

INFLAMMATORY RESPONSE IN RHEUMATOID ARTHRITIS

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Summary: The aim of the research is to determine the clinical significance of cytokines TNF-alpha, IL-12, IL-15 and IL-18 in evaluation of the activity of rheumatoid arthritis (RA). By comparing the concentrations in 30 patients with high, 14 patients with moderate and 20 patients with mild activity of RA it is established that the patients with high degree of disease activity, have significantly high ($p < 0.01$; $p < 0.05$) concentrations of examined cytokines and rheumatoid factor in blood and synovial fluid as well as C-reactive protein in serum in relation to patients with moderate and mild active disease. We have concluded that the cytokines concentrations can be good indicators of the degree of the general activity of RA. This research can contribute to interpretation of insufficiently well known views of pathogenesis role of cytokines in active disease.

Key words: cytokines, arthritis, rheumatoid factor, C-reactive protein

Introduction

Rheumatoid arthritis (RA) is chronic inflammatory disease of unknown etiology and multifactor pathogenesis, of changing clinical course and unpredictable prognosis in which base is the progressive destructive synovitis and the impairment of the joint structure (1). Symmetric pains and swelling of small peripheral joints, long-term morning stiffness, general symptoms, the occurrence of rheumatoid nodules, rheumatoid factor (RF) in blood and characteristic radiological changes in joints are the primary clinical features of the disease. The appearance of extraarticular manifestations, autoimmunoglobulin antibodies and immune complexes in the circulation with activation of the complement system imply to systemic nature of the disease, which justifies the term rheumatoid disease (2). The most epidemiological, pathophysiological, immunological and genetic aspects of the disease are well known, but the precise starter and factors, which maintain the destructive inflammatory process, still haven't been determined. Because of that it is not

unusual to claim that RA is the disease with many causes or many diseases with one cause (2, 3).

Immunopathological process in synovial tissue develops in five phases (4): in the first phase the presentation and recognition of the unknown antigen is being done by T lymphocyte; in the second phase occurs the proliferation of T and B lymphocytes, migration of inflammatory cells in to the joint and angiogenesis. The cells components of the joint react to the injury by changing the functional profile. Changes occur in endothelium of small blood vessels and hyperplastic reaction of synovia with proliferation of synovial cells – the third immunological phase of the disease. In the fourth phase of the disease the panus is formed. It is metabolically active and autonomous joint tissue. Chondrocytes and osteoclasts are activated. Cytokines interleukine (IL)-1, the tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (INF-gamma) and many others, which are produced by macrophages and activated T cells, stimulate synovial cells to create hydrolytic enzymes, proteinases. The initial damage of the joint occurs. In the fifth immunological phase many mediators such as prostaglandins, activated components of complement, proteases, free oxygen and nitrogen radicals bring to the definitive destruction of joint cartilage, bones and surrounding structures (5).

The tumor necrosis factor-alpha and IL-1 have almost identical biological activity, mutually stimulate

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production and act synergistic in the induction of inflammatory reaction (6, 7). Proinflammatory effects IL-18, IL-15 and IL-12 have been examined and demonstrated in experimental conditions *in vitro* and *in vivo* with mice on model of arthritis induced by collagen (8–13). According to us available literature great number of research with patients with RA has not been conducted. It is unknown if the production of cytokines in blood, and especially in synovial fluid (SF) is connected with the severity of RA and if their levels can reflect the disease activity, to imply to progression and foresee the course of the disease. These have just been the basic motives to conduct the research with the aim to determine clinical significance of cytokines for the evaluation of activity of rheumatic arthritis. In this region such research has not been done.

Methods

In four year period, total of 89 patients have been analyzed, 64 patients with RA, newly formed or in the phase of deterioration of the disease. The diagnosis of all patients has been set according to revised criteria for classification and diagnosis of RA (ARA/ACR, 1987). We have analyzed clinical manifestations of patients (P group) and have grouped the in relation of the disease activity in three groups: patients with highly active RA (HiA), 30 patients, patients with moderately active RA (MoA), 14 patients and patients with mildly active RA (MiA), 20 patients. The control group was consisted of 25 subjects with the arthritis of the knee during the deterioration of osteoarthritis (OA), for whom we have presumed not to have deranged immunological parameters in blood and SF. The total evaluation of activity of rheumatic arthritis is determined on the basis of total number of swollen joints (SJ), total number of tenderness joints (TJ), values on visible analogical scale for pain (VAS) and rapidity of erythrocyte sedimentation rate (ESR) by means of is calculated index Disease Activity Score 28 (DAS 28), numerical indicator of the degree of disease activity (14). High disease activity have represented the given values DAS >5.1, moderate >3.2, and mild disease activity have showed given values of DAS >2.6. In both examined groups the analyzes of the samples of serum (S) and SF has been done and the concentrations of C-reactive protein (CRP) (mg/L) have been determined by immunonephelometry method, IgM-RF (IU/mL) Latex nephelometry (DADE Behring, Germany) and concentrations of cytokines (pg/mL) TNF-alpha, IL-12, IL-15 and IL-18 immunoenzymatic (ELISA) methods by using kits for humane interleukines (R&D, USA). The value of ESR (mm/h) was determined by standard laboratory procedure according to Westergreen.

For evaluation of statistical significance of difference between characteristics of observation in control group and group of patients with RA Mann-Whitney U test was administered. For comparing between groups

of patients divided according to the degree of activity both Kruskal-Waliss test and Mann-Whitney U tests were used. The level of significance in all administered methods was on in the limit of 0.05.

Results

Comparing the number of subjects in control group and group of patients with RA in relation to sex, showed that there is highly significant difference, caused by larger number of female patients in the group of patients with RA ($p < 0.01$) (Table I).

In groups with highly and moderately active RA examined patients have had significant increase of serum values RF in relation to the group of patients with mild case of RA ($p < 0.01$) (Table II).

Activity of RA in the group of patients was determined and numerically presented on the basis of values of DAS 28 index. Clinical manifestations have been compared between the two groups of examinees, and between groups of HiA, MoA and MiA, by comparing clinical parameters, individual factors of DAS: average values of number of tenderness joints, swollen joints and average values ESR and VAS.

Values of all examined clinical parameters (SJ, TJ, VAS, DAS), have high significantly differentiated between the groups of patients with different activity of RA, with the highest values in HiA group, smaller in MoA and the least in the group of patients with mild RA activity (Table III). The examined subjects in control group have had significantly lower ($p < 0.01$) average values clinical parameters in relation to patients with RA (results are not show).

Table I General characteristics of the study population

Characteristics	Group	
	Control	Patients
Number patients (% of total number examinees)	25 (28)	64 (72)
Age (yr.)	59.2	57.8
Female	15	45
Male	10	19
Mean duration of disease (m.)	45.2	74.4
Mean duration of presently hardships (m.)	5.4	6.0

Table II The division of the group of patients in relation to disease activity and seropositivity

Group	P		HiA		MoA		MiA	
	N	%	N	%	N	%	N	%
Number of »seropositive« pat.	57	89	30	100	14	100	13	65
Number of »seronegative« pat.	7	11	0	0	0	0	7	35
Total number patients	64	100	30	100	14	100	20	100

Table III Clinical parameters values in groups of patients with RA in relation to disease activity

Group	HiA	MoA	MiA	
Parameters	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	p
TJ	9.8 ± 6.1	4.2 ± 0.9	2.4 ± 0.5	0.00**
SJ	8.4 ± 5.7	2.8 ± 0.7	1.8 ± 0.6	0.00**
ESR	78.0 ± 24.9	47.4 ± 10.7	27.2 ± 11.3	0.00**
VAS	70.3 ± 13.8	31.4 ± 4.6	12.0 ± 5.7	0.00**
DAS	10.1 ± 5.2	4.7 ± 0.5	2.9 ± 0.1	0.00**

* p < 0.05; ** p < 0.01

Table IV Comparing of mean values CRP and RF in serum and synovial fluid and ESR between control group and group of patients with RA

Group		CRP (mg/L)	CRP (mg/L)	RF (IU/mL)	RF (IU/mL)	SE (mm/h)
		S	SF	S	SF	
Patients	Mean	43.95	25.41	489.96	316.14	55.5
	SD	38.72	21.26	546.71	327.82	29.3
Control	Mean	6.67	4.77	14.08	12.36	14.8
	SD	7.15	4.46	10.02	6.98	9.6
z		5.79	5.74	6.22	5.95	6.44
p		0.00**	0.00**	0.00**	0.00**	0.00**

* p < 0.05; ** p < 0.01

Table V Cytokines concentrations (TNF-alpha, IL-18, IL-15, IL-12) in the serum samples and synovial fluid of the control group and group of patients with RA

Group		TNF α (pg/mL)		IL-18 (pg/mL)		IL-15 (pg/mL)		IL-12 (pg/mL)	
		S	SF	S	SF	S	SF	S	SF
Patients	Mean	5.59	13.96	156.69	281.11	1.77	10.39	11.80	10.52
	SD	3.53	19.36	90.39	317.23	3.03	9.78	8.09	7.58
Control	Mean	2.32	3.45	89.68	112.18	0.59	3.34	4.92	6.08
	SD	0.80	1.22	17.23	84.83	0.20	1.17	2.14	2.11
z		4.66	5.18	4.40	3.24	3.88	5.13	4.77	3.73
p		0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**

* p < 0.05; ** p < 0.01

Comparing mean values of CRP, RF and ESR between control group and group of patients with RA, indicated significant differences in the concentrations CRP, RF and values ESR (p<0.01). RF and CRP values were higher in the samples of synovial fluid and samples of RA patients' serums as well as ESR values (Table IV).

Comparing mean values of TNF-alpha, IL-18, IL-15, IL-12 has showed that the values of these cytokines are significantly higher (p< 0.01) in serum samples and synovial fluid of the patients with RA in relation to samples of the control group (Table V).

Average serum values of CRP were the highest in HiA group, significantly lower (p<0.01) in MoA group and the lowest (p<0.01) in MiA group. Average values of CRP in synovial fluid were the highest in MoA group, then in MiA group while the lowest were in the group with the highest RA activity, but without any significant differences (Figure 1). Mean values of RF in serum samples and in the synovial fluid samples were highest in HiA group, significantly lower in MoA group (p<0.01) and again significantly the lowest in MiA group (p<0.01) (Figure 2).

Comparing the mean values of cytokines of the patients with RA according to disease activity indicates that with all observed parameters exist highly statistically significant differences (p<0.01), which appear because mean concentrations of cytokines were always higher in HiA group in relation to MoA and MiA groups. Only for the IL-12 concentrations in syno-

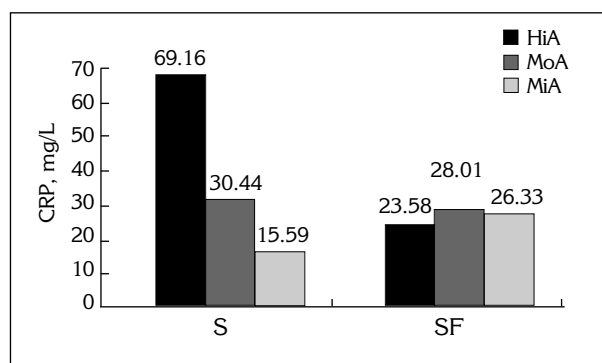


Figure 1. Concentrations of CRP in serum and synovial fluid samples of the patients according to degree of RA activity

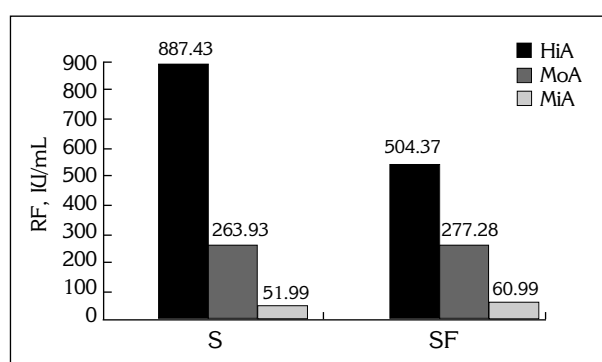


Figure 2. Concentrations of RF in serum and synovial fluid samples of the patients according to degree of RA activity

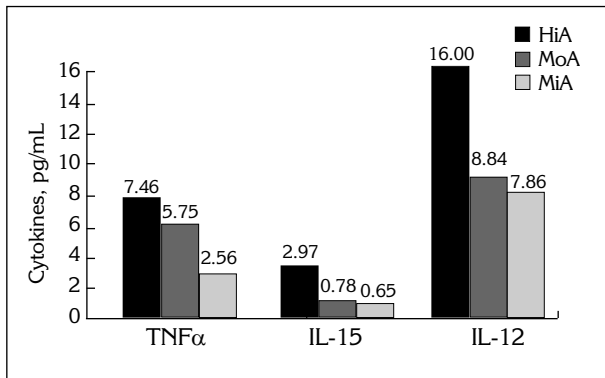


Figure 3. Concentrations of TNF alpha, IL-15 and IL-12 in serum samples of patients according to the degree of RA activity

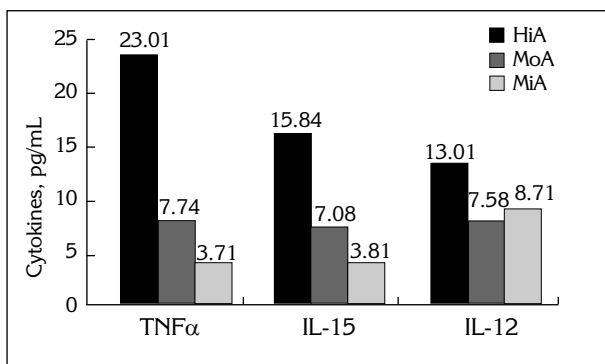


Figure 4. Concentrations of TNF-alpha, IL-15 and IL-12 in synovial fluid samples of patients according to the degree of RA activity

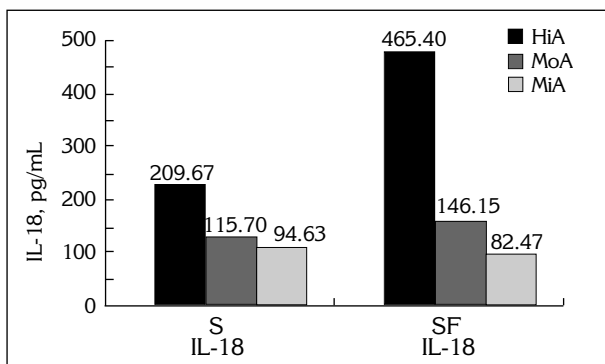


Figure 5. Concentrations of IL-18 in serum and synovial fluid samples of patients according to the degree of RA activity

vial fluid the difference was statistically significant ($p < 0.05$) (Figure 3–5).

Intergroup comparisons between three groups according disease activity within the group of patients with RA, has showed the existence of statistically significant difference in all cases and concentration of

cytokines in serum and synovial fluid have always been the highest in HiA and the smallest in MiA. Only situations when the intergroup differences haven't been significant ($p > 0.05$) are with TNF-alpha in serum between HiA and MoA, IL-12 in serum and in synovial fluid where the difference haven't been significant between HiA and MoA.

Discussion

In pathogenesis of RA TH1 immune response prevails. TH1 cells create special profile of cytokines, which together with products of synovocytes disturbs natural balance in cytokines net inside the synovial tissue. It leads at last to immunoinflammatory reaction and joint damage. Many up today researches showed that IL-12 has major role in the differentiation of TH1 cells response, by stimulating differentiation of T cell's precursor (TH0) in TH1 phenotype. In the arisen TH1 reaction IL-12 increases the proliferation of T cells and production of IFN-gamma and other T cells cytokines in synergism with IL-18. Two cytokines create synergistic effect by reciprocal increase expression of T cells receptor and by different mechanism of IFN-gamma genetic expression (on transcription level). They are produced by synovial macrophages. Lately in synovial tissue of patients with RA has been discovered the IL-15, mediator with pleiotropic effects on many immune system cells. In RA IL-15 activates T cells and stimulates their intercells contact with macrophages. That's how it indirectly induces large synthesis of TNF-alpha and other cytokines of the macrophages. With the help of IL-15 activated synovial T cells secrete TNF-alpha directly. Interleukine-15 stimulates the proliferation of T cells, induces the expression of adhesive molecules and has significant effect of chemotaxis. It stimulates the proliferation of B cells with the consequential production of immunoglobulin including RF. It intensifies the cytotoxicity of natural killer cells, activates neutrophils and stimulates the differentiation of osteoclasts and angiogenesis. Interleukine-15 is produced mainly by T cells and macrophages.

The discovery of new proinflammatory cytokine IL-18 is added to the list of mediators which stimulate activity and development of TH1 cell response with ability to induce the production of IFN-gamma in T cells and NK cells. Synergistic effect with IL-12 and IL-15 in the induction of IFN-gamma defines it as the member of the cytokine family, which induce TH1 (IFN-gamma, IL-2, IL-12 and IL-15). Interleukine-18 is the member of IL-1 family, because it shows the structural and functional similarity with this cytokine. It also owns pleiotropic effects and the role in innate and acquired immunity. Interleukine-18 is synthesized by macrophages, dendrital cells, chondrocytes, osteoclasts, keratinocytes, Kupffers cells, adrenal gland cortex cells and pituitary gland cells. The presence of IL-18 is proved in many diseases in human population, but his role in stimulating or repression the disease is

different. In RA it contributes to the development of TH1 response and induces the IFN-gamma and TNF-alpha synthesis in T and NK cells with consequential stimulation of production and secretion of proinflammatory cytokines of monocytes. The most important role of IL-18, pleiotropic proinflammatory cytokine in synovitis is most probably to facilitate and enable the creation of IFN-gamma dominant T cell response, which is induced by IL-12. There will be necessary further research to establish how much is the real contribution of IL-18 in process of immunopathogenesis of RA. The dilemma about the positive and negative effects of IL-18 in inflammation and synovitis and if there is a need to block or not to his effects because of the potentially positive effects still remains. These and other unknown facts invite us for further researches concerning IL-18.

In our research first was done the analyze of clinical manifestations of diseases inside the group of the patients with active RA, by comparing several clinical parameters which form DAS (average values of the number of tenderness joints and number of swollen joints and average values of ESR and VAS). Patients in the group with highly active disease had significantly higher number of tenderness and swollen joints in relation to group with moderate and mild activity of RA. The mean values of ESR and VAS of patients with the most severe case of the disease were significantly higher in relation to mean values in groups with lower degree of RA activity. Average values of DAS index of RA patients were 6.7. The highest DAS value was in HiA group (10.1) while the patients in MoA group (4.7) and MiA (2.9) had significantly lower DAS values. Patients with RA were divided according to the degree disease activity measured by values of DAS index, were significantly different in relation to the severity of clinical manifestations of the disease.

Between the patients with RA and OA as a control group and between the groups of patients divided according to degree of disease activity inside the group of patients with RA we have compared concentrations of TNF-alpha, IL-18, IL-15 and IL-12 in the samples of S and SF. The average values of all 4 cytokines were high significantly different between the control group and group of patients with RA. Patients with erosive arthritis have higher average concentrations of examined cytokines in blood as well in fluid of joint in relation to examinees with OA. This indicates that pathophysiological mechanism and the degree of inflammatory reaction is different in RA and OA. We have confirmed that TNF-alpha, IL-18, IL-15 and IL-12 have significant role in pathogenesis of rheumatoid synovitis.

Comparing the results of patients with different degree of RA activity significant difference was achieved. Average concentrations of TNF-alpha, IL-18, IL-15 and IL-12 in S and SF were highly and statistically different between the groups of patients with high,

moderate and mild active RA. Patients with high active RA had higher average concentrations of all examined cytokines in serum and synovial fluid in relation to patients with moderate and mildly active RA. When we want to look at the intergroup comparisons between groups of HiA and MiA patients, high and statistically significant differences were established in cytokines concentrations, except for the average values of IL-12 in S ($p=0.62$) and SF ($p=0.43$). Concentrations of TNF-alpha of HiA and MoA patients' serum have also been approximately equal ($p=0.11$), while for other examined parameters highly significant differences came up. Comparing mean values of cytokines concentrations between HiA and MiA groups, highly and statistically significant differences came up. Higher cytokines concentrations in more severe disease cases and their lower values in the disease of the lesser activity indicate that levels of TNF-alpha, IL-18, IL-15 and IL-12 in S and in SF influence clinical manifestations and severity of RA and can be good indicators of general activity of RA as well as parameters to evaluation of the disease severity. We can expect high values of cytokines in S as well in SF in the patients with active RA. We have concluded that the concentrations of cytokines can predict with great accuracy the severity of RA. Concentrations of TNF-alpha, IL-18 and IL-15 in SF reflect the degree of general activity of the disease and severity better than their serum values.

In active disease in laboratory analyzes acute phase parameters of inflammation CRP, fibrinogen and speeded ESR were usually increased. For now there isn't specific biohumoral indicator of disease activity, except of RF. On the basis of comparison of concentrations of CRP and RF in serum and synovial fluid, we have tried to estimate if there is general and local activity of the disease. Patients in HiA group had higher average concentrations of CRP and RF in S and RF in SF in relation to MoA and MiA patients. It implies that RF concentrations in blood and ST and CRP concentrations in S were good indicators of the general activity degree and severity of the disease. Local concentrations of CRP in the knee joint haven't showed any difference among patients groups and it isn't the system inflammatory reaction indicator.

Our research has indicated to the importance of determining several cytokines concentrations in blood and synovial fluid in patients with active RA and can contribute to explanation of insufficiently known views of role in pathogenesis and significance of TNF-alpha, IL-12, IL-15 and especially IL-18 cytokines in the active disease. This research confirmed that examined cytokines could be indicators of the degree of RA activity of the same value and specific quality as up to now used standard biochemical parameters. Concentrations of cytokines can be useful for the early assessment of disease activity. The research can serve as the basis for further studies and evaluation of the administered therapy's effect.

INFLAMATORNI ODGOVOR U REUMATOIDNOM ARTRITISU

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Kratak sadržaj: Cilj istraživanja je da se utvrdi klinički značaj citokina faktora nekroze tumora-alfa (TNF-alfa), interleukina (IL)-12, IL-15 i IL-18 u proceni aktivnosti reumatoidnog artritisa (RA). Poređenjem koncentracija kod 30 bolesnika sa visoko, 14 sa umereno i 20 bolesnika sa blago aktivnim RA utvrdeno je da bolesnici sa visokim stepenom aktivnosti oboljenja imaju značajno veće ($p < 0,01$; $p < 0,05$) koncentracije ispitivanih citokina i reumatoidnog faktora u krvi i sinovijalnoj tečnosti kao i C-reaktivnog proteina u serumu u odnosu na bolesnike sa umereno i blago aktivnim oboljenjem. Zaključili smo da koncentracije citokina utiču na težinu RA i mogu biti dobri pokazatelji stepena opšte aktivnosti RA. Rad može pružiti doprinos razjašnjenju nedovoljno poznatih stavova o patogenetskoj ulozi i značaju određivanja IL-18, IL-15, IL-12 i TNF-alfa u aktivnoj bolesti.

Cljučne reči: citokini, artritis, reumatoidni faktor, C-reaktivni protein

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