Serum immunoglobulin levels and risk factors for hypogammaglobulinemia during long-term maintenance therapy with Rituximab in patients with Granulomatosis with polyangiitis

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Short title: Hypogammaglobulinemia on Rituximab

ABSTRACT

Objective: Rituximab (RTX) is a B cell depleting agent used to induce and maintain remission in patients with Granulomatosis with polyangiitis (GPA). As the development of hypogammaglobulinemia in GPA patients on long-term RTX has not been addressed, the aim of this study was to investigate changes in immunoglobulin levels and risk factors for hypogammaglobulinemia during long-term RTX maintenance therapy in GPA.

Methods: Single centre cohort study of 29 GPA patients who received median total cumulative dose of cyclophosphamide (CYC) of 17g and were treated with RTX 2g followed by retreatment with either 2g once annually, 1g biannually or a combination of both. Immunoglobulin (Ig) levels were measured before each RTX re-treatment and hypogammaglobulinemia was defined as levels of total immunoglobulin < 6g/l.

Results: During a median follow-up of 4 years, patients received a cumulative dose of 9g RTX. While serum immunoglobulin levels decreased during RTX maintenance, the largest decrease occurred after the first infusion. Baseline Ig levels and the CYC cumulative dose predicted Ig levels whereas the RTX cumulative dose did not. Eight patients (28%) discontinued RTX due to hypogammaglobulinemia. Male gender (HR=8.7 p=0.044), kidney involvement (HR=6.5 p=0.083) and the 1g biannually regimen (HR=8.0 p=0.024) increased the risk to discontinue RTX due to hypogammaglobulinemia whereas orbital-subglottic involvement (HR=0.23 p=0.080) decreased it.

Conclusion: Hypogammaglobulinemia occurred in a quarter of GPA patients during RTX maintenance, independently of the RTX cumulative dose. Male gender, kidney involvement and the 1g biannually RTX regimen constitute risk factors for severe hypogammaglobulinemia necessitating withdrawal of RTX.

INTRODUCTION

Rituximab (RTX) is a chimeric human-mouse monoclonal antibody that gives rapid sustained depletion of premature and mature B-cells through antibody-dependent, complement-mediated cellular cytotoxicity and apoptosis [1]. RTX reduces auto-antibody producing

precursor plasma cells, inhibits B cells interaction with autoreactive T cells and decreases the level of soluble factors secreted by B cells [1]. B cells usually return to pre-treatment level within twelve months after RTX [2].

RTX is approved for the treatment of rheumatoid arthritis (RA) and ANCA-associated vasculitis (AAV) and used off label in a large number of autoimmune conditions [3]. In AAV, RTX is used both for induction [4,5] and maintenance [6-10] of remission through iterative RTX infusions. Relevant side effects include late onset neutropenia, hypogammaglobulinemia development and an increased risk for infections [11-14].

However the long-term safety profile of RTX in AAV is not as well documented as in RA [13-15]. RTX is associated with a greater decrease in immunoglobulin (Ig) levels in RA patients treated with a cumulative dose of 5 grams or more [16] and in AAV patients previously treated with cyclophosphamide (CYC) [17]. In the light of its multiple immune modulating mechanisms, the long-term effects of RTX on natural autoantibodies production and T and NK cells levels are of special interest.

To analyse the impact on Ig levels and lymphocyte subpopulations and to determine risk factors to hypogammaglobulinemia during long-term RTX maintenance, we investigated the course of these variables in 29 patients with Granulomatosis with polyangiitis (GPA) who received RTX 5g or more at our centre.

METHODS

Since 2001 the vasculitis disease registry (Nordnorsk Vaskulittregister) has collected information on disease presentation and course from patients with an established diagnosis of primary vasculitis followed at the University Hospital of North Norway. All patients gave informed written consent at registry inclusion according to the declaration of Helsinki. The

study did not require formal ethical approval in accordance with the standards applied in Norway.

This analysis concerns 29 GPA patients from that registry who were treated with RTX between April 2004 and September 2011. All patients satisfied the American College of Rheumatology 1990 classification and/or Chapel Hill consensus conference criteria.

The patients -15 men (52%) and 14 women (48%) with a median age of 50 (9-75) years at RTX initiation – were mostly ANCA positive (86% PR3-ANCA, 3% MPO-ANCA and 10% ANCA negative) at diagnosis and had renal (59%), pulmonary (66%) and orbital-subglottic (62%) involvements. At RTX initiation, patients had median disease duration of 57 (22-70) months and they had received a median total CYC cumulative dose of 17 (0-250) g.

RTX treatment was initiated as two 1-gram infusions 2 weeks apart with co-administration of methylprednisolone 125 mg, paracetamol 1000 mg and either cetirizine 10 mg or polaramine 4 mg (RA protocol). Due to the observed RTX efficacy and the relapsing nature of GPA, RTX was then re-administered pre-emptively either as 2g infusion (1g twice during a fortnight) annually or as 1g infusion biannually (1g every 6 months). RTX 1g biannually became the preferred maintenance regimen as it was found to better sustain B cell depletion than the 2g annually regimen. During maintenance, 12 (41%) patients received the 1g biannually regimen, 6 (21%) patients received the 2g annually regimen and 11 (38%) patients alternated between regimens.

RTX was added to other immunosuppressive drugs (ID) (other than prednisolone) in 27 patients (93%), while 2 patients (7%) received RTX in monotherapy. Nine patients (31%) were given RTX in combination with a median CYC dose of 5 (2.8-6) grams. Median duration of combination treatment of RTX with other ID was 24 (1-54) months. The timing

and pace of ID discontinuation was at the discretion of the treating physician, whereas the daily prednisolone dose was tapered from a median of 22.5 mg at baseline and discontinued in a protocolized manner. Patients received a median cumulative RTX dose of 9 (5-13) grams and were followed a median of 49 (19-88) months after RTX initiation. At last visit, 17 patients (59%) were still treated with long-term pre-emptive RTX and almost all (except for 3 patients) had discontinued other ID. Twelve patients (41%) had discontinued RTX mainly due to clinical concerns about hypogammaglobulinemia (67%) and recurrent infections (42%). One patient (3%) died during the study period.

Fixed set of blood tests (including ANCA, Ig classes' quantification and flow cytometric immunophenotyping of lymphocytes) were performed prior RTX initiation and before each new re-treatment until either 30th September 2011 (closing date of this study) regardless of ongoing therapy - or shortly before the start of intravenous immunoglobulins (IVIG) administration due to hypogammaglobulinemia and/or infections. Ig levels were measured by nephelometry with normal ranges defined as IgG 7.0-16.0g/L, IgA 0.7-4.0g/L, IgM 0.4-2.3g/L. Total Ig was defined as the sum of IgG, IgA and IgM serum levels. Lowest levels of Ig during long-term RTX maintenance – nadir Ig levels - were recorded. Decline in Ig between each RTX 2g increment at 2, 4, 6 and 8g of RTX were determined. Hypogammaglobulinemia was defined as total Ig under 6g/L.

Data were analysed with SPSS version 20.0 (SPSS Ltd, Chicago IL, USA). Descriptive studies were first performed to assess Ig levels and lymphocytes' subtypes for each 2g RTX increment. Linear regression models were afterwards used to identify factors predicting levels for each Ig class during RTX long-term pre-emptive maintenance; the linear regression fit was assessed by visual inspection of the residuals plot. Patients that discontinued due to

hypogammaglobulinemia were compared with the remaining patients from the cohort (control group). Difference in immunoglobulin serum levels and their decline between groups were analysed using Mann-Whitney U tests. Kaplan-Meier survival curves were used to identify factors responsible for RTX discontinuation due to hypogammaglobulinemia. Finally, logistic binary regression and Cox's proportional hazard models – unadjusted and multivariate analysis with backward Wald selection (variable removed if p>0.1) - were used to determine individual risk factors and the most important single risk factor for hypogammaglobulinemia. P-values <0.05 were considered significant

RESULTS

Changes in Ig levels

About 30% of the patients had low levels of IgG (<7 g/L) and/or IgM (<0.4 g/L) prior to RTX initiation. The proportion of patients with low levels IgG and/or IgM increased to about 60% after the administration of 2g RTX and to about 70% after 6g RTX. The proportion of patients with low IgA (<0.7 g/L) increased from 7% after RTX 2g to 29% after 10g RTX. None of the patients was hypogammaglobulinemic prior to RTX initiation, but 11% and 16% were hypogammaglobulinemic after respectively RTX 2g and RTX 6g (Table 1).

Median levels of total Ig and its classes showed a continuous decrease under long-term RTX maintenance (Table1). However, the largest decline was seen after the first 2g of RTX with the decrease becoming less pronounced in the following rounds (Figure1). In contrast, NK and CD4 cell counts as well as CD4/CD8 ratio increased (Table 1).

Predictors of Ig levels during RTX maintenance

Ig levels during long-term pre-emptive RTX maintenance were partly dependent of the levels in Ig at baseline – except for IgA (R^2=0.079, p=0.076) (See supplementary Table S1,

available at *Rheumatology* Online). This relation was particularly strong with IgM (R^2=0.561, p<0.001) and to a lesser extent with IgG (R^2=0.179, p=0.013).

Male gender and older age decreased Ig levels, except for IgA. Men had 2g/L lower IgG level at nadir compared to women during long-term RTX maintenance. The RTX cumulative dose did not predict the levels of any Ig classes during long-term RTX maintenance. On the other hand the cumulative CYC dose significantly decreased the IgG (R^2=0.107, p=0.047) and IgA (R^2=0.133, p=0.029) levels. CD4 cell count at baseline did not predict levels of any Ig classes, although higher CD4 cell count at baseline seemed to protect from lower IgG levels (R^2=0.065 p=0.098). Variability of Ig levels during long-term RTX maintenance – determined by multiple linear regression models with backward selection - differed in Ig classes. Age, total CYC cumulative dose and baseline IgG level explained 40% of the variability of IgG level during RTX maintenance while male gender, total CYC cumulative dose and baseline IgM level explained 67% of the variability of IgM. However the total CYC cumulative dose was the lone predictor of IgA level, only explaining 13% of its variability.

RTX discontinuation due to hypogammaglobulinemia

Patient characteristics

Eight patients (28%) discontinued RTX due to hypogammaglobulinemia. They had their first episode of hypogammaglobulinemia a median of 63 (17-212) weeks from RTX initiation before they discontinued RTX after a median of 189 (100-356) weeks.

Hypogammaglobulinemia was complicated in 6 patients: 2 patients had concomitant severe infections; 3 had recurrent infections and 2 had late onset neutropenia. These patients were mostly male, had received a higher CYC cumulative dose and received the 1g RTX biannually maintenance regimen (Table 2). Five patients had kidney involvement without orbital-subglottic involvement whereas 1 patient had orbital-subglottic involvement without

kidney involvement. Three of these 8 patients had had a previous episode of transient hypogammaglobulinemia (total Ig levels ranging from 3.7 to 5.0 g/L) before RTX initiation (a median of 3 years from GPA diagnosis) while receiving CYC.

In addition to the 8 patients that discontinued RTX, 5 other patients had hypogammaglobulinemia during RTX maintenance, without RTX being discontinued. One patient had 2 episodes of hypogammaglobulinemia, but none had transient hypogammaglobulinemia before RTX initiation. They had their first episode of hypogammaglobulinemia 108 (80-218) weeks from RTX initiation lasting 32 (16-156) weeks and they continued RTX maintenance during 291 (156-365) weeks before censoring. One patient did eventually discontinue during the study period after receiving a renal transplant. Five patients received IVIG due to hypogammaglobulinemia; four of them discontinued RTX.

RTX survival time

The mean drug survival time on RTX in our cohort was 304 weeks (95% CI 268-340 weeks). RTX survival time in men was almost 2 years shorter than in women (261 vs 347 weeks; p=0.017). Patients with kidney involvement had shorter mean RTX survival time (275 vs 346 weeks, Log rank p=0.047). On the other hand, patients with orbital-subglottic involvement had longer mean RTX survival time (334 vs 228 weeks; Log rank p=0.056). There was no difference in mean RTX survival time for patients with lung involvement (298 vs 312 weeks; Log rank p=0.696) (See supplementary Figure 1, available at *Rheumatology* Online).

RTX maintenance regimens played an important role in RTX survival time: the 1g biannually regimen decreased significantly the mean RTX survival time compared to the 2g annually regimen and the combination of both regimens (Log rank p=0.027) (Figure 2).

Risk factors to discontinue RTX due to hypogammaglobulinemia

Total Ig levels in patients that discontinued RTX due to hypogammaglobulinemia had an early decline after the first RTX 2g but also a late decline after a RTX cumulative dose superior to 6g compared to the control group (Figure 3 and supplementary Table S2, available at *Rheumatology* Online). IgA levels after RTX 2g seemed to be the single most important risk factor to discontinue RTX due to hypogammaglobulinemia during long-term RTX maintenance (OR=0.01; p=0.069) – adjusted for gender after backward Wald selection (See supplementary Table S3, available at *Rheumatology* Online).

Of interest, patients that discontinued RTX due to hypogammaglobulinemia had lower CD4 cell count after the administration of RTX 2g (0.26 vs 0.39 $x10^9$/L p=0.075) and RTX 4g (0.24 vs 0.50 $x10^9$/l p=0.010) compared to the control group (See supplementary Table S2, available at *Rheumatology* Online).

In unadjusted Cox's proportional hazard regression models, male patients and those treated with the 1g biannually regimen were 8 times more likely to discontinue RTX during long-term maintenance due to hypogammaglobulinemia (Table 3). A 10g increment in the CYC cumulative dose increased the risk to discontinue RTX due to hypogammaglobulinemia by 14% while orbital-subglottic involvement decreased its risk by 77% (p=0.080) (Table 3). Male gender and the 1g biannually regimen for RTX maintenance were the single most independent predictors to discontinue RTX due to hypogammaglobulinemia (respectively HR=8.7 p=0.050 and HR=8.4 p=0.037) after backward Wald selection (Table 3).

DISCUSSION

In GPA patients treated with long-term pre-emptive RTX maintenance, our study showed that the largest serum Ig decline occurred after the first 2g RTX and that the RTX cumulative dose did not influence Ig levels whereas the cumulative CYC dose did. Male gender and the 1g

biannually regimen were risk factors for RTX discontinuation due to hypogammaglobulinemia.

Previous studies in AAV/GPA reported a decline in Ig levels during long-term remission maintenance with RTX, but studies had different maintenance strategies [8-10]. The proportion of patients with low IgG or IgM at baseline in our study was similar to the study by Smith et al [9], but started to diverge after the administration of RTX 6g as 70% of our patients had low IgG or IgM by then. Our study included patients treated with RTX in combination with other ID whereas the study of Smith et al included mostly patient treated with RTX monotherapy (97%) [9]. Neither this study nor the study of Venhoff et al showed lower Ig levels with the concomitant use of ID during RTX maintenance [17] despite the fact that in our study patients treated longer with concomitant ID had received a higher CYC cumulative dose (data not shown).

Our study shows clearly that GPA patients are different from RA patients in term of immunoglobulin serum levels at RTX initiation and during maintenance. At baseline, 3.5% of the RA patients treated with RTX from the AIR registry had low IgG levels [14] compared to 30% in our study. RA patients had a gradual incidence with low IgM levels (10% after the first round and 40% after the fifth RTX round) [13,18] during long-term RTX re-treatment while the prevalence of low IgG levels was either stable (3-6%) [13] or increased to a lesser extent (12% after the first round to 22% in the fifth round) [18]. CYC use prior RTX administration in GPA patients might explain lower IgG and IgA levels during RTX maintenance. A 10g CYC cumulative dose increment increased the risk to discontinue RTX due to hypogammaglobulinemia confirming the synergistic effect of CYC and RTX in GPA patients [17]. Moreover CYC induced hypogammaglobulinemia before RTX initiation could

also increase the risk to discontinue RTX due to hypogammaglobulinemia during long-term maintenance.

In our study, patients that discontinued RTX due to hypogammaglobulinemia had an early and a late decline of their total immunoglobulin levels during RTX long-term pre-emptive maintenance. The type of RTX maintenance regimen could explain this double-dip in Ig levels. The 1g biannually RTX maintenance regimen increased the risk for hypogammaglobulinemia compared to the 2g-annually regimen (used in RA). Thus allowing B cells recovery during RTX maintenance could prevent hypogammaglobulinemia. Other maintenance strategies allowing B cells repopulation after each re-treatment [10] or after 2 years and a RTX cumulative dose of 6g [9] have not reported an increased risk of hypogammaglobulinemia while patients had an equivalent follow-up time and received the same RTX cumulative dose.

Low levels of Ig at initiation and especially after the first RTX 2g increased the risk of hypogammaglobulinemia. IgA levels usually remained stable under RTX maintenance [13,19] and interestingly RA patients with raised IgA level had a less robust clinical response and earlier B cells repopulation coincident with relapse [19]. In our study, IgA levels after the first RTX 2g seemed to be a reliable independent marker in identifying patient at risk for hypogammaglobulinemia during RTX long-term pre-emptive maintenance as nadir IgA levels were less dependent of their baseline levels and independent of gender and age. Low IgA levels (<1.0g/L) could reflect a deeper B cell depletion.

In our study, CD4 and NK cells levels increased while immunoglobulins decreased during long-term B cell depletion. Of interest, B-cell depletion by RTX at induction in non-sensitized

patients undergoing renal transplantation increased the risk of acute cellular rejection in the first 3 months after transplantation [20]. These consequences of B cell depletion by RTX could also be explained by non-depleted B cells as they enhanced CD4 cells proliferation in vitro [21].

GPA patients with orbital-subglottic involvement seemed to have a reduced risk to discontinue RTX due to hypogammaglobulinemia, whereas GPA patients with kidney involvement seemed to have a higher risk. Toxic effect from aggressive immunosuppressive therapies and renal capillary leakage could increase the risk for hypogammaglobulinemia in GPA patients with kidney involvement [22]. But it is also possible that GPA patients with kidney involvement are generally more immunodeficient compared to patients with orbital-subglottic involvement.

Our retrospective study had some limitations mainly due to the small sample size making subgroups analysis prone to type II errors of not detecting differences and to wide confidence interval in both the logistic binary regression and Cox's proportional hazard models analysis. But the longitudinal data was collected prospectively in a disease registry from a single tertiary centre. We also analysed patients that discontinued RTX due to hypogammaglobulinemia, reflecting a more severe phenotype complicated by infections. RTX discontinuation purely based on isolated hypogammaglobulinemia could have major consequences for ensuing remission maintenance therapy in GPA patients. Further studies are needed to determine if our findings are also valid in microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA).

In conclusion, long-term pre-emptive treatment with RTX 1g every six months increased the risk of discontinuation due to hypogammaglobulinemia in GPA patients. Male gender, kidney involvement, exposure with CYC and low Ig serum levels at RTX initiation were independent risk factors, whereas the RTX cumulative dose did not influence this risk. As hypogammaglobulinemia may limit the number of times anti-CD20 mediated B cell depletion can be carried out [3], close monitoring of immunoglobulins and lymphocytes subtypes during maintenance is warranted as hypogammaglobulinemia with low CD4/CD8 ratio are known risk factors for severe infections in AAV [23].

Key messages

- Compared to RA, rituximab maintenance in granulomatosis with polyangiitis had an increased risk for hypogammaglobulinemia

- Granulomatosis with polyangiitis men with kidney involvement and prior cyclophosphamide exposure discontinued rituximab maintenance due to hypogammaglobulinemia.

- The 1g biannually maintenance regimen in granulomatosis with polyangiitis increased the risk of hypogammaglobulinemia necessitating rituximab withdrawal

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Table 1. Median serum levels of Ig (g/L) and lymphocytes types (x10^9/L) prior to RTX and during long-term remission maintenance.

	Prior RTX	RTX 2g	RTX 4g	RTX 6g	RTX 8g	RTX 10g	RTX 12g	At nadir
No patients	29	28[#]	28[#]	25	14	7	3	29
IgG	7.7	6.1	6.4	6.0	6.3	5.4	4.4	4.9
% with low IgG	28	54	64	68	57	71	67	76
IgA	1.62	1.29	1.24	1.30	1.04	1.57	2.5	1.00
% with low IgA	0	7	7	8	14	29	33	17
IgM	0.68	0.33	0.31	0.28	0.19	0.13	0.11	0.18
% with low IgM	28	64	68	76	71	86	67	76
Total Ig	10	7.9	7.9	7.5	7.9	8.1	7.1	6.3
% with hypogammaglobulinemia	0	11	18	16	29	29	33	45
B cells	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NR
CD4 cells	0.30	0.36	0.46	0.65	0.73	0.71	1.05	NR
CD4/CD8 ratio	1.12	1.02	1.20	1.53	1.35	1.30	2.02	NR
NK cells	0.14	0.17	0.24	0.26	0.31	0.33	0.29	NR

CD: cluster of differentiation; Ig: immunoglobulins; No: number; NR: not reported; RTX: rituximab.
[#] Ig results missing in 1 patient after RTX 2g and after RTX 4g.

Table 2. Comparison between patients that discontinued RTX due to hypogammaglobulinemia and the rest of the cohort (control group)

Results expressed in median (range) and percentage (number of patients) as appropriate. ANOVA and Fisher's exact test were used appropriately to test for difference between groups.

	Patients discontinuing RTX n=8	Controls n=21	p-value
Male	**88% (7)**	**44% (9)**	**0.035**
Age years	53 (23-62)	48 (9-75)	0.753
BVAS at baseline	9.5 (4-12)	10.0 (4-21)	0.334
Kidney involvement	88% (7)	48% (10)	0.093
Lung involvement	75% (6)	62% (13)	0.675
Orbital-subglottic involvement	38% (3)	71% (15)	0.197
CYC cumulative dose g	**51 (2-250)**	**13 (0-60)**	**0.037**
RTX cumulative dose g	7.0 (5-13)	9.0 (5-13)	0.074
MTX under RTX maintenance	25% (2)	43% (9)	0.419
AZA under RTX maintenance	25% (2)	29% (6)	1.000
MMF under RTX maintenance	13% (1)	33% (7)	0.371
1g biannually RTX regimen	50% (4)	38% (8)	0.683
Both RTX regimens	50% (4)	22% (7)	0.433
2g annually RTX regimen	0% (0)	29% (6)	0.148

AZA: azathioprine; BVAS: Birmingham vasculitis activity score; CYC: cyclophosphamide; MMF: mycophenolate mofetil; MTX: methotrexate; RTX: rituximab.
Significant results ($p<0.05$) are highlighted in bold.

Table 3. Hazard ratio to discontinue hypogammaglobulinemia during long-term pre-emptive RTX maintenance.

All values were determined at RTX initiation and were analysed with unadjusted and multivariate Cox-regression model with backward Wald selection (removal if p<0.10).

|	Unadjusted analysis			Multivariate analysis		
	p-value	HR	95% CI	p-value	HR	95% CI
Male gender	**0.044**	**8.7**	**1.06-72**	**0.050**	**8.7**	**1.00-76**
Age (10 years increments)	0.311	1.3	0.79-2.1			
Kidney involvement	0.083	6.5	0.79-54	0.141		
Lung involvement	0.698	1.4	0.28-6.8			
Orbital-subglottic involvement	0.080	0.23	0.05-1.2	0.088		
Baseline Prednisolone dose (mg)	0.958	1.00	0.95-1.1			
Concomitant CYC dose w/ RTX (g)	0.807	0.97	0.74-1.3			
Total cumulative CYC dose (10g)	**0.034**	**1.14**	**1.01-1.3**	0.120		
1g biannually regimen	**0.024**	**8.0**	**1.3-49**	**0.037**	**8.4**	**1.14-63**
B cells ($x10^9$/L)	0.491	0.11	0.01-56			
CD4 cells ($x10^9$/L)	0.431	0.43	0.04-4.7			
IgG (g/L)	0.291	0.82	0.56-1.2			
IgA (g/L)	0.162	0.26	0.04-1.7			
IgM (g/L)	0.657	0.70	0.14-3.4			
Total Ig (g/L)	0.230	0.83	0.61-1.2			

CD: cluster of differentiation; CI: confidence interval; CYC: cyclophosphamide; ID: immunosuppressive drugs; Ig: immunoglobulin; OR: odds ratio; RTX: rituximab. w/: with

Significant results (p<0.05) are highlighted in bold.

Figure 1. Mean of total immunoglobulins decline after every RTX 2g increment

P-value determined by paired sample t-tests.
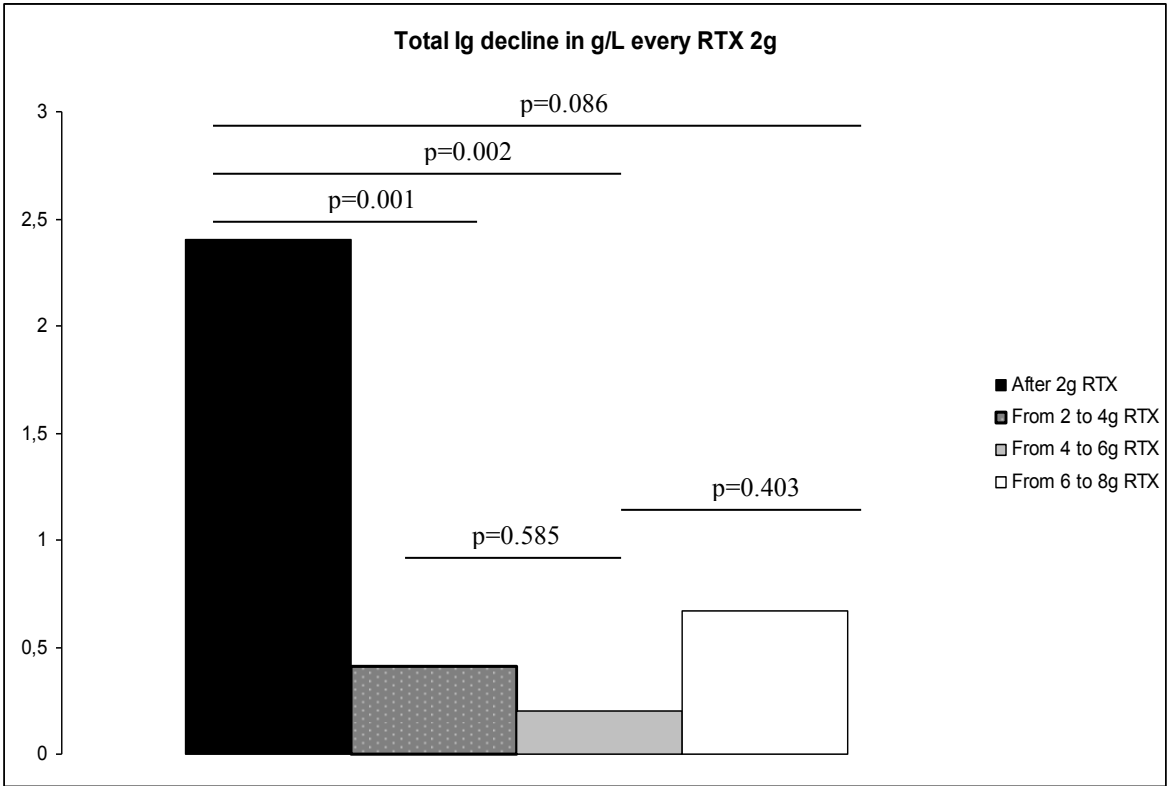
Ig: immunoglobulin; RTX: rituximab.

Figure 2. Kaplan-Meier analysis of the probability to discontinue rituximab due to hypogammaglobulinemia according to type of maintenance regimen
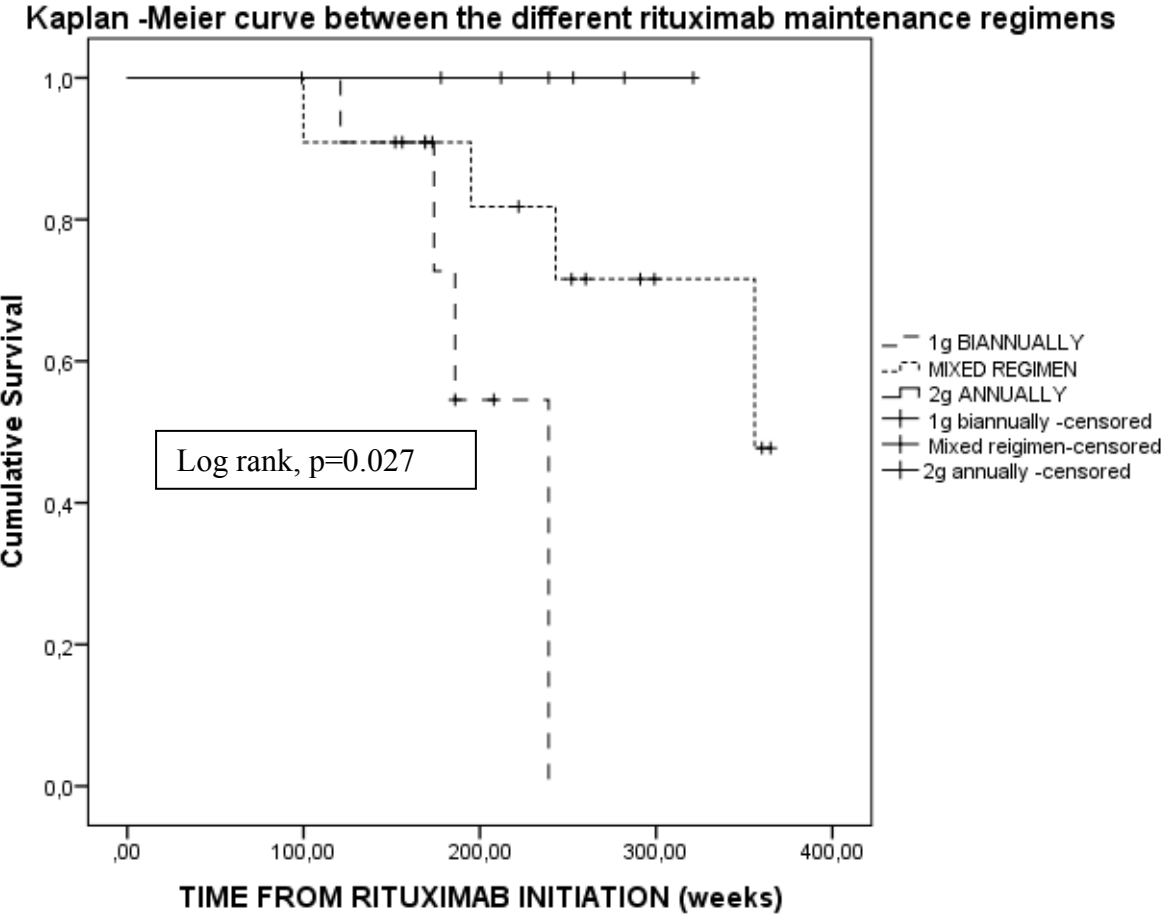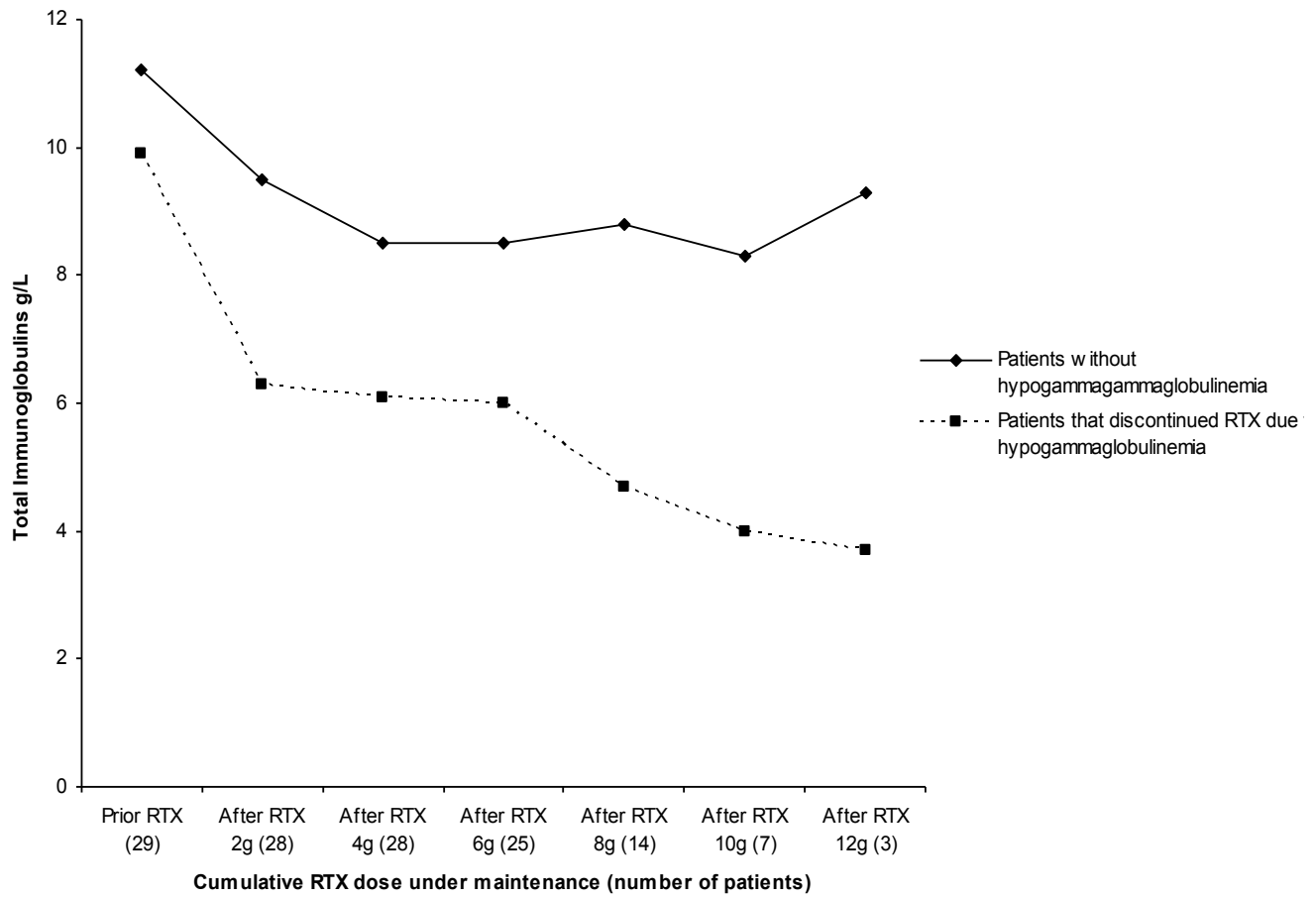

Kaplan -Meier curve between the different rituximab maintenance regimens

Figure 3. Median total Ig levels in patients that discontinued RTX due to hypogammaglobulinemia and in the control group

RTX: rituximab