Synovial Tissue Lymphoid Aggregates Are Associated With Response To Rituximab Therapy In Rheumatoid Arthritis Patients

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Research Article

Keywords: Rituximab, Rheumatoid Arthritis, Disease Activity, Synovial tissue, Lymphoid aggregates, Remission

Posted Date: February 15th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1275951/v2>

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Abstract

Background: To evaluate the clinical and laboratory factors associated with long-term responses to rituximab therapy in patients with RA.

Methods: One hundred fourteen RA patients received intravenous Rituximab between 2003-2016. Prior to treatment, arthroscopy and synovial biopsy was performed on a subgroup of this cohort who had active knee arthritis. Demographic, clinical, and outcome data were collected prospectively and immunohistology was performed on synovial tissue biopsies.

Results: In the overall cohort, 89% of patients were seropositive for either RF (rheumatoid factor) or ACPA (anti-citrullinated protein antibodies). At baseline, median disease duration was 13.5 years. Seventy-four percent of patients had received a csDMARD and two thirds had received a bDMARD before rituximab. Rituximab monotherapy was used in 34 patients, while 80 patients received rituximab-csDMARD combination therapy. Twenty-six of the 68 patients in remission (38%) received rituximab monotherapy and 42/68 (62%) received combination therapy with a csDMARD. Twenty-four of 39 (62%) biologic naïve patients achieved remission on treatment with rituximab. Forty-four patients underwent an arthroscopy and synovial biopsy prior to treatment. Synovial tissue lymphoid aggregates (LA) were observed in 21 subjects, of which 17 (81%) showed complete or partial remission in response to treatment with rituximab. The presence of LA was significantly associated with rituximab-induced remission in these patients ($p=0.007$, $OR=7.286$ [1.737-30.555]).

Conclusion: In this real world series, patients with RA demonstrate long-term, high response rates to rituximab therapy and synovial biopsy B-cell rich lymphoid aggregates are associated with a higher rate of good response.

Introduction

Rheumatoid arthritis (RA) is associated with the presence of circulating autoantibodies such as rheumatoid factor (RF) and anti-citrullinated proteins (ACPA), which may be present before clinical manifestations of disease [1]. The association with autoantibodies has led to focus on the role of activated B lymphocytes and plasma cells in RA pathogenesis. The anti-CD20 monoclonal antibody (rituximab) has been reported as a successful treatment for RA [2, 3].

The efficacy of Rituximab, both as induction and maintenance therapy, in the treatment of RA has now been the subject of several open and randomized controlled trials (RCTs) [4–13]. Rituximab has been demonstrated to be effective in early RA, as monotherapy, in combination with methotrexate or cyclophosphamide, and in tumour necrosis factor inhibitor (TNFi) inadequate responders [6–11]. Rituximab has also been shown to inhibit radiographic disease progression in RA [11, 12]. The duration of these RCTs and time to the primary endpoint, has been relatively short 24-52 weeks duration. The high quality RCTs that have been performed to date evaluating rituximab in RA provide strong evidence of efficacy and safety. However, while rightly considered the highest form of evidence on which to base

treatment decisions, there are inherent limitations to RCTs; including the short time of follow up, lack of power to assess rare outcomes, and the inclusion of patients who are generally healthier and have better outcomes than the target population [14, 15].

Therefore, evidence in the form of real world, observational studies is useful for the evaluation of long-term treatment outcomes in routine patient populations. A number of registry-based studies have been reported [16–19], with large numbers of patients and follow-up duration of 2-4 years [19]. Good response to rituximab has been associated with clinical factors such as ACPA titres and response at one year has been identified as a useful predictor of good response to TNFi bDMARDs [20–22]. The effects of rituximab on synovial tissue have been reported with some studies showing a reduction in the numbers of B cells [23–25] associated with a good clinical outcome. However, complete clearance of B cells from the synovium does not appear to be achieved following rituximab therapy [26, 27]. Importantly, while these studies report on B cell and plasma cell numbers, no studies to date have examined the presence or absence of lymphoid aggregates in the synovial tissue. More recently, studies suggest that gene profiling of synovial tissue may be useful in predicting the responses to rituximab treatment, with analysis of synovium with low numbers of B-cells associated with a transcriptomic profile that suggests an inadequate response to rituximab [28, 29]. We performed a long-term, prospective, observational cohort study of all RA patients treated with rituximab at our institution over a period of 13 years. Specifically, we have analyzed synovial tissue biopsies for lymphoid aggregates, prior to starting treatment, on a subgroup of 40 patients.

Patients And Methods

Patient Recruitment

All patients attending outpatient clinics at the Department of Rheumatology, St. Vincent's University Hospital from 2003-2016 with a diagnosis of RA and who were scheduled to receive Rituximab were included in this observational study. All patients fulfilled the 1987 revised ACR criteria for the classification of RA [30]. All subjects gave fully informed written consent as approved by the St. Vincent's Healthcare Group Medical Research and Ethics Committee and all research was performed in accordance with the Declaration of Helsinki.

Clinical Assessment

Demographic and clinical characteristics, including age, gender, disease duration, current and previous medications, and response to treatment were recorded. Laboratory investigations including ESR, CRP, RF and ACPA were recorded at baseline. The CRP, 28-joint counts for tender and swollen joints and patient global health score (0-100mm visual analogue scale (VAS)) was completed at each subsequent visit. Disease activity was calculated using the DAS28-CRP [31]. Patients were followed up to the time of their most recent outpatient visit. The data in this study was recorded prospectively and entered a secure database. Remission was defined as DAS28-CRP <2.6 or meeting the 2011 ACR/EULAR remission criteria [32]. Patients with active disease (DAS28-CRP $\geq$ 3.2) were classified as primary or secondary non-

responders based on their initial and subsequent responses. If Rituximab was discontinued due to adverse events the severity and nature of these were recorded.

Arthroscopy

Arthroscopy and synovial biopsy was performed in a sub-group of patients prior to treatment under local anaesthesia using a Wolf 2.7mm arthroscope, as previously described [33]. Patients were selected for arthroscopy based on the presence of active knee synovitis and provided written, fully informed consent for the procedure. The joint cavity was inspected, and the degree of synovitis and vascularity were scored on a VAS (0–100 mm) by experienced operators.

Synovial tissue immunohistological staining

Immunohistochemistry staining was performed using 3µm paraffin sections. Paraffin embedded sections were baked for 30 minutes at 90°C, deparaffinised in xylene and rehydrated in alcohol and deionised water. Antigen retrieval was performed by heating sections in antigen retrieval solution (15ml of 1M sodium citrate and 15ml of 1M citric acid in deionised water, pH 6.0) in a pressure cooker. Slides were washed in PBS for 5 minutes. Non-specific binding was blocked using 10% casein in PBS for 20 minutes. CD20 (DAKO, UK) and CD138 (DAKO, UK) primary antibodies were incubated on sections for 1 hours at room temperature. An IgG1 control antibody was used as a negative control. Following primary antibody incubation, endogenous peroxidase activity was blocked using 0.3% H₂O₂. Slides were incubated for 1 hour with horseradish peroxidase–conjugated secondary antibody (Dako). Colour was developed in diaminobenzidine solution (1:50; Dako) and counterstained with hematoxylin. Slides were mounted in Pertex media and analyzed using an established and validated semiquantitative scoring method 0–4 scale [34]. Lymphocyte-rich areas in synovial tissue were first described as ‘resembling tonsil’ [35]. More recently, it has been noted that lymphoid aggregates may become more highly organised into ectopic lymphoid follicle-like structures [36]. The presence or absence of lymphoid aggregates was determined in the synovial tissue, based on the original description of a lymphocyte aggregate.

Statistical Analysis

Descriptive statistics were reported as mean and standard deviation (SD), median and interquartile range (IQR), or number (n) and percentages as appropriate. For between group comparisons Chi-squared tests were used for categorical variables and independent samples t-tests for continuous variables. Student’s t-tests were used to analyse parametric data. For non-parametric data Wilcoxon signed rank test for related samples and Mann Whitney U test for unrelated samples were used.

Linear regression was used to assess the association between response to Rituximab and continuous variables. Correlations between response to Rituximab and dichotomous variables were assessed using binary logistic regression. Univariate analyses were performed to assess demographic and clinical predictors of rituximab response. Multiple logistic regression analyses were performed to model predictors of rituximab response. All factors that demonstrated $p < 0.20$ in the univariate analyses were

used to create a multivariate model and were evaluated with logistic regression analysis to model predictors of rituximab response.

Statistical significance was set at $P < 0.05$ throughout. All analyses were performed using IBM SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.) and GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Binary logistic regression was used to evaluate relationships between DAS28-CRP remission and lymphoid aggregates.

Patient and Public Involvement

Patients and public were not directly involved in the design of this research study, which was commenced over 15 years ago. However, CARD now has an active patient and public interaction, so that the analysis and results of this study have been discussed with our patient partners.

Results

There were a total of 114 RA patients (83 female and 31 male) with a mean age of 62 years and a median disease duration of 13.5 years were followed up (Table 1).

Table 1

Baseline characteristics of 114 Rituximab treated patients. Data expressed as mean (SD), n (%), or median (IQR) as indicated.

Variable	Mean ($\pm$ SD) or Median (IQR)
Age, years, mean ($\pm$ SD)	62 ($\pm$ 13)
Female, n (%)	83 (73%)
Disease duration, years, median (IQR)	13.5 (7, 24.3)
Previous csDMARDs, n (%)	84 (74%)
Previous bDMARDs, n (%)	75 (66%)
Serology, n (%)	97 (85%)
RF+	73 (64%)
ACPA+	67 (59%)
RF+ACPA+	13 (11%)
RF-ACPA-	
Tender Joint Count, median (IQR)	7 (3, 15)
Swollen Joint Count, median (IQR)	5 (1, 11)
ESR, mm/hr, median (IQR)	32 (16, 57)
CRP, mg/dL, median (IQR)	11 (4, 27)
*SD, standard deviation; IQR, interquartile range; csDMARD, conventional synthetic disease modifying anti-rheumatic drug; bDMARD, biologic disease modifying anti-rheumatic drug; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein	

Overall, 74% of patients had received a csDMARD previously and 66% had received a bDMARD. Thirty-four patients received rituximab monotherapy, while eighty received combination therapy rituximab plus a csDMARD. At baseline 85% of patients were RF positive; 64% anti-CCP antibody (ACPA) positive; 59% both RF and ACPA positive and 11% were autoantibody negative. Forty-four patients underwent an arthroscopy and synovial tissue biopsies were available to be evaluated for lymphoid aggregates (LA) in 40 subjects.

Efficacy of Rituximab

The maximum duration of follow-up was 12.8 years. DAS28-CRP remission was maintained by 68% of RA patients treated with rituximab, there were 15 primary non-responders, 24 secondary non-responders and 7 patients stopped due to adverse events (Figure 1). The patients maintained in remission on rituximab included 38% on monotherapy and 62% on combination therapy rituximab plus a csDMARD.

Rituximab achieved equivalent outcomes in patients who had previously failed a bDMARD. In the bDMARD naïve patients (n=39) remission was achieved in 62% of patients, while 59% bDMARD-experienced patients achieved remission and 41% did not. Fifty-five patients had previously failed 2 or more bDMARDs, 55% of whom achieved remission. There was no difference in any clinical or arthroscopic metrics between responders and non-responders before treatment with rituximab. In the 40 patients with evaluable synovial biopsy tissue LA positive for the B cell marker CD20 (LA+) (Figure 2A), were observed in 21 subjects, while 19 were LA negative (LA-) (Figure 2B). Sustained remission was observed in 14/21(66%) LA+ subjects and partial remission in 3/21(14%). In contrast, 19 subjects were LA-, only 3/19 (15%) of whom had a sustained response, 4/19 (21%) had a partial response and 12/19(63%) had no response.

Predictors of Rituximab Response

Binary logistic regression analysis was performed to assess predictors of response to rituximab. Variables analysed included RF positivity, RF titre, ACPA positivity and, titre, ESR, CRP, TJC, SJC, disease duration, number of previous csDMARDs failed, number of prior biologics failed, LA, and B cell marker staining. The presence of LA was the only factor significantly associated with remission in patients treated with rituximab (p=0.007, OR=7.286 [1.737-30.555]) (Table 2).

Table 2
Binary logistic regression analysis of microscopic findings in patients who underwent arthroscopy

Variable	P value	OR (95% CI)
Predictors of remission:		
CD20+	NS	
CD138+	NS	
Lymphoid Aggregates	0.007	7.286 (1.737-30.555)
*Remission is defined as Das28-CRP≤2.6.		
**OR = odds ratio, DAS28-CRP = disease activity score 28 based on C-reactive protein.		

Safety of long-term Rituximab

Seven patients stopped Rituximab due to adverse events over the course of the study. This represented 6% of all patients treated with rituximab. Of these 3 had hypersensitivity reactions, 2 stopped due to recurrent LRTIs, 1 due to neutropenia, and 1 due to severe herpes zoster infection. 4 patients had an adverse event on the first course of rituximab (3 hypersensitivity (1 on 1st infusion) and 1 herpes zoster), 3 patients developed an adverse event on the second course (2 LRTIs, 1 neutropenia).

Discussion

In this study we evaluated long term outcomes in unselected RA patients treated with rituximab at our institution. Overall, we identified a good response to rituximab treatment, 68% of patients maintained DAS28-CRP remission over a mean of 3.1 years follow up, significantly longer than reported in RCTs. These data suggest rituximab compares favorably to remission responses, in the real-world setting, that we have reported with other bDMARDs [21]. Importantly, patients who had previously failed other bDMARDs demonstrated good response rates to rituximab, regardless of the number previously tried. Remission was achieved in 59% of those having failed one and 55% of those having failed two or more bDMARDs compared with 62% of biologic naïve patients. As reported previously the unique mode of action of rituximab, depleting B cells, it may facilitate a better response in those RA patients with B cell rich synovial biopsies, especially in those with B cell rich lymphoid aggregates. Interestingly, in contrast to the many open studies which found a higher response in ACPA+ RA patients we did not find any significant differences in clinical, serological, or macroscopic arthroscopy characteristics between rituximab responders and non-responders.

Our data support the original observational and RCT studies showing a good response to rituximab in patients with RA [6–13] and the persistence of response as reported by the British Society for Rheumatology Biologics Register (BSRBR-RA) [17]. The latter study evaluated persistence with rituximab treatment after 4 years in 1629 patients with a median disease duration of 12 years, in which the majority of patients were receiving concurrent csDMARDs. The authors found 60% of patients remained on rituximab treatment after 4 years. Rituximab had similar efficacy whether used as a first or second biologic in RA in their cohort. The Belgian MIRA registry reported a continuation rate of 67% in 649 patients over 1.8 years with a mean baseline disease duration over 12 years. All patients were receiving concurrent methotrexate and had received prior biologic treatment, with 54% receiving one, 32% receiving two, and 13% having > 2 prior biologics [18]. The German RABBIT registry reported on 907 patients with a mean disease duration of 14 years. 32% of patients received monotherapy and 68% combination with methotrexate or leflunomide, the mean number of prior biologics was 1.5. They demonstrated positive outcomes with only 18% of patients switching to an alternative biologic over 3 years of treatment [16]. Using similar methodology, an Australian study analysed 167 patients treated with Rituximab over 2.7 years[19]. The median disease duration was 12 years and 78% of patients had one or more prior biologic. These data for rituximab suggest it is being used as a second or third line biologic for RA patients in the 'real world' setting. We report similar results with over 60% of RA patients obtaining a significant clinical response over long-term follow up suggesting that if we can predict those patients who will respond, then it would be useful to introduce rituximab earlier in the treatment of RA patients.

In addition, our data suggests that if B cell depletion in the synovial LA, regardless of serotype, is an important part of the mechanism of action of rituximab, then synovial tissue biopsy should be used to guide the selection of candidates for rituximab therapy.

The main strength of our data over the previous registry studies is our detailed measurement of clinical and response data at the individual patient level. We also report on long term response rates up to 13 years in some patients. In addition to our clinical data, our research provides unique target tissue data from arthroscopies in, with immunohistological features of LA associated with a higher positive response to treatment.

Several studies have attempted to establish predictors of response in RA patients receiving rituximab therapy. A combination of data obtained from 10 registries in Europe showed that positive ACPA status, fewer failed prior DMARDs, single previous biologic use, and lower baseline DAS-28 independently predicted EULAR good response at 6 months [37]. The 6-month multicenter open-label rituximab re-treatment dose study (SMART) looked at 208 refractory RA patients and found that presence of ACPA or RF, and an elevated serum IgG level were independently associated with more favorable clinical response [38]. Both studies find an association between autoantibody status and short-term response to rituximab. The BSRBR-RA reported baseline RF status as the only predictor of response to rituximab, although they did not have data on, and therefore could not evaluate, ACPA status[17]. Similarly, a study cohort comprised of 1,008 RA patients from two independent RCTs (SERENE and REFLEX) demonstrated positivity for RF or ACPA in combination with an elevated CRP at baseline to predict a more favourable clinical response to rituximab [39]. Rituximab's success in treatment has been attributed to the degree of B cell depletion, particularly in the 2 weeks following administration rather than the dose itself [40]. In contrast to previous studies, in our cohort, no baseline features were predictors of response to rituximab. In particular, neither RF or ACPA positivity (nor titer) were predictive of response, this may reflect the real-world nature of our patients or the long-term response that we used in the analysis. However, we did find significant links between B-cell staining in the synovial LA, a feature not evaluated in any previous study, and successful treatment with RTX. Previous work from our group has shown an association between ACPA positivity and both synovial B cell LA in patients with RA [41]. We do not routinely measure serum IgG or markers of circulating B-cell depletion, so were unable to evaluate changes in these markers in response to rituximab treatment.

Our findings have several important implications for clinical practice. By demonstrating high levels of long-term persistence, efficacy and safety we confirm a role for rituximab as an important bDMARD for use in the real-world setting. Rituximab has usually been utilized in seropositive patients; however, our data demonstrate that it may also be an effective option in seronegative RA patients. This data mirrors results from previous studies which show good response to rituximab in both seropositive and seronegative patients, albeit a stronger response in seropositive patients [42]. Indeed careful analysis of previous results shows that while seropositive patients have a higher good EULAR response rate of 70%, seronegative patients have a good EULAR response rate as high as 48% [37]. The use of synovial biopsy B-cell rich LA maybe a useful marker of patients who may have a good response to rituximab.

There are limitations to our study. We have evaluated a relatively small number of patients managed over a long time period in routine clinical practice. There are inherent limitations to the observational method in evaluating patients outside of the rigorously controlled setting of a RCT. In order to maximise the

duration of our follow up data we have recorded responses up to the time of last follow-up, this has resulted in a wide range of follow up duration (median 3.1 years, maximum 12.8 years). This was a single-centre study, and this may limit the external validity of the findings and applicability to other populations.

Conclusions

In conclusion we have shown that rituximab is an effective long-term treatment achieving remission, sustained in over 60% of RA patients up to 13 years. Prior bDMARD treatment does not appear to appreciably diminish the likelihood of good response. Interestingly, the presence of lymphoid aggregates in synovial tissue biopsies are significantly associated with a higher response.

List Of Abbreviations

ACPA	anti-citrullinated protein antibody
ACR	American College of Rheumatology
bDMARD	biologic disease modifying anti-rheumatic agent
CD	cluster of differentiation
CRP	C-reactive protein
csDMARD	conventional synthetic disease modifying anti-rheumatic agent
DAS	disease activity score
DMARD	disease modifying anti-rheumatic agent
ESR	erythrocyte sedimentation rate
EULAR	European Alliance of Associations for Rheumatology
IQR	interquartile range
LA	lymphoid aggregate
RA	rheumatoid Arthritis
RF	rheumatoid factor
SD	standard deviation
SJC	swollen joint count

Declarations

Ethics approval and consent to participate

This study was approved by St. Vincent's University Hospital Ethics Committee

Consent for publication

The authors consent for this article to be published.

Availability of Data and Materials

Data will be made available on reasonable request. There are no additional unpublished data.

Competing interests

The authors have no competing interests to declare

Funding

No funding was received for the work reported in this manuscript

Author's Contributions

MAT collected and analysed data, performed statistical analysis, and wrote the manuscript.

CL planned the study, collected and analysed data, and wrote the manuscript

CO, RC, KM and FY collected and analysed data, revised the manuscript for intellectual content and approved the final version.

EM, ABM, UF, and DJV planned the study, contributed to the data analysis, revised the manuscript for intellectual content and approved the final version.

Acknowledgements

We would like to thank all the patients and the staff at the Bone and Joint unit of St. Vincent's University Hospital.

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Figures

A

- RF+/ACPA+
- RF+/ACPA-
- RF-/ACPA+
- RF-/ACPA-

Rituximab Responders

B

Rituximab Non-Responders

C

Stopped Rituximab

Figure 1

Serostatus among (A) responders, (B) non-responders, and (C) those who stopped taking rituximab.

Figure 2

Immunostaining of rheumatoid arthritis synovial tissues for CD20+ B cells showing a B cell rich lymphoid aggregate in A, and a sparse diffuse B cell infiltrate without any aggregates in B. (Original magnification x 10).