Predictors of response to rituximab in patients with active rheumatoid arthritis and inadequate response to anti-TNF agents or traditional DMARDs

J. Narváez[1], C. Díaz-Torné[2], J.M. Ruiz[3], M.V. Hernández[4], V. Torrente-Segarra[2], S. Ros[3], A. Rodriguez de la Serna[2], C. Díaz-López[2], R. Sanmartí[4], J.M. Nolla[1]

[1]Department of Rheumatology, Hospital Universitario de Bellvitge - IDIBELL, Barcelona; [2]Department of Rheumatology, Hospital de la Santa Creu i Sant Pau, Barcelona; [3]Rheumatology Unit, Hospital de Viladecans, Barcelona; [4]Department of Rheumatology, Hospital Clínic de Barcelona – IDIBAPS, Barcelona, Spain.

Abstract

Objective

Identifying early predictors of response to biological agents is important for both the individual patient and health economics. The aim here was to identify clinical variables that are easily assessed in clinical practice which are associated with a major response to rituximab (moderate to good EULAR response, according to DAS28 values) in patients with active rheumatoid arthritis and inadequate response to anti-TNF agents or traditional DMARDs.

Methods

Rituximab (2x1g, two weeks apart) was administered to 108 patients in four different Spanish hospitals. The primary efficacy endpoint was the percentage of patients who achieved a major response at six months. Potential predictors of a major response were identified using multivariate binary logistic regression models.

Results

At six months of treatment 75.9% of patients achieved a major response (24% good and 52% moderate). Comparing the clinical features at baseline between patients who did or did not achieve a major response, significant differences were found in rheumatoid factor (RF) and anti-CCP positivity, as well as in the number of failed anti-TNF agents prior to rituximab. While rituximab delivers clinical benefit in seronegative patients, the presence of RF and/or anti-CCP consistently enriches clinical responses. The multivariate analysis showed that the best model for predicting a major EULAR response to rituximab was comprised of the following two variables: the anti-CCP antibody positivity (p=0.045) and the number of previous anti-TNF agents used (p=0.028). Using a cut-off level for CCP of 300 U/ml we found that patients with an anti-CCP titre >300 U/ml were 3–4 times more likely to achieve a major EULAR response [odds ratio (OR): 3.38; 95% CI: 1.025–11.17]. By contrast, those patients who had failed to respond to 2 or more anti-TNF agents had a 72.5% lower probability of achieving a moderate to good EULAR response (OR: 0.275; 95% CI: 0.087–0.871) than did patients who had only failed to respond to one such agent.

Conclusion

A lower number of previously-failed TNF blockers and high anti-CCP titre can help select the best candidates for RTX therapy in patients with RA.

Key words

rheumatoid arthritis, rituximab, EULAR response

Javier Narváez, MD, PhD
César Díaz-Torné, MD
José Miguel Ruiz, MD
Maria Victoria Hernández, MD, PhD
Vicens Torrente-Segarra, MD
Sergio Ros, MD, PhD
Arturo Rodriguez de la Serna, MD, PhD
César Díaz-López, MD, PhD
Raimon Sanmartí, MD, PhD
Joan Miquel Nolla, MD, PhD

Please address correspondence and reprint requests to:
Dr Javier Narváez,
Department of Rheumatology (planta 10-2),
Hospital Universitario de Bellvitge,
Feixa Llarga s/n,
L´Hospitalet de Llobregat,
08907 Barcelona, Spain.
E-mail: fjnarvaez@bellvitgehospital.cat
31577edd@comb.cat

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Introduction

The treatment of rheumatoid arthritis (RA) has changed dramatically with the introduction of anti-tumour necrosis factor (TNF) agents. However, anti-TNF agents are not effective in all patients: about 20–40% of cases fail to achieve an improvement of 20% in American College of Rheumatology criteria (ACR20; primary failure of inefficacy), and even more lose response over time (secondary failure or acquired therapeutic resistance) or experience adverse events following treatment with a TNF inhibitor (1-4). In this group of patients, therapeutic options include switching from one TNF inhibitor to another or the use of newer biological agents with a different mechanism of action, such as the B-cell depleting agent rituximab (RTX), the selective co-stimulation modulator abatacept, and the anti-IL-6 receptor monoclonal antibody tocilizumab. However, the optimal treatment strategy in these patients has yet to be defined because there have been no randomised, prospective, head-to-head trials comparing the available therapeutic options. Until such time as these studies are conducted, useful information may be gained from identifying reliable predictors of clinical response to the different biological agents.

RTX has proved to be an effective and safe therapy for patients with severe active RA who have shown an inadequate response to one or more TNF alpha inhibitors (5, 6). Although several methods have been assayed for the prediction of clinical response to RTX in RA patients, data regarding predictors of good response are still sparse. It has been reported that high synovial infiltration of B cells and complete B-cell depletion after the first RTX infusion are predictors of good response to this agent, but such data are not accessible at the beginning of treatment (7-12). Recently reported predictors of a better response include lower levels of type I interferons (IFN-γ), lower serum levels of B-cell activating factor (BAFF) or B lymphocyte stimulator (BLyS), lower levels of immunoglobulin free light chains in synovial fluid and synovial tissue, a favourable Fcγ receptor III genotype and the C/G-174 polymorphism of IL-6 (13-15). However, these determinations are not generally available in everyday clinical practice.

The most direct approach, *i.e.* the measurement of clinical variables that are easily assessed in clinical practice, is therefore still being investigated. Previous data showed that RTX may be beneficial in both seronegative and seropositive RA, although the presence of rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP) antibodies consistently enriches clinical responses (5, 6, 12, 16-22). As with anti-TNF agents (23-27), the patient's exact autoimmune profile, including the baseline anti-CCP titre, might be an early predictor of efficacy of RTX therapy in patients with RA, although in some studies the anti-CCP status has been found to be a weak predictor (19, 20).

The efficacy of RTX also seems to be greater when it is used in patients who have shown an inadequate response to a single TNF inhibitor, as compared with patients with failed responses to two or more TNF inhibitors (19, 28, 29). In addition, a recent observational study has demonstrated that in patients with RA who have stopped a previous anti-TNF treatment because of ineffectiveness, changing to RTX is more effective than switching to an alternative anti-TNF agent. Finally, a low baseline HAQ disability has also recently been shown to be an early predictor of response (19). However, none of these variables has been unequivocally confirmed as a predictor.

In light of the above, the present study explored the predictors of a major response [European League against Rheumatism (EULAR) moderate-to-good response, according to a disease activity score on 28 joints (DAS28)] present at treatment initiation in a real-life cohort of 108 patients with active RA and inadequate response to anti-TNF agents or traditional DMARDs treated with RTX. The possible correlation between the efficacy of RTX therapy and the titre of anti-CCP antibodies was specifically examined.

Material and methods

Data on 108 patients with active RA (all of whom met the ACR classifi-

cation criteria for RA) (31), treated with at least one cycle of RTX (1g x 2 weeks) in four different Spanish hospitals were retrospectively collected. Patients included in this analysis represent the large majority of RA patients treated with RTX in those centres, followed up for at least 6 months after RTX treatment (we only excluded 20 patients in whom complete information was not available). In accordance with the guidelines of our institutional ethics committee, formal approval for this study was not required. The local ethics committee agreed that the findings in this report were based on normal clinical practice and therefore were suitable for dissemination.

Ninety-eight patients (91%) received RTX after the failure of ≥1 anti-TNF agent. In ten patients (9%), RTX was administered as a first-line biological therapy due to contraindications for anti-TNF therapy. The local drug and therapeutics committee authorised the use of RTX in these cases and patients signed an informed consent form.

RTX treatment consisted of two intravenous infusions of 1 g per treatment cycle separated by a two-week interval (days 1 and 15), with repeated courses of therapy at least six months afterwards, depending on clinical response. All patients received premedication (methylprednisolone 100 mg by intravenous infusion) to prevent infusion reactions. RTX was administered alone in 31 (29%) patients and in combination with one DMARD (methotrexate or leflunomide) in the other 77 (71%) cases. Ninety-five (88%) patients were also receiving concomitant low-dose oral glucocorticoid treatment (≤10 mg/day of prednisone or equivalent). Patients continued DMARDs, steroids, and non-steroidal anti-inflammatory drugs (NSAIDs) at a stable dose.

Inpatient and outpatient charts of patients were reviewed comprehensively following a specifically designed protocol. Baseline data collected at the time of RTX prescription included age, sex, disease duration, presence of rheumatoid nodules, evidence of erosions (as established by radiographs of hands and feet), presence of extra-articular manifestations, details of past and present anti-rheumatic therapies, and assessment of disease activity including swollen and tender joint count in 28 joints, DAS28 score, health assessment questionnaire (HAQ), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). The same assessment of disease activity were recorded every six months during follow-up. The baseline serological status for RF and anti-CCP antibodies was also collected. Anti-CCP antibodies (IgG) were measured using commercially available second-generation ELISA kits: EliA™ CCP Assay on the ImmunoCAP250 instrument (Phadia, Germany) in three hospitals, and the Immunoscan RA™ (Euro-Diagnostica, Malmö, Sweden) in one hospital. Anti-CCP antibody levels were expressed in arbitrary units per millilitre and were considered to be positive when the concentration was higher that the cut-off value of the kit (manufacturer's cut-off: Phadia 7-10 EliA units/mL and Euro-Diagnostica 25 units/mL).

The follow-up of patients after the first infusion of RTX ranged from 6 to 24 months (median ± standard deviation: 13.5±6.6 months; range 7-25). Two cycles of RTX were administered in 44 patients (41.9%), three cycles in 26 patients (24.7%), and four or more cycles in 18 (17.1%).

The primary endpoint of this retrospective analysis was the DAS28 response rate at month 6, according to the EULAR improvement criteria (32). Good response was defined as a significant decrease in DAS28 score (>1.2) and a low level of disease activity (≤3.2). Non-response was defined as a decrease of ≤0.6 or a decrease of 0.6-1.2 with a score of >5.1 on the DAS28. Any scores between these limits were regarded as indicative of moderate responses. Secondary efficacy endpoints included the percentage of patients in remission (DAS28 <2.6) and with low disease activity (defined as DAS28 ≤3.2), the percentage of patients fulfilling the ACR50 response criteria, and the progression of functional disability as measured by change from baseline on the Stanford Health Assessment Questionnaire (HAQ) disability index.

The ACR and EULAR response considered for the analysis was the maximal clinical response obtained between the end of month +4 and the end of month +6 of follow-up after the first cycle of RTX.

Statistical analysis

Statistical analysis was performed using SAS 9.1.3 statistical software. Continuous data were described as mean ± standard deviation (SD) or median (minimum, maximum), while categorical variables were presented as number of cases with percentages. Continuous variables were compared using the Student's *t*-test or the median test. Categorical variables were analysed using the Chi-square test or by calculating the 95% confidence intervals for the differences between proportions using Newcombe's method. Potential predictors of a major response (EULAR moderate to good response) at six months were identified using multivariate binary logistic regression models (forward stepwise method). Statistical significance was defined as $p<0.05$.

Results

Baseline characteristics

Patient and treatment characteristics at baseline are shown in Table I. All patients had a history of failed treatment with at least one DMARD (mean ± SD: 2.9±1.4; range 1-7). Ninety-eight patients (91%) underwent RTX after the failure of ≥1 anti-TNF agent. Primary or secondary inefficacy, rather than development of side effects, was the reason for anti-TNF failure in the large majority of cases (70%). Seven of these patients had also failed to respond to other biological agents (abatacept or tocilizumab). In ten patients (9%), RTX was administered as a first-line biological therapy due to contraindications for anti-TNF therapy. The baseline serological status for RF and anti-CCP antibodies is presented in Table II. Sixteen percent (17/108) of patients were seronegative for both RF and anti-CCP antibodies.

Response rates

Table III shows the response rates at six and twelve months of therapy. Overall, 76% (82/108) of all patients were considered responders after six

Table I. Patient and treatment characteristics at baseline.

Number of patients	108
Women/men	86/22
Age, years	58.4 ± 12.2
Disease duration, years	9.8 (1.36)
Positive rheumatoid factor	86 (80%)
Positive anti-CCP antibodies	88 (81%)
Rheumatoid nodules	28 (26%)
Erosions in the peripheral joints	100 (93%)
Systemic extra-articular manifestations	18 (17%)
Secondary Sjögren's syndrome	8 (7%)
Pleuro-pulmonary involvement	5 (5%)
Felty's syndrome	2 (2%)
Secondary AA amyloidosis	3 (3%)
Tender joint count/28	10.2 ± 6.1
Swollen joint count/28	7.5 ± 5
DAS28	5.8 ± 1.1
HAQ (0–3)	1.5 ± 0.6
ESR (mm/h)	43 (15.103)
CRP (mg/l)	11.8 (6.36)
Number of previous DMARDs used	2.9 ± 1.4 (range.1–7)
Rituximab as a first-line biological therapy	10 (9%)
Rituximab after TNF failure	98 (91%)
Number of previous anti-TNF agents used	1.44 ± 0.81
Reason for the anti-TNF failure	
Primary or secondary inefficacy	70%
Development of side effects	30%
Other biological agents used	
Abatacept	6
Tocilizumab	1
Concomitant therapy	
DMARDs	77 (71%)
Low-dose oral glucocorticoid treatment	95 (88%)

Results are presented as mean ± standard deviation, median (minimum, maximum) or number of cases with percentages.

Table II. Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody status.

RF-positive patients	86 (80%)
Anti-CCP positive patients	88 (81%)
Anti-CCP titre (mean ± standard deviation), u/ml	466 ± 225
RF-positive / anti-CCP positive patients	83 (77%)
RF-negative / anti-CCP negative patients	17 (15.7%)
RF-positive / anti-CCP negative patients	3 (2.7%)
RF-negative / anti-CCP positive patients	5 (4.6%)

Results are presented as number of cases with percentages.

months of treatment, 24% (26/108) as having a good response and 52% (56/108) as having a moderate response. Low disease activity (DAS28 ≤3.2) was achieved in 29% of patients, remission (DAS28 <2.6) in 12%, and ACR 50 response in 20%. The median DAS28 score decreased significantly from 5.8 at baseline to 4.1, while the HAQ scores improved by 0.84. Clinical improvement was accompanied by a parallel improvement in the acute phase reactants (ESR and CRP).

After twelve months of treatment, the benefit persisted and increased slightly the percentage of major EULAR responses.

Predictors of treatment response

Comparing the clinical features at baseline between patients who did or did not achieve a major response (Table IV), significant differences were found in RF (p=0.03) and anti-CCP positivity (p=0.03), as well as in the number of failed anti-TNF agents prior to RTX (p=0.04).

While RTX delivers clinical benefit in seronegative patients, the presence of RF and/or anti-CCP consistently enriches clinical responses. As shown in Table 5, patients who were seropositive to either of these two autoantibodies were 1.5 times more likely to achieve a major EULAR response, compared to patients who did not have these autoantibodies (this difference being statistically significant).

Results of the regression analysis are presented in Table VI. The multivariate analysis showed that the best model for predicting a major EULAR response to RTX was comprised of the following two variables: the anti-CCP antibody positivity (p=0.045) and the number of previous anti-TNF agents used (p=0.028). Using a CCP cut-off level of 300 u/ml we found that patients with an anti-CCP titre >300 were 3-4 times more likely to achieve a major EULAR response [odds ratio (OR): 3.38; 95% CI: 1.025-11.17]. By contrast, those patients who had failed to respond to more than one anti-TNF agent had a 72.5% lower probability of achieving a moderate-to-good EULAR response (OR: 0.275; 95% CI: 0.087–0.871) than did patients who had only failed to respond to one such agent.

Discussion

Identification of early predictors of response to biologic agents is important regarding both the individual patient and health economics. Both clinical parameters and biomarkers are possibly predictive of a good outcome in patients undergoing RTX therapy. In our real-life cohort of RA patients treated with RTX, the univariate analysis showed that RF and anti-CCP positivity and a lower number of previously-failed TNF blockers were significantly associated with a major EULAR response at six months of therapy. In the multivariate logistic regression analysis, the anti-CCP antibody positivity and the number of previous anti-TNF agents used remained statistically significant.

Our results are in agreement with other studies. Data from clinical trials demon-

Table III. Treatment response rates at 6 and 12 months of therapy.

	6 months (n=108)	12 months (n=65)
DAS28	4.12 ± 1.48	3.63 ± 1.25
Change in DAS 28	-1.7 ± 1.46	-2.17 ± 1.36
% of change in DAS28	-28.3 ± 24.1	-36.1 ± 20.6
EULAR response		
good-moderate	82 (76%) [95% CI: 0.670 – 0.830]	55 (85%) [95% CI: 0.739 -0.914]
Good	26 (24%) [95% CI: 0.169 - 0.329]	22 (34%) [95% CI: 0.265 - 0.459]
Moderate	56 (52%) [95% CI: 0.425 – 0.610]	33 (51%) [95% CI: 0.389- 0.625]
DAS28 < 3.2	31 (29%)	19 (29%)
DAS28 < 2.6	13 (12%)	9 (14%)
ACR50	21 (20%)	18 (28%)
HAQ	0.84 ± 0.6	0.90 ± 0.79
Change in HAQ	- 0.84 ± 0.6	- 0.90 ± 0.7
% of change in HAQ	- 34.5 ± 93.6	- 43.8 ± 39.62
ESR (mm/h)	27 (5.85)	22 (2.75)
CRP (mg/l)	5.9 (0.87)	3.45 (0.74)

Results are presented as mean ± standard deviation, median (minimum, maximum) or number of cases with percentages. CI: confidence interval.

Table IV. Baseline differences between patients who did or did not achieve a major response [EULAR moderate-to-good response, according to DAS28 values).

	EULAR major response (n= 82)	No response (n= 26)	*p*-value
Women/men	62/20	24/2	0.06[1]
Age, years	58.6±12.3	57.8 ± 12.2	0.78[2]
Disease duration, years	9 (1,36)	14.6 (1.35)	0.17[3]
Positive rheumatoid factor	69 (84%)	17 (65%)	0.03[1]
Positive anti-CCP antibodies	72 (88%)	16 (61%)	0.03[4]
Rheumatoid nodules	22 (27%)	6 (23%)	0.70[2]
Erosions in the peripheral joints	74 (90%)	26 (100%)	0.09[2]
Systemic extra-articular manifestations	17 (21%)	2 (8%)	0.11[2]
DAS28	5.85±1.53	5.76±1.34	0.65[2]
HAQ (0-3)	1.5 (0.3)	1.75 (0.3)	0.44[3]
ESR (mm/h)	43 (15.103)	44.5 (16.99)	0.99[3]
CRP (mg/l)	11.7 (6.33)	12.1 (7.39)	0.65[3]
Number of previous DMARDs used	3 (1.7)	4 (1.5)	0.16[3]
Number of previous anti-TNF agents used	0 (0.3)	1 (0.3)	0.04[3]
Concomitant therapy			
DMARDs	60 (73%)	18 (69%)	0.69[1]
Low-dose oral glucocorticoid treatment	71 (87%)	24 (92%)	0.72[1]

Results are presented as mean ± standard deviation, median (minimum, maximum) or number of cases with percentages.
[1]Chi-square, [2]*t*-test, [3]Median test, [4]Fisher test.

strate that efficacy after a single course of RTX is superior when it is used in patients who have shown an inadequate response to a single TNF inhibitor, as compared with patients with a failed response to two or more TNF inhibitors (28). However, we can not exclude that this result could results from a simple selection process: patients who have failed more previous biologic agents are harder to treat and less likely to respond to any alternative treatment option. Thus, while these patients are in fact less likely to respond to RTX, they would also be less likely to respond to any other treatment option.

Importantly, at long-term follow-up, this better response seems to be maintained after repeated courses of RTX, indicating that earlier intervention with RTX was preferable (29). In this regard, it should be noted that prolonged exposure to anti-TNF therapy may induce resistance to RTX by increasing the B-cell survival factor BLyS/BAFF (33), and the overall duration of previous anti-TNF therapy, rather than the number of anti-TNF agents failed, may then be relevant.

As with anti-TNF agents (23-27) the patient's exact autoimmune profile, including the baseline anti-CCP titre, seems to be an early predictor of efficacy of RTX therapy in patients with RA. Previous data have shown that RTX may be beneficial both in seronegative and seropositive RA, although the presence of RF and/or anti-CCP antibodies consistently enriches clinical responses (5, 6, 12, 16-22). Isaacs *et al*. (17) presented the results of a post-hoc analysis based on a pooled cohort of 670 RA patients with inadequate response to DMARDs from two phase III studies of RTX. At week 24, seropositive patients were more than twice as likely to achieve an ACR response (ACR20 or ACR50) than those who were seronegative. At week 48, seropositive patients were over three times more likely to achieve an ACR70 response compared to seronegative patients. Seropositive patients also had significantly greater falls in disease activity scores, and were more likely to achieve a low disease status by week 48. The results of the SMART trial, a randomised open study designed to evaluate two strategies of re-treatment in patients responding to RTX after failure or intolerance to anti-TNF therapy, also confirm the predictive value of these autoantibodies for treatment response in this group of patients (18). It is important to note that in some of these studies the predictive value of RF seems to be much higher than that of anti-CCP antibodies (19, 20). A large-scale Italian study of 110 RA patients treated with RTX found that the variables associated with a major EULAR response were a lower HAQ score, a lower number of previously failed TNF blockers, and RF (but not anti-CCP) positivity (19).

The most striking result of the present study, however, was the link between the baseline level of anti-CCP antibodies and the clinical response to RTX. Using a cut-off level of 300 u/ml we found that patients with an anti-CCP

Table V. Comparative study of treatment response rates at 6 months of therapy between RF and/or PCC positive patients *versus* RF and PCC negative patients.

	RF and/or PCC positive patients (n=91)	RF and PCC negative patients (n=17)	*p*-value
DAS28	4.07 (1.45)	4.4 (1.67)	0.39[1]
Change in DAS 28	-1.66 (-6.2)	-1.37 (-3.1)	0.18[2]
% of change in DAS28	-32.74 (-92.36)	-25.85 (-60.25)	0.79[2]
EULAR response good-moderate	73 (80%)	9 (53%)	d = 27.3 [95% CI: 4.4, 50.3] (p<0.05)[4]
Good	23 (25%)	3 (18%)	d = 7.6 [95% CI: -17.0, 22.7] (p>0.05)[4]
Moderate	50 (56%)	6 (35%)	d = 19.7 [95% CI: -5.9, 40.1] (p>0.05)[4]
None	18 (20%)	8 (47%)	d = -27.3 [95% CI: -50.3, -4.4] (p<0.05)[4]
DAS28 < 3.2	28 (31%)	3 (17%)	0.38[3]
DAS28 < 2.6	11 (12%)	2 (12%)	0.99[3]
ACR50	19 (21%)	2 (12%)	0.51[3]
HAQ	0.75 (0.3)	0.38 (0.2)	0.13[2]
Change in HAQ	-0.63 (-3.2)	-0.53 (-2.0)	0.96[2]

Results are presented as mean ± standard deviation, median (minimum, maximum) or number of cases with percentages.
[1]*t*-test, [2]Median test, [3]Fisher test, [4]Confidence interval for the difference between means: d: differences; CI: confidence interval.

Table VI. Predictive factors of a major response [EULAR moderate to good response, according to DAS28 values) to rituximab at 6 months in the multivariate analysis.

Variable	Odds ratio	95% Confidence interval	*p*-value
Anti-CCP titre > 300	3.384	1.025 – 11.177	0.045
Previous number of anti-TNF agents >1	0.275	0.087 – 0.871	0.028

titre >300 u/ml were 3–4 times more likely to achieve a major EULAR response (OR: 3.38, 95% CI: 1.025-11.17). By contrast, various studies have reported that high anti-CCP levels predicted poorer early response to anti-TNF therapy. A large study in the UK involving 642 patients with RA demonstrated that anti-CCP antibody positivity are associated with poor response to anti-TNF therapy, as measured by the DAS28 (23). This association remained even after accounting for markers of severity, such as HAQ score and disease duration. Supporting this finding, other studies have demonstrated a positive correlation between lower baseline levels of anti-CCP and clinical response to infliximab (24, 25), etanercept (26) and adalimumab (27). If confirmed, this observation is of particular importance because the anti-CCP titre may serve as a simple and practical tool to predict response to RTX when considering the possible use of RTX rather than switching to an alternative anti-TNF agent in RA patients failing initial TNF inhibitor therapy.

Few studies have examined the effects of RTX therapy on the level of anti-CCP antibodies in RA (9, 34). Grosjean *et al*. reported a decline in the levels of both anti-CCP antibodies and RF in RA patients after the first course of RTX, although the evolution of these autoantibodies at seven months was not predictive of the clinical or biological response to RTX, since a significant decrease of both autoantibody levels was also observed in patients with an inadequate EULAR response (34).

Our study has several limitations due to its retrospective design, the relatively small sample size, and not homogeneous ELISA kits in the assessment of anti-CCP antibodies. However, the data do represent 'real-life' patients and realistic clinical practice (the strength of our study is that it is not part of corporate sponsored research).

In conclusion, the value of RTX with respect to anti-TNF therapy remains an open issue. In this observational study, certain factors that are easily assessed in clinical practice emerged as independent predictors of response to RTX in patients with mostly long-standing, refractory RA who had received this therapy either as their first biological agent or after failure of one or more TNF-antagonists. Our findings suggest that a lower number of previously-failed TNF blockers and high anti-CCP titre can help select the best candidates for RTX treatment. Further prospective studies are needed to confirm these results.

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