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RESEARCH ARTICLE

Circulating cytokine pattern and factors describing rheumatoid arthritis: IL-15 as one of the biomarkers for RA?

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Abstract

The aim of study was to examine relationship among levels of cytokines (IL-6, IL-13, IL-15, TNF-α) and chemokine (IL-8), production of autoantibodies, radiographic progression, and factors describing rheumatoid arthritis (RA). A total of 156 RA patients according to ACR criteria, and 55 control subjects were recruited into study. We observed higher levels of IL-15 within RA patients compared to healthy controls. Correlations among cytokine levels and the measures of rheumatoid factors, anti-CCP, measures of disease activity, and radiographic progression were observed. We conclude that IL-15 level in circulation could serve as one of the biomarkers for RA detection.

Keywords: Rheumatoid arthritis, cytokine, interleukin, circulating level

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease affecting at least twice as many women as men. Although it may begin at any age, its peak onset is in the fourth and fifth decades of life. Initial disease symptoms are joint pain and swelling, accompanied by joint stiffness, and in most cases with the overall response of the organism. The synovial membrane of joints, tendons and bursae are places of the pathological inflammation (Goldring 2003). The course of RA is highly variable, but generally the disease is progressive. Chronic synovitis leads to the destruction of articular cartilage, subchondral bone erosions and finally to severe deformities of the affected joints.

The pathogenetic background of RA is strongly associated with the immune mechanisms, with the key role of T-lymphocytes, B-lymphocytes, neutrophils, macrophages, fibroblasts and endothelial cells, and substances affecting the immune system including chemokines and cytokines. The great attention is paid to the role of CD4+ Th cells which can develop into at least four types of committed helper T cells: Th1, Th2, regulatory T cells (Treg), and Th17. The last mentioned lineage of the Th cells probably plays a key role in the induction of autoimmune diseases such as rheumatoid arthritis (Gaffen 2004, Afzali et al. 2007). Until recently, it was assumed that Th1 cells play the major role in inflammation with autoimmune component (Figure 1). The paradigm has been changed, and RA seems to be a result of deregulation of immune responses with preferential activity towards pro-inflammatory lineages (Th1 and Th17) and attenuation towards the anti-inflammatory ones (Treg and Th2). Essential role in the pathogenesis of RA have the Th1 cytokines (e.g. TNF-α, and interferon (IFN)-γ), Th2 cytokines (e.g. IL-10, and IL-13), pro-inflammatory cytokines (e.g. IL-6), chemokines (e.g. IL-8, macrophage

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Abbreviations

ANA: antinuclear antibodies
Anti-CCP: anticyclic citrullinated peptide
CRP: C-reactive protein
DAS28: 28-joint disease activity score
ELISA: enzyme-linked immunosorbent assay
HAQ: Health Assessment Questionnaire
IFN: interferon
Ig: immunoglobulin
IL: interleukin
MCP: macrophage chemotactic protein
NK: natural killer
RA: rheumatoid arthritis
RF: rheumatoid factor
STAT: signal transducer and activator of transcription
TNF: tumour necrosis factor
TSS: total sharp score

chemotactic protein-1 (MCP-1)), and regulators of T- and natural killer (NK) cell activation and proliferation (e.g. IL-15).

The local release of cytokines with a central role of IL-6 could be the basis of this mechanism (Afzali et al. 2007). IL-6 is a pleiotropic cytokine with various biological

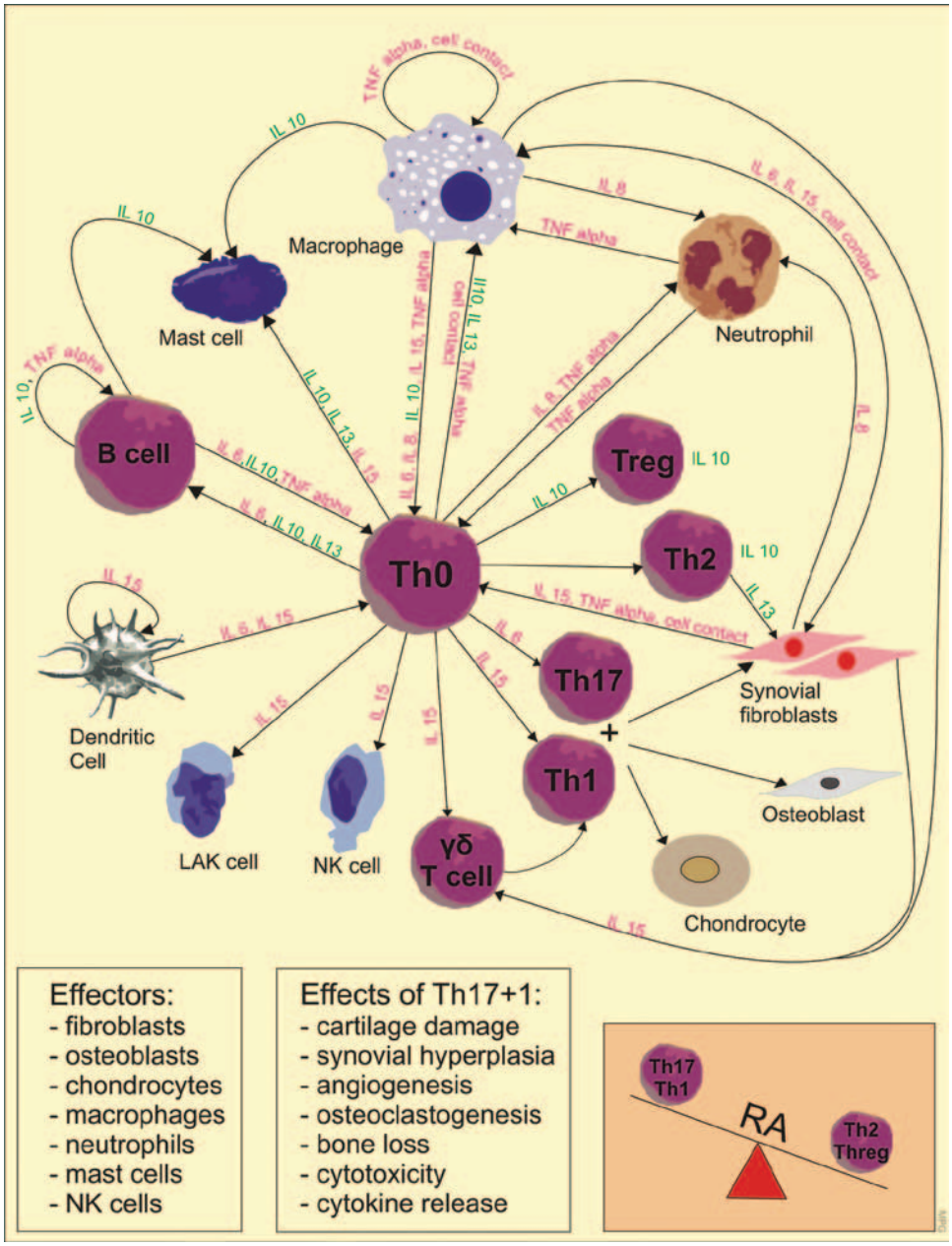

Figure 1. Modulation of effector function in rheumatoid arthritis through the release of cytokines. Only cytokines and chemokines analyzed in present study were included into the model. DC, dendritic cell; IL, interleukin; LAK cell, lymphokine-activated killer cell; NK cell, natural killer cell; Th cell, T helper cell; TNF, tumour necrosis factor.

activities, including among others control of immune responses, hematopoiesis, and inflammation, especially systemic acute phase reaction. IL-6 promotes together with TGF-β the differentiation of Th17 from the naive T cells and also inhibits TGF-β-induced differentiation of Treg (Bettelli et al. 2006, Mangan et al. 2006, Veldhoen et al. 2006). Moreover, IL-17 mediates chemotaxis of neutrophils and monocytes to sites of inflammation through the chemoattractant IL-8 and MCP-1 (Witowski et al. 2000, Laan et al. 2001, Miyamoto et al 2003), which reflects the role of IL-8 in the initiation and amplification of acute inflammatory responses and in chronic inflammation in RA. The production of the chemokines IL-8 and monocyte chemotactic protein-1 from monocytes, which plays important role in the regulation of leukocyte infiltration during inflammation, is referred to be stimulated by IL-15 (Badolato et al. 1997). IL-15, a member of the IL-2 family of cytokines, is produced by activated monocytes, macrophages, epithelial cells, fibroblasts (Badolato et al. 1997, McInnes & Liew 1998) endothelial cells (Oppenheimer-Marks et al. 1998), and chondrocytes (Woolley & Tetlow 2000).

The aim of the study was to describe the role of circulating cytokines in RA etiology and disease progression, and to investigate the cytokine pattern in association with factors describing RA.

Materials and methods

Patient population

A total of 156 patients with RA diagnosed according to the revised classification criteria of the American College of Rheumatology were consecutively recruited into the study over a 3-year period. Patients with RA were recruited from the outpatient populations of the Rheumatology Division, 2nd Department of Internal Medicine, St. Anne's University Hospital, Brno. All subjects were Caucasians from Moravian regions of the Czech Republic. Exclusion criteria were: age > 80 years; known or newly diagnosed malignancy; estimated life expectancy < 12 months; refusal to provide written consent or non-compliance; clinical signs of acute infection at time of blood sampling. The control group consists of 55 unrelated volunteers (age: median value – 47 years; 42% of postmenopausal women) of Caucasian origin from Moravian region with sex proportion 3:1 in favor of women, no clinical signs of RA, no chronic medication and with negative laboratory parameters (negative RFs, negative anti-CCP and CRP <5 mg/L).

The study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno. Inclusion of individuals to the study was conditioned by obtaining a written informed consent form. The study was in agreement with the Declaration of Helsinki approved at the World Medical Association meeting in Edinburgh.

Blood samples were obtained from all patients at the time of clinical examination for determination of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), presence of rheumatoid factors (RFs), and anti-cyclic citrullinated peptide autoantibody (anti-CCP). Serum levels of IgM RF, IgA RF, and IgG RF isotypes were determined using an ELISA assay (Aeskulisa Rf – A, G, N; Aesku Diagnostics, Wendelsheim, Germany). Serum levels of anti-CCP were measured by ELISA assay (ELISA KIT anti-CCP, Genesis Diagnostics, Cambridgeshire, UK).

Radiographic analysis

All patients enrolled in the study underwent radiographs of the hands. X-ray findings of hands were scored by one observer using the modified Sharp/van der Heijde method. The Total Sharp Score (TSS) and the annual radiographic progression rate from the disease onset (TSS/year) were calculated. Median of TSS/year was 1.11. All RA patients were classified into two groups according to the annual radiographic progression rate: those with TSS/year ≤1.11 and those with TSS/year > 1.11.

Cytokine levels in serum

IL-6, IL-13, IL-8, IL-15, and TNF α levels were measured in fasting serum samples frozen immediately after sampling and then stored in –80°C awaiting analysis. The samples were thawed and analyzed using IL-8 (sensitivity <5 pg/mL, 0–1000 pg/mL, no dilution; BioSource, Camarillo, CA, USA), IL-10 (<1 pg/mL, 0–500 pg/mL, no dilution; BioSource, Camarillo, CA, USA), IL-13 (<6 pg/mL, 0-2000 pg/mL, dilution: 1:1; BioSource, Camarillo, CA, USA), IL-15 (<10 pg/mL, 0-2500 pg/mL, dilution: 1:1; BioSource, Camarillo, CA, USA) ELISA kits. Immulite TNF α assay (2–1000 pg/mL; DPC Biermann, Bad Nauheim, Germany), Immulite IL-6 assay (2–2000 pg/mL; DPC Biermann, Bad Nauheim, Germany) were used. Intra-assay CV and inter-assay CV for all analyses were <10%.

Statistical analysis

Within the case and control groups and groups of RA patients, the Kruskal–Wallis ANOVA test was used for the evaluation of significant differences in variables. Correlation analyses were performed using two-tailed Spearman's rank correlation. Bonferroni correction was applied to adjust the α level according to the number of independent comparisons. ROC analysis was adopted for the identification of cut-offs of biomarkers for RA detection and description of their sensitivity and specificity. Predictive capability of IL-15 was analysed by means of reclassification analyses NRI (net reclassification improvement) and IDI (integrated discrimination improvement).

Statistica v. 9.0 (Statsoft Inc., Tulsa, OK) and SPSS 20.0.0 (IBM Corporation) were used to analyze the data.

Results

Serum levels and clinical parameters were obtained for the 144 patients of the study group. Baseline characteristics of RA patients are shown in Table 1.

Circulating levels of cytokines and RA

Higher circulating levels of IL-15 were observed for RA patients compared to healthy controls (IL-15: median: 167.8 pg/mL; 5% and 95% percentile: 0.0–2500.0 pg/mL versus 7.8 pg/mL; 5% and 95% percentile: 0.0–97.5 pg/mL; $p < 0.01$). No significant differences in TNF-α, IL-6, and IL-13 cytokine circulating levels between RA patients and controls were found (TNF α median value: 9.75 pg/mL versus 8.15 pg/mL; IL-13 median value: 2.06 pg/mL versus 1.3 pg/mL; IL-6 median value: 5.1 pg/mL versus 2.0 pg/mL).

Circulating levels of cytokines and clinical parameters of RA

We found the significant correlation among circulating level of IL-15 and the measures of rheumatoid factors (total and specific isotypes: IgA, IgM, IgG) and anti-CCP (Table 2). Correlation with the same parameters was found for circulating level of TNF-α. Further, the correlation of circulating level of IL-6 with almost all clinical parameters of disease activity (DAS28 and TSS) in RA patients, acute phase reactant CRP, and anti-CCP was observed (Table 2). The IL-8 level was, except the IL-6 level, the only parameter that correlated with measures of disease activity (DAS28, TSS), swollen joint count, and the measure of anti-CCP. Other cytokines did not show a significant correlation with clinically assessed disease activity; the IL-10 level correlated with the anti-CCP measurement, and the IL-13 level with radiographic progression. The correlations were strongest for IL-15 versus the laboratory-based rheumatoid factors: RF r = 0.7111 ($p < 0.001$), RF IgM r = 0.6678 ($p < 0.001$), and RF IgG r = 0.6271 ($p < 0.001$). Furthermore, for the IL-6 versus the laboratory-based marker of acute phase reaction: CRP r = 0.6078 ($p < 0.001$).

The relationship among cytokine circulating levels is shown in Table 3. According to receiver operating characteristics (ROC) analysis we identified cut-off value to predict RA for IL-15 and compared Area under the curve (AUC) for IL-15 with AUC of biomarker anti-CCP (Table 4).

When the group of RA patients was divided into three subgroups according to the EULAR classification

Table 1. Demographic, clinical, and laboratory characteristics of rheumatoid arthritis (RA) patients.

Characteristics	Values	n
Age (year)	55.6 (20.2–82.1)	144
Sex (women)	[a]112 (77.8)	144
Disease duration (year)	11.0 (2.0–50.2)	144
Swollen joints	6 (0–24)	144
Tender joints	5 (0–26)	144
DAS 28	3.88 (1.03–7.58)	144
TSS/year	1.11 (0–11.59)	144
IgM RF positivity	[a]91 (63.2)	144
IgA RF positivity	51 (35.4)	144
IgG RF positivity	71 (49.3)	144
Anti-CCP positivity	89 (61.8)	144

Values are given as median (range) or as [a]number (percentage).
anti-CCP, anticyclic citrullinated peptide; DAS28, disease activity score 28; RF, rheumatoid factor; TSS/year, the annual radiographic progression rate of the Total Sharp Score.

Table 3. Correlations among serum IL-6, TNF α, IL-8, IL-10, IL-13, and IL-15 levels*.

N = 144	IL-6	TNF-α	IL-8	IL-10	IL-13
TNF-α	**0.1921; 0.02**				
IL-8	**0.2621; 0.001**	0.0214; 0.80			
IL-10	**0.1812; 0.03**	**0.2110; 0.01**	0.0942; 0.26		
IL-13	–0.0669; 0.42	–0.1153; 0.17	0.0638; 0.45	0.1058; 0.21	
IL-15	0.1233; 0.14	**0.3700; <0.001**	–0.0356; 0.67	**0.2433; 0.003**	0.1469; 0.08

*Spearman rank order coefficients are shown in the corresponding cells, results with $p < 0.05$ are in bold; results with $p < 0.05$ with Bonferroni correction are in bold and underlined.
IL, interleukin; TNF, tumour necrosis factor.

Table 2. Correlations among clinical measurements of disease activity and serum IL-6, TNF α, IL-8, IL-10, IL-13 and IL-15 levels*.

	Acute inflammation	Auto-antibodies	RF and isotypes				Disease activity			Quality of life	
N = 144	CRP, mg/L	Anti-CCP	RF	RF IgG	RF IgA	RF IgM	TTS	DAS28	X-ray st.	HAQ	Euroqol
IL-6	**0.6078; <0.001**	**0.4149; <0.001**	0.1534; 0.15	**0.2097; 0.01**	**0.2408; <0.001**	**0.1820; 0.03**	**0.3441; <0.001**	**0.5052; <0.001**	**0.2970; <0.001**	**0.2970; <0.001**	–0.0924; 0.25
TNF-α	0.1327; 0.11	**0.2017; 0.02**	**0.2451; 0.02**	**0.3861; <0.001**	**0.4468; <0.001**	**0.4165; <0.001**	0.1481; 0.08	0.0248; 0.77	0.1212; 0.15	0.1000; 0.26	–0.0751; 0.31
IL-8	0.0924; 0.27	**0.1775; 0.03**	0.0326; 0.76	0.0942; 0.26	0.0521; 0.54	0.0781; 0.35	**0.1727; 0.04**	**0.1779; 0.03**	**0.1685; 0.05**	0.0048; 0.96	–0.0114; 0.86
IL-10	0.0535; 0.52	**0.1789; 0.03**	0.1761; 0.10	0.1374; 0.10	**0.1775; 0.03**	0.1604; 0.06	0.1368; 0.10	–0.0302; 0.72	0.1128; 0.18	0.0488; 0.59	0.0314; 0.62
IL-13	0.0560; 0.50	0.0107; 0.90	**0.2324; 0.03**	0.1209; 0.15	0.0743; 0.37	0.1390; 0.09	–0.1039; 0.22	0.0745; 0.37	**–0.1745; 0.04**	0.0293; 0.74	0.0368; 0.81
IL-15	0.0954; 0.25	**0.3309; <0.001**	**0.7111; <0.001**	**0.6271; <0.001**	**0.5825; <0.001**	**0.6678; <0.001**	0.0616; 0.46	0.0026; 0.97	0.0367; 0.66	–0.0367; 0.66	–0.0826; 0.42

*Spearman rank order coefficients are shown in the corresponding cells with p value under the coefficient, results with $p < 0.05$ are in bold; results with $p < 0.05$ with Bonferroni correction are in bold and underlined.
Anti-CCP, anticyclic citrullinated peptide; CRP, C-reactive protein; DAS28, 28-joint disease activity score; HAQ, Health Assessment Questionnaire; Ig, immunoglobulin; IL, interleukin; TSS, total sharp score; TNF, tumour necrosis factor.

Table 4. Receiver operating characteristics analysis for prediction of RA.

	AUC (95% CI)	Sig.	Cut off	Sensitivity	Specificity
IL-15 (ng/mL)	0.800 (0.740; 0.861)	<0.001	≥22.0	74.8%	70.4%
Anti-CCP (U/ml)	0.807 (0.748;0.867)	<0.001	≥0.0	69.4%	85.2%

Anti-CCP, anticyclic citrullinated peptide.

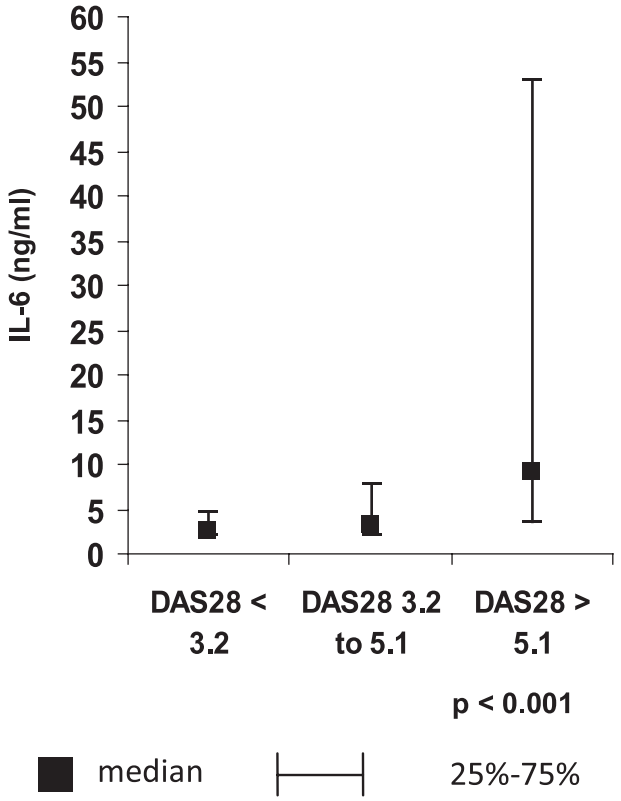

Figure 2. Levels of IL-6 in subgroups according to the EULAR classification of disease activity (low disease activity: DAS28 ≤ 3.2, moderate disease activity: DAS28 3.2 to 5.1, high disease activity: DAS28 > 5.1). DAS28, 28-joint disease activity score.

(van Gestel et al. 1996) of disease activity (low disease activity: DAS28 ≤ 3.2, moderate disease activity: DAS28 3.2 to 5.1, high disease activity: DAS28 > 5.1), increased circulating levels of IL-6 were found in the group with high disease activity ($p < 0.001$) (Figure 2). No significant differences were observed for the other cytokines.

We found significantly higher circulating level of TNF α ($p < 0.001$), and lower circulating level of IL-13 ($p < 0.01$) in patients on anti-TNF biologic agents (Figure 3A and 3B). Further cytokine profiles were similar between patients with and without treatment by biologic agents.

Discussion

Cytokines and chemokines are peptide, protein or glycoprotein mediators produced primarily by immune cells, but also by other cells and tissues in response to a specific signal, operating on the target cells via cytokine receptors through which induce intracellular signal transduction-mechanisms. In this work, the IL-6 and IL-15 levels in RA patients correlated with anti-CCP and RF IgA, which can both be detected several years before any symptoms of RA, although IgA-RF does not reach as high specificity for RA as anti-CCP antibody (Rantapää-Dahlqvist et al. 2003). In agreement with published results, we observed higher circulating IL-6 levels in RA patients with high disease activity (DAS > 5.1), the extent of acute phase reaction (CRP), and the number of swollen joints. Also, the HAQ score as a measurement of functional disability reflects the progression of RA. Overproduction of IL-6 and abnormalities in IL-6 signalling are likely, according to recent research, causal factors in the development and progression of autoimmune diseases including RA. IL-6 activates both signal transducers and activators of transcription - STAT3 and STAT1; STAT3 activation is maintained while STAT1 activation is suppressed in Th17 cells (Kimura et al. 2007). Further positive or negative regulation of Th17 development is due to activation of STAT by

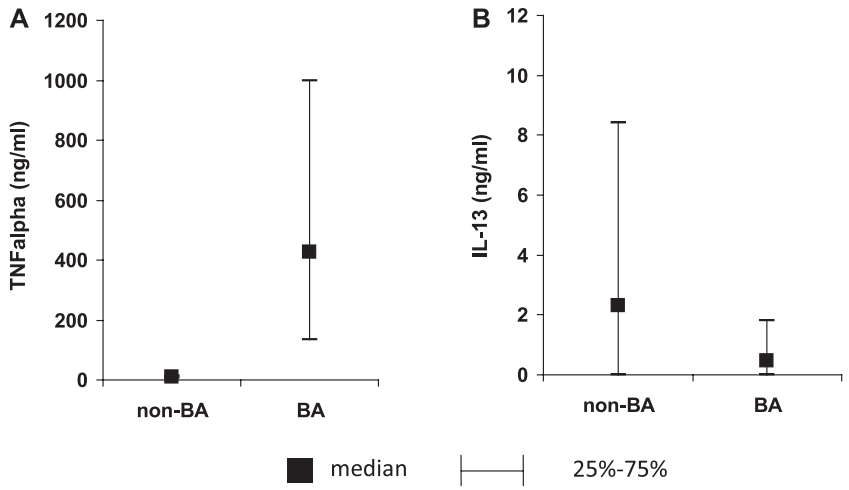

Figure 3. Levels of tumour necrosis factor (TNF) α (A) and IL-13 (B) in patients on anti-TNF biologic agents.

various cytokines. IL-17 produced by Th17 cells during active RA stimulates monocytes and macrophages to synthesize IL-1β and TNF-α, which act synergistically, and stimulate synovial cells to produce also IL-6, IL-8 (Gaffen 2004). This stimulation could explain the similar disease pattern in circulating levels of IL-8 in our study. The production of IL-6 reflects the acute phase response and disease progression in RA, the IL-15 levels show rather relation to specific inflammation. Recent studies on RA indentified production of IL-15 in $CD68^+$ lining cells (McInnes et al. 1996), macrophages, T cells and natural killer cells in affected synovial tissues (Thurkow et al. 1997). IL-15 is generally accepted as a Th1 cytokine but recently, Harris 2011 reported evidence that IL-15 promotes Th17 and Th1 responses. Generally, IL-15 regulates the activation, proliferation and cytokine release from the T cells, natural killers, mast cells (Bamford et al. 1994, Tagaya et al. 1996, Tagaya et al. 1996, Carson et al. 1997); enhances neutrophil and natural killer cell responses (enhances cytotoxicity and up-regulates NK cell survival and production of NK cell-derived cytokines -γ, and TNF-α (Becknell & Caligiuri 2005, Budagian et al. 2006); stimulates locomotion and chemotaxis of normal T cells; regulates proliferative renewal of memory $CD8^+$ T cells in periphery; contributes to B-cell proliferation and immunoglobulin (Ig) synthesis (McInnes et al. 1997). Further, IL-15 increases the production of IL-17 as well as the other cytokines except IFN-γ (Harris 2011). From the other course, regarding the Th2 role, it has been reported that IL-15 promotes the survival of B cells (Armitage et al. 1995), mast cells (Masuda et al. 2001), and eosinophils (Hoontrakoon et al. 2002, Mori et al. 1996). In accord with biological effects of Th17 and Th1 responses, our results show a strong correlation of circulating levels of IL-15 with the rheumatoid factors (RF, including isotypes IgG, IgA, IgM). IL-15 stimulates peripheral blood T cells to induce monocytic TNF-α production (McInnes et al. 1997, Sebbag et al. 1997). In consensus to published data (Park et al. 2011), we have found higher circulating levels of IL-15 in patients with RA compared to the control population. This finding can reflect important role of IL-15 in the circulation of patients with RA, with respect to presented relation to the TNF-α levels and similar pattern of relation to rheumatoid factors. The continuing influence of IL-15 on newly recruited T cells in the synovial membrane results in production of TNF-α directly or via cell contact with macrophages. This leads to amplification of inflammation by the T cells, which can be enhanced by TNF-α and IL-6 (Sebbag et al. 1997). TNF-α further up-regulates production of other cytokines, including IL-6, IL-8, and IL-10 (Feldmann et al. 1996, Brennan et al. 1998). Furthermore, the level of IL-15 in the synovial fluid was reported to be significantly higher in RA compared to serum (Park et al. 2011). The effect of IL-15 in RA is enhanced by the synovium by means of expression from fibroblast-like synoviocytes and cells in the lymphocytic infiltrates in both the lining layer and the sublining layer (Park et al. 2011). So, the IL-15 function in the circulation is primarily fundamental for the development of inflammation in the joint by lymphocytic infiltration, followed by increased local production of IL-15 in the affected joint related to the severity of RA. This is supported by the results of work by Park et al. 2011, where the count of cells producing IL-15 depend on severity of RA, and could be related to osteoclastogenesis and bone loss. On the other hand, it was reported that the local synovial fluid IL-15 concentrations do not correlate with parameters of disease activity or with the response to drug treatment in patients with RA (McInnes et al. 1996), which once more stresses the role of IL-15 in the circulation. Our hypothesis of the primary role of IL-15 derived from circulation in the first phase of joint affection is supported also by our results, indicating that the levels do not reflect the difference in the parameters of disease severity, such as radiographic progression, and disease activity measures (TSS, DAS28). Moreover, IL-15 is a potent inducer of IL-10, which increases the NK cell cytotoxicity (Park et al. 2011) and up-regulates farther cell-derived cytokine production.

Widely discussed is the relative imbalance of pro-inflammatory and anti-inflammatory cytokines in RA, with high levels of TNF-α and IL-6, and lower levels of IL-13 (Pawlik et al. 2005). In consensus with this data we observed increased circulating levels of TNF-α and decreased circulating IL-13 levels in patients treated by anti-TNF biological agents that are the most broadly used biologics for the treatment of RA, and results in rapid improvement in clinical symptoms, and delay radiographic progression. We have associated the IL-13 levels to radiographic progression, with the lowest levels in the erosive stage of RA. IL-13 acts as an anti-inflammatory cytokine and regulates B-cell and mast cell proliferation, IgG, IgE and antigen MHC class II expression, and inhibition of cytokine production by Th1-related pathway (Brombacher 2000) among others by the differential regulation of TNF-α (Brombacher 2000, Wynn 2003).

In conclusion, our results show correlation of IL-8 and IL-6 circulating levels with measures of disease activity (DAS28 and TSS), swollen joint count, and anti-CCP, and correlation of IL-13 to radiographic progression of RA. Further, we present higher levels of IL-15 in RA patients compared to healthy controls, and a correlation of circulating levels of IL-15 with the rheumatoid factors including subtypes (RF, IgG, IgA, IgM), with relation to TNF-α levels. Both IL-15 and TNF-α were correlated with RF, but TNF-α correlation was less significant. The relation disappeared completely in anti-CCP negative subgroup of RA patients, and we did not find any significant differences in levels of TNF-α between these two subgroups (anti-CCP positive subgroup: median 8.45 pg/mL; anti-CCP negative subgroup: 10.5 pg/mL; $p = 0.14$). The IL-15 correlation to RF was significant for both subgroups of RA patients (anti-CCP positive subgroup: $r = 0.738$, $p < 0.001$; anti-CCP negative subgroup: $r = 0.665$, $p < 0.001$). Although we are aware that the interaction of protein secretion of genes from family of cytokines and downstream proteins may

co-determine the disease phenotype, the IL-15 function in the circulation seems to be primarily fundamental for the development of inflammation in the affected joints by promoting lymphocytic infiltration. Regarding the IL-15 contribution to immunoglobulin synthesis, we conclude that the IL-15 level in circulation could serve as one of the biomarkers for the RA detection with cut off level 22.0 pg/mL with sensitivity of 74.8% and specificity 70.4% comparable to anti-CCP with sensitivity of 69.4% and specificity 85.2%. The sensitivity is higher even compared to RFs IgM (63 %), IgG (47 %), and IgA (34 %). Furthermore, predictive capability of IL-15 was compared to a basic model comprising anti-CCP and RF by means of reclassification analyses NRI (net reclassification improvement) and IDI (integrated discrimination improvement). In comparison to the basal model, sum of sensitivity and specificity increased by 0.046 (–0.023; 0.113), $p = 0.190$ (based on NRI) and difference in average probability of patients inclusion into RA/non-RA group increased by 0.058 (0.022; 0.094), $p = 0.002$ (based on IDI). The analyses show that IL-15 enhances the model containing two commonly used predictors: RF and anti-CCP (cut-off level 0 U/mL). On the other hand, IL-15 is a non-specific marker that is also elevated in numerous autoimmune and inflammatory diseases, and cancers. Kakumu et al. 1997 reported elevated levels of IL-15 in type C chronic liver disease with the highest levels in hepatocellular carcinoma (mean value 77.4 pg/mL), and with decreasing tendency after interferon treatment. The majority of the baseline IL-15 values in asymptomatic HCV, chronic hepatitis and liver cirrhosis were under the value 22 pg/mL. Recent study in psoriatic arthritis showed increased levels of IL-15 (median value 114 pg/mL) with association to ectopic lymphoid neogenesis (median value 180 pg/mL) (Celis et al. 2012). Moreover, regarding to the therapeutic intervention on the level of IL-15, the human anti-IL-15 monoclonal antibody has been shown to be effective in a xenograft mouse disease models (Villadsen et al. 2003) and in subjects with rheumatoid arthritis (Baslund et al. 2005), causing a decrease in NK cell proliferation and antigen-driven T-cell responses (Mortier et al. 2004, Ruckert et al. 2005).

We conclude that determination of IL-15 circulating levels could increase the likelihood of RA detection. Furthermore, according to our results, the increased level of IL-15 is not linked to the disease activity. Cytokines IL-6 and IL-13 were in our study associated to disease activity and progression.

Declaration of interest

The study was supported by a Grant of Ministry of Education 0021624202. The authors report no conflicts of interest.

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