The Journal of Clinical Pharmacology
2018, 00(0) 1–15

DOI: 10.1002/jcph.1341

FCGR2A/FCGR3A Gene Polymorphisms and Clinical Variables as Predictors of Response to Tocilizumab and Rituximab in Patients With Rheumatoid Arthritis

Alberto Jiménez Morales, PharmD[1], Mar Maldonado-Montoro, PhD[1], Juan Enrique Martínez de la Plata, PhD[2], Cristina Pérez Ramírez, PhD[1,3], Abdelali Daddaoua, PhD[3], Carolina Alarcón Payer, PhD[1], Manuela Expósito Ruiz, MSc[4], and Carlos García Collado, PharmD[1]

Abstract

We evaluated the influence of clinical, biochemical, and genetic factors on response in 142 patients diagnosed with rheumatoid arthritis, of whom 87 patients were treated with tocilizumab (61.26%) and 55 patients were treated with rituximab (38.7%;) according to the variables European League Against Rheumatism (EULAR) response, remission, low disease activity, and improvement in Disease Activity Score, 28 joints (DAS28) at 6, 12, and 18 months. A retrospective prospective cohort study was conducted. Patients carrying the *FCGR3A* rs396991-TT genotype treated with tocilizumab showed higher EULAR response (OR, 5.075; 95%CI, 1.20–21.33; $P = .027$) at 12 months, those who were naive for biological disease-modifying antirheumatic drugs (bDMARDs) at the beginning of treatment showed satisfactory EULAR response, higher remission, and greater improvement in DAS28 at 6 months. Younger age at start of tocilizumab treatment was associated with satisfactory EULAR response at 18 months and greater remission at 6 and 18 months. Subcutaneous tocilizumab administration was associated with higher remission at 6 months and improved low disease activity rate at 12 months. In patients treated with rituximab, carriers of the *FCGR2A* rs1801274-TT genotype had higher EULAR response at 6 months (OR, 4.861; 95%CI, 1.11–21.12; $P = .035$), 12 months (OR, 4.667; $p = 0.066$, 95%CI, 0.90–24.12; $P = .066$), and 18 months (OR, 2.487; 95%CI, 0.35–17.31; $P = .357$), higher remission (OR: 10.625; $p = 0.044$, $CI_{95\%}$: 1.07, 105.47) at 6 months, and greater improvement in DAS28 at 12 months ($B = 0.782$; 95%CI, −0.15 to 1.71; $P = .098$) and 18 months ($B = 1.414$; 95%CI, 0.19–2.63; $P = .025$). The *FCGR3A* rs396991-G allele was associated with improved low disease activity rate (OR, 4.904; 95%CI, 0.84–28.48; $P = .077$) and greater improvement in DAS28 ($B = -1.083$; 95%CI, −1.98 to −0.18; $P = .021$) at 18 months. Patients with a lower number of previous biological therapies had higher remission at 12 months. We suggest that the *FCGR3A* rs396991-TT genotype, higher baseline value of DAS28, subcutaneous tocilizumab administration, younger age at the beginning of treatment, and being bDMARD naive are associated with better response to tocilizumab. In patients treated with rituximab, we found better response in those patients with the *FCGR2A* rs1801274-TT genotype, the *FCGR3A* rs396991-G allele, and lower number of previous biological therapies.

Keywords

tocilizumab, rituximab, rheumatoid arthritis, FCGR3A, FCGR2A, effectiveness

Rheumatoid arthritis is a chronic inflammatory autoimmune disease that primarily affects the joints. The most common comorbidities are cardiovascular, metabolic, and bone loss.[1] The prevalence of rheumatoid arthritis is 1% of the population, being more frequent in women than men.[2] Rheumatoid arthritis patients start treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) such as methotrexate (MTX), leflunomide, or sulfasalazine, with MTX the most commonly used DMARD.[3] Patients who do not reach the therapeutic goal (clinical remission or persistently low inflammatory activity, evaluated by objective and validated indices) at 6 months and who present poor prognosis factors (failure of 2 csDMARDs, high disease activity [Disease Activity Score 28 joints, DAS28] >5.1, early erosions, or the presence of rheumatoid arthritis-associated antibodies, particularly rheumatoid factor [RF] and anticyclic citrullinated peptide antibodies [ACPA]) are candidates for treatment

[1]Pharmacy Service, UGC Provincial de Farmacia de Granada, University Hospital Virgen de las Nieves, Granada, Spain
[2]Pharmacy Service, Hospital de Poniente-El Ejido, El Ejido (Almería), Spain
[3]Department of Biochemistry, Faculty of Pharmacy, University of Granada, Granada, Spain
[4]Fundación Pública Andaluza para la Investigación Biosanitaria de Andalucía Oriental "Alejandro Otero" (FIBAO), University Hospital Virgen de las Nieves, Granada, Spain

Submitted for publication 25 July 2018; accepted 25 October 2018.

Corresponding Author:
Alberto Jiménez Morales, PharmD, Pharmacy Service, UGC Provincial de Farmacia de Granada, University Hospital Virgen de las Nieves, Avda. Fuerzas Armadas, 2, 18014 Granada, Spain
Email: alberto.jimenez.morales.sspa@juntadeandalucia.es

with biological disease-modifying antirheumatic drugs (bDMARDs).[3,4]

The bDMARD mechanism of action is based on the association of their hypervariable region (Fab) with their therapeutic target and on the binding of their constant region (Fc) with Fcγ surface receptors,[5] presented in immune system cells and triggering antibody-dependent cell-mediated cytotoxicity (ADCC).[6,7] Tumor necrosis factor inhibitors (TNFi) were the first group of drugs to appear. Despite good TNFi results, in one-third of patients an adequate response according to European League Against Rheumatism (EULAR) criteria is not reached.[8] Subsequently, new bDMARDs have emerged that act against other therapeutic targets, such as tocilizumab and rituximab. Tocilizumab is a monoclonal antibody that binds to the IL6 receptor, both soluble and membrane bound, and blocks the activity of this cytokine.[9] Similarly, rituximab is a monoclonal antibody that specifically binds to the CD20 antigen expressed by mature pre-B and B lymphocytes, producing lysis of B cells.[10] Tocilizumab is currently indicated after failure of 2 csDMARDs, whereas rituximab is approved after failure of a TNFi.[3] Naive patients who are treated with tocilizumab reach an overall response rate of 79%–85% (EULAR criteria)[11] and those treated with rituximab after failure of a previous TNFi reach an overall response rate of 54.8%.[12]

Interindividual variability in bDMARD response is influenced by clinical, biochemical, and genetic factors. Clinical factors associated with better response (EULAR response/remission) to TNFi have been reported in numerous studies: being male, younger age at beginning of treatment, concomitant treatment with csDMARDs, low level of C-reactive protein (CRP), and a high baseline value of DAS28.[13–15] Two baseline factors were associated with greater response to rituximab: a high DAS28 value and a smaller number of previous biological therapies.[16,17] Contradictory results have been shown in patients treated with tocilizumab. Some studies have reported better response in patients treated with a smaller number of previous biological therapies and higher baseline value of DAS28, whereas others have not found this association.[18,19]

The relationship between biochemical factors and TNFi response has been described in 2 meta-analyses (in 50 South American rheumatoid arthritis patients and 5146 European rheumatoid arthritis patients and in 5703 white rheumatoid arthritis patients). They did not find a correlation between TNFi response and RF or anti-CCP values.[20,21] However, the association between RF value (+) at the beginning of treatment and better response in patients treated with rituximab has been reported in a meta-analysis with 5832 white rheumatoid arthritis patients.[22] The presence of anti-CCP at the beginning of treatment has only been associated with higher rituximab response in 2019 rheumatoid arthritis patients.[16]

Genetic factors such as single-nucleotide polymorphisms (SNPs) have been shown to be associated with interindividual differences in response in rheumatoid arthritis patients treated with bDMARD. Polymorphisms of genes encoding the Fcy receptors (Fc fragment of IgG receptor 2A [FCGR2A] and 3A [FCGR3A]), which influence their affinity for the Fc region, have been linked to bDMARD efficacy.[23,24] A G>A point mutation in *FCGR2A* (rs1801274) has been identified that results in an arginine (R) to histidine (H) amino acid substitution at position 131 (R131H). The variant H131R was reported to interact differently with IgG, the H131 isoform showing higher affinity than R131.[25] Similarly, the *FCGR3A* rs396991 polymorphism is a point mutation (T>G) at nucleotide 596, which results in either a valine (V158) or phenylalanine (F158) at amino acid position 158 (F158V). The *FCGR3A* V158 isoform is considered high binding to IgG, which correlates with a greater immunological response by complement-dependent cytotoxicity, apoptosis, and cellular cytotoxicity.[23] Conflicting results have been reported for both polymorphisms in rheumatoid arthritis patients treated with TNFi. Previous studies have shown an association between these SNPs and TNFi response, whereas others have not found such an association.[24–28] However, for rheumatoid arthritis patients treated with rituximab, the *FCGR3A* rs396991-V158 allele has been associated with better response,[29,30] but the influence of *FCGR2A* rs1801274 on rituximab efficacy has not been studied. The role of genetic alterations on tocilizumab response has only been studied in a genome-wide association study[31] and 3 other studies,[32–34] the polymorphisms in *GALNT18*, *CD69*, and *IL-6* genes being statistically significant. Tocilizumab is a structural analogue of IgG1, and its constant fraction interacts with the FCGR2A and FCGR3A receptors.[9] *FCGR2A* rs1801274 and *FCGR3A* rs396991 gene polymorphisms may therefore play a crucial role in tocilizumab response.

Based on the above, the identification of genetic variants in *FCGR2A* and *FCGR3A* genes may be essential for predicting clinical outcomes of rheumatoid arthritis. To date, there have been few studies on germ line variations in *FCGR2A* and *FCGR3A* genes and rheumatoid arthritis patients treated with rituximab and tocilizumab. In this study, we aimed to evaluate the influence of clinical parameters and *FCGR2A* (rs1801274) and *FCGR3A* (rs396991) gene polymorphisms on response to tocilizumab and rituximab, European League Against Rheumatism (EULAR) response, remission, low disease activity, and

DAS28 improvement after 6, 12, and 18 months of therapy.

Material and Methods

A retrospective prospective cohort study was conducted.

Ethics Statement

This study was approved by University Hospital Virgen de las Nieves (UHVN) Ethics and Research Committee, which was conducted in accordance with the Helsinki Declaration. Patients signed a written informed consent form for saliva sample collection and genotyping analysis. Sample identification was based on nonpatient codes.

Study Population

This study included 142 white patients diagnosed with rheumatoid arthritis and recruited in the Pharmacy and Rheumatology Service at UHVN, Granada, Spain, between 2009 and 2016. Patients who were aged ≥18 years old, diagnosed with rheumatoid arthritis according to ACR classification criteria,[3,35] and treated with tocilizumab (8 mg/kg intravenous or 162 mg subcutaneous administration monthly) or rituximab (each rituximab cycle consisted of 2 intravenous infusions of 1000 mg) were suitable for inclusion in the study.

Sociodemographic and Clinical Variables

Sociodemographic data including sex, age at rheumatoid arthritis diagnosis, and age at start of treatment with tocilizumab or rituximab, concomitant corticosteroids, concomitant treatment with csDMARDs, duration of previous biological therapies, and being bDMARD naive were collected from clinical records. The bDMARD-naive clinical variable was not used in rituximab because this drug is not indicated as first-line biological treatment in Spain, unlike tocilizumab, which is indicated as first-line biological treatment after failure of csDMARDs.

Clinical data were also collected from clinical records and comprised DAS28, Health Assessment Questionnaire (HAQ) score, CRP level, erythrocyte sedimentation rate (ESR), presence of rheumatoid factor (RF) and anticyclic citrullinated peptide antibodies (anti-CCP), number of previous bDMARDs, drug administration (intravenous, subcutaneous), number of tender joints (NTJ), number of swollen joints (NSJ), and visual analog scale (physician and patient) of pain (VAS).

Genetic Variables

DNA Isolation. Saliva samples were collected in 50-mL BD Falcon conical tubes (BD, Plymouth, UK). DNA isolation was performed using a QIAamp DNA Mini Kit (QiagenGmBH, Hilden, Germany) according to the manufacturer's instructions for DNA purification from blood or saliva and stored at −40°C.

Detection of Gene Polymorphisms. FCGR2A (rs1801274) and FCGR3A (rs396991) gene polymorphisms were analyzed by real-time PCR using TaqMan probes. Genotyping methodology was previously described.[36]

The assay ID used for FCGR2A (rs1801274) is C___9077561_20, and for FCGR3A (rs396991) is C__25815666_10.

Response Variables

The effectiveness of the treatment was evaluated 6, 12, and 18 months after the start of bDMARD therapy.

EULAR response was according to the guidelines given by the European League Against Rheumatism and classified as satisfactory (presenting DAS28 < 3.2 and DAS28 improvement > 1.2) or unsatisfactory (presenting DAS28 ≥ 3.2 and DAS28 improvement ≥ 1.2).[37]

Remission and low disease activity were considered when patients achieved DAS28 < 2.4 and DAS28 < 3.6, respectively.[38]

The variations of DAS28 were calculated as the difference between the reference values and the values obtained at 6, 12, and 18 months.

Statistical Analysis

Deviation from Hardy-Weinberg equilibrium and pairwise linkage disequilibrium for each polymorphism was calculated using the free, open-source whole-genome association analysis tool set PLINK.[39]

Quantitative data were estimated as the mean ± standard deviation for normally distributed variables or median and percentiles (25th and 75th) for nonnormally distributed variables. The Shapiro-Wilks test was used to assess normality.

The bivariate association between response and demographic, clinical, and genetic variables was tested using Pearson's chi-square or Fisher's exact test for qualitative variables. For the analysis of quantitative variables, the Student *t* test was applied for normally distributed variables and the nonparametric Mann-Whitney test otherwise.

Analysis of variance factor or Kruskal-Wallis tests were applied for qualitative variables with more than 2 categories.

Multivariate analysis (logistic or linear regression) was used to estimate the adjusted odds ratio (OR) and 95% confidence interval (95%CI) for potential prognostic factors for EULAR response, remission, and low disease activity. A backward stepwise method

was applied. The goodness of fit for each model was analyzed with the Hosmer-Lemeshow test and the omnibus test of coefficients, in addition to calculating the R^2 coefficient of Cox-Snell and Nagelkerke.

All tests were 2 sided, and a probability of 0.05 or lower was considered statistically significant. Data analysis was performed using IBM (Armonk, New York) SPSS Statistics 19 software.

Results

A total of 142 patients diagnosed with rheumatoid arthritis were recruited into the study, of whom 87 (61.26%) were treated with tocilizumab and 55 (38.7%) with rituximab. Baseline characteristics are listed in Tables 1 and 2. All patients received treatment with csDMARD before starting treatment with tocilizumab or rituximab.

In the tocilizumab subgroup of 87 patients (61.2%; 87 of 142 patients), 27 of them (23.5%) were bDMARD naive, 35 of them (40.2%) started the treatment after failure of 1 TNFi, and 25 of them (28.7%) started the treatment after failure of 2 TNF inhibitors. Tocilizumab was administered intravenously in 62 of 87 patients (71.2%) and subcutaneously in 25 of 87 patients (28.7%), as monotherapy in 42 of 87 patients (48.2%), and concomitantly with a csDMARD in 45 of 87 patients (51.7%). Most patients were positive for RF (64.04%; 56 of 87 patients) and anti-CCP (69.4%; 59 of 85 patients) at baseline. Clinical parameters (DAS28, HAQ) and acute-phase reactants (CRP, ESR) at baseline and after 6, 12, and 18 months are detailed in Table 1.

In the rituximab subgroup, 10 of 55 patients (18.1%) started treatment after failure of 1 TNFi and 45 of 55 patients (81.8%) after failure of 2 TNF inhibitors. Fifty-four of 55 patients were treated with rituximab in combination with a csDMARD (98%). Fifty of 52 patients (98%) had a positive RF baseline value, and 27 of 48 patients (52.9%) had positive anti-CCP at baseline. Baseline values are detailed in Table 2.

Clinical Effectiveness of Tocilizumab

Of 87 patients, tocilizumab effectiveness was evaluated in 87 (100%), 84 (96.5%), and 75 (87.3%) at 6, 12, and 18 months, respectively (Table 3). Eight of 87 patients (9.2%) abandoned treatment because of lack of effectiveness within 12 months (37.5%; 3 of 8) and 18 months (62.5%; 5 of 8) of starting tocilizumab treatment, and 4 of 75 patients patients (5.3%) developed an adverse reaction within 18 months of starting tocilizumab treatment.

EULAR response at 6 months was 67.8%, increasing to 76.1% at 12 months, and slightly decreasing to 74.6% at 18 months. Remission increased from 50.5% at 6 months to 60.7% at 12 months and to 64% at 18 months. Low disease activity was achieved by 75.8% at 6 months, 83.3% at 12 months, and 82.6% at 18 months. DAS28 variation was 2.8 ± 0.7 at 6 months, 3.1 ± 0.6 at 12 months, and 3.07 ± 0.2 at 18 months. The results are detailed in Table 3.

Table 1. Demographics and Clinical Characteristics of Patients Treated With TCZ

	Baseline		
Variable	N	%	Mean ± SD
Sex	87		
Male	16	19.2	
Female	71	80.8	
Age at RA diagnosis	87		42.8 ± 12.5
Age at start with TCZ	87		53.4 ± 7.7
ACPA	85		
Positive	59	69.4	
Negative	26	30.5	
TCZ administration	87		
Intravenous	62	71.2	
Subcutaneous	25	28.7	
Rheumatoid factor	87		
Positive	56	64.04	
Negative	31	35.9	
Duration of previous BT (months)	87		41.1 ± 62.2
Number of previous BT	87		2 ± 1.45
Previous BT			
Naive	25	28.7	
1 TNFi	35	40.2	
2 TNFi	25	28.7	
Other lines	2	2.2	
Concomitant csDMARDs			
Methotrexate	38	43.6	
Leflunomide	8	9.1	
Sulfasalazine	–		
Concomitant corticosteroids	87		
Yes	81	93.1	
No	6	6.8	
Monotherapy			
Yes	42	48.3	
No	45	51.7	
DAS28	87		5.5 ± 1.2
HAQ	87		1.6 ± 0.9
ESR	87		35.1 ± 28.9
NAD	87		10 ± 2.1
NAT	87		5.3 ± 1.4
VAS	87		61.1 ± 21.1
CRP	87		4.9 ± 1.34

ACPA, anticitrullinated peptide antibodies; BT, biological therapy; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; EULAR, European League Against Rheumatism; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NAT, number of swollen joints; NAD, number of painful joints; RA, rheumatoid arthritis; TNFi, tumor necrosis factor inhibitor; TCZ, tocilizumab; VAS, visual analog scale (physician and patient). Qualitative variables are shown as number (percentage). Quantitative variables with a normal distribution are shown as mean ± standard deviation. Quantitative variables with a nonnormal distribution are shown as P_{50} (P_{25}, P_{75}).

Table 2. Demographics and Clinical Characteristics of Patients Treated With RTX

	Baseline		
Variable	N	%	Mean ± SD
Sex			
Male	12	21.8	
Female	43	78.2	
Age at RA diagnosis	55		43.2 ± 10.8
Age at start with RTX	55		54 ± 19.09
ACPA	48		
Positive	27	52.9	
Negative	21	47.1	
Rheumatoid factor	51		
Positive	50	98	
Negative	1	1.9	
Duration of previous BT (months)	55		49.3 ± 30.4
Number of previous BT	55		1.9 ± 1.09
Previous BT			
1 TNFi	10	18.1	
2 TNFi	45	81.8	
Concomitant csDMARDs	54		
Methotrexate	45	83.3	
Leflunomide	7	12.9	
Sulfasalazine	2	3.7	
Concomitant corticosteroids	55		
Yes	54	98.1	
No	1	2.9	
DAS28	55		5.4 ± 1.16
HAQ	55		1.82 ± 0.65
ESR	55		30.7 ± 0.5
NAD	55		10.2 ± 8.4
NAT	55		5.6 ± 0.2
VAS	55		66.01 ± 14.1
CRP	55		9.3 ± 14.7

ACPA, anticitrullinated peptide antibodies; BT, biological therapy; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; EULAR, European League Against Rheumatism; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NAT, number of swollen joints; NAD, number of painful joint; RA, rheumatoid arthritis; TNFi, tumor necrosis factor inhibitor; TCZ, tocilizumab; VAS, visual analog scale (physician and patient).
Qualitative variables are shown as number (percentage). Quantitative variables with normal distribution are shown as mean ± standard deviation. Quantitative variables with nonnormal distribution are shown as P_{50} (P_{25}, P_{75}).

Clinical Effectiveness of Rituximab

Rituximab effectiveness was evaluated in 52 of 55 patients (94.5%), 42 of 55 patients (76.3%), and 38 of 55 patients (69.1%) at 6, 12, and 18 months, respectively (Table 3). Three of 55 patients (5.4%) developed an adverse reaction within 6 months of starting rituximab treatment.

EULAR response at 6, 12, and 18 months was 21.2%, 19%, and 21%, respectively. Remission was 9.6%, 7.14%, and 10.5% at 6, 12, and 18 months, respectively, and low disease activity was achieved by 40.3%, 42.8%, and 44.7% of the patients at 6, 12, and 18 months, respectively. Variation in DAS28 was 1.5 ± 0.33 at 6 months, 1.4 ± 1.43 at 12 months, and 1.4 ± 0.7 at 18 months.

Genotype Distribution

Genotype distributions are shown in Table S1. All gene polymorphisms were in Hardy-Weinberg equilibrium. No linkage disequilibrium was found.

Predictors of Response to Tocilizumab

Predictors of Response at 6 Months.

EULAR Response. In the bivariate analysis, EULAR response was higher in patients with subcutaneous tocilizumab administration, bDMARD-naive patients, younger age at rheumatoid arthritis diagnosis, and patients with a smaller number of previous biological therapies (values are detailed in Table S2).

Multivariate analysis revealed that younger age at rheumatoid arthritis diagnosis (OR, 0.93; 95%CI, 0.89–0.98; $P = .010$; Table 4) and bDMARD-naive patients (OR, 13.66; 95%CI, 2.66–69.97; $P = .002$; Table 4) were the independent factors able to predict better EULAR response at 6 months.

Remission. The bivariate analysis showed higher remission in patients with subcutaneous tocilizumab administration, those who were bDMARD naive, those with younger age at start of tocilizumab treatment, those with elevated levels of baseline CRP, and patients with a smaller number of previous biological therapies (values are detailed in Table S3). After multivariate analysis, the factors independently associated with higher remission were subcutaneous tocilizumab administration (OR, 2.92; 95%CI, 0.91–9.31; $P = .070$; Table 4), bDMARD-naive patients (OR, 3.75; 95%CI, 1.19–11.79; $P = .024$; Table 4), and younger age at start of tocilizumab treatment (OR, 0.96; 95%CI, 0.92–0.99; $P = .044$; Table 4).

Low Disease Activity. The variables associated with improved low disease activity rates in the bivariate analysis were bDMARD-naive patients, reduced baseline value of NTJ, and patients with a smaller number of previous biological therapies (values are detailed in Table S4).

The only factors independently associated with improved low disease activity rates after multivariate analysis were lower number of previous biological therapies (OR, 0.57; 95%CI, 0.39–0.84; $P = .004$; Table 4) and reduced baseline value of NTJ (OR, 0.93; 95%CI, 0.85–1.01; $P = .101$; Table 4).

Improvement in DAS28. In the bivariate analysis, greater improvement in DAS28 was shown in patients with subcutaneous tocilizumab administration,

Table 3. Clinical Effectiveness of Tocilizumab and Rituximab

Tocilizumab									
	6 Months			12 Months			18 Months		
Response Variable	N	%	Mean ± SD	N	%	Mean ± SD	N	%	Mean ± SD
EULAR response	87	100		84	96.5		75	87.3	
Satisfactory	59	67.8		64	76.1		56	74.6	
Unsatisfactory	28	32.2		20	23.8		19	25.3	
Remission (DAS28 < 2.4)	44	50.5		51	60.7		48	64.0	
LDA (DAS28 < 3.6)	66	75.8		70	83.3		62	82.6	
Variation of DAS28	87		2.8 ± 0.7	84		3.1 ± 0.6	75		3.07 ± 0.2
Rituximab									
	6 Months			12 Months			18 Months		
Response Variable	N	%	Mean ± SD	N	%	Mean ± SD	N	%	Mean ± SD
EULAR response	52			42					
Satisfactory	11	21.2		8	19		8	21	
Unsatisfactory	41	78.8		34	81		30	79	
Remission (DAS28 < 2.4)	52	9.6		42	7.14		38	10.5	
LDA (DAS28 < 3.6)	52	40.3		42	42.8		38	44.7	
Variation of DAS28	52		1.5 ± 0.3	42		1.4 ± 1.34	38		1.4 ± 0.7

DAS28, 28-joint Disease Activity Score; EULAR, European League Against Rheumatism; LDA, low-activity disease.

bDMARD naive, younger age at start of tocilizumab treatment, and higher baseline values of DAS28, NTJ, NSJ, VAS, and ESR (values are detailed in Table S5).

Multivariate analysis revealed that bDMARD-naive patients (B = −1.118, 95%CI, −1.73 to −0.64; $P <$ .001; Table 4), younger age at start of tocilizumab treatment (B = −0.033; 95%CI, −0.05 to −0.01; $P =$ .001; Table 4), higher baseline value of DAS28 (B = 0.555; 95%CI, 0.26–0.84; $P = <$ .001; Table 4), higher baseline value of VAS (B = 0.015; 95%CI, 0.00–0.02, $P =$ .045; Table 4), and higher baseline value of ESR (B = 0.020; 95%CI, 0.007–0.03; $P =$ .002; Table 4) were the independent factors able to predict greater improvement in DAS28 at 6 months.

Predictors of Response at 12 Months.

EULAR Response. The variables associated with more satisfactory EULAR response in the bivariate analysis were patients carrying the TT genotype for *FCGR3A* rs396991 gene polymorphism (Table S2), bDMARD-naive patients, younger age at start of tocilizumab treatment and rheumatoid arthritis diagnosis, subcutaneous tocilizumab administration, and patients with a lower number of previous biological therapies (values are detailed in Table S2).

After multivariate analysis, the factors independently associated with better EULAR response were the TT genotype for *FCGR3A* ($OR_{TT/GT\text{-}GG}$, 5.075; 95%CI, 1.20–21.33; $P =$ .027; Table 4), bDMARD-naive patients (OR, 8.246; 95%CI, 1.54–44.12; $P =$ .014; Table 4), and younger age at rheumatoid arthritis diagnosis (OR, 0.949; 95%CI, 0.90–1.00; $P =$ .051; Table 4).

Remission. The bivariate analysis showed higher remission in patients with tocilizumab administered subcutaneously, who were bDMARD naive, and who had a smaller number of previous biological therapies (values are detailed in Table S3).

The only factor independently associated with higher remission after multivariate analysis was bDMARD-naive patients (OR, 12.732; 95%CI, 2.75–58.95; $P =$ .001; Table 4).

Low Disease Activity. In the bivariate analysis, improved low disease activity rate was found in bDMARD-naive patients, patients administered tocilizumab subcutaneously, patients with younger age at start of tocilizumab treatment, and patients with a smaller number of previous biological therapies (values are detailed in Table S4).

Multivariate analysis revealed that subcutaneous tocilizumab administration (OR, 6.362; 95%CI, 0.78–51.65; $P =$ .083; Table 4) was the independent factor able to predict improved low disease activity rate at 12 months.

Improvement in DAS28. Bivariate analysis revealed greater improvement in DAS28 in patients administered tocilizumab subcutaneously, those who were bDMARD naive, and those with higher baseline values of DAS28, NTJ, NSJ, and ESR (values are detailed in Table S5).

Table 4. Predictors of Response at 6, 12, and 18 Months of Treatment With Tocilizumab in Rheumatoid Arthritis Patients (Multivariable Analysis)

Response Variable	Independent Variable	B	Odds Ratio	*P* (Variable)	95% Confidence Interval	R^2	Goodness of Fit	*P*[a] (Model)
6 months remission								
	Administration subcutaneous	1.072	2.921	.070	0.91–9.31	R^2 Cox-Snell = 0.180 R^2 Nagelkerke = 0.240	$\chi^2 = 7.628$ $P = .367$	$\chi^2 = 17.238$ $P = .001$
	bDMARD-naive	1.323	3.754	.024	1.19–11.79			
	Age at TCZ start	−0.040	0.961	.044	0.92–0.99			
Low-activity disease								
	Number of previous BT	−0.556	0.573	.004	0.39–0.84	R^2 Cox-Snell = 0.054 R^2 Nagelkerke = 0.091	$\chi^2 = 17.438$ $P = .026$	$\chi^2 = 12.833$ $P = .002$
	Baseline NAD	−0.069	0.933	.101	0.85–1.01			
EULAR response								
	bDMARD-naive	2.615	13.666	.002	2.66–69.97	R^2 Cox-Snell = 0.197 R^2 Nagelkerke = 0.276	$\chi^2 = 5.537$ $P = .595$	$\chi^2 = 19.106$ $P < .001$
	Age at RA diagnosis	−0.063	0.939	.010	0.895–0.985			
Variation of DAS28								
	bDMARD-naive	−1.118		< .001	−1.73 to −0.64	$R^2 = 0.544$ R^2 corrected = 0.516		< .001
	Age at TCZ start	−0.033		.001	−0.05 to −0.01			
	Baseline DAS28	0.555		< .001	0.26–0.84			
	Baseline VAS	0.015		.045	0.00–0.02			
	Baseline ESR	0.020		.002	0.00–0.03			
12 months remission								
	bDMARD-naive	2.544	12.732	.001	2.75–58.95	R^2 Cox-Snell = 0.183 R^2 Nagelkerke = 0.248	$\chi^2 = 0.000$ $P = —$	$\chi^2 = 16.984$ $P < .001$
Low-activity disease								
	Subcutaneous administration	1.850	6.362	.083	0.78–51.65	R^2 Cox-Snell = 0.054 R^2 Nagelkerke = 0.091	$\chi^2 = 0.000$ $P = -$	$\chi^2 = 71.033$ $P = .091$
EULAR response								
	bDMARD-naive	2.110	8.246	.014	1.54–44.12	R^2 Cox-Snell = 0.174 R^2 Nagelkerke = 0.260	$\chi^2 = 13.008$ $P = .112$	$\chi^2 = 16.016$ $P = .003$
	FCGR3A							
	GT	–	1	.061	–			
	GG	1.090	2.973	.213	0.53–16.51			
	TT	1.624	5.075	.027	1.20–21.33			
	Age at RA diagnosis	−0.052	0.949	.051	0.90, 1.00			
Variation of DAS28								
	Baseline DAS28	1.006		< .001	0.83–1.17	$R^2 = 0.702$ R^2 corrected = 0.683		< .001
18 months remission								
	bDMARD-naive	1.557	4.745	.014	1.37–16.34	R^2 Cox-Snell = 0.137 R^2 Nagelkerke = 0.186	$\chi^2 = 3.504$ $P = .835$	$\chi^2 = 11.022$ $P = .004$
	Age at RA diagnosis	−0.054	0.947	.016	0.90–0.99			
Low-activity disease								
	bDMARD-naive	2.063	7.868	.054	0.96–64.17	R^2 Cox-Snell = 0.077 R^2 Nagelkerke = 0.124	$\chi^2 = 0.000$ $P = -$	$\chi^2 = 5.988$ $P = .014$
EULAR response								
	Positive factor rheumatoid	1.314	3.719	.029	1.14–12.11	R^2 Cox-Snell = 0.169 R^2 Nagelkerke = 0.249	$\chi^2 = 8.379$ $P = .300$	$\chi^2 = 13.847$ $P = .003$
	Age at TCZ start	−0.053	0.948	.039	0.90–0.99			
	Baseline NAT	0.158	1.171	.097	0.97–1.41			

(*Continued*)

Table 4. Continued

Response Variable	Independent Variable	B	Odds Ratio	*P* (Variable)	95% Confidence Interval	R^2	Goodness of Fit	*P*[a] (Model)
Variation of DAS28								
	Age at TCZ start	−0.020		.013	−0.03 to −0.00	$R^2 = 0.713$		<0.001
						R^2 corrected = 0.688		
	Baseline DAS28	0.807		< .001	0.62–0.98			

BT, biological therapy; DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; NAT, number of swollen joints; NAD, number of painful joints; RA, rheumatoid arthritis; TCZ, tocilizumab; VAS, visual analog scale (physician and patient).
[a]Likelihood ratio test.

After multivariate analysis, the factor independently associated with greater improvement in DAS28 at 12 months was higher baseline value of DAS28 (B = 1.006, 95%CI, 0.83–1.17; $P < .001$; Table 4).

Predictors of Response at 18 Months.

EULAR Response. The variables associated with more satisfactory EULAR response in the bivariate analysis were younger age at start of tocilizumab treatment and rheumatoid arthritis diagnosis, smaller number of previous biological therapies, positive baseline value of RF, and higher baseline value of NTJ (values are detailed in Table S2).

The only factors independently associated with more satisfactory EULAR response after multivariate analysis were positive baseline value of RF (OR, 3.71; 95%CI, 1.14–12.11; $P = .029$; Table 4), younger age at start of tocilizumab treatment (OR, 0.94; 95%CI, 0.90–0.99; $P = .039$; Table 4), and higher baseline value of NTJ (OR, 1.17; 95%CI, 0.97–1.41; $P = .097$; Table 4).

Remission. Bivariate analysis showed higher remission in patients without corticosteroids as concomitant treatment with bDMARDs, bDMARD naive, younger age at start of tocilizumab treatment and rheumatoid arthritis diagnosis, and smaller number of previous biological therapies (values are detailed in Table S3).

Multivariate analysis revealed that bDMARD-naive patients (OR, 4.74; 95%CI, 1.37–16.34; $P = .014$; Table 4) and younger age at rheumatoid arthritis diagnosis (OR, 0.947; 95%CI, 0.90–0.99; $P = .016$; Table 4) were the independent factors able to predict higher remission at 18 months.

Low Disease Activity. In the bivariate analysis, improved low disease activity rate was found in patients with subcutaneous tocilizumab administration, those who were bDMARD naive, and those with a smaller number of previous biological therapies (values are detailed in Table S4).

After multivariate analysis, the factor independently associated with improved low disease activity rate was bDMARD-naive patients (OR, 7.86; 95%CI, 0.96–64.17; $P = .054$; Table 4).

Improvement in DAS28. The variables associated with greater improvement in DAS28 in the bivariate analysis were bDMARD-naive patients, younger age at start of tocilizumab treatment, and higher baseline values of DAS28, NTJ, NSJ, and ESR (values are detailed in Table S5).

The only factors independently associated with greater improvement in DAS28 after multivariate analysis were younger age at start of tocilizumab treatment (B = −0.020; 95%CI, −0.036 to −0.004; $P = .013$; Table 4) and higher baseline value of DAS28 (B = 0.807; 95%CI, 0.62–0.98; $P < .001$; Table 4)

Predictors of Response to Rituximab

Predictors of Response at 6 Months.

EULAR Response. In the bivariate analysis, higher EULAR response was found in patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism, smaller number of previous biological therapies, and lower baseline value of VAS (values are detailed in Table S6).

Multivariate analysis showed that patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism ($OR_{TT/CT\text{-}CC}$, 4.86; 95%CI, 1.11–21.12; $P = .035$; Table 5) was the independent factor able to predict higher EULAR response at 6 months.

Remission. Bivariate analysis showed higher remission in patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism and a lower baseline value of VAS (values are detailed in Table S7).

After multivariate analysis, the factors independently associated with higher remission were patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism (OR, 10.62; 95%CI, 1.07–105.47; $P = .044$; Table 5) and lower baseline value of VAS (OR, 0.94; 95%CI, 0.89–0.99, $P = 0.025$; Table 5).

Table 5. Predictors of Response at 6, 12, and 18 Months of Treatment With Rituximab in Rheumatoid Arthritis Patients (Multivariable Analysis)

Response Variable	Independent Variable	B	Odds Ratio	*P* (Variable)	95% Confidence Interval	Goodness of Fit		*P*[a] (Model)
				6 months				
Remission								
	FCGR2A (TT vs C)	2.363	10.625	0.044	1.07–105.47	R^2 Cox-Snell = 0.180 R^2 Nagelkerke = 0.384	$\chi^2 = 11.196$ $P = .130$	$\chi^2 = 10.336$ $P = .006$
	Baseline VAS	−0.062	0.940	0.025	0.89–0.99			
Low-activity disease								
	Baseline VAS	−0.036	0.965	0.029	0.93–0.99	R^2 Cox-Snell = 0.102 R^2 Nagelkerke = 0.137	$\chi^2 = 4.193$ $P = .651$	$\chi^2 = 5.570$ $P = .018$
EULAR response								
	FCGR2A (TT vs C)	1.581	4.861	0.035	1.11–21.12	R^2 Cox-Snell = 0.081 R^2 Nagelkerke = 0.125	$\chi 2 = 0$ $P =$ —	$\chi^2 = 4.367$ $P = .037$
Variation of DAS28								
	Baseline DAS28	0.687		<0.001	0.34–1.03	$R^2 = 0.469$ R^2 corrected = 0.398		$P < .001$
	Baseline ESR	−0.017		0.077	−0.035 to 0.002			
	Number of previous BT	−0.328		0.033	−0.62 to −0.027			
				12 months				
Remission								
	Number of previous BT	−1.580	0.206	0.065	0.03–1.10	R^2 Cox-Snell = 0.114 R^2 Nagelkerke = 0.282	$\chi^2 = 1.324$ $P = .723$	$\chi^2 = 5.060$ $P = 0.024$
Low-activity disease								
	Sex (male)	1.886	6.590	0.042	1.06–40.62	R^2 Cox-Snell = 0.304 R^2 Nagelkerke = 0.408	$\chi^2 = 12.149$ $P = .145$	$\chi^2 = 15.231$ $P = .002$
	Baseline VAS	−0.047	0.954	0.035	0.91–0.99			
EULAR response								
	FCGR2A (TT vs C)	1.540	4.667	0.066	0.90–24.12	R^2 Cox-Snell = 0.076 R^2 Nagelkerke = 0.122	$\chi^2 = 0$ $P =$ =	$\chi^2 = 3.327$ $P = .068$
Variation of DAS28								
	FCGR2A (TT vs C)	0.782		0.098	−0.15–1.71	$R^2 = 0.352$ R^2 corrected = 0.319		p<0.001
	Baseline DAS28	0.564		0.003	0.20–0.92			
				18 months				
Remission								
	Baseline NAT	0.382	1.465	0.058	0.98–2.17	R^2 Cox-Snell = 0.105 R^2 Nagelkerke = 0.215	$\chi^2 = 5.798$ $P = .446$	$\chi^2 = 4.227$ $P = .040$
Low-activity disease								
	FCGR3A (G vs TT)	1.590	4.904	0.077	0.84–28.48	R^2 Cox-Snell = 0.230 R^2 Nagelkerke = 0.308	$\chi^2 = 0.819$ $P = .664$	$\chi^2 = 9.647$ $P = 0.008$
EULAR response								
	FCGR2A (TT vs C)	0.911	2.487	0.357	0.35–17.31	R^2 Cox-Snell = 0.179 R^2 Nagelkerke = 0.278	$\chi^2 = 8.257$ $P = .310$	$\chi^2 = 7.489$ $P = .024$
Variation of DAS28								
	FCGR2A (TT vs C)	1.414		0.025	0.19–2.63	$R^2 = 0.676$ R^2 corrected = 0.587		p<0.001
	FCGR3A (TT vs G)	−1.083		0.021	−1.98 to −0.18			
	Positive ACPA	0.942		0.087	−0.15–2.03			
	Baseline DAS28	0.793		0.016	0.16, 1.42			
	Baseline ESR	−0.029		0.068	−0.061 to 0.002			

ACPA, anticitrullinated peptide antibodies; BT, biological therapy; DAS28, 28-joint Disease Activity Score; EULAR, European League Against Rheumatism; ESR, erythrocyte sedimentation rate; NAT, number of swollen joints; RA, rheumatoid arthritis; TCZ, tocilizumab; VAS, visual analog scale (physician and patient).
[a]Likelihood ratio test.

Low Disease Activity. The variable associated with improved low disease activity rate in the bivariate analysis was lower baseline value of VAS (values are detailed in Table S8).

The only factor independently associated with improved low disease activity rate after multivariate analysis was lower baseline value of VAS (OR, 0.96; 95%CI, 0.93–0.99; $P = .029$; Table 5).

Improvement in DAS28. In the bivariate analysis, greater improvement in DAS28 was shown in patients with higher baseline values of DAS28, NTJ, NSJ, and ESR (values are detailed in Table S9).

Multivariate analysis showed that higher baseline value of DAS28 (B = 0.687; 95%CI, 0.34–1.03; $P < .001$; Table 5), higher baseline value of ESR (B = −0.017, 95%CI, −0.035 to 0.002; $P = .077$; Table 5), and smaller number of previous BT (B = −0.328; 95%CI, −0.62 to −0.027; $P = .033$; Table 5) were the independent factors able to predict greater improvement in DAS28 at 6 months.

Predictors of Response at 12 Months.

EULAR Response. Bivariate analysis showed higher EULAR response in patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism (Table S6).

After multivariate analysis, the factor independently associated with higher EULAR response was patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism ($OR_{TT/CT\text{-}CC}$, 4.66; 95%CI, 0.903–24.12; $P = .066$; Table 5).

Remission. The variable associated with higher remission in the bivariate analysis was smaller number of previous biological therapies (Table S7). This variable remained statistically associated in the multivariate analysis (OR, 0.20; 95%CI, 0.03–1.10; $P = .065$; Table 5).

Low Disease Activity. In the bivariate analysis, improved low disease activity rate was shown in patients with lower baseline value of VAS and elevated baseline CRP (Table S8).

The only factor independently associated with improved low disease activity rate after multivariate analysis was lower baseline value of VAS (OR, 0.95; 95%CI, 0.91–0.99; $P = .035$; Table 5) and male sex (OR, 6.59; 95%CI, 1.06–40.62; $P = .042$; Table 5).

Improvement in DAS28. The variables associated with greater improvement in DAS28 in the bivariate analysis were patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism and higher baseline ESR (values are detailed in Table S9).

Multivariate analysis showed that patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism (B = 0.782; 95%CI, −0.15 to 1.71; $P = .098$; Table 5) and higher baseline value of DAS28 (B = 0.564, 95%CI, 0.20–0.92; $P = .003$; Table 5) were the independent factors able to predict greater improvement in DAS28 at 12 months.

Predictors of Response at 18 Months.

EULAR Response. The variables associated with higher EULAR response in the bivariate analysis were patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism (Table S6).

After multivariate analysis, the factor independently associated with higher EULAR response was patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism (OR, 2.487; 95%CI, 0.35–17.31; $P = .357$; Table 5).

Remission. Both bivariate (Table S7) and multivariate analysis (OR, 1.465; 95%CI, 0.98–2.17; $P = .058$; Table 5) showed that the only factor independently associated with greater remission was higher baseline NSJ.

Low Disease Activity. In the bivariate analysis, improved low disease activity rate was shown in patients carrying the G allele for the *FCGR3A* rs396991 gene polymorphism (Table S8) and in patients with higher baseline NSJ (Table S8).

The only factor independently associated with improved low disease activity rate after multivariate analysis was patients carrying the G allele for the *FCGR3A* rs396991 gene polymorphism ($OR_{GG\text{-}GT/TT}$, 4.904; 95%CI, 0.84–28.48; $P = .077$; Table 5).

Improvement in DAS28. Bivariate analysis showed greater improvement in DAS28 in patients carrying the G allele for the *FCGR3A* rs396991 gene polymorphism, patients carrying the TT genotype for the *FCGR2A* rs1801274 gene polymorphism, positive baseline value of ACPA and RF, and higher baseline values of DAS28, NTJ, NSJ, and ESR (values are detailed in Table S9).

Multivariate analysis showed that patients carrying the GG genotype for the *FCGR3A* rs396991 gene polymorphism ($B_{TT/GT\text{-}GG}$ = −1.083; 95%CI, −1.98 to −0.18; $P = .021$; Table 5), the TT genotype for *FCGR2A* rs1801274 gene polymorphism ($B_{TT/CT\text{-}CC}$ = 1.414; 95%CI, 0.19–2.63; $P = .025$; Table 5), positive baseline anti-CCP (B = 0.942; 95%CI, −0.15 to 2.03; $P = .087$; Table 5), higher baseline value of DAS28 (B = 0.793; 95%CI, 0.16–1.42; $P = .016$; Table 5), and higher baseline ESR (B = −0.029; 05%CI, −0.061

to 0.002; $P = .068$; Table 5) were the independent factors able to predict greater improvement in DAS28 at 18 months.

Discussion

Tocilizumab and rituximab are 2 bDMARDs that target and block the IL6 receptor and CD20, respectively. They are used to treat rheumatoid arthritis and came on the market after the TNFi drug group. The lack of clinical experience and absence of clinical trials comparing the efficacy of tocilizumab and rituximab versus TNFi initially promoted the use of tocilizumab in patients for whom at least 1 treatment with TNFi had failed and of rituximab in patients who had received more than 2 treatment lines,[40] despite the results obtained in the clinical trials.[41–43] The combination of tocilizumab and MTX has been previously investigated in 2 pivotal studies with 1220 and 622 rheumatoid arthritis patients, respectively, which included bDMARD-naive patients. EULAR response (good or moderate) after 24 weeks of treatment was observed in 79% and 80% of patients and remission in 27% and 30% of patients, respectively.[41,42] Similar results were shown in a study (499 rheumatoid arthritis patients) that included patients refractory to TNFi treatment and treated with tocilizumab reporting good or moderate EULAR response at 12 weeks in 67.7% of patients and remission in 30.1% of patients.[44] In our study, satisfactory EULAR response at 6 months was achieved in 76.1% of tocilizumab-treated patients, which is consistent with previous studies.[41,42,44] Our patients also achieved higher remission rates than those previously described, that is, 64% at 18 months.

In our study the rituximab group reported good or moderate EULAR response at 6 and 18 months in 21.2% of patients, lower than a previous pivotal study with 311 rheumatoid arthritis patients (65% of patients with good/moderate EULAR response at 6 months). This difference may be because in the pivotal study, the percentage of patients who were refractory to 2 or more TNFi therapies was 40%, whereas among our patients this figure was 80%. This situation may therefore affect rituximab effectiveness.

The effectiveness of tocilizumab and rituximab has been compared with TNFi in numerous studies, and there is a larger body of clinical evidence available today.[11,12] They have thus been designated first-choice treatment in patients naive or refractory to TNFi, respectively.[45]

There are numerous therapeutic options for treating a candidate rheumatoid arthritis patient with bDMARDs, and therefore predictors of response are required to select the treatment to which the patient will best respond. The clinical predictors of response for TNFi are male sex, age $\leq$ 54 years old at the start of TNFi therapy, high baseline DAS28, concomitant treatment with cDMARDs, and negative or low levels of CRP.[13–15,44] Regarding other biochemical predictors, 2 meta-analyses (5561 and 5703 rheumatoid arthritis patients) did not find any association between the values of RF and anti-CCP and response to TNFi therapy.[20,21]

In our study, the multivariate analysis demonstrated that patients treated with tocilizumab reported higher response when they were bDMARD naive, when tocilizumab was administered subcutaneously, when a smaller number of previous biological therapies had been administered, when they were younger at diagnosis and beginning of treatment, and when baseline values of DAS28, NTJ, and NSJ were higher. Regarding biochemical variables, we found greater EULAR response at 6 and 18 months in tocilizumab-treated patients with higher baseline values of ESR and RF. Similar results were found in previous studies that reported satisfactory EULAR response in patients who were bDMARD naive, treated with a smaller number of previous biological therapies, with a higher baseline value of DAS28, and with a positive RF value.[18,19,22,46]

In our patients treated with rituximab, the multivariate analysis reported better response for patients with higher baseline DAS28 and smaller number of previous biological therapies. Furthermore, male sex and higher baseline value of NSJ were associated with EULAR response at 12 and 18 months, respectively. Unlike other variables, lower baseline value of VAS was associated with better response in low disease activity and remission at 6 and 12 months. Regarding biochemical variables, lower baseline value of ESR and positive value of anti-CCP were the independent factors able to predict better response at 18 months.

These results are consistent with previous studies that describe an association between satisfactory EULAR response and the clinical variables baseline DAS28 and number of previous biological therapies.[16,17] The biochemistry variable anti-CCP has also been associated with EULAR response in previous studies.[16] Similarly, positive RF has been identified as a predictor of EULAR response in previous research studies.[22] However, in our rituximab patients we did not find any association between RF status and response, although a higher remission trend was reported at 6 and 12 months.

High baseline value of DAS28, low age at rheumatoid arthritis diagnosis, and male sex are clinical predictors of response to TNFi, tocilizumab, and rituximab. These variables act as response predictors in the 3 treatments in the same way, and therefore could not be used as such for selecting one treatment over another.

In our study, high baseline values of NSJ, NTJ, and ESR were also predictors of response to tocilizumab

and rituximab, whereas this was not so for TNFi. However, it is possible that these variables might not allow an appropriate treatment to be selected because they are included in the formula for obtaining DAS28,[47] which is associated with response to TNFi therapy.

Biochemical parameters such as RF and anti-CCP were not predictors of response to TNFi, unlike tocilizumab and rituximab, as has been reported in previous studies[16,22] and in our results. For this reason, RF and anti-CCP may be useful as predictors of response to tocilizumab and rituximab. Similarly, genetic factors also play an essential role in interindividual differences in response to biological therapies.[24,31,34,48–52] Clinical, biochemical, and genetic parameters may thus be a useful tool for guiding treatment selection.

The effect of *FCGR2A* rs1801274 and *FCGR3A* rs396991 gene polymorphisms on response to tocilizumab has not been previously investigated. In our patients, carriers of *FCGR3A* rs396991-TT, which is the lower-affinity genotype, showed higher EULAR response at 12 months. However, no association was found between *FCGR2A* rs1801274 gene polymorphism and response to tocilizumab. These polymorphisms may play an essential role through binding to the Fc region of tocilizumab, modifying its clearance from circulation and therefore possibly producing different therapeutic effects. A low FCRγ receptor affinity for the Fc region of tocilizumab may thus be associated with lower clearance from circulation and higher response to tocilizumab. This hypothesis has been verified in TNFi therapy.[25] However, a meta-analysis with 3058 rheumatoid arthritis patients did not find any association between FCGR2A rs1801274 polymorphism and response to TNFi, except in the case of patients treated with adalimumab, who showed better response with the lower-affinity genotype.[53] Despite the hypothesis proposed above, a meta-analysis (899 rheumatoid arthritis patients) did not show any association between the *FCGR3A* rs396991 gene polymorphism and variation in DAS28 in response to TNFi.[54] As per the results of our study, patients who carry genotype TT for rs1801274 FCGR3A would be better candidates for treatment with tocilizumab because they obtain a better EULAR response.

These contradictory results reveal the need to carry out further studies to confirm the influence of these polymorphisms on the response to tocilizumab.

In our study, patients treated with rituximab and carrying the G allele for the *FCGR3A* rs396991 gene polymorphism had improved low disease activity rate and improvement in DAS28 at 18 months. The G allele for the *FCGR3A* rs396991 gene polymorphism promotes a high FCRγ receptor affinity for the Fc region of rituximab, which correlates with greater immunological response via ADCC, apoptosis, and cellular cytotoxicity and therefore better response to treatment.[4] The influence of the *FCGR3A* rs396991 gene polymorphism has been previously investigated in rheumatoid arthritis and other diseases such as non-Hodgkin's lymphoma, demonstrating higher response to rituximab in patients carrying the G allele.[29,30,55–57] Based on the above, the *FCGR3A* rs396991 gene polymorphism could be used for predicting response to rituximab.

The influence of *FCGR2A* rs1801274 gene polymorphism has not been previously investigated in rheumatoid arthritis patients treated with rituximab. In our study, patients carrying the *FCGR2A* rs1801274-T allele, which is the high-affinity allele, reported higher EULAR response at 6, 12, and 18 months, higher remission of the disease at 6 months, and a greater variation in DAS28 at 12 and 18 months. However, a TNFi study found a significant association between the *FCGR2A* rs1801274-CC genotype and response to adalimumab ($P = .022$) and infliximab ($P = .035$) after 3 months of therapy.[24] These contradictory results may be because the mechanism of action of rituximab involves the ADCC pathway, exhibiting greater ADCC if the affinity for the FCGR receptor is higher.

Based on the results, the *FCGR2A* rs1801274 gene polymorphism may be considered a good predictor of response to rituximab, although further studies would be required to confirm it.

The main limitation of this study is sample size. Nevertheless, this limited sample size was sufficient to demonstrate consistent associations in the *FCGR2A* and *FCGR3A* genes with response variables, positioning these SNPs as potential biomarkers for identifying patients who will benefit from treatment with tocilizumab and rituximab in clinical practice. The power of the study was 70%, not 80% which was the desired power. Further studies will be necessary to confirm these associations in other populations.

In summary, these results show that the *FCGR2A* rs1801274 and *FCGR3A* rs396991 gene polymorphisms may significantly act as predictors of response to tocilizumab and rituximab therapy.

Conclusion

Our results suggest that the *FCGR3A* rs396991-TT genotype, higher baseline DAS28, subcutaneous tocilizumab administration, younger age at the beginning of treatment, and being bDMARD naive are associated with better response to tocilizumab. For patients treated with rituximab, we found better response in patients with the *FCGR2A* rs1801274-TT genotype, patients with the *FCGR3A* rs396991-G allele, a smaller number of previous biological therapies, positive

baseline value of anti-CCP, lower baseline value of ESR, and lower baseline value of VAS.

Acknowledgments

The authors thank all the patients who participated in the study and the professionals from University Hospital Virgen de las Nieves (Granada) who contributed to sample management, particularly the nursing team. The results of this investigation are part of the doctoral thesis presented by Alberto Jiménez Morales at the University of Granada.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported and that there are no competing financial interests in relation to the work described in this article.

Funding

The authors have no private financing to report.

Data-Sharing Statement

The data sets analyzed during the current study are available from the corresponding author on reasonable request.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med*. 2011;365(23):2205–2219.
2. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet*. 2017;389(10086):2328–2337.
3. Smolen JS, Landewe R, Bijlsma J, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis*. 2017;76(6):960–977.
4. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet*. 2010;376(9746):1094–1108.
5. Ternant D, Bejan-Angoulvant T, Passot C, Mulleman D, Paintaud G. Clinical pharmacokinetics and pharmacodynamics of monoclonal antibodies approved to treat rheumatoid arthritis. *Clin Pharmacokinet*. 2015;54(11):1107–1123.
6. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther*. 2008;117(2):244–279.
7. Beenhouwer D, Wallis R, Broder M, Furst DE. Mechanisms of action of tumor necrosis factor antagonist and granulomatous infections. *J Rheumatol*. 2004;31(10):1888–1892.
8. Gottenberg JE, Brocq O, Perdriger A, et al. Non-TNF-targeted biologic vs a second anti-TNF drug to treat rheumatoid arthritis in patients with insufficient response to a first anti-TNF drug: a randomized clinical trial. *JAMA*. 2016;316(11):1172–1180.
9. Song SN, Yoshizaki K. Tocilizumab for treating rheumatoid arthritis: an evaluation of pharmacokinetics/pharmacodynamics and clinical efficacy. *Expert Opin Drug Metab Toxicol*. 2015;11(2):307–316.
10. Bornstein GG, Queva C, Tabrizi M, et al. Development of a new fully human anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. *Invest New Drugs*. 2010;28(5):561–574.
11. Backhaus M, Kaufmann J, Richter C, et al. Comparison of tocilizumab and tumour necrosis factor inhibitors in rheumatoid arthritis: a retrospective analysis of 1603 patients managed in routine clinical practice. *Clin Rheumatol*. 2015;34(4):673–681.
12. Soliman MM, Hyrich KL, Lunt M, Watson KD, Symmons DP, Ashcroft DM. Rituximab or a second anti-tumor necrosis factor therapy for rheumatoid arthritis patients who have failed their first anti-tumor necrosis factor therapy? Comparative analysis from the British Society for Rheumatology Biologics Register. *Arthritis Care Res (Hoboken)*. 2012;64(8):1108–1115.
13. Conigliaro P, Triggianese P, Sole Chimenti M, et al. Factors predicting 2 years of remission and low disease activity in rheumatoid arthritis patients treated with TNF-inhibitors. *Isr Med Assoc J*. 2017;19(8):467–472.
14. Pomirleanu C, Ancuta C, Miu S, Chirieac R. A predictive model for remission and low disease activity in patients with established rheumatoid arthritis receiving TNF blockers. *Clin Rheumatol*. 2013;32(5):665–670.
15. Atzeni F, Bongiovanni S, Marchesoni A, et al. Predictors of response to anti-TNF therapy in RA patients with moderate or high DAS28 scores. *Joint Bone Spine*. 2014;81(1):37–40.
16. Chatzidionysiou K, Lie E, Nasonov E, et al. Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: pooled data from 10 European registries. *Ann Rheum Dis*. 2011;70(9):1575–1580.
17. Quartuccio L, Fabris M, Salvin S, et al. Rheumatoid factor positivity rather than anti-CCP positivity, a lower disability and a lower number of anti-TNF agents failed are associated with response to rituximab in rheumatoid arthritis. *Rheumatology (Oxford)*. 2009;48(12):1557–1559.
18. Forsblad-d'Elia H, Bengtsson K, Kristensen LE, Jacobsson LT. Drug adherence, response and predictors thereof for tocilizumab in patients with rheumatoid arthritis: results from the Swedish biologics register. *Rheumatology (Oxford)*. 2015;54(7):1186–1193.
19. Pers YM, Fortunet C, Constant E, et al. Predictors of response and remission in a large cohort of rheumatoid arthritis patients treated with tocilizumab in clinical practice. *Rheumatology (Oxford)*. 2014;53(1):76–84.
20. Lv Q, Yin Y, Li X, et al. The status of rheumatoid factor and anti-cyclic citrullinated peptide antibody are not associated with the effect of anti-TNFalpha agent treatment in patients with rheumatoid arthritis: a meta-analysis. *PLoS One*. 2014;9(2):e89442.
21. Salgado E, Maneiro JR, Carmona L, Gomez-Reino J. Rheumatoid factor and response to TNF antagonists in rheumatoid arthritis: systematic review and meta-analysis of observational studies. *Joint Bone Spine*. 2014;81(1):41–50.
22. Maneiro RJ, Salgado E, Carmona L, Gomez-Reino JJ. Rheumatoid factor as predictor of response to abatacept, rituximab and tocilizumab in rheumatoid arthritis: Systematic review and meta-analysis. *Semin Arthritis Rheum*. 2013;43(1):9–17.
23. Hatjiharissi E, Xu L, Santos DD, et al. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism. *Blood*. 2007;110(7):2561–2564.
24. Avila-Pedretti G, Tornero J, Fernandez-Nebro A, et al. Variation at FCGR2A and functionally related genes is associated with the response to anti-TNF therapy in rheumatoid arthritis. *PLoS One*. 2015;10(4):e0122088.
25. Canete JD, Suarez B, Hernandez MV, et al. Influence of variants of Fc gamma receptors IIA and IIIA on the American College of Rheumatology and European League Against Rheumatism responses to anti-tumour necrosis factor alpha therapy in rheumatoid arthritis. *Ann Rheum Dis*. 2009;68(10):1547–1552.

26. Tutuncu Z, Kavanaugh A, Zvaifler N, Corr M, Deutsch R, Boyle D. Fcgamma receptor type IIIA polymorphisms influence treatment outcomes in patients with inflammatory arthritis treated with tumor necrosis factor alpha-blocking agents. *Arthritis Rheum*. 2005;52(9):2693–2696.
27. Rooryck C, Barnetche T, Richez C, Laleye A, Arveiler B, Schaeverbeke T. Influence of FCGR3A-V212F and TNFRSF1B-M196R genotypes in patients with rheumatoid arthritis treated with infliximab therapy. *Clin Exp Rheumatol*. 2008;26(2):340–342.
28. Sarsour K, Greenberg J, Johnston JA, et al. The role of the FcGRIIIa polymorphism in modifying the association between treatment and outcome in patients with rheumatoid arthritis treated with rituximab versus TNF-alpha antagonist therapies. *Clin Exp Rheumatol*. 2013;31(2):189–194.
29. Ruyssen-Witrand A, Rouanet S, Combe B, et al. Fcgamma receptor type IIIA polymorphism influences treatment outcomes in patients with rheumatoid arthritis treated with rituximab. *Ann Rheum Dis*. 2012;71(6):875–877.
30. Quartuccio L, Fabris M, Pontarini E, et al. The 158VV Fcgamma receptor 3A genotype is associated with response to rituximab in rheumatoid arthritis: results of an Italian multicentre study. *Ann Rheum Dis*. 2014;73(4):716–721.
31. Wang J, Bansal AT, Martin M, et al. Genome-wide association analysis implicates the involvement of eight loci with response to tocilizumab for the treatment of rheumatoid arthritis. *Pharmacogenomics J*. 2013;13(3):235–241.
32. Maldonado-Montoro M, Cañadas-Garre M, González-Utrilla A, Plaza-Plaza JC, Calleja-Hernández MÁ. Genetic and clinical biomarkers of tocilizumab response in patients with rheumatoid arthritis. *Pharmacol Res*. 2016;111:264–271.
33. Maldonado-Montoro M, Canadas-Garre M, Gonzalez-Utrilla A, Angel Calleja-Hernandez M. Influence of IL6R gene polymorphisms in the effectiveness to treatment with tocilizumab in rheumatoid arthritis. *Pharmacogenomics J*. 2016.
34. Enevold C, Baslund B, Linde L, et al. Interleukin-6-receptor polymorphisms rs12083537, rs2228145, and rs4329505 as predictors of response to tocilizumab in rheumatoid arthritis. *Pharmacogenet Genomics*. 2014;24(8):401–405.
35. Walker UA, Jaeger VK, Chatzidionysiou K, et al. Rituximab done: what's next in rheumatoid arthritis? A European observational longitudinal study assessing the effectiveness of biologics after rituximab treatment in rheumatoid arthritis. *Rheumatology (Oxford)*. 2016;55(2):230–236.
36. Jimenez-Varo E, Canadas-Garre M, Henriques CI, Pinheiro AM, Gutierrez-Pimentel MJ, Calleja-Hernandez MA. Pharmacogenetics role in the safety of acenocoumarol therapy. *Thromb Haemost*. 2014;112(3):522–536.
37. van Gestel AM, Anderson JJ, van Riel PL, et al. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. *J Rheumatol*. 1999;26(3):705–711.
38. Aletaha D, Ward MM, Machold KP, Nell VP, Stamm T, Smolen JS. Remission and active disease in rheumatoid arthritis: defining criteria for disease activity states. *Arthritis Rheum*. 2005;52(9):2625–2636.
39. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analysis. *Am J Hum Genet*. 2007;81(3):559–575.
40. Smolen JS, Landewé R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann Rheum Dis*. 2010;69(6):1519.
41. Genovese MC, McKay JD, Nasonov EL, et al. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum*. 2008;58(10):2968–2980.
42. Smolen JS, Beaulieu A, Rubbert-Roth A, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet*. 2008;371(9617):987–997.
43. Cohen SB, Emery P, Greenwald MW, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum*. 2006;54(9):2793–2806.
44. Emery P, Keystone E, Tony HP, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis*. 2008;67(11):1516–1523.
45. Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis*. 2014;73(3):492–509.
46. Narvaez J, Magallares B, Diaz Torne C, et al. Predictive factors for induction of remission in patients with active rheumatoid arthritis treated with tocilizumab in clinical practice. *Semin Arthritis Rheum*. 2016;45(4):286–390.
47. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*. 1995;38(1):44–48.
48. Davila-Fajardo CL, van der Straaten T, Baak-Pablo R, et al. FcGR genetic polymorphisms and the response to adalimumab in patients with rheumatoid arthritis. *Pharmacogenomics*. 2015;16(4):373–381.
49. Marotte H, Arnaud B, Diasparra J, Zrioual S, Miossec P. Association between the level of circulating bioactive tumor necrosis factor alpha and the tumor necrosis factor alpha gene polymorphism at -308 in patients with rheumatoid arthritis treated with a tumor necrosis factor alpha inhibitor. *Arthritis Rheum*. 2008;58(5):1258–1263.
50. Maxwell JR, Potter C, Hyrich KL, et al. Association of the tumour necrosis factor-308 variant with differential response to anti-TNF agents in the treatment of rheumatoid arthritis. *Hum Mol Genet*. 2008;17(22):3532–3538.
51. Morales-Lara MJ, Canete JD, Torres-Moreno D, et al. Effects of polymorphisms in TRAILR1 and TNFR1A on the response to anti-TNF therapies in patients with rheumatoid and psoriatic arthritis. *Joint Bone Spine*. 2012;79(6):591–596.
52. Swierkot J, Bogunia-Kubik K, Nowak B, et al. Analysis of associations between polymorphisms within genes coding for tumour necrosis factor (TNF)-alpha and TNF receptors and responsiveness to TNF-alpha blockers in patients with rheumatoid arthritis. *Joint Bone Spine*. 2015;82(2):94–99.
53. Lee YH, Bae SC. Associations between PTPRC rs10919563 A/G and FCGR2A R131H polymorphisms and responsiveness to TNF blockers in rheumatoid arthritis: a meta-analysis. *Rheumatol Int*. 2016;36(6):837–844.
54. Montes A, Perez-Pampin E, Joven B, et al. FCGR polymorphisms in the treatment of rheumatoid arthritis with

Fc-containing TNF inhibitors. *Pharmacogenomics*. 2015;16(4): 333–345.
55. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood*. 2002;99(3):754–758.
56. Kim DH. FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma, *Blood.* 2006;108:2720–2725.
57. Liu D, Tian Y, Sun D, Sun H, Jin Y, Dong M. The FCGR3A polymorphism predicts the response to rituximab-based therapy in patients with non-Hodgkin lymphoma: a meta-analysis. *Ann Hematol*. 2016;95(9):1483–1490.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.