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The effect of continuous B cell depletion with rituximab on pathogenic autoantibodies and total IgG levels in ANCA vasculitis

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Abstract

Objective—To evaluate the effect of rituximab on pathogenic autoantibodies and total immunoglobulin levels, and to identify serious adverse events, in patients with ANCA vasculitis treated with continuous B cell depletion.

Methods—We conducted a retrospective analysis of 239 patients with ANCA vasculitis treated with rituximab-induced continuous B cell depletion. Two treatment cohorts were analyzed: an induction group (n= 53) and a maintenance group (n=237). The change in ANCA titers and total immunoglobulin levels over time were evaluated using mixed-effects models. Risk factors for serious infections during maintenance were evaluated with Poisson regression.

Results—During induction, IgG levels fell at a rate of 6% per month (95% CI, 4 to 8%), while ANCA levels declined at 47% per month (95% CI, 42 to 52%) and 48% per month (95% CI, 42 to 54%) for anti-MPO and anti-PR3 titers, respectively. During maintenance treatment, with a median duration of 2.4 (IQR, 1.5- 4.0) years, IgG levels declined at 0.6% per year (95% CI, -0.2 to 1.4%). New significant hypogammaglobulinemia (IgG < 400 mg/dL) during maintenance occurred in 4.6% of patients, all of whom were in the lowest baseline IgG quartile. Serious infections during maintenance occurred at a rate of 0.85 [95% CI, 0.66 to 1.1] per 10 patient years and were independently associated with an IgG level < 400mg/dL.

Conclusion—B cell targeted therapy causes a preferential decline in ANCA titers relative to total IgG levels. Despite prolonged maintenance therapy with rituximab, IgG levels remain essentially constant. Serious infections were rare.

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Introduction

The anti-CD20 monoclonal antibody, rituximab, has emerged as a useful immunosuppressive agent for the treatment of several antibody-mediated autoimmune diseases (1, 2). An important immunosuppressive mechanism of rituximab is to attenuate the production of pathogenic autoantibodies (3-5). The potential downside of this therapy is suppression of the protective benefits of the humoral immune system, thus predisposing to infectious complications (6, 7). The degree to which treatments using rituximab can suppress pathogenic autoantibodies, compared to suppression of protective antibodies, may be a major determinate of the clinical value of these treatments. Characteristics of the disease, variations of the treatment regimen, and patient characteristics may heavily influence this balance.

Rituximab has become an important component of the treatment of antineutrophil cytoplasmic antibody (ANCA) vasculitis (2, 8, 9). The Rituximab in ANCA-Associated Vasculitis (RAVE) trial suggested that an induction of remission regimen using rituximab with glucocorticoids was equivalent to one using cyclophosphamide with glucocorticoids (2). More recently, the Maintenance of Remission using Rituximab in Systemic ANCA-Associated Vasculitis (MAINRITSAN) trial suggested that rituximab with low-dose prednisone is superior to azathioprine with low-dose prednisone as maintenance therapy to prevent disease relapse (9). An additional randomized controlled trial evaluating rituximab for maintenance of remission is ongoing (10).

Unfortunately, relapse is common in ANCA vasculitis following cessation of rituximab, and is often heralded by B cell reconstitution (11, 12). With the aim of preventing disease relapse, our practice has evolved to treat patients for extended periods of time with scheduled rituximab dosing to maintain B cell depletion (13). Data on the complications of such a treatment regimen, however, are sparse. One particular concern is the development of hypogammaglobulinemia and predisposition to serious infections.

In this study, we retrospectively evaluate the impact of continuous B cell depletion with rituximab on immunoglobulin levels during induction and maintenance of remission therapy for ANCA vasculitis. In particular, we compare the relative effect of treatment on the levels of pathogenic antibodies versus the effect on total immunoglobulin levels. In addition we describe serious adverse events and identify predictive factors for the development of hypogammaglobulinemia and serious infections.

Materials and Methods

Study Population and Treatment Groups

We performed a single-center retrospective analysis of 239 patients with ANCA vasculitis treated with rituximab-induced continuous B cell depletion at the Massachusetts General Hospital Vasculitis and Glomerulonephritis Center from April of 2006 to August of 2015. Patients were considered to have ANCA vasculitis if they had a positive test for antibodies to proteinase 3 (PR3) or myeloperoxidase (MPO) together with clinical and laboratory features

consistent with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), or one of the other related forms of vasculitis (14).

Starting in 2006, our practice evolved into a consistent treatment regimen for induction and maintenance of remission in the majority of patients. For induction therapy, patients receive combination therapy with rituximab, a 2 month course of low-dose oral cyclophosphamide, and a short course of high dose steroids with a rapid taper to low dose. The rationale for combining rituximab with cyclophosphamide is to allow for rapid tapering of high-dose glucocorticoids, such that the prednisone dose is reduced to 15 mg daily by the fifth week of treatment.

For maintenance therapy, patients receive scheduled rituximab with or without low-dose (7.5 mg/day) prednisone. Prednisone is slowly tapered to discontinuation except as limited by adrenal insufficiency. Rituximab is initially administered as two 1 gm doses intravenously (IV) separated by approximately 2 weeks. Thereafter, rituximab is administered as a 1 gm dose IV every 4 months for 2 years, followed by a 1 gm dose IV every 6 months. B cell depletion, defined as $< 10 \text{ CD20}^+ \text{ cells/mm}^3$, is confirmed with peripheral flow cytometry prior to each rituximab infusion. In the rare event that B cell return was detected, the dosing interval for that patient was shortened.

During induction of remission therapy, all patients received prophylaxis for *Pneumocystis pneumonia* (PCP) with trimethoprim-sulfamethoxazole. Patients with an allergy to sulfonamides were administered atovaquone as an alternative. During maintenance therapy, PCP prophylaxis was continued until prednisone was completely discontinued and patients were on rituximab monotherapy. Patients with a positive hepatitis B core antibody were given prophylaxis to prevent hepatitis B reactivation with either entecavir or lamivudine.

Two treatment groups were analyzed in this study: an induction group and a maintenance group (Figure 1). Patients were included in the induction group if they had newly diagnosed and active (BVAS-WG ≥ 3) ANCA vasculitis, were treated with the induction regimen described above, and had baseline and followup immunoglobulin G (IgG) and ANCA levels during the induction period. Patients with relapsing disease and patients treated with plasma exchange (PLEX) were excluded. The induction phase commenced with the first dose of rituximab during induction therapy and ended when the patient achieved complete remission (defined as BVAS-WG=0, a prednisone dose of $\leq 7.5 \text{ mg/d}$, and the absence of immunosuppressants other than rituximab and prednisone).

Patients were included in the maintenance group if they were in complete remission, were being treated with rituximab maintenance therapy as described above, and had baseline and follow up IgG and ANCA levels during the maintenance period. For patients who had received PLEX, inclusion started 180 days after initiation of therapy or after complete remission was attained, whichever was later. Patients who received alternative maintenance regimens were excluded from this analysis. In the event of a disease relapse, patients remained in the maintenance cohort provided they continued receiving scheduled rituximab. Patients who ultimately had rituximab withdrawn were censored at the time of B cell return.

Cases of ANCA vasculitis associated with levamisole were excluded. Likewise, patients with simultaneous membranous nephropathy or anti-GBM disease were excluded. The study was approved by the Partners HealthCare Human Research Committee.

Ig levels and ANCA levels

Immunoglobulin and ANCA levels were typically measured approximately every 4-6 months in patients on continuous B cell depletion as part of clinical care. ANCA titers were measured by ELISA as previously described (15, 16). In brief, all samples are tested in comparison to a standard reference serum by creation of a standard curve generated from serial 1:2 dilutions. Test samples are diluted 1:16 followed by serial 1:8 dilutions. Concentrations are read off the standard curve and multiplied by the relative dilution to determine the relative concentration. Only dilutions with reactivity falling on the steep part of the reference curve are used for determining concentration.

For the induction of remission analysis, changes in levels were determined using values from the first measurements closest to the time of initiation of induction treatment (values more than 2 months before the first dose were excluded) up to 150 days after the first dose of rituximab. In the maintenance of remission analysis, changes in levels were determined using values from the first measurement on or after the date of full remission until the last measurement with documented B cell depletion (< 10 B cells/mm³).

Serious Adverse Events and Hypogammaglobulinemia

Serious adverse events (SAEs) were defined as events that were life threatening, led to a hospitalization, caused persistent disability or permanent damage, or resulted in death. SAEs were determined by review of our electronic medical records and flow sheets maintained as part of patient management. Significant hypogammaglobulinemia was defined as an IgG level < 400 mg/dL. This level was chosen based on prior studies that demonstrated a higher infectious risk below this threshold (17, 18).

Statistical Analyses

All analyses were carried out using Stata version 14 (College Station, TX). Patient baseline characteristics were stratified by treatment group. Continuous variables were presented as mean $\pm$ SD or median (interquartile range) as appropriate, and categorical variables were presented as percentages. P values for baseline characteristics are not provided because many patients were included in both the induction and maintenance groups, and the observations were not independent.

Mean population changes in Ig levels and ANCA titers were evaluated using linear mixed-effects modeling by regressing log(ANCA titer or Ig level) against time. In the maintenance group, the model was constructed with a random patient-specific intercept and random time effect. Given the limited number of observations per patient in the induction group, models were constructed only with a random patient-specific intercept.

Time to hypogammaglobulinemia during maintenance treatment stratified by baseline IgG level was described using Kaplan-Meier curves. Predictors of hypogammaglobulinemia were

evaluated using Cox proportional hazards models. Confidence intervals and predictors of serious adverse events/infections were evaluated using Poisson regression, treating serious events as a count outcome. For both Cox and Poisson regression, the following covariates were prespecified based on scientific relevance: age, sex, ANCA serotype, baseline IgG level, history of plasma exchange, time from diagnosis of ANCA vasculitis to initiation of rituximab, and duration of continuous B cell depletion prior to remission. Due to the limited number of patients who developed hypogammaglobulinemia, only univariable Cox models were assessed. For Poisson regression, both univariable and multivariable models were constructed. The multivariable model was adjusted for all prespecified covariates listed above.

Results

Baseline Characteristics

Between April of 2006 and April of 2015, 52 and 237 patients met inclusion criteria for the induction and maintenance groups, respectively. The induction group was comprised of patients with newly diagnosed and active (BVAS-WG ≥ 3) ANCA vasculitis who received a standardized induction regimen. The maintenance group consisted of patients in complete remission (BVAS=0 on prednisone ≤ 7.5 mg/d) treated with rituximab-induced continuous B cell depletion. Patients in the maintenance group included those treated with a standardized induction regimen who achieved complete remission and those who were already receiving maintenance therapy with an alternative agent and were transitioned to rituximab (Figure 1). Patients transitioned from an alternative maintenance regimen were diagnosed with ANCA vasculitis at a median of 4.3 (IQR, 1.1 to 8.1) years before entering the maintenance cohort. At entry into the maintenance cohort, 92% of patients were receiving prednisone (median 7.5 [IQR, 5-7.5] mg/day). Thereafter, prednisone was tapered to discontinuation as possible such that by the 8th month of maintenance therapy the median prednisone dose was 0 (IQR, 0-4) mg/day.

Baseline characteristics stratified by treatment group are presented in Table 1. The median BVAS-WG in the induction group was 6 (IQR, 4-8) and, by definition, was 0 at entry into the maintenance group. The median (IQR) IgG level at initiation of induction therapy was 797 (643-1052) mg/dL compared with 678 (534-811) mg/dL at entry into the maintenance group. Baseline maintenance IgG levels were lower in patients who received PLEX with the standardized induction regimen (559 mg/dL [IQR, 445 to 642]) compared with those who received the induction regimen alone (665 mg/dL [IQR, 554 to 792]) or were transitioned from an alternative maintenance regimen (738 mg/dL [IQR, 570 to 880]); ($P < 0.01$ for both comparisons).

Among the 50 patients who were included in both groups, the mean within person decrease in IgG levels from the beginning of induction to the beginning of maintenance therapy was 226 mg/dL (95% confidence interval [CI], 155 to 298 mg/dL; $P < 0.001$). No patients in the induction group had significant baseline hypogammaglobulinemia (IgG < 400 mg/dL), compared with 9% of patients in the maintenance group.

Change in Immunoglobulin Levels and ANCA Titers with Treatment

The population mean change in total Ig levels and ANCA titers during induction therapy is given in Table 2 and depicted graphically in Figure 2. IgG levels declined at mean rate of 6% per month (95% CI, 4 to 8%). ANCA titers had a more precipitous decline at 47% per month (95% CI, 42 to 52%) and 48% per month (95% CI, 42 to 54%) for anti-MPO and anti-PR3 titers, respectively.

Patients in the maintenance group were in a state of continuous B cell depletion for a median of 2.4 years (IQR 1.5- 4.0), with the longest duration being 7.6 years. At entry into the maintenance group, 139 patients (59%) continued to have an ANCA titer above the cutoff for the assay. The change in ANCA titers in this subset of patients was compared with the change in IgG levels across the entire maintenance group (Table 2 and Figure 2). During maintenance therapy with rituximab-induced continuous B cell depletion, IgG levels fell by a mean of 0.6% per year (95% CI, -0.2 to 1.4%). Conversely, in patients with a positive ANCA titer at the initiation of maintenance treatment, anti-MPO and anti-PR3 titers fell by a mean of 42% per year (95% CI, 32 to 50%) and 73% per year (95% CI, 58 to 83%), respectively.

Ig levels and ANCA titers fell more rapidly in induction than in maintenance. When standardized to a common time unit, IgG levels fell 52% per year (95% CI, 39 to 61%) during induction and 0.6% per year (95% CI, -0.2 to 1.4%) during maintenance. Likewise, anti-MPO titers fell by 47% per month (95% CI, 42 to 52%) during induction compared with 4% per month (95% CI, 3 to 6%) during maintenance among patients who still had a positive ANCA titer. A similar trend was seen for anti-PR3 titers.

In the maintenance group, 13 of 237 patients (5.5%) suffered a major relapse (BVAS-WG > 2). All patients were treated with prednisone and continued rituximab, save one patient who additionally received two months of oral cyclophosphamide. A sensitivity analysis demonstrated that continued inclusion of these patients in the maintenance cohort did not influence the results.

Predictors of Hypogammaglobulinemia

At entry into the maintenance group, 21 patients (9%) had significant hypogammaglobulinemia (IgG < 400 mg/dL). Of the remaining 216 patients with a baseline maintenance IgG level > 400mg/dL, 10 patients (4.6%) developed significant hypogammaglobulinemia over a median duration of rituximab-induced maintenance continuous B cell depletion of 2.4 (IQR, 1.5- 4.0) years. There was no difference in the proportion of patients developing hypogammaglobulinemia among patients treated for disease relapse compared with those who did not (P= 0.44).

In univariate Cox proportional hazard models, the baseline IgG level at entry into the maintenance group was the only factor associated with subsequently developing significant hypogammaglobulinemia during maintenance therapy with rituximab (HR 0.25 per 50 mg/dL increase in IgG level; 95% CI, 0.11 to 0.56; P= 0.001). Duration of continuous B cell depletion prior to entry in the maintenance group was not associated with future significant hypogammaglobulinemia. Likewise, time since the initial diagnosis of ANCA vasculitis,

which should serve as a rough surrogate for cumulative non-rituximab immunosuppression, lacked a significant association with developing an IgG level < 400 mg/dL.

Only patients with a baseline maintenance IgG level in the bottom quartile (408-559 mg/dL) developed significant hypogammaglobulinemia (Figure 3). The median baseline IgG level of patients who subsequently developed an IgG level < 400 mg/dL was 448 (IQR, 428-503) mg/dL. Despite being in the lowest baseline IgG quartile, 82% of patients maintained an IgG level > 400 mg/dL during continuous maintenance rituximab therapy.

Serious Adverse Events

Cumulative immunosuppressive exposure was 23.8 and 725.4 patient years for the induction and maintenance groups, respectively. During induction therapy, there were 5.9 (95% CI, 3.2 to 9.6) SAEs per 10 patient years compared with 1.8 (95% CI, 1.5 to 2.2) SAEs per 10 patient years during maintenance. Serious infections occurred at a greater rate during induction (2.9 [95% CI, 1.2 to 6.0] per 10 patient years) than in maintenance (0.85 [95% CI, 0.66 to 1.1] per 10 patient years). Pulmonary infections accounted for the majority of serious infections in both groups.

During maintenance therapy, 5 patients (2.1%) received treatment with intravenous immunoglobulin (IVIG) for recurrent infections: 1 patient had recurrent sinus infections, 3 patients developed bronchiectasis with recurrent pulmonary infections, and 1 had recurrent pulmonary infections without bronchiectasis. The median IgG level at initiation of IVIG was 408 (IQR, 196- 586) mg/dL.

There were no deaths during induction. However, the induction group inclusion criteria could result in an underreporting of mortality. To address this issue, we analyzed all patients (n=89) treated with the standardized induction regimen regardless of whether they met criteria for inclusion in the induction group. The majority of the additional 37 patients were not included in the induction group due to the absence of ANCA and IgG levels required for analysis. Among these 89 patients, 2 deaths occurred during the induction period (0.6 [95% CI, 0.1 to 2.4] deaths per 10 patient years). One patient died from septic shock in the setting of pneumonia and the other died of a myocardial infarction. During maintenance, there were 0.17 deaths per 10 patient years (95% CI, 0.09 to 0.29). Two of the 12 deaths in the maintenance group were attributed to an infectious complication, and none were attributed to a vasculitis flare.

In multivariable models, increasing age and a baseline maintenance IgG level < 400 mg/dL were associated with developing a serious infection during maintenance therapy with rituximab-induced continuous B cell depletion (Table 3). There was no association of serious infection with the duration of ANCA vasculitis prior to rituximab treatment or the duration of rituximab-induced continuous B cell depletion prior to entering maintenance. In the 216 patients who entered maintenance with an IgG level > 400 mg/dL, there was no association with IgG level and risk of subsequent serious infections in a univariate and adjusted model (incident rate ratio 1.02 [95% CI, 0.95 to 1.09; P= 0.65 for the adjusted model).

Discussion

This retrospective analysis of patients with ANCA vasculitis treated with rituximab provides insight into several interesting features of the effect of B cell targeted therapy on antibody-mediated autoimmune diseases. During induction therapy with combination glucocorticoids, cyclophosphamide, and rituximab, the levels of both pathogenic ANCA and total immunoglobulin levels decline. The decline in ANCA, however, occurs at a more rapid rate. There was a 6% (95% CI, 4 to 8%) per month decline in IgG levels compared with a 47% (95% CI, 42 to 52%) and 48% (95% CI, 42 to 54%) per month decline in anti-MPO and anti-PR3 antibodies, respectively. During maintenance therapy with prolonged rituximab-induced continuous B cell depletion, total IgG levels remained essentially constant with a mean decline of 0.6% per year (95% CI, -0.2 to 1.4%). Furthermore, the rate of serious infections during maintenance therapy was relatively low at 0.85 [95% CI, 0.66 to 1.1] per 10 patient years.

A preferential decline in pathogenic ANCA allows for a therapeutic window to treat disease while preserving protective humoral immunity. A similar paradigm has been observed in rheumatoid arthritis, whereby treatment with rituximab leads to a sharper decline in rheumatoid factor and antibodies to cyclic citrullinated peptide compared to total immunoglobulin levels (3). Given that plasma cells do not express CD 20, it is possible that certain disease-associated autoantibodies are produced by plasmablasts and plasma cells that are inherently short-lived. Alternatively, autoantibody producing cells may be more sensitive to cyclophosphamide, prednisone, or other induction agents used in conjunction with rituximab. Regardless of which mechanism predominates, the autoantibody-producing plasmablasts and plasma cells are unable to be replenished following B cell depletion. Conversely, disease states in which rituximab fails to induce a preferential decline in disease-associated autoantibodies may be relatively resistant to B cell depletion treatments. For example, treatment of the antiphospholipid syndrome with rituximab has little effect on the levels of autoantibodies and appears to be of limited efficacy in most cases (19).

Given the long term, relapsing nature of many antibody mediated autoimmune diseases, the development of hypogammaglobulinemia is a particular concern with repeated courses of rituximab (6, 7). While prior reports evaluating the effect of B cell targeted therapy on immunoglobulin levels have included heterogeneous populations with varying cumulative rituximab exposure and dosing frequency, our analysis was restricted to patients undergoing continuous B cell depletion. During maintenance treatment, patients receiving scheduled doses of rituximab at 4 to 6 month intervals showed essentially no change in the mean IgG level despite 25% of patients receiving scheduled rituximab for greater than 4 years. Furthermore, our results suggest developing hypogammaglobulinemia during maintenance therapy with rituximab is unlikely unless the baseline IgG level is low. Indeed, among patients who entered maintenance therapy in the top three IgG quartiles, no patients developed an IgG level < 400 mg/dL. Therefore, declines in IgG levels are seen primarily during induction therapy, with little change thereafter. We attribute the stability of IgG levels during maintenance to a long-lived plasma cell population that is resistant to immunosuppressive agents and is able to maintain the IgG pool. In agreement with prior reports, the rate of IgM decline we observed was significantly greater than that of IgG (20,

21). IgA levels fell at a rate similar to IgG during induction therapy, but at a rate slightly greater than IgG during maintenance therapy.

This stability of IgG levels independent of repeated rituximab doses agrees with prior reports in the literature. In a large analysis of 2,578 patients with rheumatoid arthritis treated with rituximab in clinical trials (1,043 of whom received 3 rounds of rituximab treatment), 5% of patients developed an IgG level less than the lower limit for the laboratory and less than 1% developed an IgG level of less than 300mg/dL (20). Importantly, the rate of hypogammaglobulinemia was similar despite the number of rituximab treatments received. In contrast, low IgM levels were observed more commonly, with 23% of patients developing an IgM level below the lower limit of normal for the assay. In another analysis of 243 patients comprised predominantly of ANCA vasculitis, approximately 16% of patients developed moderate hypogammaglobulinemia (IgG level 300-500 mg/dL) following treatment with rituximab (21). The pre-rituximab IgG concentration correlated with the development of hypogammaglobulinemia, but the total number of rituximab doses did not. Low IgM levels were significantly more common, with 58% of patients developing a level < 30 mg/dL.

The immunosuppressive effects of rituximab extend beyond a potential reduction in total IgG levels. Despite the relative stability of total IgG levels, the ability to manufacture antibodies against new antigens may be impaired. Indeed, patients undergoing treatment with rituximab have an impaired ability to respond to vaccination with pneumococcal polysaccharide or novel antigens (22). B cell depletion has also been demonstrated to modulate T cell response to infections, which would impair immunity independent of the IgG concentration (23).

To evaluate the safety of long-term continuous B cell depletion, we reviewed all serious adverse events within our cohort. The rate of serious infections observed in our induction cohort (2.9 [95% CI, 1.2 to 6.0] per 10 patient years) is comparable to the rate of grade 3 or 4 infections that occurred in patients receiving induction therapy in the RAVE trial (1.4 per 10 patient years) (2). In our maintenance cohort, serious infections occurred at a rate of 0.85 [95% CI, 0.66 to 1.1] per 10 patient years, the vast majority of which were pneumonia. Our results are similar to the rate of grade 3 or 4 infections observed in patients receiving rituximab maintenance therapy in the MAINRITSAN trial (0.82 per 10 patient years) (9).

A baseline IgG level < 400 mg/dL and increasing patient age were associated with an increased risk of serious infections in our maintenance cohort. The association of infectious complications with an IgG level < 400 mg/dL has been observed in other patient populations. For example, a serum IgG level < 400mg/dL was associated with a higher rate of infections compared with more mild reductions in both solid organ and stem cell transplant populations (17, 18). Therefore, particular caution should be exercised in using rituximab in patients with IgG levels approaching this level. Of note, low IgM levels following rituximab were not associated with an increased risk of infections in a prior investigation (20). Likewise, IgA levels do not appear to be significantly associated with infectious events (24). The association of prior plasma exchange with a lower risk of serious infections does not have a plausible scientific explanation. Furthermore, other studies

evaluating the role of plasma exchange in ANCA vasculitis have not demonstrated this association (25).

Our study has several strengths and weaknesses. The greatest strengths are the large size for an ANCA vasculitis cohort, long duration of follow up, and uniformity of treatment. To our knowledge, this cohort provides data on the longest duration of continuous B cell depletion published to date. The main weaknesses are inherent to data collection in retrospective studies. We were only able to accurately record serious adverse events and were not able to provide data on infections that did not require hospitalization. In addition, the exact cumulative dose of cyclophosphamide was unknown for a large percentage of patients transitioned to rituximab from an alternative maintenance regimen, thus precluding a detailed analysis of the effect of cyclophosphamide exposure on IgG levels. Some insight, however, can be derived from the baseline IgG levels of different groups at entry into the maintenance cohort. The 109 patients transitioned to rituximab from an alternative maintenance regimen were diagnosed with ANCA vasculitis at a median of 4.3 (IQR, 1.1 to 8.1) years before entry into the maintenance cohort and received a minimum of 3 to 6 months of oral cyclophosphamide for induction of remission. Conversely, those patients who received our standard induction regimen received 2 months of oral cyclophosphamide. There was no difference in baseline maintenance IgG levels or significant hypogammaglobulinemia between these groups, suggesting that cumulative cyclophosphamide exposure alone was not the primary driver of declining IgG levels. Additional investigation is needed to more accurately define the importance of cumulative cyclophosphamide exposure on hypogammaglobulinemia in this setting.

Rituximab has become an important therapy for maintenance of remission in ANCA vasculitis. The results of the MAINRITSAN trial show superior disease control with continuous rituximab compared to maintenance azathioprine. These results await confirmation by the ongoing Rituximab Vasculitis Maintenance (RITAZAREM) trial (10). In addition, the MAINRITSAN 2 trial is currently underway to compare fixed-dosing of rituximab versus dosing contingent upon B cell reconstitution (26). A previous analysis of our cohort demonstrated that maintenance therapy with rituximab-induced continuous B cell depletion was extremely effective at preventing relapse with a major disease flare rate of 5% over a median of 2 years (13).

As the use of extended courses of rituximab are likely to increase, it is essential to understand the long-term implications of this treatment. This retrospective analysis of 239 patients with ANCA vasculitis undergoing treatment with scheduled rituximab provides several salient insights. While both ANCA and IgG levels decline during induction, pathogenic ANCA fall at a more rapid rate. During maintenance, IgG levels remain essentially constant despite prolonged continuous B cell depletion. Serious infections with long-term rituximab therapy are relatively rare, and are associated with patient age and significant hypogammaglobulinemia. Further studies are required to help balance the risk of prolonged maintenance therapy against the risk of disease relapse.

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References

1. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med*. 2004 Jun 17; 350(25):2572–81. [PubMed: 15201414]
2. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med*. 2010 Jul 15; 363(3):221–32. [PubMed: 20647199]
3. Cambridge G, Leandro MJ, Edwards JC, Ehrenstein MR, Salden M, Bodman-Smith M, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum*. 2003 Aug; 48(8):2146–54. [PubMed: 12905467]
4. Smith KG, Jones RB, Burns SM, Jayne DR. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: Remission, relapse, and re-treatment. *Arthritis Rheum*. 2006 Sep; 54(9):2970–82. [PubMed: 16947528]
5. Cambridge G, Leandro MJ, Teodorescu M, Manson J, Rahman A, Isenberg DA, et al. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. *Arthritis Rheum*. 2006 Nov; 54(11):3612–22. [PubMed: 17075806]
6. Besada E, Koldingsnes W, Nossent JC. Long-term efficacy and safety of pre-emptive maintenance therapy with rituximab in granulomatosis with polyangiitis: results from a single centre. *Rheumatology (Oxford)*. 2013 Nov; 52(11):2041–7. [PubMed: 23934313]
7. Venhoff N, Effelsberg NM, Salzer U, Warnatz K, Peter HH, Lebrecht D, et al. Impact of rituximab on immunoglobulin concentrations and B cell numbers after cyclophosphamide treatment in patients with ANCA-associated vasculitides. *PLoS One*. 2012; 7(5):e37626. [PubMed: 22629432]
8. Specks U, Merkel PA, Seo P, Spiera R, Langford CA, Hoffman GS, et al. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med*. 2013 Aug 1; 369(5):417–27. [PubMed: 23902481]
9. Guillevin L, Pagnoux C, Karras A, Khouatra C, Aumaitre O, Cohen P, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med*. 2014 Nov 6; 371(19):1771–80. [PubMed: 25372085]
10. Jones RB. Rituximab in the treatment of anti-neutrophil cytoplasm antibody-associated vasculitis. *Nephron Clin Pract*. 2014; 128(3-4):243–9. [PubMed: 25401382]
11. Cartin-Ceba R, Golbin JM, Keogh KA, Peikert T, Sanchez-Menendez M, Ytterberg SR, et al. Rituximab for remission induction and maintenance in refractory granulomatosis with polyangiitis (Wegener's): ten-year experience at a single center. *Arthritis Rheum*. 2012 Nov; 64(11):3770–8. [PubMed: 22730028]
12. Smith RM, Jones RB, Guerry MJ, Laurino S, Catapano F, Chaudhry A, et al. Rituximab for remission maintenance in relapsing antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*. 2012 Nov; 64(11):3760–9. [PubMed: 22729997]
13. Pendergraft WF 3rd, Cortazar FB, Wenger J, Murphy AP, Rhee EP, Laliberte KA, et al. Long-term maintenance therapy using rituximab-induced continuous B-cell depletion in patients with ANCA vasculitis. *Clin J Am Soc Nephrol*. 2014 Apr; 9(4):736–44. [PubMed: 24626432]
14. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*. 2013 Jan; 65(1):1–11. [PubMed: 23045170]

15. Han WK, Choi HK, Roth RM, McCluskey RT, Niles JL. Serial ANCA titers: useful tool for prevention of relapses in ANCA-associated vasculitis. *Kidney Int.* 2003 Mar; 63(3):1079–85. [PubMed: 12631091]
16. Hellmark T, Niles JL, Collins AB, McCluskey RT, Brunmark C. Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol.* 1997 Mar; 8(3):376–85. [PubMed: 9071706]
17. Florescu DF, Kalil AC, Qiu F, Schmidt CM, Sandkovsky U. What is the impact of hypogammaglobulinemia on the rate of infections and survival in solid organ transplantation? A meta-analysis. *Am J Transplant.* 2013 Oct; 13(10):2601–10. [PubMed: 23919557]
18. Norlin AC, Sairafi D, Mattsson J, Ljungman P, Ringden O, Remberger M. Allogeneic stem cell transplantation: low immunoglobulin levels associated with decreased survival. *Bone Marrow Transplant.* 2008 Feb; 41(3):267–73. [PubMed: 17994123]
19. Erkan D, Vega J, Ramon G, Kozora E, Lockshin MD. A pilot open-label phase II trial of rituximab for non-criteria manifestations of antiphospholipid syndrome. *Arthritis Rheum.* 2013 Feb; 65(2):464–71. [PubMed: 23124321]
20. van Vollenhoven RF, Emery P, Bingham CO 3rd, Keystone EC, Fleischmann R, Furst DE, et al. Longterm safety of patients receiving rituximab in rheumatoid arthritis clinical trials. *J Rheumatol.* 2010 Mar; 37(3):558–67. [PubMed: 20110520]
21. Roberts DM, Jones RB, Smith RM, Alberici F, Kumaratne DS, Burns S, et al. Rituximab-associated hypogammaglobulinemia: incidence, predictors and outcomes in patients with multi-system autoimmune disease. *J Autoimmun.* 2015 Feb; 57:60–5. [PubMed: 25556904]
22. Bingham CO 3rd, Looney RJ, Deodhar A, Halsey N, Greenwald M, Coddling C, et al. Immunization responses in rheumatoid arthritis patients treated with rituximab: results from a controlled clinical trial. *Arthritis Rheum.* 2010 Jan; 62(1):64–74. [PubMed: 20039397]
23. Lund FE, Randall TD. Effector and regulatory B cells: modulators of CD4+ T cell immunity. *Nat Rev Immunol.* 2010 Apr; 10(4):236–47. [PubMed: 20224569]
24. Furst DE. Serum immunoglobulins and risk of infection: how low can you go? *Semin Arthritis Rheum.* 2009 Aug; 39(1):18–29. [PubMed: 18620738]
25. Walsh M, Casian A, Flossmann O, Westman K, Hoglund P, Pusey C, et al. Long-term follow-up of patients with severe ANCA-associated vasculitis comparing plasma exchange to intravenous methylprednisolone treatment is unclear. *Kidney Int.* 2013 Aug; 84(2):397–402. [PubMed: 23615499]
26. Tanna A, Pusey C. Clinical trials. Rituximab for maintenance of remission in AAV. *Nat Rev Nephrol.* 2015 Mar; 11(3):131–2. [PubMed: 25599619]
27. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999 Mar 16; 130(6):461–70. [PubMed: 10075613]

Figure 1. Composition of induction and maintenance groups

The induction group was comprised of 52 patients who received treatment with combination rituximab, cyclophosphamide, and prednisone, and had baseline and follow up ANCA titers and IgG levels. The maintenance group was comprised of 237 patients who achieved remission after induction therapy or were transitioned from an alternative maintenance regimen.

Figure 2. Change in immunoglobulin levels and ANCA titers with treatment

Shown are data from linear mixed-effects models examining the percent change in ANCA titers and immunoglobulin levels over time during induction (panel A) and maintenance (panel B) therapy. Slopes with accompanying 95% confidence intervals are shown in Table 2.

Figure 3. Kaplan-Meier estimate of significant hypogammaglobulinemia in the maintenance group

Shown are Kaplan-Meier curves for the top 3 IgG quartiles (range 560-1657 mg/dL) compared to the lowest IgG quartile (range 408-559) in patients who entered maintenance therapy with an IgG level > 400 mg/dL. Significant hypogammaglobulinemia was defined as an IgG level < 400 mg/dL. Abbreviations: w/o, without

Table 1**Characteristics by group**

Data are presented as mean $\pm$ SD or median (Interquartile range) unless otherwise specified. Fifty patients from the induction group entered the maintenance group. P values are not provided as a large subset of observations are not independent.

Characteristic	Induction [†] (n=52)	Maintenance [*] (n=237)
Age (years)	62 $\pm$ 14	61 $\pm$ 16
Female, n (%)	30 (57)	140 (59)
MPO at diagnosis, n (%)	36 (69)	142 (60)
Baseline IgG (mg/dL)	797 (643-1052)	678 (534-811)
IgG < 400	0 (0)	21 (9)
Baseline IgA (mg/dL) [†]	170 (134-224)	129 (82-191)
Baseline IgM (mg/dL) [*]	94 (68-139)	40 (23-67)
Baseline Positive ANCA, n (%)	52 (100)	139 (59)
μ Baseline eGFR (ml/min)	45 (28-71)	50 (28 to 65)
eGFR > 60 ml/min	18 (35)	79 (33)
eGFR 30-60 ml/min	20 (38)	90 (38)
eGFR < 30 ml/min	14 (27)	68 (29)
PLEX, n (%)	0	45 (19)
Baseline BVAS-WG	6 (4-8)	0
Time from ANCA vasculitis diagnosis (mo)	0.7 (0.4-1.3)	14 (4.4-68)
Cumulative RTX during treatment group (g)	2 (2-2)	7 (5-10)

[†]38 patients had values for IgA and IgM.

^{*}216 patients had values for IgA and IgM.

μ eGFR was determined using the four-variable Modification of Diet in Renal Disease (MDRD) equation (27).

Abbreviations: BVAS-WG, Birmingham Vasculitis Activity Score for Wegener's granulomatosis; eGFR, estimated glomerular filtration rate; Ig, immunoglobulin; MPO, myeloperoxidase; mo, months; PLEX, plasma exchange; RTX, rituximab

Table 2
Decline in immunoglobulin levels and ANCA titers

The mean population change in immunoglobulin levels and ANCA titers estimated from linear mixed effects models are presented as mean percent decline (95% confidence intervals).

Antibody	Induction Monthly % Decline (n=52)	Maintenance Yearly % Decline (n=237)
IgG	6 (4 to 8)	0.6 (-0.2 to 1.4)
IgA	5 (2 to 9)	5 (3 to 6)
IgM	16 (13 to 19)	9 (8 to 11)
Anti-MPO	47 (42 to 52)	42 (32 to 50)
Anti-PR3	48 (42 to 54)	73 (58 to 83)

Mean percent decline in ANCA titers presented for the 139 patients who had positive titers at entry into the maintenance group.

Abbreviations: Ig, immunoglobulin; MPO, myeloperoxidase; PR3, proteinase 3.

Table 3
Risk factors for serious infections

Incident rate ratio (IRR) obtained from univariable and multivariable Poisson regression.

Variables	Univariable IRR (95%CI)	P value	Adjusted IRR (95% CI)	P value
Age (per 10 yr)	1.49 (1.24 to 1.80)	<0.001	1.46 (1.19 to 1.78)	<0.001
Male	0.78 (0.46 to 1.32)	0.34	0.83 (.50 to 1.42)	0.51
MPO	1.76 (1.03 to 3.02)	0.04	1.32 (0.73 to 2.37)	0.36
Time from diagnosis to rituximab (per yr)	0.97 (0.92 to 1.03)	0.30	0.97 (0.91 to 1.04)	0.41
Duration of continuous rituximab before remission (per mo)	0.79 (0.53 to 1.17)	0.24	0.99 (0.69 to 1.41)	0.94
PLEX	0.64 (0.29 to 1.41)	0.27	0.42 (0.19 to 0.96)	0.04
IgG < 400 mg/dL	2.40 (1.22 to 4.72)	0.01	2.13 (1.04 to 4.36)	0.04

Abbreviations: CI, confidence interval; Ig, immunoglobulin; mo, month; MPO, myeloperoxidase; PLEX, plasma exchange; yr, year.