Rituximab is an effective treatment for lupus nephritis and allows a reduction in maintenance steroids

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Abstract

Background. Lupus nephritis is a life-threatening complication of SLE. Treatment regimes include steroids and cyclophosphamide, both associated with significant morbidity. Newer regimes include mycophenolate mofetil (MMF). We report our outcomes in a prospectively monitored cohort of patients receiving our new standard treatment protocol, comprising rituximab induction therapy and
MMF maintenance in patients already taking maintenance immunosuppression for SLE who developed lupus nephritis. We then attempted steroid reduction/withdrawal.

Methods. Patients with class III/IV/V lupus nephritis were included. All patients were on steroids prior to the development of lupus nephritis. Eighteen patients have reached at least 1 year follow-up. These patients received rituximab induction therapy and MMF maintenance therapy. Steroid reduction/withdrawal was guided by clinical response.

Results. Fourteen of 18 (78%) patients achieved complete or partial remission with a sustained response of 12/18 (67%) at 1 year, with 2 patients having a relapse of proteinuria. Four patients did not respond. There was a significant decrease in proteinuria from a mean protein:creatinine ratio (PCR) of 325 mg/mmol at presentation to 132 mg/mmol at 1 year ($P = 0.004$). Serum albumin significantly increased from a mean of 29 g/L at presentation to 34 g/L at 1 year ($P = 0.001$). The complication rate was low with no severe infections. Following treatment with rituximab, 6 patients stopped prednisolone, 6 patients reduced their maintenance dose and 6 patients remained on the same dose (maximum 10 mg).

Conclusion. This data demonstrates the efficacy of a rituximab and MMF based regime in the treatment of lupus nephritis, allowing a reduction or total withdrawal of corticosteroids.

Keywords: immunosuppression; lupus nephritis; mycophenolate mofetil; rituximab; steroids

Introduction

Lupus nephritis is a major cause of mortality and morbidity in patients with systemic lupus erythematosus (SLE) [1], with renal involvement occurring in up to two-thirds of patients [2]. Conventional treatment with cyclophosphamide and corticosteroids resulted in complete or partial remission in 71% of patients in a European trial [3]. A significant number of patients will fail to respond or subsequently relapse following treatment. The combination of steroids and cyclophosphamide results in a significant morbidity particularly from ovarian failure and major infections [4,5]. Steroids alone also have a morbidity including obesity, diabetes, osteoporosis and infection. Additionally, steroids may also be linked with cardiovascular complications in this group of patients who are already at increased cardiovascular risk. [6–8]. Steroids also increase the infective complications when used in combination with cyclophosphamide [9].

Attempts to reduce the morbidity from the adverse effects of treatment of other immunosuppressant regimes have been trialled in SLE. In pilot studies, mycophenolate mofetil (MMF) proved effective in the treatment of lupus nephritis in patients unresponsive to other immunotherapy [10,11]. Induction of disease remission with intravenous (IV) cyclophosphamide followed by mycophenolate mofetil therapy for maintenance of disease remission was shown to be more efficacious and safer than long-term use of IV cyclophosphamide [12]. Subsequently, a large randomized controlled trial indicated that MMF was also more effective than pulsed IV cyclophosphamide in the induction of disease remission [13]. A recent meta-analysis of five randomized controlled trials supported MMF as a preferential induction agent compared to IV cyclophosphamide both with respect to efficacy in the induction of remission in severe lupus nephritis and with a better side effect profile [14]. In particular, MMF does not impair fertility, which is particularly important as SLE is relatively common in young women of childbearing age. However, although these regimes now avoid cyclophosphamide, most still include high-dose steroids.

Rituximab is a chimeric monoclonal antibody directed against CD20, a transmembrane protein present on the B-cell lineage from the pre-B cells to memory cells but not plasma cells. B cells are thought to play a crucial role in the pathogenesis of SLE including the production of autoantibodies, the regulation of T-cell activation and the production of cytokines involved in disease [15]; therefore, rituximab would seem a logical therapeutic choice in SLE. However, most studies to date have used rituximab in combination with corticosteroids [16–19].

We assessed the use of rituximab and MMF in the treatment of lupus nephritis in a cohort of patients who were already receiving steroids prior to the diagnosis of nephritis. Following induction therapy with rituximab, the patients were maintained on MMF, and steroids were then reduced or withdrawn.

Patients and methods

The patients were included in the new treatment protocol if they had a diagnosis of lupus nephritis, either ISN/RPS Class III (<50% glomeruli with proliferative lesions), IV (>50% of glomeruli with proliferative lesions) or V (membranous) [20] on a current renal biopsy and were already taking corticosteroids for SLE at the time of diagnosis. The patients were excluded if they had previously received rituximab or were currently receiving cyclophosphamide therapy. Those patients with life-threatening complications of lupus, such as cerebritis or renal failure requiring haemodialysis, or a rapidly progressive glomerulonephritis, were also excluded. Induction therapy consisted of two doses of rituximab, 1 g, given at Day 1 and Day 15. The patients were additionally given 500 mg methylprednisolone IV with each dose of rituximab, at the discretion of the admitting physician, if they felt that it was clinically necessary. Pre-medication, of hydrocortisone 100 mg and chlorpheniramine 10 mg, was given to all patients. Maintenance therapy was with MMF initially at a dose of 1 g/day. Those patients not receiving MMF were commenced on MMF, and all patients had their dose adjusted to target MPA levels of 1.5–3 mg/L if leucocyte count and gastrointestinal symptoms allowed [21]. The rate of steroid withdrawal/reduction was guided by a clinical response and the presence of extra-renal manifestations of lupus. Angiotensin receptor blockers and angiotensin-converting enzyme inhibitors were used in combination at the highest doses tolerated without inducing symptomatic/postural hypotension.

The outcome was defined as complete remission [normal values of serum creatinine, normal serum albumin and minimal proteinuria defined as the protein:creatinine ratio (PCR) <50 (equivalent to <500 mg/24 h)]. Partial remission was defined as ≥50% improvement in proteinuria, together with stabilization or normalization of serum creatinine. Relapse was defined as an increase in any renal parameter, serum creatinine or proteinuria, by >30%. Treatment failure was defined as a failure to reduce proteinuria by 50% and/or a sustained increase in serum creatinine by >30%. The secondary end-point was the reduction in the dose of prednisolone. B-cell counts were measured by CD19 flow cytometry with B-cell depletion defined by CD 19+ <5 cells/µL [22–24]. For statistical analysis, the sign test (a non-parametric test) was used to compare...
Rituximab is an effective treatment for lupus nephritis.

Table 1. Characteristics of 18 patients at the start of therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Ethnic origin</th>
<th>Disease duration (years)</th>
<th>Previous nephritis</th>
<th>Previous therapies</th>
<th>Class</th>
<th>Steroid dose (mg)</th>
<th>Induction therapy</th>
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<td>V</td>
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<td>10</td>
<td>Rituximab MP</td>
</tr>
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<td>Oriental</td>
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<td>CS, MMF, CYC, AZA</td>
<td>IV+V</td>
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<td>Rituximab</td>
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<tr>
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<td>CS, CYC, AZA</td>
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CS, corticosteroids; MMF, mycophenolate mofetil; CYC, cyclophosphamide; AZA, azathioprine; MP, methylprednisolone.

differences between biopsy and 6 months after treatment and also between biopsy and 12 months following treatment, for albumin, creatinine and PCR (proteinuria).

Results

Of the 20 patients who were started on this treatment protocol, 2 patients were later excluded from analysis for the following reasons: 1 patient had an unplanned pregnancy 6 months into treatment having entered a complete remission and required a change of immunosuppression from MMF to azathioprine because of the pregnancy. The second patient died 1 month following commencement of treatment. She had a background of severe hypertension and non-compliance with medications and presented with a hypertensive intracranial bleed and died. Therefore, 18 patients have reached at least 1-year follow-up. Table 1 demonstrates the patient characteristics at baseline. The majority of patients had had lupus for several years, were of Black or Asian ethnicity and had had cyclophosphamide, MMF or azathioprine for prior renal or systemic flares. The renal biopsies demonstrated ISN/RPS Class III lupus nephritis in three patients, Class IV in eight patients, Class IV+V in three and Class V in four patients. No patient withdrew from treatment or was lost to follow-up. The mean time in days from time of biopsy to the first dose of B-cell depletion therapy was 21 days (range 4–55 days).

Ten patients received rituximab alone for induction and the remaining 8 additionally received methylprednisolone 500 mg with each dose of rituximab. Twelve out of 18 were not taking MMF at the time of renal biopsy though 2 patients had a history of previous MMF use. All 12 were started on MMF for maintenance therapy, initially at a dose of 1 g/day. The dose of MMF was altered according to MPA levels. The remaining six patients already taking MMF had the dose altered during the follow-up as per trough MPA levels. Table 2 demonstrates the mean MPA level measured during the first 12 months of treatment and the dose of MMF achieved at 12 months.

Clinical response

At 12 months, 6 out of 18 (33.3%) patients are in complete remission (2 Class III, 1 Class IV, 1 Class V, 2 Class IV+V). A further 6 out of 18 patients have achieved partial remission (1 Class III, 3 Class IV, 2 Class V). Four of these six patients in partial remission have a normal serum creatinine, have normalized their albumin but have persistent proteinuria (PCR range 78–152). Two patients in partial remission (patients 1 and 3) have not achieved a complete remission due to abnormal serum creatinine. Both patients had abnormal renal function due to chronic kidney disease prior to the development of the current episode of active
lupus nephritis. In these two patients, there was deterioration in serum creatinine at time of the latest relapse. In both patients, renal function had improved and returned to baseline by 12 months. At 12 months, these two patients also had a normal albumin, with negligible proteinuria in one patient (PCR < 50); the other patient had a urine PCR of 79 at 1 year, but by 18 months this was also negligible (PCR < 50). One of the patients in partial remission at 12 months achieved complete remission at 2 years (patient no. 2).

A further 2 out of 18 patients achieved a partial response by 9 months, but at 12 months have relapsed with an increase in proteinuria. One of these patients had a chronically elevated serum creatinine prior to this episode of nephritis, which remained stable throughout treatment.

Therefore, 14/18 (78%) patients have achieved a complete or partial remission with a sustained response of 12/18 at 1 year (67%) with 2 patients having a relapse with an increase in proteinuria.

Four patients did not respond to treatment (patients 6, 11, 15, 18). All four had Class IV nephritis at presentation with a normal serum creatinine. All 4 have subsequently been re-biopsied and still show active Class IV nephritis (Table 3). At 1 year, two patients (patients 15 and 18) have increased their serum creatinine from 67 to 167 µmol/L, and 83 to 105 µmol/L. These two patients have received further immunosuppression. Patient 18 who had karyorrhexis on original biopsy, with re-biopsy demonstrating active Class IV and crescents, has now been treated with pulsed cyclophosphamide. The original biopsy of patient 15 demonstrated karyorrhexis and a crescent, and the re-biopsy also demonstrated active disease (with crescents) but additionally with extensive scarring. The patient received further treatment with plasma exchange, intravenous immunoglobulin and rituximab. Despite the escalation in immunosuppression, these two patients have failed to respond to treatment so far. The remaining two patients have stable creatinine within the normal range (with no crescents on re-biopsy). One of these patients has received further treatment (rituximab and methylprednisolone).

The addition of methylprednisolone to rituximab as induction therapy did not result in more patients entering a complete remission. The initial doses of methylprednisolone did not appear to significantly alter outcome (Table 4), although the numbers are small. Fifteen out of 18 patients were hypoalbuninaemic (serum albumin < 33 g/L, range 22–32 g/L) at presentation. The median albumin at presentation was 29 g/L, increasing to 34 g/L at 1 year. There was a significant increase in albumin between biopsy and 12 months (P = 0.015) and biopsy and 12 months (P = 0.001) (Table 5).

Seventeen patients had significant proteinuria at presentation (PCR > 50). The median protein:creatinine ratio (PCR) at presentation was 212 mg/mmol decreasing to 78.5 mg/mmol at 1 year. There was a significant decrease in PCR between biopsy and 6 months (P = 0.007) and between biopsy and 12 months (P = 0.004) (Table 5).

In 3 out of 18 patients, the serum creatinine was elevated prior to presentation (162–210 umol/L). In one of these, the creatinine remained stable throughout treatment. Two patients had an increase in baseline creatinine at the time of the latest relapse that returned to baseline following treatment. There was no significant change in serum creatinine between biopsy and 12 months.

Two patients had persisting systemic clinical features of disease (haemolytic anaemia in one patient, rash and arthralgia in another) despite immunosuppression. Both these patients were also classified as non-responders with respect to renal disease. During the course of follow-up, five other patients experienced extra-renal symptoms (rash, arthralgia, fevers and headaches), which were of a short duration.

At time of biopsy, all 18 patients were already receiving steroid therapy. The mean and median doses of prednisolone were both 10 mg. At 12 months, four patients had stopped their prednisolone and a further two had stopped by...
Rituximab is an effective treatment for lupus nephritis

24 months. Six patients were maintained on a lower dose of prednisolone (reduced from a mean of 12 mg to 6 mg at 1 year) with six patients remaining on the same dose (maximum 10 mg). During the period of follow-up, a total of five patients required temporary increases in prednisolone for extra-renal manifestations. Both mean and median doses decreased to 5 mg at 1 year.

**B cells**

Full B-cell depletion was defined as a CD19 count of <5 cells/µL [22–24]. B-cell depletion was variable. Ten patients depleted fully; eight of these patients depleted fully; within the first 3 months and one depleted at 4.5 months. The 10th patient did not have initial lymphocyte subset monitoring, but demonstrated depletion (4 cells/µL) at 18 months following treatment. There were three patients in whom the B cells decreased significantly but not fully: one patient had a B-cell count of 6 cells/µL at 9 months post-treatment (no earlier data); therefore, it is likely that full depletion had been achieved at an earlier time point. The other two patients reached nadir counts of 8 cells/µL and 9 cells/µL, respectively. A further two patients did not deplete, although the B-cell counts did decrease below the normal reference range (100–600 cells/µL) to 63 and 53 cells/µL, respectively. There are no early B-cell data on three patients. Of these three patients, one patient demonstrated a B-cell count of 59 at 7 months, with the two remaining patients demonstrating counts of 43 and 153 at 1 year, respectively. Of the patients who fully depleted, depletion persisted for a mean of 8.2 months (range 3–15.5 months).

Of the patients who achieved complete remission, three out of six depleted, one out of six partially depleted (9 cells/µL), and there are no early data on two patients. Of the four patients who did not respond to treatment, one failed to deplete their B cells, two depleted and there are no early data on one patient whose first B-cell count demonstrated reconstitution at 6 months. Of the two patients who flared, this was associated with B-cell reconstitution, with one of the patients demonstrating rapid reconstitution by 4.5 months post-rituximab, with the second patient demonstrating B-cell return (although still below normal range) at the time of relapse.

**Serology**

IgG and IgM double-stranded DNA (dsDNA) antibodies were measured by both ELISA and immunofluorescence. At entry, 11/18 patients had elevated levels of anti-dsDNA antibodies. The median at time of biopsy was 107.5 u/mL (range from 0 to 1670 u/mL). There was some evidence of a difference in the dsDNA antibody between time of biopsy and 12 months \( (P = 0.035). \) Eleven of the 18 patients were hypocomplementaemic at entry. Four of these patients remained hypocomplementaemic throughout, with seven patients normalizing complement between 3 and 18 months post-treatment. There was weak evidence of an increase in C3 (measured by nephelometry) between time of biopsy and at 12 months \( (P = 0.048) \) (data not shown).

**Immunoglobulin levels**

Levels of IgG were maintained in the majority of patients. Four patients had elevated levels of IgG following treatment; two of these patients had elevated IgG prior to rituximab and the levels of IgG remained high despite B-cell depletion. One patient had low IgG levels between 8 and 10 months post-rituximab in the context of replete B cells, followed by normalization of IgG. None of the patients had decreased levels of IgA. Thirteen patients had low IgM levels during follow-up despite B-cell reconstitution in 12/13 (data not shown).

**Adverse events**

The number of treatment-related adverse events was low. There was one death, related to hypertension and non-compliance with medication 1 month into treatment. There were three infection-related admissions within the first year of treatment (16.7% of patients). One patient developed cannula site cellulitis 3 days post-rituximab and required treatment with intravenous antibiotics. One patient required two hospital admissions, the first with chest pain in association with a small pericardial effusion that was thought to be related to disease activity, followed by an admission with urosepsis. One patient developed a chest infection in the context of an extrarenal lupus relapse and an increase in steroids from 10 mg to 20 mg. A further patient developed shingles just after 1 year post-rituximab and was treated with acyclovir and a temporary decrease in the dose of MMF.

**Discussion**

SLE is a relapsing/remitting autoimmune disease predominantly affecting women of childbearing age. Lupus nephritis is one of the most serious manifestations. Since treatment often requires prolonged therapy, it is crucial not only to define regimes that are effective but that minimize toxicity both in the short and long term.

Provisional reports of two large double-blind control trials in non-renal (Explorer) [25] and renal (LUNAR) [26] lupus have failed, disappointingly, to meet their end-points, though the full explanation as to why these failures have occurred (including the probable use of concomitant steroids) has yet to be published. A previous multicentre phase I/II trial of rituximab for SLE involved 15 patients with active and refractory SLE, of whom 7 had lupus nephritis. Following treatment with prednisolone and rituximab, three of the patients with renal disease demonstrated improvements at 28 weeks as assessed by BILAG scores [27]. Our data demonstrate a role for rituximab in reducing the immunosuppressive burden of patients by enabling long-term steroid therapy to be reduced or withdrawn completely. Open-label studies have demonstrated disease response even in highly refractory patients. In a study of six patients with refractory SLE, an improvement was seen in all patients, with B-cell reconstitution occurring prior to disease relapse that was treated with further rituximab [18]. Other studies have demonstrated that patients treated...
with rituximab for autoimmune disease can continue to show a response even when B cells have re-populated [28]. In another study, seven patients with cyclophosphamide-resistant proliferative lupus nephritis were treated with rituximab and cyclophosphamide demonstrating both clinical and histological response by 6 months [19]. A further study of patients with proliferative lupus nephritis demonstrated a sustained remission at 1 year in 4 out of 10 patients [16]. However, most of these pilot studies used other potent immunosuppressive agents such as cyclophosphamide or high-dose steroids in combination with the rituximab.

Our cohort included patients with lupus nephritis already receiving steroid therapy prior to the latest relapse. Most of these patients have relapsing disease with a significant history of immunosuppressive treatment. We have demonstrated an effective treatment regime for the remission of lupus nephritis in these patients. This regime was well tolerated and allowed a decrease or withdrawal of steroid therapy in the majority of patients. Others have also found rituximab to be a steroid-sparing agent. In a recent study, three patients with Class IV lupus nephritis were induced with rituximab and steroids and then only had rituximab for maintenance treatment. The dose of steroids was decreased to 5 mg 6 months after the start of treatment, with two patients subsequently stopping steroids altogether. All three patients achieved complete remission [29].

In our cohort, the non-responders (4/18) all had Class IV lupus nephritis that is known to be a poor prognostic factor in this disease. Three patients were of African Caribbean or Asian ethnicity and one Caucasian. This is consistent with previous reports showing Black ethnicity to be associated with a worse outcome [30]. The majority of patients deemed partial responders were thus classified due to persistent, though in most cases modest, residual proteinuria. It is entirely possible that the proteinuria at this stage represents healing with scarring, glomerulosclerosis, rather than active disease. In this group, a repeat renal biopsy will be valuable to define whether there is ongoing active disease or histological remission.

All patients had regular monitoring of MPA levels, and the dose of MMF was adjusted according to the drug levels as well as gastrointestinal side effects and leucopenia. The target level (1.5–3 mg/L) was based on previous experience in the renal transplant population that demonstrated an association between higher levels and toxicity. In this population, toxicity was more likely in patients with significant renal impairment or hypoalbuminaemia [21,31]. However, another recent study, which included six patients with SLE, demonstrated differences in the pharmacokinetics of mycophenolic acid in transplant patients and in those patients with autoimmune disease, with factors such as renal function, enterohepatic recirculation as well as other immunosuppressant use such as calcineurin inhibitors, influencing the pharmacokinetics [32]. Both this study and a later study have demonstrated the association between the MPA trough levels and MPA exposure [32,33]. This latter study included 13 patients with stable SLE receiving MMF for maintenance therapy, none of whom had nephrotic range proteinuria. Compared to the transplant population [31], MPA trough levels predicting either disease recurrence or MMF toxicity in autoimmunity were higher (<3 and >4.5 mg/L) for autoimmune patients compared with transplant patients. The ideal level in those patients treated with a rituximab-based regime remains to be fully determined.

Not all our patients were able to achieve a level of 1.5–3 mg/L, and it is interesting to note that three of the patients who achieved sub-optimal levels were receiving at least 2 g/day of MMF. Additionally, two of the patients classified as non-responders did not achieve the desired MPA level. Further studies would be useful to determine the ideal MPA level for induction of disease remission and for maintenance of remission in lupus nephritis in patients treated with rituximab.

There remain a number of unanswered questions. Despite two infusions of rituximab 2 weeks apart, a number of patients failed to adequately deplete their B cells. The addition of two doses of intravenous cyclophosphamide in those patients who have not fully depleted their B cells maybe a technique that may be employed to achieve B-cell depletion. We did not assess the formation of human anti-chimeric antibodies (HACAs), but it would be interesting to explore the relationship between the formation of these antibodies and inadequate depletion in this group of patients. In a study of idiopathic membranous nephropathy, proteinuria had an effect on both pharmacodynamics and pharmacokinetics of rituximab and this was associated with lower rates of B-cell depletion, suggesting altered pharmacokinetics of rituximab, although there was no clear relationship between response and the initial amount of proteinuria [34]. Again, in our cohort, there was no clear relationship between adequate depletion and degree of proteinuria. The exact mechanism by which B-cell depletion therapy results in the alteration of autoimmunity is yet to be fully determined. A relationship between B-cell depletion and the FcγR3a genotype, an FcR important in antibody-dependent cell-mediated cytotoxicity, has been demonstrated [35]. Despite inadequate depletion, some patients still achieved a complete response to treatment, while other patients failed to respond despite full depletion. These areas need further investigation, as does the re-dosing of patients. Should patients have further courses of rituximab when B cells have reconstituted despite remaining in disease remission? Many patients may achieve long-term remission, even when B cell replete which may be related to the continued use of MMF. The long-term effects of repeated courses of B-cell depletion are as yet unknown. Infusion reactions may prevent repeated dosing although the newer fully humanized anti-CD20 antibody, Ocreluzimab, may ameliorate this problem.

In summary, rituximab induction and MMF maintenance therapy provide a safe and effective combination treatment in lupus nephritis. It results in disease remission in a significant number of patients. It is well tolerated and allows long-term steroids to be stopped or reduced in many patients.

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Rituximab is an effective treatment for lupus nephritis

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