## Original Article

# New Protocols for Treatment of Class IV Lupus Nephritis with Emphasis on Rituximab as the Sole Maintenance Therapy

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Kuwait Medical Journal 2018; 50 (3): 343 - 350

**ABSTRACT**

**Objective**: A safe and effective treatment for lupus nephritis (LN)
**Design**: An 8-year prospective study
**Setting:** Hospital-based
**Subjects**: Three groups of patients with class IV LN; comparison of 2 new treatment-protocols for class IV LN with a retrospective group of patients who had received the standard treatment for LN
**Intervention**: The 2 treatment groups had received an induction phase of monthly intravenous Cyclophosphamide, Mycophenolate (MP) and Prednisone (P). The maintenance phase in the first group was only MP and P, while patients in the second group had received only yearly Rituximab infusions.
**Main outcome measures**: Morbidity and mortality
**Results**: Patients in the first group did not have significant relapses, yet had 10 episodes of infections during the maintenance phase. In the second group, there were five treatment failures, yet none had renal deterioration, infections or death. In the third group, seven relapses occurred during the induction period and three in the maintenance one. Moreover, complications included 1 death of disseminated sepsis, 12 cases of chronic renal failure, three kidney losses, 16 episodes of major infections, two cases of aseptic necrosis, two cases of gonadal failure, two cases of hemorrhagic cystitis and 2 cases of retinal deposits.
**Conclusions**: Rituximab infusions, used once yearly, are effective and a safe maintenance therapy for most patients with LN after a short course of three anti-proliferative agents. In those who failed to respond, MP and P are more effective and safer than the standard protocol.

**KEY WORDS**: nephritis, rituximab, systemic lupus erythematosus, treatment

## INTRODUCTION

Lupus nephritis (LN) is a common disease in our area, as it represents 12% of those biopsied for glomerulopathy with a calculated incidence of 6.5 per 100,000 in adult female Kuwaiti nationals[1]. It is one of the most serious complications of systemic lupus erythematosus (SLE) since it is a major predictor of poor prognosis[2]. Previous and standard treatment protocols included 3 - 6 months of high dose corticosteroids and either cyclophosphamide (Cyclo) or mycophenolate mofetil (MP), followed by maintenance therapy with MP, small dose prednisone (P) and hydroxychloroquine[3-8]. Those protocols were associated with malignant potentials and significant infectious complications viz. bacterial, fungal and cytomegalovirus[9-11]. Gonadal failure was a particular risk with Cyclo use, and osteoporosis as well as aseptic necrosis with P[9-11]. Since LN is a chronic disease, induction and maintenance therapy should be safe, efficient and tolerable to improve the patient's compliance and survival. Those were the basis of testing two new protocols of using all the three anti-proliferative agents (P, Cyclo and MP) for a relatively short induction phase of 3 months to limit their

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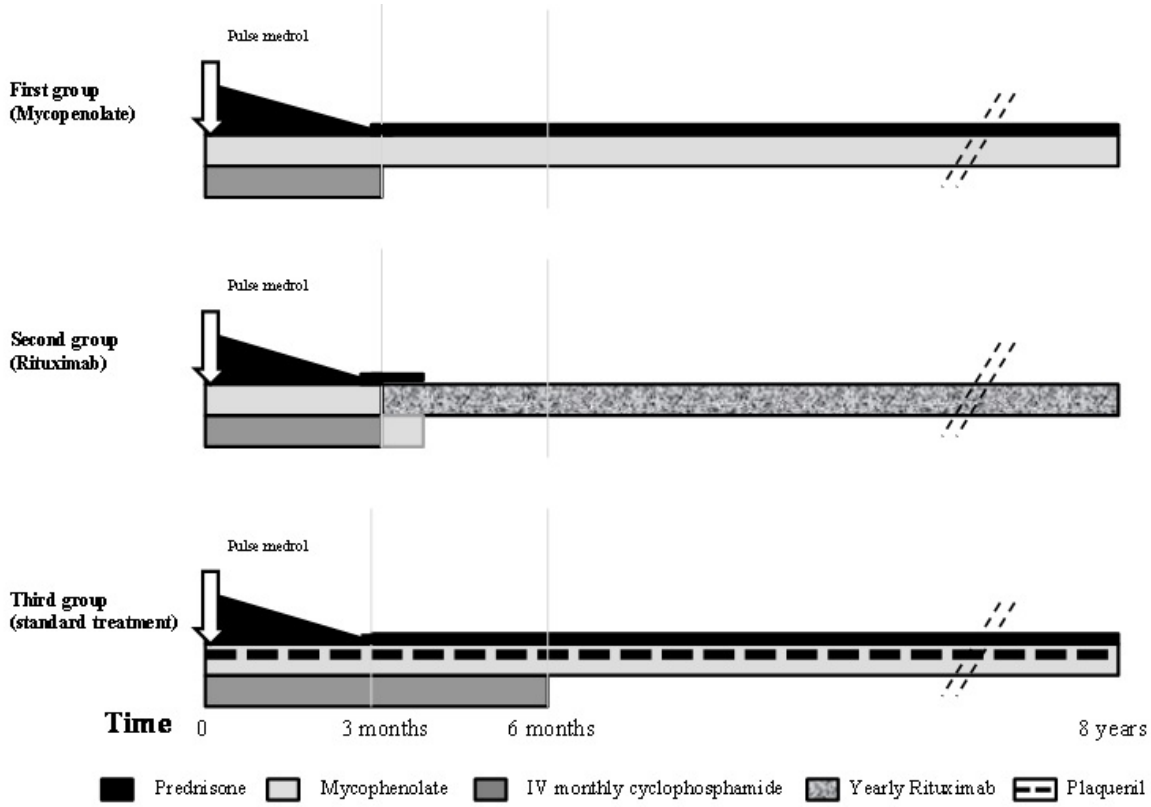


**Fig 1:** Study design of the 3 treatment groups

cumulative long-term side effects, followed by either MP with a small dose of P or yearly Rituximab (R) infusions as the sole maintenance in the second.

## SUBJECTS AND METHODS

The study started from 1st January, 2008 until 31st December, 2015. It included all patients who had their first attack of class IV LN. The latter was defined as: (a) kidney biopsy showing diffuse segmental or global endo-or extracapillary glomerulonephritis involving >50% of all glomeruli; (b) all patients showed diffuse wire loop deposits with or without fibrinoid necrosis and/or cellular crescents; (c) immunofluorescent stains were positive for immunoglobulin G (IgG), IgM, IgA and complement C3; (d) patients with already high chronicity index at the time of biopsy viz. sclerotic glomeruli, fibrous crescents, tubular atrophy, and interstitial fibrosis were excluded from the study[12].

### Study design

All patients received induction therapy with 1 g of intravenous solumedrol for 3 days, followed by P at 1 mg/kg/day for 1 month to be tapered down to 5 mg daily by the 3rd month. Moreover, they received Cyclo infusions at 1 g/m$^2$ every month for 3 consecutive months and oral MP. The latter was started at a dose of 250 mg every 12 hours and was adjusted higher gradually, upto a maximum of 1 g twice daily, if total peripheral leucocytic count remained above 4x10$^9$/L. Subsequently, patients were assigned, at random, to either of two maintenance groups. In the first (MP) group, the maintenance regimen consisted of P 5 mg daily in addition to 1 g of MP twice daily. In the second (R) group, two infusions of 1 g of R were given two weeks apart. One month later and once CD20 levels were confirmed to be <0.5% of total lymphocytes by flow cytometry[13], MP and P dose was reduced to 1/2 and both drugs were discontinued one month later. Patients in the R group received the same R dose every year as the sole maintenance immunosuppressive therapy. Moreover, these two groups were compared with a third retrospective group of 63 of our previous patients, with the same inclusion criteria, who had received a standard treatment for LN and were followed for eight years prior to the current study. Their standard treatment consisted of six months of 1 g/m$^2$ of IV Cyclo and 1 g solumedrol for 3 days, followed by 1 mg/kg/day of P for 1 month to be tapered down to 5 mg daily by the 3rd month. This induction phase was followed by a similar maintenance dose of MP, P and hydroxychloroquine. The latter was administered as 200 mg twice daily. The study design is summarized in

Fig 1. The study was approved by the Health Science Center Ethical Committee of Kuwait University and Ministry Of Health (Re: VDR/EC/2508).

**Technique of R administration**

Patients were pre-medicated with two 500 mg Paracetamol and one Piriton tablets, followed by an infusion of 125 mg of Solumedrol in 50 ml of D5W over 30 minutes before infusions. The 100 ml of 1 g of R was diluted in 400 ml of normal saline leading to a concentration of 2 mg/ml. The first infusion rate was 20 ml/h for the first 30 minutes, followed by 20 ml increments/30 minute until reaching 100 ml/h, until the total dose is given.

**Periodic assessment**

Patients were reassessed every two months for disease activity both clinically and by routine laboratory investigations, which included complete blood count and serum estimates of glucose, renal, liver and lipid function tests as well as urine routine. Twenty-four hour urine collections for assessment of creatinine clearance (Cr Cl) and daily urinary protein output (UPO) were done after the end of the induction phase, and every six months during the maintenance phase. Testing for serum complements (C3 & C4) and anti-double stranded DNA (dsDNA) was conducted at months 1, 4, 8 and 12 for the first year only. In a similar fashion, CD20 level was tested for patients in R group. In the subsequent years, serum complements and anti-dsDNA tests were done twice per year, or if clinical or laboratory assessment indicated new activity. Statistical analysis of repeated measurements of Cr Cl and UPO was used to assess kidney function, and C3 as well as anti-dsDNA are measures of immunological activity of LN[2].

Renal deterioration was defined as any 20% decrease in Cr Cl or 50% increase in serum creatinine and/or doubling of daily urinary protein excretion.

**Statistical analysis**

The SPSS statistical package version 21 was used for data entry and processing. The p-value <0.05 was used as the cut-off level for significance. Mean and standard deviation were used to describe the normally distributed variables viz. age and duration of lupus disease before LN. Since duration of treatment was not normally distributed, it was expressed as a median (interquartile range) and the Kruskal-Wallis test was used to assess the difference between the 3 groups. Analysis of variance (ANOVA) with repeated measures was used to examine the difference between the groups and within the groups on follow up, using means, of the normally distributed C3, anti-dsDNA and Cr Cl, while the non-parametric Kruskal-Wallis and Wilcoxon Signed Ranks test were used for the difference between the groups of UPO since it was not normally distributed.

## RESULTS

The demographical data on patients in the 3 groups are summarized in Table 1. They were all adult Asians. There was no statistical difference between the three groups with regards to gender, age, duration of disease before development of LN, and their length of treatment.

**Table 1:** Baseline characteristics of patients with class IV LN in the three treatment groups

| Characteristic of groups * | Group MP n = 56 | Group R n = 58 | Retrospective n = 63 |
|---|---|---|---|
| Gender (Female/Male) | 52/4 | 54/4 | 59/4 |
| Age (years) | 25.6 ± 5 | 25.8 ± 5 | 25.6 ± 4 |
| Duration of SLE prior to LN | 12.7 ± 4 | 12.4 ± 4 | 11.7 ± 5 |
| Duration of treatment | 49 (23-75) | 48 (20-76) | 45 (13-77) |

* No statistical difference between the three groups
SLE: systemic lupus erythematosus; LN: lupus nephritis; R: rituximab; MP: mycophenolate

**Periodic assessment**

The details of the means of Cr Cl, C3 and anti-dsDNA titers, as well as medians of UPO of the 3 groups at baseline, 3 months, 2 years and 5 years is displayed in Table 2. Statistical analysis of the differences between the groups and response to treatment at those time-intervals is shown in the lower row of the table. Moreover, Fig 2 shows the changes in the activity markers (C3 and anti-dsDNA) as well as the kidney function (Cr Cl and UPO) over the study period. As seen in both, there was no statistical difference between the 3 groups with regards to their initial Cr Cl, UPO, C3 and anti-dsDNA titers. However, subsequent follow up data showed the following results:

1. The initially high activity parameters of LN viz. C3 and anti-dsDNA declined at 3 months in all patients, yet the decline was more in those in groups 1 and 2 compared to group 3 ($p < 0.001$). As expected, the difference between patients in groups 1 and 2 who had received similar induction therapy was not significant.
2. The initially low Cr Cl and high UPO improved by the 3rd month, yet such improvement was more in groups 1 and 2 compared to group 3 ($p < 0.001$). The difference between patients in groups 1 and 2 was not significant.
3. Improvement in Cr Cl and lowering of anti-dsDNA levels were maximal in all groups by the 3rd month. Further improvement in C3 and UPO were evident upto 1 year in patients in groups 1 and 2. Patients

**Table 2:** Results of laboratory and serological assessment during follow up of the three groups of LN

| Variable* | Baseline | | | | 3 months | | | | 2 years | | | | 5 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cr Cl | UPO | C3 | DNA | Cr Cl | UPO | C3 | DNA | Cr Cl | UPO | C3 | DNA | Cr Cl | UPO | C3 | DNA |
| Group I | 47 ± 1 | 2500 (700 - 3900) | 55 ± 1 | 139 ± 2 | 91 ± 1 | 1250 (200 - 1800) | 95 ± 2 | 13 ± 7 | 96 ± 1 | 300 (100 - 400) | 121 ± 1 | 11 ± 3 | 101 ± 8 | 150 (100 -160) | 125 ± 1 | 11 ± 2 |
| Group II | 48 ± 1 | 2200 (800 - 3800) | 56 ± 9 | 138 ± 2 | 92 ± 1 | 1250 (250 - 1900) | 93 ± 1 | 12 ± 6 | 96 ± 9 | 155 (100 - 200) | 119 ± 1 | 10 ± 2 | 102 ± 7 | 130 (100 - 150) | 122 ± 2 | 11 ± 3 |
| Group III | 46 ± 1 | 2500 (1200 - 3950) | 54 ± 9 | 138 ± 2 | 68 ± 2 | 1700 (800 - 2000) | 84 ± 2 | 28 ± 1 | 70 ± 7 | 900 (400 - 1100) | 85 ± 5 | 22 ± 6 | 63 ± 1 | 1000 (800 - 1600) | 84 ± 6 | 21 ± 6 |
| ** Significance (p) | NS | | | | ←---------- GI vs II: NS, GI & II vs GIII: < 0.001 ----------→ | | | | | | | | | | | |

Cr Cl: creatinine clearance (ml/min.), UPO: urinary protein output (mg/day), C3: serum complement 3 (mg/dl), DNA: anti-dsDNA (IU/ml), NS: not significant
Normal levels of C3: > 79 mg/dl, anti-dsDNA: < 20 IU, Cr Cl: > 80 ml/minute and UPO: < 150 mg/day.
* All variables are expressed in mean ± SD except for UPO in median (range)
** Significance: if p-value is < 0.05

in group 3 had similar improvement in UPO by year 1, but not in C3 levels.

4. Further improvement in Cr Cl, UPO, C3 and anti-dsDNA levels persisted until the end of the study in groups 1 and 2. However, in group 3, Cr Cl and UPO deteriorated with time. Their C3 levels and anti-dsDNA remained stable, yet remained above normal (Fig 2).

Cr Cl decreased by >20% in 12 patients in group 3 compared to none in the two prospective groups.

**Complications in the MP group**

As seen in Table 3, only one minor relapse occurred in a patient during the induction phase that was easily treated with a second 3-day treatment with 1 g IV solumedrol, followed by an increase in the dose of P to 1 mg/kg/day for 1 month to be tapered gradually over three months again. Subsequently, all patients enjoyed a stable treatment without relapse, significant renal damage or death during the maintenance phase. However, 10 episodes of infections were encountered. They were herpes zoster (n = 3), Epstein Barr virus (n = 2), cytomegalovirus (n = 4), and fungal chest infection (n = 1).

**Table 3:** Complications in the three treatment groups of class IV LN

| Complications | Group MP n = 56 | Group R n = 58 | Retrospective n = 63 |
|---|---|---|---|
| Induction relapse | 1 | 1 | 3* |
| Maintenance relapse | 0 | 5* | 7* |
| Treatment failure | 0 | 3* | 3* |
| Side effects: | | | |
| Autoimmune | 0 | 2* | 0 |
| Infections | 10* | 0 | 16* |
| Renal failure | 0 | 0 | 12* |
| ESRD | 0 | 0 | 3* |
| Death | 0 | 0 | 1* |
| Aseptic necrosis | 0 | 0 | 2* |
| Gonadal failure | 0 | 0 | 2* |
| Hemorrhagic cystitis | 0 | 0 | 2* |
| Retinal deposits | 0 | 0 | 2* |

*: major incidents
R: rituximab, MP: mycophenolate, ESRD: end stage renal disease

**Complications in R-group**

Again, only 1 minor relapse occurred in a patient during the induction phase that was easily treated as in the first group. Unfortunately, three patients could not tolerate the initial R infusions for allergic reactions. They were treated as patients in the first group, yet were excluded from the study. Out of the 58 patients who tolerated R initially, 5 had minor complications. One had an allergic reaction to the R infusion given one year later, and hence was shifted to maintenance therapy with MP and P and was excluded from the study. Two patients developed active SLE 3 and 4 months after R infusions in the first year, and hence were considered R-failure and excluded from the study. They were retreated with the same initial induction therapy yet without Cyclo and were kept on the same maintenance protocol of the first group. The 4$^{th}$ patient relapsed within 1 month following conception that developed two months into her second year of R therapy after an unplanned pregnancy. She was retreated with 1 g solumedrol for three days, followed by 1 mg/kg/day of P for 1 month to be tapered down to 5 mg daily by the 3$^{rd}$ month, followed by a tapering dose of P in addition to Imuran 100 mg daily. Subsequently, she was retreated with R after delivery without any relapse for 48 months. The 5$^{th}$ patient had a minor relapse after her self-medication with an oral contraceptive. She also had received induction therapy without Cyclo for three months followed by R-maintenance, without any subsequent relapse

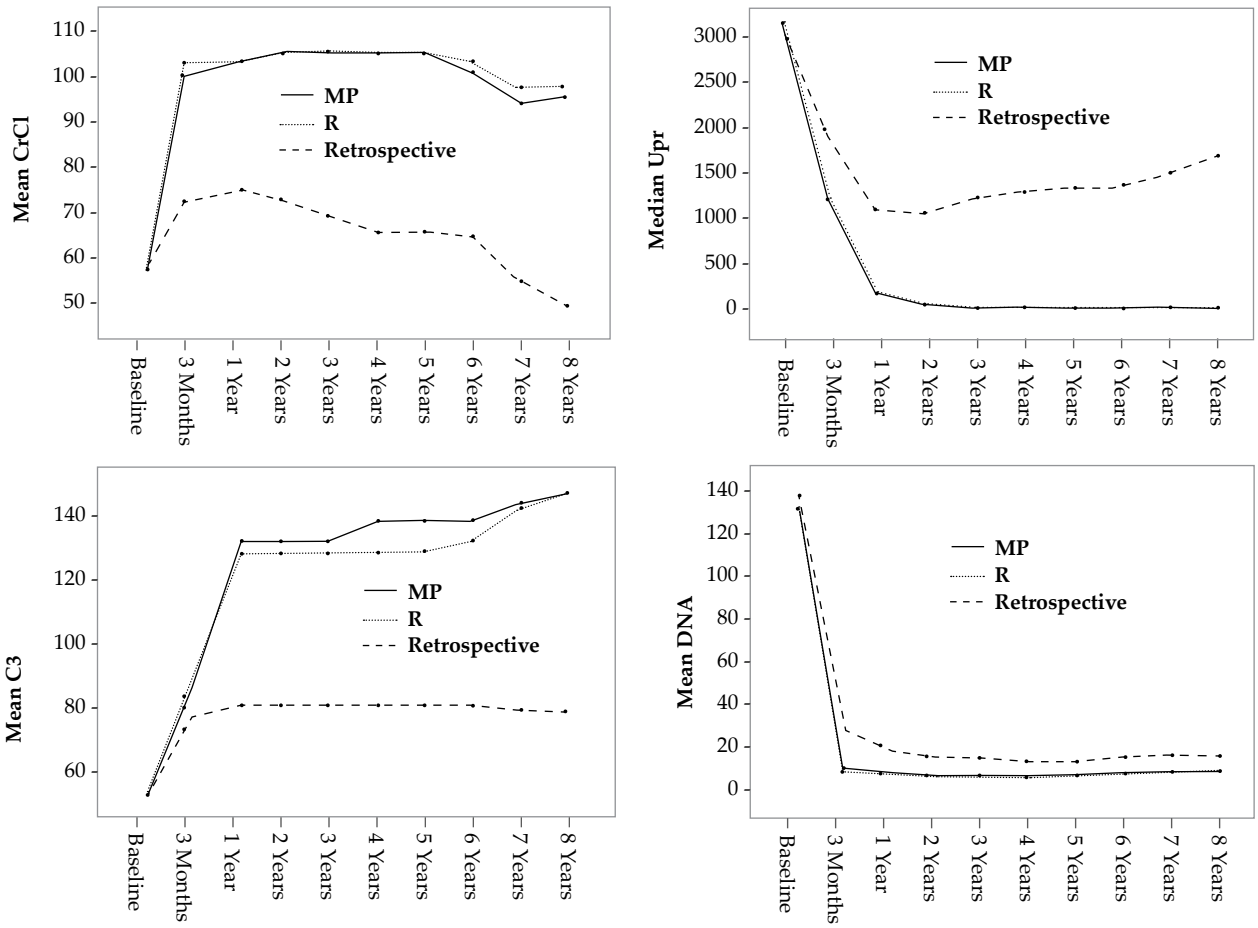


**Fig 2:** Changes from baseline to the end of the study (year 8) in C3, anti-dsDNA, Cr Cl and UPO in the 3 treatment groups of LN. Normal levels of C3: > 79 mg/dl, anti-dsDNA: < 20 IU, Cr Cl: > 80 ml/minute and UPO: < 150 mg/day.

for nearly 36 weeks. The cumulative number of patients at the end of each year was as follows: 7+15+22+28+32+40+46+55. Hence, 46 patients had completed 2 years and 28 had completed five years on such prophylaxis without signs of SLE activity, renal deterioration, infections or serious side effects of treatment, as described in Table 2. However, two patients had autoimmune side effects within four weeks of R infusions viz. arthritis and hemorrhagic colitis. They responded to four weeks treatment with P without any recurrence. In the R group, five patients also had antiphospholipid syndrome. They were controlled with R and warfarin without any difference from others.

**Lymphocyte subpopulation in R group**

As expected, none of the patients had a significant reduction in their total circulating lymphocyte counts, yet all had achieved decline of their initially normal CD20 to <0.5% one month after the first infusion. CD20 cells were not adequately suppressed in those who had developed active LN after R treatment, while the rest had achieved adequate suppression at 8 months (n = 32) and at 12 months (n = 14).

**Pregnancy while on R prophylaxis**

Despite our clear instructions to avoid pregnancy as well as oral contraceptives and to use intrauterine contraceptive devices in married females, four pregnancies occurred during the study. Two patients had conceived in the second year after rituximab treatment and one in the fourth. Of the first two women, one had relapsed one month later and her management was described previously. The other one did not have complications, and she conceived again 2 years later. The first two conceptions occurred two months after the last R infusions, while the other two were four and six months later. In those patients, azathioprine at a dose of 1-2 mg/kg/day was added to avoid relapse of LN. Except for the one patient who had developed a relapse, the other three pregnancies progressed normally without flare of LN and all were delivered, as planned, at 36 weeks. Their siblings were healthy and remained so until the end of the study.

After delivery, the R infusions were resumed as scheduled initially.

**Complications in the retrospective group**

In this group, three relapses occurred in the induction phase and seven in the maintenance phase. They required an additional IV solumedrol, major increment of P and even repeating Cyclo pulses for an extra 3 months. Despite those efforts, 12 patients had renal failure, three patients lost their kidneys and one had died of fulminant sepsis. Infections were common and included herpes zoster (n = 5), severe bacterial sepsis (4), pneumocystis carnii (3), fungal infection (n = 3) and unlocalized fulminant sepsis in one patient. Moreover, gonadal failure was confirmed by persistent high luteinizing hormone and follicle stimulating hormone in two patients who had a total dose of nine months of Cyclo infusions. Those two patients also had recurrent hemorrhagic cystitis. In this group, osteoporosis was common despite early treatment with calcium and vitamin D. Moreover, one patient had a bilateral hip replacement and another one had bilateral knee replacement for aseptic necrosis. Two patients had significant retinal deposits which were attributed to hydroxychloroquine.

**Ancillary medications**

The diuretics (lasix ± aldactone) were rarely used beyond the induction period (first three months) in the two prospective groups contrary to the retrospective one. In the prospective groups, antihypertensive drugs used in the initial induction period were discontinued on follow up except in 2 patients. However, angiotensin converting-enzyme inhibitors or angiotensin II receptor antagonists were used in 28 patients during the induction period and were kept subsequently for kidney protection.

## DISCUSSION

Knowledge of the specific immunopathogenesis of LN is essential in planning its induction and maintenance therapy. In susceptible individuals suffering from SLE, *in situ* formation and deposition of immune complexes (ICs) from apoptotic bodies occur in the kidneys due to an amplified epitope immunological response. IC glomerular deposits generate the release of pro-inflammatory cytokines and cell adhesion molecules, causing inflammation. This leads to monocytes and polymorphonuclear cells chemotaxis. Their subsequent release of proteases generates endothelial injury and mesangial proliferation. The presence of ICs promotes adaptive immune response and causes dendritic cells to release type I interferon. The latter induces maturation and activation of infiltrating T cells, and amplification of Th2, Th1 and Th17 lymphocytes. Each of them amplify B cells and activates macrophages to release more pro-inflammatory molecules, generating effector cells that promote kidney epithelial proliferation and fibrosis[14]. Hence, in our two prospective groups, three potent anti-proliferative (cytotoxic) agents viz. high dose corticosteroids, Cyclo and MP were used simultaneously to treat active LN in the induction phase, since multiple effector cells are involved. MP was added to the standard protocol since it was shown to be as effective as Cyclo as an antiproliferative agent in induction phase of non-renal lupus[15]. The combination of the three agents proved to be a potent tool in limiting disease activity (decline in C3 to <0.97 mg/dl and high anti-dsDNA levels >20 IU/ml) and preservation of kidney function (Cr Cl >80 ml/minutes and low UPO). This combination therapy resulted in less early and late relapses in the two prospective groups as compared to the retrospective one. Limiting such relapses was clearly associated with better kidney survival and less need for repeating corticosteroid and even Cyclo pulses. The latter may explain the lack of infections in the R group and limited non-infectious complications in the MP group.

Since B lymphocytes are the most essential in maintenance of SLE, glomerular damage and fibrosis, R was tested solely for such phase in one group, since it is a potent cytotoxic agent only to mature B-lymphocytes[16]. R is a chimeric human/mouse monoclonal antibody that binds avidly to CD20 antigen expressed on normal differentiated B-lymphocytes, but not stem cells or plasma cells. It destroys the B-cell by multiple mechanisms, including complement-dependent cellular cytotoxicity, induction of apoptosis and sensitization to other chemotherapeutic agents[16]. Such a potent therapeutic effect is associated with limited mild to moderate infusion-related reactions; hence, it has to be administered slowly over hours[17]. Moreover, the clinical remission induced by R may persist beyond the effect of peripheral B-cell ablation, since alteration of memory cells has been reported up to six years[18]. The latter character, its long-lasting efficacy (months) and safety were the bases of our protocol for selecting such an agent as a sole maintenance therapy in one group of our study. However, R-use was limited in three patients by its infusion reactions. Interestingly, one more patient developed allergic reactions one year later, indicating the possibility of encountering the rare antibody formation[19]. Overall, two patients failed to achieve sustained remission with R and had to be shifted to maintenance treatment with

MP and small dose P. The latter indicates the limited efficacy of R in certain patient's population and confirms the heterogeneity of SLE disease in different patient population[20]. Despite our explicit instruction regarding contraception, few accidental pregnancies were encountered after the second month of treatment with R. Fortunately, there were no significant side effects on the mother or fetus[21]. However, one patient developed relapse after conception and another one after an accidental use of oral contraceptives, indicating the risk of disease activation in those situations.

Hydroxychloroquine was recommended for treatment of LN[22], yet it was avoided in our two prospective groups, since we believed that our protocol could offer adequate maintenance immunosuppression without the added risk of its retinal disease[23].

Two previous randomized and controlled trials in extra-renal SLE (The EXPLORER study) and LN (LUNAR study) have questioned the role of R in treatment of SLE and claimed its failure to improve the clinical outcomes after 1 year of therapy[24,25]. Contrary to our study, the drug was used in the induction phase, which may explain its limited efficacy. Interestingly, despite its limited role in their induction therapy, the authors have described fewer flares in those treated with R in the first study, and more responders as well as greater reductions in anti-dsDNA and C3/C4 levels in the second. In our protocol, we limited R-use to the maintenance phase, based on the essential role of B-lymphocytes in disease progression and not in the active phase of LN[26-29].

## CONCLUSION

Our study describes an efficient and safe three-month induction therapy with three anti-proliferative agents in treatment of active LN. Moreover, it has shown that R is an effective maintenance therapy for this chronic autoimmune disease. With its ease of administration, as once yearly, the drug will improve patient's compliance and will limit the long-term infectious and non-infectious side-effects of Cyclo, MP, azathioprine, P and hydroxychloroquine. However, nephrologists should be aware and patients should be informed about its limitations in some patient population. If so, the MP-protocol as in the first treatment group is the best alternative.

## ACKNOWLEDGMENT

**Declaration of interest**: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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