Long-term efficacy and safety of pre-emptive maintenance therapy with Rituximab in Granulomatosis with polyangiitis: results from a single centre.

Introduction

Granulomatosis with polyangiitis (GPA) is a clinicopathologic variant of anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), with a distinct association with the presence of ANCA, directed against proteinase 3 (PR3) and myeloperoxidase (MPO) [1]. The spectrum of GPA comprises necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract, a necrotizing vasculitis affecting predominantly small to medium vessels and commonly a necrotizing glomerulonephritis [1].

GPA can rapidly lead to the development of severe organ damage and death but the overall prognosis of GPA has improved dramatically since the introduction of immunosuppressive drugs (ID) in induction and maintenance therapy. Cyclophosphamide (CYC) has evolved as the standard of care but has serious side effects – from infertility, typical and atypical infections, solid and non-solid malignancies, bone marrow toxicity, to hemorrhagic cystitis - and therefore other treatments are actively sought [2]. TNFα inhibitor agents have been unsuccessful and even detrimental in treating GPA [3]. Rituximab (RTX), a chimeric monoclonal antibody against CD20 that induces selective antibody-dependent and complement- mediated cellular cytotoxicity with subsequent apoptosis and elimination of B-cells, has demonstrated efficacy in both the induction [4,5] and maintenance of remission in GPA [6-10]. While seemingly having few side effects, the long-term outcome after RTX therapy has not yet been described and safety issues concerning hypogammaglobulinemia and serious infections seen with re-treatment are still a major concern [11]. Herein we present

efficacy and safety data from a single centre in GPA patients treated during a median of

almost 4 years with long-term pre-emptive maintenance therapy with RTX.

Methods

This analysis concerns 35 GPA patients from that registry, who were consecutively treated with RTX between April 2004 and September 2011. All patients satisfied the American College of Rheumatology 1990 classification criteria and/or Chapel Hill consensus conference criteria [12,13]. RTX was initially administered 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 (rheumatoid arthritis protocol). Due to the observed RTX efficacy and the relapsing nature of GPA, RTX was subsequently re-administered in a pre-emptive attempt to maintain B cell depletion. Patients received either 1g-infusion biannually or two 1-gram infusions 2 weeks apart annually. The 1g-infusion biannually became the preferred regimen as this was found to better sustain B cell depletion.

Prior to RTX, most patients were treated with intravenous methylprednisolone 750-1000 mg daily for 3 days, followed by a daily oral prednisolone dose of 0.5-1mg/kg that was tapered to 5-10 mg by 6 months. RTX was usually added to an ID (other than prednisolone) – mostly methotrexate (MTX), azathioprine (AZA), mycophenolate mofetil (MMF) and CYC. Discontinuation of prednisolone and ID during RTX remission maintenance relied upon the discretion of the treating physician. Ongoing treatments at RTX initiation and at last visit as well as the cumulative doses of both CYC and RTX were recorded.

Patients were seen 3 months after diagnosis and then every 6 months with extra visits in case of suspected relapse. Remission was assessed at every patient visit. Clinical examination, disease activity determined by the Birmingham Vasculitis Activity Score (BVAS 2003), a fixed set of blood tests (including ANCA, immunoglobulin (Ig) classes quantification and flow cytometric immunophenotyping of lymphocytes) as well as urine examinations were performed at all patient visits. If needed, radiology investigation of the involved organs and others system-specific clinical investigation were performed.

Complete remission was defined as the absence of disease activity attributable to active vasculitis based on clinical judgement and with a BVAS score of 0. Partial remissions were defined as a decrease of at least 50% from baseline BVAS score without any signs of deterioration. Relapses were defined by the recurrence, worsening or first appearance of ≥1 BVAS items attributable to GPA after remission. Grumbling disease was defined clinically as persisting symptoms not attributable to damage (such as arthralgia, fatigue, low-grade nasal crusting) in patients seemingly in remission [14].

Immunoglobulins levels at last visit reflect data recorded either at last visit before 30th September 2011 (closing date of this database) regardless of ongoing therapy - or shortly before the first administration of intravenous immunoglobulins (IVIG). Nadir levels of total Ig, IgG, IgA and IgM during RTX maintenance were also recorded. Ig decline after the first RTX round (difference between levels before the first and the second RTX round) and their overall decline during maintenance (difference between levels at baseline and at last visit) were calculated. Hypogammaglobulinemia was defined as total Ig under 6 g/L and decision to start supplemental treatment with IVIG was based on clinical judgement taking in account if the patient had either ongoing infections or was considered at an increased risk to develop infections.

Severe adverse events – including infections, malignancy and bone marrow toxicity - were recorded, irrespective of a possible association with vasculitis and/or therapy. Infections were regarded as either severe or chronic infections. Severe infections were defined as necessitating hospitalisation and intravenous antibiotics treatment. Chronic infections were defined as symptomatic localized infections lasting 3 months or more and requiring several antibiotic courses.

Trimetoprim-sulfamethoxazole (TMP-SMX) was not prescribed routinely during RTX maintenance.

Data were analysed with SPSS version 20.0 (SPSS Ltd, Chicago, IL, USA). Results are expressed as actual values for categorical variables and as median (range) for continuous variables. Pearson chi-square, Mann-Whitney U tests were used as appropriate. Significant predictor variables for infections in univariate analysis were entered into a multivariate binary (backward stepwise, $p < 0.05$ to enter, $p < 0.10$ to stay) logistic regression model. P-values < 0.05 were considered significant.

Results

Patients' description

Thirty-five patients with GPA were included with disease duration of 55 months (1-270) before RTX initiation (Table 1). Thirty patients (86%) were ANCA positive at diagnosis. Twenty-one patients (60 %) had renal involvement; 22 patients (63%) had lung involvement and 20 patients (57%) had either orbital, subglottic or both involvements. Prior to RTX initiation, patients had used 3 (1-8) ID and a total cumulative dose CYC of 14.8 (0-250) grams. The relapse rate prior to RTX treatment was 30.9 / 100 patient-years observation. Patients were given RTX for disease relapse (28 patients), new onset disease (6 patients) and maintenance in one patient because of increasing high PR3-ANCA titer 6 months after induction with methotrexate (MTX). Almost all the patients received RTX in combination with another ID: only three patients were treated with RTX in monotherapy for relapse. Baseline BVAS was 9 (0-22). Ten patients (29%) had low IgM (<0.4 g/L) and 8 patients (23%) had low IgG (<7.0 g/L) serum levels while none had low IgA (<0.7g/L) levels or hypogammaglobulinemia (<6.0 g/L). Sixteen patients (46%) were still PR3-ANCA positive. Patients received a cumulative RTX dose of 8 (2-13) grams given over five (1-10) rounds and were followed for 47 (2-88) months after RTX initiation.

Patients' outcome

    Remission and treatment failures

Six months after RTX initiation, 29 patients (83%) had achieved complete remission and 4 patients (11%) partial remission with an overall BVAS score of 0 (0-8) (See supplemental Table 1). Two patients were not in remission at 6 months but achieved complete remission at 12 months. At last visit, 97% were in remission and one patient had grumbling disease with a BVAS of 1.

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Nine relapses occurred during 1636 months (136 years) of follow-up translating into a flare rate of 6.6 /100 patient-years observation. Relapses appeared 18 (7-75) months after RTX initiation and 15 (6-24) months after last RTX round. Five relapses occurred after the first RTX round, 2 after the second round and 2 after the fourth round. Five relapses occurred in patients using concomitant ID (3 patients on MMF, 1 on MTX and 1 on AZA). B cells were undetectable ($<0.01 \times 10^9$/L) in 2 relapses and both patients received concomitant ID. B cells had returned at the time of relapse in the remaining 7 cases with count of 0.16×10^9/L (0.03-0.60×10^9L). ANCA rose prior to 3 out of 9 relapses: two patients seroconverted to ANCA positivity and the titer almost quadrupled in the third patient. All 9 relapses were re-treated with RTX inducing again remission. Two patients were also retreated with RTX for grumbling disease (BVAS =1) 40 months after its initiation while B-cells were still depleted and ANCA titers were negative.

RTX discontinuation

At last visit, RTX had been discontinued in 13 patients (37%) for the following reasons (some patients had multiple causes): 8 patients for hypogammaglobulinemia (total Ig 4.71 g/L (3.51-5.46)), 3 for severe infections, 4 for renal transplantation, 2 for malignancy, 2 for late-onset neutropenia, 1 for colitis and 1 for pregnancy. RTX was discontinued a median of 41 (12-78) months after its initiation and it had been discontinued a median of 14.5 (2-28) months at last visit. None of the patients that discontinued RTX had relapsed at last visit.

Four of the 8 patients that discontinued RTX because of hypogammaglobulinemia received IVIG: total Ig before IVIG was lower in patients treated with IVIG 4.1 g/L (3.51-5.24) compared with 5.43 g/L (4.14-6.22) in those not treated with IVIG even though it was not statistically significant (p=0.118).

Two patients died, both 28 months after RTX initiation. One patient died from bowel obstruction and Escherichia coli sepsis secondary to colonic cancer. The other patient died from sepsis without an identifiable agent complicating a myeloproliferative malignancy secondary to a high cumulative CYC dose (250 grams).

Other immunomodulating medication at last visit:

At last visit, 22 patients (63%) were still treated with RTX. Nineteen patients (86%) were maintained on RTX monotherapy while 3 patients (14%) were on ID: one patient received MMF 500mg daily, one patient used MTX 15mg weekly for control of his skin psoriasis and another used IVIG for hypogammaglobulinemia complicated with infection at last visit. Patients had used concomitant ID under RTX maintenance for 22 (1-54) months but had discontinued their ID 28 (1-50) months before last visit.

In contrast, 8 of the 13 (62%) of patients that had discontinued RTX were still treated with other ID regimens at last visit. Four patients with renal transplantation received either tacrolimus or cyclosporine, in combination with MMF for 3 of them. Four patients were receiving IVIG at last visit for hypogammaglobulinemia. Two patients had died and 3 patients were off ID at last visit: 2 because of ongoing infection and 1 due to pregnancy.

The median daily oral dose of Prednisolone decreased from 22.5mg (0-60) at RTX initiation to 5mg (0-30) at last visit. Seven patients (21%) had been off Prednisolone for 31 (8-60) months at last visit.

Of interest, a total of five patients were treated with IVIG in the study period 41 (37-82) months from RTX initiation and between 6 and 12 months after the last RTX infusion. Total Ig was 4.51g/L (3.51-5.46) in these patients prior to IVIG. Hypogammaglobulinemia was complicated by severe infection in 3 patients and chronic infection in one, while the last

patient received preventive IVIG because of severe hypogammaglobulinemia (total Ig 3.51 g/L). Two of these patients were re-treated with RTX after receiving IVIG.

Risk for infections

Severe infections

Nine patients (26%) had severe infections necessitating hospitalization and intravenous therapy 29 (4-59) months after RTX initiation. The rate for severe infection was 6.6 /100 patient-years observation. Five patients had pneumonia - due to cytomegalovirus, influenza B virus, Pneumocystis jiroveci, Staphylococcus aureus and an unidentified agent in one patient each. Two patients had gastrointestinal infections: one with Clostridium difficile colitis, the other with Escherichia coli complicating bowel obstruction. One patient developed Herpes-Zoster infection and one was treated for Pseudomonas aeruginosa sinusitis.

Patients with severe infections were older at RTX initiation (60 years vs 48.5 years; p=0.031) and all had renal involvement (OR=11.2; p=0.001), whereas the risk for severe infection was decreased in patients with orbital and subglottic involvements (OR=0.16; p=0.013). Patients with severe infections had received a higher cumulative CYC dose and had lower levels of total Ig during RTX maintenance and at last visit. Patients with severe infections had more profound decline in total Ig after the first RTX round and during RTX maintenance. Patients with severe infections had lower CD4 cell count at last visit, lower CD4/CD8 ratio both prior RTX and at last visit. (See supplemental Table 2 for the complete analysis with Mann-Whitney U test).

A higher cumulative dose of CYC, larger decreases in IgG, IgM and total Ig after the first RTX round as well as lower CD4 levels at last visit increased the risk for severe infections by univariate analysis (Table 2). Multivariate analysis using binary logistic regression showed that higher cumulative CYC dose (p=0.037), lower CD4 cell levels at last visit (p=0.045) and

larger decrease of total Ig after the first RTX round (p=0.040) were independently associated with severe infections. Larger decrease of total Ig after the first round was the most important independent risk factor for severe infections (OR=2.38 p=0.040) (Table 2). A 10 grams increase of the cumulative dose of CYC gave a proportionate change in odds of 2.38

Chronic infections

Ten patients (29%) had chronic and relapsing infections (some patients had 2 chronic infections): 5 had bronchitis, 3 sinusitis, 2 pneumonia, 1 urethritis, 1 prostatitis, 1 gastroenteritis with chronic diarrhea.

Chronic infections were not increased with older age and in patients with either renal, pulmonary or orbital-subglottic involvement.

Neither the cumulative CYC dose, the daily oral Prednisolone dose at last visit, the cumulative RTX dose nor the number of rounds of RTX was associated with chronic infections. Lower serum levels of total Ig, IgG, and IgM at nadir and at last visit increased the risk for chronic infections. In patients with chronic infections B cells counts at RTX initiation were lower, although with borderline significance. (See supplemental Table 3 for the complete analysis with Mann-Whitney U test).

Lower IgM, IgG, total Ig both at nadir under RTX maintenance and at last visit - and possibly the cumulative dose and number of rounds of RTX - were risk factors for chronic infections by univariate analysis (Table 3). But low IgG at nadir was the only independent risk factor for chronic infections (OR=2.33 p=0.026) by multivariate analysis (Table 3).

Discussion

In this Norwegian GPA cohort study RTX was an effective therapy for remission induction and long-term pre-emptive RTX maintenance reduced the relapse rate from 30.9 to 6.6 relapses /100 patient-years. Our relapse rate under RTX maintenance is lower than the flare rates of 8 and 18/100 patient-years under AZA maintenance observed in the Cyclops study with induction with either daily oral or intravenous pulse of CYC [15]. It is also lower than the flare rate of 13.8 from the study of Cartin-Ceba et al [10]; however patients in this study had only relapsed after the first remission induction and did not relapse subsequently under a long-term RTX maintenance strategy based on serial B lymphocytes and PR3-ANCA testing [10]. In the study of Smith et al eighteen out of 61 patients (30%) with refractory/relapsing AAV had relapsed at 48 months during a 2 years fixed interval RTX re-treatment strategy [9]. Although fewer patients (23%) relapsed in our study, long-term pre-emptive six monthly re-treatment with RTX does not seem to be greatly superior to the two-year fixed interval RTX re-treatment strategy [9] and the long-term pre-emptive RTX re-treatment strategy based on serial B lymphocytes and PR3-ANCA testing [10] in term of relapse.

However our relapse rate under RTX maintenance is higher when compared to other RTX maintenance studies: Roubaud-Baudron et al [8] observed flare rate of 2.0 and Rhee et al of 5.0 [7]. This difference is likely explained by a higher prevalence of granulomatous disease with orbital and subglottic involvements among our patients (57%), which are generally more prone to relapses [16].

Maintenance therapy with RTX permitted both the withdrawal of concomitant ID and the reduction of the daily prednisolone dose in almost all our patients. This clinically important result is similar to other studies [6-9]. The return of B cells and the rise of ANCA did not predict relapse in our study, comparable to other studies [6,7,9].

In our study, 13 patients (37%) had discontinued RTX treatment after a median of 41 months mainly due to the occurrence of hypogammaglobulinemia and / or infections. These patients have been followed for 14.5 months (2-28) after discontinuation and none have relapsed so far. This confirms the findings of Smith et al that the risk for relapses remains reduced after RTX withdrawal [9].

Given the importance of B cells in the surveillance against microbes, it is not surprising that long term B cell depletion will result in an increased risk of infectious complications as clearly shown here. Smith et al reported that 27% of the patients receiving routine RTX re-treatment for 2 years had a total of 44 infections – a total of 2.6 infections per patients and found that the risk of severe infections during RTX maintenance was similar to that observed in patients treated with mycophenolate mofetil, alemtuzumab and deoxyspergulin [9]. In our study we distinguished between severe and chronic infections and 26% of our patients had severe infections alike the study of Smith et al. Thus long-term pre-emptive re-treatment with RTX does not seem to increase the risk for infections compared to the two-year fixed interval RTX re-treatment strategy proposed by Smith et al [9]. Nine patients (29%) were defined clinically as having a chronic infection that often lacked microbiological evidence and for which no comparative data are available.

In our study the risk of severe infections was increased with older age, renal involvement, a high CYC cumulative dose, T cells defect and low total of immunoglobulins. These findings are not new as these same risk factors are known to increase the risk for severe infections when using CYC especially early in the course of the disease [17]. Of interest, the infectious causes of pneumonia in our study reflected a cell-mediated deficiency rather than a humoral deficiency.

Our severe infection rate is similar to the result from the German registry (6.5 per 100 patient-years observation) that has a shorter follow-up time [18] and it has not increased during long-term pre-emptive RTX maintenance therapy in our study. The risk for severe infections seems mostly conditioned by important decreases in Ig especially after the first RTX round (as shown in our study) and concurrent medication such as CYC and MMF. Two studies report severe infection rates of 15.5 and 22.5 per 100 patient-years with a much shorter observation time with respectively 66% of the patients using CYC [19] and 75% using either CYC or MMF in combination with RTX [20]. Although there is no clear evidence for an increased risk of severe infections during long-term pre-emptive RTX maintenance, repeated treatment with RTX by further decreasing total immunoglobulins level as shown in our study and by influencing T cell immunity [21] can still potentially increase the risk for severe infections. The risk for chronic infections seems to be associated with higher cumulative dose of RTX and increases with lower serum levels of total Ig, IgG and IgM during RTX maintenance. But other factors, a high cumulative dose of CYC and the prolonged exposure to prednisolone, can also contribute in the genesis of the dysgammaglobulinemia prior to RTX [22-23]. Even though our study was retrospective and lacked a control group, the longitudinal data was collected in a prospective manner in the form of a disease registry from a single tertiary care center. The relatively small numbers on the other hand make subgroup analyses prone to type II errors of not detecting significant differences.

In conclusion, long-term pre-emptive RTX is efficacious in reducing the risk for relapse and allows withdrawal of other ID in GPA patients with a high prevalence of granulomatous disease and a high flare rate before RTX. However, long-term pre-emptive RTX maintenance had to be discontinued in 37% of our patients due to hypogammaglobulinemia and infections. Limiting the use of CYC prior to long-term pre-emptive RTX maintenance strategy could be

beneficial in preventing hypogammaglobulinemia [24] and infections, not only at induction but also over time. The inherent trade-off between relapse and infection risks in GPA management seems to be more predictable and manageable with RTX than with other ID as low levels of total immunoglobulins are the strongest predictors for infections.

Key messages

Long-term pre-emptive rituximab is efficacious in reducing the risk for relapse.

After four years, 37% of the patients discontinued rituximab due to hypogammaglobulinemia and infections.

Low levels of immunoglobulins and high cyclophosphamide cumulative dose increase the risk for infection.

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Table 1

Characteristics of study cohort patients with Granulomatosis with polyangiitis (GPA) treated with long term Rituximab. All figures indicate median values (range) unless otherwise indicated

No. of patients	35
No. Male / Female	16 / 19
Age (years) at diagnosis at RTX initiation	 48 (12-68) 50 (14-79)
Disease duration before RTX (months)	55 (1-270)
No of patients with ANCA positive at diagnosis Type PR3 / MPO	30 (86%) 29 / 1
No of patients with ANCA positive prior RTX Type PR3 / MPO	16 / 35 (46%) 16 / 0
No of patients with orbital or subglottic involvement	20 (57%)
No of patients with renal involvement No of patients with pulmonary involvement	21 (60%) 22 (63%)
Number of previous immunosuppressive drugs prior RTX	3 (1-8)
Prednisolone dose at RTX initiation (mg)	22.5 (0-60)
Cumulative Cyclophosphamide dose (g)	14.8 (0-250)
No of RTX for maintenance / new disease / relapse	1 / 6 / 28
BVAS at RTX initiation	9 (0-22)
RTX cumulative dose (g) No of RTX rounds	8 (2-13) 5 (1-10)
Follow-up after RTX initiation (months)	47 (2-88)

ANCA: anti-neutrophil cytoplasmic antibodies; BVAS: Birmingham Vasculitis Activity Score; MPO: myeloperoxydase; No: number; PR3: anti-proteinase3; RTX: rituximab

Table 2

Risk factors for severe infections in our study cohort determined by univariate and multivariate (backward stepwise) binary logistic regression analysis. All predictor variables in this analysis are continuous.

	Univariate analysis			Multivariate analysis		
	Odds ratio	p-value	Lower and upper 95% CI	Odds ratio	p-value	Lower and upper 95% CI
Age (years)	1.06	0.059	0.99-1.14			
Cumulative dose of CYC (g)	1.04	0.036	1.00-1.08	1.09	0.037	1.01-1.18
Prednisolone dose at LV (mg)	1.18	0.055	0.99-1.39			
IgA level at nadir (g/L)	0.07	0.050	0.01-1.00		0.341	
CD4/CD8 ratio prior RTX	0.24	0.076	0.05-1.16			
CD4/CD8 ratio at LV	0.31	0.070	0.09-1.10			
Total CD4 cells at LV (x10^9/L)	0.002	0.016	0.001-0.33		0.045	
IgG decline after 1. round (g/L)	1.78	0.038	1.03-3.06			
IgM decline after 1. round (g/L)	53.3	0.026	1.63-1740			
Total Ig decline after 1. round (g/L)	1.81	0.023	1.09-3.01	2.38	0.040	1.04-5.46
Total Ig overall decline (g/L)	1.33	0.064	0.98-1.78			

CI: confidence interval; CYC: cyclophosphamide; CD: cluster of differentiation; Ig: immunoglobulins; LV: last visit; RTX: rituximab

Table 3

Risk factors for chronic infections in our study cohort determined by univariate and multivariate (backward stepwise) binary logistic regression. All predictor variables in this analysis are continuous.

	Univariate analysis			Multivariate analysis		
	Odds ratio	p-value	Lower and upper 95% CI	Odds ratio	p-value	Lower and upper 95% CI
Number of RTX rounds	1.43	0.061	0.98-2.07			
Cumulative RTX dose (g)	1.30	0.075	0.97-1.73	1.40	0.095	0.94-2.06
IgG level at nadir (g/L)	0.48	0.023	0.26-0.91	0.43	0.026	0.21-0.90
IgM level at nadir (g/L)	0.001	0.039	0.001-0.67		0.264	
Total Ig level at nadir (g/L)	0.62	0.038	0.39-0.98			
IgG level at LV (g/L)	0.53	0.036	0.30-0.96			
IgM level at LV (g/L)	0.001	0.025	0.001-0.39			
Total Ig level at LV (g/L)	0.64	0.042	0.41-0.98			

CI: confidence interval; Ig: immunoglobulins; LV: last visit; RTX: rituximab.

Online supplementary data

Supplementary Table 1

Patients' outcome after long-term pre-emptive rituximab maintenance therapy. All figures indicate median values (range) unless otherwise indicated

At 6 months No. of patients in remission* No of complete remission – partial remission BVAS	33/35 (94%) 29 (83%) – 4 (11%) 0 (0-8)
At last visit No. of patients in remission# No of complete remission – partial remission BVAS	32/33 (97%) 25 (76%) – 7 (21%) 0 (0-5)
Prednisolone dose at last visit (mg)	5 (0-30)
No. of patients treated with ID at last visit (other than RTX and prednisolone)	7 / 33 (21 %)
No. of patients with relapse under RTX	8 / 35 (23%) 1 patient with 2 relapses.
No. of patients with RTX discontinuation at last visit No. of patients that discontinued due to hypogammaglobulinemia (<6g/L)	13 / 35 (37%) 8 / 13 (62%)
No of patients treated with IVIG	5 / 35 (14%)
No. of patients with Chronic infections Severe infections Death	 10 / 35 (29%) 9 / 35 (26%) 2 / 35 (5.7%)

BVAS: Birmingham Vasculitis Activity Score; ID: immunosuppressive drugs; IVIG: intra-venous immunoglobulins; RTX: rituximab.

* Two patients were not in remission at 6 months, but were in remission at 12 months.

One patient had grumbling disease at last visit.

Supplementary Table 2

Difference in medians between patients with and without severe infections; p-values calculated by non-parametric significance test -Mann Whitney U test. All predictor variables in this analysis are continuous.

	Patients with severe infection	Patients without severe infection	p-value
CYC cumulative dose (g)	51.7	13.0	0.045
Daily prednisolone dose at LV (mg)	5	5	0.024
RTX cumulative dose (g)	6	8.5	0.110
Number of RTX rounds	5	5.5	0.446
Total Ig at nadir (g/L)	4.7	6.7	0.018
IgG at nadir (g/L)	3.7	5.7	0.015
IgA at nadir (g/L)	0.82	1.1	0.038
Total Ig at LV (g/L)	5.4	7.4	0.031
IgG at LV (g/L)	4.4	6.0	0.046
IgG decline after 1. round• (g/L)	3.0	1.4	0.013
IgM decline after 1. round• (g/L)	0.71	0.21	0.001
Total Ig decline after 1. round• (g/L)	4.3	1.7	0.005
Total Ig overall decline* (g/L)	5.6	2.9	0.001
IgG overall decline* (g/L)	4.3	2.2	0.016
CD4/CD8 ratio prior RTX	0.84	1.26	0.023
CD4/CD8 ratio at LV	0.72	1.67	0.025
CD4 cells count at LV (x10^9/L)	0.25	0.67	0.002

CD: cluster of differentiation; CYC: cyclophosphamide; Ig: immunoglobulins; LV: last visit; RTX: rituximab

* Ig overall decline corresponds to the absolute change in Ig levels between baseline and last visit.

•Ig decline after 1. round corresponds to the absolute change in Ig levels before the first and before the second RTX round.

Supplementary Table 3

Difference in medians between patients with and without chronic infections; p-values calculated by non-parametric Mann Whitney U test. All predictor variables in this analysis are continuous.

	Patients with chronic infection	Patients without chronic infection	p-value
CYC cumulative dose (g)	17.1	14.8	0.867
Daily prednisolone dose at LV (mg)	5.0	5.0	0.669
RTX cumulative dose (g)	8.5	7	0.113
Number of RTX rounds	6.5	5	0.113
Total Ig at nadir (g/L)	5.7	7.5	0.019
IgG at nadir (g/L)	4.1	6.2	0.013
IgM at nadir (g/L)	0.13	0.33	0.012
Total Ig at LV (g/L)	5.7	8.7	0.019
IgG at LV (g/L)	4.4	6.2	0.013
Total IgM at LV (g/L)	0.16	0.35	0.007
B cells count prior RTX (x10^9/L)	0.035	0.09	0.065

CYC: cyclophosphamide; Ig: immunoglobulins; LV: last visit; RTX: rituximab