Tohoku J. Exp. Med., 2017, **241**, 89-95

# Elevated Serum Levels of Interleukin-29 Are Associated with Disease Activity in Rheumatoid Arthritis Patients with Anti-Cyclic Citrullinated Peptide Antibodies

**Qiong-jie Chang,[1] Cheng Lv,[1] Feng Zhao,[1] Ting-shuang Xu[1] and Ping Li[1]**

[1]Department of Rheumatology and Immunology, China-Japan Union Hospital of Jilin University, Changchun, Jilin, China

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that may lead to progressive joint destruction. The anti-cyclic citrullinated peptide (anti-CCP) antibody is an essential marker for the diagnosis of RA and has a crucial role in the bone destruction in RA. Recent studies have shown that interleukin (IL)-29, a vital member of type III interferon (IFN) family, could enhance proinflammatory cytokine production and might be involved in the joint destruction in RA. Therefore, in this study, we aimed to examine the role of IL-29 in RA patients with anti-CCP antibodies. The result showed that the serum IL-29 levels were higher in RA patients (n = 68) compared with healthy controls (HC, n = 68, P = 0.019). Correlation analysis demonstrated a significant positive correlation among serum IL-29 level, rheumatoid factor (RF, P < 0.001) and anti-CCP antibodies (P = 0.042). However, when RA patients were divided into two groups according to anti-CCP antibodies, the serum IL-29 levels were significantly higher in anti-CCP-antibodies positive RA patients (n = 54) than those in HC (n = 68) and anti-CCP-antibodies negative RA patients (n = 14). Furthermore, the serum IL-29 levels were positively correlated with the disease activity (P < 0.05) and significantly declined after 6 months of treatment (P < 0.01) in the anti-CCP-antibodies positive RA patients, whereas no significant change was found in the anti-CCP-antibodies negative RA patients (P > 0.05). The findings indicate that IL-29 is a potential biomarker for disease activity in anti-CCP-antibodies positive RA patients.

**Keywords:** anti-cyclic citrullinated peptide antibody; autoimmune disease; cytokine; interleukin-29; rheumatoid arthritis

Tohoku J. Exp. Med., 2017 February, **241** (2), 89-95. 

## Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammatory cell infiltration of the synovium, synovial hyperplasia, angiogenesis, and cartilage and bone erosion (Turk et al. 2014; Ning et al. 2015). Proinflammatory cytokines play a central role in the generation of the inflammatory and destructive response in RA (Abe and Takeuchi 2001; Ruscitti et al. 2015).

Interferon (IFN) is a kind of cytokine with antiviral, anti-tumor and immune regulatory functions. As a newly described member of the IFN family, Type III IFNs include IFN-$\lambda$1, IFN -$\lambda$2, and IFN-$\lambda$3, which are also known as interleukin (IL)-29, IL-28A, and IL-28B respectively (Sheppard et al. 2003; Uze and Monneron 2007; Dickensheets et al. 2013). Through binding to respective receptors, IFN-$\lambda$s activate the Janus kinase-signal transducer and activator of transcript (JAK-STAT), mitogen-activated protein kinase (MAPK) or Akt signaling pathways to induce antiviral, anti-proliferative, antitumor and immune responses (Donnelly and Kotenko 2010; Zhang et al. 2011; Liu et al. 2012). IL-29, as the most active cytokine among all type III IFNs (Xu et al. 2015a), has been implicated in protecting against viral infection and modulating autoimmune inflammation (He et al. 2010, 2011; de Groen et al. 2014). Specifically, IL-29 is able to upregulate the levels of IL-6, IL-8 and IL-10 secreted by monocytes (Jordan et al. 2007) and enhance the IL-2-dependent proliferation of $CD4^+CD25^+Foxp3^+$ T cells induced by dendritic cells (Mennechet and Uze 2006), Which indicates that IL-29 may play a crucial role in modulating immune response. However, the immune-regulatory role of IL-29 in RA patients remains unclear.

In this study, we compared the serum levels of IL-29 in RA patients with healthy controls (HC) and further determined the correlations of serum IL-29 levels with disease activity and clinical parameters in these RA patients. We note that this is the first study to investigate the effect of

Received October 21, 2016; revised and accepted January 10, 2017. Published online February 1, 2017; doi: 10.1620/tjem.241.89.

Correspondence: Ping Li, Department of Rheumatology and Immunology, China-Japan Union Hospital of Jilin University, No.126 Xiantai Street, Changchun, Jilin 130033, China.

e-mail: lipingzt@163.com

traditional disease modifying anti-rheumatic drugs (DMARDs) based treatment on the serum level of IL-29, and the relationship between anti-cyclic citrullinated peptide (anti-CCP) antibody and the serum level of IL-29 in RA patients.

## Materials and Methods

*Patients and controls*

Sixty-eight patients with RA diagnosed according to the 1987 revised criteria of the American Rheumatism Association (Arnett et al. 1988) were enrolled in our study. Individuals with infections, diabetes, heart failure, renal failure, hepatitis, hepatic failure or malignant tumors and other rheumatic diseases were excluded from the study. Sixty-eight age- and sex-matched healthy individuals were also enrolled as HC. The individuals of HC had no history of rheumatic disease, heart failure, renal failure, hepatitis, hepatic failure, heart valve disease and infectious disease. All RA patients were recruited from the baseline and had not yet been treated with glucocorticoid and DMARDs before the blood samples were collected, besides, then all RA patients received the prescribed DMARDs including iguratimod, methotrexate, leflunomide, sulfasalazine or two of them and were followed up for six months. The study was in accordance with the Helsinki Declaration and was approved by the ethics committee of China-Japan Union Hospital of Jilin University. The informed consents in written were approved by all patients and volunteers.

The parameters, including age, sex, disease duration, rest pain, morning stiffness, visual analogue score (VAS), the number of tender and swollen joints, Health Assessment Questionnaire (HAQ), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity score (DAS) with 28 joints using ESR [DAS28 (ESR)], DAS with 28 joints using CRP [DAS28 (CRP)], anti-CCP antibodies, rheumatoid factor (RF) and serum platelet counts were assessed through questionnaires and the review of medical records. The platelets were counted by impedance. The serum RF and CRP levels were determined by immunoturbidimetry. The levels of serum anti-CCP antibodies were detected by commercial enzyme-linked immunosorbent assays (ELISA). The Westergren method was utilized to detect ESR.

*Blood samples*

Blood samples were taken after 12 hours overnight fast between 08:00 am and 09:00 am from four separate visits: prior to starting DMARDs (at baseline), 1, 3 and 6 months after the onset of treatment. Serum samples were stored at −80ºC until the level of IL-29 was determined.

*Detection methods*

The serum level of IL-29 was determined by ELISA (eBioscience, BMS2049) according to the manufacturer's instructions. Reading was performed on a microplate reader.

*Statistical analysis*

Results were presented as the median (interquartile range, IQR) or mean ± standard deviation (SD). For non-normal distributions, Mann-Whitney U-test and the Wilcoxon Signed Ranks Test were applied. For normal distributions, the *t* test was employed. Associations between the general characteristics and serum IL-29 levels were analyzed using the Spearman rank correlation test for non-normal distributions and Pearson's correlation test for normal distributions. Statistical analyses in our study were performed with Statistical Package for the Social Science (SPSS) version 17.0. (SPSS, Inc., Chicago, IL, USA). The Graphpad Prism 5.0 software (Graphpad Software, La, Jolla, CA, USA) was used for figure production. All tests were two-tailed. P values less than 0.05 were considered to be statistically significant.

## Results

*Clinical parameters and serum IL-29 levels of RA patients and HC*

The general parameters and serum IL-29 levels of RA patients and HC were summarized in (Table 1). The level of serum IL-29 in RA patients at baseline was 965.7 (371.0-3118.0) pg/ml, while that in HC was 468.1 (349.9-726.4) pg/ml. The serum IL-29 levels were significantly higher in RA patients than those in HC ($P = 0.019$).

*The serum IL-29 levels in RA patients with or without anti-CCP-antibodies*

Mustila et al. (2011) have shown that the RA patient with a higher serum level of anti-CCP antibodies tends to have a more severe disease course and a high risk of joint destruction and disability. Knowing that anti-CCP-antibodies positive and anti-CCP-antibodies negative RA patients represent different disease entities, the sixty-eight patients with RA in our study were divided into two groups according to their reactions to anti-CCP antibodies. The clinical parameters and serum IL-29 levels of anti-CCP-antibodies positive and anti-CCP-antibodies negative RA patients were shown in (Table 1), with no difference observed between anti-CCP-antibodies positive RA patients and anti-CCP-antibodies negative RA patients regarding age, disease duration, morning stiffness, rest pain, VAS, the number of tender and swollen joints, HAQ, ESR, DAS28 (ESR), CRP, DAS28 (CRP) and serum platelet counts ($P > 0.05$). Interestingly, the serum IL-29 levels were significantly different between anti-CCP-antibodies positive RA patients and anti-CCP-antibodies negative RA patients at baseline (Table 1 and Fig. 1). Specifically, the serum IL-29 levels were significantly higher in RA patients with anti-CCP-antibodies [1288.0 (392.7-3975.0) pg/ml] than those in HC ($P = 0.004$) and RA patients with anti-CCP-antibodies ($P = 0.005$) (Table 1 and Fig. 1). However, the serum IL-29 levels are comparable between anti-CCP-antibodies negative RA patients (517.9 ± 260.5 pg/ml) and HC ($P = 0.660$) (Fig. 1).

*Correlations between serum IL-29 levels and clinical parameters in RA patients*

As shown in (Table 2), serum IL-29 levels, when compared in 68 RA patients along with other clinical parameters at baseline, showed positive correlation only with RF ($P < 0.001$) and anti-CCP antibodies ($P = 0.042$), but not with the other clinical parameters examined. Interestingly, when RA patients were further divided based on their reactions to

Table 1. Clinical and laboratory parameters in patients with RA before treatment and HC subjects.

| Parameter | All RA (n = 68) | Anti-CCP+ RA (n = 54) | Anti-CCP- RA (n = 14) | HC (n = 68) |
|---|---|---|---|---|
| Sex (male/female) | 8/60 | 6/48 | 2/12 | 8/60 |
| Age (y) | 50.1 ± 8.6 | 48.9 ± 8.0 | 53.6 ± 10.2 | 49.6 ± 7.8 |
| Disease duration (m) | 15.0 (7.3 - 19.0) | 13.1 ± 6.6 | 14.8 ± 5.8 | - |
| Morning stiffness (min) | 140.0 (90.0 - 240.0) | 125.0 (90.0 - 240.0) | 183.0 ± 88.8 | - |
| Rest pain | 75.0 (60.0 - 86.3) | 72.5 (60.0 - 83.8) | 79.0 ± 11.0 | - |
| Doctor VAS | 72.5 (60.0 - 81.3) | 70.0 (60.0 - 80.0) | 78.0 ± 11.1 | - |
| Patients VAS | 72.5 (60.0 - 85.0) | 71.4 ± 14.8 | 78.5 ± 11.6 | - |
| Number of tender joints | 17.0 (12.0 - 25.0) | 16.5 (11.3 - 24.0) | 17.3 ± 6.2 | - |
| Number of swollen joints | 10.0 (7.0 - 17.3) | 9.0 (6.0 - 15.8) | 14.4 ± 5.1 | - |
| HAQ | 21.5 (9.0 - 33.3) | 20.1 ± 13.0 | 27.3 ± 15.7 | - |
| ESR (mm/h) | 46.0 (12.0 - 62.0) | 44.0 (10.5 - 62.0) | 37.8 ± 26.5 | - |
| DAS28 (ESR) | 6.7 ± 1.3 | 6.5 ± 1.3 | 7.1 ± 1.2 | - |
| CRP (mg/l) | 19.1 (4.1 - 44.4) | 15.8 (4.1 - 37.1) | 27.4 (3.2 - 72.5) | - |
| DAS28 (CRP) | 6.0 (5.3 - 7.3) | 5.8 (5.3 - 7.2) | 6.9 ± 1.1 | - |
| RF positive (%) | 69% | 84% | 10% | - |
| Serum platelet counts ($\times 10^9$ /L) | 329.0 ± 93.6 | 315.4 ± 100.1 | 354.3 ± 79.5 | - |
| Anti-CCP-antibodies positive (%) | 79% | 100% | 0% | - |
| Serum IL-29 levels (pg/ml) | 965.7 (371.0 - 3118.0)* | 1288.0 (392.7 - 3975.0)† | 517.9 ± 260.5 | 468.1 (349.9 - 726.4) |

Categorical variables are given as the %, normally distributed data are shown as the mean ± SD, other continuous variables are shown as the median (IQR). The significance levels are shown as *P < 0.05 when comparing all RA patients and HC and when comparing anti-CCP-antibodies positive RA patients and anti-CCP-antibodies negative RA patients shown as †P < 0.05. There was no significant difference in age, disease duration, morning stiffness, rest pain, VAS, the number of tender and swollen joints, HAQ, ESR, DAS28 (ESR), CRP, DAS28 (CRP) and serum platelet counts between anti-CCP-antibodies positive RA patients and anti-CCP-antibodies negative RA patients.
Anti-CCP+, anti-CCP-antibodies positive; anti-CCP−, anti-CCP-antibodies negative.

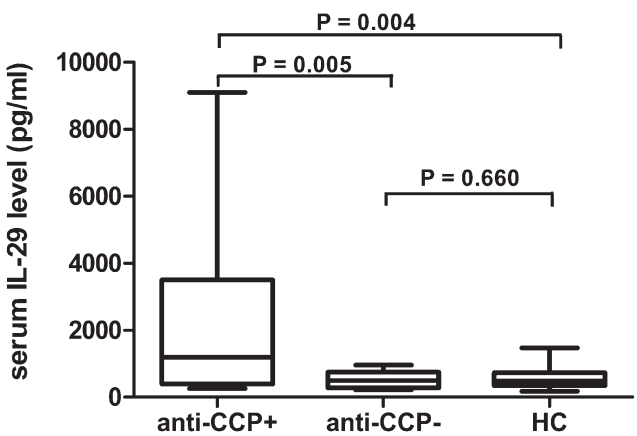


Fig. 1. Comparison of serum levels of IL-29 among anti-CCP-antibodies positive RA patients, anti-CCP-antibodies negative RA patients and HC.
The serum IL-29 levels of anti-CCP-antibodies positive (anti-CCP+) RA patients (n = 54) were significantly higher than HC (n = 68, P = 0.004) and anti-CCP-antibodies negative (anti-CCP−) RA patients (n = 14, P = 0.005). However, there was no significant difference between anti-CCP-antibodies negative RA patients and HC (P = 0.660).

anti-CCP antibodies, the serum IL-29 levels of anti-CCP-antibodies positive RA patients were positively correlated with HAQ (P = 0.033), ESR (P = 0.033), CRP (P = 0.001), DAS28 (CRP) (P = 0.011), RF (P < 0.001) and serum platelet counts (P = 0.042). However, there was no correlation observed between serum IL-29 levels and the clinical parameters measured in anti-CCP-antibodies negative RA patients (Table 2).

*Changes in serum levels of IL-29 in RA patients treated with DMARDs*

The changes in serum levels of IL-29 in RA patients following DMARDs treatment were shown in Table 3 and Fig. 2. Notably, the serum IL-29 levels of all RA patients (Table 3 and Fig. 2a) were declined after 3 months of treatment with DMARDs (P < 0.05), and declined more significantly after 6 months of treatment (P < 0.01). Comparing the anti-CCP-antibodies positive group and the anti-CCP-antibodies negative group, we found that the serum IL-29 levels of patients in the anti-CCP-antibodies negative group did not change after DMARDs treatment (P > 0.05) (Table

Table 2. Correlations between serum IL-29 levels and clinical characteristics.

| Parameter | P-value (All RA) | P-value (Anti-CCP+ RA) | P-value (Anti-CCP- RA) |
|---|---|---|---|
| Disease duration | 0.220 | 0.867 | 0.202 |
| Morning stiffness | 0.369 | 0.225 | 0.687 |
| Rest pain | 0.708 | 0.532 | 0.239 |
| Doctor VAS | 0.901 | 0.437 | 0.536 |
| Patients VAS | 0.909 | 0.543 | 0.822 |
| Number of tender joints | 0.545 | 0.118 | 0.889 |
| Number of swollen joints | 0.369 | 0.077 | 0.844 |
| HAQ | 0.413 | 0.033 | 0.333 |
| ESR | 0.199 | 0.033 | 0.402 |
| DAS28 (ESR) | 0.310 | 0.055 | 0.347 |
| CRP | 0.063 | 0.001 | 0.385 |
| DAS28 (CRP) | 0.214 | 0.011 | 0.293 |
| RF | < 0.001 | < 0.001 | 0.873 |
| Serum platelet counts | 0.490 | 0.042 | 0.058 |
| Anti-CCP antibodies | 0.042 | 0.863 | - |

Table 3. Changes in serum levels of IL-29 in RA patients after treatment.

| Time | All RA | Anti-CCP+ RA | Anti-CCP- RA |
|---|---|---|---|
| Baseline (pg/ml) | 965.7 (371.0 - 3118.0) | 1288.0 (392.7 - 3975.0) | 517.9 ± 260.5 |
| 1 month (pg/ml) | 873.0 (322.2 - 2221.2) | 1226.8 (442.4 - 3354.3) | 423.7 ± 231.8 |
| 3 months (pg/ml) | 706.8 (281.9 - 1483.1)* | 1071.1 (252.9 - 2089.9)* | 492.1 ± 282.9 |
| 6 months (pg/ml) | 706.8 (297.4 - 1109.4)** | 777.7 (301.4 - 1240.1)** | 470.1 ± 269.2 |

Normally distributed data are shown as the mean ± SD, other continuous variables are shown as the median (IQR). The significance levels are shown as *P < 0.05, **P < 0.01 compared with baseline.

3 and Fig. 2b), while the serum IL-29 levels of the anti-CCP-antibodies positive RA patients declined significantly ($P < 0.05$) after only 3 months of treatment with DMARDs (Table 3 and Fig. 2c). Further comparing parameters obtained during different treatment period, we found no correlation between the reduction of serum IL-29 levels and the reduction of disease activity in the first treatment period (0 to 1 month). However, the changes of the serum IL-29 levels in anti-CCP-antibodies positive RA patients are well correlated with the reductions of DAS28 (CRP) and DAS28 (ESR) ($P < 0.05$) during the treatment periods of 1 to 3 months and 3 to 6 months (Table 4).

*The comparison of serum IL-29 levels at baseline between the effective treatment patients and ineffective treatment patients*

The American College of Rheumatology (ACR) response criteria (Felson et al. 1995) was used to judge the treatment response. After 6 months of treatment with DMARDs, 40 patients out of 68 had an ACR20 response (20% improvement in tender and swollen joint counts and 20% improvement in 3 of the 5 remaining ACR core set measures: patient and physician global assessments, pain, disability, and an acute-phase reactant). We also compared the serum IL-29 levels at baseline between the ACR20 positive group (1,051.8 (387.8-5,337.7) pg/ml) and the ACR20 negative group (777.7 (347.9-1,874.9) pg/ml) with no significant difference ($P > 0.05$).

## Discussion

IL-29, as a new member of type III IFN family of cytokines, is involved in multiple immune responses including inhibition of viral infection and proliferation of tumor cells as well as regulation of immune system (Kotenko 2011; Liu et al. 2012). Examples of the latter include the connective tissue disorders such as RA and systemic lupus erythematosus (Wu et al. 2011). Our results showed that serum IL-29 levels in patients with RA were significantly higher compared to HC, suggesting a role for IL-29 in the pathogenesis of RA. Our results are in agreement with a previous work by Wang et al. (2012), where the serum IL-29 levels of 54 RA patients and 60 HC were measured and the serum levels of circulating IL-29 were found to be significantly higher in RA patients than in HC (Wang et al. 2012). Moreover, we have examined the correlations between serum IL-29 levels and clinical parameters in RA.

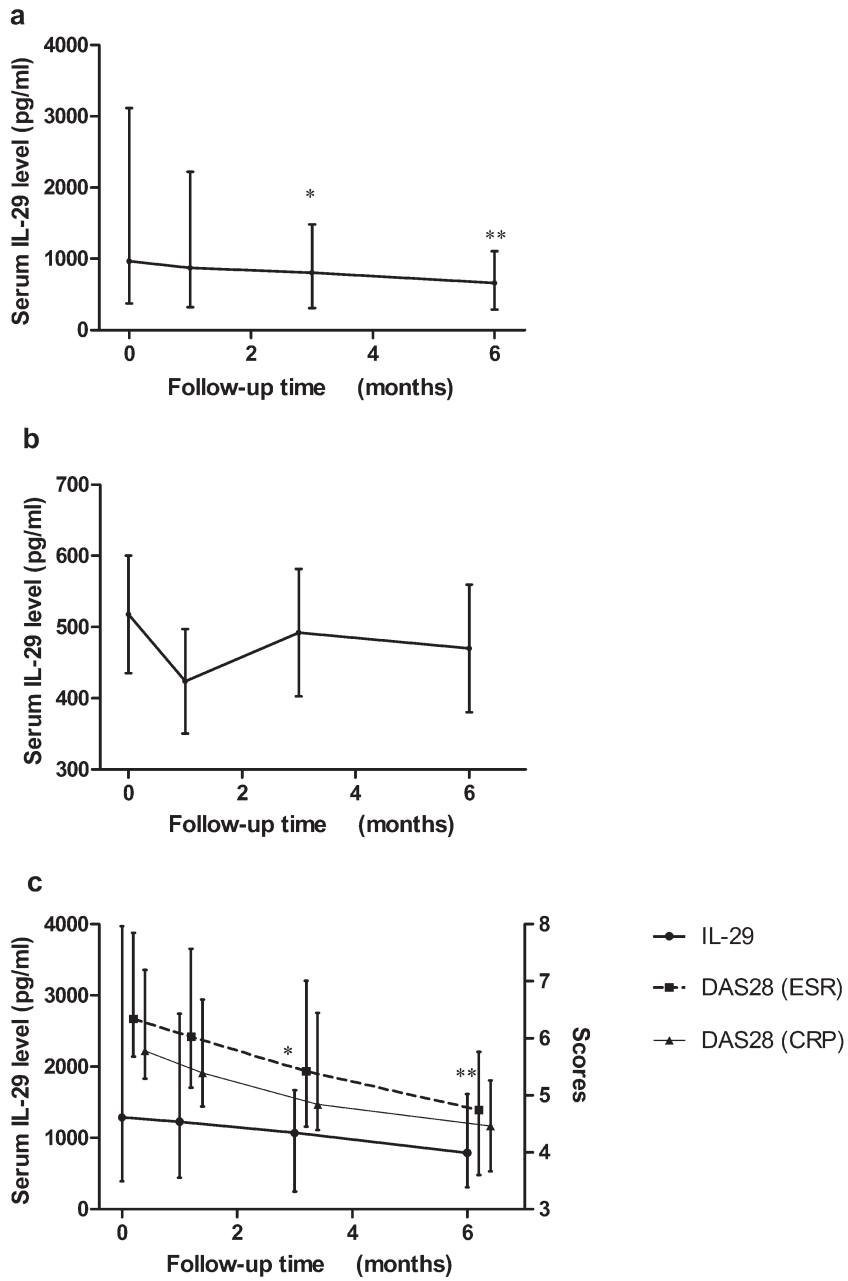


Fig. 2. Changes in serum levels of IL-29 and DAS28 in RA patients after treatment.
With treatment of DMARDs, the serum IL-29 levels of all RA patients (a, n = 68) were declined after 3-month treatment ($P < 0.05$), and declined more significantly after 6-month treatment ($P < 0.01$). When the RA patients were divided into anti-CCP-antibodies positive group (n = 54) and anti-CCP-antibodies negative group (n = 14), the changes of anti-CCP-antibodies negative RA patients (b) did not have statistical significance after treatment ($P > 0.05$), however, the results of serum IL-29 levels of anti-CCP-antibodies positive RA patients (c) indicated significant decline as a consequence of treatment with DMARDs ($P < 0.01$). The changes of disease activity parameters including DAS28 (ESR) and DAS28 (CRP) in anti-CCP-antibodies positive RA patients after 1, 3 and 6 months of treatment were shown in (c). Data are presented as median (IQR). The significance levels are shown as $^{*}P < 0.05$, $^{**}P < 0.01$ compared with baseline.

Our results suggested that IL-29 was positively associated with RF and the level of anti-CCP-antibody. However, Wang et al. (2012) did not find any association between IL-29 levels and clinical characteristics. It should be noted that the RA patients in their study fulfilled the ACR/European League Against Rheumatism (EULAR) 2010 diagnostic criteria (Aletaha et al. 2010), and the DAS28 (4.58 ± 2.08) of their RA patients were quite lower than our patients (6.7 ± 1.3). Furthermore, the disease duration of their RA patients (36.0 (3.0-444.0) months) was longer than ours (15.0 (7.3-19.0) months). Therefore, we attribute the discrepancy in our results to the disease status of RA patients at the time of blood sampling and the differences in the protocol used.

Table 4. Correlation between the reduction of serum IL-29 levels and the reduction of disease activity after the treatment.

| | P-value (DAS28 (ESR) ) | P-value ( DAS28 (CRP) ) |
|---|---|---|
| 1 month - Baseline | 0.509 | 0.380 |
| 3 months - 1 month | 0.040 | 0.008 |
| 6 months - 3 months | 0.031 | 0.048 |

The levels of anti-CCP antibodies and RF are associated with more severe disease characterized by earlier onset, faster progression, more extra-articular manifestations and worse outcome (Nell et al. 2005). An early work from Mustila et al. (2011) has shown that RA patients with high serum levels of anti-CCP antibodies have a more severe disease course and a high risk of joint destruction and disability (Mustila et al. 2011). Thus, we hypothesized that the level of IL-29 correlates with the level of anti-CCP antibodies. Therefore, RA patients in our study were further divided into two subgroups: anti-CCP-antibodies positive RA patients and anti-CCP-antibodies negative RA patients. Surprisingly, for the first time, we found that the serum IL-29 levels of anti-CCP-antibodies positive RA patients were significantly higher than anti-CCP-antibodies negative RA patients and HC, and the serum IL-29 levels positively correlated with HAQ, ESR, CRP, DAS28 (CRP), RF and serum platelet counts in anti-CCP-antibodies positive RA patients. On the other hand, there was no difference of the serum IL-29 levels between anti-CCP-antibodies negative RA patients and HC, and the serum IL-29 levels of anti-CCP-antibodies negative RA patients did not correlate well with clinical parameters. It is tempting to speculate that IL-29 may be involved in the occurrence and development of anti-CCP-antibodies positive RA patients. A previous study showed that IL-29 enhanced Toll-like receptor (TLR)-induced proinflammatory cytokine production in RA fibroblast-like synoviocytes (FLS) via upregulating TLRs (Xu et al. 2015a). IL-29 could directly induce nuclear factor-kB ligand (RANKL) expression in RA -FLS via MAPK signaling pathway, which might facilitate osteoclastogenesis (Xu et al. 2015b). Moreover, serum IL-29 levels were significantly higher in RA patients with knee joint involvement than in RA patient without knee joint involvement (Wu et al. 2013). These reports coincide with our speculation. Based on these findings, we hypothesize that IL-29 has a special role in pathogenesis of anti-CCP-antibodies positive RA and it may be involved in bone destruction around the joints. One possibility is that IL-29 can induce RANKL expression so as to enhance proinflammatory cytokine production in anti-CCP-antibodies positive RA patients. More evidence is needed to prove the mechanism.

Longitudinal follow-up of our RA patients showed that serum IL-29 levels declined after 3 months of treatment with DMARDs, and further after 6 months of treatment. When the RA patients were divided into anti-CCP-antibodies positive group and anti-CCP-antibodies negative group, we found that the serum IL-29 levels of anti-CCP-antibodies negative group did not change after treatment, while the serum IL-29 levels of the anti-CCP-antibodies positive RA patients significantly declined after the 3 and 6 months of treatment with DMARDs. Furthermore, during 1 to 3 months of treatment and 3 to 6 months of treatment, the serum IL-29 levels of anti-CCP-antibodies positive RA patients indicated decline after treatment and paralleling the reduction of disease activity. There was no correlation between the reduction of serum IL-29 levels and the reduction of disease activity at the first month of treatment, which can be explained if a lagging period is needed for the reciprocal changes in the serum IL-29 levels upon the decrease in disease activity. The serum IL-29 levels of anti-CCP-antibodies negative RA patients remained stable after treatment. These results confirm our hypothesis that IL-29 has a special pathogenic role in anti-CCP-antibodies positive RA patients, which renders IL-29 a potential biomarker or therapeutic target for anti-CCP-antibodies positive RA patients. To the best of our knowledge, this is the first time to compare serum IL-29 levels in RA patients before and after the treatment with DMARDs.

To further examine the role of IL-29 in RA, we have compared the serum IL-29 levels at baseline between the ACR20 positive group and the ACR20 negative group. Unfortunately, there was no significant difference between these two groups. Thus, whether IL-29 level can be used as a biomarker for the response to DMARDs remains unclear. We note that this negative result may be due to small sample size (68 RA patients and 68 HC), which is a major limitation of this study. Statistically, a small sample size may lead to a higher deviation of the measurements. We also note that the dose level of DMARDs in the treatment varied among participants.

In conclusion, the serum IL-29 levels of anti-CCP-antibodies positive RA patients were significantly higher than anti-CCP-antibodies negative RA patients and HC. For the first time, the serum IL-29 levels of anti-CCP-antibodies positive RA patients have been found to correlate significantly with the parameters of disease activity and declined significantly after the treatment with DMARDs. The findings indicate that IL-29 is a potential biomarker for disease activity in anti-CCP-antibodies positive RA patients, and they will not only shed light on the pathogenesis of anti-CCP-antibodies positive RA patients, but also aid the development of new therapeutic regimens.

## Acknowledgments

The authors acknowledge all members for their participation in this research.

## Conflict of Interest

The authors declare no conflict of interest.

## References

Abe, T. & Takeuchi, T. (2001) Rheumatoid arthritis and tumor necrosis factor alpha. *Autoimmunity*, **34**, 291-303.

Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham, C.O. 3rd., Birnbaum, N.S., Burmester, G.R., Bykerk, V.P., Cohen, M.D., Combe, B., Costenbader, K.H., Dougados, M., Emery, P., Ferraccioli, G., et al. (2010) 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*., **62**, 2569-2581.

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., Medsger, T.A., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*., **31**, 315-324.

de Groen, R.A., Liu, B.S. & Boonstra, A. (2014) Understanding IFNlambda in rheumatoid arthritis. *Arthritis Res. Ther*., **16**, 102.

Dickensheets, H., Sheikh, F., Park, O., Gao, B. & Donnelly, R.P. (2013) Interferon-lambda (IFN-lambda) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. *J. Leukoc. Biol*., **93**, 377-385.

Donnelly, R.P. & Kotenko, S.V. (2010) Interferon-lambda: a new addition to an old family. *J. Interferon Cytokine Res.*, **30**, 555-564.

Felson, D.T., Anderson, J.J., Boers, M., Bombardier, C., Furst, D., Goldsmith, C., Katz, L.M., Lightfoot, R. Jr., Paulus, H., Strand, V., Tugwell, P., Weinblatt, M., Williams, H.J., Wolfe, F. & Kieszak, S. (1995) American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum.*, **38**, 727-735.

He, S., Li, T., Chen, H., Ma, W., Yao, Q., Yang, H., Wang, H., Wang, F., Zhao, C. & Yang, P. (2011) CD14+ cell-derived IL-29 modulates proinflammatory cytokine production in patients with allergic airway inflammation. *Allergy*, **66**, 238-246.

He, S., Zhang, H., Chen, H., Yang, H., Huang, T., Chen, Y., Lin, J., Wang, F., Chen, X., Li, T.L. & Yang, P. (2010) Expression and release of IL-29 by mast cells and modulation of mast cell behavior by IL-29. *Allergy*, **65**, 1234-1241.

Jordan, W.J., Eskdale, J., Boniotto, M., Rodia, M., Kellner, D. & Gallagher, G. (2007) Modulation of the human cytokine response by interferon lambda-1 (IFN-lambda1/IL-29). *Genes Immun.*, **8**, 13-20.

Kotenko, S.V. (2011) IFN-lambdas. *Curr. Opin. Immunol.*, **23**, 583-590.

Liu, M.Q., Zhou, D.J., Wang, X., Zhou, W., Ye, L., Li, J.L., Wang, Y.Z. & Ho, W.Z. (2012) IFN-lambda3 inhibits HIV infection of macrophages through the JAK-STAT pathway. *PloS One*, **7**, e35902.

Mennechet, F.J. & Uze, G. (2006) Interferon-lambda-treated dendritic cells specifically induce proliferation of FOXP3-expressing suppressor T cells. *Blood*, **107**, 4417-4423.

Mustila, A., Korpela, M., Haapala, A.M., Kautiainen, H., Laasonen, L., Mottonen, T., Leirisalo-Repo, M., Ilonen, J., Jarvenpaa, S., Luukkainen, R. & Hannonen, P. (2011) Anti-citrullinated peptide antibodies and the progression of radiographic joint erosions in patients with early rheumatoid arthritis treated with FIN-RACo combination and single disease-modifying anti-rheumatic drug strategies. *Clin. Exp. Rheumatol.*, **29**, 500-505.

Nell, V.P., Machold, K.P., Stamm, T.A., Eberl, G., Heinzl, H., Uffmann, M., Smolen, J.S. & Steiner, G. (2005) Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. *Ann. Rheum. Dis.*, **64**, 1731-1736.

Ning, X., Jian, Z. & Wang, W. (2015) Low serum levels of interleukin 35 in patients with rheumatoid arthritis. *Tohoku J. Exp. Med.*, **237**, 77-82.

Ruscitti, P., Cipriani, P., Di Benedetto, P., Liakouli, V., Berardicurti, O., Carubbi, F., Ciccia, F., Alvaro, S., Triolo, G. & Giacomelli, R. (2015) Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1beta via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3 (NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. *Clin. Exp. Immunol*., **182**, 35-44.

Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E., Kuestner, R., Garrigues, U., Birks, C., Roraback, J., Ostrander, C., Dong, D., Shin, J., Presnell, S., Fox, B., et al. (2003) IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol.*, **4**, 63-68.

Turk, S.A., van Beers-Tas, M.H. & van Schaardenburg, D. (2014) Prediction of future rheumatoid arthritis. *Rheum. Dis. Clin. North Am.*, **40**, 753-770.

Uze, G. & Monneron, D. (2007) IL-28 and IL-29: newcomers to the interferon family. *Biochimie*, **89**, 729-734.

Wang, F., Xu, L., Feng, X., Guo, D., Tan, W. & Zhang, M. (2012) Interleukin-29 modulates proinflammatory cytokine production in synovial inflammation of rheumatoid arthritis. *Arthritis Res. Ther*., **14**, R228.

Wu, Q., Yang, Q., Lourenco, E., Sun, H. & Zhang, Y. (2011) Interferon-lambda1 induces peripheral blood mononuclear cell-derived chemokines secretion in patients with systemic lupus erythematosus: its correlation with disease activity. *Arthritis Res. Ther*., **13**, R88.

Wu, Q., Yang, Q., Sun, H., Li, M., Zhang, Y. & La Cava, A. (2013) Serum IFN-lambda1 is abnormally elevated in rheumatoid arthritis patients. *Autoimmunity*, **46**, 40-43.

Xu, D., Yan, S., Wang, H., Gu, B., Sun, K., Yang, X., Sun, B. & Wang, X. (2015a) IL-29 Enhances LPS/TLR4-Mediated Inflammation in Rheumatoid Arthritis. *Cell. Physiol. Biochem.*, **37**, 27-34.

Xu, L., Feng, X., Shi, Y., Wang, X., Kong, X., Zhang, M., Liu, M., Tan, W. & Wang, F. (2015b) Interleukin-29 induces receptor activator of NF-kappaB ligand expression in fibroblast-like synoviocytes via MAPK signaling pathways. *Int. J. Rheum. Dis.*, **18**, 842-849.

Zhang, L., Jilg, N., Shao, R.X., Lin, W., Fusco, D.N., Zhao, H., Goto, K., Peng, L.F., Chen, W.C. & Chung, R.T. (2011) IL28B inhibits hepatitis C virus replication through the JAK-STAT pathway. *J. Hepatol.*, **55**, 289-298.