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Concentrations of IL-18 in patients with rheumatoid arthritis — a preliminary study

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ABSTRACT

Objectives. Interleukin-18 (IL-18) is a representative proinflammatory cytokine that plays an important role in the pathogenesis of rheumatoid arthritis (RA). The objective of this study was to evaluate the concentration of IL-18 in patients with RA in relation to RF IgM and anti-cyclic citrullinated peptide antibodies (anti-CCP2).

Design and methods. 75 patients with diagnosed RA (aged 20–80 years) treated with non-steroidal anti-inflammatory (NSAIDs) and disease modifying anti-rheumatoid drugs (DMARDs) and 52 age-matched controls were included.

Results. IL-18 concentration was almost sixfold higher in RA patients in comparison with the reference group (235 pg/mL v. 39 pg/mL). The association between IL-18 and RF IgM and anti-CCP was found.

Conclusions. Serum IL-18 level seems to be positively related to the increased RF IgM and anti-CCP concentrations in patients with established rheumatoid arthritis.

Key words: interleukin-18, rheumatoid arthritis, anti-cyclic citrullinated peptide antibody

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by a chronic inflammation of the synovial joints that leads to a progressive degradation of the articular cartilage and bone. The etiology of RA remains still unknown, yet many studies have indicated that cytokines and inflammatory mediators are significantly involved in the pathogenesis of this disease. It is believed that the initiation of RA is associated with CD45RO+ T lymphocytes response and subsequent releasing of proinflammatory cytokines. The imbalance between pro- and anti-inflammatory cytokines in RA affects the active chronic inflammation and the tissue destruction in the joints of RA patients [1, 2].

Interleukin-18 (IL-18) is a representative proinflammatory cytokine and its role in RA has been extensively studied recently [3, 4]. It is acknowledged that IL-18 is a member of the IL-1 superfamily, which is synthesized as a precursor form (pro-IL-18), and its biological activity depends on the secreted IL-18 binding protein (IL-18BP) and its receptor (IL-18R) [5]. IL-18, first identified as an interferon gamma-inducing factor (IFN-γ IF), stimulates the secretion of IFN-γ and other inflammatory cytokines (TNF-α, IL-1β, IL-6) as well as chemotactic factors participating in the acute-phase response, that play a key role in the regulation of immunity and inflammation in patients with RA [6, 7].

In particular, IL-18 is produced in RA by a number of cells such as macrophages, fibroblasts, articular chondrocytes and osteoblasts, therefore, it is suggested that IL-18 is involved in the destruction of cartilage and bone [8]. Additionally, the administration of IL-18 increased the development of erosive, inflammatory arthritis in a mice model [1, 8]. Moreover, the increased concentrations of IL-18 were demonstrated in the serum, synovial fluid and synovial tissue of RA patients [3]. Notwithstanding these findings, the use of IL-18 as a possible biomarker of disease activity in RA is still controversial [9, 10].

Therefore, the aim of this study was to evaluate the serum concentration of interleukin-18 in patients diagnosed with RA. In addition, we examined the relationship between the concentration of IL-18 and the presence of rheumatoid factor IgM/ and anti-cyclic citrullinated peptide antibodies as well as the serum concentration of high-sensitivity C-reactive protein.

Materials and methods

Patients

The study group consisted of 75 patients (68 females and 7 males, aged 20–80 years) with the established diagnosis of RA. Patients were recruited in the Rheumatology Outpatient Clinic of the University Hospital in Bydgoszcz, Poland. All these patients met the classification criteria of the American College of Rheumatology (ACR). Taking into account the positive or the negative result of RF IgM or anti-CCP, the patients were divided into two groups: seropositive and seronegative. The patients were also divided according to the Steinbrocker criteria into groups at four stages of the disease advancement: I (early) — 11 patients, II (moderate) — 35, III (severe) — 21 and IV (terminal) — 8 patients. In the entire study group the duration of the disease ranged from 1 to 25 years. Most RA patients received disease-modifying anti-rheumatic drugs (DMARDs), i.e. methotrexate, sulfasalazine, gold salts and non-steroidal anti-inflammatory drugs (NSAIDs) as well as glucocorticoids. 13 subjects were treated with DMARDs for the period ranging from 2 months to 25 years, and 10 patients with NSAIDs for the period ranging from 8 months to 14 years. The combination therapy was applied in 11 patients for the period ranging from 3 months to 20 years (DMARDs and NSAIDs), in 19 patients (DMARDs and corticosteroids) and 9 patients (DMARDs, corticosteroids and NSAIDs) for the period ranging from 1 month to 20 years. 13 RA patients received no pharmacological treatment. The clinically healthy volunteers (34 women and 18 men, aged 24–60 years) with no evidence of present inflammatory rheumatic diseases served as the reference group.

The study protocol was approved by the Bioethics Committee at the Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz and it complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects. From all patients and controls an informed written consent was obtained.

Sample collection and analysis

Fasting venous blood samples from patients and controls were collected in the morning. The samples were centrifuged at 3000 g for 15 min, aliquoted, and stored at –80°C until assayed.

The serum concentration of IL-18 was determined by the enzyme-linked immunosorbent assay (ELISA) (human IL-18 ELISA; Bender MedSystems, Vienna, Austria) in compliance with the manufacturer's protocol. The method utilized anti-IL-18 monoclonal antibodies against human IL-18. Samples were run at a 1:2 dilution in an assay with standards ranging between 78.1 pg/mL and 5000 pg/mL. The sensitivity of the assay was 9.2 pg/mL and the reference range was from 55 to 280 pg/mL. The overall intra- and interassay coefficients of variation (CV) were 6.5% and 8.1% respectively. IL-18 concentrations above 280 pg/mL were considered positive.

C-reactive protein (CRP) was measured with a high sensitivity assay using the BN II System nephelometer (N High-Sensitivity CRP; Siemens Healthcare Diagnostics, Deerfield, IL, USA), providing excellent precision with the CV, reported by the manufacturer, of less than 10%. CVs for hsCRP estimated in our laboratory were below 3.5% and < 4.5% for the hsCRP concentrations below 1 mg/L and above 3 mg/L respectively. The lower limit of hsCRP detection was 0.175 mg/L. The normal range of hsCRP was ≤ 3 mg/L. The results above 3 mg/L were considered positive.

IgM rheumatoid factor was measured in serum using the commercially available enzyme immunoassay (IMTEC-IgM-RF; IMTEC Immundiagnostica GmbH, Berlin, Germany). The standards ranged from 12.5 U/mL to 200 U/mL. The results above 15 U/mL for RF IgM were considered positive.

Anti-CCP2 antibodies were determined by ELISA (Anti-CCP ELISA [IgG]; Euroimmun, Luebeck, Germany) in accordance with the manufacturer's instruction. This assay detects human autoantibody of the IgG class against the second generation cyclic citrullinated peptide (CCP2). The serum samples were assayed at a 100-fold dilution with standards ranging from 0 RU/mL to 100 RU/mL (intraassay CV 2.9–7.3%, interassay CV 4.0–8.3%). The lower detection limit of the anti-CCP2 ELISA was 0.05 RU/mL and values over 5 RU/mL were considered positive.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of distribution of the investigated parameters. Data were expressed as medians with the interquartile range (25th–75th percentiles) according to the distributions of continuous variables. The differences between the RA and control groups were analyzed by the Mann-Whitney U-test and the Kruskal-Wallis test for independent samples of non-parametric data, and then with the post-hoc Dunn's test. The association between the variables was calculated with the Spearman correlation coefficient. P values lower than 0.05 were considered statistically significant. The statistical analysis and the sample size calculation were performed using Statistica 10.0 for Windows (StatSoft, Tulsa, OK, USA).

Table 1. Clinical and laboratory characteristics of the study participants

Parameter	RA patients (n = 75)	Reference group (n = 52)	p
Age (years)	55 (46–65)	43.5 (28–50)	< 0.0001
Females n (%)	68 (91)	34 (65)	0.0001
Males n (%)	7 (9)	18 (35)	0.0001
Family history of RA, positive n (%)	42 (56)	0	< 0.0001
Other diseases n (%)	36 (48)	0	0.0003
Disease duration (years)	9.5 (5–16)	NA	NA
Duration of treatment (years)	7 (0.2–11.5)	NA	NA
Stage (Steinbrocker) n (%)			
I	11 (15)	NA	NA
II	35 (47)	NA	NA
III	21 (28)	NA	NA
IV	8 (10)	NA	NA
Treated with: n (%)			
DMARDs	13 (17)	NA	NA
NSAIDs	10 (13)	NA	NA
DMARDs and NSAIDs	11 (15)	NA	NA
DMARDs and corticosteroids	19 (25)	NA	NA
DMARDs, corticosteroids and NSAIDs	9 (12)	NA	NA
Non-treated	13 (17)	NA	NA
IL-18 [pg/mL]	235 (88–428)	39 (20–68)	< 0.0001
CRP [mg/L]	2.86 (1.0–11.6)	0.62 (0.2–1.2)	< 0.0001
RF IgM [IU/mL]	16.8 (9.5–40.7)	3.8 (2.7–6.1)	< 0.0001
Anti-CCP [RU/mL]	1.99 (1.13–83.53)	< 0.05*	< 0.0001

Anti-CCP — anti-cyclic citrullinated peptide antibody; CRP — C-reactive protein; DMARDs — disease-modifying anti-rheumatic drugs; IL-18 — interleukin 18; NA — not applicable; NSAIDs — non-steroidal anti-inflammatory drugs; RA — rheumatoid arthritis; RF — rheumatoid factor; RF IgM — rheumatoid factor in class M; *Anti-CCP below test's sensitivity

Results

Baseline characteristics and results of basic statistics

Baseline characteristics of the RA patients and the control group including demographic data, clinical and laboratory parameters are shown in Table 1. All quantitative variables differed significantly between both investigated groups. Patients with RA, in comparison with the reference group, had significantly higher concentrations of IL-18 and hsCRP as well as higher levels of RF IgM and anti-CCP2. Positive values of IL-18 were found in 43%, RF IgM in 55%, whereas positive anti-CCP2 and hsCRP were found in 48% of patients.

In the course of further analysis, RA patients were divided into groups on the basis of the laboratory parameters. RF IgM (+) patients showed significantly higher concentrations of IL-18, hsCRP and anti-CCP2 than RF IgM (–) patients. However, statistically significant differences between the anti-CCP2 (+) and the anti-CCP2 (–) groups were found only for IL-18 and RF IgM (Tab. 2).

The correlation analysis showed significant relationships between serum IL-18 concentration and other variables in the RA patients. Positive correlation was found for RF IgM ($R = 0.54$, $p < 0.00001$), age ($R = 0.26$, $p = 0.007$), hsCRP ($R = 0.26$, $p = 0.02$) and anti-CCP2 ($R = 0.35$, $p = 0.002$).

Discussion

Our study clearly demonstrated almost sixfold higher median concentration of IL-18 in patients with RA compared to the subjects in the reference group. Additionally, in almost half of the patients, the concentrations of IL-18 were above the levels accepted as normal. The concentration of IL-18 was elevated in seropositive RA patients. Moreover, our study revealed the association between IL-18 and other serological and inflammatory biomarkers of disease activity (RF IgM, anti-CCP2 and hsCRP serum levels).

These results are in line with studies conducted by Shao [6] and Munakata [11]. The increased levels of

Table 2. The values of measured parameters in RF IgM/anti-CCP positive and negative RA patients

Parameter	RF IgM(+) (n = 40)	RF IgM(–) (n = 35)	p
IL-18 [pg/mL]	368 (93–540)	132 (73–245)	0.0005
CRP [mg/L]	5.46 (1.88–14.25)	1.59 (0.68–4.84)	0.01
anti-CCP [RU/mL]	47.02 (1.81–106.36)	1.60 (0.65–1.69)	< 0.0001
Parameter	**anti-CCP(+) (n = 36)**	**anti-CCP(–) (n=39)**	**p**
IL-18 [pg/mL]	392 (179–533)	133 (63–248)	0.0001
CRP [mg/L]	4.96 (1.46–10.65)	2.36 (0.64–11.9)	NS
RF IgM [IU/mL]	39.10 (21.5–110.4)	9.90 (7.8–16.0)	< 0.0001

Anti-CCP — anti-cyclic citrullinated peptide antibody; Anti-CCP(+) — positive anti-cyclic citrullinated peptide antibody; Anti-CCP(–) — negative anti-cyclic citrullinated peptide antibody; CRP — C-reactive protein; IL-18 — interleukin 18; NS — not significant; RA — rheumatoid arthritis; RF IgM — rheumatoid factor in class M; RF IgM: RF IgM(+) — positive rheumatoid factor in class M; RF IgM(–) — negative rheumatoid factor in class M

serum IL-18 in patients with RA have also been observed in other studies [12, 13].

The increased levels of IL-18 are primarily associated with chronic inflammation in the autoimmune diseases [14]. IL-18, described for the first time by Gracie et al. [15, 16], was significantly elevated in the rheumatoid synovium, which may confirm its contribution to the development of arthritis in RA and the destruction of cartilage [17, 18].

According to Joosten et al. [19], elevated IL-18 levels are detected in 80% of patients with RA, which may result from the association between IL-18 gene polymorphisms and the occurrence of individual susceptibility to RA [1, 8, 12], but the exact contribution of genetic predisposition to the RA development has not been well recognized and needs further research. Additionally, the markedly higher IL-18 concentration in RA than in OA [20] and psoriatic arthritis [21] indicates the increased local production of this cytokine [20]. High expression of IL-18 by synoviocytes in RA may regulate the production of pathogenic cytokines responsible for the local inflammatory process [10].

In our study, seropositive RA patients were characterized by higher concentrations of IL-18 than seronegative RA patients, which is consistent with other reports and may suggest that IL-18 can be a sensitive marker for monitoring disease activity [20, 21]. Autoantibody production in patients is a common symptom of RA [22]. Currently, RF and anti-CCP antibodies are the gold standard in the diagnosis of RA, and, due to the high sensitivity and specificity, are incorporated into the ACR classification criteria for RA [22–24]. Also, the synovial tissue from anti-CCP positive patients expresses higher concentration of immune cytokines and shows a greater degree of joint destruction than the tissue from anti-CCP negative patients [25, 26]. These findings are in agreement with our observation that IL-18 levels correlate with the serological markers of disease activity (anti-CCP and RF IgM). On the contrary, Chang et al. [27] have not found any significant relationship between serum IL-18 and RF, thus the role of IL-18 as a marker of disease activity is still controversial and requires further, extended studies.

Currently, CRP is one of the best indicators of the acute phase response to inflammation [28]. Indeed, it is widely acknowledged that the serum CRP concentrations are typically increased during a disease, making this inflammatory biomarker very useful for the diagnosis and monitoring of RA.

Some researchers did not present a statistically significant correlation between the serum IL-18 levels and the CRP concentration, thus suggesting that IL-18 may have a limited ability to induce acute phase proteins and other mediators of the inflammation. Furthermore, the slight decrease in the concentration of IL-18 following anti-rheumatic drug therapy, in contrast to CRP, may suggest that the acute phase response may be largely independent of IL-18 [21]. However, we demonstrated a statistically significant correlation between the level of IL-18 and hsCRP concentration. In patients with RA the inflammatory process of insignificant intensity may occur, thus it seems reasonable to measure CRP with a high sensitivity assay [29, 30]. The correctness of the choice of this assay for the determination of CRP is corroborated by the fact that the concentration of hsCRP above 3 mg/l was found in only 48% of patients with RA. Our results suggest that the determination of CRP, which is, in fact, the most recommended marker of the acute inflammation in RA, by means of using a high sensitivity assay may be useful to monitor the course of disease in RA patients, which was also previously suggested by other authors [22, 29, 31].

According to Chang et al. [17], IL-18 levels may correlate with the indicators of local inflammatory response. Moreover, in patients with advanced RA, in addition to IL-18 and CRP, the increased concentrations of other

proinflammatory cytokines, such as IL-15 and TNF-α, were found in the serum and the synovial fluid [21]. Rooney et al. [32] have also observed an increased expression of IL-18 in the synovium of RA patients compared to patients with OA. Furthermore, the expression of IL-18 in the articular synovial tissue does not correlate with the serum IL-18 concentration [33]. In contrast, in another study, a significant relationship was found with other cytokines, such as IL-1β and TNF-α, and also CRP, which may suggest that the expression of IL-18 is associated with other cytokines and with the local inflammatory process in the synovium of RA patients [32, 34]. Additionally, its increased concentration in the synovium correlates with the acute phase response, which demonstrates that IL-18 is a major proinflammatory cytokine in RA, thus leading to a local production of IL-1β and TNF-α involved in the synthesis of C-reactive protein [19].

Although among inflammatory biomarkers only CRP is recommended as a useful parameter of disease activity and treatment response, it is recognized that the combination of biomarkers will greatly facilitate the diagnosis of RA. International guidelines recommend the use of anti-CCP, RF and CRP as the most useful combination of biomarkers in RA [23, 25]. The fact whether the addition of IL-18 to a combination of anti-CCP and RF IgM might have an added value and a beneficial impact on the diagnosis and evaluation of disease activity still requires further studies. However, our results indicate that this marker has certain potential and its diagnostic value in RA has not been comprehensively used so far.

Our study has some limitations to be considered. Our results in this study should be carefully interpreted because the number of subjects included was relatively small. However, this is a preliminary study which we intend to continue further.

Conclusion

This study implies that IL-18 is associated with the joint inflammation and the cartilage destruction in RA patients, but the exact mechanism of this pathway is not well understood and needs further studies. The elevated IL-18 concentration in RA, especially in seropositive individuals, suggests that IL-18 could possibly represent an important biomarker, which may be clinically relevant for the development of the new diagnostic strategy.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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