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Clinical and Serological Response to Tocilizumab in Patients with Rheumatoid Arthritis

Oscar Epis[1]*, Claudia Alpini[2], Sara Marceglia[3], Cinzia Casu[1], Luca Giacomelli[4] and Eleonora Bruschi[1]

[1]*Rheumatology Unit, Niguarda "Ca' Granda" Hospital, Milan, Italy*
[2]*ClinicalChemistry IRCCS Policlinico San Matteo, Pavia, Italy*
[3] *Dipartimento di Bioingegneria, Politecnico di Milano, Milan, Italy*
[4] *Free Researchers, Milan, Italy*

Abstract

Objective: The role of rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) in the response to treatment for rheumatoid arthritis (RA) such as tocilizumab (TCZ) is still not completely understood. This study investigates the relationship between the presence and levels of RF and anti-CCP and clinical response to TCZ in patients with RA.

Methods: This was an observational longitudinal study in 27 patients with active, long-standing RA despite previous treatment with >2 Disease-Modifying Anti Rheumatic Drugs (DMARDs) and/or steroids. Patients were treated with TCZ 8 mg/kg every 4 weeks. The following parameters were assessed: Erythro Sedimentation Rate (ESR), C - reactive protein (CRP), Health Assessment Questionnaire (HAQ), Disease Activity Score of 28 joints (DAS28), Clinical Disease Activity Index (CDAI) and Simplified Disease Activity Index (SDAI). IgM-, IgA- and IgG-RFs and anti-CCP antibodies were measured using ELISA at baseline, 3 months (T1), 6 months (T2), and 12 months (T3).

Results: All patients showed significant and sustained clinical response to TCZ treatment. All clinical scales with the exception of HAQ significantly decreased. There was a significant correlation (p=0.03) between anti-CCP and SDAI changes from baseline at T1 and T2. However, no significant correlation was measured between antibody count at T0 and changes in the DAS-28 ESR at T1 and at T2. Also, there was no correlation between clinical scales and antibody levels RF-IgG, IgA, IgM as well as between clinical scales and anti-CCP levels.

Conclusions: Tocilizumab is effective in treating the clinical symptoms of RA, and the efficacy of this molecule was not correlated with either RF or anti-CCP levels.

Keywords: Tocilizumab; Rheumatoid arthritis; Serological markers; Antibodies to citrullinated proteins; IgG- Rheumatoid factor; IgA-Rheumatoid factor

Introduction

Rheumatoid arthritis (RA), a chronic systemic autoimmune disease with complex genetic and environmental origins, involves inflammation of the synovium with progressive erosion of bone leading to joint damage and loss of function. A proactive approach with the early introduction of treatment is generally recommended to control pain and prevent disability with its extensive economic and social consequences. A recent European League against Rheumatism (EULAR) task force for the management of RA recommended that the majority of patients should receive synthetic DMARDs as a first-line therapy [1]. The task force concluded that patients with a severe and aggressive disease course often do not respond sufficiently well to monotherapy/combination with a synthetic DMARD without the addition of biological DMARDs [1].

There are a range of different biological DMARDs agents approved for the treatment of RA including tocilizumab (TCZ), a humanised monoclonal interleukin-6 (IL-6) receptor antibody that has shown clinical efficacy in the treatment of patients with moderate to severe RA with previous inadequate responses to methotrexate (MTX) and one or more anti-TNFα agent [2]. However, not all patients achieve an adequate response and the ability to predict which patients will respond to a given therapy is central to the successful management of RA.

This element, together with the fact that an early diagnosis is fundamental in RA to prevent joint damage, calls for the evaluation of predictive and prognostic biomarkers for the diagnosis and management of RA.

Until recently the diagnosis and clinical course of RA was more or less entirely based on clinical manifestations and levels of rheumatoid factor (RF); however relying on assay of RF is no longer considered to be adequate [3,4].

Although RF assay to diagnose RA is relatively sensitive(up to 90%) it has a low specificity for RA (70-90%) and patients with other conditions such as systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, polymyositis/dermatomyositis, tuberculosis and hepatitis C have positive RF assays but do not have RA [5,6]. To address these limitations a new test for RA, involving the assay of anti-cyclic citrullinated peptide antibodies (anti-CCP), has shown efficacy in the early diagnosis of RA and in the prediction of disease severity/

***Corresponding author:** Oscar Epis, Rheumatology Unit, Niguarda Ca' Granda Hospital Piazza Ospedale Maggiore 3, 20162Milan, Italy, Tel: +39 0264442168, Fax: +390264443277; E-mail: oscar.epis@ospedaleniguarda.it

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joint damage [4,7-16]. The anti-CCP enzyme linked immunosorbent assay (ELISA) has a high specificity (up to 96%) and a reasonable sensitivity (up to 88%) and these antibodies are frequently detected early suggesting they play an important role in the pathogenesis of RA [6,17-20]. The anti-CCP assay has sensitivity similar to that of RF but its specificity is much higher which may result in a more accurate diagnosis [4,12-13, 21-22].

The precise correlation between these biomarkers in the response to treatment is not fully understood: in particular, the mechanism by which biological agents could lead to a decrease in the titre of autoantibodies is still matter of debate. The down-regulation of proinflammatory processes and/or the modulation of apoptosis have been suggested to play a role in the synthesis of autoantibodies or in protein citrullination, that eventually might trigger the B cell response (Ziolkowska M, Maslinski W, 2003, J Rheumatol, Lugering A, Schmidt M, Gastroenterology 2001). However, studies investigating changes in the levels of RF in response to synthetic and biological DMARDs have not been able to confirm a definitive relationship between decreased RF subtypes and clinical response, while studies investigating changes in anti-CCP levels have yielded conflicting results [23-29]. In addition, to our knowledge the correlation between autoantibodies and TCZ treatment has been poorly investigated to date.

Number of patients	T0, T1, T2	27
	T0, T1, T2, T3	19
Age (years)	Mean	56.44
	Median	56
	Standard deviation	10.75
	Range (min, max)	(35-78)
Gender	Males	3
	Females	24
Disease duration (years)	Mean	9.96
	Median	10
	Standard deviation	6.30
	Range (min, max)	(1,31)
RF	Positive	16
	Negative	11
CCP	Positive	14
	Negative	9 (3 not defined)
Months of therapy at May 2012	Mean	19.52
	Median	18
	Standard deviation	7.84
	Range (min, max)	(9,35)
Previous biological treatments*	No	7
	Yes	20
	Abatacept *Adalimumab* *Etanercept* *Infliximab* *Rituximab*	5 11 18 4 3
Past syntethic DMARDs (>2)*	No	11
	Yes	16
	Cyclosporine *Hydroxychloroquine* *Leflunomide* *Methotrexate* *Gold salts* *Sulfasalazine*	11 20 12 27 1 1
Current DMARDs*	No	7
	Yes	20
	Cyclosporine *Hydroxychloroquine* *Leflunomide* *Methotrexate* *Gold salts* *Sulfasalazine*	2 4 0 18 0 0
Steroids	Mean	5.42
	Median	5
	Standard deviation	3.78
	Range (min, max)	(0, 15)

* Patients could be in treatment with >1 agent.

CCP: Cyclic Citrullinated Peptide; DMARDs: Disease-Modifying Anti Rheumatic Drugs; RF: Rheumatoid Factor

Table 1: Demographic and clinical characteristics of patients included in the study.

The current study was performed to investigate the relationship between the presence and levels of RF and anti-CCP (including their different isotypes) and clinical response to TCZ in patients with RA.

Patients and Methods

This was an observational longitudinal study in 27 patients (24 females, 3 males, mean age 56.4 ± 10.7 years) with long-standing RA (Table 1). All patients had active disease despite having previously received treatment with >2 DMARDs and/or steroids and 20/27 were receiving DMARDs at study entry (Table 1). All previous biological DMARDs were interrupted for poor efficacy. All patients gave written informed consent and the study was approved by the local ethics committee.

Patients were treated with TCZ 8 mg/kg once every 4 weeks as a 60 minute single intravenous drip infusion. Patients were studied at baseline (T0) and at follow-up visits 3 months (T1), 6 months (T2), and 12 months (T3) after the beginning of treatment. Non-steroidal anti-inflammatory drugs and oral steroids were permitted during the study period.

Autoantibody analysis

Serum samples for autoantibody assessment were collected and stored at -70°C immediately before the first administration of TCZ (T0) and thereafter at 3 months (T1), 6 months (T2), and 12 months (T3).Testing for the different autoantibodies was carried out on serum samples at the end of the study.

Rheumatoid factors

RF was measured by immunonephelometry using the quantitative N Latex RF system(Dade Behring, Marburg, Germany).The different rheumatoid factor isotypes (IgM, IgA and IgG)were assessed using an indirect solid-phase ELISA (Orgentec Diagnostika, Mainz, Germany) involving the binding of Fc fragments of highly purified human IgG to the microwells. The quantitative test system for IgM, IgG and IgA RF is calibrated in relative arbitrary units related to the 1st British Standard Preparation 64/2 as reported in the kit insert. Manufacturer reference values for RF and RF IgG, IgA and IgM were <20 U/ml which was considered to be negative and >20 U/ml to be positive.

Anti-cyclic citrullinated peptides

Anti-CCP antibody reactivity was tested using a commercially available automated ELISA (EliA™ CCP Assay; Phadia GmbH, Freiburg, Germany) on an ImmunoCAP100 automatic analyzer (Phadia AB, Uppsala, Sweden) according to the manufacturer's recommendations. Values of 10.0 U/ml or greater were considered to be positive and <7.0 U/ml to be negative.

Statistical analyses

Univariate and bivariate analyses were carried out. Patients were assessed at each time point (T0, T1, T2, T3) using clinical scales [ESR, CRP, SW 28 (SWollen joints), TEN 28(TENder joints), GH(Global Health), PGA (Patient Global Assessment), MGA(Medical Global Assesment), HAQ, DAS28-ESR, DAS28-CRP, CDAI, and SDAI] and antibodies levels (RF IgG, RF IgA, RF IgM, and anti CCP). In the univariate analysis, each assessed variable was considered as independent. The probability distribution of the variable was tested against normality. If the distribution resulted close to normality, a one-way ANOVA was used to disclose significant differences between the values of the variable at the different time points ($p<0.05$). Otherwise, a Kruskal-Wallis test was used ($p<0.05$).

As antibodies had a bimodal distribution indicating that patients could be stratified in two classes (low and high count), bivariate analyses were conducted to determine the empirical relationship between them. Variables were stratified according to a range of patients' characteristics, including gender, length of RA, previous treatments etc.

Results

Clinical response

Data sets were available for all 27 patients at baseline and T1 and T2 and for 19 patients at T3. All patients showed significant and sustained clinical response to TCZ treatment during the observation period. All clinical scales with the exception of HAQ significantly decreased during the observation period (Table 2). Figure 1 shows box plots of clinical variables at T0, T1, and T2 showing the significant decrease in clinical scales (except HAQ) over time. The time point T3 was not included as the dataset at this time was incomplete.

Baseline and treatment-induced changes in antibody profile

Levels of IgM-, IgA- and IgG-RFs and anti-CCP antibodies at the time points measured are shown in table 3. At baseline, there are gender effects on TEN28, HAQ, and also on RF-IgG count and there was a trend towards higher SW28 scores in patients with high antibodies count compared with those with low antibodies count. Levels of RF and anti-CCP at baseline were different in patients with previously positive or negative RF and anti-CCP. Figure 1 shows box plots of changes in antibodies over time (T0, T1, and T2but not T3 due to an incomplete dataset). There was a significant correlation ($p=0.03$) between anti-CCP and SDAI changes from baseline at T1 and T2. Likewise there were no significant correlations between antibody count at T0 and changes in the DAS-28 ESR at T1 and at T2. No significant relationship between clinical scales and antibody levels RF-IgG, IgA, IgM as well as between clinical scales and anti-CCP levels were observed (Figure 2 a-d).

Discussion

TCZ has been demonstrated in seven large scale phase III trials to be effective as both as monotherapy and in combination with DMARDs in adult patients with moderate to severe RA [30]. Furthermore, three recent studies (TAMARA, REACTION and DANBIO) confirmed the efficacy of TCZ in the treatment of RA in every-day real world practice [31-35]. However, despite the demonstrated efficacy of TCZ not all patients respond to TCZ and given that early introduction of effective therapy for RA is the key to preventing joint destruction, it is important to be able to differentiate between those patients likely to respond to TCZ and those not. In order to do so, accurate biomarkers are required. For many years RF was the standard diagnostic and prognostic variable in RF but it has low specificity for RA and other tests including Antinuclear Antibody (ANA) Test, anti-CCP antibodies and CRP are now a routine part of rheumatologists' armamentarium.

Clinical scale	Time point	Mean	P value
ESR	T0	37.04	0.0042
	T1	16.67	
	T2	16.85	
	T3	11.16	
CRP	T0	16.24	0.0011
	T1	10.40	
	T2	8.42	
	T3	1.34	
SW 28	T0	10.78	0.0000
	T1	4.37	
	T2	3.89	
	T3	2.84	
TEN 28	T0	12.00	0.0008
	T1	6.26	
	T2	6.37	
	T3	4.42	
GH	T0	37.30	0.0216
	T1	58.04	
	T2	60.78	
	T3	65.16	
PGA	T0	7.01	0.0091
	T1	4.77	
	T2	3.97	
	T3	4.21	
MGA	T0	5.66	0.0004
	T1	3.03	
	T2	3.68	
	T3	2.43	
HAQ	T0	1.55	0.2957
	T1	1.61	
	T2	1.13	
	T3	1.24	
DAS 28-ESR	T0	5.77	0.0003
	T1	3.57	
	T2	3.61	
	T3	3.15	
DAS 28-CRP	T0	5.34	0.0001
	T1	3.49	
	T2	3.45	
	T3	2.92	
CDAI	T0	35.45	0.0003
	T1	18.42	
	T2	17.91	
	T3	13.91	
SDAI	T0	37.07	0.0003
	T1	19.46	
	T2	18.76	
	T3	14.16	

CDAI: Clinical Disease Activity Index; CRP: C-Reactive Protein; DAS28: Disease Activity Score of 28 joints; ESR: Erythro Sedimentation Rate; GH: Growth Hormone; HAQ: Health Assessment Questionnaire; MGA: Medical Global Assesment; PGA: Patient Global Assessment; SDAI: Simplified Disease Activity Index; SW 28: SWollen joints; TEN 28:TENder joints

Table 2: Clinical response: changes in clinical scales over the study period.

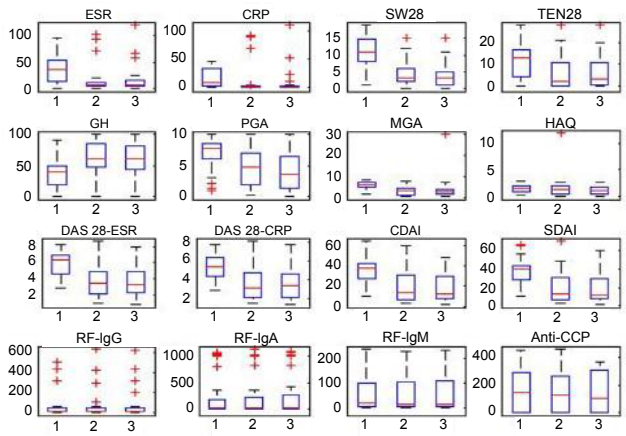

Figure 1: Clinical response: changes in clinical scales over the study period. All clinical variables (except HAQ) and antibody titers significantly decreased from T0 to T2 (values at T3 are not reported due to an incomplete dataset).a

Antibody	Time point	Mean	P value
RF IgG	T0	62.13	0.8970
	T1	67.48	
	T2	71.98	
	T3	33.89	
RF IgA	T0	227.51	0.8727
	T1	229.08	
	T2	237.79	
	T3	222.54	
RF IgM	T0	62.65	0.9561
	T1	55.56	
	T2	59.21	
	T3	37.70	
Anti CCP	T0	146.83	0.9994
	T1	147.34	
	T2	143.88	
	T3	118.27	

CCP: Cyclic Citrullinated Peptide; RF: Rheumatoid Factor

Table 3: Changes in antibody levels over the study period. Both RF and anti-CCP were tested in all 27 patients.

In our study, TCZ was effective in treating the clinical symptoms of RA in patients with different serological profiles, and the efficacy of this molecule does not seem to be affected by the presence of either RF or anti-CCP (or both).However, we must point out that our study cannot lead to definitive conclusions, due to the reduced number of patients and the overall short observation period: only 19 patients were followed for the entire follow-up (12 months). Although these limitations should be taken into account when evaluating the results of our study we believe that they may not preclude the significance of the conclusions reached, also considering that studies with similar or even smaller sample size have been published before and led to valid results [25,26,28]. For instance, Bobbio-Pallavicini et al. evaluated a cohort of 30 patients and showed that infliximab treatment was associated, over a 78-week period, with a reduction in RF titre [25]. De Rycke et al. reached similar conclusions in a slightly larger sample (n=62) [26], and Yazdani-Biuki et al. when analysing 12 patients - showed that etanercept may have a pivotal effect on RF-producing B cells [28].

There is now a growing body of evidence suggesting that markers associated with clinical response may not be the same biomarkers that predict risk of further joint damage [36]. At the EULAR 2012 meeting, data from Bay-Jensen et al. discussing new biomarkers for early differentiation between likely TCZ responders and non responders caused much interest. These markers included matrix metalloproteinase-mediated degradation of type II collagen (C2M) or type III collagen (C3M); matrix metalloproteinase-mediated C-reactive protein (CRPM); citrullinated and matrix metalloproteinase-degraded

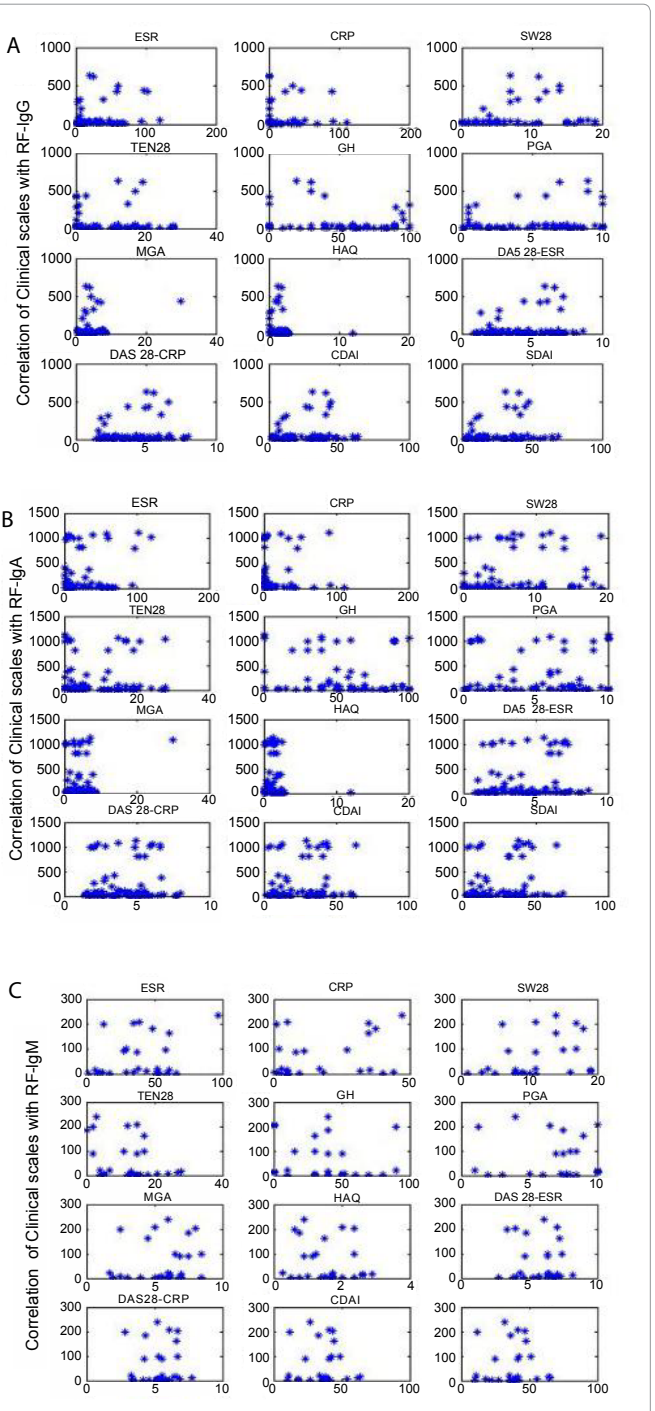

Figure 2: Clinical response: changes in clinical scales over the study period. All clinical variables (except HAQ) and antibody titers significantly decreased from T0 to T2 (values at T3 are not reported due to an incomplete dataset).a

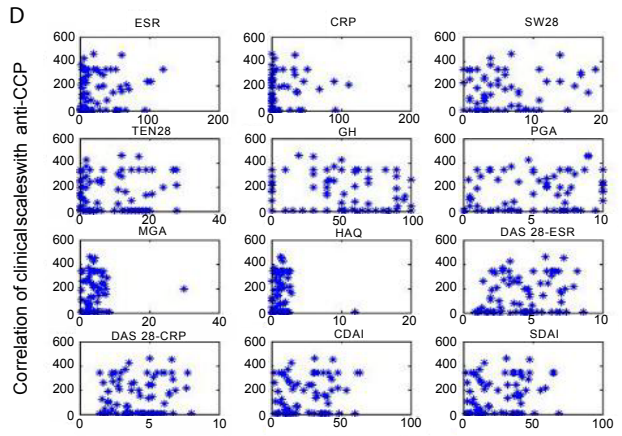

Figure 2: Clinical response: changes in clinical scales over the study period. All clinical variables (except HAQ) and antibody titers significantly decreased from T0 to T2 (values at T3 are not reported due to an incomplete dataset).a

vimentin (VICM); and matrix metalloproteinase-destroyed type I collagen (ICTP). The major difference is that CRP is produced in the liver while CRPM is produced in the joints and reflects joint-specific tissue inflammation and as such might provide a more accurate picture of what is going on in patients with RA [36]. Levels of these 'new' markers decreased significantly following TCZ treatment with levels of CRPM dropping by 33% following 4 weeks of therapy in responders and by significantly lesser amounts in the nonresponders. In contrast, the conventional marker of systemic inflammation, high-sensitivity CRP, dropped by about 35% in both responders and nonresponders showing it had little predicative value. With the move towards personalised healthcare, biochemical markers that can accurately detect ongoing joint damage may help the treating physicians to select therapeutic agents to which a patient is most likely to respond.

In conclusion, despite the limited number of patients observed and the overall short follow-up, our study suggests that TCZ is effective in treating the clinical symptoms of RA, and the efficacy of this molecule was not affected by the levels of either RF or anti-CCP (or both). We believe that our study may pave the way for further, larger studies with a longer follow-up aimed at further assessing the efficacy of TCZ in patients with different serological profiles and therefore either accept or discard the present findings.

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