Influence of rheumatoid factor on serum drug levels of TNF inhibitors with different structures in rheumatoid arthritis

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Abstract

Objective

Certolizumab pegol (CZP), an Fc-free antibody fragment, has shown stable serum levels and steady efficacy in the treatment of RA patients, irrespective of RF levels at baseline. Here, we examine, in clinical practice, the effect of baseline RF and ACPA levels on serum drug levels of IFX, ADL and CZP an Fc-free antibody fragment.

Methods

This is a retrospective study performed in real-world patients. We assessed 170 patients with RA: 90 (53%) received IFX, 48 (28%) ADL and 32 (19%) CZP. Demographic and clinical variables, RF and ACPA levels were obtained at the baseline visit (T0), and patients were stratified based on negative, low, medium, or high levels. After 6 months (T6) serum drug levels and anti-drug antibodies (ADAb), were computed.

Results

While CZP serum levels did not differ across RF groups at T6, high baseline RF was linked to lower serum drug levels compared to RF negative status in treatment with complete monoclonal antibodies IFX and ADL. No differences in disease activity measured by DAS28 at baseline were observed across RF quartiles in patients treated with IFX or ADL. ADAb was observed in 26 patients with IFX, 3 with ADL and 1 with CZP, following 6 months of treatment. Patients with high baseline RF levels dropped out more frequently by secondary non-response in IFX or ADL than CZP (80% vs. 75% vs. 33%, p=0.002).

Conclusion

In this real word data evaluation, CZP serum levels were independent of RF levels in patients however patients with high baseline RF levels who obtained IFX or ADL had lower serum drug levels at 6 months than baseline RF-negative patients. In addition, secondary non-response was more frequent in patients with high RF levels treated with IFX and ADL.

Key words

rheumatoid arthritis, rheumatoid factor, TNF inhibitors, infliximab, adalimumab, certolizumab pegol, monoclonal antibodies

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Introduction

Rheumatoid arthritis (RA) is a systemic and destructive inflammatory joint disease that can cause chronic disability (1). The worldwide occurrence of RA is approximately 0.5–1% (2). It has an autoimmune aetiology, having both genetic and environmental factors (3, 4) The immunological response to RA is characterised by presence of autoantibodies, mainly rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) (5), which are used as diagnostic biomarkers and can also indicate a higher likelihood of more severe disease (6, 7) RF is an antibody against the Fc portion of IgG_1 and different RFs can recognise different parts of the IgG-Fc.

Currently, methotrexate (MTX), is the first-line treatment for RA (8), and when MTX is unsuccessful, biological disease-modifying anti-rheumatic drugs (bDMARDs) including tumour necrosis factor-alpha inhibitors (TNFi), (adalimumab [ADL], certolizumab pegol [CZP], infliximab [IFX], golimumab and etanercept [ETA]), are considered second-line treatment. TNFi are the most widely used biologics (8) because they were the first to be developed and clinical trials have extensively indicated their efficacy with a reasonable safety profile (9).

For many decades, RF has been considered a risk factor for more aggressive RA and thus rapidly progressive destructive disease. Increased RF in patients having RA is linked to increased disease activity and is regarded as a risk factor for disease progression (10-12). This association is mainly, but not only, mediated by greater disease activity in RF-positive compared to RF-negative patients. Additionally, high RF is suggested to be predictive of the discontinuation of TNFi owing to their ineffectiveness (13, 14) and is related to decreased response to TNFi (*e.g.* IFX, ETA and ADL) response to TNFi (*e.g.* IFX, ETA and ADL) compared to negative or low RF levels (15-18). It has been hypothesised that when RF binds with the Fc region of TNFi, immune complexes are produced that are subsequently cleared from the circulation, leading to lower levels of circulating drug and secondarily in clinical inefficacy (19, 20).

CZP is a polyethylene glycol-conjugated humanised, Fc-free anti-TNF antibody fragment (9). *Post-hoc* analyses of phase 3 and non-interventional studies consistently demonstrate that CZP has comparable efficacy in RA patients despite RF status, with a specifically consistent influence on patients with very high RF (21, 22). Here we aim to investigate the effect of RF levels on serum drug levels of 3 TNFi, one of them with different molecular structures (IFX, ADL and CZP), in clinical practice.

Patients and methods

Study design

This was a real-world study carried out at the Complex Therapies Unit of the Rheumatology Department in La Paz University Hospital, involving patients with RA who initiated biologic treatment with IFX, ADL, or CZP between 1999 and 2019. All patients enrolled fulfilled the ACR/EULAR 2010 classification criteria for RA, were over 18 years, had a moderate or high disease activity (DAS28>3.2) and satisfied the criteria of the Spanish Society of Rheumatology recommendations concerning the use of biological therapies in RA. No patient patients with other concomitant inflammatory immune-mediated disease were included in this study. All participating patients gave their informed consent.

Methods

Demographic and clinical variables (age, sex, BMI, smoking status, duration of disease before initiation of bDMARDs, baseline DAS28-ESR, RF, ACPA, C-reactive protein (CRP) in addition to concomitant and preceding treatment were obtained at baseline (T0) in all patients. DAS28-ESR was employed to monitor disease activity.

RF and ACPA titres were measured at baseline using nephelometry (Siemens®) and ACPA by a commercial ELISA kit CCPImmunoscan (Menarini®). Serum drug levels of TNFi (IFX, ADL) alongside anti-drug antibodies (ADAb) at 6 months (T6) were measured via commercial ELISA kits

Table I. Baseline demographic and clinical characteristics of patients treated with infliximab, adalimumab and certolizumab.

Characteristics	Total (n=170)	Infliximab (n=90)	Adalimumab (n=48)	Certolizumab (n=32)	*p*-value*
Age (years), median (IQR)	55.5 (45.3–66)	57 (46–65)	50⁺ (42–64)	61⁺ (47–70)	0.08
BMI, median (IQR)	24.5 (21.7–29.0)	24.2 (21.8–27.7)	24.7 (21.5–30.3)	24.6 (22.2–30.3)	0.3
Male, n (%)	28 (16.7)	14 (15.6)	9 (18.8)	5 (15.6)	0.2
Disease duration (years)	8.7 (4.5–14.3)	8.4 (4.4–14.3)	8.8 (3.9–16)	9.7 (5–12)	0.940
Smoking status, n (%)					
Current or previous smoker	66 (39.3)	29 (32.2)	22 (45.8)	16 (50.0)	0.03
Nonsmoker	96 (57.1)	61 (67.8)	24 (50.0)	12 (37.5)	
RF positive, n (%)	128 (76.2)	75 (83.3)	28 (58.3)	25 (78.1)	0.002
ACPA positive, n (%)	134 (79.8)	73 (81.1)	35 (72.9)	27 (84.4)	0.3
DAS28-ESR, mean ± SD	5.1 ± 1.3	5.4 ± 1.3^	4.5 ± 1.3^	4.9 ± 1.3	0.002
CRP level (mg/dl), median (IQR)	7.8 (3–21.8)	10.3 (3.2–25.2)	5.1 (1.4–10.1)	7.8 (2.3–18.2)	0.1
Previous bDMARDs, n (%)	26 (15.5)	10 (11.1)	10 (20.8)	6 (18.75)	0.2
Monotherapy, n (%)	16 (9.5)	8 (8.9)	8 (16.7)	0	0.2
Methotrexate, n (%)	112 (66.7)	64 (71.1)	33 (68.8)	17 (53.1)	0.2
Prednisone, n (%)	85 (50.6)	49 (54.4)	21 (43.8)	16 (50.0)	0.6

IQR: interquartile range; RF: rheumatoid factor; ACPA: anti-citrullinated peptide antibodies; BMI: body mass index; DAS28: disease activity score 28; CRP: C-reactive protein; bDMARDs: biologic disease-modifying drugs; csDMARDs: conventional synthetic disease-modifying drugs.
*Differences between groups were assessed by parametric test one-way ANOVA.
⁺Statistical differences were observed only between the ADL group *vs*. CZP by Turkey test (p=0.042).
^Statistical differences were observed only between the IFX group *vs*. ADL by Turkey test (p=0.002).

(Promonitor and Grifols®). CZP levels and anti-CZP antibodies were assessed utilizing in-house, fluorometric assays automated on the AutoDELFIA (PerkinElmer, Waltham, MA, USA) immunoassay platform (23). All samples to analyze serum drug and ADAb levels were collected at trough time before the next injection. During the first 6 months of follow-up, all TNFi were administered at standard doses for rheumatoid arthritis according to the technical information sheet (IFX at 3 mg/kg/iv at weeks 0, 2, 6 and then every 8 weeks; ADL 40 mg sc every 2 weeks; CZP 400 mg sc in weeks 0, 2 and 4 and then every 2 weeks). According to RF and ACPA levels at baseline, patients were divided in quartiles: RF negative (<20.0 IU/ml), low (LL: 20.0–57.0 IU/ml), medium (ML: 57.0–380.0 IU/ml) and high (HL: >380.0 IU/ml) levels and ACPA negative (<25.0 IU/ml), low (LL: 25.0–167 IU/ml), medium (ML: 167–1582 IU/ml) and high (HL: >1582 IU/ml) levels. Clinical data related to drug discontinuation (date and reason) were followed until December 2022.

Statistical analysis

The continuous variable results were expressed as the median and interquartile range (IQR) or mean and standard deviation (SD), whereas categorical ones were described with absolute and relative frequencies, and the differences based on the different treatment groups were evaluated by one-way test ANOVA. *Post-hoc* analysis (Turkey test) was performed when more than 2 groups were compared in the ANOVA test. The non-parametric test, Mann-Whitney, was employed to assess the relationship between RF/ACPA values and serum levels of TNFi and between RF/ACPA values and DAS28 at baseline as well. In patients with IFX, we used the Chi-square test to examine the differences in the percentage of ADAb-positive and -negative patients and drug discontinuation based on the RF/ACPA values. Statistical significance was established with $p \leq 0.05$. All statistical procedures were performed with the Statistical Package for the Social Sciences (SPSS 24, Chicago, IL, USA). Graphs were developed using GraphPad Prism 6.0 software (GraphPad Prism Inc., La Jolla, CA, USA).

Results

Baseline clinical and demographic characteristics

Data from 170 patients were retrospectively evaluated: 90 (53%) received IFX, 48 (28%) ADL and 32 (19%) CZP. Table I describes the patient's demographic and baseline characteristics. All patients had active disease at the initiation of treatment (DAS28 = 5.1±1.3). The IFX group exhibited the highest disease activity compared to ADL and CZP groups (5.4 *vs*. 4.5 and 4.9, respectively, p=0.002). RF was positive in 76.2% of patients, with significant differences between the treatment groups, (IFX 78.1%, ADL 58.3% and CZP 83.3%, respectively, p=0.02). In all, 39% of patients were previous or current smokers, while 57% never smoked. A higher proportion of patients in the IFX group (77%) had both RF+ and ACPA+ status followed by CZP (75%) and ADL (54%) groups (Supplementary Table S1). Twenty-six (15.5%) patients had previously received biological treatment, 22 were treated with TNFi, 1 with abatacept, 2 with tocilizumab and 1 patient with a JAK inhibitor.

Disease activity (DAS28) at T0 stratified by RF and ACPA baseline levels

No differences in disease activity quantified by DAS28 were detected between RF quartiles at baseline in patients treated with IFX or ADL. However, patients having high levels of RF treated with CZP had higher disease activity than those with negative/low levels of RF (DAS28: 6.1 (5.5–7.3) *vs*. 4.47 (3.9–4.90), p<0.05 and DAS28: 6.1 (5.5–7.3) *vs*. 4.21 (3.27–5.32), p<0.05, respectively) (Suppl. Fig. S1). No differences in disease activity were

Table II. Drug levels (µg/ml) at 6 months based on rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) quartiles

Baseline RF levels	Seronegative (<20.0 IU/ml)	Low levels (20–57 IU/ml)	Medium levels (57–380 IU/ml)	High levels (>380 IU/ml)
Infliximab (n=90)				
Median (IQR)	1.1 (0.3–4.5)	0.2 (0–2.0)	0.3 (0–1.6)*	0 (0–0.8) ^
Adalimumab (n=48)				
Median (IQR)	5.4 (2.7–9.0)	2.7 (0.3–9.9)	2.4 (0.7–5.4)	0.1 (0.01–2.2)*
Certolizumab (n=32)				
Median (IQR)	29 (17.7–48.9)	37.6 (29–47.7)	27.7 (7.7–45.9)	31.5 (21.4–34.5)
Baseline ACPA levels	Seronegative (<25 IU/ml)	Low levels (25-167 IU/ml)	Medium levels (167–1582 IU/ml)	High levels (>1582 IU/ml)
Infliximab (n=90)				
Median (IQR)	1.2 (0.2–3.4)	0 (0–1.0)*	0.3 (0–2.4)	0.5 (0–1.0)*
Adalimumab (n=48)				
Median (IQR)	5.5 (1.0–12.1)	3.7 (0.1–9.6)	2.6 (0.4–4.9)	3.9 (0.9–5.8)
Certolizumab (n=32)				
Median (IQR)	23.8 (9.7–29.4)	32 (23.0–47.3)	36.6 (15.7–46.4)	35.8 (31.5–40.2)

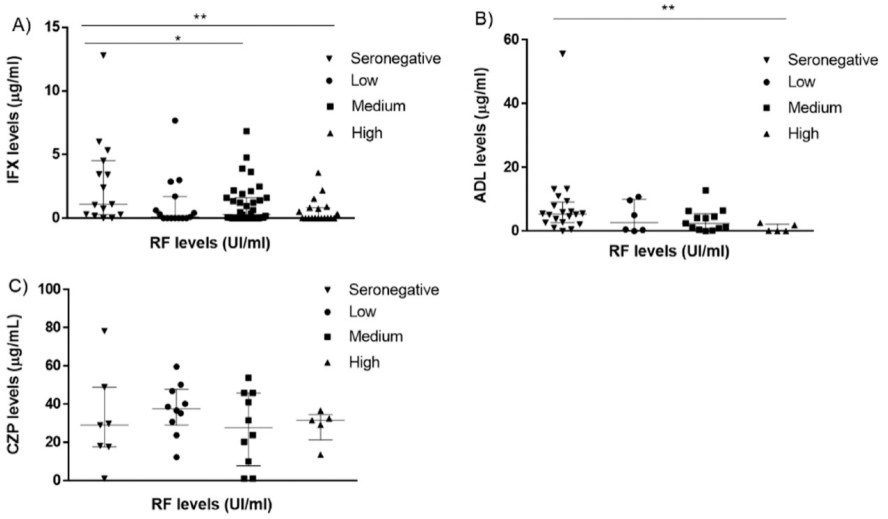
*p<0.05, comparator group: RF or ACPA seronegative, non-parametric test, Mann-Whitney.
^p<0.001, comparator group: RF seronegative, non-parametric test, Mann-Whitney.

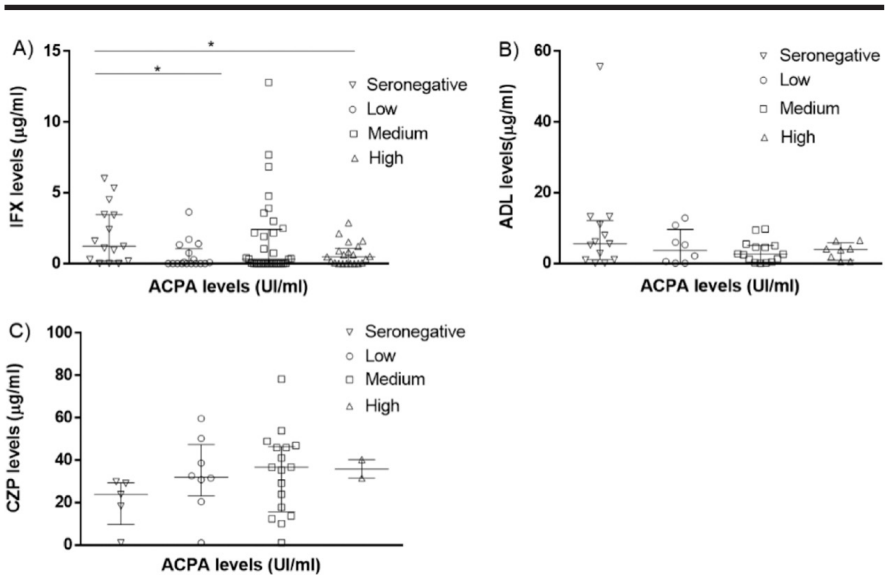
observed in patients treated with any TNFi when stratified by ACPA quartiles at baseline (Suppl. Fig. S2).

RF and ACPA levels and serum levels of anti-TNFi at T6

Serum levels of IFX, ADL and CZP grouped based on RF and ACPA quartiles are shown in Tables II and III. In IFX-treated patients, drug levels were significantly higher in RF-negative patients as compared to medium [median 1088 (IQR 288–4519) *vs*. 288 (IQR 0–1600), p<0.05] and high [median 1088 (IQR 288–4519) *vs*. 0 (IQR 0–824), p<0.01]. In ADL-treated patients, drug levels were statistically higher in RF-negative patients as compared to high RF levels [median 5371 (IQR 2720–9036) *vs*. 128 (IQR 14–2233), p<0.01] (Fig. 1 and Table II). Contrarily, no clear differences in drug levels were detected between RF groups at 6 months in patients treated with CZP (Fig. 1 and Table II).

In the case of serum drug levels and ACPA quartiles, we found differences only in patients with IFX. Serum IFX levels were statistically higher in ACPA-negative patients as compared to low [median 1218 (IQR 184–3456) *vs*. 0 (IQR 0–1048), p<0.05] and high [1218 (IQR 184–3456) *vs*. 465 (IQR 0–1056), p<0.05]. (Fig. 2 and Table II). Noteworthy, most patients with ACPA titres in the high quartile had also medium or





high levels of RF levels: 11/22 (50%) patients and 1/22 (32%), respectively.
A subanalysis was performed only including patients without previous biologic treatment (n=144) and similar findings were observed (Suppl. Table S2).

Immunogenicity of anti-TNFi drugs at T6

Since there is a relationship between drug levels and immunogenicity, we examined the development of anti-drug antibodies (ADAb) regarding RF and ACPA quartiles. At 6 months of treatment, ADAb was observed in 26 patients with IFX, 3 with ADL and 1 with CZP. The frequency of ADAb detection based on RF and ACPA quartiles are demonstrated (Suppl. Table S3). In the case of IFX, the major percentage of patients with ADAb positive were RF medium and high levels patients (39% and 38%, respectively) compared to RF-seronegative and RF low levels patients (4% and 19%, respectively). Statistical analysis cannot be conducted owing to the low number of patients. ADAb negative patients were more frequent in RF and ACPA seronegative groups (RF: 1 ADAb+ *vs*. 37 ADAb-, p=0.04 and ACPA: 2 ADAb+ *vs*. 30 ADAb-, p=0.049).

Association between therapy discontinuation and baseline RF and ACPA levels

Overall, 128 (75.3%) patients discontinued TNFi treatment for different reasons: 24 (18.8%) due to primary non-response, 66 (51.5%) due to secondary non-response, 8 (6.3%) due to adverse effects, and 30 (23.4%) due to other causes (neoplasms, change of address, infections, surgeries and pregnancy). The dropout proportion was higher in IFX patients (60.9% in IFX *vs*. 23.4% in ADL *vs*. 15.6% in CZP, p=0.001).
When comparing the baseline RF status with the reasons for discontinuation, we observed that the majority of patients with high RF levels dropped out due to secondary non-response compared to the other patient groups (82.8% with HL *vs*. 57.4% with ML *vs*. 34.8% with LL *vs*. 24.1% seronegative, p=0.002). This effect is mainly observed in patients treated with IFX (80%) or ADL (75%) and not in CZP (33%), being statistically significant only in IFX group (p=0.002).
In the case of ACPA levels, no statistically significant differences are observed between dropout due to secondary non-response and baseline ACPA status (70% with HL *vs*. 45% with ML *vs*. 64.7% with LL *vs*. 41.7% seronegative, p=ns). On the other hand, in the analysis separated by drugs, more patients with high levels dropped out due to secondary non-response in IFX (75%) and ADL (70%) than in CZP (50%), being statistically significant only in IFX (p=0.039).

Discussion

This is the first study to evaluate serum drug levels following baseline RF and ACPA levels in RA patients treated with TNFi with and without the Fc region. In this retrospective cohort, patients with high baseline RF levels treated with IFX or ADL exhibited lower serum drug levels at 6 months as compared to baseline RF-negative patients. Whereas, patients treated with CZP demonstrated no differences in serum drug levels regardless of baseline RF levels.
TNFi treatment has altered the course of RA, but some patients still do not respond properly. To be effective, TNFi should achieve sufficient adequate circulating drug levels as there is an association between serum drug concentration and the clinical response. Factors that may influence drug levels of biologicals are complex and diverse. Adherence to treatment, immunogenicity, concurrent treatment as methotrexate, obesity and disease activity are the best common factors (24-26), where immunogenicity and disease activity are the most significant (27-29).
Consistently, some recent publications highlight a significantly different efficacy of TNF-alpha blockers (IFX and ADL) in RF positive *versus* negative subpopulations (16, 30-33). Complete monoclonal antibodies, alongside the fusion protein etanercept, demonstrate lower efficacy in RA patients with RF positive compared with low or negative RF subgroups (20). This decreased efficacy has been described by the greater disease activity with higher acute phase reactant levels and more structural damage in these patients (13-18, 34) and it has been demonstrated that IgM RF amplifies the production of TNF from macrophages triggered by the immune complex containing ACPA (35). A high inflammatory burden induced by high expression of TNF in the inflamed tissue will presumably result in tissue retention of the TNFi, thereby elevating drug concentration in the joint and lowering it in the blood. Nevertheless, in this cohort, no significant differences in disease activity at baseline were detected regarding RF or ACPA levels, and differences in drug levels at 6 months could hence be probably attributed to other factors. Different authors have shown lowered clinical efficacy of TNFi in RA patients with high RF titres. Takeuchi *et al*. have reported that TNFi without Fc may be more efficacious than TNFi with Fc in RA patients with high RF titres, and higher doses of TNFi with Fc regions may be needed to regulate RA disease activity in high RF titre patients (36). In our cohort, clinical scores data at 6 months were not available. On the other hand, when evaluating the reasons for discontinuation, we observed that the majority of patients treated with IFX and ADL who had high levels of autoantibodies (RF and ACPA) dropped out due to secondary non-response. However, this was not as striking in patients treated with CZP. This fact could be explained in part by the observed findings of a lower drug concentration in patients treated with IFX or ADL with high levels of RF or ACPA.
In the current research, we detected that high RF titres were inked to lower serum drug levels of IFX and ADL but not CZP. In addition, high levels of ACPA were associated with lower serum IFX levels. The results pointed out that this could be mainly due to the RF as we observed that patients under IFX with high levels of ACPA had also medium-high levels of RF. There are various possible explanations regarding the effect of RF over serum TNFi levels. One is that RF (pentameric IgM) binds to the IgG-Fc of the TNFi that are monoclonal antibodies (or receptor fusion proteins) and

forms large immune complexes rapidly cleared by endocytosis, causing low serum drug levels and eventually loss of efficacy. The same phenomenon has been proposed with *in vitro* methods to identify the presence of ADAb in RA patients. As RF can bind the Fc region of IgG monoclonal TNFi and ADAb, it is essential to block the RF present in serum by adding IgG before ADAb determination (37). It is hypothesised that the Fcγ-receptor mediated elimination could be the dominant mechanism, in cases where antibody is able to form soluble immune complexes containing three or more IgG molecules, as occurs with FR, carried out by macrophages and on other phagocytic who express Fcγ receptors (38). Because CZP lacks the Fc region, its clearance is presumably unaffected by the presence of RF. Finally, another reason could be an effect of different disease activity, although no differences in disease activity were detected between groups with different RF levels.

One of the most significant factors that influence the efficacy of biologicals is their potential for the induction of ADAb development, which averts the binding of the drug to its target. Clinically significant ADAb are those that are present at high concentrations resulting in a significant reduction in serum drug concentrations. Immunogenicity is an important pharmacodynamic factor and is related to the structure and composition of the monoclonal antibodies, its use in terms of dosage, route of administration, and co-medication (26). However, this effect does not seem to be so clear in the case of CZP. In a recent publication including 40 RA patients treated with CZP, most cases where ADAb were detected, high CZP concentrations were also present (39). In our cohort, the frequency of ADAb to IFX and ADL is similar to what has been identified in the literature (40) and anti-CZP antibodies in clinical trials of RA patients range from 5% to 8% (21, 22, 41, 42) and in the NOR-DMARD study, at 3 months the incidence was 6% (43).

Contradictory findings between the relationship between RF seropositivity and the development of immunogenicity have been explained in the literature (44). In our cohort, we revealed that the highest percentage of patients with anti-IFX antibodies were in the medium and high levels of RF- patients. This is in agreement with the research reported by Sakane *et al*. in patients with RA treated with IFX in which they demonstrated that in patients in the ADAb-positive group, the value of RF was significantly higher than that in the ADAb-negative group (44). This fact could be associated with the prior outcomes. If the presence of high RF levels is linked to decrease drug levels, it is easier to observe the presence of ADAb by drug-sensitive methods like ELISA.

This study has various limitations. The retrospective design with a relatively small number of patients, with differences in sample sizes between the individual TNFi, is a limitation. Additionally, there were no clinical data available at 6 months to indicate that lowered serum drug levels had clinical consequences. Furthermore, numerous reports have demonstrated that TNFi with Fc fragments exhibit better responses in patients with negative *versus* positive and low *versus* high baseline RF titres (15-18).

In conclusion, the outcomes introduced herein show that baseline RF levels do not impact serum drug levels of Fc-free anti-TNF (CZP) whereas baseline RF levels are significantly linked to low serum drug levels of complete monoclonal antibodies (IFX and ADL) in clinical settings of RA patients. Furthermore, secondary non-response was more frequent in patients with high RF levels treated with IFX and ADL. These differences in serum drug concentration along the treatment, associated with baseline RF levels might aid physicians to select suitable treatment for the management of RA.

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