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Short communication

Outcomes after single-cycle rituximab monotherapy in patients with anti-MAG polyneuropathy: A bi-center experience with an average follow-up of 11 years

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ABSTRACT

Rituximab is efficacious in myelin-associated glycoprotein (MAG) polyneuropathy, but the question on timing of retreatments is open. We studied 21 anti-MAG polyneuropathy patients who responded to a first cycle of rituximab, were followed-up for an average of 11.2 years, and were retreated only when relapsing. Baseline serum B-cell-activating factor (BAFF) levels were measured.

Clinical improvements lasted on average 6 years, and as many as 71% of the patients resulted long-lasting responders. Severity of disease and high serum BAFF levels (cut-off ≥860 pg/mL for relapse risk) at onset seemed to predict worse prognosis. Measurements of these variables could help deal with the issue of maintenance rituximab therapy in MAG polyneuropathy.

1. Introduction

Myelin-associated glycoprotein (MAG) polyneuropathy is an immune-mediated demyelinating disorder mainly characterised by sensory ataxia, possibly combined with motor involvement (Dalakas, 2018). Open-label studies indicate that 30–50% of patients respond to rituximab, a B-cell-depleting monoclonal antibody (Dalakas, 2018), but two double-blind placebo-controlled trials confirmed these data only partially (Dalakas et al., 2009; Léger et al., 2013). Important open questions regard: a) benefit duration of single courses; b) when and with which schedule to repeat courses; c) availability of clinico-laboratory predictors of therapeutic response. Few studies addressed this topic, with smaller sample sizes and shorter follow-up (Benedetti et al., 2008; Iancu Ferfoglia et al., 2016; Gazzola et al., 2017).

In MAG polyneuropathy patients that initially responded to rituximab monotherapy, and that were followed-up for very long periods and retreated only when they relapsed, we investigated the duration of clinical benefit, searching for predictors of drug efficacy and of timing for re-treatment.

2. Patients and methods

Twenty-one previously-untreated patients (13 men; mean age, 74.7 years; range, 55–86) with MAG polyneuropathy, who responded to a first cycle of rituximab (375 mg/m^2, 4 consecutive weekly intravenous infusions), were prospectively followed-up for an average of 11.2 years (range, 7–15) (Table 1). All the patients showed clinical pictures typical of MAG polyneuropathy, with distal, symmetric, sensory involvement, and gait ataxia at onset. In 13 out of 21 of them, weakness manifested later. Postural tremor in the upper limbs was present in 7/21 patients. Electrophysiology studies disclosed symmetrically prolonged distal latencies with Terminal Latency Index < 0.25

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Table 1
Demographic, clinical and laboratory features pre-therapy of polyneuropathy associated with anti-myelin-associated glycoprotein (MAG) antibodies.

Patients (sex, age)	Haematological condition	Disease duration (years)	INCAT score	MRC score	ISS score	Anti-MAG antibody titer[§]	BAFF (pg/mL)	FU (years)
1 (m, 44)	thrombocytosis	1	3	54	11	1/100,000	1083	15
2 (m, 61)	Waldenström	5	4	54	12	1/800,000	931	14
3 (f, 60)	IgM MGUS	4	2	52	10	1/100,000	1367	13
4 (f, 69)	IgM MGUS	1	0	60	2	1/51,200	609	14
5 (f, 53)	IgM MGUS	2	1	59	7	1/3200	841	13
6 (m, 65)	IgM MGUS	3	1	57	6	1/1600	568	13
7 (f, 61)	IgM MGUS	16	2	57	6	1/51,200	469	12
8 (m, 60)	IgM MGUS	1	1	60	3	1/51,200	475	11
9 (f, 65)	IgM MGUS	15	2	58	6	1/3200	834	11
10 (m, 67)	IgM MGUS	4	4	52	9	1/51,200	214	11
11 (m, 74)	IgM MGUS	4	2	60	6	1/800,000	891	11
12 (m, 68)	IgM MGUS	3	3	59	8	1/51,200	314	11
13 (m, 77)	IgM MGUS	8	2	59	7	1/12,800	NA	9
14 (m, 75)	IgM MGUS	2	0	60	2	1/100,000	766	8
15 (m, 54)	IgM MGUS	1	0	60	0	1/51,200	763	8
16 (m, 48)	IgM MGUS	2	4	58	6	1/12,800	599	11
17 (f, 65)	IgM MGUS	7	0	60	2	1/3200	879	10
18 (f, 73)	IgM MGUS	6	5	40	14	1/100,000	NA	9
19 (f, 71)	IgM MGUS	1	2	60	4	1/3200	NA	7
20 (f, 70)	IgM MGUS	1	1	60	4	1/51,200	NA	11
21 (m, 73)	Waldenström	10	5	44	8	1/25,600	NA	13

INCAT, Inflammatory Neuropathy Cause and Treatment disability scale; MRC, Medical Research Council scale; ISS, INCAT sensory scale; BAFF, B-cell-activating factor; FU, follow-up; m, male; f, female; Waldenström, Waldenström macroglobulinemia; MGUS, monoclonal gammopathy of uncertain significance; NA, not available; [§]measured with Western blot.

(Cocito et al., 2001), and reduced nerve conduction velocities without conduction blocks in all patients. A detailed description of these features in 10/21 patients has been reported elsewhere (Benedetti et al., 2007).

The following clinical scales were administered before the treatment and every six months: INCAT disability scale, MRC sum score, and ISS. All patients were responders, showing improvements of at least one point on two of the three scales at month 12 (Benedetti et al., 2008). "Clinical relapse" was defined as a worsening by one point in at least two of the three scales.

Given the absence of guidelines, in 2003 we empirically decided to perform no maintenance therapy, and to treat only relapses with additional rituximab cycles, using the initial schedule. Number of rituximab cycles, benefit duration for each cycle, and variations of anti-MAG antibody titer after each cycle *vs* pre-cycle values were recorded (Table 2). The patients were classified as: a) "responders", when showing an equivalent response, in terms of clinical improvement, *vs* the response shown after the previous cycle (Benedetti et al., 2008); b) "partially-responders", when showing improvements in one of the three scales, or when improvement scores on any scale were lower than those recorded on the previous cycle; c) "non-responders", when no clinical improvement occurred.

Blood CD19+ B-cell number (flow cytometry, Becton Dickinson), and serum anti-MAG antibody titers [Western blot (Nobile-Orazio et al., 1994) until 2015, ELISA (Bühlmann, Switzerland) after 2015] were measured before treatment and every six months. Given the different methods for antibody determinations, which imply different ways of quantification (*i.e.*, titers for Western blot, Bühlmann titer units for ELISA), pre- and post-therapy testing for each patient at each rituximab cycle was performed with the same method. Moreover, to allow the comparison between pre-therapy mean anti-MAG antibody titer and the other variables, serum samples of 6 patients (#16 to #21) were tested with Western blot, too (Table 1). B-cell-activating factor (BAFF) was measured with ELISA (R&D Systems, USA) in the pre-therapy serum samples of 16/21 patients. Electrophysiology studies were performed only at the time of diagnosis. Type of response and clinical benefit duration were correlated with age, sex, pre-therapy disease duration, scale scores, anti-MAG antibody titer, and serum BAFF levels.

Quantitative variables were expressed as means ± standard errors. Mann-Whitney test was used for quantitative variables, chi-square/Fisher's test for qualitative variables, Spearman test for correlations between biomarker levels and clinical data, receiver operating characteristic (ROC) curve analysis for the optimum cut-off of BAFF. *P*-values ≤ .05 (two-tailed) were considered as statistically significant.

Written informed consents were obtained from the patients.

3. Results

Table 2 summarizes: a) the number of rituximab cycles; b) the responses to, and benefit durations of each cycle; c) serum anti-MAG antibody titer variations. The mean follow-up period was 11.2 years (range, 7–15). Clinical improvements after the first rituximab cycles lasted two years at least in all the patients (mean duration, 6 years; range, 2–12). Four/21 patients (19%) had no relapse after an average 9.5-year follow-up (range, 8–11). Seventeen (81%) presented at least one relapse after 5.1 years (mean value; range 2–12) from the first cycle: they underwent repeated drug cycles (2 in 11 patients; 3 in 4; 4 in 2) that resulted efficacious in 13/17 (76.5%). As a whole, the number of partial responses to each cycle was 4, as well as that of no response (Table 2).

Benefit duration of the first rituximab cycles correlated with the following pre-therapy items: a) lower scores of INCAT ($p = .019$, Fig. 1A), and ISS scales ($p = .021$, Fig. 1B); b) lower serum anti-MAG antibody titers ($p = .033$), but significance was due to outliers (not shown), whereas the differences between the values in patients who relapsed after four years *vs* those who relapsed before were not significant ($p = .52$); c) lower serum BAFF levels ($p = .028$, Fig. 1C). Interestingly, when the cohort was split between patients relapsing after 2–3 years (serum BAFF concentrations, 1017.0 ± 117.2 pg/mL) *vs* those relapsing at 4 year or later (627.9 ± 70.5 pg/mL; $p = .012$, Fig. 1D), ROC curve analysis showed a cut-off of 860 pg/mL for serum BAFF values (AUC, 0.937; 95%CI, 0.810–1.065; $p = .010$; likelihood ratio, 12.0) that identify the optimal decision threshold. No significant correlation between response to therapy and all the other variables was found. Serum anti-MAG antibody titer decreased after 31 out of the 46 rituximab cycles (67%), and increased in one "responder", and in one "non-responder" (Table 2), without correlations with rituximab response. CD19+ B cells were undetectable one month after therapy initiation, returning to pre-treatment values in 20/21 patients after 12–18 months, without correlations with relapses, or BAFF levels. In

Table 2
Effects of rituximab cycles on clinical and autoantibody parameters.

Patients (#)	Number of cycles	Response to therapy	Clinical benefit duration (years)	Anti-MAG Ab values[§] (at onset and after 1 year of therapy)	Anti-MAG Ab variation (%)
1	1	R	4	1:100,000–1:12,800	−87
	2	R	3	1:400,000–1:100,000	−75
	3	PR	3	1:400,000–1:200,000	−50
	4	NR	NA	1:400,000–1:100,000	−75
2	1	R	2	1:800,000–1:25,600	−97
	2	NR	NA	1:100,000–1:6400	−93
3	1	R	3	1:100,000–1:51,200	−49
	2	R	2	1:200,000–1:100,000	−50
	3	R	2	1:200,000–1:100,000	−50
	4	R	6, on FU	1:200,000–1:100,000	−50
4	1	R	7	1:51,200–1:6400	−87
	2	R	7, on FU	1:6400–1:1000	−84
5	1	R	12	1:3200–1:1000	−69
	2	R	1, on FU	neg-neg	NA
6	1	R	12	1:1600-neg	−100
	2	PR	1	neg-neg	NA
7	1	R	8	1:51,200–1:51,200	0
	2	PR	3	1:51,200–1:51,200	0
	3	PR	1, on FU	1:51,200–1:51,200	0
8	1	R	4	1:51,200–1:1000	−98
	2	R	7, on FU	neg–neg	NA
9	1	R	11	1:3200-neg	−100
10	1	R	5	1:51,200–1:12,800	−75
	2	NR	NA	234,131-234,100	0
11	1	R	2	1:800,000–1:800,000	0
	2	NR	NA	1:51,200–1:200,000	+25
12	1	R	4	1:51,200–1:51,200	0
	2	PR	7, on FU	1:200,000–1:100,000	−50
13	1	R	2	1:12,800–1:1000	−92
	2	PR	7, on FU	1:6400–1:6400	0
14	1	R	8	1:100,000–1:51,200	−49
15	1	R	8	1:51,200–1:6400	−87
16	1	R	5	70,000–68,000	−3
	2	R	4	68,000–53,140	−22
	3	R	2, on FU	78,635–77,161	−2
17	1	R	2	70,000–55,600	−21
	2	R	8, on FU	57,000–19,380	−66
18	1	R	3	76,000–63,000	−17
	2	R	2	64,341–64,000	−1
	3	R	4, on FU	64,000–70,000	+10
19	1	R	7	38,000–38,000	0
	2	R	0.5, on FU	47,318–47,200	0
20	1	R	11	12,670–neg	−100
21	1	R	7	63,000–29,100	−54
	2	R	6	neg–neg	NA
	3	R	0.5, on FU	12,276–neg	−100

Ab, antibody; [§]expressed as titers (1:), or as arbitrary titer units (Bühlmann titer units) (see text); R, responder; PR, partially-responder; NR, non-responder; neg, negative; NA, not applicable; FU, follow-up.

one patient, B cells remained suppressed up to 6 years after the first treatment. Rituximab caused no remarkable/severe adverse effect over the very long follow-up.

4. Discussion

Rituximab and other B-cell-targeting agents represent promising treatments in MAG polyneuropathy (Dalakas, 2018), but criteria for deciding time of retreatment are needed.

Our data confirm that, after the first rituximab cycles, clinical improvements can last for two years at least (Benedetti et al., 2008), persisting, as a novelty, for longer periods, up to an average of 6 years. One fifth of the patients were relapse-free after an average 9.5-year follow-up, and the remaining four fifths had a relapse only after an average of 5.1 year from the first cycle.

Few studies addressed this topic, with smaller sample sizes and shorter follow-ups. RIMAG study failed to find significant changes in most outcome measures, but schedules were heterogeneous and intervals between the rituximab cycles not reported (Iancu Ferfoglia et al., 2016). In our previous study, clinical improvements in 6/10 patients lasted 3 years, and 4/10 relapsed over 2–3 years (Benedetti et al., 2008). Gazzola et al. retrospectively found that rituximab was effective in 10/33 patients, in line with the seminal trial (Dalakas et al., 2009), and that benefit duration lasted 42 ± 23 months after an average 5-year follow-up (Gazzola et al., 2017).

About 40% of our patients became “partially-/non-responders” over years. Lower scores on the INCAT and/or ISS scales, and serum BAFF levels were pre-therapy predictors of longer benefit duration, although the significance of the association was likely influenced by outliers. BAFF values ≥860 pg/mL might help identify patients needing maintenance protocols due to higher relapse risk. This finding confirms and expands our previous data on the usefulness of measuring baseline serum BAFF levels as a biomarker of clinical response in rituximab-treated patients with MAG polyneuropathy (Benedetti et al., 2011). Our longitudinal monitoring of serum anti-MAG antibody titer showed no correlation between their values and risk of relapse, in line with previous evidence of little correlation between reductions in disease activity and changes in titers of pathogenic autoantibodies in patients

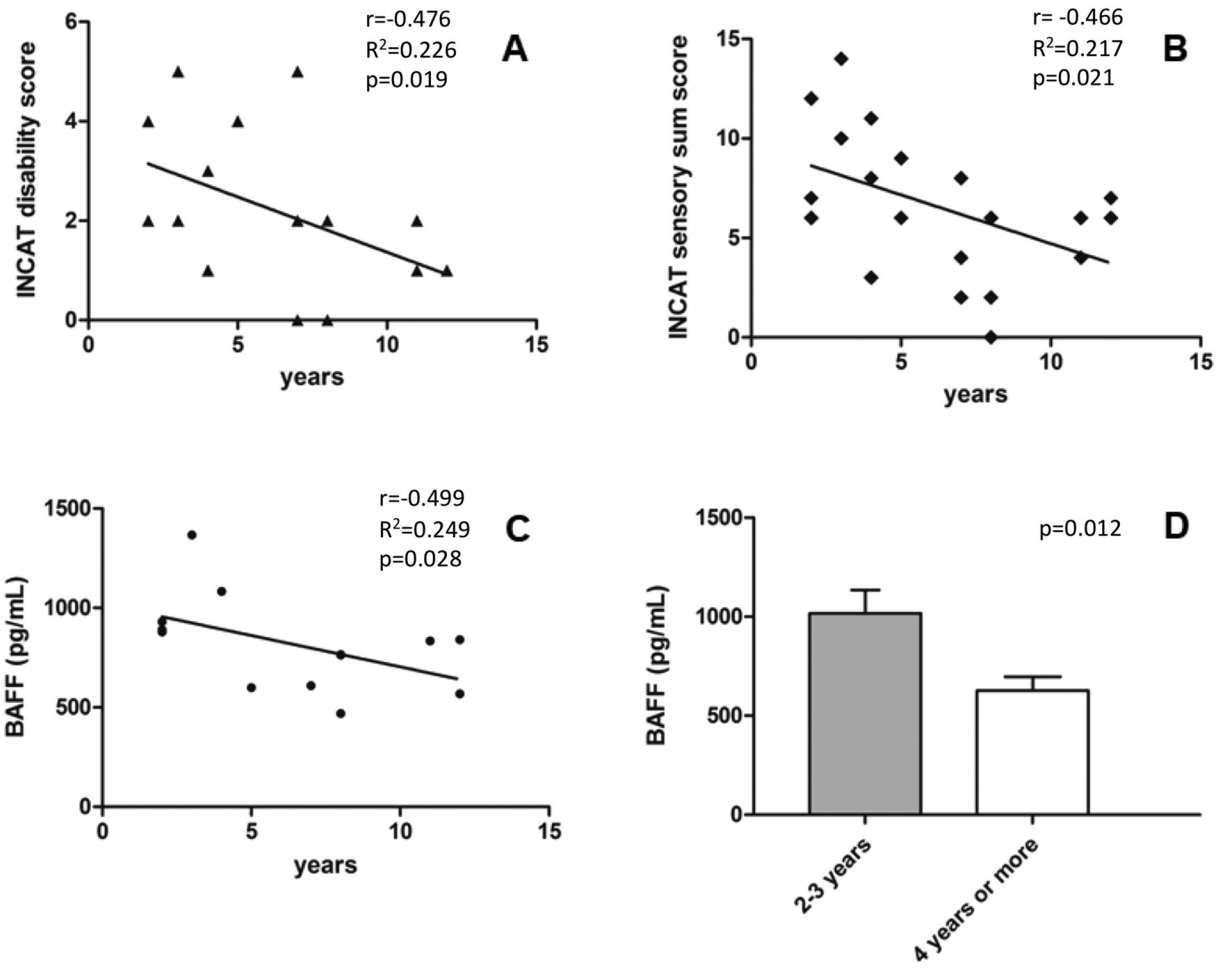

Fig. 1. Clinical and biological correlations in rituximab-treated patients with anti-MAG polyneuropathy.
Benefit duration correlates with the following features measured at onset: INCAT scores ($p = .019$; $R^2 = 0.226$; $r = -0.476$; A); ISS scale scores ($p = .021$; $R^2 = 0.217$; $r = -0.466$; B); serum BAFF levels ($p = .028$; $R^2 = 0.249$; $r = -0.499$; C). Mean serum BAFF levels in patients relapsing after 2–3 years were higher than those in patients relapsing at 4 year or later (2–3 years = 1017.0 ± 117.2 pg/mL; 4 years or more = 627.9 ± 70.5 pg/mL; $p = .012$; D).

with rituximab-treated autoimmune diseases (Taylor and Lindorfer, 2008), likely due to the persistence of long-lived plasma cells.

Unlike what has happened in rheumatology, there is no wide consensus on the schemes of maintenance therapy for rituximab in neurology. In neuromyelitis optica spectrum disorders, for instance, single rituximab re-infusions whenever blood CD27+ memory B cells re-emerge at given percentages have been proposed (Kim et al., 2013). In MAG polyneuropathy too, this B-cell subset might be a promising biomarker of re-infusion especially when coupled with early signs of clinical relapse (Dalakas, 2018; Dalakas et al., 2009). The lack of maintenance therapy likely favored irreversible axonal damage and accumulation of disability in some "partially−/non-responders", but as many as 71% of our patients were long-lasting responders. This extraordinarily high frequency challenges the schemes of rituximab re-treatments 6–12 months after the first infusion to stabilize clinical pictures (Nobile-Orazio et al., 2017). Therefore, both serum BAFF measurements and the severity of clinical pictures at onset of MAG polyneuropathy might help select patients deserving maintenance therapy. Finally, our long-lasting monitoring confirms the excellent safety profile of rituximab.

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Competing interests

None declared.

Ethics approval

The study was approved by the local ethics committees, and written informed consents obtained from all the patients.

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