DE GRUYTER OPEN

Folia Medica 2014; 56(4): 245-252

doi: 10.1515/folmed-2015-0003

ORIGINAL ARTICLES

Clinical Investigations

CLINICAL AND MORPHOLOGICAL IMPROVEMENT OF LUPUS NEPHRITIS TREATED WITH RITUXIMAB

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КЛИНИЧЕСКОЕ И МОРФОЛОГИЧЕСКОЕ УЛУЧШЕНИЕ ПРОЯВЛЕНИЙ ВОЛЧАНОЧНОГО НЕФРИТА НА ФОНЕ ЛЕЧЕНИЯ РИТУКСИМАБОМ

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ABSTRACT

Aim: To assess the effects of rituximab (RTM) therapy on clinical and morphologic activity of lupus nephritis (LN). **Material and methods**: The study included 45 patients with confirmed diagnosis of systemic lupus erythematosus (SLE), unaffected by previously received standard therapy with glucocorticoids (GCs) and cytostatics. The disease activity was assessed using Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI 2K); to assess the LN activity we used the SLICC RA/RE index. Forty-five patients with LN were given puncture renal biopsy prior to prescribing RTM; 16 patients had repeated renal biopsy 1 year and more after beginning the anti-B-cell therapy. LN was graded histologically in accordance with the WHO classification (2003) with indices of activity (AI) and chronicity (CI). **Results**: The predominant number of patients had class III – IV of LN. The repeated renal biopsies demonstrated that LN had undergone a transition into a more favourable morphologic class, which was associated, in most of these cases, with a positive therapeutic effect. The follow-up dynamics showed a statistically significant reduction of AI (p=0.006), and no statistically significant changes in the CI (p = 0.14). **Conclusion**: The long-term follow-up in the study has showed that repeated courses of anti-B-cell therapy with RTM have a positive effect both on SLE activity and generally on the renal process. The reduction of the morphologic class of LN as assessed in the repeated renal biopsies is a convincing proof for this. Eleven out of 16 patients experienced transition of the morphologic class into a more favourable type, which in most cases was combined with lower AI (p = 0.006). We found no evidence of increase in the CI (p = 0.14).

Key words: *systemic lupus erythematosus, lupus nephritis, rituximab, morphology of lupus nephritis, renal biopsy*

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РЕЗЮМЕ

Цель: Оценить влияние терапии ритуксимабом (РТМ) на морфологические показатели активности волчаночного нефрита (ВН) по результатам повторных биопсий почки.

Материал и методы: В исследование включены 45 пациентов с достоверным диагнозом системной красной волчанки (SLE) рефрактерной к стандартной терапии глюкокортикоидами (ГК) и цитостатиками. Клиническая оценка активности СКВ осуществлялась с использованием индекса активности SLEDAI 2K, активность волчаночного нефрита в соответствие с индексом SLICCRA/RE. 45 больным ВН проведена пункционная нефробиопсия до назначения курса РТМ и 16 из них - повторная биопсия через год и более от начала терапии анти-В-клеточной терапии. Гистологический класс ВН, а также индексы активности и хронизации (ИА и ИХ) определяли в соответствие с классификацией Всемирной Организации Здравоохранения (2003г.).

Результаты: Преобладали больные с III и IV классом волчаночного нефрита. Повторные биопсии показали трансформацию морфологического класса нефрита в более «благоприятный» вариант в сочетание у

Article's history: Received: 23 June 2014; Received in a revised form: 7 Aug 2014; Accepted: 11 Oct 2014
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большинства больных с положительным общим терапевтическим эффектом. Динамическое наблюдение не показало достоверное изменение ИХ (p = 0.14).

Заключение: Длительное наблюдение показало, что повторные курсы анти-В-клеточной терапией РТМ оказывают положительное действие как на активность SLE, так и на почечный процесс. Уменьшение морфологической активности ВН при повторной биопсии является убедительным доказательством этого. У 11 из 16 пациентов выявлено трансформация морфологического класса в более благоприятный вариант с понижением ИА (p = 0.006). Нам не удалось выявить достоверное снижение ИХ (p = 0.14).

Ключевые слова: *системная красная волчанка, волчаночный нефрит, ритуксимаб, морфология волчаночного нефрита, биопсия почки*

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of unknown etiology. It is characterised with overproduction of organ-specific autoantibodies against the cell nuclear components and development of immune inflammation damage of tissues and internal organs.[1,2] LN is one of the most serious manifestations of SLE with adverse prognosis. Long-term administration of GCs and cytostatics is often associated with a risk of adverse events and complications as a result of such a therapy.

A promising development in the SLE therapy has been offered by medication that block B-cell proliferation – RTM is one such agent that is quite well studied. RTM is a chimeric anti-CD20 monoclonal antibody which kills normal and malignant B-cells that express the cell-surface molecule CD20. CD20 molecules are expressed at all stages of the B-cell differentiation, with the exception of the earliest precursor cells and plasma cells.[3]

Several clinical trials have found certain dynamics in the clinical and laboratory data of activity of LN during RTM therapy.[4-6] M Condon et al. have demonstrated that RTM in combination with intravenous infusions of 6-methylprednisolone and per oral mycophenolate mofetil, but no oral steroids, shows good results.[2] In spite of the promising results obtained from observational studies and registries the randomised clinical LUNAR study failed to find any differences between the LN patients on RTM and those receiving only placebo.[7]

Repeated biopsy plays an important role in the assessment of LN activity. I Gunnarsson et al.[8,9] analyzed all publications with data about the morphologic activity of LN treated with RTM. Almost all studies in this respect show a positive change in the AI while the CI undergoes no substantial dynamics.

Despite improvement of clinical and laboratory confirmed manifestations of LN during the therapy with RTM, evidence demonstrating positive dynamics in the morphologic activity with RTM treatment is scantily reported in the relevant literature. It is therefore quite necessary to perform a study where we could investigate the effect of RTM on the morphologic activity of LN.

AIM

To assess the effect of RTM in treating LN as evidenced by the morphologic AI and CI which are scored at repeated renal biopsies.

MATERIAL AND METHODS

The study was performed in the Intensive Care Department (head, Prof. SK Soloviev, DMSc) and the Rheumatic Diseases Morphogenesis Laboratory (head, Prof. SG Radenska-Lopovok, DMSc) affiliated with the Nasonova Research Institute of Rheumatology in Moscow (Russia). The study included 45 patients [39 women (86.7%), 6 men (13.3%)] with confirmed SLE which was refractory to the standard therapy with GCs and cytostatics (mean age 27 years, range 23 to 36 years, and disease duration of 48 months, range 21 to 72 months) (Table 1). Most of the patients (80%) were found to have class IV of LN. The clinical activity assessment was done using Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI 2K)[10-12]; the LN activity was assessed using the newer Systemic Lupus International Collaborating Clinics Criteria (SLICC RA/RE)[10]. The activity score as assessed by SLEDAI 2K was 20.5 (16-24 points), while the SLICC RA/RE gave a score of 9 (6-9 points). All patients received GCs, and 27 of them were given high doses of the steroids (more than 40 mg/day). The therapy they had received previously was combined: GCs with cyclophosphamide, mycophe-

nolate mofetil, azathioprine, hydroxychloroquine, cyclosporine A (Table 2).

Forty-five LN patients were given puncture renal biopsy under ultrasound control prior to prescribing RTM. Repeated renal biopsies were done in 16 patients 1 year and more after beginning the anti-B-cell therapy. The histologic class of LN, the AI and CI were determined in accordance with the WHO classification (2003).[13]

The AI is determined on a three-point scale depending on how pronounced the morphologic alteration signs are. The maximum score is 24 points.
- Proliferation of mesangial cells (1 – 3 points);
- Leukocyte infiltration (1 – 3 points);
- Hyaline deposits (1 – 3 points);
- Fibrinoid necrosis and Karyorrhexis (1 – 3 points) x 2;
- Cellular crescents (1 – 3 points) x 2;
- Interstitial inflammation (1 – 3 points).

Because of the prognostic significance and severity of karyorrhexis and cellular crescents, the assigned points were multiplied by two. The scores were determined by the extent of involvement of the sign measured in percentages of the biopsy specimen area: a score of 1 point is assigned if the sign involves less than 25% of the glomeruli and stroma, 2 points – between 25% and 50% of glomeruli and stroma, and 3 points – if the sign involves more than 50% of the glomeruli and stroma.

The CI was determined in the same manner and reflects the chronic morphologic signs.
- Glomerular sclerosis (1 – 3 points);
- Fibrous crescents (1 – 3 points);
- Tubular atrophy (1 – 3 points);
- Interstitial fibrosis and necrosis (1 – 3 points).

Statistica 6.0 (StatSoft, USA) was used in the statistical analysis of various parametric and non-parametric variables; a p value of less than 0.05 was considered statistically significant.

Table 1. General characteristics of patients with LN

Characteristic	$\overline{X}$ (X_{min} – X_{max})
Age, years	27 [23-36]
Sex, man / woman, n (%)	6/39 (86.7)/(13.3)
Disease duration, mos	48 [21-72]
SLEDAI2K, score [points]	20.5 [16-24]
SLICC RARE, score [points]	9 [6-9]
Activity, n (%)	
class II	9 (20)
class III	36 (80)
Course of the disease, n (%)	
Acute	16 (35.6)
Subacute	20 (44.4)
Chronic	9 (20)

Table 2. Previous therapy received by patients with LN (n = 45)

Therapy	Number of patients	
	n	%
High doses of glucocorticoids (< 40 mg/day)	27	61.6
Cyclophosphan	34	61.2
Mycophenolate mofetil,	10	22.2
Azathioprine	4	8.9
Hydroxychloroquine	10	22.2
Cyclosporine A	3	6.7
GC monotherapy	2	4.4

RESULTS

Most of the patients (n = 32, 71%) had diffuse proliferative glomerulonephritis (class IV), 8 patients had focal proliferative glomerulonephritis (class III), 2 patients presented with mesangial proliferative glomerulonephritis (class II) and 1 patient had only minimal alterations (class I). Class V of LN (membranous glomerulonephritis) was diagnosed in two cases. LN-associated morphologic signs were found in all cases, which was the reason we specified the diagnoses as LN. Two patients with SLE had no clinical and laboratory confirmed renal symptoms, but the renal biopsy before prescribing the anti-B-cell therapy clearly showed the presence of LN. In one female patient with LN class III (AI = 4, CI = 1) the activity of SLE was seen in skin lesions, articular syndrome, vasculitis, alopecia, Raynaud syndrome, pulmonary syndrome (the findings at CT were fibrous and interstitial alterations of lung tissue in the middle and lower lung lobes, bronchiectasis, frosted glass appearance), immunological activity (anti-dsDNA – 35 IU/ml, C3 - 0.57 g/l, cryoglobulinemia). Ten days after a second course of RTM therapy the patient suffered from left hemisphere ischemic stroke with pyramidal injuries. The therapy included 4 courses of RTM (total dose of 4000 mg).

The activity of SLE in another female patients

with class IV LN was manifested with arthritis, enanthema, mouth ulcers, alopecia, polyserositis (pleuritis and pericarditis), immunological activity (anti-dsDNA ≥ 200 IU/ml, C3 - 0.35 g/l, C4 - 0.03 g/l). The high doses of GCs the patients received induced mental and behavioral disorders, avascular necroses in the femur, and the methotrexate therapy was ineffective. We also found certain cyclophosphamide intolerance. The first course of RTM in this patient failed – the mouth ulcers were unaffected as were the livedoid vasculitis, cryoglobulinemia, and the immunological activity. At 6 months the RTM therapy was repeated. At the start of the infusion of 500 mg of RTM the patients reported feeling dyspneic and her blood pressure dropped down to 80/50. We detected wheezing in the lungs, the oxygen saturation dropped down to 85%, and the patient developed erythematous rash on body and limbs. The infusion was discontinued.

RTM was prescribed in various dosage regimens: 24 patients received the maximum dose of 2.0 g, the dose given to 18 patients was 1.0 g, 1 female patient received RTM in a dose of 1.5 g, and the remaining 2 patients received RTM in a single dose of 500 mg (Table 3). All patients received 6-methylprednisolone in a patient-tailored doses of 250 to 1000 mg immediately prior to RTM infusion.

Sixteen patients received RTM infusion simultaneously with pulse therapy with 6-methylprednisolone and cyclophosphamide; 29 patients received monotherapy with RTM.

Repeated renal biopsy was done in 16 LN patients at different intervals after RTM therapy. In 5 patients the LN changed from class IV to class II with complete response (CR) for 3 to 5.5 years of follow-up, in 3 patients – class IV of LN became class III with CR to RTM therapy for 5.5 years, in 1 patient we registered regression of the morphologic class from class IV to class I over a period of 3 years, and in 1 patient the disease went from class III to class I with the histological renal status becoming normal and CR for 6 years of follow-up.

In 5 female patients the morphologic class of LN underwent no changes in spite of the administered RTM therapy; in 4 female patients the AI declined while the CI became higher (3 patients with CR, 1 patient unaffected by therapy) and LN relapsed in one woman with higher AI, retaining the same CI for a period of 1.5 year after the first RTM infusion.

Thirteen patients received RTM in the total dose of 2 g for 1 course of therapy, 2 patients received 3 g over 2 courses and 1 female patient – a total dose of 4 g for 2 courses of treatment. In 8 cases the RTM infusions were simultaneous with pulse therapy with 6- methylprednisolone and cyclophosphamide. For the duration of the follow-up 13 patients out of 16 (81.2%) had a CR to therapy, in 1 patient it was a partial response, in 1 patient the therapy with RTM failed and 1 female patient had a flare of LN during the 1.5 years of follow-up. All patients received GCs in maintenance doses of 5 to 15 mg/day. Out of the 13 patients with CR to the therapy, 6 received mycophenolate mofetil, 3 patients received azathioprine, and the remaining patients were on monotherapy with GCs in maintenance doses. Until the anti-B-cell therapy was commenced, 45 LN patients had a morphologic AI of 6.0 (range 4.0 – 8.0) points, and CI 1.0 (range 1.0 – 3.0) points.

In 16 patients on RTM therapy the AI was found to decrease statistically significantly from 8.0 (range 5.0 - 9.0) to 3.5 (range 1.0 - 6.5) ($p = 0.006$) (Fig. 1). The change in the CI could not reach statistical significance ($p = 0.14$) (Figs 2, 3, 4). The CI became higher in 4 patients with class IV LN: repeated renal biopsy was conducted in one female patient six months after the second RTM course combined with cyclophosphamide and mycophenolate mofetil, and this therapy was found to increase her CI score from 2 to 5 points with simultaneous decline of the AI score from 12 to 4 points. This patient had a CR to therapy for 5 years. Another female patient on a combined therapy of RTM, cyclophosphamide and azathioprine got a higher score for the CI (from 1 point

Table 3. First course regimen of RTM therapy (n = 45)

Infusion regimen and doses	Number of patients	
	n	%
Rituximab 2000 mg	24	53.4
- 500 mg in 4 infusions	13	
- 1000 mg in 2 infusions	11	
Rituximab 1000 mg	18	40
- 500 mg in 2 infusions	16	
- 1000 mg in 1 infusion	3	
Rituximab 500 mg in 3 infusions	1	2.2
Rituximab 500 mg in 1 infusion	2	4.4

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to 4 points), while the AI score declined from 10 points to 4 points after a 12-month-long combined therapy and at the end of the year we registered a CR to therapy.

In another case the CI score increased from 1 point to 4 points over 3 years of follow-up despite the CR to the administered therapy. One female patient also had a higher score for the CI which increased from 1 to 4 points during 12 months after the second course with RTM without getting an overall improvement as a result of the therapy.

Eleven patients achieved a CR to the RTM therapy with their LN becoming lower morphologic class which we consider as therapy's favourable effect.

DISCUSSION

Lupus nephritis is one of the commonest and most severe clinical manifestations of SLE. It usually

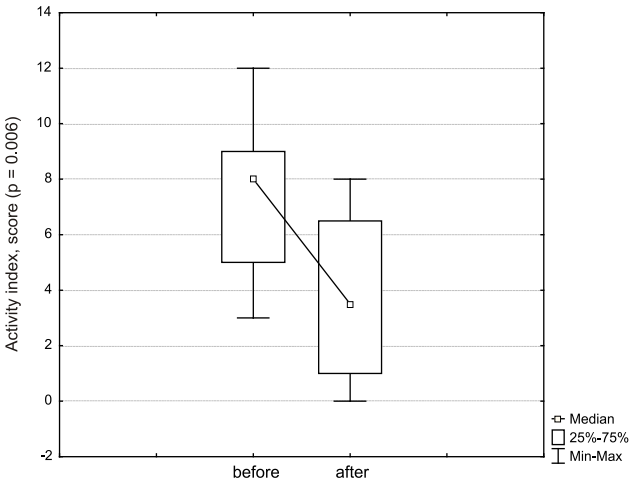

Figure 1. Changes in the AI after RTM therapy.

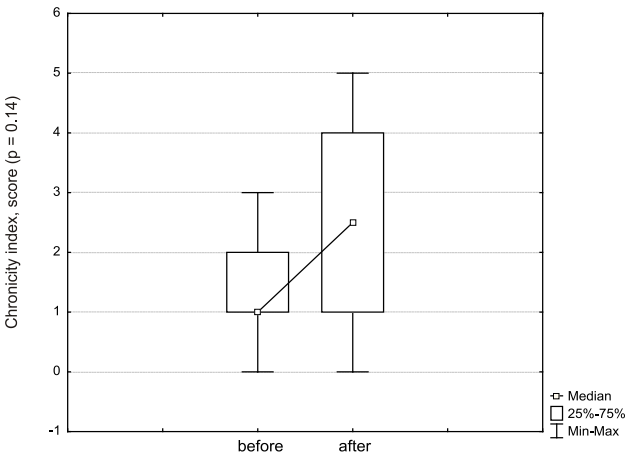

Figure 2. Changes in the CI after RTM therapy.

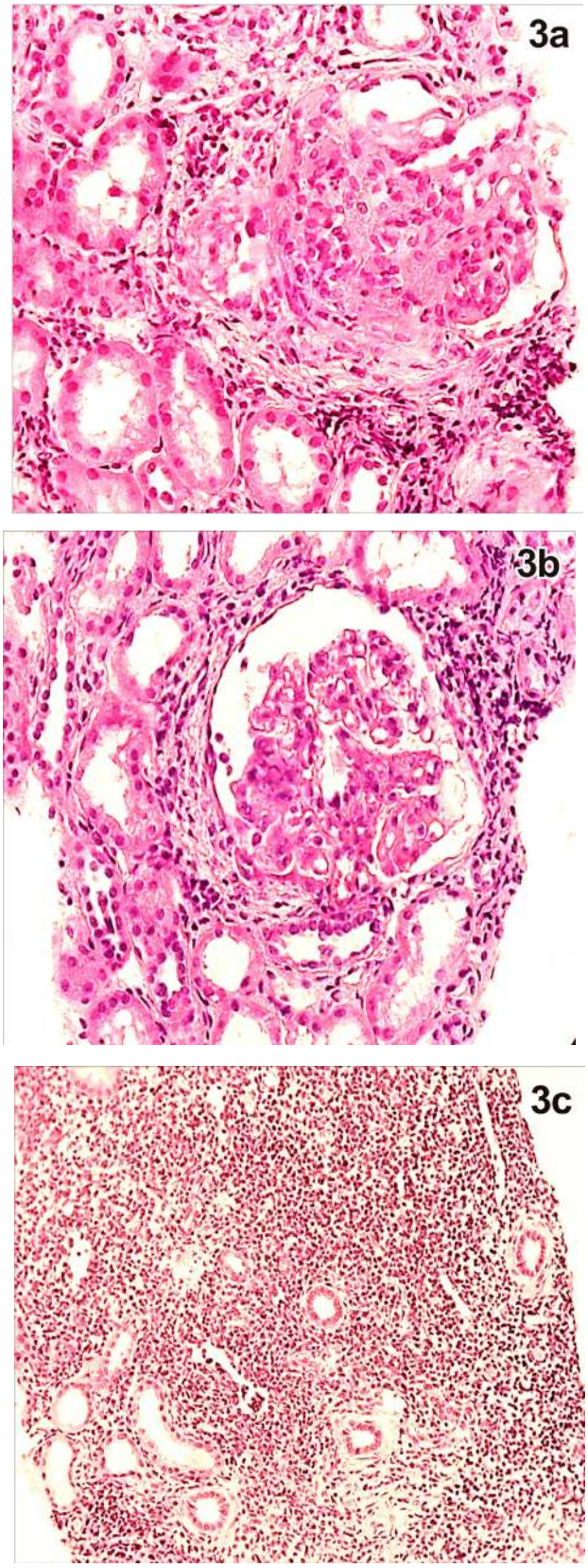

Figure 3. Before RTM therapy. Class IV LN, subclass T/A, AI = 9, CI = 1. (a) and (b) - Moderate total proliferation of mesangial cells. Fibrinoid necrosis. Cellular crescents. Lymphocytic interstitial infiltration; (c) Diffuse lymphocytic infiltration into the medullar renal area. Staining H&E, magnification x400.

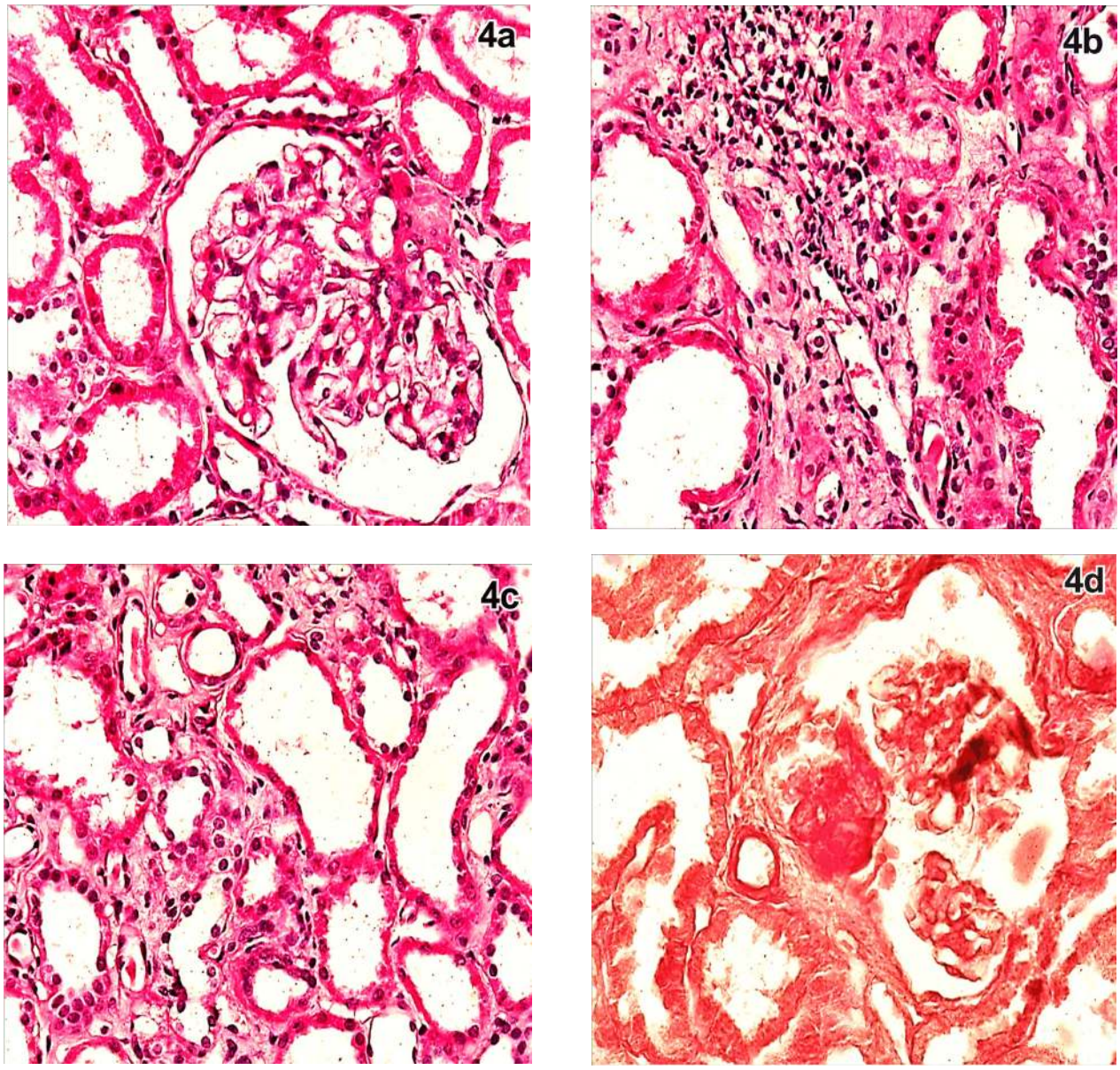

Figure 4. After three years of successful therapy with rituximab. Class I LN, , AI = 1, CI = 3. (a) Mild proliferation of mesangial cells. Dilated capillaries. (b) Inflammatory Lymphohistiocytic interstitial infiltration. Tubular atrophy; (c) Tubular thyroidization; (d) Glomerular sclerosis and fibrosis of the stromata. Staining: picrofuxin according to Van Gieson; magnification x200.

requires a long-term aggressive therapy to manage with the ultimate goal of arresting the disease progression and normalizing laboratory parameters. Clinical practice is currently employing therapy with high doses of GCs and cyclophosphamide, and mortality rate due to renal pathology keeps being very high. Recently developed biological agents help in reducing the side effects and the risk of developing complications from GCs and cytostatic therapy.

Rituximab is widely discussed in relevant literature as a therapeutic option in the treatment of LN patients.[14,15] Despite the negative results of the randomized LUNAR study, many other observational studies discuss the high efficiency of the drug when used in the treatment of LN patients that were refractory to standard immunosuppressive therapy.[4,7]

The therapeutic effect of RTM consists actually in reducing not only the clinical and laboratory confirmed activity of LN but also its morphologic activity. Sixty-eight percent of repeated renal biopsies clearly show that LN changes into a more favourable type, the transformation being consistent in most cases with complete response to the

rituximab therapy. Furthermore, we found in our study a statistically significant reduction of AI and statistically non-significant rise of CI (p = 0.006, p = 0.14, respectively). Similar results were obtained by T Jonsdottir et al.[16] Many publications underlie the correlation between reduction of morphologic AI and the clinical improvement in patients.[9,10,15]

RJ Davies et al. included 18 patients with LN who were refractory to the standard therapy with GCs and immunosuppressants.[17] All studied patients received RTM (1000 mg) in two infusions, 2 weeks apart, and a pulse therapy with cyclophosphamide (500 mg) plus 6- methylprednisolone (500 mg). At 6 months of follow-up 13 patients (72%) achieved CR to the therapy. The remaining five patients, despite the B-lymphocyte depletion, developed a rapidly progressing LN and were transferred to hemodialysis. Three patients had LN class IV, and two other patients - class V and class III, respectively. One of the patients was on a long-term therapy with hemodialysis and died of sepsis. For the entire long-term follow-up, 5 patients had flares of the LN at different times (at 6 months, and at 2, 3, 6 years after RTM infusions); in 5 patients the flares were manifested by extrarenal events 5 and 6 years after baseline. Apart from these cases, 5 out of 7 patients (71%) were found to have a change of the morphologic class of LN into a more favourable type with reduction of the AI. We should point out that the researchers did not find changes in the AI in the repeated renal biopsies; based on this finding they made the conclusion that RTM inhibits any irreversible processes in the kidneys.

CONCLUSIONS

The long-term follow-up in the study showed that repeated courses of anti-B-cell therapy with RTM have a positive effect both on SLE activity and generally on the renal process. The reduction of the morphologic class of LN as assessed in the repeated renal biopsies is a convincing proof for this. Eleven out of 16 patients experienced transition of the morphologic class into a more favourable type, which in most cases was combined with lower AI (p = 0.006). We found no evidence of increase in the CI (p = 0.14).

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