Short running head: Long-term rituximab in Sjögren

Title:

Long-term safety of rituximab in primary Sjögren's syndrome: the experience of a single centre

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Abstract:

Objective: This work aims to evaluate the long-term safety of rituximab (RTX) in primary Sjögren's syndrome (pSS) to determine the safety and the efficacy of long-term treatment with B cell depleting therapy in pSS patients with active systemic disease.

Methods: An historical cohort study, enrolling 35 pSS patients treated with RTX, between 2008 and 2019, in a single Rheumatologic Unit was performed. When patients experienced adverse events, the treatment was suspended, and patients' data were recorded.

Results: The included patients were mainly female (91%) with a mean age of 54 years. During the observation, 13 patients (37.1%) suspended RTX treatment (10 cases per 100 patient-years, 95% CI: 0.061-0.17). Baseline demographics, disease characteristics, ESSDAI values, and treatment were comparable across RTX-suspended and non-suspended. Patients exposed to RTX had been followed for 35.82 ± 32.56 months, and the time of observation varied from 96 to 6 months. All the patients, except one, experienced a significant and persisting meaningful improvement of their ESSDAI (≥ 3 points) during the long-time follow-up. For the duration of the follow-up, 13 (37%) patients discontinued RTX treatment. Four out of 13 (30.8%) discontinued the treatment after the first infusion due to infusion-related reactions. During subsequent courses, the main cause of withdrawal was hypogammaglobulinemia onset (7 patients). In 2 patients, hypogammaglobulinemia was associated with severe infections.

Conclusion: RTX long-term administration showed to be a safe, well-tolerated, and effective treatment in patients with active systemic disease, significantly reducing ESSDAI, and the control of the disease activity last for years.

Introduction

Primary Sjogren's syndrome (pSS) is an autoimmune disorder characterized by lymphoplasmacytic infiltration of the exocrine glands, affecting predominantly salivary and lacrimal ones, responsible for sicca syndrome and extra-glandular clinical features. pSS mainly affects middle-aged women, and its incidence ranges between 3 and 11 per 100,000 individuals per year [1,2]. More than half of the affected patients develop systemic involvement [3,4]. In severe patients, the excess of mortality, is mainly related to the development of B cell lymphoma and visceral involvement, like interstitial lung disease, renal failure, hypokalaemic paralysis, and severe cryoglobulinaemic vasculitis [5].

Even if not unlicensed, over the last 2 decades, B-cell targeted therapy with the anti-CD20 monoclonal antibody rituximab (RTX) has been used for the management of selected pSS patients, after the failure of conventional treatment, based on observational studies and randomized clinical trials (RCTs) results [6]. Furthermore, the use of RTX, in pSS patients with severe and refractory systemic disease, is supported by the EULAR guidelines [7].

The rationale for B cell depletion therapy use relies upon the well-established pivotal role of B cell hyperactivity in pSS immune-pathogenesis, as confirmed by the presence of autoantibodies, hypergammaglobulinemia, elevated levels of rheumatoid factors (RF), and the increased risk of non-Hodgkin B cell lymphoma [8].

Despite the long-term experience in pSS, to date RTX right timing of administration is unclear and there are no guidelines regarding the "treat-to-target approach". As far as safety is concerning, data on repeated peripheral B cell depletion with RTX and long-term therapy are missing. Thus, the incidence of hypogammaglobulinemia, associated risk factors, and adverse events following RTX long-term administration in routine clinical practice are mostly unexplored.

This work aims to evaluate, in pSS, the safety of long-term RTX treatment and determine the consequences of repeated treatment with a peripheral B cell depleting therapy over eight years in severe pSS patients.

Materials and methods

Study design and participants

A historical cohort study, enrolling 35 pSS patients treated with RTX between 2008 and 2019 in the Rheumatologic Unit of San Salvatore Hospital, L'Aquila were performed. All patients were ≥18 years old, fulfilled the American–European Consensus Criteria for pSS [9], and received at least one RTX infusion. Disease activity assessment was performed, at the baseline and during follow-up, using the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) [7]. Systemic disease was defined as a disease that affects or has affected any of the organs/systems included in the clinESSDAI score, and active systemic disease was defined as patients with clinESSDAI score ≥1 [7]. Patients with ESSDAI score >14 or high activity in any of the ESSDAI domains with a definition of high activity were defined as patients with severe systemic disease [7]. Patients were selected to be treated with RTX if they have a moderate activity (5≤ESSDAI≤13) or high activity (ESSDAI≥14) [7,10], or they have enlarged parotid or arthritis for at least 3 months not responsive to glucocorticoids and immunosuppressive agents [7]. Patients received an infusion of 1,000 mg RTX on day 1 and on day 15 to complete a single course of therapy. RTX administration was repeated every 24 weeks. Treatment was considered effective if patients experienced a clinically meaningful improvement, defined as an improvement in ESSDAI of at least 3 points, as reported before and/or if parotid enlargement and arthritis improved [7,10]. When patients experienced side effects or lack of efficacy the treatment was discontinued, according to clinical judgment, and the data were recorded. Hypogammaglobulinemia was defined as IgG <500 mg/dL [11]. Patients' files were analysed to collect demographic characteristics, disease-specific features, and concomitant treatment. Patients with missing data were excluded from the study. The local ethics committees approved this study (Ethical

Committee Azienda Sanitaria Locale 1 Avezzano/Sulmona/L'Aquila, L'Aquila, Italy; protocol number 17660/19). The study was performed according to the Good Clinical Practice guidelines, and written informed consent was obtained from all patients according to the Declaration of Helsinki. However, since the retrospective nature of the study, for those patients who were not anymore followed-up (lost to follow-up or died during assessment), after having made every reasonable effort to contact them, we used the fully anonymized clinical data according to the Italian Law on privacy only for research purposes without any other intended aim (*Garante per la protezione dei dati personali, Autorizzazione n. 9/2016 – Autorizzazione generale al trattamento dei dati personali effettuato per scopi di ricerca scientifica - 15 dicembre 2016* [5805552]).

Statistical analysis

The statistical analysis provided descriptive statistics. Continuous variables, normally distributed, were expressed as mean ± standard deviation (SD). Data distribution was tested by using the Shapiro-Wilk test. To compare the clinical characteristics of RTX-suspended and RTX-non suspended parametric or non-parametric t-tests were used for all the continuous variables and Chi-squared test or McNemar's test with Yates'correction was used for the categorical variables, as appropriate.

Results

Baseline demographics, disease characteristics and duration of exposure

The patients included in our cohort were mainly female (91%) with a mean age of 54 years (SD ± 11.23). During the time of observation 13 patients (37.1%) discontinued RTX treatment (10 cases per 100 patient-years, 95% CI: 0.061-0.17). Baseline demographics, disease characteristics, ESSDAI values, and treatment were comparable across the included populations, as shown in Table 1. As of October 2020, 35 pSS patients had been treated with up to 16 courses of RTX for up to 8 years. Patients exposed to RTX had been followed for 35.82 ± 32.56 months, and the time of observation varied from 96 to 6 months. In detail, 6 patients

(17.1%) received 16 RTX courses, 2 patients (5.7%) received 11 RTX courses, 1 patient (2,8%) received 10 RTX courses, 1 patient (2.8%) received 8 RTX courses, 1 patient (2.8%) received 7 RTX courses, 2 patients (5.7%) received 6 RTX courses, 1 patient (2.8%) received 5 RTX courses, 3 patients (8.6%) received 4 RTX courses, 4 patients (11.4%) received 3 RTX courses, 6 patients (17.1%) received 2 RTX courses. Finally, 8 (22,8%) patients received 1 RTX infusion. At baseline, patients mean ESSDAI was 5.56 (SD ± 5.38), without difference between RTX suspended and non-suspended as shown in Table 1. All the patients, except one, experienced a significant meaningful improvement of their ESSDAI (≥ 3 points) after RTX during long-time follow-up, as showed in Figure 1. In RTX non-suspended group, the long-term treatment significantly reduced the number of pSS patients who showed constitutional, glandular, pulmonary, and nervous manifestations, as reported in supplementary table 1.

Treatment withdrawal

During the follow-up 13 (37%) patients suspended RTX treatment. Four out of 13 (30.8%) discontinued the treatment straight after the first infusion due to infusion-related reactions during the first drug administration. During subsequent courses the main cause of withdrawal was hypogammaglobulinemia onset. This adverse event (AE) occurred in 7 (53.9%) patients, and in 2 patients was associated with severe infections. One patient (7.7%) discontinued the treatment because of patients 'decision after 4 RTX courses, and 1 (7.7%) patient discontinued the treatment because after 4 courses of treatment there was no improvement and insufficient therapeutic response. These data are summarized in Table 2.

Hypogammaglobulinemia-related withdrawal

Among the 7 patients who discontinued the RTX treatment due to hypogammaglobulinemia, the time from RTX beginning and withdrawal was 35.86 ± 28.79 months, with an average of 7 treatment courses. Two patients out of seven had concomitant severe infection. One patient developed pyelonephritis and one patient developed an ocular infection caused by

Cytomegalovirus. Patients with hypogammaglobulinemia did not differ from patients without for age, age at pSS diagnosis, age at pSS onset, delay in diagnosis, ESSDAI and FS.

Discussion

This report is the first work, at the best of our knowledge, summarizing the safety and efficacy of prolonged RTX treatment (6 to 96 months) in pSS. In our cohort, the RTX long-term administration showed to be a safe, and well tolerated treatment in patients with active systemic disease. Furthermore, treatment with RTX improve disease management, significantly reducing ESSDAI, during the long-term observation. In fact, in our cohort, all the treated patients, except for one, achieved clinically meaningful improvement and maintained this result during the long-term follow-up. As far as safety is concerned, infusion-related treatment and hypogammaglobulinemia were the most common AEs observed. Four RTX-treated pSS patients (11.4%) developed infusion-related reactions, during the first administration. All of them experienced mild symptoms shortly after the beginning of the first infusion, usually regressed with corticosteroids and antihistamine drugs, after RTX withdrawal. The treatment was discontinued in these 4 patients while all the other did not experience any infusion-related reactions after the first course. The incidence of this AE, in our cohort, did not differ from what already reported. In fact, *Mejier et al.* described infusion-related reactions in 20% of treated patients, and, among them, one patient developed mild serum sickness-like disease [12]. *Devauchelle-Pensec et al.* reported a lower rate of infusion-related reactions (8%), and no serum sickness-like disease cases were reported [13]. During long-term follow-up the most common AE reported was the insurgence of hypogammaglobulinemia, observed in 7 RTX-treated patients (14.2%), with an average insurgence time of 36 months. Hypogammaglobulinemia is reported to be one of the most common side effects of RTX administration, in non-malignant hematological disorders [14]. Of interest, the rate of hypogammaglobulinemia in our cohort is lower when compared to the incidence of hypogammaglobulinemia during long-term follow-up of RTX-treated RA patients, which has been established to be up of 40% [15]. No difference

in the demographic baseline features was outlined between RTX-suspended and non-suspended. Furthermore, pSS baseline features, such as disease presentation, FS, autoantibodies, and concomitant therapies did not influence the development of hypogammaglobulinemia, mirroring what already reported in RA [16].

In our cohort, only 2 patients (5,7%) developed severe infection related to hypogammaglobulinemia induced by RTX treatment, confirming the RTX safety profile during long-term use [15-18]. The data observed in our cohort are similar to the results observed during long-term follow-up in RTX treated RA patients, in which severe infections varied from 5% to 10%, mainly associated with hypogammaglobulinemia [15-18]. As suggested, immunoglobulin levels monitoring before RTX and throughout treatment courses with B-cell depleting agents is recommended [16].

None of our patients developed lymphoma during the long-term follow-up. We are aware of some limitations of this study. Its retrospective nature, together with the relatively small number of included patients, limits the study power. However, our study is the first work analyzing the effect, in a large pSS cohort, of a very long RTX treatment, confirming its efficacy and safety in patients with systemic manifestations, thus endorsing its use in moderate to severely active disease, more than to treat sicca-related symptoms. The main reason of RTX discontinuation is hypogammaglobulinemia, while severe infections seem to be an uncommon AE in RTX-treated pSS patients, even during a long-term follow-up. Finally, pSS characteristics, disease presentations, and concomitant therapies do not to influence RTX suspension rate. Prospective long-term studies, enrolling a larger number of patients, would confirm and better clarify what we observed about the RTX efficacy and safety during long-term treatment in pSS. Due to the lack of specific disease-modifying drugs for pSS patients, and the conflicting results between specific designed trials using RTX in pSS, which failed to reach the primary outcome, and the large number of observational trials showing clinical benefits in pSS patients after RTX treatment, mirroring what already observed for RTX treatment in systemic lupus

erythematous, these efforts are necessary in order to understand if this treatment may be helpful for those pSS patients with systemic manifestations and severe disease, which are more prone to develop lymphoma [6]. Furthermore, due to the improvement of the systemic score, which evaluates several domains concerning different organs, our data may suggest new perspectives in order to treat specific-organ manifestations, unresponsive to conventional drugs as well as a possible "treat-to-target approach" in pSS, as in other rheumatic diseases. In fact, it has been already suggested that modification of ESSDAI values may be helpful to assess the efficacy of specific pSS treatments, pre-identifying possible targets to reach in clinical trials, thus opening a new avenue for the therapy of this disease.

Key messages

- RTX treatment is effective and safe in pSS patients, even during long-term treatment.
- The main cause of RTX withdrawal during long-term treatment in pSS is hypogammaglobulinemia.
- Severe infections related to hypogammaglobulinemia are rare in pSS patients treated with RTX.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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Figures legend:

Figure 1. Rituximab effects on ESSDAI in treated pSS patients.

ESSDAI values before and after RTX treatment in A) all the patients, B) in RTX non-suspended, and in RTX suspended. Student t test. D) ESSDAI values during RTX long-term follow-up (22 patients). T0, baseline; T1, after 6 months; T2, after 12 months; T3, at last observation.

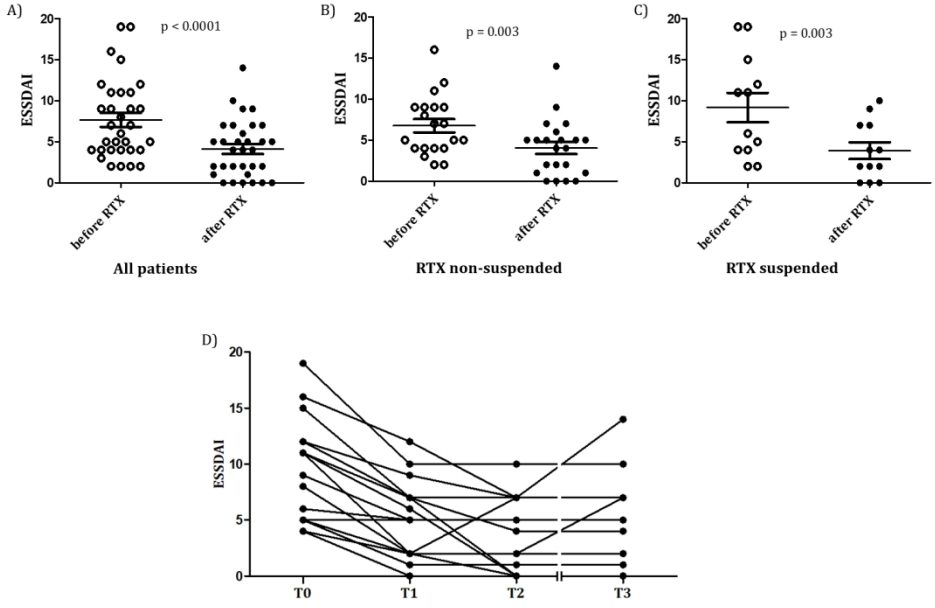

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Table 1. pSS patients' characteristics at baseline.

	All pSS (35)	RTX-suspended (13)	RTX-non suspended (22)	p value (suspended Vs non-suspended)
Female, n (%)	32 (91%)	12 (92%)	20 (91%)	1
Age at diagnosis, years (mean ± SD)	53.71 ± 11.23	55.36 ± 10.92	50.92 ± 11.64	0.26
Delay from symptoms onset and diagnosis, years (mean ± SD)	5.06 ± 6.38	6.23 ± 7.57	3.08 ± 2.90	0.16
Age at RTX first administration, years (mean ± SD)	60.40 ± 10.60	62.00 ± 10.08	57.69 ± 11.32	0.25
Clinical manifestations				
Xerostomia, n (%)	32 (91%)	12 (92%)	20 (91%)	1
Xeroftalmia, n (%)	34 (97%)	12 (92%)	22 (100%)	0.37
Constitutional symptoms, n (%)	9 (26%)	3 (23%)	6 (27%)	1
.....Lymphoadenopathy, n (%)	8 (23%)	2 (15%)	6 (27%)	0.68
Parotidomegaly, n (%)	5 (14%)	1 (8%)	4 (18%)	0.63
Arthritis, n (%)	23 (66%)	9 (69%)	14 (64%)	1
Skin involvement, n (%)	1 (3%)	0 (0%)	1 (4%)	1
Lung involvement, n (%)	2 (6%)	1 (8%)	1 (4%)	1
Kidney involvement, n (%)	0 (0%)	0 (0%)	0 (0%)	-
Muscular involvement, n (%)	0 (0%)	0 (0%)	0 (0%)	-
PNS involvement, n (%)	6 (17%)	2 (15%)	4 (18%)	1
CNS involvement, n (%)	1 (3%)	1 (8%)	0 (0%)	0.37
ESSDAI (mean ± SD)	5.56 ± 5.38	6.54 ± 6.59	5.05 ± 5.38	0.46
RF positivity, n (%)	9 (26%)	2 (15%)	7 (32%)	0.43
ANA positivity, n (%)	13 (37%)	4 (31%)	9 (41%)	0.72
antiRo/SSA positivity, n (%)	12 (34%)	5 (38%)	7 (32%)	0.73
antiLa/SSB positivity, n (%)	6 (17%)	2 (15%)	4 (18%)	1

IgG, mg/dL (mean ± SD)	1223 ± 267.3	1269 ± 310.8	1148 ± 163.8	0.30
Therapies				
GCs, n (%)	22 (63%)	8 (62%)	14 (64%)	1
HCQ, n (%)	11 (31%)	3 (23%)	8 (36%)	0.48
MTX, n (%)	6 (17%)	1 (8%)	5 (23%)	0.38
Focus score (mean ± SD)	2.01 ± 2.28	1.55 ± 1.00	2.36 ± 2.88	0.34

RTX, rituximab; n, number; SD, standard deviation; RF, rheumatoid factor; ANA, anti-nuclear antibodies, GCs, glucocorticoids; HCQ, hydroxychloroquine; MTX, methotrexate.

Table 2. Rituximab discontinuations during the study period.

Variable	RTX-treated patients (35)	RTX-suspended (13)
Infusion related reactions	4 (11.4%)	4 (30.8%)
Hypogammaglobulinemia	5 (14.2%)	5 (38.5%)
Hypogammaglobulinemia and CMV infection	1 (2.8%)	1 (7.7%)
Hypogammaglobulinemia and pyelonephritis	1 (2.8%)	1 (7.7%)
No improvement	1 (2,8%)	1 (7.7%)
Patient's decision	1 (2.8)	1 (7.7%)

RTX, rituximab.