CHANGES IN DISEASE ACTIVITY AND SERUM CYTOKINE (IL-1B, IL-6, IL-10, TNF-A) CONCENTRATIONS IN RHEUMATOID ARTHRITIS PATIENTS FOLLOWING IL-6 INHIBITOR TREATMENT

Phung Anh Duc[1], Nguyen Dinh Khoa[1], Doan Van De[2]

SUMMARY

Objectives*: To evaluate changes in disease activity and serum cytokine including tumor necrosis factor alpha (TNF-α) and interleukins (IL-1β, IL-6 and IL-10) concentrations in rheumatoid arthritis (RA) patients following IL-6 inhibitor treatment.*

Subjects and methods: *This was a pre-treatment observational and comparison study on 46 patients who fulfilled the classification criteria of American College of Rheumatology (ACR) (1987). Patients who did not meet their treatment target with methotrexate (MTX) and those who also had severe prognostic factors were indicated for biological treatment with Tocilizumab (TCZ), dose 4 - 8 mg/kg/month in combination with MTX. All these patients were assessed for disease activity and serum cytokines (IL-1β, IL-6, IL-10 and TNF-α) concentration every 4, 8, 12, and 24 weeks after treatment.*

Results: *46 patients including 43 females and 3 males with average age of 51.54 ± 10.68 were enrolled in this study. The indicators used to evaluate disease activity including number of tender joints (TJ28), number of swollen joints (SJ28), erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP) concentration, DAS28-ESR and DAS28-CRP all decreased right after the first follow-up visit. There was a statistically significant difference in parameters between pre-treatment and re-examination ($p < 0.05$). After 12 weeks, the percentage of patients who achieved treatment target (with remission or low activity) was 36% according to DAS28-ESR and 42% according to DAS28-CRP. After 24 weeks, these ratios were 33% and 36%. The mean concentrations (pg/mL) at the time of pre-treatment of IL-1β, IL-6, IL10 and TNF-α were 21.90 ± 41.43; 41.51 ± 45.37; 21.04 ± 47.24 and 18.99 ± 31.69. After 12 weeks of treatment, these concentrations were 6.04 ± 9.61; 23.05 ± 21.62; 5.17 ± 9.07; 27.15 ± 77.61. The IL-1β, IL-6 and IL10 was decreased statistically significantly after 12 weeks ($p < 0.05$; Wilcoxon Signed - Rank).* ***Conclusion****: TCZ has decreased the disease activity immediately after 4 weeks and continues to be effective in the next 12 - 24 weeks of treatment. Serum IL-1β, IL-6 and IL10 concentration reduced statistically significantly after 12 weeks of treatment. Serum TNF-α concentration was not decreased after 12 weeks of treatment.*

**Keywords: Rheumatoid arthritis; Interleukin; Tumor necrosis factor alpha; TNF-α; DAS28; Methotrexate; Tocilizumab.*

[1]*Department of Rheumatology, Cho Ray Hospital*

[2]*Military Hospital 103,* ***Vietnam Military Medical University***

Corresponding author: Phung Anh Duc (anhducbs03@gmail.com)

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INTRODUCTION

Rheumatoid arthritis (RA) is a typical autoimmune disease characterized by chronic inflammation of the synovial membrane that destroys the joint resulting in adhesion and deformity of the joint leading to disability. The disease can be found in every country in the world with the prevalence of 0.3 - 1.1% and a female/male ratio of 2.5 - 3/1 [10].

The cause of the disease is unknown. However, autoimmune disorders, infections and genetics were involved in the the the progenesis of disease. B cells, T cells and cytokines are thought to play important roles in the RA pathogenesis [10]. In RA patients, there is a predominance of pro-inflammatory cytokine over anti-inflammatory cytokine. IL-1, IL-6 and TNF-α are inflammatory cytokines that have crucial role in the pathophysiology of RA. The main action of these cytokines in patients with RA includes stimulation of production of other cytokines, inflammatory cell chemotaxis, stimulation of the production of matrix metalloproteinases (MMPs), cells adhesion and osteoclasts and involvement in angiogenesis [6]. In contrast, IL-10 is a classical anti-inflammatory cytokine thought to play a role in the RA pathogenesis. The main action of IL-10 in RA is to inhibit inflammatory cytokines production and other activities on the immune to downregulate the inflammatory [6, 10].

The basic treatment of RA with disease modifying antirheumatic drugs (DMARDs) with anti-rheumatic drugs has shown some efficacy. However, many patients do not achieve targeted therapeutic efficacy with DMARDs and the introduction of biologic agents is seen as revolutionary in the treatment of RA [10]. Tocilizumab (TCZ), an IL-6 receptor monoclonal antibody, has been shown to be highly effective and safe in studies worldwide. However, studies have not yet elucidated all the biological effects of TCZ on patients with RA [6]. In Vietnam, TCZ has been used effectively in many hospitals. However, the studies on this drug are purely to evaluate its effectiveness and safety but no mention the effect of this drug on the alteration of cytokine concentrations [2, 3].

Therefore, our study was carried out: *To assess the changes in disease activity and serum concentration of IL-1β, IL-6, IL-10 and TNF-α in RA patients treated with TCZ*.

SUBJECTS AND METHODS

1. Subjects

Patients over 18 years old who fulfilled the classification criteria of ACR 1987 [4] were indicated for treatment of TCZ as recommended by the Vietnam Association of Rheumatology [1] and ACR [9] (patients do not meet treatment target with MTX or patients have a severe prognostic factor).

The study was conducted at Department of Rheumatology, Cho Ray Hospital from June 2015 to October 2019.

2. Methods

This is a study of observation with comparison pre- and post-treament.

All patients were treated with TCZ (Actemra); at a dose of 4 - 8 mg/kg, once every 4 weeks with or without MTX based

on standard treatment regimen [1, 9]. Patients were evaluated immediately before treatment (T0) and 4 weeks (T1), 8 weeks (T2), 12 weeks (T3) and 24 weeks (T6) after the first dose of TCZ infusion. The assessment includes clinical and laboratory changes namely: the number of tender and swollen joints in 28 peripheral joints (TJ28 and SJ28); patient global assessment on the VAS scale; (ESR); plasma CRP concentration; DAS28-ESR and DAS28-CRP and quantification of serum IL-1β, IL-6, IL10 and TNF-α concentration. All tests were performed at Cho Ray Hospital. The quantification of serum cytokine concentration was performed by biochips simultaneous quantitative method based on sandwich chemiluminescent immunoassay principle (Randox system).

* *Data processing:* Using SPSS 20.0 software

RESULTS

1. Patients characteristics

Table 1: The number of patients at follow-up visits.

Follow-up visits	T0	T1	T2	T3	T6
Number of patients	46	46	43	40	14
Female/male ratio	43/3	43/3	40/3	37/3	13/1

The number of patients in the study decreased gradually from the second follow-up visit (after 8 weeks of TCZ treatment). All patients who did not continue follow-up were due to voluntary discontinuation of therapy but not due to serious side effects of the drug.

Table 2: The characteristics of pre-treatment patients.

The characteristics of pre-treatment patients	n	$\overline{X}$ ± SD	Median [quartile]
Age (years)	46	51.54 ± 10.68	52.50 [42.75 - 60.25]
Duration of disease (years)	46	6.22 ± 6.07	36.00 [12.00 - 99.00]
Methylprednisolone dose (mg/day)	36	7.44 ± 4.05	8.00 [4.00 - 8.00]
Duration of corticosteroid treatment (months)	36	31.56 ± 44.21	12.00 [6.00 - 34.50]
MTX dose (mg/week)	45	14.52 ± 3.51	12.50 [12.50 - 17.50]
Duration of MTX treatment (months)	45	24.84 ± 35.36	12.00 [5.00 - 27.00]
TJ28	46	11.50 ± 7.40	9.50 [5.00 - 17.00]
SJ28	46	4.04 ± 3.29	3.00 [2.00 - 5.25]
ESR (mm)	46	52.48 ± 36.38	40.00 [23.75 - 91.50]
DAS28-ESR (points)	46	5.55 ± 1.09	5.70 [4.41 - 6.30]
CRP (mg/L)	46	45.10 ± 59.80	30.55 [7.60 - 71.07]
DAS28-CRP (points)	46	5.10 ± 0.99	5.13 [4.32 - 5.96]

The average duration of patients treated with TCZ was quite high (6.22 ± 6.07 years). Most of the patients were treated with MTX (45/46 patients) with average dose of 14.52 ± 3.51 mg/week. The average duration of MTX treatment was quite long (24.84 ± 35.36 months). All disease activity index demonstrated the severity of disease, of which, mean disease activity was high (5.55 ± 1.09 for DAS28-ESR and 5.10 ± 0.99 for DAS28-CRP).

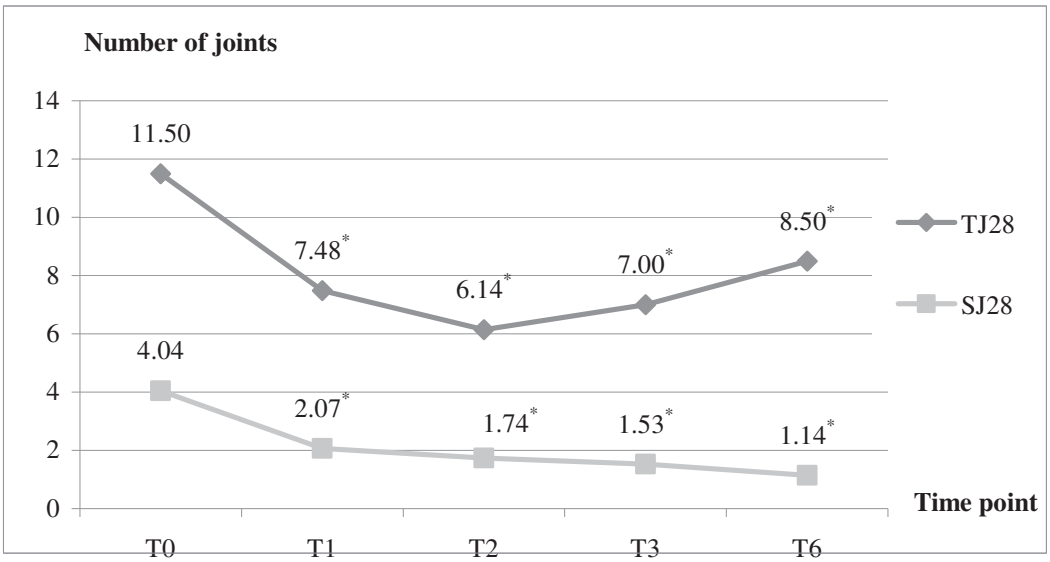

Chart 1: Changes of TJ28 and SJ28 of RA patients following TCZ treatment.
**p < 0.05; paired T-test*

The results (*chart 1*) showed that both TJ28 and SJ28 (mean values) were decreased right after TCZ treatment. At all times of follow-up visit, TJ28 and SJ28 were statistically significantly decreased compared to all time-points before TCZ treatment (p < 0.05). Contrary to SJ28, TJ28 tended to be not decreased continuously after 8 weeks of TCZ treatment.

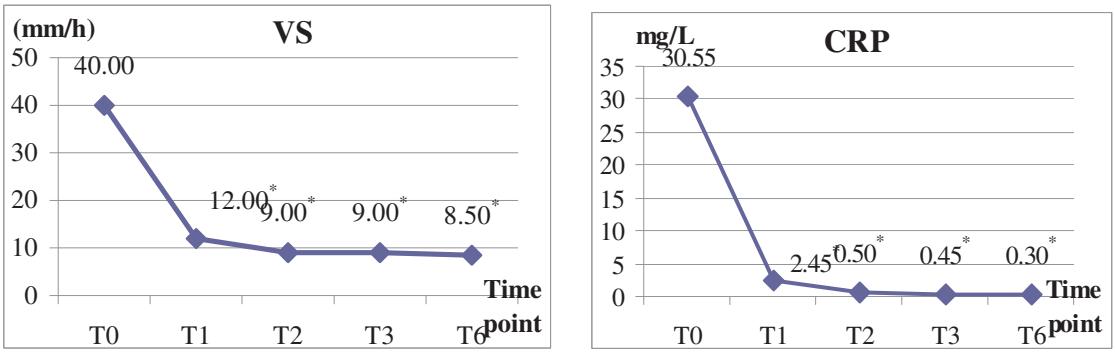

Chart 2: Changes in ESR and CRP of RA patients following TCZ treatment.
**p < 0.05; Wilcoxon Signed - Rank Test*

It can be seen from chart 2, ESR and CRP (median values) decreased right after TCZ treatment. At all times of follow-up visit, ESR and CRP were statistically significant decreased compared to all time-points before TCZ treatment (p < 0.05)

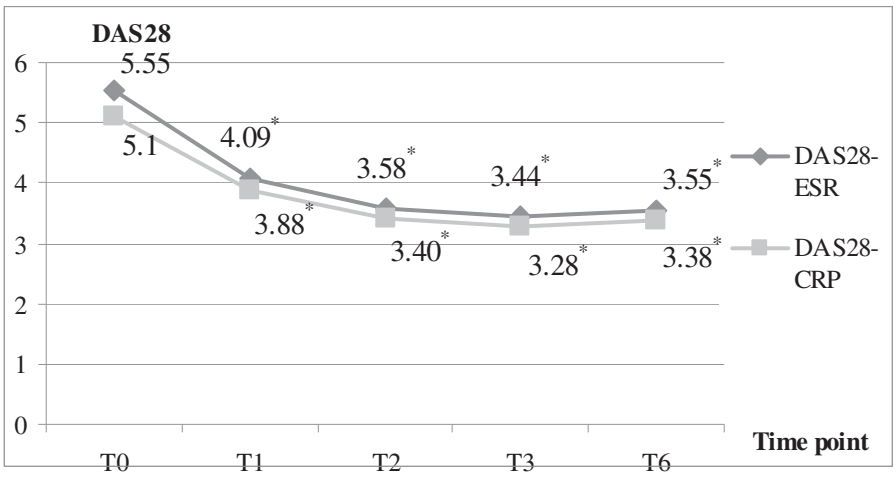

Chart 3: Changes in DAS28-ESR and DAS28-CRP of RA patients following TCZ treatment.

**p < 0.05; paired T-test*

The results (*chart 3*) showed that DAS28-ESR and DAS28-CRP (mean values) were decreased right after TCZ treatment. However, after 12 weeks, this did not occur. At all times of follow-up visit, DAS28-ESR and DAS28-CRP were statistically significantly decreased compared to all time-points before TCZ treatment (p < 0.05).

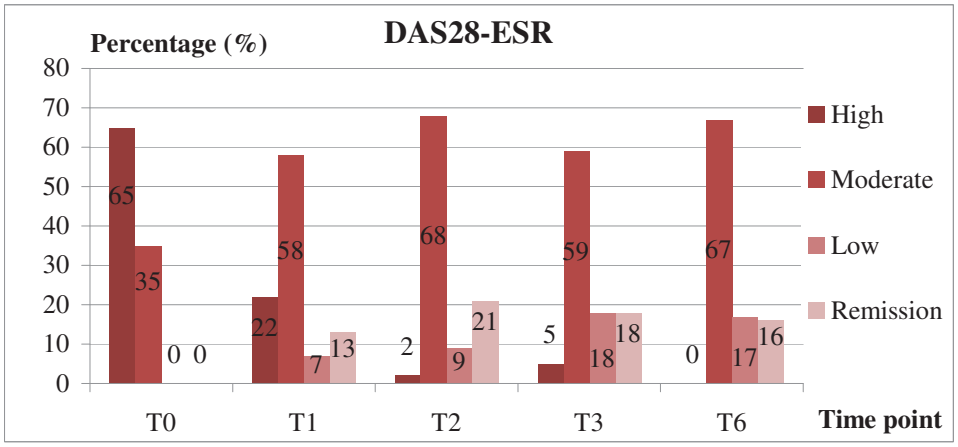

Chart 4: Changes in disease activity classification according to DAS28-ESR following TCZ treatment.

The results (*chart 4*) showed that disease activity was decreased at each follow-up visit. By the 6th follow-up visit, there were no longer RA patients with high disease activity. However, after 12 weeks, this downtrend did not occur. After 12 weeks, the percentage of patients who achieved the treatment target according to DAS28-ESR (low disease activity or remission) was 36% and 33% after 24 weeks.

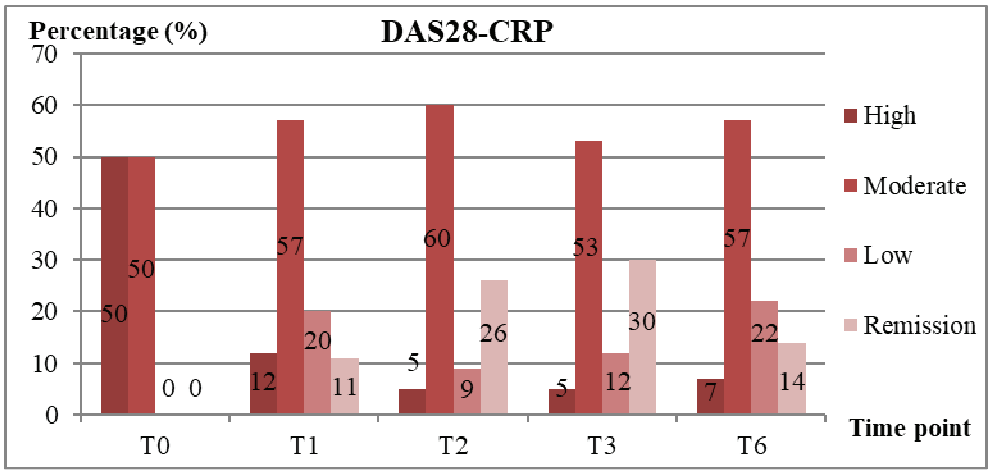

Chart 5: Changes in disease activity classification according to DAS28-CRP following TCZ treatment

The results (*chart 5*) showed that disease activity decreased at each follow-up visit. However, after 12 weeks, this downtrend did not occur. After 12 weeks, the percentage of patients who achieved the treatment target according to DAS28-CRP (low disease activity or remission) was 42% and after 24 weeks it was 36%.

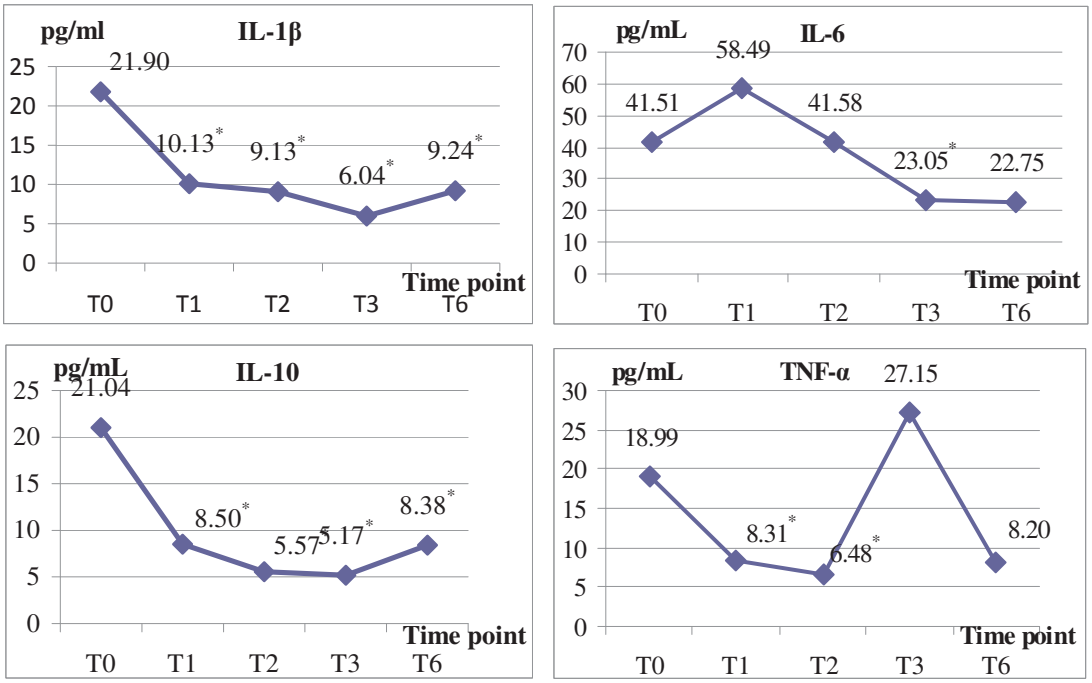

Chart 6: Changes of serum cytokine concentrations in RA patients following TCZ treatment

**p < 0.05; Wilcoxon Signed - Rank Test*

The results (*chart 6*) showed the changes of serum cytokine concentrations (mean values) of RA patients during treatment with TCZ. IL-1β and IL-10 concentration were decreased right after TCZ treatment. IL-6 concentration tended to be increased after 4 weeks and be decreased after 8 weeks of treatment. After 12 weeks, IL-1β, IL-6 and IL-10 concentration decreased significantly compared to all time-points before treatment ($p < 0.05$). In contrast, TNF-α concentration was decreased right after treatment but increased after 8 weeks of treatment. After 12 weeks, the changes of TNF-α concentration was not significant compared to all time-points before treatment.

DISCUSSION

Tocilizumab has been proven effective and safe through many studies in the world [6] as well as in Vietnam [2, 3]. Similar to these results, our research has shown a good result of efficacy. All indicators of disease activity including TJ28, SJ28, ESR, CRP, DAS28-ESR and DAS28-CRP were decreased significantly from the first visit and continued to be decreased at the next visits during TCZ treatment. At 24 weeks of treatment (T6), performance indicators did not continue to be declined compared to 12 weeks of treatment (T3). Thus, this study shows that the effect of TCZ was achieved at the first follow-up visit (4 weeks after treatment) and seemed to be maximized after 12 weeks of treatment. While the majority of studies around the world showed that TCZ reduces disease activity after each follow-up visit lasting up to 24 or 52 weeks [6]. Then, our results are closer to the findings by Yazici (2013, the ROSE study) [11]. This author's study showed that TCZ was effective from the first week and continued to be effective for the following weeks. But after 16 weeks, the effect of TCZ no longer continued to be improved. However, because the number of patients by the time of 24 weeks treatment was small (14 patients), we may have further studies with larger sample sizes and longer follow-up to get the more appropriate assessment.

Due to the small number of patients at 24 weeks of treatment, we mainly evaluated changes of cytokine concentrations after 12 weeks of treatment. As the result, IL-1β and IL-10 concentration were decreased right after TCZ treatment meanwile IL-6 concentration tended to be increased after 4 weeks and be decreased after 8 weeks of treatment. After 12 weeks, these cytokine concentrations decreased significantly. The decrease of IL-1β and IL-10 reflect the impact of TCZ on the immune system. TCZ inhibits the binding of IL-6 to receptors leading to inhibition of the effects of this multifunctional cytokine and reduction in inflammatory responses and production of pro- and anti-inflamatory cytokines. Regarding the increase in IL-6 concentration after 4 weeks of TCZ treatment, our results were similar to Nishimoto's [7] and Shimamoto's findings [8]. Nishimoto concluded that IL-6 concentration was increased after 14 days of treatment and remained constant at high concentration after 42 days of TCZ

treatment. Meanwhile Shimamoto confirmed that the IL-6 concentration was increased after 4 weeks of treatment. Nishimoto also proposed a "bathtub model" based on action mechanism of TCZ to explain this change of IL-6 concentration. Accordingly, the author argues that the serum IL-6 concentration is determined by the production of new IL-6 in combination with the elimination of circulating IL-6. There are two eliminations of circulating IL-6: one is the elemination of IL-6 that bound its receptor (IL-6R) and the other is the free IL-6 degradation. When it is assumed that IL-6 production is constant, the serum IL-6 concentration will be increased due to inhibition of the excretion of IL-6 in combination with IL-6R. Similar finding among these researches was that they only assessed IL-6 concentration after a short period of 42 and 28 days of treatment. Our results showed that IL-6 concentration increased after 28 days of treatment and were almost similar to these results. However, to explain for the results that the IL-6 was decreased after 8 weeks of treatment and continued to be decreased for up to 12 - 24 weeks, we may rely on the immune response effect of inhibition of IL-6. After a period of treatment, TCZ results in inhibiting the production of cytokines including IL-6. The results of the IL-6 reduction from the 8th week of treatment in our study were similar to Kashama's finding [5]. This author concluded that the IL-6 concentration decreased after 3 months of TCZ treatment. Thus, the assessment of the changes in IL-6 concentration after TCZ treatment should be observed according to the duration of treatment.

Our result showed that TNF-α concentration were decreased right after TCZ treatment similar to IL-1β and IL-10. However, this concentration was not decreased after 12 week of treatment. This change are required to be further assessment in next studies.

CONCLUSION

Tocilizumab effectively decreased disease activity in RA patient right after 4 weeks of treatment and continued to be effective for the next 12 - 24 weeks. IL-1β, IL-6 and IL-10 concentration decreased significantly after 12 weeks of TCZ treatment. In contrast, TNF-α concentration was not decreased after 12 weeks of TCZ treatment.

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