

Long-Term Results of Triple Immunosuppression With Tacrolimus Added to Mycophenolate and Corticosteroids in the Treatment of Lupus Nephritis



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Introduction: Addition of a calcineurin inhibitor (CNI) to corticosteroids and mycophenolate increased the renal response rate in lupus nephritis (LN) because of proteinuria reduction, but there is little long-term efficacy and safety data on this triple immunosuppressive regimen.

Methods: This is a cohort study of patients with class III/IV/V LN whose proteinuria persisted despite initial standard therapy with mycophenolate mofetil (MMF) and prednisolone (PRED), in whom tacrolimus (TAC) was added (target 12-hour trough TAC plasma levels of 4–6 µg/l).

Results: A total of 22 patients with LN treated with triple immunosuppression were included, with follow-up of 61.1 ± 28.1 months. Achieved trough levels of TAC and mycophenolic acid (MPA) were 3.8 to 5.7 µg/l and 1.3 to 2.1 mg/l respectively. Significant proteinuria reduction occurred after 6 months and was sustained up to 5 years. Complete response (CR) and partial response (PR) rates at 12, 24, and 36 months was 59.1%, 72.7%, and 77.3% respectively. The slope of estimated glomerular filtration rate (eGFR) over time did not change after TAC was added. A total of 7 patients (31.8%) showed progressive chronic kidney disease (CKD). Two patients reached end-stage kidney disease during follow-up. Renal survival rate at -, 3, and 5 years was 100.0%, 95.0%, and 88.7% respectively. Two patients (9.1%) had renal relapse after 8.5 ± 0.7 months. A total of 5 patients (22.7%) showed worsening of hypertension, and 3 (13.6%) had worsened hyperlipidemia. Other key adverse events included infection ($n = 16$, 1 in 7 patient-years) and gastrointestinal upset ($n = 6$).

Conclusion: Triple immunosuppression with the addition of TAC to mycophenolate and PRED resulted in further proteinuria reduction and sustained disease quiescence in patients with LN whose proteinuria did not respond optimally to standard therapy.

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KEYWORDS: long-term; lupus nephritis; mycophenolate; tacrolimus; triple

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LN is a common and serious organ involvement in patients with systemic lupus erythematosus and remains an important cause of CKD associated with much morbidity and still significant mortality.^{1,2} The current standard-of-care treatments for severe LN include moderate- to high-dose corticosteroids combined with MMF or cyclophosphamide as initial

therapy for active nephritis,^{3–7} followed by low-dose corticosteroids combined with MMF or azathioprine as long-term maintenance immunosuppression.^{3–5,8} With these treatment regimens, renal response rates were 30.4% to 66.2% within the first year of treatment.^{9–12} A favorable renal response is associated with better long-term renal survival.^{13–16}

CNIs exert their immunosuppressive actions on T lymphocytes via inhibition of interleukin-2 secretion. In addition, CNIs also have a direct effect on the podocyte actin cytoskeleton, resulting in lowering of proteinuria in various glomerulopathies.¹⁷ The immunosuppressive efficacy of CNI is well proven, and it has a pivotal role in the standard prophylactic immunosuppressive

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treatment to prevent organ transplant rejection. Both cyclosporine (CYA) and TAC have been used in the treatment of LN at various points in the course of management, as part of initial treatment for active nephritis or as maintenance therapy. When used together with corticosteroids in dual immunosuppressive regimens, CNIs showed comparable short-term efficacy as cyclophosphamide or MMF in studies with relatively small sample size.^{18–22} In these studies, CNI treatment was not guided by regular therapeutic drug monitoring (TDM), and in many reports, the duration of follow-up was relatively short.^{18–21} Compared with CYA, TAC is associated with fewer cosmetic adverse effects and less severe gingival hyperplasia and is therefore often the preferred CNI in patients with LN. Preliminary data on TAC in Japanese patients, incorporated in various immunosuppressive regimens, suggest that it can be used as long-term therapy in the LN population.^{23,24}

Recent major clinical trials on CNI-containing triple immunosuppression in LN include a study in China that compared “multi-target treatment” (MTT) comprising fixed-dose TAC, low-dose MMF, and corticosteroids against controls treated with sequential high-dose i.v. cyclophosphamide then azathioprine in combination with corticosteroids, and the international multicenter trials comparing voclosporin against placebo added to background immunosuppression with corticosteroids and MMF.^{25–28} Results of the former showed superior renal response rates in the MTT group at 24 weeks but similar cumulative response rates of around 90% in the 2 groups in the following year, and similar flare rates (5.47% and 7.62%) in the 2 groups in the 18-month maintenance phase of the trial.^{29,30} The voclosporin phase 2 and phase 3 trials showed higher renal response rates at 6 months and 1 year in patients treated with triple immunosuppression that comprised voclosporin, MMF, and corticosteroids, compared with controls treated with placebo, MMF, and corticosteroids.^{25–28} Nephrotoxicity is a major concern with the use of CNI, and the risk is higher in patients with significant CKD at baseline. Acute CNI nephrotoxicity is often associated with excessive exposure and is largely reversible on dose reduction. In contrast, chronic CNI nephrotoxicity portends progressive CKD and is characterized by irreversible renal fibrosis and vasculopathy. In view of marked individual variations in pharmacogenomics and pharmacokinetics, it is standard practice to perform TDM when using CYA or TAC, to ensure that drug exposure is within the desired target range. Despite this, CNI nephrotoxicity is still common in kidney transplant recipients.^{31,32} It is pertinent to note that the MTT trial excluded patients with serum creatinine >265 µmol/l, while the voclosporin trials included only patients with baseline eGFR >45 ml/min/

1.73 m², therefore excluding those with CKD stage 3b or above. To date, there is little long-term data on CNI-containing triple immunosuppressive regimens in LN, and little data on the relationship between drug exposure and clinical outcomes. In this context, we analyzed the results of 22 patients with LN in whom TAC, titrated according to TDM, was added when proteinuria did not respond satisfactorily to treatment with PRED and MMF.

METHODS

Patients

The case records of all biopsy-proven patients with LN followed up at the Nephrology and Rheumatology Divisions of the Department of Medicine, University of Hong Kong, at Queen Mary Hospital, Hong Kong during the period 2012 to 2019 were reviewed. Patients who had received triple immunosuppression with PRED, TAC, and MMF for a minimum duration of 3 months were identified and included for analysis. The data on patient characteristics, laboratory parameters, and clinically significant events were analyzed. This study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong West Cluster Hospitals (approval number: UW11-115).

Immunosuppression and Follow-Up Schedule

Patients with biopsy-proven active class III/IV/V LN were managed with a standard immunosuppressive protocol that included corticosteroids and MMF, with gradual tapering doses, continuously from the early phase to the maintenance phase. All patients received pulse methyl-PRED at 0.25 to 0.5 g/d for up to 3 days followed by oral PRED (0.6–0.8 mg/kg/d) that was gradually tapered to 5 mg/d after 18 to 24 weeks, then further tapered slowly in stable patients so that in the second and third year the PRED dose was 2 to 5 mg/d. Discontinuation of PRED was not standard practice. MMF dose was 1 g twice daily for at least 6 months, then tapered to a total daily dose of 1.25 to 1.5 g in the second year and 1.0 to 1.25 g in the third year, with further reductions thereafter.^{10,33,34} MPA blood level 12 hours after dose was measured approximately once every 6 to 9 months to ensure that the level was in the target range of 1.5 to 2.5 mg/l during the second and third year of treatment, before attempting to gradually withdraw treatment in stable patients.

TAC was given as an add-on treatment to PRED and MMF when there was persistence of urine protein excretion at ≥2 g/d after 6 months of standard immunosuppressive treatment, despite already optimized blood pressure control and renin–angiotensin–aldosterone system blockade. TAC was started at

0.07 mg/kg/d (Prograf with twice daily dosing), with TDM to aim for a 12-hour trough plasma TAC level of 4 to 6 µg/l. In patients with MMF total daily dose ≥ 1.5 g, the dose would be reduced by 500 mg when TAC was added, to aim for lower readings in the target range of MPA blood level.

Patients were seen every fortnight initially, and the interval between successive follow-up visits was progressively lengthened to 3 months in stable patients. In addition to clinical parameters, investigations at each visit included complete blood count, renal and liver biochemistry, anti-double-stranded DNA antibody and C3 levels, trough TAC level, and urine protein-to-creatinine ratio. The 24-hour urine protein excretion was measured at least once every 6 months, or more frequently when clinically indicated. Patients were treated with hydroxychloroquine unless contraindicated or refused by patient, renin-angiotensin-aldosterone system blockade, and antihypertensive medications as required to target a diastolic blood pressure of ≤ 80 mm Hg. Lipid-lowering treatment was given to patients with low density lipoprotein cholesterol >3.4 mmol/l. Patients were given cotrimoxazole prophylaxis against pneumocystis for 1 year. HBsAg-positive patients are treated with entecavir long-term. Antiviral prophylaxis is not routine. Suspected renal flares were confirmed with repeat kidney biopsy, and patients were treated with increased dose of PRED and MMF, while the dose and target exposure for TAC were unchanged. Extrarenal flares were managed with increase in PRED dose of up to 20 mg daily, depending on clinical manifestations.

Study Outcomes and Statistical Analysis

Renal CR was defined as reduction of proteinuria to ≤ 0.5 g/d with improved or stable renal function defined as serum creatinine level $<115\%$ of the baseline level at initiation of induction immunosuppressive treatment. Renal PR was defined as decrease in urine protein excretion by at least 50% and non-nephrotic (<3 g/d) range proteinuria with improved or stable renal function as defined above. Other study outcomes included blood pressure control, clinical events, serologic parameters, glycemic and lipid profiles, disease relapse, and adverse events especially infective complications and CNI nephrotoxicity. Disease relapse would be considered when there was active extrarenal disease manifestations, or proteinuria increased by ≥ 1 g/d, or urine sediment became active, or at deterioration of lupus serology, and all suspected renal relapses required confirmation with kidney biopsy. To examine the impact on the rate of CKD progression, the slopes of eGFR over time before or after the initiation of triple immunosuppression (“pre-treatment slope” and “post-

treatment slope,” respectively) were compared, excluding the period starting from 3 months before the kidney biopsy that demonstrated active nephritis to 6 months after this kidney biopsy, to avoid the inclusion of acute changes in kidney function due to acute kidney injury and the effect of induction immunosuppression confounding the determination of eGFR slopes. Continuous variables were expressed as mean (SD) or median (range) and compared with Mann-Whitney *U* test or Wilcoxon-signed rank test where appropriate. Categorical variables were expressed as frequency (percentages) and compared with χ^2 or Fisher exact test where appropriate. Longitudinal values were compared with Friedman test and pairwise multiple comparisons were performed by Dunn’s test. All statistical analyses were performed with SPSS version 23.0, and 2-sided $P < 0.05$ were considered statistically significant.

RESULTS

Patient Characteristics

A total of 22 patients with LN (16 class III/IV \pm V, 6 class V) received triple immunosuppression with PRED, TAC, and MMF for 61.1 ± 28.1 months (median 70.2 months, interquartile range 39.8–86.5 months), giving a total treatment exposure duration of 1345.8 patient-months (Table 1). The actual prescribed dose of TAC was 2.9 ± 1.5 mg/d, 3.7 ± 1.0 mg/d, 3.7 ± 0.4 mg/d, 3.6 ± 0.9 mg/d, 4.0 ± 1.4 mg/d, and 4.6 ± 2.1 mg/d, at 6, 12, 24, 36, 48, and 60 months after commencement of triple immunosuppression, respectively, and the corresponding trough TAC level was 5.7 ± 2.1 µg/l, 4.6 ± 1.1 µg/l, 4.5 ± 1.7 µg/l, 4.0 ± 0.5 µg/l, 3.8 ± 1.2 µg/l, and 3.9 ± 1.4 µg/l, respectively. The time to achieve the target TAC exposure was 2.2 ± 1.0 months after initiation of treatment. The actual prescribed dose of MMF was 1511.9 ± 515.2 mg/d, 1229.2 ± 678.1 mg/d, 1159.1 ± 615.1 mg/d, 1000.0 ± 534.5 mg/d, 1166.7 ± 930.9 mg/d, and 1083.3 ± 736.0 mg/d at 6, 12, 24, 36, 48, and 60 months, respectively, and the corresponding MPA C12 levels were 1.3 ± 0.1 mg/l, 1.7 ± 1.4 mg/l, 1.6 ± 1.4 mg/l, 2.1 ± 0.9 mg/l, 1.8 ± 1.0 mg/l, and 1.3 ± 0.7 mg/l, respectively. The mean dosages of PRED were 8.8 ± 3.5 mg/d, 8.1 ± 3.8 mg/d, 6.5 ± 3.0 mg/d, 5.8 ± 2.0 mg/d, 5.3 ± 2.1 mg/d, and 5.0 ± 2.3 mg/d at 6, 12, 24, 36, 48, and 60 months, respectively. PRED dose in stable patients at last follow-up was 4.8 ± 0.4 mg/d.

Renal Outcomes

At initiation of triple immunosuppression (baseline), 24-hour urine protein excretion was 5.4 ± 4.1 g/d, and it decreased to 2.1 ± 2.3 g/d, 1.9 ± 2.4 g/d, 1.6 ± 1.8 g/d, 1.0 ± 1.9 g/d, 1.0 ± 1.8 g/d, and 1.2 ± 1.7 g/d,

Table 1. Clinical characteristics of 22 patients with lupus nephritis who were treated with a triple immunosuppressive regimen comprising corticosteroids, tacrolimus, and mycophenolate mofetil

Clinical characteristics	Value
Patient demographics	
Age, yrs	43.9 ± 11.7
Sex, F/M	17/5
Duration of follow-up (mo)	61.1 ± 28.1
Class of LN at presentation	
III ± V or IV ± V, n (%)	16 (72.7)
V, n (%)	6 (27.3)
History of hypertension, n (%)	9 (40.9)
History of diabetes mellitus, n (%)	2 (9.0)
Number of renal relapses before the use of triple immunosuppression	1.5 ± 1.4
Clinical parameters at commencement of triple immunosuppression	
Proteinuria (g/d)	5.4 ± 4.1
Serum creatinine (μmol/l)	88.9 ± 51.2
eGFR (ml/min per 1.73 m ²)	75.2 ± 20.1
Anti-dsDNA level (IU/ml)	39.4 ± 71.1
C3 level (mg/dl)	72.7 ± 26.2
Dose of immunosuppressant at commencement of triple immunosuppression	
Dose of PRED (mg/d)	21.1 ± 12.1
Dose of MMF (mg/d)	1511.9 ± 515.2
Dose of TAC (mg/d)	2.9 ± 1.5

dsDNA, double-stranded DNA; eGFR, estimated glomerular filtration rate; F, female; LN, lupus nephritis; M, male; MMF, mycophenolate mofetil; PRED, prednisolone; TAC, tacrolimus.

respectively after 6, 12, 24, 36, 48, and 60 months ($P = 0.001, 0.004, 0.005, 0.007, 0.008, \text{ and } 0.018$, respectively, compared with baseline) (Figure 1a). eGFR was 75.2 ± 20.1 ml/min per 1.73 m^2 at baseline, and 71.5 ± 22.1 ml/min per 1.73 m^2 , 71.4 ± 24.1 ml/min per 1.73 m^2 , 69.3 ± 25.6 ml/min per 1.73 m^2 , 68.4 ± 27.1 ml/min per 1.73 m^2 , 66.9 ± 24.5 ml/min per 1.73 m^2 , and 66.3 ± 26.8 ml/min per 1.73 m^2 , respectively at 6, 12, 24, 36, 48, and 60 months ($P = 0.158, 0.116, 0.025, 0.047, 0.068, \text{ and } 0.069$, respectively, compared with baseline) (Figure 1b).

The overall (CR + PR) renal response rate was 59.1%, 72.7%, and 77.3% after 12, 24, and 36 months of triple immunosuppressive treatment (CR rate 36.4%, 45.5%, and 45.5%; PR rate 22.7%, 27.2%, and 31.8%, respectively). The time to achieve CR (time-to-CR) after starting triple immunosuppressive treatment was 11.1 ± 8.3 months. A total of 5 patients (22.7%) did not achieve CR or PR, and the level of proteinuria in these patients was 3.1 ± 1.3 g/d at baseline and 1.3 ± 1.2 g/d after 24 months. Membranous nephropathy was more frequently observed in the baseline kidney biopsy of patients who achieved CR or PR (responders) compared with those who did not achieve CR or PR (nonresponders) (64.7% vs. 0.0%, $P = 0.011$). Responders and nonresponders did not differ in age, sex, serum creatinine, C3, or anti-double-stranded DNA level at baseline ($P > 0.05$, for all)

The slope of eGFR over time did not change significantly after the addition of TAC (-2.5 ± 2.7 ml/min per $1.73 \text{ m}^2/\text{yr}$ before vs. -2.0 ± 3.1 ml/min per $1.73 \text{ m}^2/\text{yr}$ after treatment with triple immunosuppression, respectively, $P = 0.832$, for the whole group; -1.8 ± 3.1 ml/min per $1.73 \text{ m}^2/\text{yr}$ vs. -1.4 ± 3.3 ml/min per $1.73 \text{ m}^2/\text{yr}$, respectively, $P = 0.92$, for responders; -3.5 ± 2.5 ml/min per $1.73 \text{ m}^2/\text{yr}$ vs. -3.0 ± 1.3 ml/min per $1.73 \text{ m}^2/\text{yr}$, respectively, $P = 0.644$, for nonresponders). The rate of eGFR decline was numerically faster in nonresponders (-3.0 ± 1.3 ml/min per $1.73 \text{ m}^2/\text{yr}$ vs -1.4 ± 3.3 ml/min per $1.73 \text{ m}^2/\text{yr}$ in responders, $P = 0.632$). Nonresponders showed a higher percentage of progressing to CKD stage 3 or above after 24 months compared with responders (60.0% vs. 11.7%, $P = 0.024$).

A total of 3 patients (13.6%) had stage 3 or above CKD before the initiation of triple immunosuppression (Table 2). The rate of eGFR decline in these patients was -5.2 ± 2.4 ml/min per $1.73 \text{ m}^2/\text{yr}$ and -3.2 ± 3.8 ml/min per $1.73 \text{ m}^2/\text{yr}$ before and after treatment with triple immunosuppression, respectively ($P = 0.480$). The 2 patients with CKD stage 3 achieved PR after commencing triple immunosuppressive treatment, while the patient with CKD stage 4 at baseline was a nonresponder. Two patients (9.1%) developed end-stage kidney disease after 25.5 ± 16.9 months of follow-up, and 5 patients (22.7%) had new-onset CKD stage 3 or above after 13.2 ± 7.8 months. The 2 patients who progressed to end-stage kidney disease had prior CKD stage 3b and stage 4, respectively, with significantly lower eGFR at baseline (25.5 ± 5.0 ml/min per 1.73 m^2 , $P < 0.001$ compared with other patients). While the numerical value appeared lower, the renal survival rate in patients who received triple immunosuppression was statistically similar to that in patients whose proteinuria responded to dual immunosuppression with corticosteroids and MMF (100.0% vs. 100.0%, 95.0% vs. 100.0%, and 88.7% vs. 98.4% at 1-, 3-, and 5-year respectively, $P = 0.075$) (Figure 2). Two patients (9.1%) had renal relapse (both class III + V), which occurred after 8.5 ± 0.7 months. At the time of renal flare, the TAC dose and trough levels were 3.0 ± 0.7 mg/d and 4.1 ± 1.6 μg/l, and the MMF dose and trough levels were 1250.0 ± 353.6 mg/d and 1.3 ± 0.1 mg/l, respectively, while the dose of PRED was 7.5 mg/d and 6.0 mg/d, respectively. Both renal flares were confirmed with repeat kidney biopsy, and treated with increased dose of PRED and MMF, while the dose and target exposure for TAC were unchanged. Two patients had extrarenal flares with skin and joint manifestations after 1 and 5 years of follow-up, respectively, when the concomitant PRED dose was 10.0 mg/d and 7.5

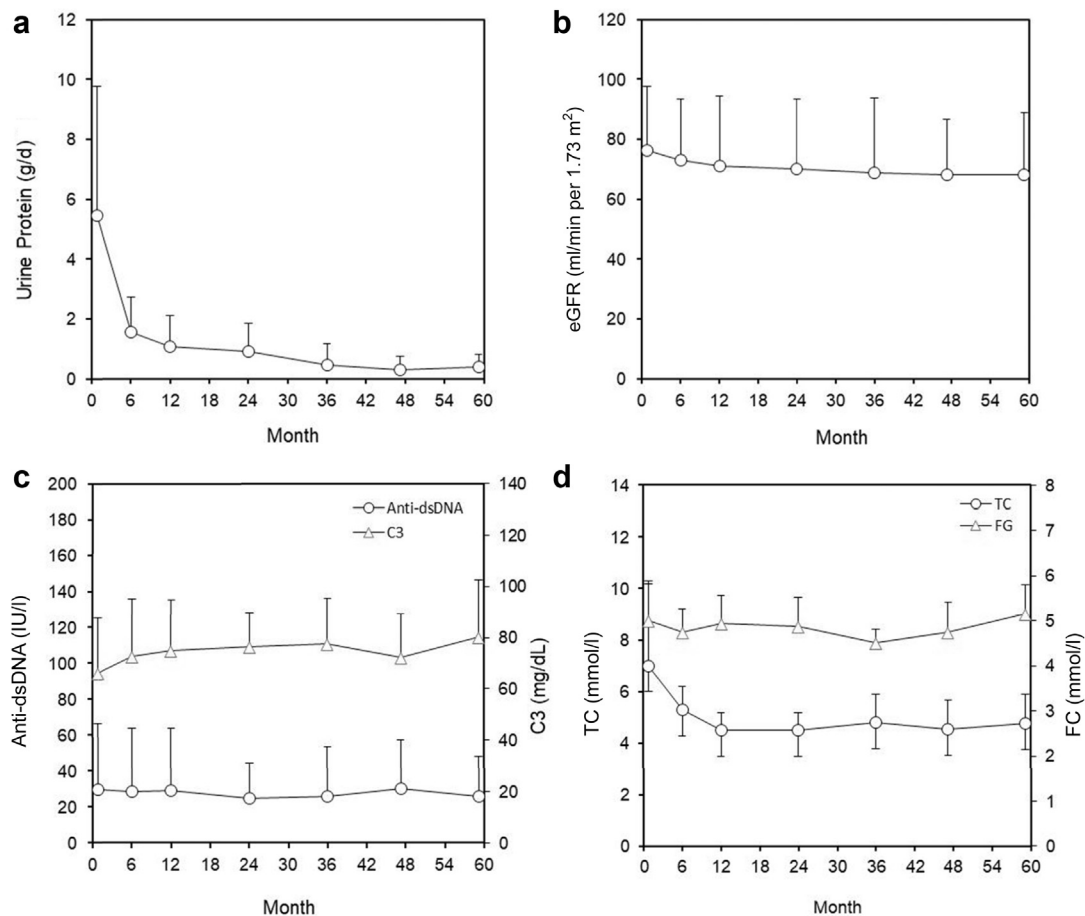


Figure 1. Serial changes in (a) proteinuria (b) eGFR (c) anti-dsDNA and C3 levels, and (d) FG and TC in 22 patients with lupus nephritis who were treated with triple immunosuppressive regimen comprising corticosteroids, tacrolimus, and mycophenolate mofetil. dsDNA, double-stranded DNA; eGFR, estimated glomerular filtration rate; FG, fasting glucose; TC, total cholesterol.

mg/d. Both were managed with increase in dose of PRED up to 20 mg daily.

Serologic Parameters

The level of anti-double-stranded DNA remained stable over time -39.4 ± 71.1 IU/ml (normal <30 IU/ml) at baseline, and 38.6 ± 46.3 IU/ml, 32.0 ± 22.9 IU/ml, 23.7 ± 26.2 IU/ml, 22.9 ± 26.4 IU/ml, and 25.9 ± 29.0 IU/ml after 12, 24, 36 and 48, and 60 months, respectively ($P = 0.730, 0.414, 0.263, 0.263,$ and 0.176 , respectively, compared with baseline) (Figure 1c). C3 level also remained stable over time: 72.7 ± 26.2 mg/dl at baseline, and 73.8 ± 17.5 mg/dl, 79.1 ± 14.4 mg/dl,

82.3 ± 12.7 mg/dl, 82.4 ± 12.6 mg/dl, and 90.0 ± 22.4 mg/dl after 12, 24, 36, 48, and 60 months, respectively ($P = 0.334, 0.074, 0.123, 0.069,$ and 0.018 , respectively, compared with baseline) (Figure 1c).

Adverse Events and Metabolic or Blood Pressure Changes

Triple immunosuppression was well-tolerated in general (Table 3). A total of 16 episodes of infections occurred (occurrence rate of 1 in 7 patient-years), and all responded to 1 course of antimicrobial treatment. A total of 10 of these infective episodes required hospitalization (4 gastroenteritis, 4 pneumonia, 1 acute

Table 2. Clinical characteristics and outcomes of 3 patients with CKD stage 3 or above

Patient	Stage of CKD and renal function at the time of initiation of triple immunosuppression		Treatment response	Rate of eGFR decline after triple immunosuppression (ml/min per 1.73 m ² /yr)	Renal outcomes at last follow-up
	CKD stage	eGFR (ml/min per 1.73 m ²)			
Patient 1	3a	50	PR	-7.0	Stage 4 CKD
Patient 2	3b	30	PR	-6.0	ESKD
Patient 3	4	22	NR	-2.5	ESKD

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; NR, no response; PR, partial response.

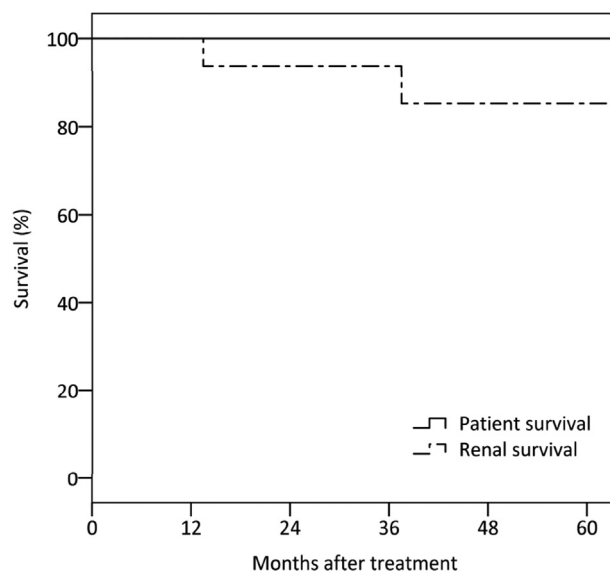


Figure 2. Rate of renal and patient survival in 22 patients with lupus nephritis who were treated with triple immunosuppressive regimen comprising corticosteroids, tacrolimus, and mycophenolate mofetil.

pancreatitis, 1 urinary tract infection; hospitalization rate of 1 in 11 patient-years). A total of 6 episodes of gastrointestinal symptoms occurred, 4 resolved after reduction of MMF dose and MMF was discontinued in 2 patients. There was no death during follow-up (Figure 2).

Fasting blood glucose level and lipid profile remained stable (Figure 1d). Fasting blood glucose was 5.2 ± 1.2 mmol/l at baseline and 4.9 ± 0.5 mmol/l after 60 months ($P = 0.750$), while that of total cholesterol/low density lipoprotein cholesterol was $6.3 \pm 2.3/4.1 \pm 2.3$ mmol/l at baseline and $4.6 \pm 1.0/2.2 \pm 1.0$ mmol/l at 60 months, respectively ($P = 0.280$ and 0.080 for total cholesterol and low density lipoprotein when compared with baseline values, respectively). There was no new-onset diabetes mellitus. Two patients (9.1%) had diabetes mellitus before the addition of TAC, and the dose of oral

Table 3. Adverse events experienced by 22 lupus nephritis patients who received treatment with triple immunosuppressive regimen comprising corticosteroids, tacrolimus, and mycophenolate mofetil

Adverse events	Number of episodes
Infections	16
Gastroenteritis	4
Pneumonia	4
Urinary tract infection	6
Herpes zoster	1
Acute pancreatitis	1
Worsening of hypertension	5
Worsening of hyperlipidemia	3
Hand tremor	2
Gastrointestinal disturbance	6
Hematological abnormalities	2
Leucopenia	1
Anemia	1

hypoglycemic drugs was not altered afterward. A total of 14 patients (63.6%) were already on lipid-lowering therapy before addition of TAC. A total of 3 patients (13.6%) showed worsening of hyperlipidemia, which responded to an increase in the dose of statin. A total of 5 patients (22.7%) required an increase in antihypertensive medication dose after addition of TAC. Two patients (9.1%) had hand tremor, one occurring when TAC trough blood level exceeded the target range, and both did not require discontinuation of TAC therapy. There was no acute kidney injury due to CNI nephrotoxicity. TAC treatment was discontinued in 2 patients—one because of deteriorating renal function and the other because of financial reasons.

Costs and Medical Expenditures Related to the Use of Triple Immunosuppression

We estimated the total medical expenditure related to the use of triple immunosuppression by including the cost of medications, hospitalization, and management of complications arising from triple therapy. In Hong Kong, the unit prices of PRED, TAC, and MMF were US \$0.015 (5 mg tablet)/US \$0.024 (1 mg tablet), US \$2.23 (1 mg tablet)/US \$1.36 (0.5 mg tablet), and US \$1.06 (500 mg tablet)/US \$0.520 (250 mg tablet), respectively. The average nominal cost of hospitalization in public hospital was US \$653.8/d. In this cohort, the average medical expenditures in patients receiving triple therapy were US \$5075.0/patient-year, US \$4179.5/patient-year, US \$4012.6/patient-year, US \$4143.4/patient-year, and US \$4407.7/patient-year in the first, second, third, fourth, and fifth year, respectively.

DISCUSSION

On the basis of the generally favorable experience with CYA, TAC, and more recently voclosporin, CNIs are increasingly used in the management of patients with LN. CNIs present a useful substitute for mycophenolate in stable patients planning for conception. CNIs are also used in dual or triple immunosuppressive regimens, either as initial treatment for active nephritis or in patients whose response to standard therapies is considered suboptimal.^{18–22} Earlier studies reported the use of CYA or TAC in triple immunosuppressive regimens (often described as MTT) in adult or pediatric patients with LN, which was associated with favorable renal response rates and tolerability.^{30,35–44} However, there was substantial heterogeneity in patient selection, corticosteroid exposure, and other immunosuppressive drugs used in the combination. Moreover, most of the studies did not include rigorous TDM to guide the dosing of CNIs. The follow-up durations were highly variable, and there is little long-term efficacy or safety data. While the data to date support a role of CNIs in the

management of LN because of their immunosuppressive potency, direct effect on podocytes, and acceptable safety profile, there are uncertainties on treatment indications, optimal exposure, treatment duration, and necessary precautionary measures. CNIs are potent inhibitors of T cell mediated immune response and, in addition to metabolic side-effects, these drugs are potentially nephrotoxic. Long-term experience is especially important in view of the potential risk of chronic CNI nephrotoxicity. Results from major clinical trials showed that triple immunosuppressive regimens that included corticosteroids and mycophenolate in combination with TAC or voclosporine were associated with higher renal response rates in the first year compared with standard therapies.^{25–29} However, in the study that compared MTT (that included corticosteroids, low-dose MMF, and TAC) against controls treated with corticosteroids and sequential cyclophosphamide followed by azathioprine, the cumulative renal response rate was similar in the 2 arms in the second year of follow-up.³⁰ There is little data on the long-term outcomes of patients with LN treated with CNI-containing triple immunosuppressive regimens.

This study analyzed the long-term outcomes of patients with LN in whom TAC was added because of proteinuria that persisted after these patients had received standard therapy for active nephritis, which included corticosteroids and MMF. The data showed that addition of TAC was effective in further reducing proteinuria, resulting in a response rate in over 70% of patients, with nearly 50% of patients achieving CR eventually. Most of the patients remained stable while on triple immunosuppression. Renal and extrarenal flares each occurred in 2 of 22 patients, respectively, over a median follow-up of over 5 years. These results cannot be compared directly with previous studies of TAC or voclosporine added to a dual immunosuppressive regimen,^{25–28,35,38,41–44} because in those studies, the triple immunosuppressive treatment was started upfront as initial treatment for active nephritis, whereas in the present series, the patients were selected based on inadequate proteinuria reduction. These patients could have been described as refractory to standard therapy by other investigators. As reported previously,^{10,33,34} Chinese patients in our locality show a high response rate to PRED and MMF combination, and those with persistent heavy proteinuria at 6 months account for <30% of the total number of patients with LN. On the basis of clinical data alone, it is not possible to assess the relative contributions of the immunosuppressive action of TAC versus its direct effect on podocytes in bringing about the proteinuria reduction.¹⁷ It is our policy to repeat kidney biopsy in patients suspected to have ongoing uncontrolled

nephritic activity. In view of the sustained serologic stability during follow-up, it is likely that the effect of TAC on podocytes has a major contribution to proteinuria reduction, while its immunosuppressive action also contributes to sustained disease quiescence. The observed association between membranous histopathologic features and proteinuria response serves as a corroborative evidence, which was also reported by other investigators,^{35,45–47} but how much the immunosuppressive actions of a CNI has helped to reduce immune-mediated injury to the kidney would be difficult to delineate, especially because the dose of PRED and MMF was slowly tapered over time.

Despite this being a group selected based on unsatisfactory proteinuria response after standard immunosuppression, their renal survival rate appeared relatively favorable at 88.7% after 5 years. It is noteworthy that the addition of TAC did not change the slope of eGFR over time, suggesting that TAC exposure with 12-hour trough blood level of 4 to 6 µg/l did not accelerate CKD progression. Nevertheless, caution is required in patients with CKD stage 3 or above at baseline, as many of these patients are already on a trajectory of progressive kidney function loss moving gradually toward end-stage kidney disease, as illustrated by the 3 patients in this series. The benefit versus risk balance of CNI treatment in these patients, and the optimal exposure if these drugs were used, remains uncertain.

We observed a low flare rate of 1 in 56 patient-years. While the risk of disease flare varies considerably between patients, the apparent stability as observed in this series and corroborated by the longitudinal serologic profile is likely related to the immunosuppressive efficacy due to the triple regimen. The 2 flares, both renal, occurred when the MPA exposure was relatively low (trough MPA blood level at around 1.3 mg/l) while the PRED dose was still at 7.5 mg/d and 6 mg/d, respectively, highlighting the need for caution when attempting to minimize the corticosteroid dose.

With regard to safety, our experience showed that long-term treatment with triple immunosuppression was generally well-tolerated, with low incidence rates of adverse events such as infections or metabolic complications. That there was no new-onset diabetes mellitus was probably related to the fact that in our treatment regimen, the CNI was not given upfront at the time when patients presented with acute nephritis, when they were exposed to high-dose corticosteroids. The avoidance of introducing CNI during the early treatment phase, when complications including infections are more common,⁴⁸ and careful attention to TDM are likely reasons for the successful prevention of acute kidney injury related to acute CNI nephrotoxicity.

While infections are the most common adverse events in this cohort, the incidence rate compares favorably with kidney transplant recipients receiving similar triple immunosuppression, and adverse outcomes are extremely rare.^{49,50} The incidence rates of worsening hypertension or dyslipidemia were similar to those in renal transplant recipients.^{51–53} Limitations of this study include the small patient sample size and lack of protocol follow-up biopsy to document histologic remission and chronic CNI nephrotoxicity.

CONCLUSION

Triple immunosuppressive treatment, with the addition of TAC in patients with suboptimal proteinuria reduction after standard therapy with PRED and MMF, resulted in significant reduction of proteinuria in 77.3% of patients. Long-term treatment with this triple immunosuppressive regimen, with TDM, is generally well-tolerated and associated with stability of disease quiescence.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

STROBE Statement.

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