persist proteinuria, relapse, or advents events, treatment regimens were replaced with either MMF or TAC monotherapies. Prior to initiation of MMF and TAC combination therapy, 12 (20%) patients had a TAC monotherapy and 50 (80%) patients had a MMF monotherapy for LN treatment. At the time of enrolment, the mean daily dose of MMF and TAC was 1.81 g and 2.25 mg, respectively. As a combination therapy, TAC was initiated at a mean dose of 1.22 mg/day and was titrated up to 2.37 mg/day in patients with prior MMF monotherapy, and MMF was initiated at a mean dose of 0.96 g/day and titrated up to 1.67 g/day in patients with prior TAC monotherapy. The initial mean dose of prednisolone at the time of MMF and TAC combination therapy was 17.6±18.7 mg/day. Compared to the initial dose, the mean doses of prednisolone 6 and 12 months after combination therapy were reduced to 9.27±7.75 and 8.20±6.60 mg/day, respectively (both $p<0.005$).

Table II. Treatment outcomes in patients with LN.

	6-month response (n=62)	12-month response (n=55)
Complete response [a]	14 (22.6%)	20 (36.4%)
Partial response [b]	21 (33.9%)	18 (32.7%)
Non-response [c]	27 (43.5%)	17 (30.9%)
End-stage renal disease or dialysis	1 (1.6%)	2 (3.6%)
Death	0	0

[a] Complete response is defined as a normal glomerular filtration rate (GFR) (≥90 ml/min/1.73 m^2) or >25% increase from baseline if baseline eGFR was abnormal; or as an urine protein-to-creatinine ratio of <0.5; or as a dipstick test of 0 to trace, and inactive urinary sediment (≤5 RBCs/high-power field [HPF], ≤5 WBCs/HPF, and no cellular casts).
[b] Partial response is defined as having a normal eGFR (≥90 ml/min/1.73 m^2) or >25% increase from baseline if the baseline eGFR was abnormal, and >50% reduction in the urinary protein-to-creatinine ratio, ranging between 0.5 and 2.0.
[c] Non-response is defined as not meeting any remission criteria. LN: lupus nephritis.

Renal response after combination treatment

After 6 months of treatment, CR was achieved in 14 of 62 patients (22.6%) treated with combination therapy, and an overall response (combined CR and PR) was achieved in 35 (56.5%) patients (Table II). Of the 62 LN patients, 55 were followed up to 12 months. After the end of follow-up, the CR rate increased to 36.4 % (20 of 55 patients), and the overall response rate (n = 38, 69.1%) was also increased from 6 months. Despite combination treatments, 1 (0.16%) of 62 patients had progressed to ESRD by 6 months, while 2 (0.36%) of 55 patients had progressed in 12 months. There were no mortalities in our cohort. A significant reduction in UPCR and lupus disease activity (SLEDAI-2K) score was observed at 3 months, and these reductions were maintained over the reporting period (Fig. I). Both the renal SLEDAI (6.51±0.59 to 4.65±0.62; $p=0.002$) and extra-renal SLEDAI score (3.44±0.59 to 2.42±0.62) exhibited significant improvement after 6 months of combination treatment compared to baseline. Furthermore, improvements were still evident at 12 months for both the renal SLEDAI (4.07±0.55; $p=0.001$) and extra-renal SLEDAI score (2.38±0.5; $p=0.048$).

Subgroup analysis

Table III compares the treatment response between the ISN/RPS class III+IV (±V) and pure class V LN. There was no difference in treatment responses at 6 and 12 months between

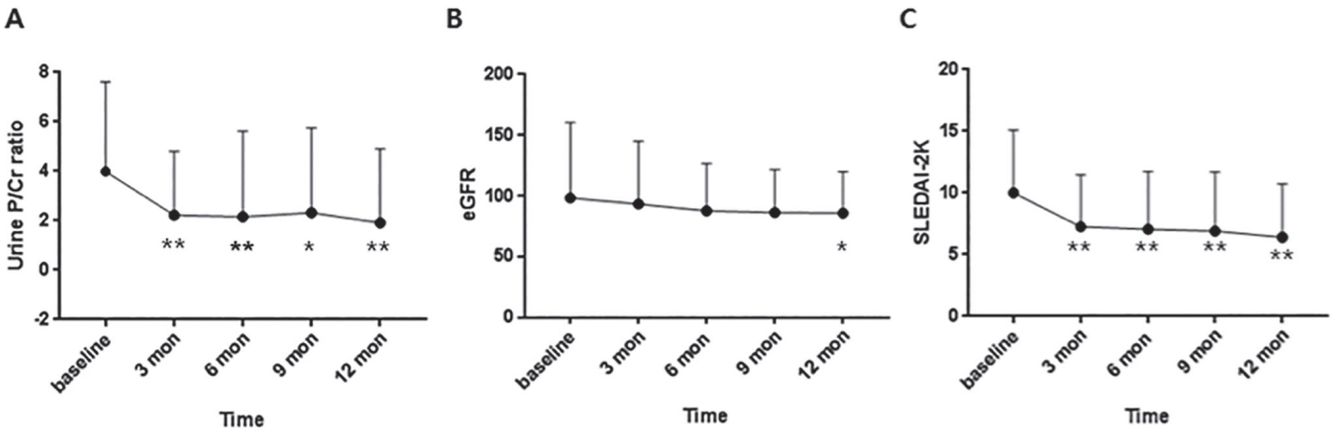

Fig. 1. Changes in renal parameters associated with lupus activity over time: **A**: urine protein to creatinine ratio; **B**: creatinine clearance; **C**: SLEDAI-2K score. *p<0.05; **p<0.001 (relative to baseline value). P: protein; Cr: creatinine; SLEDAI: systemic lupus erythematosus disease activity index.

Table III. Comparison of treatment outcomes by histological classification.

	6- month response (n=62)	12-month response (n=55)
III/IV±V LN	(n=48)	(n=43)
Complete response [a]	10 (20.8%)	15 (34.9%)
Partial response [b]	17 (35.4%)	12 (27.9%)
Non-response [c]	21 (43.8%)	16 (37.2%)
Pure V LN	(n=14)	(n=12)
Complete response [a]	4 (28.6%)	5 (41.7%)
Partial response [b]	4 (28.6%)	6 (50.0%)
Non-response [c]	6 (42.9%)	1 (8.3%)
P value	0.803	0.132

[a] Complete response is defined as a normal glomerular filtration rate (GFR) (≥90 ml/min/1.73 m^2) or >25% increase from baseline if baseline eGFR was abnormal; or as an urine protein-to-creatinine ratio of <0.5; or as a dipstick test of 0 to trace, and inactive urinary sediment (≤5 RBCs/high-power field [HPF], ≤5 WBCs/HPF, and no cellular casts).

[b] Partial response is defined as having a normal eGFR (≥90 ml/min/1.73 m^2) or >25% increase from baseline if the baseline eGFR was abnormal, and >50% reduction in the urinary protein-to-creatinine ratio, ranging between 0.5 and 2.0.

[c] Non-response is defined as not meeting any remission criteria. LN: lupus nephritis.

the groups. While not statistically significant (p=0.132), more patients with pure class V achieved CR or overall response than those with class III+IV (±V) at 12 months. Furthermore, as shown in Table IV, treatment regimens (MMF *vs*. TAC) prior to combination treatment did not affect the CR rate at 6 or 12 months.

Adverse events

During follow-up, 29 adverse events were reported in 21 LN patients (Table V). Infectious adverse events included infection requiring hospitalisation (n=3, 10.3%), infection not requiring hospitalisation (n=2, 6.9%), and herpes zoster infection (n=4, 13.8%). Transient increases in SCr and serum potassium levels were observed in three (10.3%) and one (3.4%) patients, respectively. Of the gastrointestinal side effects, three (10.3%) patients reported transient dyspepsia and two (6.9%) patients reported diarrhoea. Only one patient developed leukopenia. We observed no effects on blood pressure and glycaemic control.

Discussion

After 6 months of combined MMF and TAC therapy, 14 of 62 LN patients (22.6%) achieved CR and over 50% of patients showed at least PR. Our findings suggest that a combined MMF and TAC treatment, with a favourable adverse-event profile, may be a good option for LN patients with inadequate response to either MMF or TAC monotherapies.

For decades, combination treatments of immunosuppressants with different mechanisms of action have provided favorable immunosuppressive effects in clinical practice. In particular, combination treatment with modified dosage could demonstrate synergistic effects while offering a reduced side-effect profile (27). Synergistic combination regimens of two or more immunosuppressive agents could compensate or overcome toxicity and other side effects associated with high doses of single drugs by countering biological compensation, lowering dosages of single compounds, or accessing context-specific multitarget mechanisms (27-29). Combined treatment of MMF and TAC has been commonly used in organ transplantation. A lower dose of TAC reduces concern for complications, including nephrotoxicity (29). Furthermore, arterial intimal thickening and angiogenesis, which is induced by chronic use of TAC, could be counterbalanced by the antiproliferative properties of MMF (30). For these reasons, researchers have great interest in the combination of MMF and TAC for the treatment of refractory LN.

MMF and TAC combination therapy has shown synergistic effects in the induction treatment of LN. Bao *et al*. (31) first described the efficacy of MMF/TAC combination regimens for mixed class IV+V LN. In that study, 40 Chinese patients with LN were randomly assigned to induction with either intravenous pulse CYC or low-dose combination of MMF (1 g/day) and TAC (4 mg/day). After 6 months of induc-

Table IV. Comparison of treatment outcomes by order of treatment.

	6- month response (n=62)	12-month response (n=55)
MMF→MMF+TAC	(n = 50)	(n = 45)
Complete response [a]	12 (24.0%)	16 (35.6%)
Partial response [b]	17 (34.0%)	16 (35.6%)
Non-response [c]	21 (42.0%)	13 (28.9%)
TAC→TAC+MMF	(n = 12)	(n = 10)
Complete response [a]	2 (16.7%)	4 (40.0%)
Partial response [b]	4 (33.3%)	2 (20.0%)
Non-response [c]	6 (50.0%)	4 (40.0%)
p-value	0.829	0.614

[a] Complete response is defined as a normal glomerular filtration rate (GFR) (≥90 ml/min/1.73 m^2) or >25% increase from baseline if baseline eGFR was abnormal; or as an urine protein-to-creatinine ratio of <0.5; or as a dipstick test of 0 to trace, and inactive urinary sediment (≤5 RBCs/high-power field [HPF], ≤5 WBCs/HPF, and no cellular casts).
[b] Partial response is defined as having a normal eGFR (≥90 ml/min/1.73 m^2) or >25% increase from baseline if the baseline eGFR was abnormal, and >50% reduction in the urinary protein-to-creatinine ratio, ranging between 0.5 and 2.0.
[c] Non-response is defined as not meeting any remission criteria.
MMF: mycophenolate mofetil; TAC: tacrolimus; CYC: cyclophosphamide.

Table V. Reported adverse events.

Adverse events	Number of events (%)
Infection requiring hospitalisation	3 (4.8)
Pneumonia	1
Urethritis	1
Episcleritis	1
Infection not requiring hospitalisation (excluding herpes)	2 (3.2)
Herpes zoster	4 (6.5)
Transient increase in serum creatinine	3 (4.8)
Diarrhoea	2 (3.2)
Transient increase in liver function test	1 (1.6)
Dyspepsia	3 (4.8)
Mood change	1 (1.6)
Hyperkalaemia	1 (1.6)
Leukopenia	1 (1.6)
Skin rash	1 (1.6)
Headache	1 (1.6)
Other	6 (9.7)
Myalgia	2
Angina-like symptoms	1
Respiratory symptoms	3
Total	29 (46.8)

tion therapy, the combined MMF/TAC treatment group achieved significantly higher rates of CR compared with the CYC group (50% *vs*. 5%, respectively). These results were also maintained at 9 months (65% *vs*. 15%, respectively). Although three (15%) patients developed new-onset hypertension, most adverse events were less frequent in the combined MMF/TAC group (31). After the pilot study, the same group performed a large multicentre RCT study comprised of 368 Chinese LN patients (17). The short-term (24 week) outcomes of a low-dose combination of MMF (1 g/day) and TAC (4 mg/day) were compared to those of an intravenous pulse of CYC (0.5–1.0 g/m^2) in patients with class III/IV/V LN. The combination regimen was superior to the intravenous pulse of CYC in terms of a complete renal response rate at 6 months (46% *vs*. 26%; $p<0.001$) (17). While more patients in the combination group dropped out of the study due to adverse events compared with the intravenous CYC group, the overall incidence of adverse events was similar in both groups (17). Collectively, combination therapy with MMF and TAC was well tolerated and superior to intravenous CYC for inducing CR in patients with active LN.

Other studies have investigated the efficacy of the addition of TAC to MMF treatment in LN patients who were resistant to induction therapies, including MMF. In an observational study performed by Cortes-Hernandez *et al*. (18), of 70 white European patients with Class III/IV/V LN who were initially treated with MMF as an induction/maintenance therapy, 17 (24%) experienced treatment failure or renal flare. Those patients received TAC (mean dose: 4.25 mg/day) in addition to MMF (mean dose: 1.5 g/day) as a rescue therapy, and after a follow-up period of 24±3 months, 12 patients (70%) achieved clinical response (35% CR and 35% PR). Moreover, a significant reduction in proteinuria was observed within 3 months, and the additional TAC treatment was well-tolerated. Lantana *et al*. (19) also reported the efficacy of the addition of TAC in seven patients with LN (3 class III+V, 2 class V, 1 class V+II , and 1 class IV+V) who did not respond to MMF. The addition of TAC (2–8 mg/day) to MMF was effective in terms of reducing 24-h proteinuria, and four patients (57%) achieved PR. However, five patients reported side effects such as diabetic ketoacidosis (n=1), pneumonia (n=2) and muscle pain (n=2). In conclusion, both studies showed that combination treatment could be a suitable alternative for MMF-resistant LN patients.

Mok *et al*. (32) also evaluated the efficacy of combined MMF and TAC, without augmentation of corticosteroids, in the treatment of LN patients who failed to respond adequately to at least two induction immunosuppressive regimens, including MMF, CYC, TAC, AZA, and CSA. Of the 21 patients with class III, IV, or V LN, 14 (67%) achieved at least PR after 12 months of combination treatment with MMF and TAC. This study demonstrated the short-term efficacy of combination treatment for LN in patients that did not respond well to standard therapies. Another retrospective study showed that combination treatment of MMF and TAC resulted in a higher CR rate than TAC alone in the treatment of 16 Japanese patients with new-onset or flared class III-V LN (33). In the current study, combination treatment with MMF and TAC was effective in achieving CR in patients with proliferative or membranous LN who did not adequately respond to either MMF or TAC monotherapies. Most previous studies evaluating the efficacy of combination treatment were conducted in populations of less than 20 LN patients (18, 19, 33). Therefore, we performed a nationwide multicentre study consisting of a relatively large sample size. Here, 14 of 62 (22.6%) patients with LN achieved CR, and over 50% of patients demonstrated an overall renal response after 6 months. Patients who responded to the combination therapy

did so relatively soon after the initiation of treatment. Furthermore, the percentage of patients achieving both CR and overall renal response increased after 1 year of treatment. Subgroup analyses determined that neither prior treatment (MMF *vs*. TAC) nor combination order influenced a patient's ability to achieve CR. Data on the efficacy of combination treatment in LN patients who did not respond to TAC is limited compared with similar data for MMF. Due to increasing evidence showing that TAC is at least as effective at inducing CR as MMF or CYC (14-16), our study provides valuable information for combination treatment in the case of inadequate response to TAC.

While statistically insignificant (p=0.138), we were interested to find that patients with pure class V LN were more likely to achieve CR and overall renal response compared with those with III/IV±V after 1 year of treatment. Previous open-label studies have shown CNIs (TAC and cyclosporine) to be effective in treating patients with pure class V LN (34-36). Moreover, in their subgroup analysis, Mok *et al*. (14) showed that TAC appeared to be more effective than MMF at reducing proteinuria in pure class V LN. Although a clear pathological mechanism has not yet been established, our study suggests that combination treatment with MMF and TAC may have additional benefits to the treatment of LN patients with membranous components. Further prospective studies are needed to confirm these results.

Combination therapy with MMF and TAC was well-tolerated with regard to adverse events by most patients with LN during the study. Similar to other studies (17, 19), infection complications were the most frequent adverse events in our study, which is likely due to the increased immunosuppressive effect of drug combination. Here, severe infection requiring hospitalisation was reported in three (10.3%) patients that were successfully treated with appropriate antibiotics. Moreover, herpes zoster was reported in four (13.8%) patients. Interestingly, although it did not occur in our patients, cytomegalovirus infection was reported in 40% of enrolled patients in the Japanese study (33). These findings suggest that clinicians should be aware of the potential for the occurrence of opportunistic infections in patients undergoing combination therapy. While there was a transient increase in SCr, new-onset hypertension and refractory nephrotoxicity did not occur in our patients.

This study has several limitations that require consideration. First, because we conducted this study during routine clinical practice, we could not strictly control the treatment of LN. Second, significant uncertainty remains regarding the dosage of combination drugs. To date, the optimal doses for MMF and TAC combination therapy is unknown, due in part to the wide range of concentrations used in different studies. Although several studies have suggested a fixed-dose combination of MMF (1 g/day) and TAC (4 mg/day) as an induction treatment for LN (17, 31, 33), other investigators did not administer MMF and TAC in fixed-dose regimens. Measuring drug concentrations in plasma may guide clinicians to determine the optimal dose for treatment of individual LN patients, though further studies will be necessary to confirm this. Third, due to the inherent limitations of a retrospective study, we were unable to include follow-up renal biopsies as part of this analysis. Given the importance of repeat renal biopsies in the evaluation of immunosuppressive treatments in LN (37), a prospective study with follow-up renal biopsies may be necessary to better assess the use of MMF and TAC-based combination therapies. Fourth, we are aware of concerns regarding the use of CNIs in patients with considerable chronicity/fibrotic kidney lesions, as these have been shown to cause microvascular and glomerular damage, arteriolar hyaline deposition, tubular atrophy, and striped interstitial fibrosis (38). Unfortunately, due to the retrospective nature of this study, we were unable to collect data on the activity and chronicity indices. Despite these limitations, we believe that our results are useful in that they highlight the additive effect of a combined MMF and TAC treatment in LN patients with inadequate response to either MMF or TAC treatments. Fifth, in our study, the mean duration of monotherapy prior to combination therapy was 9.34±3.32 months. Such a duration is consistent with current ACR guideline, which recommend a change in immunosuppressive agents for patients who fail to respond after 6 months based on the treating physician's clinical impression (39). Similarly, EULAR guidelines recommend switching to an alternative agent in patients who fail to improve within 3–4 months, do not achieve an at least partial response after 6–12 months, or have not experienced a complete response after 2 years of treatment (40). Based on these recommendations, we enrolled patients with LN who did not respond to either MMF or TAC monotherapies after at least 6 months of treatment, based upon the treating physician's judgment. Nevertheless, additional studies may be necessary to determine optimal timing of MMF and TAC-based combination therapies. Finally, because this study was conducted in one homogenous population, results should be interpreted with caution, and further prospective studies of heterogeneous populations are required to verify our results.

In summary, our study demonstrated that the combination treatment of MMF and TAC has synergistic effects in terms of achieving CR and is generally well-tolerated by patients. Therefore, combined MMF and TAC treatments, with a favorable adverse-event profile, may be a good option for LN patients with inadequate response to either MMF or TAC treatments. Nevertheless, the long-term efficacy and prognosis of combination treatment should be determined in future studies.

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