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Original Article

Evaluation of relationship between cytokine and chemokine levels measured by using multiplex laboratory method before and after treatment and clinical course and treatment response in rheumathoid arthritis patients receiving TNF-α blocker therapy

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Abstract

Aim: The laboratory findings are non-specific in Rheumatoid arthritis (RA). We have based our study on this idea and chose cytokines and chemokine to evaluate ; we then tried to establish a relationship between the progress of the disease and the levels of these molecules before and 3-6 months after the TNF-α blocker treatment.

Material and Methods: A total of 34 RA patients (28 females, 6 males) who were diagnosed with RA and received TNF-α blocker therapy were included in the study.Blood samples were drawn from the patients before the TNF-α treatment began and then in the following 3rd and 6th months of the treatment.

Results: The mean age of the study group is 49.15±11.03. the mean disease period is 9.77±6.29 years and the mean treatment period is 6.88±4.33 years. 84.6% (n=22) of the study group is female and 15.4% (n=4) is male.Patients with good treatment response to TNF-α blocker treatment were younger than the patients with medium treatment response. The increase of mean hemoglobin (Hgb) levels between 0 and 6 months were statistically meaningful (both $p<0.05$).

Conclusion: In RA patients receiving TNF-α blocker therapy. cytokine levels decreased with treatment.The relationship between IL-6 and DAS28 change in the pre-treatment and 6-month period may help the clinician to show the disease activity and to guide the course of the disease.

Keywords: Rheumatoid Arthritis; TNF-α blocker treatment; cytokines; DAS28; disease activity.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic, autoimmune disease that primarily affects the synovial joints and causes progressif destruction around the joints (1-5). The diagnosis of RA can be made based on the 1987 ACR and 2010 ACR/EULAR diagnostic criteria (6,7). 1% of the world's population is diagnosed with RA, and thus it is the most common inflammatory arthritis. RA is 2-3 times more common in females than in males, and it is most frequent in 4th-5th decades.The etiologyof the disease is unknown but there are several genetic and environmental risc factors that have been speculated (8-14). The pathophysiological chain of events starts with predominantly mononuclear cell clusters around the synovium, this is followed by the secretion of certain cytokines and chemokines from several cell types. The disturbance of the balance between the pro and anti-inflammatory cytokines plays an important role in the typical pathological progress of RA. Abnormal production of certain proinflammatory cytokines (IL-6, IFN-γ, IL-12, IL-13, IL-15, IL-17, GM-CSF) and proinflammatory chemokines (MCP-1,IL-8 ve ENA-78) takes part in the inflammatory process and among all these factors macrophage originated IL-1 and TNF-α are known to play

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the most crucial role(15,16). The laboratory findings are non-specific in RA, and they are rather used to support the diagnosis made by the clinical symptoms and findings and to assess the progress of the disease. RF, anti-CCP antibodies and serum CRP and sedimentation rates can be useful in confirming the diagnosis and assessing the progress of the disease, never the less several studies have shown that most of the laboratory findings are not sensitive or specific enough in determining the diagnosis, progress or the prognosis of the disease. Therefore different molecules that can provideuseful information about the diagnosis, the progress, and the prognoses of the disease have been sought(8,9). We have based our study on this idea and chose 9 cytokines (IL-1β, IL-1ra, IL-6, IL-10, IL-12, IL-13, TNF-α, VEGF, IFN-γ) and 1 chemokine (MCP-1) to evaluate using a multiplex laboratory technique, we then tried to establish a relationship between the progress of the disease and the levels of these molecules before and 3-6 months after the TNF-α blocker treatment.

MATERIAL and METHODS

The study has been held by the Marmara University, Medical School, Internal Medicine department, in Rheumatology department policlinics, between the dates June 2009 and December 2010. It is a prospective study. A total of 34 RA patients (28 female and 6 male), who have been diagnosed with RA between the dates June 2009 - December 2010 in the Marmara University Medical School Hospital Rheumatology policlinics, based on the criteria published in 1987 by the American College of Rheumatology (ACR). The patients have been indicated to take TNF-α blocker treatment and 8 of the patients have not been able to complete the study due to several reasons, and thus a total of 26 patients (22 female, 4 male) have been able to complete the study. As the TNF-α blocker treatment 3 patients were given infliximab (remicade), 9 patients were given enbrel (etanercept) and 14 patients were given adalimumab (humira). Blood samples were drawn from the patients before the TNF-α treatment began and then in the following 3rd and 6th months of the treatment. Ten cc venouse blood samples were centrifuged on the same day for 10 minutes at 1500 rpm, in room temperature and then they were stored at -20°C temperature. 3 serum samples were stored for each patient and from these samples the levels of 9 cytokines (IL-1β, IL-1ra, IL-6, IL-10, IL-12p40, IL-13, TNF-α, VEGF ve IFN-γ) and 1 chemokine (MCP-1) were measured using the multiplex/luminex technology. The multiplex/luminex technology requires a multiplex IS 200 device and Panomics-Multiplex kits and it enables us to measure the levels of several cytokines at the same time from a small amount of blood sample. The results were expressed in pg/ml units. At the beginning of the study and through the 6 month follow-up control evaluations were made in proper intervals. The control evaluation process included the detailed history and physical examination of each patient. The patients were tested for hepatitis serology (HbsAg, AntiHbs, AntiHCV), AntiHIV, RF, anti CCP. Their PA lung graphies were taken and they were tested with PPD and/or with quantiferon if necessary and Isoniaside (INH) treatment was given as an anti-tuberculosis prophilaxy to some patients.

RESULTS

Our study was initiated with 36 RA patients who were eligible for TNF-α blocker treatment indication at the beginning but 26 RA patients completed the study due to discontinuation of 8 patients. The patients were followed up between June 2009 and December 2010. The mean age of the study group is 49.15±11.03, the mean disease period is 9.77±6.29 years and the mean treatment period is 6.88±4.33 years. 84.6% (n=22) of the study group is female and 15.4% (n=4) is male. Patients with good treatment response to TNF-α blocker treatment were younger than the patients with medium treatment response. The treatment responses were evaluated based on the EULAR's clinical response criteria. During the comparison of average DAS28, ESR, CRP levels and Hb values of 26 patients in our study group measured before TNF-α blocker treatment (0th month) and in the 3rd and 6th months of treatment, statistically significant differences were determined (Table 1).

Table 1. Some laboratory and monitoring of the clinical response of study group before treatment (0th Month) and after treatment (3rd and 6th Months)

	0th Month (before treatment)	3^{td} Month	6th Month
DAS28	5.35±0.85 5.13 (4.74-5.58)	3.83±1.07* 3.71 (3.18-4.40)	3.35±1.27** 3.12 (2.24-4.50)
ESR (mm/h)	36.08±15.98 33.00 (24.00-45.75)	24.04±16.02* 21.00 (13.25-30.25)	24.96±20.02** 23.00 (10.75-30.50)
CRP (mg/dL)	21.19±28.75 12.45 (6.33-23.28)	14.10±31.87* 4.07 (2.91-9.11)	10.39±26.67** 3.35 (2.91-7.48)
Hb (g/dL)	12.09±1.20 12.20 (11.35-13.00)	12.49±1.32 12.60 (11.75-13.42)	12.78±1.23** 12.75 (12.25-13.37)

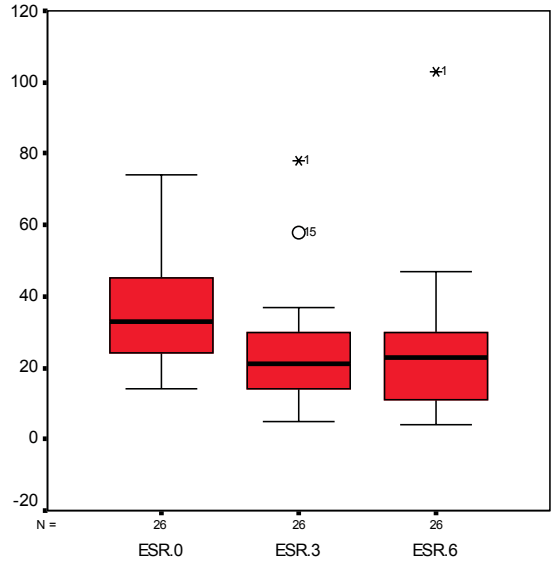
Measurement values were summarized as mean, standard deviation, median and 1st, and 3rd quartiles.
Wilcoxon sign test was used for statistical analysis of the repeated measurements.
* Data showing significance between 0-3 months measurements ($p<0.05$).
** Data showing significance between 0-6 months measurements ($p<0.05$).

Table 2. Demographic characteristics and some laboratory data of the groups with a good and moderate response to TNF-α blocker therapy in study group according to EULAR criteria for the clinical response before treatment

	Patients with a GOOD RESPONSE according to EULAR criteria for the clinical response (n=14)	Patients with a MODERATE RESPONSE according to EULAR criteria for the clinical response (n=12)
Age (mean, standard deviation, median, 1st, and 3rd Quartiles)	45.86±10.71 (29-60) 44.50 (34.75-57.25)	53.00±10.53 (30-69) 53.00 (46.50-59.75)
Gender (Female/Male) Female%	12/2 (85.7%)	10/2 (83.3%)
Disease duration (mean, standard deviation, median, 1st, and 3rd Quartiles)	8.00±3.30 (3-13) 8.00 (5.75-12.00)	11.83±8.27 (2-32) 11.50 (4.50-16.00)
DAS28 (mean, standard deviation, median, 1st, and 3rd Quartiles)	5.35±0.79 (4.50-7.59) 5.16 (4.86-5.58)	5.35±0.96 (4.37-7.73) 5.01 (4.72-5.80)
RF (positive/negative) positive%	10/4 (71.4%)	8/4 (66.7%)
AntiCCP (positive/negative) positive%	6/8 (42.9%)	6/6 (50.0%)

Additionally, significant decreases ($p<0.05$) were determined in average ESR and CRP levels measured before treatment compared to average ESR and CRP levels at 0-3 months and 3-6 months (Table 2, Figure 1, Figure 2). Also, average Hb values measured before treatment were increased with treatment and again a statistical significance ($p<0.05$) was determined in average Hb values between 0-6 months.

Even though our findings were not statistically meaningfull we did observe that the mean levels of IL-1β, IL-6, IL-12p40, IL-13, TNF-α, IFN-γ, VEGF and MCP-1 measure before the treatment were higher in patients with good response to the treatment , based on the EULAR clinical response criteria, compared to those with medium response. Another result we obtained in our study group was the comparison of initial average cytokine values of patients groups with good and intermediate response according to EULAR treatment response criteria; no statistically significant difference was determined due to reasons such as a small number of patients, variables not distributed normally or absence of our control group (Table 4). As it is seen in Table 4, although they do not reach statistical significance, it is observed that average



While the mean value of the group at 0th month ESR measurement was 36.08±15.98 [33.00 (24.00-45.75)], it was determined to be 24.04±16.02 [21.00 (13.25-30.25)] at 3rd month ESR measurement. While the mean value of the group at 3rd month ESR measurement was 24.04±16.02 [21.00 (13.25-30.25)], it was determined to be 24.96±20.02 [23.00 (10.75-30.50)] at 6th month ESR measurement. While the mean value of the group at 0th month ESR measurement was 36.08±15.98 [33.00 (24.00-45.75)], it was determined to be 24.96±20.02 [23.00 (10.75-30.50)] at 6th month ESR measurement. A statistically significant difference was determined between mean values of 0th and 3rd months measurements and 0th and 6th months measurements after using Wilcoxon sign test for the repeated measurements (p=0.000 and p=0.002).

Figure 1. Mean ESR (mm/h) changes in the study group measured before treatment (0th month) and after treatment (3rd and 6th Months).

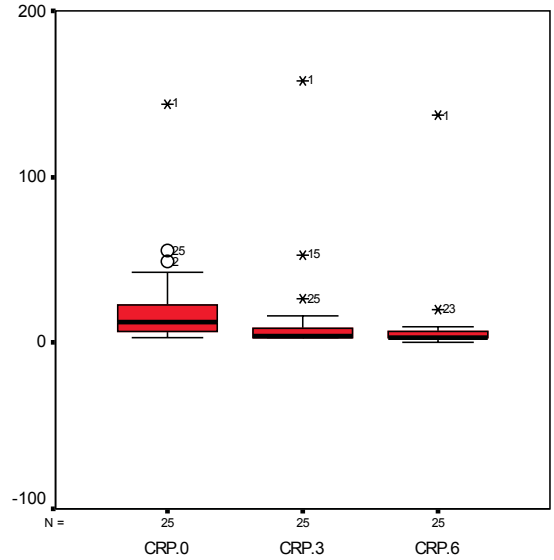


Figure 2. While the mean value of the group at 0th month CRP measurement was 21.19±28.75 [12.45 (6.33-23.28), it was determined to be 14.10±31.87 [4.07 (2.91-9.11)] at 3rd month CRP measurement. While the mean value of the group at 3rd month CRP measurement was 14.10±31.87 [4.07 (2.91-9.11)], it was determined to be 10.39±26.67 [3.35 (2.91-7.48)] at 6th month CRP measurement. While the mean value of the group at 0th month CRP measurement was 21.19±28.75 [12.45 (6.33-23.28)], it was determined to be 10.39±26.67 [3.35 (2.91-7.48)] at 6th month CRP measurement. A statistically significant difference was determined between mean values of 0th and 3rd months measurements and 0th and 6th months measurements after using Wilcoxon sign test for the repeated measurements (p=0.007 and p=0.001).

Figure 2. Mean CRP (mg/dL) changes in the study group measured before treatment (0th month) and after treatment (3rd and 6th Months).

Table 3. Mean cytokine (IL-1β, IL-1ra, IL-6, IL-10, IL-12, IL-13, TNF-α, VEGF, IFN-γ) values and mean chemokine (MCP-1) values of the study group measured by using multiplex laboratory method before treatment (0th month) and after treatment (3rd and 6th Months)

	0. Month	3. Month	6. Month
IL-1β (pg/mL)	39.66±153.73 4.96 (4.64-9.00)	7.31±4.79 5.58 (4.87-6.79)	6.65±3.70 5.15 (4.64-6.63)
IL-1ra (pg/mL)	85.74±285.62 2.40 (2.40-53.35)	34.73±64.83 2.40 (2.40-32.93)	21.70±60.78 2.40 (2.40-2.40)
IL-6 (pg/mL)	36.54±118.18 11.68 (10.83-13.89)	22.80±34.43 10.51 (10.51-11.84)	25.90±54.43 10.51 (10.08-11.79)
IL-10 (pg/mL)	5.01±5.36 3.27 (3.09-3.98)	3.10±0.56 3.27 (2.56-3.37)	**2.92±0.42**** **3.27 (2.56-3.27)**
IL-12p40 (pg/mL)	15.64±14.37 9.70 (5.55-24.54)	17.45±10.16 17.07 (10.73-24.45)	**13.62±10.58**** **12.93 (3.58-20.14)**
IL-13 (pg/mL)	20.11±8.03 18.45 (17.12-18.45)	17.72±1.40 17.12 (17.12-18.45)	17.79±1.59 17.12 (17.12-18.45)
VEGF (pg/mL)	97.28±70.00 68.62 (52.42-134.31)	83.79±61.01 67.47 (46.00-92.63)	80.98±82.80 61.02 (34.98-95.72)
TNF-α (pg/mL)	6.58±9.15 3.95 (3.12-4.97)	5.57±4.24 3.95 (3.12-6.19)	5.46±4.29 3.95 (3.12-5.28)
IFN-γ (pg/mL)	91.77±142.01 18.89 (17.17-86.79)	27.30±36.21 17.17 (17.17-18.98)	**25.28±31.80**** **17.17 (17.17-18.98)**
MCP-1 (pg/mL)	67.68±151.59 22.07 (13.14-65.21)	22.60±19.45 19.40 (11.62-24.65)	**21.37±20.13**** **14.40 (11.19-23.00)**

Measurement values were summarized as mean, standard deviation, median and 1st, and 3rd quartiles. Wilcoxon sign test was used for statistical analysis of the repeated measurements

*** Data showing significance between 0-3 months measurements (p<0.05)**

**** Data showing significance between 0-6 months measurements (p<0.05)**

Table 4. Mean cytokine (IL-1β, IL-1ra, IL-6, IL-10, IL-12, IL-13, TNF-α, VEGF, IFN-γ) values and mean chemokine (MCP-1) values in the study group measured before treatment (0th month) and in patient groups with a GOOD and MODERATE response according to EULAR criteria for the clinical response

Cytokines and Chemokines measured Before Treatment (0th Month)	Patients with a GOOD response according to EULAR criteria for the clinical response (mean, standard deviation, median, 1st, and 3rd Quartiles) (n=14)	Patients with a MODERATE response according to EULAR criteria for the clinical response (mean, standard deviation, median, 1st, and 3rd Quartiles) (n=12)
IL-1β (pg/mL)	66.62±209.10 4.69 (4.50-10.24)	8.21±5.77 5.15 (4.87-13.78)
IL-1ra (pg/mL)	33.82±64.61 2.40 (2.40-53.35)	146.32±415.97 2.40 (2.40-70.92)
IL-6 (pg/mL)	57.65±160.68 11.79 (10.83-17.47)	11.90±1.40 11.47 (10.61-13.27)
IL-10 (pg/mL)	4.80±5.49 3.27 (2.56-3.44)	5.27±5.43 3.27 (3.27-5.54)
IL-12p40 (pg/mL)	17.43±14.51 9.70 (4.74-29.78)	13.70±14.60 9.70 (6.16-17.15)
IL-13 (pg/mL)	21.61±10.70 18.45 (16.78-19.76)	18.36±2.26 18.12 (17.12-18.45)
VEGF (pg/mL)	99.88±69.02 81.34 (51.76-134.31)	94.23±74.07 62.21 (49.88-137.97)
TNF-α (pg/mL)	8.88±12.17 3.95 (3.95-7.20)	3.9±0.83 3.95 (3.12-4.66)
IFN-γ (pg/mL)	93.00±157.39 18.98 (16.71-77.73)	90.33±128.67 18.96 (17.17-214.50)
MCP-1 (pg/mL)	93.13±205.05 18.80 (12.97-48.62)	37.99±27.38 22.79 (12.84-70.16)

IL-1β, IL-6, IL-12p40, IL-1, TNF-α, IFN-γ, VEGF, and MCP-1 levels of patient group with good treatment response according to EULAR treatment response criteria are higher than the average levels of patient group with intermediate treatment response.

Further more the levels of IL-1ra and IL-10 measured before the treatment were observed to be higher in patients with medium treatment response compared to those in patients with good treatment response.

DISCUSSION

In our study significant decrease was determined in average ESR and CRP levels measured before treatment compared to average ESR and CRP levels. Also average Hb values measured before treatment, ıt was increased with treatment .There was a study obtaining a similar result to our result in the literature. In this study performed by Macias et al. and published in The Journal of Rheumatology in 2005, a patient group including 25 patients with RA received MTX treatment in one arm and MTX + infliximab treatment in another arm and MTX group and MTX + infliximab group was compared during follow-ups. Significant decreases were determined in DAS28 and MHAQ scores of MTX + infliximab group compared to ESR and CRP levels of MTX arm and initial average DAS28, HAQ, ESR and CRP levels of MTX + infliximab arm (17).

Also in our study group, according to Multiplex measurements, the average values of each cytokine and chemokine in the patients showed a decrease after treatment compared to the values before anti-TNF treatment .But since there was a small number of patients and the variables were not distributed normally, there was a statistically significant difference in 3 cytokines and chemokines including IL-10, IL12p40, IFN-γ, and MCP-1 after treatment .

On the other hand, it is seen that initial average IL-1ra, IL-10 levels of patient group with intermediate treatment response are higher than the average levels of the patient group with good treatment response.

TNF-α blocker treatment acts with various immune interactions. With reference to this approach, we think that treatment response can be predicted by looking these cytokines and chemokines rapidly using Multiplex method before TNF-α blocker treatment and it may help regarding treatment choice or making changes in treatment follow-ups. For example, higher levels of anti-inflammatory cytokines such as IL-10 and IL-1ra before treatment may be suggestive of try of another therapy instead of TNF-α blocker treatment. However, it is clear that extensive studies that will be performed since our study has not reached statistical significance due to the reasons stated earlier will provide more elucidative information about this subject.

In a study with a similar design with our study; usability of plasma levels of inflammatory proteins as biochemical markers which can show disease activity was investigated by Inmacula Rioja et al. (18).In the study, 3 groups as MTX group, control group and the group receiving TNF-α blocker treatment were investigated. When serum levels of these proteins of the patients in MTX arm were compared with those of healthy controls in the second group, serum levels of IL-6, IL-12, Oncostatin M, M-CSF, TNF receptor superfamily member 9, CCL23 (Human Chemokine (C-C motif) ligand 23), TGF-α and CXCL13 (Human Chemokine (C-X-C motif) ligand 13) were found to be quite high in the patients with higher disease activity compared to the patients with intermediate disease activity and healthy controls and these were shown to have a positive correlation with serum DAS28, CRP, ESR, RF levels. Also in the third group receiving TNF-α blocker treatment, again it was determined that serum CXCL13, CCL-23, M-CSF levels to have a positive correlation with DAS28. While serum cytokine levels measured 2 weeks before treatment especially in this group were quite high, remarkable decreases were determined in serum levels of CXCL13 and almost in all molecules just beginning from the 1st and 7th day of treatment. In this study, ELISA, RCA and Multiplex laboratory methods were used to measure the plasma proteins and it was determined that each of three methods showed similar results. Also in our study, statistically significant decreases were determined in most cytokine levels at the 3rd month of therapy. For example, serum levels of IL-1β, IL-6, IL-12p40, IL-13, TNF-α, IFN-γ, VEGF, and MCP-1 at the beginning of TNF-α blocker treatment decreased in all patients. Decreases were determined to be markedly higher in the patients with good treatment response according to EULAR response criteria compared to those with intermediate treatment response, it was determined that 14 patients were in the group with good treatment response and 12 patients were in the group with intermediate treatment response but it did not reach statistical significance due to the reasons stated earlier.

However, another relationship investigated by us in our study group is the comparison of change rates observed in average levels of cytokines between 0-6 months and change rates in average levels of CRP, ESR, and DAS28 between 0-6 months. In consequence of this analysis, we determined an intermediate positive correlation between change rates in average levels of IL-6 and DAS28 between 0-6 months. The relationship between serum levels of IL-6 and disease activity determined in the patients receiving MTX treatment in the study performed by Inmacula Rioja et al. was shown in patients receiving TNF-α blocker treatment in our study. This result is suggestive of that the most helpful cytokine for the clinician to show disease activity in RA patients followed up with TNF-α blocker treatment and to guide therapy is IL-6.

If we mention about a study in the literature similar to ours; in a study performed by Clio P. Mavragani et al. with 38 patients began to receive TNF-α blocker treatment and 50 healthy control group and published in February 2010 (19), blood samples were taken from the patients before and during treatment and plasma IL-1ra, the rate of IFNβ/α

and the levels of Type-1 IFN activity were measured. The patients with, good, intermediate and poor response were determined by comparing the measured values with a change in DAS28 scores which was response criterion of EULAR. Plasma IL-1ra levels and Type-1 IFN activity (Type-1 IFN activity was calculated with the rate of IFN-β/α and thus it was a value directly proportional to IFN-β) were measured to be higher in all patients compared to control group at the beginning and it was determined these values were higher in the patients with good response to treatment compared to the patients with intermediate and poor response. This result showed us that plasma IL-1ra levels and Type-1 IFN activity which would be measured before TNF-α blocker treatment could predict treatment response. This study supports the usability of higher Type-1 IFN activity and higher plasma IL-1ra levels which will be measured before TNF-α blocker treatment as a positive predictive measurement for response to TNF-α blocker treatment. From another point of view, it was stated that alternative treatments other than TNF could be planned in the patients with lower levels of IL-1ra, IFN-β or Type-1 IFN activity which would be measured before treatment (19). Contrary to the study performed by Clio P. Mavragani et al., in our study group, it is observed that even though not statistically significant IL-1ra values determined to be higher at the beginning are higher in the patients with intermediate response not in the patients with good response.

A similar study to ours in the literature performed by Wolfgang Hueber et al. determined the blood cytokines in RA patients followed with Anti TNF treatments by using Multiplex and other laboratory methods and investigated their relationship with treatment response. Three different RA cohorts were investigated in the study. The first group was comprised of "ABcON cohort" including 29 Caucasian patients living in North America, the second group was comprised of "Swedish cohort" including 43 Caucasian patients living in Sweden and the third group was comprised of "Japanese cohort" including 21 Japanese patients living in Japan. "Etanercept" treatment was started in all patients as TNF-α blocker treatment. Blood samples taken before treatment and at least 3 months after initiation of treatment were investigated. According to ACR response criteria as treatment response in the patients; recovery more than 50% was considered to be good response and recovery less than 20% was considered to be poor response or unresponsiveness. Cytokines investigated in the blood samples were measured by using Luminex X 200 platform, RA antigen microarrays and ELISA methods. TNF-α, IL-1α, IL-1β, IL-12p40, IL-12p70, IL-15, G-CSF, MCP-1, FGF-2 (Fibroblast growth factor), Eotaxin, and IFN-γ-inducible protein values measured before treatment by using Multiplex/Luminex method were determined to be correlated with early RA and considered as proinflammatory molecules. In the group with good response to treatment, a decrease was determined in these molecules together with the treatment. While higher levels of TNF-α and IL-15 measured before treatment were correlated with good response to treatment, lower levels were correlated with unresponsive patients (20). Also in our study group, all cytokines and chemokines we investigated before treatment were measured by using Multiplex method and decreases were observed in these molecules together with TNF-α blocker treatment. Similar to the result of the study performed by Wolfgang Hueber et al., marked decreases are observed in IL-1β, IL-6, IL-12p40, TNF-α, and MCP-1 values measured before TNF-α blocker treatment. In parallel to the results of the study performed by Wolfgang Hueber et al., TNF-α values measured at the beginning of treatment were detected to be higher in the group with good response to TNF-α blocker treatment (in our study, group with good response according to EULAR and in aforementioned study group with good response according to ACR response criteria) and they were determined to be correlated with good response, but our results did not reach statistical significance due to the reasons stated by us earlier.

In conclusion, in our study, the clinical efficacy of TNF-α blocker treatment was shown with changes in DAS28 scores which was response criterion of EULAR and significant changes in CRP, ESR, Hb values as laboratory tests.Although they do not reach statistical significance due to the reasons stated earlier, marked higher levels of IL-1β, IL-6, IL-12p40, TNF-α, and MCP-1 determined before treatment in the patient with good treatment response according to EULAR treatment response criteria might be suggestive of TNF-α blocker treatment will be successful.Similarly, higher levels of IL-1ra and IL-10 which will be measured at the beginning of treatment may be suggestive of an intermediate response can be obtained according to EULAR treatment response criteria .Possibly one of the most important results obtained from our study is as follows: higher levels of IL-6 were determined in the group with good treatment response before TNF-α blocker treatment and as well as change in DAS28 and change in IL-6 levels was statistically correlated during treatment and this highlighted the significance of IL-6 regarding follow up of RA.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

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REFERENCES

1. McCarty DJ: Clinical picture of rheumatoid arthritis.In: Arthritis and allied conditions. McCarty DJ. (Eds). Lea and Febiger pp 781-809, 1993.
2. Guidelines for the Management of Rheumatoid Arthritis 2002 Update American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, Arthritis

Rheum 2002;46:328–46.
3. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. Guidelines for monitoring drug therapy in rheumatoid arthritis. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. Arthritis Rheum 1996;39:723-31.
4. American College of Rheumatology Task Force on Osteoporosis Guidelines. Recommendations for the prevention and treatment of glucocorticoid induced osteoporosis. Arthritis Rheum 1996;39:1791-801.
5. Harris ED Jr. Rheumatoid arthritis: pathophysiology and implication for therapy. N Engl J Med 1990;322:1277–89.
6. Rheumatoid Arthritis: Diagnosis and Management, Arthritis Rheumatism 1988;31:315–24.
7. 2010 Rheumatoid Arthritis Classification Criteria An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative, Arthritis &Rheumatism. 2010;62:2569–81.
8. Hamuryudan V. İ.Ü.Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri, Türkiyede sık karşılaşılan hastalıklar dizisi, Enfeksiyon Hastalıkları, Romatizmal Hastalıklar, Afetlerde Ezilme Yaralanmaları Sempozyum Dizisi 2007 55; 69-86.
9. Hamuryudan V. İ.U.Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri Romatolojik Hastalıklar, Sempozyum Dizisi No:34, Nisan 2003; s. 19-29, Romatoid Artrit.
10. Epidemiology of rheumatoid arthritis, S Akar, N Akkoç, Turkiye Klinikleri, J Int Med Sci 2006.
11. Hochberg MC. Adult and juvenile rheumatoid arthritis: current epidemiologic concepts. Epidemiol Rev 1981;3:27-44.
12. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.
13. O'Dell JR. ED, Rheumatoid arthritis. Rheum Dis Clinics North Ame.Philadelphia: Saunders WB Company, May 2001.
14. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. Guidelines for the management of rheumatoid arthritis. Arthritis Rheum 1996;39:713–22.
15. Saxne T Palladino MA Heinegard D et al. Detection of tumor necrosis factor alfa but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum. Arthritis Rheum 1988;31:1041-5.
16. Sewell KL Trentham DE. Pathogenesis of rheumatoid arthritis. Lancet 1993;341:283-90.
17. Macías I1, García-Pérez S, Ruiz-Tudela M, et al. Modification of pro- and antiinflammatory cytokines and vascular-related molecules by tumor necrosis factor-a blockade in patients with rheumatoid arthritis. J Rheumatol 2005;32:2102-8.
18. De Jager W, te Velthuis H, Prakken BJ, et al. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 2003;10:133-9.
19. Mavragani CP, La DT, Stohl W, Crow, Association of the response to tumor necrosis factor antagonists with plasma type I interferon activity and interferon-beta/alpha ratios in rheumatoid arthritis patients: a post hoc analysis of a predominantly Hispanic cohort. Arthritis Rheum. 2010;62:392-401.
20. Hueber W, Tomooka BH, Batliwalla F, et al. Blood autoantibody and cytokine profiles predict response to anti-tumor necrosis factor therapy in rheumatoid arthritis. Arthritis Res Ther 2009;11:R76