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Review

A 4-year observation in lupus nephritis patients treated with an intensified b lymphocyte depletion without immunosuppressive maintenance treatment—Clinical response compared to literature and immunological re-assessment

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ABSTRACT

Background: B cells (BC) play a critical role in systemic lupus erythematosus (SLE). BC depletion therapy still remains an attractive option, despite the disappointing results of randomized controlled trials (RTCs).
Methods: Twelve patients with SLE [3 males, mean age 43.8 yrs (25–55)] with severe multiorgan involvement all including kidney (3 patients with Class IV, 4 with Class III/V and 5 with Class V, according to the International Society of Nephrology/Renal Pathology Society glomerulonephritis classification), skin lesions [10], severe polyarthralgias with arthritis [10], polyserositis [2], and lymphadenopathy [5] have been prospectively treated with an intensified B cell depletion therapy (IBCDT) protocol due to their resistance or intolerance to previous therapy (six cases) or as a front line immunosuppressive treatment in 6 women with unsatisfactory therapeutic compliance or as a specific request of a short-time immunosuppression for gestational perspectives. Protocol: Rituximab (RTX) 375 mg/sm on days 1, 8, 15, 22, and 2 more doses after 1 and 2 months, associated with 2 IV administrations of 10 mg/kg of cyclophosphamide and 3 methylprednisolone pulses (15 mg/kg) followed by oral prednisone (0.8 mg/die, rapidly tapered to 5 mg/day by the end of the 3rd month after RTX). No further immunosuppressive maintenance therapy has been given.
Results: Patients had been followed-up for a mean of 44.5 (24–93) months. Significant decreases ($p < 0.05$) were found in the levels of ESR (baseline mean value: 55.0 mm; 3 months: 36; end of follow-up: 13), anti-dsDNA antibodies (baseline: 185 U; 3 months: 107; end of follow-up: 15), and proteinuria (baseline: 4.9 g/24 h; 3 months: 0.97; end of follow-up: 0.22). C4 values (baseline 11 mg/dl) significantly increased ($p < 0.05$) after 3 months (22 mg/dl) and at the end of the follow-up (20 mg/dl). Of the 12 patients, 9 (75%) have remained well after one cycle of IBCDT, with no flare (mean 51.6 months [25–93]). Three patients relapsed after 36, 41, and 72 months, respectively. Following re-treatment, they again showed complete remission over 18–48 months of observation.
Conclusions: A promising role of RTX in an intensified protocol of induction therapy can be envisaged in patients for whom avoiding immunosuppressive maintenance therapy and sparing steroids are particularly appealing. Moreover, our data confirm in one of the longest follow-up available, the opportunity to reconsider the regimens of BL depletion in the treatment of the most severe or refractory forms of SLE despite the disappointing results of RCTs.

Abbreviations: RTX, rituximab; IS, immunosuppression; GC, glucocorticoid; F/U, follow-up; PCS, prospective color study; RCS, retrospective cohort study; CR, complete remission; PR, partial remission; LN, lupus nephritis; NM, not mentioned; CYC, cyclophosphamide; RCT, randomized controlled trials; MTP, methylprednisolone; AZA, azathioprine; MMP, mycophenolate; Cys, cyclosporine; UTI, urinary tract infection.

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Contents

1. Introduction

B cells are thought to play an important role in the pathogenesis of systemic lupus erythematosus (SLE) [1,2]. B cell depletion therapy (BCDT), based on rituximab (RTX), a chimeric monoclonal antibody specific for CD20, has proved to be promising in the treatment of patients with SLE [3] and other autoimmune conditions [4–10]. We have previously published the favorable outcome of 8 patients with severe SLE treated with an intensive short-term treatment with RTX, cyclophosphamide, and methylprednisolone pulses [11]. Our approach was able to avoid further immunosuppressive maintenance therapy [12].

BCDT is generally well tolerated, but its long-term safety profile is still debatable as most studies have follow-up data of less than 1 year.

We are now reporting on the very long-term outcome of 12 prospectively enrolled patients with renal involvement SLE (the original cohort plus an additional 7 patients) treated with the intensified BCDT (IBCDT) at our center.

Additionally, the observed results were compared to those emerging from the updated reviews of the literature on this topic focusing on study with a minimum follow-up of 24 months.

2. Methods

2.1. Patients

Twelve patients, nine women and three males, mean age 43.8 years (range 25–55 years), with severe multiorgan involvement, including kidney (3 patients with Class IV, including a case of rapidly progressive glomerulonephritis, with 60% of florid crescents), 4 with Class III/V and 5 with Class V Lupus Nephritis (LN) according to the International Society of Nephrology/Renal Pathology Society Glomerulonephritis Classification), skin lesions [10 patients], severe polyarthralgias with arthritis [10], polyserositis [2], and lymphadenopathy [5], were considered eligible for RTX therapy due to their resistance or intolerance to previous therapy (6 cases) or as a front line treatment in 6 women with unreliable therapeutic compliance or gestational perspectives.

In non-naive patients, prior immunosuppressive therapy included methylprednisolone pulses and oral steroids (all 6 previously treated patients), I.V. cyclophosphamide (2 patients), and both intravenously and orally administered cyclophosphamide for a cumulative dose of 9 g in one patient, azathioprine in 2 patients, mycophenolate mofetil in 2 patients, cyclosporine A in four cases, hydroxychloroquine in all 6 previously treated cases, and thalidomide in 1 patient.

RTX was administered intravenously as previously described [11] at a dose of 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 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 3 months.

Response was evaluated by assessing the changes in clinical signs and symptoms and laboratory parameters. SLEDAI score was separately assessed by two investigators (S.S. and M.A.).

Circulating B cells in the peripheral blood were investigated by detection of CD20$^+$ B cells and analyzed by flow cytometry at baseline, month 1, month 2, and every other month thereafter.

We examined changes in T cell homeostasis following rituximab-induced B cell depletion in two patients. Analysis included flow cytometry studies at baseline (before the first RTX infusion), at months 3, 6, and 9. Whole blood samples obtained in the morning, in EDTA, were stained with monoclonal antibodies against CD45 (APC 100 eBioscience Bender Medsystems, CA, USA), CD3 (FITC eBioscience Bender Medsystems, CA, USA), CD4 (PC7 Beckman Coulter, CA, USA), CD19 (Pacific Blue™, Beckman Coulter, CA, USA), CD20 (PE Beckman Coulter, CA, USA), and CD25 (PerCP-eFluor 710 eBioscience/Bender Medsystems, CA, USA), FOXP3 (PE Staining set, eBioscience Bender Medsystems, CA, USA).

2.2. Renal response and relapse

For the evaluation of the renal response, the Joint European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA-EDTA) consensus statement was used [13].

In detail, a complete renal response (CR) has been defined as proteinuria <0.5 g/24 h and normal or near-normal (within 10% of normal GFR if previously abnormal) GFR and, additionally, negative anti-DNA antibodies and normal levels of C_3 and C_4. Partial response (PR) has been defined as ≥50% reduction in proteinuria to subnephrotic levels and normal or near-normal GFR.

The definition of renal flares included a reproducible increase of serum creatinine by ≥30% (or decrease in GFR by ≥10%) and/or an increase of proteinuria >0.5 g/24 h if CR was initially achieved, or ≥50% in cases of PR.

2.3. Statistics

For comparison of variables at baseline and follow-up, Student's *t*-test was used for normally distributed parameters and the non-parametric Mann–Whitney test for non-normally distributed parameters. Correlations were calculated and significance determined by Fisher's test. Multivariable logistic regression analysis was used to identify any

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