Concise Report

Long-term observation of patients with anti-neutrophil cytoplasmic antibody-associated vasculitis treated with rituximab

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Objective. Rituximab, a chimeric anti-CD20 monoclonal antibody, has been shown to be quite effective in the treatment of immune disorders resulting from autoantibodies. We prospectively studied the long-term effects of rituximab in 10 patients with anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis refractory to conventional therapy ($n = 3$) or in second or subsequent relapse ($n = 7$).

Methods. The median age of patients was 53 yrs (range 38–70 yrs). Eight were classified as Wegener's granulomatosis, and two as microscopic polyangiitis. Clinical activity was assessed using the Birmingham Vasculitis Activity Score modification for Wegener's granulomatosis. Treatment consisted of intravenous infusions of rituximab given at the dose of 375 mg/m^2 weekly for four consecutive weeks.

Results. All patients experienced a rapid clinical improvement following the administration of rituximab, with nine complete responses and one partial response at 6 months. With a median follow-up of 33.5 months (range 26–45 months), three patients have thus far relapsed. Retreatment with the monoclonal antibody at the same dose and schedule resulted in a new sustained response in all these patients. Rituximab therapy resulted in prolonged B-cell depletion. The ANCA titres decreased significantly in all patients, with eight out of 10 becoming ANCA-negative and three remaining ANCA-negative even after B-cell recovery. Infusion-related side effects were observed in one patient, but were of mild intensity and did not require discontinuation of treatment.

Conclusions. Rituximab is an effective and well-tolerated treatment for patients with ANCA-associated vasculitis and should be strongly considered in severely affected patients who do not respond to standard therapy or in those in whom cytotoxic therapy bears a high risk of morbidity.

KEY WORDS: Anti-neutrophil cytoplasmic antibody, Vasculitis, Rituximab, Therapy.

Introduction

Within the spectrum of primary vasculitic syndromes, the anti-neutrophil cytoplasmic antibody (ANCA)-related syndromes form a distinct group with overlapping features. ANCA-related small-vessel vasculitides are potentially life-threatening diseases with high mortality. The introduction of steroids and cyclophosphamide results in disease remission in 77% of patients by 3 months and in 93% by 6 months [1]. However, there is a considerable morbidity related to current regimens, on which at least 25% of the patients experience severe drug-related adverse effects. Furthermore, 50% of patients experience disease relapse, resulting in accumulating damage from disease scars and treatment [2]. Thus, there is a clear need to achieve more effective remission induction and to reduce therapy-related toxicity.

Recently, selective B-cell depletion with rituximab, a chimeric anti-CD20 monoclonal antibody, has been shown to be quite effective in the treatment of immune disorders resulting from autoantibodies, including rheumatoid arthritis [3], idiopathic thrombocytopaenic purpura [4], non-familial thrombotic thrombocytopaenic purpura [5], autoimmune haemolytic anaemia [6, 7] and mixed cryoglobulinaemia [8]. The use of this immunotherapy has been described recently also in patients with ANCA-associated vasculitis [9–16]. In these small series or case reports, the use of rituximab and steroids, with or without the use of other immunosuppressive agents, resulted in a complete or partial remission in all cases. However, patients' follow-up was often equal or less than 1 yr, and the findings about ANCA titres in relation to both rituximab therapy and disease activity were discordant [13, 14]. In this article, we present the long-term results of a study in which we used an approach with rituximab for frequently relapsing or refractory patients with ANCA-associated vasculitis.

Patients and methods

Patients

The study group comprised 10 consecutive patients (five men and five women) who received treatment for ANCA-associated

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vasculitis refractory to conventional therapy ($n = 3$) or in second or subsequent relapse ($n = 7$). More specifically, refractory disease was defined as an active disease that had not been controlled by the maximally tolerated cyclophosphamide dose given in conjunction with glucocorticoids. Patients' clinical and laboratory characteristics are summarized in Table 1. Additional details about cyclophosphamide doses and other immunosuppressive treatments at the time of rituximab therapy are reported in Table 2. The median age of patients was 53 yrs (range 38–70 yrs). Eight patients had cytoplasmic (c)-ANCA and two patients had perinuclear (p)-ANCA. The former were classified as Wegener's granulomatosis (WG) and the latter as microscopic polyangiitis (MPA) according to the definitions of the Chapel Hill Consensus Conference [17]. The review of these cases was approved by the Institutional Review Board. All patients were considered to have active, severe disease (see subsequently). Six patients had active renal disease, which was documented by red blood cell (RBC) casts on urine microscopy; four of these patients had active glomerulonephritis confirmed by renal biopsy. Five patients had lung involvement with cough and dyspnea; in these cases, alveolar haemorrhage was documented by bronchoscopy with broncho-alveolar lavage. One patient had polyarticular arthritis, with swelling and pain of the knees, elbows and wrists. Three patients suffered from nasal obstruction with serosanguineous discharge. One patient presented a purpuric rash over the lower extremities.

Study design

Local hospital Ethical Committee approval and patients' informed consent was obtained. Rituximab (Mabthera; Roche, Milan, Italy) at a dose of $375\,mg/m^2$ was administered intravenously once weekly for a total of four infusions (days 1, 8, 15 and 22). The drug was reconstituted in normal saline to a concentration of no more than 4 mg/ml. The initial infusion rate was 50 mg/h, with subsequent infusion-rate increase if no toxicity was seen. Pre-medication with oral acetaminophen 500 mg and diphenhydramine 50 mg was given to all patients. Patients who experienced any treatment-related nausea or vomiting with the first treatment received subsequent pre-medication with a serotonin receptor antagonist. All patients also received oral prednisone (up to 2 mg/kg/day), which was tapered once disease activity improved.

Clinical activity assessment

Clinical activity was assessed using a disease-specific activity index, the Birmingham Vasculitis Activity Score modification for WG (BVAS/WG) [18]. The BVAS/WG was validated specifically for use in WG, but not for MPA. 'Severe disease' was defined as a disease that posed an immediate threat to the patient's life or the function of a critical individual organ (items classified as 'major' on the BVAS/WG instrument and generally deemed to require therapy with cyclophosphamide). Complete remission was defined as a BVAS/WG score of 0, indicating the absence of signs of new or worse disease activity. Partial remission was defined by persistent disease activity for no more than one item. Major relapse was defined by the recurrence or first appearance of at least one of the 24 items on the BVAS that are indicative of threatened function of a vital organ (kidneys, lungs, brain, eye, motor nerve or gut) attributable to active vasculitis. Minor relapse was defined by the recurrence or first appearance of at least three other items in the BVAS.

TABLE 1. Clinical characteristics of 10 patients with ANCA-associated vasculitis at the time of rituximab therapy

Case no.	Age	Sex	Diagnosis	Duration of vasculitis (months)	Affected organs since the onset of vasculitis	Active organ involvement at the time of rituximab	No. of relapses	BVAS/WG score	Follow-up duration (months)	Creatinine clearance (ml/min)	ESR (mm/h)	CRP (mg/l)
1	51	F	WG	35	ENT	ENT, L	3	3	45	92	12	8
2	70	M	WG	19	ENT, K	K	2	5	41	51	59	26
3	48	F	MPA	42	K, S	K, S	0	7	38	22	97	153
4	63	M	WG	74	K, J, L	K, J	2	11	36	17	63	94
5	38	F	WG	5	ENT, L	ENT, L	0	4	35	105	53	47
6	55	F	WG	39	ENT, L	L	4	3	32	73	85	66
7	42	M	WG	21	K, J, L	K, L	5	8	30	41	74	78
8	66	M	WG	56	K, L	K	3	6	29	35	43	31
9	47	F	MPA	12	K, S	K	2	4	27	49	26	18
10	61	M	WG	29	ENT, L	ENT, L	0	7	26	84	35	24

WG, Wegener's granulomatosis; MPA, microscopic polyangiitis; BVAS/WG, Birmingham Vasculitis Activity Score modification for Wegener's granulomatosis; ENT, ear, nose and throat; J, joints; K, kidneys; L, lungs; S, skin; ESR, erythrosedimentation rate; CRP, C-reactive protein.

TABLE 2. Cumulative doses of cyclophosphamide and doses of immunosuppressive drugs at the time of rituximab therapy

Case no.	Previous medication (besides steroids)	Cumulative cyclophosphamide dose (gm)	Failing therapy at the time of rituximab (dose)
1	Cy, Az, MTX, IVIG, CSA	17.5	CSA (200 mg/day)
2	Cy, Az, TMP/SMX	6	TMP/SMX (800/160 mg × 2/day)
3	Cy, IVIG	36	Cy (100 mg/day)
4	Cy, Az, TMP/SMX	52	TMP/SMX (800/160 mg × 2/day)
5	Cy, IVIG	4	Cy (100 mg/day)
6	Cy, Az, MTX, IVIG, CSA	13.5	CSA
7	Cy, Az, MTX, IVIG, TMP/SMX	11	TMP/SMX (800/160 mg × 2/day)
8	Cy, Az, IVIG, TMP/SMX	15	TMP/SMX (800/160 mg × 2/day)
9	Cy, Az, IVIG	9	Cy (2 g/month I.V.)
10	Cy, IVIG	31	Cy (100 mg/day)

Cy, cyclophosphamide; IVIG, intravenous immunoglobulins; Az, azathioprine; TMP/SMX, trimethoprim/sulfamethoxazole; CSA, cyclosporine A; MTX, methotrexate.

Laboratory studies

Patients were assessed before the first rituximab infusion, after 1 month, 2 months and every 2 months thereafter. Laboratory investigations included a complete haemogram, serum chemistry profiles, immunoglobulin levels, erythrosedimentation rate (ESR), plasma C-reactive protein (CRP), lymphocyte subsets, rheumatoid factor, antinuclear antibodies, anticardiolipin antibodies and lupus anticoagulant. Glomerular filtration rates were estimated with creatinine clearance through 24 h urine collection.

The ANCA testing was performed with the use of indirect immunofluoresence on ethanol-fixed neutrophils. Each ANCA titre was determined by two independent readers, and in case of disagreement, by a third reader. Further characterization of ANCA specificity was performed by antigen-specific enzyme-linked immunosorbent assay (ELISA) for antimyeloperoxidase (MPO-ANCA) and antiproteinase 3 (PR3-ANCA) with commercially available test kits (DLD Diagnostika GmbH; Hamburg, Germany).

Statistical analysis

Data collection was censored on 31 March 2005. Student's paired t-tests were used when similar measurements were obtained in the same patient at different time intervals. Data are presented as the mean $\pm$ s.d. P-values < 0.05 were considered significant. All P-values are two-tailed.

Results

Response

All patients experienced a rapid clinical improvement following the administration of rituximab (Fig. 1). A BVAS/WG score of 0 was reached within 6 months in 9 cases. One patient (patient 10) had persistent bloody nasal discharge but no evidence of active disease in other organs, and he was classified as a partial remission. In the six patients with active renal disease, the microscopic haematuria with RBC casts resolved completely, and the calculated mean $\pm$ s.d. creatinine clearance increased from 32.8 ± 13.2 ml/min at baseline to 40.8 ± 7.3 ml/min at 6 months. The time course of individual serum creatinine clearances are reported in Fig. 2.

The efficacy of rituximab allowed a gradual steroid withdrawal. The mean $\pm$ s.d. prednisone dose was 24.75 ± 18.90 mg (range 10–62.5) at the time of the first rituximab infusion,
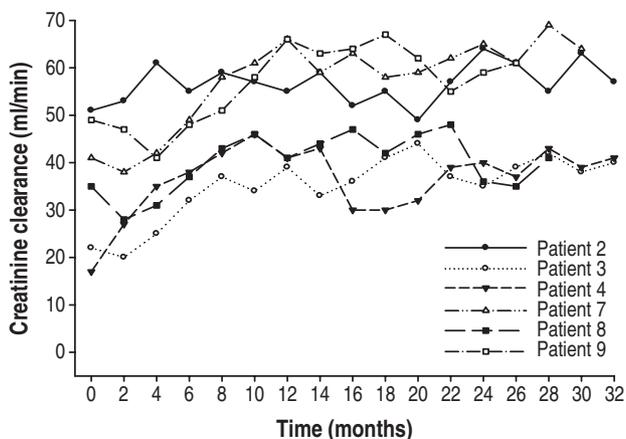
18.75 ± 14.63 mg (range 5–50) at 1 month, 11.25 ± 11.68 mg (range 0–37.5) at 3 months and 2.50 ± 5.40 mg (range 0–15) at 6 months. Only two patients were still receiving prednisone at 6 months.

Significant reductions in the ESR and CRP levels were observed in all patients. The mean $\pm$ s.d. ESR fell from 54.7 ± 26.5 mm/h to 18.1 ± 9.2 mm/h ($P < 0.001$), and the mean $\pm$ s.d. CRP level fell from 54.5 ± 44.4 mg/l to 12.0 ± 3.9 mg/l ($P = 0.010$).

Duration of response

With a median follow-up of 33.5 months (range 26–45 months), three patients have thus far relapsed (cases 4, 8 and 10). Relapse occurred in the same organs that were involved before rituximab therapy. The time to relapse was 16, 24 and 12 months, respectively. Relapsed patients were rechallenged with rituximab at the same dose and schedule. Repeat treatment resulted in a new sustained response in all these patients (Fig. 2).

Monitoring of immunological parameters

Peripheral blood B-cells, evaluated by flow cytometry as CD19-positive cells, had a median pre-treatment count of $72 \pm 45 \times 10^6$/l (range 28–165×10^6/l). Following rituximab treatment, the B-cell counts fell to $< 5 \times 10^6$/l within 4 weeks in all patients. In relapsed individuals recovery of circulating B-cells to concentrations greater than 20×10^6/l occurred in 4 (case 4), 5 (case 8) and 6 months (case 10), respectively (Fig. 3). In continuously responding patients, B-cell recovery occurred between 6 and 10 months (median 6 months). Median absolute T-cell counts in peripheral blood, using CD3, CD4 and CD8 as well as natural killer cell counts, remained stable during the study period. ANCA-titres decreased significantly in all patients, with 8 of the 10 patients becoming ANCA negative (Fig. 4). Four of these 8 patients (cases 1, 3, 5 and 7) remained ANCA-negative after B-cell recovery, and experienced disease in remission. The three relapses were preceded by elevations in ANCA titres (Fig. 3). Re-treatment with rituximab resulted in sustained reconversion to ANCA-negative in two patients.

There were no significant changes in mean immunoglobulin G (IgG) or IgM levels after rituximab. Patients with normal IgM levels before treatment had their levels remain normal; two patients with low initial IgM levels (case 2 and 8) and one with a low initial IgG level (case 10) maintained their IgM and IgG levels.

FIG. 1. Time course for serum creatinine clearance values in patients with renal vasculitis after rituximab therapy.
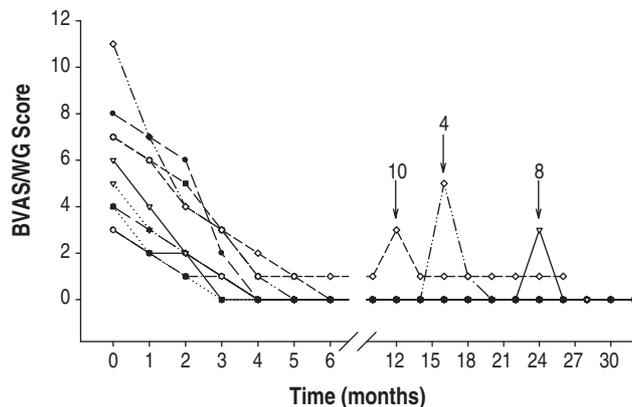

FIG. 2. Changes in scores on the Birmingham Vasculitis Activity Score Index modified for Wegener's granulomatosis (BVAS/WG) following initiation of rituximab therapy. Arrows indicate re-treatment with rituximab in the three patients with disease relapse (cases 4, 8 and 10).
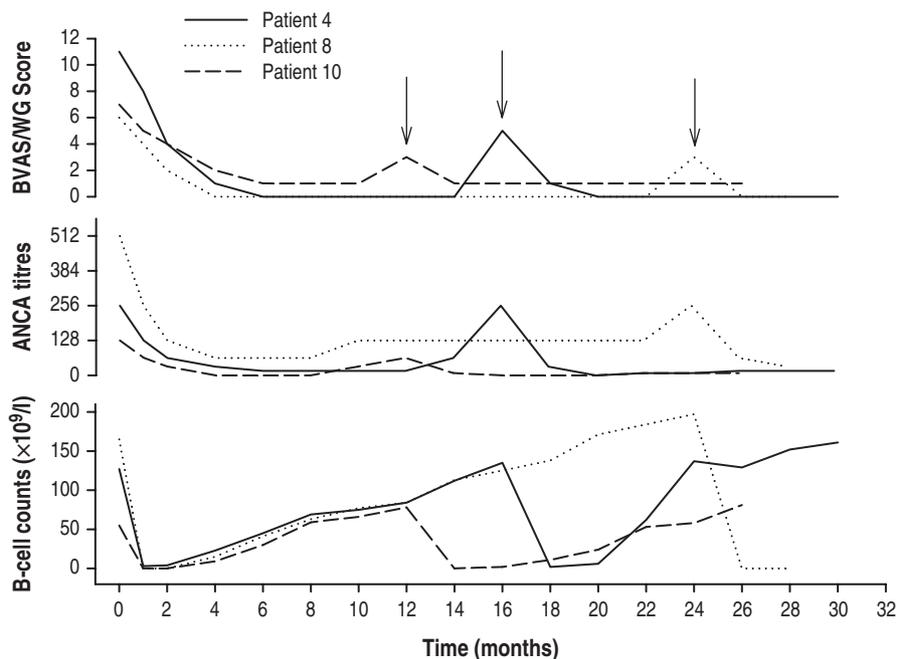
FIG. 3. Time course for ANCA titres and B-cell counts in patients relapsed after rituximab therapy.
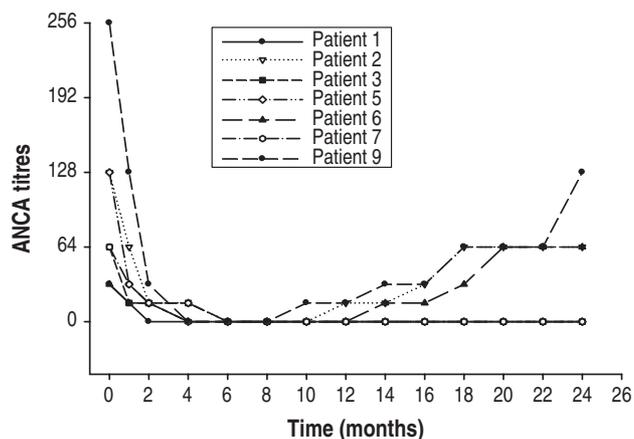

FIG. 4. Time course for ANCA titres in patients with a sustained response after rituximab therapy. Arrows indicate re-treatment with rituximab.

One patient (case 2) had his IgG level decrease from normal (910 mg/dl) to less than normal; the week 12 level was 713 mg/dl (normal, 800–1800 mg/dl) and week 52 was 689 mg/dl.

In three patients, the presence of ANCA was associated with the presence of lupus anticoagulant activity; two of these patients also tested positive for anticardiolipin antibodies. Both lupus anticoagulant and anticardiolipin antibodies were no longer detectable 2 months after the last rituximab infusion.

Adverse events

One patient experienced first-infusion reactions, consisting of fever, chills and nausea. Reactions were of mild intensity (grade 1, according to the National Cancer Institute criteria) and did not require discontinuation of antibody infusion. No other acute or delayed relevant side effects attributable to rituximab occurred.

Discussion

Following the dramatic successes with the treatment of lymphoma, rituximab has become an appealing candidate for the treatment of non-malignant diseases involving B-cells. The central concept has been the removal of the cellular source of pathogenic autoantibodies, but effective targeting of B-cells may also affect pathogenesis by removing the many functional contributions of B-lymphocytes to the cell–cell interactions that drive the disease process [19]. The promising results obtained in rheumatoid arthritis and idiopathic thrombocytopaenic purpura have warranted the use of rituximab in several other disorders in which a pathogenic role for autoantibodies is generally accepted or demonstrated.

The present study confirms the long-term efficacy and safety of rituximab therapy in patients with ANCA-associated vasculitis suggested by previous reports. The use of rituximab resulted in a dramatic and rapid clinical improvement in all patients, which was sustained in seven patients. In addition to the durability of responses, rituximab compares favourably to other therapies because of the substantial lack of significant toxicity, with only one moderate infusion-related reaction. Because this agent depletes normal B-cells, it is also notable that no relevant infectious episodes were observed during the follow-up period. Conversely, immunosuppressive therapy bears potential complications such as neutropaenic fever, herpes zoster, myelodysplasia and cataracts; deaths as a result of sepsis have also been described [1, 20, 21].

Not unexpectedly, peripheral B-cell depletion was found in all cases, and the pattern of B-cell depletion appears comparable to that seen in other autoimmune disorders treated with this antibody [3, 4, 8]. Likewise, ANCA titres decreased significantly following treatment, becoming undetectable in eight of the 10 patients. This is in good agreement with the report of Keogh et al. [14], whereas Eriksson [13] reported that ANCA did not change in seven of nine patients. In three of our patients, ANCA testing remained negative even after the reconstitution of B-lymphocytes. Interestingly, the three relapses were preceded by elevations in ANCA titres. However, in four other cases, the increase in ANCA titres was not associated with signs or symptoms of disease activity. These findings do not fully support the practice of pre-emptive therapy in patients with increasing

ANCA titres that has been advocated in other reports [14]. In fact, even though rituximab has a favourable toxicity profile, pre-emptive therapy may result in over-treatment for many patients. Besides, once disease activity has been demonstrated, the effects of rituximab are so rapid that a significant organ deterioration is not likely to occur. The speed of therapeutic onset is especially important in serious renal involvement where scarring can result in chronic renal failure and dialysis dependency.

In conclusion, our results indicate that rituximab therapy has a valuable effect in patients with ANCA-associated vasculitis. However, a routine recommendation for its use in this autoimmune disorder should await the outcome of larger and controlled trials. Some issues about this agent, such as the optimum dose and treatment schedule, as well as the exact mechanisms of action, remain to be explored and warrant further investigation.

Rheumatology	Key messages
	• The use of rituximab results in a rapid and sustained clinical improvement in most patients with ANCA-associated vasculitis.
	• Compared with other conventional therapies for ANCA-associated vasculitis, rituximab lacks of significant toxicity.

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