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CITOKINI U REUMATOIDNOM ARTRITISU I OSTEOARTROZI

CYTOKINES IN RHEUMATOID ARTHRITIS AND OSTEOARTHROSIS

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Sažetak - 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. Kod 64 bolesnika ocenjena je aktivnost bolesti pomoću indeksa *Disease Activity Score* 28 i određene su koncentracije citokina u uzorcima seruma i sinovijske tečnosti koristeći ELISA kitove za humane interleukine. Kontrolnu grupu činilo je 25 ispitanika sa osteoartrozom. Poređenjem koncentracija citokina kod 30 bolesnika sa visoko, 14 sa umereno i 20 bolesnika sa blago aktivnim reumatoidnim artritisom utvrđeno je da bolesnici sa visokim stepenom aktivnosti oboljenja imaju značajno veće ($p<0,01$; $p<0,05$) koncentracije sva 4 citokina u krvi i sinovijskoj tečnosti u odnosu na bolesnike sa umereno i blago aktivnim oboljenjem. Zaključili smo da koncentracije citokina utiču na kliničke manifestacije, težinu bolesti i mogu biti dobri pokazatelji stepena opšte aktivnosti i prediktori težine reumatoidnog artritisa. Rad može pružiti doprinos razjašnjenju nedovoljno poznatih stavova o patogenetskoj ulozi i značaju određivanja citokina u aktivnoj bolesti.
Ključne reči: Citokini; Reumatoidni artritis; Interleukini; Faktor nekroze tumora; Osteoartritis

Summary *- The aim of this research was to determine the clinical significance of tumor necrosis factor-alpha (TNF-alpha), IL-12, IL-15 and IL-18 in evaluation of the activity of rheumatoid arthritis. Cytokine concentrations in serum samples and synovial fluid were measured by immunoenzymatic methods using kits for human interleukins and the Disease Activity Score 28 in 64 patients with active disease. The control group consisted of 25 subjects with arthritis of the knee and osteoarthrosis. Patients with rheumatoid arthritis have significantly high ($p<0.01$) concentrations of examined cytokines in relation to patients with osteoarthritis. By comparing concentrations in 30 patients with high, 14 patients with moderate and 20 patients with mild activity of rheumatoid arthritis, it was established that patients with high degree of disease activity have significantly high ($p<0.01$; $p<0.05$) concentrations of examined cytokines in the blood and synovial fluid in relation to patients with moderate and mild disease. We have concluded that cytokine concentrations are good indicators of the degree of rheumatoid arthritis activity. This research is a contribution to understanding the insufficiently known pathogenetic mechanisms of cytokines, especially IL-18, in active disease.*
Key words: *Cytokines; Arthritis, Rheumatoid; Interleukins; Tumor Necrosis Factor; Osteoarthritis*

Uvod

Reumatoidni artritis (RA) je idiopatsko, hronično oboljenje u čijoj osnovi je perzistentni, progresivni i destruktivni sinovitis autoimunske prirode sa kliničkom odlikom simetričnog poliartritisa diartrotičnih zglobova [1,2]. U patogenzi RA preovladava imunski odgovor helper (pomoćničkih) T-ćelija (TH)1. Ćelije TH1 stvaraju poseban citokinski profil koji zajedno sa produktima sinoviocita remeti prirodnu ravnotežu u citokinskoj mreži unutar sinovijskog tkiva, što uzrokuje inflamatornu reakciju i oštećenja zgloba [3,4]. Proinflamatorni citokini poreklom iz sinovijskih makrofaga i fibroblasta privlače leukocite iz krvi, regulišu ekspresiju adhezivnih molekula na endotelu i adheziju leukocita i endotelnih ćelija, stimulišu angiogenezu, aktiviraju fibroblaste, pospešuju fibrozu i pojačavaju resorpciju kosti. Antiinflamatorni citokini imaju suprotne efekta [5]. Citokinski milje koga proizvodi sinovijsko tkivo stimuliše maturaciju T-ćelija i funkcionalno ih usmerava prema reakcijama TH1 tipa, ali i relativni nedostatak TH2 citokina doprinosi pojačanom oslobađanju inflamatornih medijatora i pogoršanju sinovitisa. Reumatoidni arthritis je pokrenut kompleksnim mehanizmom u kome učestvuju citokini makrofaga i fibroblasta u kombinaciji sa defektnim reakcijama T-ćelija [6]. Glavni medijator inflamatorne reakcije sa primarnom ulogom u patogenezi RA je faktor nekroze tumora-alfa (TNF-alfa)

Introduction

Rheumatoid arthritis (RA) is an idiopathic, inflammatory, chronic disease, characterized by persistent, progressive, destructive autoimmune synovitis clinically manifested as symmetric polyarthritis of diarthrotic joints [1,2]. In the pathogenesis of RA, TH1 immune response is predominant. A special cytokine profile is created by TH1 cells, and with products of synoviocytes it disturbs the natural balance of the cytokine network within the synovial tissue, which evenually leads to inflammatory reaction and joint damage [3,4]. Proinflammatory cytokines which produce macrophages and fibroblasts, function as chemotaxis; they regulate the expression of adhesion molecules on endothelium, stimulate angiogenesis, activate fibroblasts causing fibrosis and enhance bone resorption and cartilage degradation. Anti-inflammatory cytokines have opposite effects [5]. Cytokine network produced by the synovial tissue stimulates maturation of T cells and promotes TH1 response. A relative lack of TH2 cytokines in RA contributes to enhanced release of inflammatory mediators and exacerbation of synovitis. Rheumatoid arthritis may be triggered by complex mechanisms involving macrophage and fibroblast cytokine networks in combination with defective T cell responses [6]. The tumor necrosis factor-alpha (TNF-alpha) and interleukin (IL-1) are

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Skraćenice

RA	- reumatoidni artritis
TNF-alfa	- faktor nekroze tumora-alfa
IL	- interleukin
GM-CSF	- faktor stimulacije kolonija granulocita i makrofaga
ST	- sinovijska tečnost
OA	- osteoartroza
BOZ	- broj bolno osetljivih zglobova
BOSZ	- broj bolno otečenih zglobova
VAS	- vizuelna analogna skala za bol
DAS 28	- *Disease Activity Score 28*
ELISA	- imunoenzimska laboratorijska metoda
VA	- visoka aktivnost bolesti
UA	- umerena aktivnost bolesti
BA	- blaga aktivnost bolesti
IFN-gama	- interferon-gama
NK ćelije	- ćelije prirodne ubice
MMP	- matrična metaloproteinaza

Abbreviations

RA	- rheumatoid arthritis
TNF-alfa	- tumor necrosis factor alpha
IL	- interleukin
GM-CSF	- granulocyte macrophage colony stimulating factor
ST	- synovial fluid
OA	- osteoarthrosis
NPSJ	- number of pain sensitive joints
NPSSJ	- number of pain sensitive swollen joints
VAS	- visual analogue scale for pain
DAS 28	- *Disease Activity Score 28*
ELISA	- enzyme-linked immunosorbent assay
HDA	- high disease activity
MDA	- moderate disease activity
MiDA	- mild disease activity
IFN-gama	- interferon-gama
NK cells	- natural killer cells
MMP	- matrix metalloproteinase

[7]. Zajedno sa interleukinom (IL)-1 stimuliše mezenhimske ćelije u zglobu da oslobađaju destruktivne enzime, metaloproteinaze [8]. Istovremeno oba citokina sprečavaju proizvodnju inhibitora proteinaza u sinovijskim fibroblastima i na taj način pojačavaju svoj inflamatorni efekat. Kao autokrini i parakrini stimulator ostalih citokina TNF-alfa povećava sekreciju IL-1, IL-6, IL-8 i faktora stimulacije kolonija granulocita i makrofaga (GM-CSF), indukuje proizvodnju IL-11 i osteoklastogenezu, stimuliše ekspresiju adhezivnih molekula na endotelnim ćelijama, čime povećava migraciju inflamatornih ćelija u zglob [9]. Nedostatak ili smanjeni efekat antiinflamatornih citokina kao što su IL-10 i IL-4 u reumatoidnom artritisu takođe može narušiti prirodnu imunološku ravnotežu. Interleukin-10 smanjuje proizvodnju IL-1 i TNF-alfa i proliferaciju T-ćelija čime sprečava degradaciju hrskavice. Interleukin-4 inhibiše aktivaciju TH1 ćelija, a oba citokina povećavaju ekspresiju antagonista IL-1 receptora [6].

Najnovija istraživanja bazirana na eksperimentalnim rezultatima *in vitro* na humanom sinovijskom tkivu i *in vivo* na mišjem modelu artritisa koji je indukovan kolagenom pokazala su značajnu ulogu citokina IL-12, IL-15 i IL-18 u nastanku erozivnog inflamatornog artritisa [10,11]. Prema dostupnoj literaturi nije sproveden veliki broj istraživanja *in vivo* na humanoj populaciji. Nije nam poznato da su rađene studije u kojima su istovremeno određivane koncentracije četiri citokina TNF-alfa, IL-18, IL-15 i IL-12 kod bolesnika koji su imali različitu težinu RA. Nepoznato je da li njihovi nivoi u sinovijskoj tečnosti (ST) i/ili krvi mogu odražavati stepen aktivnosti RA i koji je od navedenih citokina najbolji pokazatelj i prediktor visoke aktivnosti bolesti. Ovo su bili osnovni motivi da se sprovede istraživanje sa ciljem da se utvrdi klinički značaj citokina za procenu aktivnosti reumatoidnog artritisa.

Materijal i metode

Kod 64 bolesnika sa RA, novonastalim ili u fazi pogoršanja i 25 ispitanika sa osteoartrozom (OA)

major proinflammatory cytokines which affect all aspects of RA [7]. The tumor necrosis factor-alpha with IL-1 stimulates production of proteinase and prostaglandin E2 by synovial fibroblasts [8]. At the same time, both cytokines inhibit production of proteinase inhibitors in synovial fibroblasts and in that way enhance their inflammatory effect. The tumor necrosis factor-alpha is an autocrine and paracrine stimulator of other cytokines. It enhances secretion of IL-1, IL-6, IL-8 and GM-CSF and induces IL-11 production and osteoclastogenesis. It stimulates expression of adhesion molecules on endothelial cells and increases migration of inflammatory cells into joints [9]. Lack or reduced effect of anti-inflammatory cytokines IL-10 and IL-4 may also disorganize the natural immunological balance in RA. Interleukin-10 inhibits IL-1 and TNF-alpha production and proliferation of T cells and inhibits cartilage destruction. Interleukin-4 inhibits TH1 cells activation and both cytokines stimulate expression of IL-1 receptor antagonist [6].

The most recent researches based on experimental results *in vitro* on human synovial tissue and *in vivo* on mouse model of collagen induced arthritis have shown considerable role of some new cytokines, IL-12, IL-15 and IL-18 in development of erosive inflammatory arthritis [10,11]. According to the available literature, an insignificant number of researches have been conducted on humans *in vivo*. We are not familiar if there were studies determining all four cytokines TNF-alpha, IL-18, IL-15 i IL-12 in patients with various steges of RA. It is not known if their levels in the synovial fluid (SF) and/or blood, can reflect the degree of RA activity and which of the stated cytokines is the best indicator and predictor of high activity of the disease. These were the main motives for conducting a research with the aim to determine the clinical role of cytokines in evaluation of RA activity.

Material and methods

We analyzed the clinical manifestations of 64 patients with newly developed or aggravated RA

(kontrolna grupa), za koje smo pretpostavili da nemaju poremećene imunološke parametre u krvi i ST analizirane su kliničke manifestacije bolesti. Svi ispitanici su u kliničkoj slici imali artritis kolenog zgloba, pa je artrocentezom dobijen uzorak ST kod svih ispitanika. Bolesnici sa RA su grupisani u odnosu na aktivnost oboljenja u tri grupe, bolesnici sa visoko, umereno i blago aktivnim RA. Ukupna ocena aktivnosti RA određena je na osnovu parametara, zbira broja bolno otečenih (BOZ), zbira broja bolno osetljivih zglobova (BOSZ), vrednosti na vizuelnoj analognoj skali za bol (VAS) i brzine sedimentacije eritrocita (SE) pomoću kojih je računat indeks *Disease Activity Score* 28 (DAS 28), numerički pokazatelj stepena aktivnosti bolesti [12]. Visoku aktivnost bolesti predstavljale su dobijene vrednosti DAS >5,1, umerenu >3,2, a blagu aktivnost bolesti davale su dobijene vrednosti DAS >2,6. U obe grupe ispitanika urađena je analiza uzoraka seruma (S) i ST i određene su koncentracije (pg/ml) citokina TNF-alfa, IL-12, IL-15 i IL-18 imunoenzimskim (ELISA) metodama korišćenjem kitova za humane interleukine (R & D, SAD).

Za procenu statističke značajnosti razlike između pojedinih obeležja posmatranja u kontrolnoj i grupi bolesnika sa RA primenjen je *Mann-Whitney* U test. Za poređenje među grupama bolesnika podeljenim prema stepenu aktivnosti koristio se *Kruskal-Wallis*-ov testom i *Mann-Whitney*-ev U test. Nivo značajnosti u svim primenjenim metodama bio je u granici od 0,05.

Rezultati

Poređenje broja ispitanika u kontrolnoj i grupi bolesnika sa RA u odnosu na pol pokazalo je da postoji visoko značajna razlika, uzrokovana većim brojem bolesnica u grupi bolesnika sa RA (χ^2 = 5,447; df = 1; p < 0,01) (Tabela 1).

Bolesnici sa RA su podeljeni u odnosu na aktivnost bolesti u tri grupe: visoku aktivnost bolesti (VA) imalo je 30 (47%) bolesnika, umerenu (UA) 14 (22%) i blagu aktivnost bolesti (BA) 20 (31%) bolesnika. Od 64 bolesnika sa RA 26 je lečeno, dok preostalih 38 bolesnika nije dobijalo imunomodulatornu terapiju. Vrednosti svih ispitivanih kliničkih parametara (BOSZ, BOZ, SE, VAS, DAS) visoko

Taela 1. Opšte karakteristike ispitanika
Table 1. *General characteristics of the study population*

Karkateristike *Characteristics*	Grupa/*Group* Kontrolna *Control*	Bolesnici *Patients*
Broj bolesnika/*Number of patients*	25	64
Broj bolesnika (%)/*Number of patients (%)*	28	72
Starost (godine)/*Age (year)*	59,2	57,8
Žene/*Female*	15	45
Muškarci/*Male*	10	19
Trajanje bolesti (meseci) *Mean duration of the disease (months)*	45,2	74,4
Trajanje sadašnjih tegoba (meseci)/*Mean duration of present difficulties (months)*	5,4	6

and 25 patients with aggravated osteoarthrosis (OA) (control group). It was presumed that these patients were without immunologic disorders. Patients with RA were divided into three groups with reference to the disease activity: patients with highly, moderately (fairly) and mildly active RA. The *Disease Activity Score index* - DAS28 - a numeric indicator of the degree of disease activity) was established based on the total number of swollen joints, number of painful joints, visual analogue score (VAS) value and the erythrocyte sedimentation rate (ESR) [12]. A high disease activity was defined as DAS >5.1, moderate disease activity was defined as DAS >3.2 and mild disease activity as DAS >2.6. Serum sample (S) and SF analysis was performed in all groups of TNF-alpha, IL-12, IL-15 and IL-18 concentrations (pg/ml) were determined by enzyme-linked immunosorbent assay (ELISA) using kits for human interleukins (R & D, USA).

The Mann-Whitney U test was used to examine the statistical significance of differences between certain parameters in the control group and in patients with RA. The Kruskal-Wallis and Mann-Whitney U tests were used for comparison of groups with different disease activities. The significance level in all applied methods was within the limits of 0.05.

Results

Sex distribution of subjects in control group and RA group showed a highly significant difference, caused by larger number of female patients in the group of patients with RA (χ^2 = 5.447; df = 1; p < 0.01) (Table 1).

Patients with RA were divided into three groups in regard to the disease activity: 30 patients with highly active RA (HDA), (47%); 14 patients with moderately active RA (MDA), (22%); 20 patients with mildly active RA (MiDA), (31%). Only 26 out of 64 patients (41%) were treated in the previous period, while the majority of RA patients - 38 (59%) did not receive immune-modulator therapy. Values of all examined clinical parameters (the average value of pain sensitive and swollen joints and the average value of ESR, VAS and DAS), showed a high statistically significant difference (p<0.01) in groups of patients with di-fferent RA activity, with highest values in HDA group, smaller in MDA and the least in the group of patients with mild RA activity.

Tabela 2. Koncentracije citokina (TNF-alfa, IL-18, IL-15, IL-12) u uzorcima seruma kontrolne i grupe bolesnika sa RA
Table 2. *Cytokines concentrations (TNF-alpha, IL-18, IL-15, IL-12) in the serum samples of the control group and in patients with RA*

Grupa/*Group*	TNF-α (pg/ml)	IL-18 (pg/ml)	IL-15 (pg/ml)	IL-12 (pg/ml)
Bolesnici/*Patients*	5,6 ± 3,5	156,7 ± 90,4	1,8 ± 3	11,8 ± 8,9
Kontrolna/*Control*	2,3 ± 0,8	89,7 ± 17,2	0,6 ± 0,2	4,9 ± 2,1
p	0,00**	0,00**	0,00**	0,00**

** p < 0,01

značajno (p<0,01) su se razlikovale između grupa bolesnika sa različitom aktivnošću RA, sa najvećim vrednostima u VA grupi, manjim u UA grupi i najmanjim u grupi bolesnika sa blagom aktivnošću RA.

Poređenje srednjih vrednosti TNF-alfa, IL-18, IL-15, IL-12 pokazalo je da su vrednosti svih ispitivanih citokina značajno veće (p<0,01) u uzorcima seruma i sinovijske tečnosti bolesnika sa RA u odnosu na uzorke kontrolne grupe (tabele 2 i 3).

Poređenje prosečnih vrednosti citokina kod bolesnika sa RA prema aktivnosti oboljenja pokazuje da kod svih posmatranih parametara postoje visoko statistički značajne razlike (p<0,01), koje nastaju zato što su prosečne koncentracije citokina uvek bile veće u grupi VA u odnosu na UA i BA (Slike 1-3). Jedino za koncentracije IL-12 u sinovijskoj tečnosti razlika je bila statistički značajna (p<0,05).

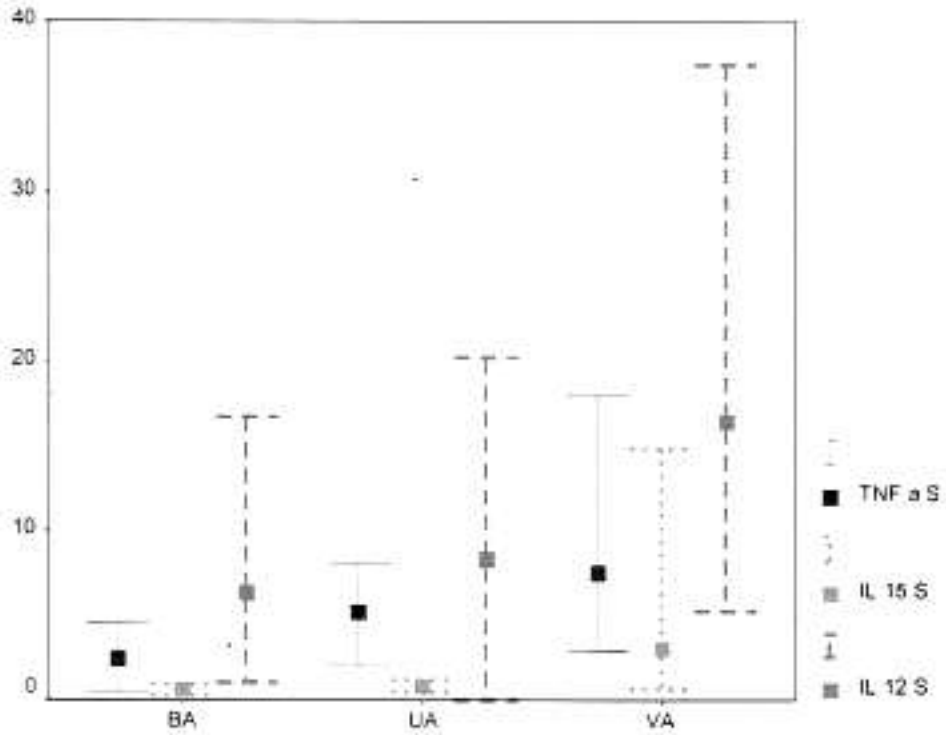
Tabela 3. Koncentracije citokina (TNF-alfa, IL-18, IL-15, IL-12) u uzorcima sinovijske tečnosti kontrolne i grupe bolesnika sa RA

Table 3. *Cytokine concentrations (TNF-alpha, IL-18, IL-15, IL-12) in the synovial fluid samples of the control group and in patients with RA*

Grupa/*Group*	TNF-α (pg/ml)	IL-18 (pg/ml)	IL-15 (pg/ml)	IL-12 (pg/ml)
Bolesnici/*Patients*	14.0 ± 19.4	281.1 ± 317.2	10,4 ± 9,8	10,5±7,6
Kontrolna/*Control*	3,5 ± 1.2	112.2 ± 84,8	3,3 ± 1,2	6,1±2,1
p	0,00**	0,00**	0,00**	0,00**

** p < 0,01

Međugrupna poređenja između tri grupe prema aktivnosti bolesti unutar grupe bolesnika sa RA, pokazala su postojanje statistički značajne razlike u svim slučajevima i uvek su koncentracije citokina u serumu i sinovijskoj tečnosti bile najveće u VA, a najmanje u BA. Jedine situacije kada međugrupne razlike nisu bile značajne (p>0,05) je kod TNF-alfa u serumu između VA i UA, IL-12 i u serumu i



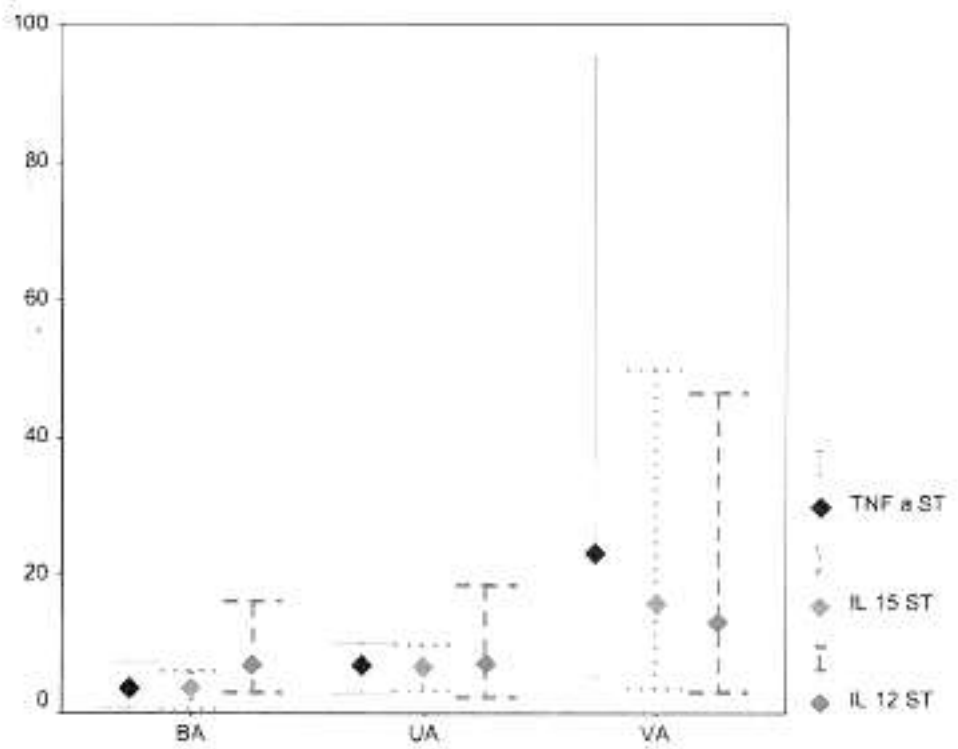
Grafikon 1. Koncentracije TNF-alfa, IL-15 i IL-12 u uzorcima seruma bolesnika prema stepenu aktivnosti RA

Fig. 1. *Concentrations of TNF alpha, IL-15 and IL-12 in serum samples of patients in regard to the degree of RA activity*

Comparison of mean values of TNF-alpha, IL-18, IL-15, IL-12 has showed that values of these cytokines are significantly higher (p< 0.01) in serum samples and synovial fluid of RA patients in relation to control group samples (Table 2,3).

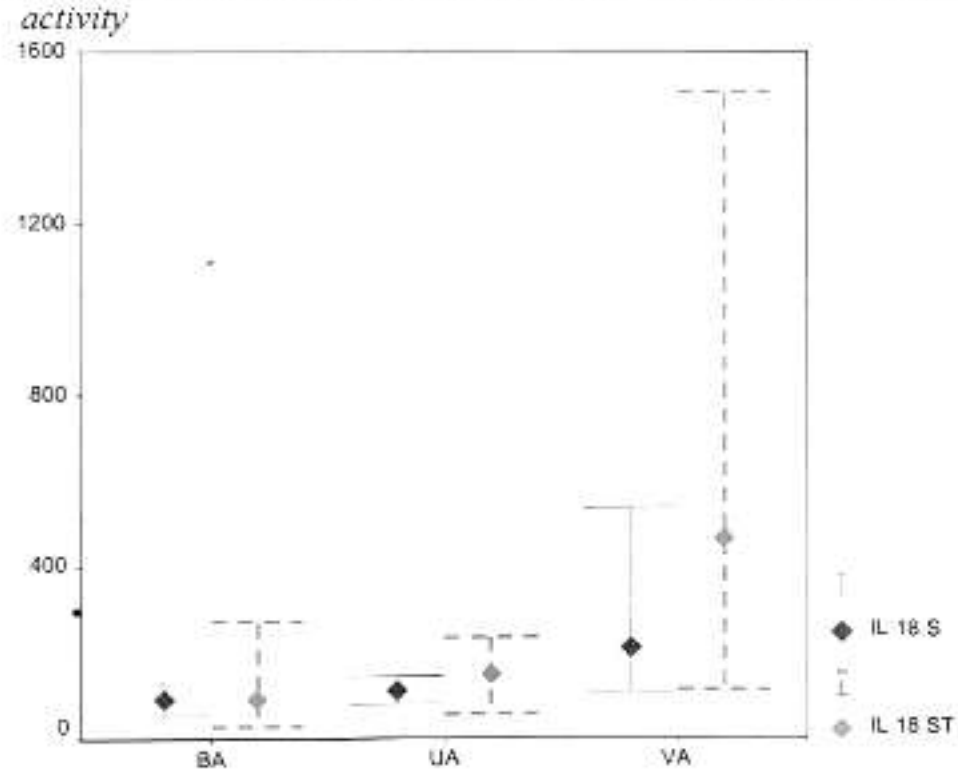
Comparison of mean values of cytokines in patients with RA in regard to disease activity indicates that all observed parameters presented with highly statistically significant differences (p<0.01), because mean concentrations of cytokines were always higher in HDA group in relation to MDA and MiDA groups (Figure 1-3). The difference was statistically significant (p<0.05) only for IL-12 concentrations in synovial fluid.

Intergroup comparisons between three groups in regard to disease activity within the group of pati-



Grafikon 2. Koncentracije TNF-alfa, IL-15 i IL-12 u uzorcima sinovijske tečnosti bolesnika prema stepenu aktivnosti RA.

Fig. 2. *Concetrations of TNF-alpha, IL-15 and IL-12 in synovial fluid samples of patients in regard to the degree of RA activity*



Grafikon 3. Koncentracija IL-18 u serumu i sinovijskoj tečnosti bolesnika prema stepenu aktivnosti RA

Fig. 3. *Concentrations of IL-18 in serum and synovial fluid samples of patients in regard to the degree of RA activity*

sinovijskoj tečnosti gde razlika nije bila značajna između UA i BA.

Diskusija

Do sada urađena ispitivanja na eksperimentalnim modelima potvrdila su da citokini TNF-alfa, IL-12, IL-15 i IL-18 učestvuju u patogenezi reumatoidnog artritisa [13]. Interleukin-12 ima presudnu ulogu u diferencijaciji TH1 ćelijskog odgovora stimulišući diferencijaciju prekursora ćelija T (TH0) u TH1 fenotip. U nastaloj TH1 reakciji IL-12 povećava proliferaciju ćelija T i proizvodnju interferona-gama (IFN-gama) i ostalih citokina T-ćelija u sinergizmu sa IL-18 [14]. Dva citokina ostvaruju sinergistički efekat recipročnim povećavanjem ekspresije receptora na T-ćelije i različitim mehanizmom genske ekspresije IFN-gama na transkripcionom nivou [15]. Interleukin-15 je medijator sa pleiotropnim efektima na mnoge ćelije imunskog sistema [16]. U RA IL-15 aktivira T-ćelije i stimuliše njihov međućelijski kontakt sa makrofazima, čime posredno indukuje veliku sintezu TNF-alfa i ostalih citokina makrofaga. Pomoću IL-15 aktivirane sinovijske T-ćelije luče TNF-alfa direktno [17-19]. Otkriće novog proinflamatonog citokina, IL-18, dopunilo je listu medijatora koji stimulišu aktivnost i razvoj TH1 ćelijskog odgovora sposobnošću da indukuje proizvodnju IFN-gama u ćelijama T i NK (ćelije prirodne ubice, engl. *natural killer*) [20]. Faktor koji reguliše proizvodnju TNF-alfa, IL-1 i matričnih metaloproteinaza (MMP) je pozitivna povratna sprega citokina, imunskih kompleksa i ćelijskog kontakta sa T-ćelijama koje su aktivirane citokinima [13]. Slično IL-15 i IL-18 potencira međućelijski kontakt u sinovitisu kod RA. Na ovaj način IL-18 zajedno sa IL-12 i IL-15 može stalno održavati i obnavljati TH1 imunološki odgovor [21,22]. Njihova uloga u kliničkom ispoljavanju RA nije do danas dovoljno ispitana. U našem istraživanju uporedili smo prosečne koncentracije četiri citokina u uzorcima S i ST između bolesnika sa reumatoidnim artritisom i osteoartritisom kao kontrolnom grupom za koje smo pretpostavili da nemaju poremećene imunološke parametre u krvi i ST. Poređene su koncentracije citokina i među grupama bolesnika sa različitim stepenom aktivnosti bolesti unutar grupe bolesnika sa RA. Prosečne koncentracije TNF-alfa, IL-18, IL-15 i IL-12 su se visoko statistički značajno razlikovale između kontrolne i grupe bolesnika sa RA. Bolesnici sa erozivnim artritisom imali su veće prosečne vrednosti ispitivanih citokina i u krvi i u zglobnoj tečnosti u odnosu na ispitanike sa OA. To pokazuje da je patofiziološki mehanizam i stepen inflamatorne reakcije različit u RA i OA. Zaključili smo da TNF-alfa, IL-18, IL-15 i IL-12 imaju značajnu ulogu u patogenezi reumatoidnog sinovitisa.

Upoređujući rezultate bolesnika sa različitim stepenom aktivnosti RA dobijena je značajna razlika. Prosečne koncentracije TNF-alfa, IL-18, IL-15 i IL-12 i u S i u ST su se visoko i statistički značajno razlikovale između grupa bolesnika sa visoko,

tients with RA, showed a statistically significant difference in all cases, whereas concentration of cytokines in serum and synovial fluid were always highest in HDA and the smallest in MiDA. The intergroup differences were not significant ($p>0.05$) only with TNF-alpha in serum between HDA and MDA, IL-12 in serum and in synovial fluid where the differences were not significant between MDA and MiDA.

Discussion

Researches conducted so far on experimental models have confirmed that cytokines TNF-alpha, IL-12, IL-15 i IL-18 take part in the pathogenesis of RA [13]. IL-12 has the main role in the differentiation of TH1 cell response, by stimulating differentiation of precursors of T cells (TH0) in TH1 phenotype. In the TH1 reaction IL-12 increases proliferation of T cells and production of IFN-gamma and other T cells cytokines in synergism with IL-18 [14]. Two cytokines have a synergistic effect and by reciprocity increase the expression of receptors on T cells and different mechanisms of gene expression of IFN-gamma on transcription level [15]. IL-15 is a mediator with pleiotropic effects on many cells of immune system [16]. In RA, IL-15 activates the T cells and stimulates their intercellular contact with macrophages, and thus indirectly induces synthesis of TNF-alpha and other macrophage cytokines. With IL-15 the activated syinovial T cells directly secrete TNF-alpha [17-19]. The discovery of new proinflammatory cytokine IL-18 has completed the list of mediators which stimulate the activity and development of TH1 cell response with ability to induce production of IFN-gamma in T and NK cells [20].

Production of TNF-alpha, IL-1 and metaloproteinase is regulated by a mechanism of positive feedback loop of cytokines, immune complexes and direct cell-cell contact (T cells/macrophages and fibroblasts) [13]. Interleukun-18, just like IL-15, stimulates the intercellular contact in synovitis and RA. In this way IL-18 in synergy with IL-12 and IL-15 can maintain and promote TH1 immune response [21, 22].

The role of cytokines in clinical manifestations of RA has not been sufficiently investigated yet. In our research, we compared the average concentrations of four cytokines in serum and synovial fluid among patients with rheumatoid arthritis and osteoarthritis (control group). It was presumed that patients with osteoarthritis had no immune disorders. We compared the concentrations and patients were divided into groups in regard to the degree of RA activity. The average concentrations of TNF-alfa, IL-18, IL-15 i IL-12 were highly significantly different between the control group and patients with RA. Patients with erosive arthritis had higher average values of monitored cytokines in blood and joint fluid compared to those with osteoarthrosis. This indicates that the pathological mechanism and the degree of inflammatory reaction differ in rheumatoid arthri-

umereno i blago aktivnim RA. Bolesnici sa visoko aktivnim RA imali su veće prosečne koncentracije svih ispitivanih citokina u serumu i sinovijskoj tečnosti u odnosu na bolesnike sa umereno i blago aktivnim RA. Kada su u pitanju međugrupna poređenja između grupa bolesnika UA i BA utvrđene su visoko i statistički značajne razlike u koncentracijama citokina, osim za prosečne vrednosti IL-12 u S (p=0,62) i ST (p=0,43). Koncentracije TNF-alfa u serumu bolesnika VA i UA takođe su bile približno jednake (p=0,11), dok su za ostale ispitivane parametre postojale visoko značajne razlike. Upoređujući srednje vrednosti koncentracija citokina između grupa VA i BA dobijene su visoko i statistički značajne razlike. Veće koncentracije citokina u težim oblicima bolesti i njihove niže vrednosti u bolesti manjeg stepena aktivnosti pokazuju da nivoi TNF-alfa, IL-18, IL-15 i IL-12 u S i ST utiču na kliničke manifestacije i težinu RA i mogu biti dobri pokazatelji opšte aktivnosti reumatoidnog artritisa i parametri procene težine bolesti.

Zaključak

Naš rad je ukazao na značaj određivanja koncentracija pojedinih citokina u krvi i sinovijskoj tečnosti kod bolesnika sa aktivnim RA i može pružiti doprinos razjašnjenju nedovoljno poznatih stavova o patogenetskoj ulozi i značaju određivanja citokina TNF-alfa, IL-12, IL-15 i posebno IL-18 u aktivnoj bolesti. Ovim istraživanjem je potvrđeno da ispitivani citokini mogu biti pokazatelji stepena aktivnosti RA veće vrednosti i specifičnosti u odnosu na do sada korišćene standardne biohemijske parametre i da mogu veoma pouzdano predvideti težinu RA. Koncentracije citokina mogu biti korisne za ranu procenu aktivnosti bolesti. Istraživanje može poslužiti kao osnova za dalje studije i procenu efekta primenjene terapije.

tis and in osteoarthrosis. We have confirmed that TNF-alpha, IL-18, IL-15 and IL-12 have a significant role in pathogenesis of rheumatoid synovitis.

Comparison of results of patients with different RA activity revealed highly significant differences. Average concentrations of TNF-alpha, IL-18, IL-15 and IL-12 in S and SF were highly and statistically different between groups of patients with high, moderate and mild RA. Patients with highly active RA had highest average concentrations of all examined cytokines in serum and synovial fluid in relation to patients with moderate and mild RA. In regard to intergroup comparisons between MDA and MiDA 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 HDA and MDA patients' sera 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 HDA and MiDA groups, highly and statistically significant differences were found. Higher values of cytokines in more severe cases and their lower concentrations in less severe cases indicate that levels of TNF-alpha, IL-18, IL-15 and IL-12 in serum and synovial fluid affect clinical manifestations and severity of RA and can be good indicators of general RA activity as well as parameters to evaluation of disease severity.

Conclusion

Our research has demonstrated the importance of determining cytokine concentrations in blood and synovial fluid in patients with active RA and it can contribute to explanation of their role in RA pathogenesis and significance of TNF-alpha, IL-12, IL-15 and especially IL-18 cytokines in its activity. This research has confirmed that the examined cytokines can be indicators of the degree of RA activity as much as standard biochemical parameters. Cytokine concentrations can be useful in early assessment of disease activity and this research provides a basis for further studies and evaluation of therapeutic effects.

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