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Original Article

Serum interleukin-6 in seropositive rheumatoid arthritis and response to tocilizumab: An observational study

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Abstract

Objective: There is no clinically useful biomarker as a predictor of response to any class of biological disease-modifying antirheumatic drugs (bDMARD). Serum interleukin-6 (IL-6) has a major role in the pathogenesis of rheumatoid arthritis (RA) and its serum level in patients of RA may predict response to treatment with IL-6 receptor (IL-6R) antagonist tocilizumab.

Methods: Biological DMARD naïve patients of seropositive RA, fulfilling American College of Rheumatology/European League Against Rheumatism classification criteria 2010, were treated with 06 doses of tocilizumab (8 mg/kg) at monthly interval. Baseline and post-treatment serum IL-6 levels were measured and correlated with response to treatment measured by disease activity score-28 joints erythrocyte sedimentation rate (DAS28 ESR) after treatment.

Results: The study included 34 patients and 26 (70%) of them achieved DAS-28 remission (DAS28 ESR < 2.6). The baseline serum IL-6 did not correlate with post-treatment DAS28 ESR (R −0.197, P = .264). Though, statistically not significant (P = .085) more patients with comparatively lower baseline serum IL-6 attained DAS28 remission (16 out of 17, P = .085). There was an increase in the serum IL-6 level (median 40.5 pg/ml [IQR 130.2] to 72.6 pg/ml [IQR 162.5]) after tocilizumab treatment and the change in IL-6 level also did not correlate with post-treatment DAS28 ESR (R −0.240, P = .172).

Conclusion: Higher number of patients with comparatively lower serum IL-6 level attained DAS28 remission in this study; however, it was not statistically significant. It requires further evaluation in larger studies to make any conclusion on the role of serum IL-6 as a predictor of response to tocilizumab in seropositive RA.

Keywords: Drug response biomarkers, tocilizumab, interleukin-6, rheumatoid arthritis, antirheumatic agents

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Introduction

Rheumatoid arthritis (RA) frequently leaves disabling deformities in inadequately treated patients.[1] It renders about half of the patients significantly disabled by the end of the first decade of illness and another 30% are disabled by the second decade and also results in reduced life expectancy.[2,3] A remarkable development in understanding the molecular basis of disease over the last few decades has led to the development of several biologic agents which has improved outcome for the treatment of patients who respond inadequately to conventional disease-modifying antirheumatic drugs (DMARD). Use of biological DMARDs (bDMARD) acting on a different pathway like tocilizumab, rituximab, and abatacept has resulted in better clinical response; nevertheless, optimal clinical response is not achieved in about 20-40% patients.[4-7] Despite the available guidelines, the choice of bDMARD is largely arbitrary today. Finding a biomarker to predict clinically significant response to a bDMARDs will help in rationalizing the bDMARD therapy with an evidence-based approach. Interleukin-6 (IL-6) is elevated in serum as well as the synovial fluid of the RA patients and it correlates with the level of the acute phase reactants and disease activity in RA patients.[8,9] This study has been conducted to assess if serum IL-6 level can predict clinical response to treatment with IL-6 receptor (IL-6R) antagonist tocilizumab.

The primary objective of this study was to assess the correlation between baseline serum IL-6 levels in patients of seropositive RA and clinical response to IL-6R antagonist tocilizumab as measured by disease

activity score in 28 joints erythrocyte sedimentation rate (DAS 28-ESR).[10] Change in serum IL-6 level from baseline to post-treatment level was also assessed and correlated with clinical response.

Methods

It was a prospective observational single-center study conducted at a rheumatology center in India between June 2014 and December 2015. Assuming a correlation coefficient of 0.55, we estimated that a sample size of 34 patients will have 80% power to detect a difference of 20% in the mean DAS28 ESR. Study patients were recruited from the rheumatology outpatient department as well as from inpatients admitted to the rheumatology ward of the hospital over 18 months.

Inclusion criteria

1. All patients of seropositive RA (positive rheumatoid factor [RF] and/or anti-citrullinated protein antibodies [ACPA], fulfilling the American College of Rheumatology/European League Against Rheumatism classification criteria 2010, and more than 16 years in age.[11]
2. Presence of active disease, defined by a DAS 28-ESR ≥ 3.2
3. Inadequate response to at least two non-biological DMARDs in optimal doses (methotrexate 20 mg weekly or leflunomide 20 mg once daily or sulfasalazine 1 gm twice daily or hydroxychloroquine 200 mg once daily) for at least 3 months. Glucocorticoids (prednisolone or equivalent (<10 mg/day) and non-steroidal anti-inflammatory drugs were permitted if their doses had remained stable for at least 4 weeks and 2 weeks respectively, before enrollment.

Main Points

- More RA patients with relatively lower baseline serum IL-6 attained DAS28 remission following tocilizumab treatment as compared to those with higher baseline serum IL-6, although it did not reach statistical significance.
- In a proportion of seropositive RA patients, the level of serum IL-6 increased following tocilizumab treatment, while it decreased in the remaining patients.
- There is divergent evidence for the role of serum IL-6 as a predictor of response to tocilizumab in seropositive RA patients; however, further evaluation is required to establish a definite clinical role.

Exclusion criteria

1. Active infection
2. Evidence of hepatitis B or hepatitis C infection
3. Alanine transaminase (ALT) and aspartate transaminase (AST) levels >1.5 times the upper level of normal (ULN) or any evidence of liver disease
4. Evidence of active tuberculous infection in radiogram of the chest or latent tuberculous infection as detected by positive TB QuantiFERON Gold test
5. Neutropenia defined as absolute neutrophil count <2000 cells/mm^3
6. Thrombocytopenia defined as platelet count <100,000/mm^3
7. Previous exposure to any bDMARD therapy
8. Pregnancy and lactation.

Patients were given injection tocilizumab 8 mg/kg intravenous every month for 06 consecutive doses. Clinical and laboratory evaluation was done at baseline and every visit final assessment was done one week following the last dose of tocilizumab.

C-reactive protein (CRP), ESR, complete blood count, AST, and ALT were measured at baseline and each visit. ESR was estimated using the Westergren method and CRP was done using nephelometry (Beckmen & Coulter system). The cutoff for the normal value of CRP was 10 mg/L.

Serum IL-6 level was measured at baseline and 24 weeks using the enzyme immunoassay method. All samples were initially measured with Diaclone human IL-6 ELISA kit (solid-phase sandwich ELISA for the *in vitro* quantitative determination of IL-6 in supernatants, serum and plasma samples) which had an assay range from 6 to 200 pg/mL. All those samples who had less than 6 pg/mL were tested again with high sensitivity Diaclone human IL-6 ELISA kit (assay range 1.56 to 50 pg/mL). The IL-6 values lower than detectable were fixed at 1.56 pg/mL and those higher than detectable were fixed at 200 pg/mL. Blood was collected at the time of clinical assessment in pyrogen-free sterile tubes.

Blood samples were permitted to clot at −40 °C for one hour and then sera were separated by centrifugation at 1000 rpm for 10 minutes as mentioned in manufacture's instruction. Collected sera were aliquoted and stored at −80 °C until analysis.

Clinical response to treatment was evaluated by the estimation of disease activity using the DAS28-ESR score. For the DAS28-ESR, cutoff points for high disease activity, moderate disease activity, low disease activity, and remission were 5.1, 3.2, 2.6, and <2.6, respectively.[12] DAS28 ESR scores were analyzed at baseline and every visit.

Approval of Ethics Committee of Army Hospital (Research and Referral), Delhi (No 20/2014 dated 18 Jun 2014) was taken and written informed consent for participation in the study was obtained from all enrolled subjects.

Statistical analysis

Baseline characteristics of study participants were described as mean (SD) for continuous measures that were normally distributed and median (interquartile range) for measures that were not normally distributed. Spearman correlation coefficient was used to test the correlation between IL-6 values and clinical variables. Paired *t*-test and Wilcoxon signed-rank tests were used to analyze the change between baseline and post-treatment levels of the different variables. Fisher's exact test was used to analyze the proportion of patients attaining remission of disease activity in different subgroups and Mann–Whitney U test was used for nonparametric comparison. All analyses were performed in SPSS version 23 (IBM Corp., Armonk, NY, USA) and a two-sided *P* value of <.05 was used to indicate statistical significance for all tests.

Results

Totally, 36 patients were enrolled and 34 patients completed the study. Treatment was withdrawn in two patients due to a rise in serum transaminases (more than five times the ULN). The demographic profile with baseline and follow-up clinical data is summarized in Table 1. There were 22 female patients and 12 male patients with the female: male ratio of 1.8:1.

At the end of the study, 24 (70%) patients attained DAS28 remission and 5 (14.7%) patients had moderate and low disease activity each.

The results of correlation analyses between IL-6 and clinical parameters have been summarized in Table 2. Baseline serum IL-6 did not correlate with the baseline DAS28 ESR, ESR, and CRP. Similarly, baseline serum IL-6 or change in serum IL-6 (Δ IL-6) level after 06 doses of tocilizumab did not correlate with post-treatment DAS28 ESR (Figures 1 and 2).

Eleven (32.3%) patients had a decrease in serum IL-6 level from their baseline value while 22 (64.6%) patients experienced a rise in IL-6 levels at the end of study (Figure 3). Ten (90.9%) of the 11 patients who experienced reduced IL-6 level attained DAS28 remission (<2.6) whereas, 17 (73.9%) patients out of 23 who did not experience a reduction in IL-6 level

Table 1. Baseline and follow up clinical parameters and IL-6 levels in study patients.

	Baseline		At 24 weeks			
	Mean (SD)		Mean (SD)		95% CI	*P*
Age (years)	42.2	(12.54)	-			
Disease duration (years)	7.88	(4.13)	-			
SJC	5.2	(3.2)	0.4	(0.9)	3.7 to 6.0	.0001
TJC	6.6	(3.7)	0.7	(1.4)	4.5 to 7.2	.0001
VAS	8.2	(0.9)	0.7	(1.5)	6.8 to 7.9	.0001
ESR (mm)	65.1	(25.1)	20.7	(16.9)	36.5 to 52.2	.0001
CRP (mg/dl)	31.3	(18.6)	4.6	(2.9)	20.2 to 33.3	.0001
DAS28 ESR	5.0	(0.5)	2.3	(1.0)	2.3 to 3.1	.0001
	Median (IQR)		Median (IQR)			
IL6 (pg/mL)	40.5	(130.2)	72.6	(162.5)		.034

CRP, C-reactive protein; DAS28 ESR, disease activity score-28 joints erythrocyte sedimentation rate; IL, interleukin; SJC, swollen joints count; TJC, tender joint count; VAS, visual analogue scale.

Table 2. Correlations between serum IL-6 and various primary and secondary outcomes.

	R	*P*
Baseline IL-6 and baseline DAS28 ESR	0.146	.411
Baseline IL-6 and baseline CRP	0.199	.260
Baseline IL-6 and baseline ESR	0.16	.365
Post-treatment IL-6 and post-treatment DAS28 ESR	-0.17	.337
Baseline IL-6 and post-treatment DAS28 ESR	-0.091	.337
Change in IL-6 and post-treatment DAS28 ESR	0.263	.132

CRP, C-reactive protein; DAS28 ESR, disease activity score-28 joints erythrocyte sedimentation rate; IL, interleukin

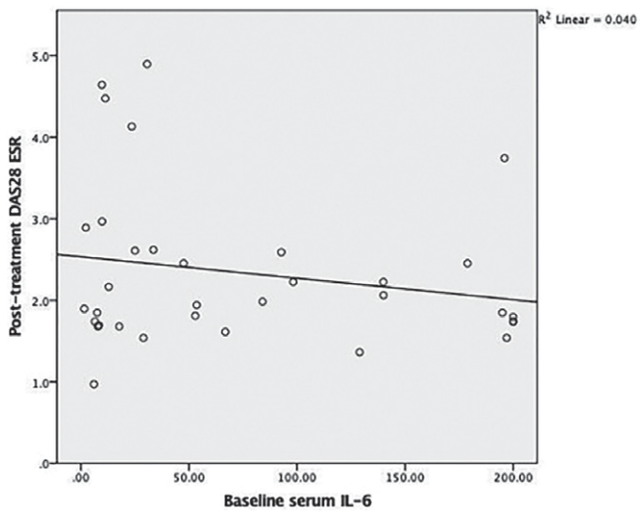

Figure 1. Scatter plot correlating baseline IL-6 with post-treatment DAS28 ESR (IL-6: interleukin 6; DAS28 ESR: disease activity score-28 joints erythrocyte sedimentation rate).

attained DAS28 remission. However, it was statistically not significant (*P*-value .384).

In a further subset analysis, we divided the subjects into high and low IL-6 groups with cutoff at the median level of serum IL-6 (40.1 pg/mL). Eleven (64.7%) out of 17 subjects with high baseline IL-6 above the median attained remission while 16 (94.1%) of the 17 subjects with low IL-6 values attained remission (*P*-value .085).

Discussion

Tocilizumab is one of the standard of care bDMARD for treatment of RA due to its proven safety and efficacy.[13] With 70% of the subjects achieving DAS 28 remission in this study, tocilizumab matches the efficacy reported in other studies worldwide which have reported the DAS28 remission in 30 to 87.9% patients.[14,15] Serum IL-6 levels have been found to correlate with acute phase reactants and disease activity in RA patients in previous studies.[8,9] However, in our study, baseline serum IL-6 did not correlate with baseline acute phase reactants (ESR and CRP). This finding will require to be validated by another study with a larger sample size before drawing any conclusion or extrapolating it to another set of population.

IL-6 as a predictor of response to tocilizumab in RA

There are a few studies to have assessed the potential of serum IL-6 as a predictor of response to tocilizumab therapy in patients with RA. In one such study by Shimamoto

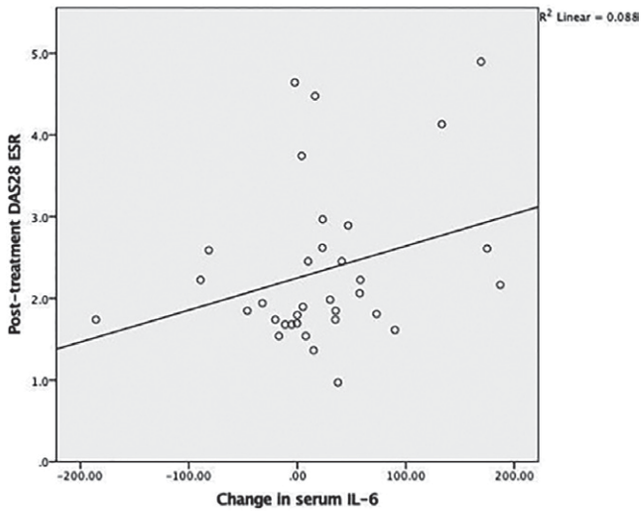

Figure 2. Scatter plot correlating change in IL-6 at 24 weeks with DAS28 ESR at 24 weeks (IL-6: interleukin 6; DAS28 ESR: disease activity score-28 joints erythrocyte.

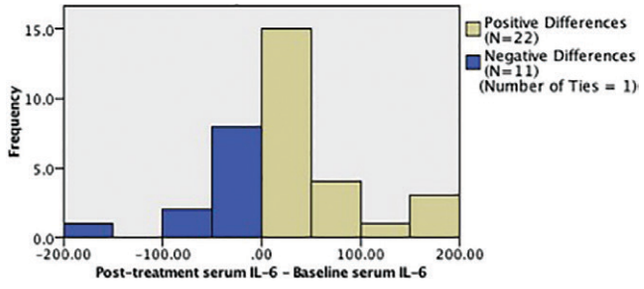

Figure 3. Change in interleukin IL-6 levels (pg/mL) from baseline to post-treatment level (measured by subtracting baseline level from the post-treatment level). In 22 subjects, an increase in serum IL-6 was observed and 11 patients revealed decrease. Remaining one patient had serum IL-6 level beyond the maximum assay range at baseline as well as in post-treatment phase.

et al[16] patients were analyzed for various serum cytokines levels as markers of disease activity and their correlation with response to tocilizumab and infliximab (IFX). In this study, 32 patients with mean DAS28 ESR of 4.69 ± 1.24 were treated with tocilizumab, and DAS28 ESR after 4 weeks was correlated with pretreatment serum IL-6 level. The pretreatment serum IL-6 levels correlated positively with post-treatment DAS28ESR score (*R* 0.427, *P* .026). In the same study, subjects were divided into high and low IL-6 groups, with the mean value of the serum IL-6 as the cutoff, and response to treatment with tocilizumab and infliximab was compared in both the groups separately. In lower IL-6 group, subjects treated with tocilizumab had lower disease activity (DAS28 ESR 2.39 ± 1.2) as compared to those treated with infliximab (DAS28 ESR 3.88 ± 1.37, *P* .02) at the end of 04 weeks. Contrary to this, in the high IL-6 group, there was no difference in disease activity of the subjects treated with tocilizumab (3.78 ± 0.74) in comparison with those treated with infliximab (DAS28 ESR 3.85 ± 1.27, *P* = .862). Based upon these findings, the author concluded that serum IL-6 level in patients with RA, particularly at low serum IL-6 levels, may potentially predict the response to tocilizumab treatment and higher serum IL-6 levels may signify resistance to tocilizumab.

In our study, there was no significant correlation observed between baseline IL-6 levels and post-treatment DAS28 ESR. However, a higher number of patients in low serum IL-6 level (16 out of 17) as compared to high serum IL-6 level (11 out of 17) achieved low disease activity, although it was statistically not significant (*P* .085). Further analysis in the study by Shimamoto et al[16] revealed that good responders to tocilizumab (defined by DAS28 ESR <3.2 after treatment) had significantly lower baseline IL-6 level (mean 20.37 ± 22.39, *n* 16) as compared to poor responders (mean 95.33 ± 128.7 pg/mL, *n* 16, *P* .01). Such subgroup analysis was untenable in our study due to the large difference in the number of patients in the subgroups as only 5 out of the 34 patients could be placed in the poor responder group.

In another study conducted by Wang et al[17] baseline serum IL-6 and change in serum IL-6 level following tocilizumab therapy at 12 weeks was not found to correlate with clinical response to tocilizumab as measured by DAS28 ESR at 24 weeks ($R < 0.3$). They conducted the same analysis using clinical disease activity index (CDAI) as the measure of disease activity, thus obviating the confounding effect of IL-6 on acute phase reactants and, resultantly, the DAS28 ESR and DAS28 CRP values, but no meaningful association was found.

Thus, despite the elevated baseline serum IL-6 in the majority of patients with RA, the evidence for its putative role as a biomarker for response to tocilizumab is presently divergent. This is possibly due to the heterogeneity of RA pathogenesis and the fact that the inflammatory response generated by IL-6 is resultant of the complex interplay among IL-6, IL-6 receptors, and other molecules down the signaling pathway.[18-20] This aspect is beyond the objectives of this study, and hence is not being discussed here. However, from the available pieces of evidence, it also appears that the lower baseline serum IL-6 level in seropositive RA patient may predict response to tocilizumab if this observation is substantiated further in larger studies. The numerically better response in our patients with low serum IL-6 levels might have not reached statistical significance due to the small sample size.

Effect of tocilizumab therapy on serum IL-6 level

In the study by Shimamoto et al[16] the mean serum IL-6 level of the RA patients raised from the mean baseline of 50.7 ± 95.1 pg/mL to 111 ± 122 pg/mL. Similarly, Wang et al[17] also observed the rise in serum IL-6 level following tocilizumab therapy from the baseline value of 40.1 ± 59.5 pg/mL. In our study also, the serum IL-6 level was found to increase following tocilizumab therapy. The plausible explanation for the rise in IL-6 level is the hypothesis propounded by Nishimoto et al[21] that metabolism of IL-6 is dependent on the availability of IL-6R and when IL-6R is consumed by tocilizumab treatment, the free IL-6 accumulates in circulation. However, an increase in serum IL-6 is not universal and many individuals experience a net decline in serum IL-6 level as seen in the DREAM study.[22] In our study also, 11 patients experienced a decline in serum IL-6 level following tocilizumab therapy. The clinical implication of this finding is yet to be known as there was no statistically significant difference in response to tocilizumab in patients who experienced an increase or decrease in serum IL-6 level at the end of the study.

There is insufficient evidence at present to suggest the role of serum IL-6 level as a biomarker to predict the response to tocilizumab therapy in patients with RA; however, patients with low baseline serum IL-6 level tend to respond better and further studies are required to consolidate the evidence. In DREAM study, it was found that patients who experienced a net decline in serum IL-6 levels had a longer duration of drug-free remission than the other subgroup of patients.[22] The follow-up data of our patients were not available to corroborate this finding and this leaves a prospect for future research for finding a clinically useful predictor of drug-free remission and a possible guideline for tapering the biological DMARD therapy. However, it has also been observed in our study that 10 out of 11 (90.9%) patients, who experienced a post-treatment decline in serum IL-6, attained DAS28 remission as compared to only 17 of the 23 (73.9%) other patients. Once again, the difference is not statistically significant, but it may draw the attention of the researchers toward a possible target in the quest for the biomarkers for clinical response to biological DMARDs.

The small sample size of our study limits the extrapolation of its findings to any different subset of the population. Nevertheless, this study is one of the few studies conducted so far exploring the role of serum IL-6 level as a biomarker of response to tocilizumab therapy in patients of seropositive RA. In the background of equivocal reports in previous studies, our findings do open up avenues for further research in the possible role of low baseline serum IL-6 level as a predictor of a favorable response to tocilizumab in seropositive RA. The inclusion of healthy controls to assess the normal level of serum IL-6 level in healthy population would have further added to the information on characteristics of serum IL-6.

Neither baseline serum IL-6 levels nor the post-treatment change in serum IL-6 levels correlated with clinical response to tocilizumab. However, more individuals with low baseline serum IL-6 level attained DAS28 remission, although it did not reach statistical significance. Hence, further research is required to explore the role of serum IL-6 for clinical response to tocilizumab in seropositive RA.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Army Hospital (Research and Referral), Delhi (Approval Date: June 18, 2014; Approval Number: 20/2014).

Informed Consent: Written and verbal consent was obtained from the individuals who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.K.; Design - K.S.; Supervision - D.S.B.; Materials - V.V., A.M.N.; Data Collection and/or Processing - A.M.N.; Analysis and/or Interpretation - K. Singh; Literature Review - A.K.; Writing - A.K.; Critical Review - A.K., A.H., K. Singh

Conflict of Interest: The authors have no conflict of interest to declare.

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