Long-term data on corticosteroids and mycophenolate mofetil treatment in lupus nephritis

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Abstract

Objective. We investigated the long-term outcome of patients with proliferative LN treated with CSs and MMF.

Methods. This was a single-centre retrospective study on patients with biopsy-proven class III/IV ± V LN treated with prednisolone and MMF continuously as both early and maintenance immunosuppression.

Results. Sixty-five patients were included, and followed for 91.9 (47.7) months. All received prednisolone and MMF as induction immunosuppression. In 31 patients, maintenance immunosuppression comprised prednisolone and MMF only (MMF-MMF group). MMF was replaced with AZA in 23 patients (MMF-AZA), and with calcineurin inhibitors (CNIs) in 11 patients (MMF-CNI) at sometime during follow-up. Ten-year patient and renal survival rates were 91% and 86%, respectively, and were similar in the three groups. MMF-MMF group showed better relapse-free survival than MMF-AZA and MMF-CNI patients (76% vs 56% vs 43%, respectively at 5 years; 69% vs 32% vs 0%, respectively at 10 years; MMF-MMF vs MMF-AZA or MMF-CNI, \( P = 0.049 \) or 0.019, respectively; MMF-AZA vs MMF-CNI, \( P = 0.490 \)). Patients treated with MMF for >24 months had better relapse-free survival than those treated for shorter durations (88% vs 48% at 5 years; 81% vs 28% at 10 years; \( P < 0.001 \)). Renal function at 10 years was better in the MMF-MMF group. Anaemia was associated with MMF treatment. Other adverse events were comparable and relatively minor with MMF, AZA or CNI as maintenance.

Conclusion. Long-term treatment with CSs and MMF from induction to maintenance phase is associated with relatively favourable long-term outcome in Chinese LN patients. Discontinuation of MMF before 24 months may increase the risk of flares.

Key words: lupus nephritis, mycophenolate mofetil, maintenance immunosuppression, renal survival.

Introduction

LN is a common and important cause of renal failure, particularly in some racial/ethnic groups including Asians. Severe proliferative LN, classes III and IV according to the 2003 ISN/RPS classification, presents with acute nephritic syndrome and if not treated promptly and effectively leads to acute and/or chronic renal failure [1, 2]. Combination immunosuppressive therapy with CSs and CYC has been the standard of care for severe proliferative LN, but long-term data showed suboptimal renal and patient survival [3, 4]. In recent years, MMF has gradually replaced CYC as induction immunosuppressive treatment, since the CSs and MMF combination has been proven efficacious and less toxic compared with CSs and CYC [5-7]. As for maintenance therapy, data from the Aspreva Lupus Management Study (ALMS) maintenance phase showed superiority of MMF over AZA while the Mycophenolate Mofetil Versus Azathioprine for Maintenance Therapy of Lupus Nephritis (MAINTAIN) trial showed similar efficacy between MMF and AZA in preventing flares [8, 9]. This apparent discrepancy could be due to a genuine but non-substantial difference in outcome between patients on MMF or AZA maintenance, the demonstration of which required a large sample size. Consequently, in place of a distinct induction phase lasting 4-6 months followed by a prolonged maintenance
Long-term outcome of MMF in LN

phase with CYC replaced by another immunosuppressive medication, in patients treated with MMF the change from induction to maintenance is gradual, with progressive dosage tapering of both CSs and MMF. The CSs and MMF combination is a relatively recent treatment and long-term data are awaited. We have previously reported the favourable short-term response rate and a high tolerability in Chinese patients with severe proliferative LN treated with prednisolone and MMF [5]. Data from an extension study that included 32 patients showed that the MMF-based induction–maintenance regimen was associated with a renal survival rate of 100% and a cumulative flare rate of 34% at 63 months, which were comparable to that of patients treated with a sequential CYC–AZA regimen [10]. We hereby present data from a retrospective study to investigate the long-term results of LN patients treated with prednisolone and MMF.

Subjects and methods

Patients

This study included patients with biopsy-proven class III or IV LN, with or without concomitant class V lupus nephropathy, who had received prednisolone and MMF as initial immunosuppression. The records of these patients were reviewed. The diagnosis of SLE was according to the revised ACR classification [11] and histological classification of proliferative LN was based on the 2003 International Society of Nephrology/Royal Pathology Society (ISN/RPS) classification [2].

Immunosuppression and adjunctive treatment

Initial immunosuppressive treatment for active nephritis comprised CSs and MMF. MMF was given for a minimum of 12 months, after which some patients continued with MMF as long-term immunosuppression (MMF-MMF group). During the maintenance phase some changed from MMF to AZA for financial reasons or consideration of pregnancy (MMF–AZA group), and some changed from MMF to calcineurin inhibitors (CNIs) when there was persistent proteinuria associated with concomitant class V nephropathy (MMF–CNI group). The CS regimen was identical in the three groups. Prednisolone was commenced at 0.8 mg/kg/day orally and tapered to reach 7.5 mg/day at ~6 months. The dose of prednisolone was tapered further to 5–7.5 mg/day at 12–15 months and maintained at the same dose afterwards. Patients with cellular or fibrocellular crescents that affected more than half of the glomeruli were given i.v. pulse methylprednisolone 500 mg/day for 3 days. The dose of MMF was 1 g twice daily for the first 6 months. The total MMF dose per day was 1.5–2 g for the next 6 months, 1–1.5 g in the second year and then tapered gradually according to clinical status. In the MMF–AZA group, the latter was given at 1.5–2 mg/kg/day. The MMF–CNI group included patients with concomitant membranous lupus nephropathy in the renal biopsy and who showed persistent proteinuria of >2 g/day in the maintenance phase despite MMF treatment. MMF was replaced with a CNI. Target trough blood levels were 70–100 µg/l for ciclosporin or 4–6 ng/l for tacrolimus. Angiotensin-converting enzyme or angiotensin-receptor blockade was used in all patients with persistent proteinuria >1 g/day before the addition of CNI. The target blood pressure was 110–130 mmHg for systolic reading and 70–85 mmHg for diastolic reading.

Patients were seen at intervals ranging from 2 to 14 weeks depending on their clinical status. Clinical parameters, urinalysis, blood pressure, complete blood picture, renal and liver biochemistry, anti-dsDNA and C3 levels were monitored at every visit. Anti-dsDNA was measured with an in-house ELISA and complement levels were determined by nephelometric methods (Synchron LX®, Beckman Coulter, Brea, CA, USA). Quantification for proteinuria, fasting glucose and lipid profile was measured at least once every 6 months.

Study outcomes

The primary objective was to examine the long-term renal outcome, including renal survival (i.e. free from end-stage renal failure), serum creatinine and doubling of baseline serum creatinine. Other parameters investigated included disease flares, patient survival and adverse events. Response to induction immunosuppression was defined as decrease in urine protein excretion to <0.5 g/day together with improved or stable renal function, the latter indicated by a serum creatinine level not higher than 115% of the level at baseline. Partial remission was defined as decrease in urine protein excretion by >50% but proteinuria remained in the range of >0.5 to <3 g/day, together with improved or stable renal function. Renal relapse is defined by clinical manifestations such as an increase in proteinuria >1 g/day or rise in serum creatinine of >15% compared with baseline that was not explained by other factors such as drug nephrotoxicity, with or without serological reactivation, and was confirmed with renal biopsy. Extra-renal flare referred to a measurable increase in disease activity in organ systems other than the kidney, involving new or worsened clinical findings and laboratory measurements that are clinically significant and required change of treatment [12]. Patients with class III or IV LN relapse received another course of induction therapy with CSs and MMF as stated previously. Treatment of extra-renal flares was according to the local standard of care, mostly with an increase in the dose of prednisolone. The glomerular filtration rate was estimated with the Modification of Diet in Renal Disease Study (MDRD) equation validated in Chinese patients [13].

Statistical analysis

Continuous variables were expressed as mean (s.d.) unless otherwise specified. Comparison of continuous variables was by t-test or Mann-Whitney U-test where appropriate. Pearson’s χ²-test or Fisher’s exact test was used for the comparison of categorical variables. Relapse-free survival was estimated by Cox-regression models, and patient and renal survival rates were assessed by actuarial analysis and compared by log-rank test. Proportionality assumption was tested by plotting
log [−log (survival function)] against time [14]. Mixed models analysis was used to look for time effect and the significance of between-group difference for each of the continuous variable with repeated measures over time [15]. This method has the advantage of handling missing values. Between-group difference of least squares means was calculated for each parameter. Akaike’s information criterion was used to assess the model fitness [16–17]. All statistical analyses were performed with Predictive Analytic Software (PASW) for Windows 18.0 (SPSS Inc., Chicago, IL, USA) and two sided \( P < 0.05 \) was considered statistically significant.

**Results**

Sixty-five patients were included (Table 1). The follow-up duration from the time of starting induction treatment for active nephritis was 5975 patient-months [mean 91.9 (47.7) months]. Forty-seven patients had class III/IV LN [class IV(G)] \( n = 37, \) class (S) \( n = 10, \) 10 had class III nephritis, while eight patients showed mixed class III/IV and V nephropathy. The duration of MMF treatment was 39.3 (32.7) months. The duration of MMF treatment exceeded 24 and 36 months in 33 (50.8%) and 26 (40.0%) patients, respectively, and the longest treatment duration was 120 months. Thirty-one patients were treated with prednisolone and AZA, respectively, and the longest treatment duration was 120 months. Thirty-one patients were treated with prednisolone and only MMF throughout the maintenance phase (MMF–MMF group). In 23 patients, MMF was replaced with AZA after 16.5 (11.9) months (MMF–AZA group), and in 11 patients MMF was replaced with CNI after 23.9 (20.4) months (MMF–CNI group).

**Long-term renal outcomes**

The proportion of patients who responded to induction immunosuppression was 50.8% at 12 months, 63.1% at 24 months and 70.8% at 36 months. The complete-or-partial remission rate was 66.2% at 12 months, 80.0% at 24 months and 86.2% at 36 months. Proteinuria decreased progressively, and there was no significant difference between the three groups (difference between least square means MMF–MMF vs MMF–AZA = −0.28 g/day, 95% CI −1.09, 0.49, \( P = 0.467; \) MMF–MMF vs MMF–CNI = 0.16 g/day, 95% CI −0.42, 0.75; \( P = 0.557 \) ). Serum creatinine remained stable except in patients subsequently treated with CNI. These patients had significantly higher serum creatinine level at baseline, and showed progressive deterioration of renal function during follow-up. At 10 years, patients in the MMF–MMF group showed a lower serum creatinine level [53.0 (9.2) \( \mu \) mol/l vs 68.8 (15.6) \( \mu \) mol/l in MMF–AZA group and 259.0 (215.0) \( \mu \) mol/l in MMF–CNI group; MMF–MMF vs MMF–AZA, \( P = 0.047; \) MMF–MMF vs MMF–CNI, \( P = 0.049; \) MMF–AZA vs MMF–CNI, \( P = 0.059 \) ], and also a higher estimated glomerular filtration rate (eGFR) [118.7 (21.7) ml/min vs 93.0 (20.3) ml/min and 31.9 (28.9) ml/min, respectively; MMF–MMF vs MMF–AZA, \( P = 0.049; \) MMF–AZA vs MMF–CNI, \( P = 0.001; \) MMF–AZA vs MMF–CNI, \( P = 0.007 \) ] (Fig. 1). Three patients (two in the MMF–CNI group and one in the MMF–MMF group) had doubling of baseline serum creatinine at 10 years (\( P = 0.510 \) ). By Cox-proportional hazard model, the risk factors for doubling of baseline serum creatinine during follow-up included the age at first renal presentation (hazard ratio 1.16, 95% CI 1.06, 1.27, \( P = 0.01 \) ), baseline anti-dsDNA level (hazard ratio 1.01, 95% CI 1.00, 1.01, \( P = 0.014 \)) and serum creatinine level after 6 months of treatment (hazard ratio 1.014, 95% CI 1.01, 1.02, \( P < 0.001 \) ). Urine protein (UP) excretion and serum C3 level at baseline and 6 months were similar between patients with or without doubling of serum creatinine during follow-up (\( P = 0.36 \) and 0.47 for UP at baseline and 6 months, respectively; \( P = 0.54 \) and 0.14 for C3 at baseline and 6 months, respectively). Six patients developed end-stage renal failure after 114.5 (44.2) months. The renal survival rate was 93% and 86% after 5 and 10 years, respectively. Renal survival at 10 years did not differ between MMF–MMF, MMF–AZA and MMF–CNI patients (87%, 89% and 82%, respectively; MMF–MMF vs MMF–AZA, \( P = 0.707; \) MMF–MMF vs MMF–CNI, \( P = 0.299; \) MMF–AZA vs MMF–CNI, \( P = 0.345 \) ) (Fig. 2A).

**Other longitudinal data**

For patients who remained relapse-free during follow-up, their level of anti-dsDNA antibodies decreased over time (slope = −0.911 IU/ml/month in MMF–MMF group, \( P = 0.03; \) −1.394 IU/ml/month in MMF–AZA group, \( P < 0.001 \); and −1.833 IU/ml/month in MMF–CNI group, \( P < 0.001 \) ) with insignificant between-group difference (\( P = 0.17 \) ). C3 level increased over time in all three groups (slope = 0.516 mg/dl/month in MMF–MMF group, \( P < 0.001 \); 0.184 mg/dl/month in MMF–AZA group, \( P = 0.007; \) 0.241 mg/dl/month in MMF–CNI group, \( P = 0.043 \) ) with insignificant between-group difference (\( P = 0.799 \) ) (Fig. 1).

**Disease flares**

Thirty-three episodes of disease flares occurred during follow-up, with 26 renal flares (24 were class IV and 2 were classes III + V) and 7 non-renal flares. Compared with 6 months before disease flare, the level of anti-dsDNA antibodies increased by 48.2 (94.5) IU/ml (\( P = 0.039 \)) and C3 decreased by 8.2 (15.8) mg/dl (\( P = 0.027 \) ) at the time of flare. The timing of renal flares was 39.6 (17.4), 37.3 (33.2) and 32.1 (23.9) months from baseline in the MMF–MMF group (\( n = 7 \)), MMF–AZA group (\( n = 13 \)) and MMF–CNI group (\( n = 6 \)), respectively (MMF–MMF vs MMF–AZA, \( P = 0.86; \) MMF–MMF vs MMF–CNI, \( P = 0.64; \) MMF–AZA vs MMF–CNI, \( P = 0.74 \) ). Patients who continued with prednisolone and MMF treatment throughout (MMF–MMF) showed a significantly lower incidence of flare compared with the other groups. The relapse-free survival at 5 and 10 years was 76% and 69% in the MMF–MMF group, compared with 56% and 32%, respectively, in the MMF–AZA group (\( P = 0.049 \), and 43% and 0% in MMF–CNI group (\( P = 0.019 \) ). The incidence rate for disease flare was 1 in 9.9 patient-years for the group as a whole, and 1 in 13.8 patient-years for the MMF–MMF group, 1 in 8.3 patient-years for the MMF–AZA group and 1 in 8.9 patient-years for the MMF–CNI group. The hazard ratio of relapse for the MMF–AZA group was 2.69 (95% CI 1.03, 7.04, \( P = 0.043 \) ) and 3.63 for the MMF–CNI group.
(95% CI 1.21, 10.90, \( P = 0.021 \)) when compared with the MMF–MMF group. Patients who received MMF for >24 months showed a significantly better relapse-free survival than those treated for <24 months. The relapse-free survival at 5 and 10 years in patient treated with >24 months of MMF was 88% and 81%, respectively, compared with 48% and 28% in those treated with <24 months of MMF treatment (\( P = 0.001 \)) (Fig. 3). The hazard ratio for relapse in the latter group was 5.94 when compared with patients who received MMF for >24 months (95% CI 1.98, 17.78, \( P < 0.001 \)). Regarding non-renal flares, five episodes (four with joint and cutaneous disease; one with cerebral lupus) occurred while patients were on low-dose prednisolone and MMF. Two episodes, both being cutaneous flares, occurred while patients were on low-dose prednisolone and AZA. All episodes of joint/cutaneous flares responded to an increase in CSs dose. The patient with cerebral lupus responded to pulse steroids and escalation of MMF dose.

**Adverse events**

The side-effects profile in the three groups was summarized (Table 2). Anaemia was more common during MMF treatment compared with the other two agents (MMF vs AZA, \( P = 0.029 \); MMF vs CNI, \( P = 0.279 \); AZA vs CNI, \( P = 0.419 \)). The incidence of leucopenia or thrombocytopenia did not differ between the different treatments. The higher number of gastro-intestinal intolerance with MMF treatment did not reach statistical significance (MMF vs AZA, \( P = 0.127 \); MMF vs CNI, \( P = 0.323 \); AZA vs CNI, \( P = 1.00 \)). Infection rates were similar during treatment with the different medications [MMF vs AZA, relative risk (RR) = 1.38, 95% CI 0.58, 3.65, \( P = 0.444 \); MMF vs CNI, RR = 2.32, 95% CI 0.56, 20.5, \( P = 0.213 \); AZA vs CNI, RR = 1.68, 95% CI 0.33, 16.2, \( P = 0.463 \)]. Five patients died—two in the MMF–MMF group, two in the MMF–AZA group and one in the MMF–CNI group. The causes of death included pneumonia (\( n = 3 \)), malignancy (\( n = 1 \)) and acute myocardial infarction (\( n = 1 \)). One patient died after reaching end-stage renal failure. Patient survival rate at 5 and 10 years was 91% and 91%, respectively. Patient survival did not differ between the three groups (10-year survival rate 93%, 91% and 88% in MMF–MMF, MMF–AZA and MMF–CNI, respectively;
**Discussion**

The combination of CSs and MMF is a relatively recent immunosuppressive regimen for LN. We have previously reported the high short-term response rate and the relatively favourable outcomes after \( \sim 5 \) years of follow-up [5, 10]. The data from this cohort study give further evidence that the combination of CSs and MMF given continuously as both initial and long-term treatment is relatively well tolerated and can lead to favourable long-term outcomes in Chinese patients with severe LN.

We have previously reported that renal failure is the strongest risk factor for excessive mortality in Chinese LN patients and hence preventing renal failure could potentially improve the overall clinical outcomes [18]. In the present study, doubling of baseline serum creatinine occurred in 1 out of 31 patients in the MMF-MMF group, and renal survival for these patients was 87\% at 10 years. When all patients were considered, those who showed progressive renal impairment had significantly higher baseline anti-dsDNA and higher serum creatinine after 6 months of induction treatment. Proteinuria at baseline or 6 months and serum C3 levels, however, were not associated with long-term renal outcome.

In this study, renal relapse was suspected whenever there was an increase in proteinuria of \( > 1 \) g/day or a rise in serum creatinine of \( > 15\% \) compared with baseline that was not explained by other factors, in the presence or absence of serological activity. Some of the episodes were associated with active urinary sediment, but we have not found these data from our local laboratory of additional diagnostic value. Also, repeat renal biopsy provided confirmatory evidence in all renal relapses. It is not uncommon that, in investigations of induction immunosuppression regimens, treatment responses were often assessed after 6 months of therapy [6, 7]. However, as...
TABLE 2 Adverse events experienced by 65 proliferative LN patients while on maintenance treatment with MMF, AZA or CNI

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>MMF</th>
<th>AZA</th>
<th>CNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infections requiring hospitalization, n</td>
<td>19</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Rate of infections requiring hospitalization (number of patient-months)(a)</td>
<td>1 in 130</td>
<td>1 in 180</td>
<td>1 in 318</td>
</tr>
<tr>
<td>Leucopenia (white blood cell count &lt;3 x 10^9/l)(b)</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Anaemia (haemoglobin &lt; 10 g/dl)(c)</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia (platelet count &lt; 100 x 10^9/l)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal upset(d)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>New-onset diabetes mellitus</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
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\(a\)MMF vs AZA: RR 1.38 (95% CI 0.58, 3.65), \(P = 0.444\); MMF vs CNI: RR 2.32 (95% CI 0.56, 20.5), \(P = 0.213\); AZA vs CNI: RR 1.68 (95% CI 0.33, 16.2), \(P = 0.463\). \(b\)MMF vs AZA, \(P = 0.634\); MMF vs CNI, \(P = 0.270\); AZA vs CNI, \(P = 0.195\). \(c\)MMF vs AZA, \(P = 0.029\); MMF vs CNI, \(P = 0.279\); AZA vs CNI, \(P = 0.419\). \(d\)MMF vs AZA, \(P = 0.127\); MMF vs CNI, \(P = 0.323\); AZA vs CNI, \(P = 1.00\).

has been observed by independent investigators, the achievement of maximum improvement often occurs after 6–12 months. The data from the present study show that while >50% of patients had responded by 1 year, the response rate continued to increase after the first year. Unless the prevailing level of proteinuria is high and judged unlikely to be able to come down to an acceptable level, patients who show evidence of continuous improvement may continue to be observed before the consideration of alternative treatment such as CNI.

Patients in the MMF–MMF group had favourable long-term outcomes provided that they did not relapse. Their renal function remained relatively stable, with mean serum creatinine at 53.0 \(\mu\)mol/l and eGFR at 118.7 ml/min at 10 years. In contrast, patients who showed a high baseline serum creatinine and persistent proteinuria seemed to do worse, with 10-year serum creatinine and eGFR at 259.0 \(\mu\)mol/l and 31.9 ml/min, respectively. The prognostic value of serum creatinine level at baseline and after induction treatment on long-term renal outcome is not surprising, since it reflects the nephron mass and renal reserve.

Prevention of relapse is important in the management of LN as repeated renal flares would result in loss of nephrons and subsequent chronic renal impairment [19, 20]. The treatment of severe lupus flares entails the use of potent immunosuppression, which also increases the risk of complications that could result in significant morbidity or even mortality. The incidence of renal flares in the MMF–MMF group was ~24% at 5 years. Since this was not a randomized study, it is inappropriate to make conclusive remarks on the comparison between the three groups. Nevertheless, the data suggested that patients who continued with MMF throughout first year, the response rate continued to increase after the first year. Unless the prevailing level of proteinuria is high and judged unlikely to be able to come down to an acceptable level, patients who show evidence of continuous improvement may continue to be observed before the consideration of alternative treatment such as CNI.

The optimal duration of MMF maintenance remains undetermined. It is likely that the choice of prior induction immunosuppressive medications has a bearing on the requirements of subsequent immunosuppressive dose and potency. In a recent study, patients who received induction therapy with pulse steroids and AZA had more renal flares than those treated with steroids and i.v. cyclophosphamide (CYC), while both groups had CSs and AZA maintenance [24]. It is of interest to note that the relapse-free survival was significantly higher in patients who had received MMF for >24 months, compared with those who were treated with MMF for shorter durations. Indeed in the survival analysis one observes a distinct separation between the two groups between 1 and 24 years, indicating that a considerable number of flares occurred following the discontinuation of MMF before 24 months. The results thus suggest that, for patients who have received MMF as induction treatment, it is advisable to continue MMF maintenance for at least 2 years.

Our data suggested that the overall tolerability of the three maintenance agents were favourable and similar. The present data also show that long-term treatment with low-dose CSs and MMF was not associated with an excessive risk of infections. Anaemia was more common while patients were on MMF, but it was not severe enough to necessitate discontinuation of treatment.

We conclude that long-term continuous treatment with CSs and MMF as both initial and maintenance immunosuppression for severe proliferative LN results in relatively favourable renal and patient outcomes. Due to
considerations of pregnancy, tolerability and cost, other treatment options such as CNI and AZA would be considered during long-term maintenance.

**Rheumatology key messages**

- CSs and MMF as induction–maintenance treatment confer favourable long-term outcomes in Chinese LN patients.
- For LN, MMF treatment for ≥24 months reduces the risk of relapse.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**