

Serum Levels of IL-17, IL-6, TNF- α and Disease Activity in Patients with Rheumatoid Arthritis

Serumspiegel von IL-17, IL-6 und TNF- α und Krankheitsaktivität bei Patienten mit rheumatoider Arthritis

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Key words

interleukin-17, interleukin-6, tumor necrosis factor- α , rheumatoid arthritis, disease activity

Schlüsselwörter

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Bibliography

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ABSTRACT

Objective The aim of this study was to evaluate serum levels of interleukin (IL)-17, IL-6, and tumor necrosis factor alpha (TNF- α) in RA patients and to assess the correlation of these cytokines with clinical and laboratory parameters.

Materials and Methods 48 patients with RA and 35 healthy volunteers were enrolled in the study. Disease activity was determined by disease activity score (DAS28) in patients with RA. Patients with RA were categorized as mild ($DAS28 \leq 3.2$), moderate ($3.2 < DAS28 \leq 5.1$), and severe ($5.1 < DAS28$) according to DAS28. The serum levels of IL-17, IL-6 and TNF- α cytokines were measured by enzyme-linked immuno sorbent assay.

Results The mean serum IL-17 and TNF- α levels did not differ between RA patients and controls ($P > 0.05$). Serum IL-6 levels were significantly elevated in RA patients compared with controls ($P < 0.001$). The increasing trend in mean serum IL-6 levels across group with mild, moderate, and severe disease activity was significant ($P < 0.001$, respectively). In RA patients, serum IL-6 concentrations were significantly correlated with ESR, CRP, DAS28, and VAS ($r = 0.371$, $P = 0.009$; $r = 0.519$, $P < 0.001$; $r = 0.536$, $P < 0.001$; $r = 0.539$, $P < 0.001$, respectively). Also, Serum IL-17 concentrations demonstrated significant correlations with ESR, CRP, but not DAS28 ($r = 0.349$, $P = 0.015$; $r = 0.299$, $P = 0.039$; $r = 0.274$, $P = 0.060$, respectively). Serum TNF- α showed no significant correlation with disease activity indices.

Conclusions This study showed that patients with RA had significantly increased cytokine level for IL-6, but not IL-17 and TNF- α and high level of serum IL-6 cytokine was associated with disease activity. However, further follow-up studies involving large samples are required to clarify precise role of these cytokines in disease development and progress.

ZUSAMMENFASSUNG

Zielsetzung Ziel dieser Studie war es, bei RA-Patienten die Serumspiegel von Interleukin (IL)-17, IL-6 und Tumornekrosefaktor-alpha (TNF- α) zu untersuchen und die Korrelation dieser Zytokine mit klinischen und laborchemischen Parametern zu beurteilen.

Material und Methoden Es wurden 48 Patienten mit RA und 35 gesunde Probanden in die Studie aufgenommen. Bei den

Patienten mit RA wurde anhand des Disease Activity Score (DAS28) die Krankheitsaktivität ermittelt. Diese wurde gemäß DAS28 als leicht ($DAS28 \leq 3,2$), mittelschwer ($3,2 < DAS28 \leq 5,1$) und schwer ($5,1 < DAS28$) eingestuft. Die Serumspiegel der Zytokine IL-17, IL-6 und TNF- α wurden mithilfe eines ELISA [Enzyme-linked Immunosorbent Assay] bestimmt.

Ergebnisse Bei den mittleren Serumspiegeln von IL-17 und TNF- α fand sich zwischen den RA-Patienten und den Kontrollen kein signifikanter Unterschied ($p > 0,05$). Die Serumspiegel von IL-6 waren bei den RA-Patienten im Vergleich zu den Kontrollen signifikant erhöht ($p < 0,001$). In allen Patientengruppen mit leichter, mittelschwerer und schwerer Krankheitsaktivität fand sich eine signifikante Tendenz zur Zunahme der mittleren Serumspiegel von IL-6 (jeweils $p < 0,001$). Bei den RA-Patienten korrelierten die Serumkonzentrationen von IL-6

signifikant mit ESR, CRP, DAS28 und VAS ($r = 0,371$, $p = 0,009$; $r = 0,519$, $p < 0,001$; $r = 0,536$, $p < 0,001$ bzw. $r = 0,539$, $p < 0,001$). Darüber hinaus zeigten die Serumkonzentrationen von IL-17 eine signifikante Korrelation mit ESR und CRP, nicht aber mit DAS28 ($r = 0,349$, $p = 0,015$; $r = 0,299$, $p = 0,039$ bzw. $r = 0,274$, $p = 0,060$). Die Serumspiegel von TNF- α zeigten keine signifikante Korrelation mit den Krankheitsaktivitätsindizes.

Schlussfolgerungen Diese Studie zeigte, dass bei RA-Patienten der Zytokinpiegel von IL-6 signifikant erhöht war, der von IL-17 und TNF- α hingegen nicht. Außerdem ergab die Studie, dass der hohe Serumspiegel des Zytokins IL-6 mit der Krankheitsaktivität assoziiert war. Die genaue Funktion dieser Zytokine bei der Entwicklung und Progression der Erkrankung muss jedoch in weiteren Folgestudien mit großen Stichproben geklärt werden.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with progressive joint destruction, functional disability, reduced healthy-related quality of life, systemic complications, premature mortality, and a high economic burden [1].

Management goals for RA are to prevent joint damage and disability, which are best achieved by attaining disease remission [2]. Cytokine networks are critical for the initiation and perpetuation of both systemic and local inflammatory responses seen in chronic inflammatory arthritis. These cytokine networks are a key component of the immunopathology of inflammatory arthritis and likely influence whether the synovitis is destined to be self-limited or sustained. Proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin (IL)-1, and IL-6 are derived primarily from macrophages and fibroblasts and dominate RA synovitis. The critical importance of these cytokines in mediating and perpetuating inflammatory arthritis is evident in the dramatic clinical responses seen in patients treated with anticytokine-directed therapies [3]. However, approximately 30% of patients have inadequate responses to TNF blockers, placing them at risk of further cartilage and joint damage [4]. Currently available cytokine blockers were developed when RA was considered a predominantly a T helper (Th)-1-mediated immunoinflammatory disease. Since then, additional subsets of CD4+ T cells, beyond the Th1/Th2 paradigm, have been identified that play important roles in autoimmune diseases, including RA [5].

Interleukin-17 (IL-17) (also known as IL 17 A) is the hallmark cytokine of a novel Th cell population termed Th17, which has altered the Th1/Th2 paradigm in immune biology. IL-17 is produced by memory CD4+ T lymphocytes [6]. However, evidence is accumulating that cells of the innate immune system, like neutrophils, mast cells, and innate lymphoid cells are the main source of IL-17, rather than T cells [7, 8]. Transforming growth factor (TGF)- β , IL-1 β , IL-6, and IL-23 are important for the polarization of Th17 cells from naive human CD4+ T cells, and the absence of TGF- β mediates a shift from a Th17 profile to a Th1-like profile. IL-17 induced the release of proinflammatory cytokines (e. g., IL-1b, TNF and IL-6) and chemokines (e. g., IL-8 driving neutrophil recruitment; CCL20 driving recruitment of CCR6+ cells, including Th17 cells and dendritic cells) from rheumatoid synovial cells [5].

High concentrations of IL-17 in blood and synovial fluid (SF) are associated with disease severity in RA and with disease markers such as anti-cyclic citrullinated protein (CCP) antibodies, suggesting elevated IL-17 signifies a more severe clinical course in RA [9–12]. To the best of our knowledge, there is no available study evaluating serum IL-17, TNF- α , and IL-6 levels simultaneously in the same cohort of patients with RA. Therefore, the aim of this study was to evaluate IL-17 levels along with IL-6 and TNF- α levels by enzyme-linked immunosorbent assay (ELISA) in RA patients compared to healthy controls and to assess association of these cytokines with clinical and laboratory parameters.

Materials and Methods

Patients

48 consecutive patients (age, 57.7 ± 12.1 years; 44 women and 4 men) who fulfilled the 1987 revised criteria of the American College of Rheumatology [13] for RA were recruited in this cross-sectional study. All patients were taking conventional synthetic disease-modifying antirheumatic drugs (DMARD) including methotrexate, sulfasalazine, leflunomide, hydroxychloroquine either as monotherapy or in combination. In addition, 31 patients were taking prednisolone (PSL, range from 2.5–10 mg/day). Patients with concomitant other rheumatic disease, severe infection, chronic autoimmune disease which may affect laboratory and cytokine profile were excluded from the study. 35 age- and sex-matched healthy volunteers (age, 56.5 ± 4.5 years; 5 men and 30 women), who had no rheumatic disease, were included as normal controls. The study was performed in accordance with the principles stated in The Helsinki Declaration and written informed consent was obtained from all participants prior to the study. The study protocol was approved by the local Ethics Committee of our institution.

Clinical assessment

Disease activity was assessed by the 28-joint disease activity score (DAS28) in RA patients [14]. Based on the DAS 28, the patients were subdivided into 3 subgroups: mild ($DAS28 \leq 3.2$), moderate ($3.2 < DAS28 \leq 5.1$), and severe ($DAS28 > 5.1$). Pain intensity was evaluated using a 100-mm horizontal visual analog scale (VAS). Erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) were recorded.

Laboratory analysis

Determination of the anti-CCP antibody was performed by ELISA using commercial kit according to the instructions provided by the manufacturer. A result was considered positive for anti-CCP antibodies if the titer was above 15 U/mL. Serum levels of rheumatoid factor (RF)-IgM were measured by nephelometric method and a result was considered positive for RF when the concentration was above 20 IU/mL.

Blood samples of patients and controls were collected and put in a sterile plain tube and stored frozen at -20°C until analysis. We used commercially available human ELISA kits for TNF- α (eBioscience, Vienna, Austria), IL-6 (Assaypro, MO, USA), and IL-17 (R&D systems MN, USA). The procedure for the ELISA method was performed according to the instructions provided by the manufacturer. Absorbance was measured at a wave length of 450 nm using ELISA reader. The levels of cytokines were recorded as a pg/mL. The intra-assay and inter-assay coefficient of variation were 6 and 7.4% for TNF- α , 4.9 and 7.3% for IL-6, 5.2 and 8.5% for IL-17, respectively. The minimum detectable dose of TNF- α , IL-6, IL-17 are 2.3, 6, and 15 pg/mL, respectively.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 13.0, for Windows (SPSS, Chicago, IL, USA). Continuous variables are presented as the mean \pm standard deviation or median (95% confidence interval [CI]). The normality of the distribution for all variables was assessed by the Kolmogorov-Smirnov test. Intergroup comparisons were made using the Student's t-test for normally distributed variables

and Mann-Whitney U test for non-parametric variables. Analyses of serum cytokine concentrations in subgroups with RA were performed using one-way analysis of variance test and Tamhane post-hoc analysis. To assess the correlations between variables, Spearman's rank or Pearson's correlation analysis were used according to data distribution. Values of $p < 0.05$ were considered statistically significant.

Results

The demographic and clinical characteristics of patients and controls are shown in ► **Table 1**. Patients and controls did not significantly differ in age or sex. The mean disease duration in RA patients was 6.4 ± 4.3 years.

The mean serum IL-17 and TNF- α levels did not differ between RA patients and controls (16.86 ± 6.28 vs. 18.37 ± 7.91 pg/mL, $P = 0.262$; 12.3 ± 5.1 vs. 13.28 ± 4.4 pg/mL, $P = 0.067$, respectively), whereas serum IL-6 levels were significantly elevated in patients with RA compared with controls (11.62 ± 5.25 vs. 7.69 ± 0.79 pg/mL, $P < 0.001$) (► **Table 2**). Analysis of patients according to DAS28, the increasing trend in mean serum IL-6 levels across group with mild, moderate, and severe disease activity was significant (8.9 ± 2.7 , 10.5 ± 3.7 , 16.1 ± 6.6 pg/mL, $P < 0.001$, respectively). According to Tamhane post-hoc analysis, this resulted from differences between subgroups with moderate and severe disease activity, and mild and severe disease activity ($P = 0.036$; $P = 0.007$, respectively). But, there were no significant differences in the mean serum IL-17 and TNF- α levels among subgroups ($P > 0.05$) (► **Table 3**). Also, there were no significant differences in the serum IL-17, TNF-

► **Table 1** Demographic and clinical characteristics of rheumatoid arthritis patients and control.

	Rheumatoid Arthritis group (n = 48)	Control group (n = 35)
Mean age \pm SD, years	57.7 \pm 12.2	56.5 \pm 4.5
Sex, female/male, n	44/4	30/5
Mean disease duration (years) \pm SD, (range)	6.4 \pm 5.3 (0.0–20)	–
Mean disease activity score 28 \pm SD, (range)	4.3 \pm 1.6 (1.2–8.3)	–
Mild (n) (%)	12 (25)	–
Moderate (n) (%)	23 (47.9)	–
Severe (n) (%)	13 (27.1)	–
VAS (mm)	47.9 \pm 25.6	–
ESR (mm/h)	27.4 \pm 20	–
CRP (mg/dL)	1.7 \pm 1.9	–
RF positivity, n (%)	29 (60.4)	–
Anti-CCP positivity, n (%)	23 (47.9)	–
Drug medication		
MTX, SSZ	15 (31.3)	–
MTX, HQ	16 (33.3)	–
LF, HQ	5 (10.4)	–
MTX	12 (25)	–

SD standart deviation, VAS visual analog scale, ESR erythrocyte sedimentation rate, CRP C reactive protein, RF rheumatoid factor, CCP cyclic citrullinated peptide, MTX methotrexate, SSZ sulfasalazine, HQ hydroxychloroquine, LF leflunomide

α , IL-6, and DAS28 between patients with either RF positive and negative RA (16.1 ± 6.0 vs. 17.9 ± 6.6 pg/mL, $P=0.333$; 11.5 ± 4.7 vs. 13.5 ± 5.6 pg/mL, $P=0.169$; 12.3 ± 5.4 vs. 10.6 ± 4.9 pg/mL, $P=0.264$; 4.5 ± 1.2 vs. 3.8 ± 2 , $P=0.193$, respectively), or anti-CCP positive and negative RA (17.2 ± 6.6 vs. 16.6 ± 6.0 pg/mL, $P=0.763$; 11.9 ± 12.7 vs. 12.7 ± 5.6 pg/mL, $P=0.590$; 12.7 ± 5.7 vs.

10.6 ± 4.6 pg/mL, $P=0.165$; 4.4 ± 1.1 vs. 4.1 ± 1.9 , $P=0.529$, respectively).

In RA patients, serum IL-6 concentrations were significantly correlated with ESR, CRP, DAS28, and VAS ($r=0.371$, $P=0.009$; $r=0.519$, $P<0.001$; $r=0.536$, $P<0.001$; $r=0.539$, $P<0.001$, respectively) (► Fig. 1). Serum IL-17 concentrations also demonstrated significant correlations with ESR, CRP, but not DAS28 ($r=0.349$, $P=0.015$; $r=0.299$, $P=0.039$; $r=0.274$, $P=0.060$, respectively). However, serum TNF- α showed no significant correlation with other studied parameters (all $P>0.05$)(data not shown).

► **Table 2** The comparison of serum levels of cytokines.

	Patients (n=48)	Controls (n=35)	P
Serum IL-17, (pg/mL)	16.86 ± 6.28	18.37 ± 7.91	0.262
Serum TNF- α , (pg/mL)	12.3 ± 5.1	13.28 ± 4.39	0.067
Serum IL-6, (pg/mL)	11.62 ± 5.25	7.69 ± 0.79	<0.001

IL interleukin, TNF tumor necrosis factor

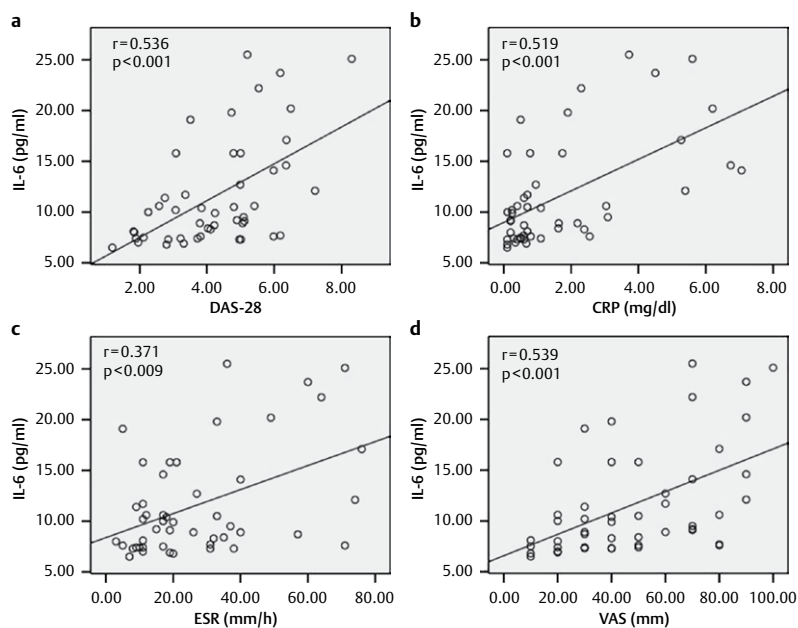
Discussion

In the present study, we evaluated serum levels of IL-17, IL-6, TNF- α cytokines in patients with established RA, and associations of these cytokines with clinical and laboratory parameters. There was no significant difference in serum IL-17 between RA patients and controls, whereas serum IL-17 levels showed significant positive correlations with ESR, CRP, but not DAS28.

► **Table 3** The comparison of rheumatoid arthritis patients based on disease activity score 28 for cytokines and visual analog scale.

	Mild (n=12)	Moderate (n=23)	Severe (n=13)	p
IL-17, (pg/mL)	14.65 ± 6.66	16.80 ± 5.43	19.00 ± 7.04	0.226
TNF- α , (pg/mL)	13.23 ± 6.28	10.50 ± 3.72	10.61 ± 3.89	0.375
IL-6, (pg/mL)	8.86 ± 2.69	12.76 ± 5.02	16.12 ± 6.62	<0.001
VAS, (mm)	18.33 ± 7.17	44.34 ± 13.42	81.53 ± 9.87	<0.001

IL interleukin, TNF tumor necrosis factor, VAS visual analog scale



► **Fig. 1** Correlation of interleukin (IL)-6 levels with **a** disease activity score (DAS) 28, **b** C- reactive protein (CRP), **c** erythrocyte sedimentation rate (ESR), and **d** visual analog scale (VAS).

In a study by Mrabet et al., IL-17 levels were similar in the serum and joint of patients with RA, psoriatic arthritis, and osteoarthritis (OA) [15]. Interestingly, comparison of paired serum and SF IL-17 levels showed that IL-17 concentrations were significantly higher in serum than in SF within the 3 study groups. Also, disease activity parameters showed no significant correlation with serum or SF IL-17 levels in their study. Similar to our results, plasma IL-17 levels showed no significant difference between each group including ankylosing spondylitis, RA, OA, and healthy subjects in a study by Zhang et al. [16]. Plasma IL-17 level also failed to show a statistical correlation with CRP level or DAS28 in that study. By contrast our results, a study by Roşu et al. found significantly higher IL-17 levels in serum from treatment naive early RA patients compared with OA [17]. Strong correlations of serum IL-17 levels with ESR, CRP, and DAS28 have also been demonstrated in their studies. They noted higher serum IL-17 levels in patients with seropositivity for RF and anti-CCP, especially in cases with elevated disease activity score. In our study, we found no significant difference in serum IL-17 levels among subgroups according to disease activity, also between anti-CCP positive and negative, or RF positive and negative RA patients. In fact, our study population consisted of patients with established RA and they have been taking treatment including DMARDs. This treatment regimens could influenced serum IL-17 levels and correlation with disease activity markers. Contrary our results, significantly higher serum IL-17 levels than healthy controls and strong correlation of serum IL-17 with disease activity were also demonstrated in other studies [12, 18].

There is conflicting observations in literature regarding the IL-17 levels in serum sample and synovial fluid of patients with RA. The importance of IL-17 relative to other cytokines in RA (e. g., TNF, IL-6, IL1 β) as well as the relative importance of the Th17 vs. Th1 pathways may differ between patients or disease stages [5]. It was shown that IL-17 was detected at higher levels in early disease compared with late, established disease [19–21]. In a study by Raza et al., it was demonstrated that patients with early inflammatory arthritis who subsequently developed RA had distinct but transient SF cytokine profile [20]. Patients with early RA had significantly elevated synovial levels of IL-17 compared with patients late disease in that study. Kokkonen et al. also evaluated whether cytokines, cytokine-related factors, and chemokines are upregulated prior to development of RA, and they found that the concentration of IL-17 in individuals before disease onset was significantly higher than that in patients after disease onset [21]. A study by Gullick et al. demonstrated that IL-17 expressing CD4+ T cells and serum IL-17 were increased in peripheral blood from patients with early inflammatory arthritis [22]. Another study by Hitchon et al. also found higher serum IL-17 level in patients with early untreated RA compared to healthy controls [3]. These studies supports the concept that T cells play a role disease initiation that is different from their role in maintaining persistent inflammation.

Interleukin-6 is a cytokine that causes an acute inflammatory response, and it is well-documented that IL-6 plays a crucial role in the pathogenesis of various inflammatory diseases including RA [23, 24]. In the current study, serum IL-6 levels were significantly higher in RA patients compared to controls, and in patients with high disease activity compared with those of low and moderate disease activity RA patients. A study by Chung et al. demonstrated

that the serum concentrations of IL-6 were significantly elevated in patients with RA and IL-6 levels showed a significant correlation with CRP levels but not with DAS28 [25]. In our study, significant correlations of serum IL-6 level with DAS28, ESR, CRP, and VAS were observed. It has been reported that IL-6 was a significant predictor for progression of bone destruction in early stages of RA in a study by Saeki et al. [26]. In present study, all patients were on DMARD treatment, and the presence of high disease activity in some of these patients may reflect insufficiency of drug response. So our results suggest that high serum level of IL-6 might reflect the activity of disease, and be good candidate for anti-IL6 therapy, especially in patients with established RA. In consistent of our observation, Nishina et al. reported that the post-treatment IL-6 level was a strong indicator of radiographic progression [27].

Besides IL-6, TNF- α is one of the key cytokines in the pathogenesis of RA, and TNF inhibitors are major biologics in the treatment of RA. In our study, serum TNF- α levels showed no significant difference between patients and controls. Also, no significant correlations of TNF- α with studied clinical, and laboratory parameters were found. A study by Saeki et al. also found that all of the serum TNF- α levels of the patients were within the range of the healthy volunteers [26]. So they did not include TNF- α as a candidate marker in that study.

Our study has some limitations. The sample size of patients was relatively small, and the patients were on drug treatment including DMARDs. Treatment regimes might influence on the serum expression of cytokines. In fact, our study had a cross-sectional design, and cytokines profile could not evaluated compared to patients with early treatment naive RA.

In conclusion, this study demonstrated higher levels of cytokine for IL-6 but not IL-17 and TNF- α in patients with RA compared to healthy controls and statistically significant correlation of serum IL-6 levels with disease activity parameters. While TNF- α levels showed no significant correlation with disease activity parameters, IL-17 levels were significantly correlated with ESR and CRP. However, further follow-up studies involving large samples are required to clarify the precise role of these cytokines in disease development and in maintaining persistent inflammation.

Conflict of Interest

No

References

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