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Intensive short-term treatment with rituximab, cyclophosphamide and methylprednisolone pulses induces remission in severe cases of SLE with nephritis and avoids further immunosuppressive maintenance therapy

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Abstract

Background. B cells play a central role in systemic lupus erythematosus (SLE). Rituximab is expected to induce apoptosis of all the CD20-positive B cells. A proportion of patients are refractory or intolerant to standard immunosup- pression. These are candidate to new therapeutic options.

Methods. Eight patients [six women, two men, mean age 41- year-old (27-51), with severe multiorgan involvement (kidney, skin, nervous system, polyarthritis, polyserositis, antiphospho- lipid antibody syndrome)] were considered eligible for an in- tensive combination therapy including rituximab. Rituximab was administered (dose 375 mg/m^2) on Days #2, 8, 15 and 22. Two more doses were administered 1 and 2 months following the last weekly infusion. This treatment was combined with two pulses of 750 mg cyclophosphamide (Days #4 and 17) and three pulses of 15 mg/kg (Days #1, 4 and 8) methylpredniso- lone followed by oral prednisone, 50 mg for 2 weeks rapidly tapered until 5 mg in 2 months. Response was evaluated by assessing the changes in clinical signs and symptoms [Sys- temic Lupus Erythematosus Disease Activity Index (SLEDAI score)] and laboratory parameters for at least 12 months.

Results. Levels of erythrocyte sedimentation rate and anti- double-strand DNA antibodies significantly decreased ($P < 0.01$ at 12 months), whereas C3 and mainly C4 values increased at 6 months ($P < 0.01$ for C4). Proteinuria im- proved in the cases with renal involvement ($P < 0.01$ at 3, 6 and 12 months). SLEDAI score improved moving from the mean 17.3 (12-27) before therapy to 3.1 (1-5) after ritux- imab treatment. Constitutional symptoms including arthral- gia, weakness and fever disappeared in all the previously affected patients; paresthesia improved in the four patients with polyneuropathy and skin lesions gradually resolved in the patients with necrotizing skin ulcers at presentation. Drug side effects were negligible.

Conclusions. Long-lasting remissions were obtained in patients with severe SLE and major organ involvement by this intensive administration of rituximab combined with low doses of intravenous cyclophosphamide and methylprednisolone pulses followed by a rapid tapering of prednisone to 5 mg/day as a sole maintenance therapy.

Introduction

Systemic lupus erythematosus (SLE) is a multisystem auto- immune disease characterized by the production of several autoantibodies against a variety of self-antigens. B cells play a central role in SLE. Targeting the B-cell compart- ment is therefore an attractive alternative to current avail- able therapies. Rituximab is a human/mouse chimeric monoclonal antibody that specifically reacts with the CD20 antigen, which is expressed on pre-B cells, imma- ture, mature naı¨ve and mature B cells but not plasma cells. Rituximab is expected to induce apoptosis of all the CD20- positive B cells. Rituximab has been investigated in SLE because of the potentially serious toxicities of immunosup- pressive agents currently in use. Some trials in adults and children with SLE suggested that rituximab—although given in combination with other immunosuppressive drugs—may improve several manifestations of SLE, in- cluding skin rash, alopecia, arthritis, haemolytic anaemia and thrombocytopenia [1-4]. The role of rituximab in the more severe forms of SLE is still being debated. Results of two randomized placebo-controlled studies [5, 6] devoted to evaluate the efficacy and safety of rituximab were dis- appointing and are still object of debate [6, 7]. These trials were conducted to test the superiority of rituximab compared to conventional immunosuppressive treatment, while the major target of uncontrolled studies was testing its role in refractory cases or in patients who were intolerant to conventional treatments.

The present study focuses on the effects of an intensive course of therapy, using rituximab in combination with two intravenous pulses of cyclophosphamide and three pulses of methylprednisolone and followed by a short course of prednisone, given prospectively to a selected cohort of severe patients. This intensive short-time treatment gave prolonged remissions even though maintenance therapy consisted of only 5 mg prednisone since the second month. This represents the novelty of this open single-center study.

Methods

Baseline data are summarized in Table 1.

Eight patients, six women and two males, six white and two black subjects, mean age 41 years (range 27-51 years), with severe multiorgan involvement including kidney (five cases, including three patients with Class IV and two with Class V International Society of Nephrology/Renal Pathology Society glomerulonephritis), skin lesions (six cases, with ne- crotizing ulcers in four), involvement of central nervous system [2], poly- neuropathy [4], severe polyarthralgias with arthritis [8], polyserositis [3], lymphadenopathy [4], secondary antiphospholipid antibody syndrome (APS) (two cases), were considered eligible for rituximab therapy due to their resistance or intolerance to previous therapy (six cases) or as a front line immunosuppressive treatment in two women with unsatisfactory therapeutic compliance (#1) or as a specific request of a short-time im- munosuppression for gestational perspectives (#7).

Previous immunosuppressive therapy included steroids in every case (three intravenous pulses of 15 mg/kg methylprednisolone followed by prednisone 1 mg/kg/day for at least 4 weeks with subsequent tapering according to clinical features), cyclophosphamide (given in a monthly intra- venous dose of 1 g for 6 months in Patient #4 and both intravenously and orally for a cumulative dose of 9 g in Patient #3) in two nephritis patients, azathioprine in three patients, mycophenolate mofetil in three patients, methotrexate in one non-nephritis patient, cyclosporine A in four cases, hydroxychloroquine in six cases and thalidomide in one patient.

Rituximab was administered intravenously at a dose of 375 mg/m2 on Days #2, 8, 15 and 22. Two more doses were administered 1 and 2 months following the last weekly infusion. This treatment was combined with two pulses of 750 mg cyclophosphamide (Days #4 and 17) and three intravenous pulses of 15 mg/kg (Days #1, 4 and 8) methylprednisolone followed by oral prednisone, 50 mg for 2 weeks rapidly tapered until 5 mg in 2 months.

Response was evaluated by assessing the changes in clinical signs and symptoms and laboratory parameters for at least 12 months. SLEDAI score was separately assessed by two investigators (S.S. and M.A.) specifically trained for activity score processing in rheumatology.

In order to prevent possible interferences in biological detection due to complex formation of rituximab and antibody, serum samples were collected 6 months after the last drug administration.

Human anti-chimeric antibodies directed against rituximab were quantified using validated antigen-binding tests radio immuno assay (RIA), while levels of therapeutic antibodies were assessed using validated enzyme-linked immunosorbent assay, both performed at Sanquin Diag- nostic Services (Amsterdam, NH) on a routine base.

Due to the low number of patients, mainly descriptive statistical anal- yses were performed. For comparing means between SLEDAI scores at the beginning and at 12 months of study, the paired t-test was used. Differ- ences among study groups were analyzed by multifactorial analysis of variance. Differences were considered statistically significant when two- sided P-values were <0.05. Statistical analyses were carried out using StatView 5.0.1 for Macintosh (SAS Institute, Cary, NC).

This study was performed according to the local rules of off-label therapy in Piedmont Region (Northwest Italy).

Results

Treatment with rituximab resulted in complete depletion of B cells in peripheral blood, as shown by the percentage of CD20- positive cells, diminishing from the pre-treatment mean value of 18% (5.6-25%) to 0.1% 7 days after the fourth infusion. No CD20-positive cells were found to be detectable at 12 months. A definite reappearance of CD20- positive cells was observed only after 18 months (mean value 14.2%, range 4.6-22%).

Rituximab blood levels were invariably undetectable 6 months after the last drug administration.

Anti-rituximab antibody levels were undetectable (i.e. <12 AU/mL) in all cases but one (Patient #5) showing very high levels (1500 AU/mL).

As shown in Figure 1, levels of erythrocyte sedimenta- tion rate and anti-double-strand (ds) DNA antibodies sig- nificantly decreased ($P < 0.01$ at 12 months), whereas C3 and mainly C4 values increased at 6 months ($P < 0.01$ for C4) and remained within the normal range at 12 months.

Before treatment, only two patients had an increase in serum creatinine [#3, serum creatinine 2.30 mg/dL, estimated glomerular filtration rate (eGFR)-Modification of Diet in Re- nal Disease (MDRD) 33 mL/min; #4, serum creatinine 1.20 mg/dL, eGFR-MDRD, 73 mL/min]. The general profile of eGFR, evaluated by MDRD [8], is shown in Figure 1.

Proteinuria improved in all five cases with renal involve- ment ($P < 0.01$ at 3, 6 and 12 months). SLEDAI score ameliorated moving from the mean 17.3 (12-27) before therapy to 3.1 (1-5) after rituximab treatment (Figure 2).

Constitutional symptoms including arthralgia, weakness and fever disappeared in all the previously affected pa- tients, paresthesia improved in the four patients with poly- neuropathy and skin lesions gradually resolved in the four patients with necrotizing skin ulcers at presentation.

No acute side effects were shown apart from mild bra- dycardia solved by reducing infusion speed in Patient #8. Asymptomatic urinary tract infections were detected in

Patients #1, 5 weeks after the first infusion of rituximab.

At the beginning of the third month, every patient received only 5 mg prednisone/day. The mean follow-up was 36.2 months (12-59 months). Two patients relapsed. Fourty-one months after the last rituximab infusion, patient # 5 presented with pericarditis, polyarthritis and diffuse myalgia. Her serologic profile revealed haemolytic anaemia, leukopenia and decreased platelet count. She had a positive gravindex. She was given 50 mg prednisone, 400 mg hydroxicloro- quine, low doses of aspirin and low-molecular weight hep- arin because of her known antiphospholipid syndrome APS (Table 1). Nevertheless, she not only had a sponta- neous abortion at the 10th week but symptoms persisted despite continuous administration of 1 mg/kg/day predni- sone. She was the only patient of our cohort with high levels of anti-rituximab antibodies.

A dramatic metrorrhagia due to the appearance of an antibody-dependent factor VIII deficiency was observed 36 months after rituximab in Patient #7. Proteinuria, pre- viously undetectable, suddenly increased to 1.7 g. She also complained of a severe polyarthritis.

Because of the good results previously obtained, retreat- ment with the combined scheme of rituximab, cyclophos- phamide and methylprednisolone was proposed in both cases. Both patients showed a complete clinical response. Retreatment was safe also in Patient #5, who had been found to have anti-rituximab antibodies.

A representative case is shown in Figure 3. This is a 41-year-old male (Table 1, #3) with a 25-year duration of SLE and 25-year story of lupus nephritis with focal lesions at the first biopsy when he was 17 years old, a III plus V lupus nephritis 16 years later. At the age of 39 years, he presented acute nephritic syndrome with rapidly progres- sive renal deterioration. The renal biopsy (Figure 4) showed a diffuse extracapillary proliferation (Class IV) with 60% of florid crescents and focal necrosis. He also had four joints with pain and swelling, a recurrence of inflammatory type rash, fever, decreased platelet count, hypocomplementaemia and high levels of anti-dsDNA with a total SLEDAI score of 28. By that time, he had reached a cumulative dose of cyclophosphamide (9 g), which imposed the search of a rescue therapy which re- duced further administration. Figure 3 shows the profiles of serum creatinine, proteinuria and serum proteins. Notably, proteinuria decreased from 10 to 2 g/24 h and creatinine from 2.8 to 1.5 in 1 month, dropping to normal range in 9 weeks (not shown). This patient, a night-working taxi-driver, interrupted his activity just for 5 days following renal biopsy.

Discussion

A few SLE patients prove to be refractory to standard ther- apy with steroids and immunosuppressive drugs. A greater proportion shows haematologic intolerance or cannot be satisfactorily treated because of elevated cumulative doses of cyclophosphamide. All these patients are candidate to new therapeutic options.

Results of an intensive scheme using rituximab combined with low doses of intravenous cyclophosphamide and pulses of methylprednisolone are presented in this open uncontrolled study in a few severe cases. The main interest of these data resides in the relatively short time of standard immunosup- pression, which strongly limits the possible adverse effects of steroids and cyclophosphamide assuring a long-lasting remis- sion without immunosuppressive maintenance therapy. Besides, this scheme might also be useful in low-compliance patients and avoid prolonged hospitalization. All patients, in- cluding a case with rapidly progressive glomerulonephritis, were allowed to restart their own work within 2 weeks.

Biochemical parameters, symptoms and SLEDAI score proved that patients reached long-lasting remission. The complete reversal of urinary abnormalities and functional improvement convinced us not to perform a second renal biopsy. Some other aspects need to be discussed.

A decrease in anti-dsDNA antibody levels was observed in this sample of patients in the long run. In patients with SLE, treatment with rituximab was associated with decline in anti- dsDNA antibody levels in most [2, 3, 9-13] but not all studies [1, 4, 14], implying that self-reactive B cells do play patho- genetic roles beyond the production of autoantibodies [15]. Rituximab-induced improvements may be related to effects on antigen presentation [16], cytokine production [17] and cell-to-cell interactions with T cells [18]. Of interest, a small population of CD20-expressing T cells was depleted after rituximab [19]. Moreover, a rituximab-induced increase in numbers of peripheral T-regulatory cells, known to be low in active SLE patients, was reported too [12]. Finally, Anolik et al. [11] found that peripheral blood B-cell abnormalities in patients with SLE, including naive B-cell lymphopenia and increased memory B cells and plasmoblasts, were corrected by rituximab treatment.

Our patients did neither show significant mild-to- moderate infusion reactions nor clinically relevant infec- tion sequelas. Rituximab has proven to be generally well tolerated. However, it has been rarely associated with se- rum sickness, agranulocytosis, fatal infections especially progressive multifocal leukoencephalopathy [20-24]. The incidence of serious infections could be quantified in a representative cohort of 1053 rheumatoid arthritis patients as 5.4 events per 100 patient-year [25]. Fatal infections were invariably associated to the combination of rituximab with conventional immunosuppressants, especially gluco-corticoids in moderate to high doses, azathioprine, myco- phenolate mofetil and cyclophosphamide, rather than rituximab alone [26].

A steroid and immunosuppressant-sparing strategy is probably mandatory to avoid the rare but fatal complica- tions of combination therapy. A combination of rituximab with a short-term intensive steroid treatment and low doses of intravenous

cyclophosphamide was used in this open study in order to offer the patients an effective therapy limiting the effects of a prolonged immunosuppression vir- tually abolishing immunosuppressive maintenance treat- ment. This scheme was adapted from Leandro's experience [2] with some modifications, mainly consisting of a four plus two infusion protocol of rituximab, which was associated with a delayed occurrence of relapses in our own experience in other immune-mediated diseases [27- 30]. Since the beginning of the third month of therapy, patients were given 5 mg prednisone. The mean post-in- duction follow-up was as long as 36 months (range 12-59 months). Two patients needed a reinduction after 36 and 41 months and had a complete remission.

Our results are similar to Gunnarsson's experience in seven cyclophosphamide-resistant female patients treated with a combination of rituximab and cyclophosphamide [13]. SLEDAI score and anti-dsDNA significantly drop- ped, while on repeat renal biopsy, improvement in the his- topatologic class of nephritis with a decrease in the renal activity index occurred in the majority of patients.

It is generally accepted that there is a relationship between serum rituximab concentration and degree of B-cell deple- tion in patients with SLE and between B-cell depletion and reduction in disease activity score, i.e., only SLE patients with B-cell depletion equal or greater than <5 cells/lL did experience significant reduction in disease activity scores [13]. Our patients experienced a complete B-cell depletion within 5 weeks following the first injection. This is expected in rheumatoid arthritis [31] but is not the rule in SLE [32]. An early CD20-positive cell depletion has been found to affect long-term clinical outcome [32]. Moreover, patients who had the shortest duration of B-cell depletion showed a trend toward a less favourable outcome [13]. Besides, at least in mice, despite depletion of B cells from the circulation, lymph nodes, peritoneal cavity, germinal centers and Peyer's patches were relatively spared from anti-CD20 antibodies [33]. The four plus two rituximab administration protocol, used in the present study in order to accelerate steroid taper ing and avoid prolonged administration of conventional immunosuppressant drugs, obtained long-lasting responses without relapse in patients with cryoglobulinaemic vasculitis because of a more extensive tissue depletion of CD20- positive cells [27-29]. Accordingly, reappearance of CD20-positive cells in circulation was found in our SLE patients only after 18 months.

This is an uncontrolled single-center study in severe SLE patients showing promising results similar to those obtained in Lu's large series in comparably difficult patients [34]. These results are in contrast with two recently concluded controlled trials. Both 'Explorer' [5] and 'Lunar' [6] were randomized, double-blind placebo-controlled studies addressed to deter- mine the additional efficacy of rituximab added to standard immunosuppressive therapy in moderate or severe SLE with [6] or without nephritis [5]. Despite a significant improvement in anti-dsDNA and complement levels in rituximab-treated patients of both studies, and a definite trend toward a greater control of disease activity of the rituximab arm in the 'Lunar' study, both trials failed to demonstrate statistically significant differences between rituximab and placebo groups with re- gard to efficacy on clinical activity. Background treatment, concomitant therapies and ethnic factors have been empha- sized as relevant factors in explaining the different outcomes of patients examined in controlled and uncontrolled studies [8, 31, 35]. As recently emphasized [7], the possible synergistic effect of rituximab in combination with cyclophosphamide, which has been suggested by some authors to have significant advantages in complicated, refractory SLE cases [34, 36], was not evaluated in randomized controlled trials.

Moreover, open uncontrolled studies focussed on pa- tients either refractory or intolerant to standard immuno- suppressive drugs who are not exceptionally observed in clinical practice. Actually, it is unlikely that these patients may be included in randomized controlled studies.

Although patients treated with rituximab are B-cell de- pleted and definitely immunosuppressed, drug safety pro- file seems to be better than standard immunosuppression.

In conclusion, although this prospective study had major limits (especially in the absence of control patient group), it brings additional evidence of a role of rituximab as an off- label drug in severe cases of SLE (with or without neph- ritis) who are intolerant to conventional therapy and need alternative therapeutic options. Used in an intensive short- term course in combination with intravenous cyclophos- phamide and methylprednisolone, rituximab obtained a long-lasting remission, which was maintained with mini- mal doses of prednisone by the beginning of the third month of therapy, avoiding the need of prolonged immu- nosuppression and minimizing the devastating effects of steroids. This scheme could have a place in the context of the acknowledged opportunity to offer patient treatments tailored to the individual needs based on the severity of the disease but also ethnicity, or desire to have children [37].

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