Rituximab-associated hypogammaglobulinaemia in ANCA-associated vasculitis and connective tissue diseases: a longitudinal observational study

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Key words: ANCA-associated vasculitis, connective tissue diseases, rituximab, hypogammaglobulinaemia, infections

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ABSTRACT

Objective. *The burden of hypogammaglobulinaemia following rituximab (RTX) treatment in rheumatic diseases has not been fully elucidated yet. Our aim was to evaluate the frequency and predictors of hypogammaglobulinaemia in patients affected by ANCA-associated vasculitis (AAV) and connective tissue diseases (CTD).*

Methods. *We retrospectively reviewed prospectively collected data of patients receiving RTX. Immunoglobulins (Ig) levels and lymphocyte subsets were recorded at RTX administration and 3-6 months later. We assessed frequency of hypogammaglobulinaemia (serum IgG<6 g/L) and its related events. Univariate and multivariable analysis were performed using SPSS 20.0 package.*

Results. *Sixty-eight patients (30 AAV, 25 systemic lupus erythematosus, 9 systemic sclerosis and 4 idiopathic inflammatory myopathies) were treated with RTX (95 infusions, median 2 [2-6]). Following RTX, IgG<6 g/L were observed in 15/68 patients (15.8%), IgM<0.4 g/L in 28/68 (41%) and IgA<0.7 g/L in 7/68 (10.2%). Hypogammaglobulinaemia was more common in patients with AAV (p=0.008), short disease duration (p=0.001), low IgG levels at baseline (p=0.008), high cyclophosphamide exposure (p=0.018), high daily and cumulative prednisone dosage (p=0.001 and p=0.006). At multivariate analysis, cumulative cyclophosphamide dosage (OR 1.1 [1.0-1.3] p=0.045), daily prednisone intake >15mg (OR 9.5 [2.2-41.7] p=0.03) and IgG levels before RTX (OR 0.74 [0.59-0.93] p=0.009) were independent predictors of hypogammaglobulinaemia. Five patients experienced severe infections within 12 months, more frequently in those with IgG<6 g/L (26.7% vs 1.9%, p=0.007).*

Conclusion. *Hypogammaglobulinaemia following RTX is uncommon in AAV and CTD and is more likely in patients with high glucocorticoids and cyclophosphamide exposure and low IgG levels at baseline.*

Introduction

Rituximab (RTX) is a chimeric human-mouse monoclonal antibody against CD20, widely used in the treatment of B-cell lymphomas and several other autoimmune conditions, including rheumatoid arthritis (RA), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and connective tissue diseases (CTD) (1-10), where a relapsing-remitting disease course often requires repeated drug infusions leading to high cumulative doses (11-13).

RTX causes a rapid depletion of CD20-expressing B-cell precursors and mature B-cells, which can persist for several months (14). The depletion is induced by antibody-dependent, complement-mediated cellular cytotoxicity and apoptosis (15). The rationale of using RTX in malignant and autoimmune diseases is to eradicate malignant or autoreactive B cells (16, 17). RTX is also able to modulate the T cell compartment, through the decrease in antigen presentation to pathogenic auto-reactive T cells as well as through the modulation of the regulatory T cell compartment (16, 17).

From 20% to 40% of patients treated with RTX might develop hypogammaglobulinaemia (18-22). RTX-associated persistent hypogammaglobulinaemia is more common in patients with malignancy, but it can also be observed in patients with autoimmune diseases (20, 23, 24). In autoimmune diseases, hypogammaglobulinaemia occurring early after anti-CD20 treatment can be multifactorial, due to active disease and the effect of other drugs, *e.g.* glucocorticoids, cyclophosphamide (CYC)

or mycophenolate mofetil (MMF) (25, 26), and usually transient, with a minimal increase in the risk of infections (27).

However, hypogammaglobulinaemia occurring late (after 12 months) after RTX may be sustained in a minority of patients and can be related to prolonged B-cell depletion. It can occur even after peripheral B-cells recovery, sharing some similarities with common variable immune deficiency (CVID) (28, 29). Severe or recurrent infections occur less frequently in patients with RTX induced hypogammaglobulinaemia compared with CVID patients (20). Infections are typically sino-pulmonary, mostly related to encapsulated bacteria, and might benefit from immunoglobulin (Ig) replacement therapy (25). Indeed, AAV patients with IgG <3.75 g/L after RTX treatment had an increased risk of hospitalisation due to infection [odds ratio (OR) 21.1] (30), likewise RA patients with IgG <6 g/L after RTX were at higher risk of severe infections (26.1% *vs*. 6.3%, p=0.033) (31) and systemic lupus erythematosus (SLE) patients with low IgM levels after RTX developed severe infections in 7% of the cases (32).

The aim of our study was to evaluate the frequency, severity and predictors of hypogammaglobulinaemia in patients affected with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), SLE, systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) treated with RTX.

Methods

We retrospectively reviewed prospectively-collected data from all patients with AAV, SLE, SSc and IIM treated with RTX between 2007 and 2016 in a single tertiary referral Rheumatologic Centre. Inclusion criteria were the fulfillment of disease-specific classification criteria [the American College of Rheumatology (ACR) criteria or 2012 Chapel Hill Consensus definitions for GPA/MPA (33, 34), the 2017 ACR criteria or 2019 EULAR (European League Against Rheumatism) criteria for SLE (35, 36), the ACR 1980 or the ACR/EULAR 2013 classification criteria for SSc (37, 38) and the Bohan and Peter's criteria for IIM (39, 40)], and the measurement of lymphocyte subsets and serum Ig levels at the time of RTX administration (maximum 2 months before) and 3 to 6 months later, consistently with previous studies (26). The minimum required follow-up was 1 year after RTX administration.

The indication of RTX treatment was remission-induction of organ-threatening or life-threatening in AAV patients (41) and persistent active disease, refractory to conventional immunosuppressants, for all other CTD.

Hypogammaglobulinaemia was defined as moderate (serum IgG levels <6 g/L) and severe (IgG <4 g/L), in keeping with other authors (22).

Demographic, clinical, laboratory and treatment variables, including previous and concomitant immunosuppressive and glucocorticoid dosage were analysed. Serological markers included white blood cell count, Ig serum levels (measured by standard nephelometry; normal ranges: IgG 7.0–16.0 g/L, IgM 0.4–2.4 g/L, IgA 0.7–4.0 g/L) and lymphocyte subset counts (measured by flow cytometry assay), assessed as part of routine clinical care.

Number and types of serological or culture-confirmed infections were registered. Severe infections were defined as requiring hospitalisations and/or parenteral antimicrobial therapy. All data were entered into an anonymised electronic sheet.

Our observational study was conducted in accordance with the National Health Research Ethics guidelines.

Statistical analysis

Data are expressed as median and interquartile ranges for continuous variables, if not stated otherwise, and as number (%) for categorical ones. Student's t-test was used to compare the differences between the means of normally distributed variables, and the Mann-Whitney U-test for non-parametric variables. Chi-squared test or Fisher's exact test were used to compare differences in proportions. Spearman's rank-order correlation was used to evaluate strength and direction of association between two ranked nonparametric variables. A two-tailed p-value ≤0.05 was considered statistically significant.

The associations between IgG concentrations and potential risk factors were first determined by univariate analyses. Significant predictors at univariate analysis were entered in a multivariable binary logistic regression model with backward selection (p<0.05 to enter and p<0.10 to stay). Data were analysed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and SPSS v. 20.0 (SPSS, Chicago IL, USA).

Results

Demographic data

We identified 80 patients who received RTX (113 infusions) between 2007 and 2016, out of them twelve were excluded due to incomplete data. We therefore considered 95 RTX infusions, including 41 (43.1%) retreatments [median 2 (2-6)].

Thirty patients were diagnosed with GPA/MPA (44.1%) and 38 (54.9%) with CTD: 25 with SLE (36.8%), 9 with SSc (13.2%) and 4 with IIM (5.9%). They were mostly female (50, 73.5%) and Caucasian (66, 97.1%). The mean age at the time of first RTX treatment was 44.3±14.8 years. Median follow-up was 28 [IQR 6–131] months. All patients had received previous treatment with at least one immunosuppressant, including CYC in the majority of cases (30, 44.1%) with a median CYC cumulative dosage of 5.4 g [3.8–8]. The median disease duration was 74.5 months (IQR 14–141 months).

RTX regimen was used at the dosage of 1000 mg twice, two weeks apart, in 47 patients (49.5%) and at the dosage of 375 mg/m^2 weekly for 4 weeks in 48 patients (50.5%).

At the time of RTX no patients had severe hypogammaglobulinaemia.

The characteristics of the study population are shown in Table I.

Effect of rituximab on serum IgG

We observed a significant decrease of IgG serum levels between baseline and 3-6 months after RTX infusion in all patients (10.0 [8.3–12.6] *vs*. 9.0 [6.4–12.0] g/L, p=0.001). IgG<6 g/L was observed in 15 cases (15.8%), out of them 6 (6.3%) had IgG<4 g/L. Two

Table I. Comparison between AAV and CTD patients treated with RTX.

	All (n=68)	GPA/MPA (n=30)	CTD (n=38)	*p*-value
No. of infusions with complete data	95	46	49	ns
Female (n, %)	50 (73.5)	21 (70)	38 (78.9)	ns
Age†, years (mean ± SD)	44.3 ± 14.8	50.1 ± 15.3	40.3 ± 13.8	0.002
Disease duration†, months	74.5 [14-141]	23 [8.5-63]	128 [74.5-160]	<0.001
Cumulative PDN dosage†, g/year	4.5 [2.9-8.9]	8.15 [5.60-13.60]	3.28 [1.49-4.53]	<0.001
Type of previous IS†				
MTX (%)	28 (41.2)	10 (33.3)	18 (47.4)	ns
AZA (%)	32 (47.1)	18 (60)	14 (36.8)	0.05
MMF (%)	38 (55.9)	10 (33.3)	28 (73.7)	<0.001
CYC (%)	30 (44.1)	17 (56.7)	13 (34.2)	0.06
Cumulative CYC dosage†, g	5.4 [3.8-8]	6 [4.4-12.8]	4.5 [3.6-5.8]	0.04
Number of total previous IS†	2 [1-3]	3 [1.75-3]	2 [2-3]	ns
Nephrotic syndrome† (%)	17 (17.9)	4 (8.9)	17 (34.7)	0.003
IgG at baseline, g/L	10.0 [8.2-12.5]	8.8 [6.7-10.3]	11.1 [9.7-13.9]	<0.001
T CD4+†, /μL	1000 [601-1245]	605 [421-1275]	1000 [850-1155]	ns
B CD19+†, /μL	200 [22-300]	134 [21-253]	220 [200-300]	0.03
RTX regimen (1000 mg twice)	47 (49.5)	19 (41.3)	43 (95.6)	<0.001
Time between RTX and Ig dosage, months	4 [3-6]	3.25 [2.25-4.75]	3.75 [2.75-5.75]	ns
PDN between RTX and Ig dosage, mgs/day	12.5 [5-23.75]	17.2 [9.5-27.9]	6.4 [0.5-14.6]	0.01
IgG after RTX, g/L	9.2 [6.8-13.0]	7.6 [6.1-9.6]	10 [8.0-14.0]	<0.001
ΔIgG%	3.4 [0-10.3]	11.3 [3.8-29.7]	2.3 [0-18.2]	0.047
IgG<6 after RTX, g/L (%)	15 (15.8)	12 (36.4)	3 (8.6)	0.008
IgG<4 after RTX, g/L (%)	6 (6.3)	6 (18.2)	0 (0)	<0.001
Nephrotic syndrome‡ (%)	9 (9.5)	1 (2.9)	8 (23.5)	0.027
T CD4+‡, /μL	825 [679-1193]	769 [555-1174]	900 [800-1075]	ns
B CD19+‡, /μL	0 [0-0]	0 [0-0]	0 [0-0]	ns
Severe infections‡ (%)	5 (5.2)	3 (7.3)	2 (4)	ns
PJP prophylaxis (%)	23 (24.2)	22 (50)	1 (2.6)	<0.001

†At RTX infusion; ‡At first serum Ig determination after RTX infusion.
If not otherwise specified, data are expressed as median and interquartile range (IQR).
GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiits; CTD: connective tissue disease; PDN: prednisone; IS: immunosuppressant; MTX: methotrexate; AZA: azathioprine; MMF: mycophenolate mofetil; CYC: cyclophosphamide; PJP: Pneumocystis jiroveci Pneumonia.

patients (2.9%) had low IgG (IgG <6 g/L) before treatment.

In 11 cases (73.4%) hypogammaglobulinaemia was transient and improved spontaneously, while in 4 patients it was persistent, requiring intravenous immunoglobulin replacement and RTX discontinuation.

Low IgM serum levels were observed after RTX in 28 patients (41%), with a significant decrease from baseline (0.55 [0.4–1] *vs*. 0.41 [0.25–0.74], *p*=0.001). Nineteen patients (28%) had low levels of IgM (<0.4 g/L) before treatment. Low IgA serum levels were seen after RTX in 7 patients (10.2%), with a significant decrease from baseline (2 [1.22–2.6] *vs*. 1.5 [1–2.2], *p*=0.03). Five patients (7%) had low levels of IgA (<0.7 g/L) before RTX.

The median percent decrease after RTX was significantly greater for IgM (16.6 [4–33] %) than for IgA (8 [0–22] %, *p*=0.04) or IgG (6.7 [0–24.3] %, *p*=0.01).

AAV patients treated with RTX developed more frequently hypogammaglobulinaemia than CTD treated: 12 patients (80%) *vs*. 3 patients (20%), *p*=0.008. Patients who developed hypogammaglobulinaemia, compared to those who did not, were more likely to have a shorter disease duration (22 [3–76] *vs*. 83 [26.5–154] months, *p*=0.001) and lower IgG levels at baseline (6.8 [5.5–10.3] *vs*. 11 [9–13.2] g/L, *p*=0.008). No differences were noted in age and sex.

We observed a significant higher yearly prednisone (PDN) cumulative dosage before RTX (8.6 [5.7–23-6] *vs*. 3.9 [2.3–6.2] g, *p*=0.006) and higher daily PDN dosage following RTX (27.1 [15.8–32.6] *vs*. 8.3 [4.9–14.9] mg/day, *p*=0.001) in patients who developed hypogammaglobulinaemia. There was a negative correlation between yearly cumulative PDN before RTX (r = -0.56 [-0.77 to -0.27], *p*=0.001) and daily PDN exposure after RTX (r = -0.54 [-0.74 to -0.24], *p*=0.001) and IgG levels. A significant higher CYC cumulative dosage was observed (16.4 [5-18] *vs*. 4.9 [3.8–6] g, *p*=0.018) in patients who developed hypogammaglobulinaemia. No significant differences in the decline of serum IgG concentrations between patients treated with MMF and those receiving other immunosuppressants were observed. Nephrotic range proteinuria was not associated with low levels of IgG. Baseline variables in the two groups are summarised in Table II.

After RTX, all patients developed complete B-cells depletion (undetectable or below 50 cells/μL) after a median time of 4 [3–6] months.

Only 5 patients experienced severe infections within 12 months, requiring hospitalisation. Severe infections were more common in patients with IgG<6 g/L than in those with IgG ≥6 g/L (4 *vs*. 1, *p*=0.007). There was no difference in the proportion of patients with infections according to IgM and IgA

Table II. Clinical characteristics of patients according to development of hypogammaglobulinaemia following RTX treatment.

	IgG<6 g/L (n=15)	IgG≥6 g/L (n=53)	*p*-value
Age†, years (mean±SD)	49.1 ± 16.2	42.7 ± 14.2	ns
Disease duration†, months	22 [3-76]	83 [26.5-154]	0.001
Cumulative PDN dosage†, g/year	8.6 [5.7-23.6]	3.9 [2.3-6.2]	0.006
Number of total previous IS†	2 [1-3]	2 [1.5-3]	ns
Previous CYC (%)	5 (33.3)	20 (37.7)	ns
Cumulative CYC dosage†, g	16.4 [5-18]	4.9 [3.8-6]	0.018
Current IS at RTX infusion†			
MMF (%)	5 (33.3)	20 (37.8)	ns
Others IS (%)	10 (66.7)	33 (62.2)	ns
Nephrotic syndrome† (%)	5 (30)	12 (22.6)	ns
IgG‡, g/L	6.8 [5.5-10.3]	11 [9-13.2]	0.008
RTX regimen (1000 mg twice)	7 (46.7)	40 (78.4)	0.025
RTX dosage, g	2 [2-2]	2 [2-2]	ns
PDN between RTX and Ig dosage, mg/day	27.1 [15.8-32.6]	8.3 [4.9-14.9]	<0.001
Nephrotic syndrome‡ (%)	1 (6.7)	7 (13.5)	ns
Severe infections‡ (%)	4 (26.7)	1 (1.9)	0.007
PJP prophylaxis (%)	8 (53.3)	11 (23.4)	ns

†At RTX infusion; ‡At first serum Ig determination after RTX infusion.
If not otherwise specified, data are expressed as median and interquartile range (IQR).
GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiits; CTD: connective tissue disease; PDN: prednisone; IS: immunosuppressant; RTX: rituximab; MMF: mycophenolate mofetil; CYC: cyclophosphamide; PJP: Pneumocystis jiroveci Pneumonia.

serum levels. Three were pulmonary infections, one complicated urinary tract infection and a case of progressive multifocal leukoencephalopathy in one patient with SLE, who never developed hypogammaglobulinaemia and had a positive cerebrospinal fluid test for JC-virus. Only two cases of opportunistic infections were reported, both oral/oesophageal Candida Albicans infections. Prophylactic sulphamethoxazole-trimethoprim was administered to 19 patients (30.6%). No deaths were reported.

Predictive factors for the development of hypogammaglobulinaemia

At univariate analysis, IgG<6 g/L was significantly associated with CYC cumulative dosage before RTX (OR 1.2 [1.1–1.3], $p<0.001$), RTX 375 mg/m^2 weekly regimen (OR 4.1 [1.2–13.9], $p=0.017$), yearly PDN cumulative dosage (OR 6.6 [1.3–33.6], $p<0.001$), daily PDN intake >15 mg/day after RTX (OR 12.7 [3.1–52.5], $p<0.001$) and IgG levels before RTX (OR 0.67 [0.55–0.83], $p<0.001$).

At multivariate analysis, CYC cumulative dosage before RTX (1.1 [1.0– 1.3], $p=0.045$), daily PDN intake >15 mg/day after RTX (OR 4.7 [1.1–21.5], $p=0.043$) and IgG levels before RTX (OR 0.74 [0.59-0.93], $p=0.009$) were independent predictors of hypogammaglobulineamia (Table III).

Differences between AAV and CTD

Comparison between patients affected with GPA/MPA and CTD treated with RTX is reported in Table I. At the time of infusion GPA/MPA patients were older ($p=0.002$), had a shorter disease duration ($p=0.001$) and lower median IgG levels ($p<0.001$) despite a lower rate of nephrotic syndrome ($p=0.003$). During the follow-up, AAV patients developed a significantly greater decrease in IgG levels (11.3 [3.8–29.7] *vs*. 2.3 [0–18.2] g/L, $p=0.047$), resulting in significantly higher frequency of IgG <6 g/L (12 *vs*. 3 patients, $p=0.008$) (Fig. 1).

Moreover, patients with GPA/MPA compared to CTD were more frequently treated with Azathioprine ($p=0.007$), CYC (<0.001), RTX 375 mg/m^2 weekly regimen ($p<0.001$), and displayed a higher cumulative and daily PDN dosage ($p<0.001$ and $p=0.01$, respectively) (Fig. 2).

Discussion

We assessed 68 patients who were treated with RTX due to systemic autoimmune diseases. During the therapeutic course, low levels of IgG, IgM and IgA occurred in 15.8%, 41% and 10.2% of patients, respectively, in keeping with previous data (20, 22). Different effects on Ig classes have been demonstrated in patients with rheumatoid arthritis, SLE or lymphoma (20, 42, 43) and could be related to the type of B cells affected by RTX. Serum IgG and IgA are mainly produced by long-lived CD20- plasma cells, dwelling in the bone marrow, which are not directly targeted by RTX (20). Conversely, serum IgM are de-

Table III. Univariate and multivariable analysis for variables associated to development of hypogammaglobulinaemia following RTX treatment.

Variables	Univariate			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Cumulative CYC dosage before RTX	1.2	1.1 – 1.3	<0.001	1.1	1.0 – 1.3	0.045
RTX 375 mg/m^2 weekly regimen	4.1	1.2 – 13.9	0.017	-	-	-
Cumulative PDN yearly dosage before RTX	6.6	1.3 – 33.6	<0.001	-	-	-
PDN intake >15 mg/day after RTX	12.7	3.1 – 52.5	<0.001	9.5	2.2 – 41.7	0.031
IgG levels before RTX (per 100-mg increase)	0.67	0.55 – 0.83	<0.001	0.74	0.59 – 0.93	0.009

CI: confidence intervals; CYC: cyclophosphamide; GPA: granulomatosis with polyangiitis; IgG: immunoglobulins G; MPA: microscopic polyangiits; OR: odds ratio; PDN: prednisone; RTX: rituximab.

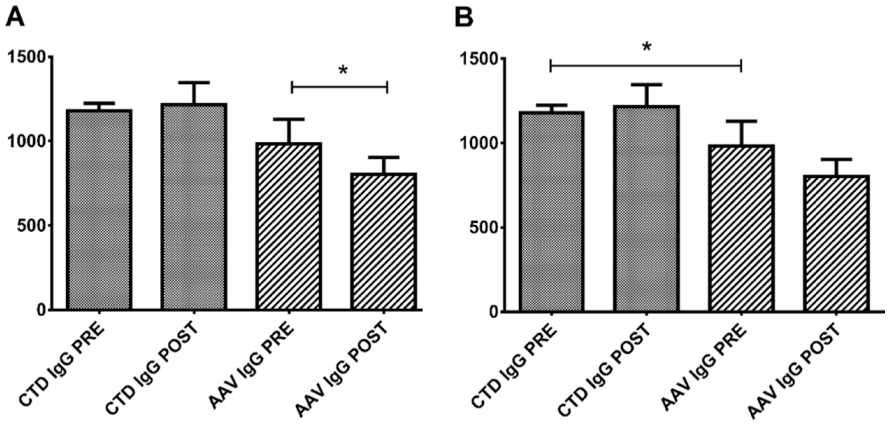

Fig. 1: (**A**) Significant reduction of IgG (mg/dl) in AAV patients. (**B**) Significant lower IgG (mg/dl) at baseline in AAV patients compared to CTD.
*p<0.05. AAV: ANCA-associated vasculitis; CTD: connective tissue diseases.

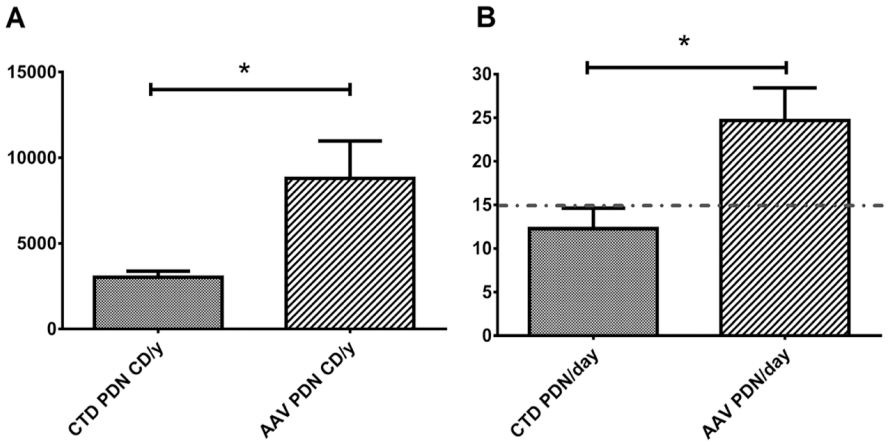

Fig. 2. Significantly higher PDN (prednisone) cumulative (A) and daily (B) dosage in AAV patients compared to CTD. The red line marks the 15 mg PDN daily dosage.
*p<0.05. AAV: ANCA-associated vasculitis; CTD: connective tissue diseases.

rived from short-lived peri-follicular B cells and un-switched marginal zone B cells, which are preferentially depleted by RTX (44, 45). Hypogammaglobulinaemia was in the majority of the cases transient with spontaneous recovery/improvement, in keeping with previous studies (25, 46, 47).

Data on the prevalence and incidence of hypogammaglobulinaemia following RTX mostly derive from retrospective observational cohort studies, both in malignant (48, 49) and non-malignant conditions (21, 22, 50), yet the frequency of hypogammaglobulinaemia and/or impaired B-cell reconstitution has never been evaluated in prospective studies.

The prevalence of hypogammaglobulinaemia was reported to be 39–42.9% in patients with lymphoma, although the rate of symptomatic hypogammaglobulinaemia, presenting with recurrent infections was significantly lower (6.6%) (19). An observational study carried out in 119 RA patients reported a prevalence of 11.8% of hypogammaglobulinaemia after one course of B-cell depleting therapy (20). In patients with autoimmune diseases (SLE and AAV), the prevalence of hypogammaglobulinaemia ranged between 14.9% and 26.3% (21, 32), in keeping with our data.

Interestingly, in our cohort hypogammaglobulinaemia occurred more frequently in AAV than in CTD. However, our AAV patients were older, treated with higher cumulative and daily PDN dosage and higher CYC cumulative dosage and had lower IgG levels before RTX treatment, compared with CTD patients. Notably, IgG fluctuations in AAV patients were reported to be related to multiple factors, such as the older age, concomitant treatments, disease severity and comorbidities (51).

Importantly, low serum IgG, daily and cumulative PDN dosages and cumulative CYC dosage were significantly associated with the occurrence of low IgG levels after RTX in our study, thus accounting for AAV patients to be more susceptible to hypogammaglobulinaemia development.

Immunosuppressive treatment, namely CYC and MMF, was associated with hypogammaglobulinaemia in patients with autoimmune diseases (21, 22). In our study, cumulative CYC dosage before RTX and daily PDN intake above 15 mg per day after RTX were independent predictors of hypogammaglobulinaemia, supporting a major synergistic effect of CYC and glucocorticoids on serum IgG concentration (26), whereas no association was retrieved for MMF.

Glucocorticoids, besides impairing T-cell function, have been reported to decrease B-cell proliferation leading to a general redistribution of lymphocytes from the intravascular compartment into tissues (52) and causing secondary hypogammaglobulinaemia, even though the underlying mechanism remains unknown (53, 54).

As CYC can deplete B lymphocytes, an addictive effect with RTX might cause a prolonged depletion of B cells, resulting in a significant decline of serum immunoglobulins. Long-lasting decrease of serum Ig and delayed B cell reconstitution were observed in a group of AAV patients treated with sequential CYC and RTX (26), but the synergistic mode of action has not been completely understood yet.

Besides the effects of treatment, hypogammaglobulinaemia in patients with autoimmune diseases, may be related also to active disease and inflammation and may recover over 6-12 months following better disease control and withdrawal of immunosuppressants and glucocorticoids (21). In contrast, later onset hypogammaglobulinaemia is more likely to be sustained (> 6 months) and may be a result of prolonged B cell depletion (28, 29). In our study hypogammaglobulinaemia was transient in the majority of the cases

and improved spontaneously. This was particularly evident in patients with hypogammaglobulinaemia occurring within the first 6 months, where a multifactorial aetiology is likely to occur. IgG monitoring at least after 6 months may be useful in identifying moderate to severe hypogammaglobulinaemia, as also suggested by EULAR recommendations (41).

A substantial increase in the incidence of infections was reported in patients treated with RTX (55), especially a subtype of non-neutropenic infections (56). The clinical impact of hypogammaglobulinaemia following RTX treatment is still debated, especially when hypogammaglobulinaemia is not directly associated with an increased risk of infections (57). In our study only 5 severe infections requiring hospitalisation were observed within 12 months after RTX infusions, more frequently in patients with severe hypogammaglobulinaemia *i.e.* IgG <4 g/L. The low rate of infections could be related also to the extensive use of Pneumocystis jiroveci pneumonia prophylaxis, as suggested by EULAR recommendations for AAV (41).

The need and the duration of immunoglobulin replacement therapy have to be tailored to each patient, taking into account that secondary hypogammaglobulinaemia may recover (58).

Our study has some limitations, mainly the small sample size and the Caucasian ethnicity of the study cohort, which prevents global inference. The retrospective design could be also a limit, but data were prospectively collected from a single tertiary centre.

Conclusions

In conclusion, moderate or severe hypogammaglobulinaemia following RTX treatment is quite uncommon and seems more likely in patients in whom PDN cannot be successfully tapered, suggesting a more severe or refractory disease requiring prolonged glucocorticoids and immunosuppressants exposure, especially CYC. IgG monitoring at least 6 months after RTX treatment may be useful in stratifying patients in order to identify those who require closer monitoring and immunoglobulin replacement therapy.

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