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IMMUNE COMPLEXES AND COMPLEMENT IN SERUM AND SYNOVIAL FLUID OF RHEUMATOID ARTHRITIS PATIENTS

IMUNSKI KOMPLEKSI I KOMPLEMENT U SERUMU I SINOVIJALNOJ TEČNOSTI KOD BOLESNIKA SA REUMATOIDNIM ARTRITISOM

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Summary: Rheumatoid arthritis (RA) is predominantly an intraarticular inflammatory and autoimmune disease that involves different autoantibodies and effector mechanisms. The aim of the study was to determine the utility of Circulating Immune Complexes (CIC) and complement components (C3c, C4) as possible markers for the disease activity in laboratory diagnostics. In a cross-section study 59 patients, according to the clinical criteria, were categorized into two groups: group with moderate (MA, n=24), and group with severe activity (SA, n=35) of RA. The concentration of CIC, C3c and C4 in sera (S) and synovial fluids (SF) was examined by an immunonephelometric method in both groups and compared with values in the control group (n=15) of patients with lesions of the menisci. Obtained results showed that there was no statistical significance in the values of C3c and C4, in both biological fluids, among all tested groups. Significant differences were found in the levels of CIC in both fluids, while testing the parameters ($\bar{x} \pm$ SD, IU/mL) in the sera of groups with SA and MA of RA: 7.43 ± 13.40; 3.01 ± 2.92 ($p<0.05$) and SF: 13.47 ± 21.1, 5.33 ± 7.53 ($p<0.001$), respectively. These differences were higher between the group with SA and CG. Results for the concentrations of CIC were significantly higher in SF compared to sera: in the RA group with SA by 77% and group with MA by about 82%. These data could provide a confirmation of the

Kratak sadržaj: Reumatoidni artritis (RA) jeste predominantno intraartikularna zapaljenska i autoimunska bolest u koju su uključena različita autoantitela i efektorni mehanizmi. Cilj ispitivanja je bio da se ustanovi značaj cirkulišućih imunskih kompleksa (CIK) i komponenti komplementa (C3c, C4), kao pokazatelja stepena aktivnosti RA za laboratorijsku dijagnostiku. U studiji preseka stanja je ispitano 59 bolesnika koji su prema kliničkim kriterijumima za aktivnost RA podeljeni u dve grupe: grupu sa umerenom (UA, n=24) i grupu sa visokom aktivnošću (VA, n=35) RA. Koncentracije CIK, C3c i C4 u serumu i sinovijalnoj tečnosti (ST) određivane su imunonefelometrijskom metodom u obe grupe ispitanika i upoređene sa vrednostima u kontrolnoj grupi od 15 pacijenata s povredama meniskusa. Rezultati su pokazali da nije bilo statistički značajnih razlika u koncentracijama za C3c i C4 u oba biološka uzorka između ispitivanih grupa. Statistički značajne razlike u koncentracijama CIK utvrđene testiranjem vrednosti ($\bar{x} \pm$ SD, IU/mL) u serumu između grupe sa VA i grupe sa UA RA bile su: 7,43 ± 13,40; 3,01 ± 2,92 ($p < 0,05$) i za vrednosti u ST: 13,47 ± 21,1, 5,33 ± 7,53 ($p < 0,001$). Razlike su bile više izražene između grupe sa VA RA i KG. Rezultati koncentracija CIK su bili značajno viši u ST u odnosu na serum u obe grupe bolesnika: u grupi sa UA za 77% a u grupi sa VA RA za 82%. Ti podaci idu u prilog potvrdi hipoteze o lokalnoj, intraartikularnoj produkciji autoantitela, odnosno

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hypothesis about local, intraarticular autoantibodies and subsequent CIC production. It can be concluded that the examination of CIC concentration in serum, and where it is possible in SF, is a useful marker of disease activity in RA patients, in contrast to the tested components of the complement. This statement does not exclude their consumption within immune effector mechanisms, but elicits the possibility that lower molecular fragments (C3d, C4d), as well as the novel activation products, could be better disease activity markers in RA patients.

Keywords: rheumatoid arthritis, circulating immune complexes, complement components, disease activity

CIK. Može se zaključiti da je laboratorijsko određivanje koncentracije CIK korisan pokazatelj stepena aktivnosti RA, što se ne odnosi na ispitivane komponente komplementa. To ne isključuje njihovu aktivnost u okviru efektornog imunskog mehanizma, ali ukazuje na to da bi manji molekulski fragmenti (C3d, C4d) i novi aktivacioni produkti mogli biti bolji pokazatelji stepena aktivnosti RA.

Ključne reči: reumatoidni artritis, cirkulišući imunski kompleksi, komponente komplementa, aktivnost bolesti

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune synovitis affecting about 1.0% of the world's population (1), but the mechanisms underlying the initiation and progression of RA are not yet completely understood. The presence of immune complexes (ICs) in the blood and synovial fluid of patients with RA has been well described (2, 3), and there is evidence that they are involved in the activation of the complement cascade in RA synovial tissue (5). However, apart from rheumatoid factor (RF-IgM) and different RF isotypes (6–8), other autoantibodies have been added to the growing list of autoantigens such as: filaggrin (10), Sa (11, 12), calpastin (13), RA33 (14), collagen type II (15) and a number of citrullinated proteins (16–18). Due to their ability to form ICs, they may promote complement activation. The ICs from RA synovial fluid can induce cytokine and RF production (19), but the identity of antigens involved in ICs remains obscure. In addition to dietary factors, speculation on and investigation of RA etiology has also been focused on genetic susceptibility, abnormal bowel permeability and microorganisms. Therefore, RA is a classic example of a multifactorial disease in which a combination of genetic and environmental factors contributes to the disease progression.

The aim of this study was to evaluate the concentrations of circulating immune complexes (CIC), C3 and C4 complement components of the classical pathway as possible biochemical parameters for disease activity and progression of RA. Taking into account that RA is predominantly an articular disease, it was interesting to compare the levels of mentioned parameters in synovial fluid (SF) and sera of different RA activity groups with the control group (CG) with non-inflamed synovitis.

Patients and Methods

In this multicentric study fifty-nine RF positive sera and SF were assayed in RA patients for the level of CIC, C3c and C4 components of the complement and compared with the same biological fluids of the control group of 15 patients with lesions menisci. All RA patients satisfied the American Rheumatism Association criteria (20) and were classified into two groups: one with moderate (n=24) and another with severe (n=35) disease activity. The RA activity was classified according to adequate clinical (number of painful and swollen joints, Steinbrocker's radiographic changes, Ritchie articular index, Disease Activity Score) and biochemical inflammatory parameters: erythrocyte sedimentation rate, fibrinogen, C-reactive protein and serum amyloid A concentration.

The levels of CIC and complement components in both fluids were assayed by an immunonephelometric (DADE Behring) method. Prior to testing, all SF samples were pretreated with hyaluronidase (Streptomyces hyalurolyticus). Statistical analyses were performed using the Basic Statistic software for the comparison of means (Mann-Whitney U test), standard deviation and distribution data (21).

Results

Average values of C3c and C4 components in both RA activity groups as well as in the control group were approximately lower by 50 to 70 percent in SF as compared to the sera values *(Table I)*, with the statistical significance of $p<0.001$.

In the groups with moderate and severe RA activity, the average values of CIC were more than 80 percent higher in SF, which is also statistically highly significant ($p<0.001$). The comparison of complement components and CIC concentration in the tested biological fluids in two different RA activity groups and the control group is presented in *Tables II–IV*.

There were no significant differences for the examined complement components in the three tested groups. High statistical significance was only found for the CIC concentrations in both fluids (serum and SF) between moderate and severe activity RA groups. The level of statistical significance in RA patients with MA and SA was higher in SF as compared to sera values. There were no significant differences between MA and CG *(Table II, Figure 1)*.

Table I Comparison of C3c, C4 and CIC levels in sera and SF in three tested groups

Control group (N=15)			$\bar{x} \pm SD$ (g/L)	$\bar{x} \pm SD$ (g/L)	Statistical significance	% $\bar{x}$, C3,C4, CIC in SF vs. serum
Serum	vs.	SF				
C3c	vs.	C3c SF	1.12 ± 0.26	0.28 ± 0.16	$p < 0.001$	– 75 %
C4	vs.	C4 SF	0.25 ± 0.09	0.07 ± 0.06	$p < 0.001$	– 72 %
CIC	vs.	CIC SF (IU mL)	1.81 ± 0.76	2.42 ± 0.66	$p < 0.05$	+34 %
Moderate activity N=24			serum	SF		
C3c	vs.	C3c SF	1.22 ± 0.37	0.41 ± 0.28	$p < 0.001$	– 66 %
C4	vs.	C4 SF	0.25 ± 0.08	0.12 ± 0.18	$p < 0.001$	– 52 %
CIC	vs.	CIC SF (IU mL)	3.01 ± 2.92	5.33 ± 7.53	$p < 0.05$	+ 77 % *
Severe activity N=35			serum	SF		
C3c	vs.	C3c SF	1.33 ± 0.36	0.44 ± 0.3	$p < 0.001$	– 67 %
C4	vs.	C4 SF	0.29 ± 0.13	0.10 ± 0.1	$p < 0.001$	– 69 %
CIC	vs.	CIC SF (IU mL)	7.43 ± 13.4	13.5 ± 21.1	$p < 0.001$	+ 82 % *

Abbreviations: CIC – circulating immune complexes; SF – synovial fluid; vs. – versus; N – number of patients; $\bar{x}$ – arithmetic mean; SD – standard deviation; p – statistical significance, NS – not significant by Mann Whitney U test. * – $p < 0.05$ in spite of the adequate value in control group (t-test proportion).

Table II Comparison of C3c, C4 levels vs. CIC in serum and SF between groups with moderate activity of RA and control group.

RA activity groups Moderate vs. Control (N = 24) (N = 15)	$\bar{x} \pm SD$ (g/L)	$\bar{x} \pm SD$ (g/L)	Statistical significance
C3c vs. C3c	1.22 ± 0.37	1.12 ± 0.26	NS
C3c SF vs. C3c SF	0.41 ± 0.28	0.28 ± 0.16	NS
C4 vs. C4	0.25 ± 0.08	0.25 ± 0.08	NS
C4 SF vs. C4 SF	0.12 ± 0.18	0.07 ± 0.06	NS
CIC vs. CIC (IU/mL)	3.01 ± 2.92	1.81 ± 0.76	NS
CIC SF vs. CIC SF (IU/mL)	5.33 ± 7.53	2.42 ± 0.66	NS

Table III Comparison of C3c, C4 levels vs. CIC in serum and SF among groups with moderate and severe activity of RA.

RA activity groups Moderate vs. Severe (N = 24) (N = 15)	$\bar{x} \pm SD$ (g/L)	$\bar{x} \pm SD$ (g/L)	Statistical significance
C3c vs. C3c	1.33 ± 0.40	1.22 ± 0.37	NS
C3c SF vs. C3c SF	0.44 ± 0.30	0.41 ± 0.28	NS
C4 vs. C4	0.29 ± 0.10	0.25 ± 0.08	NS
C4 SF vs. C4 SF	0.10 ± 0.10	0.12 ± 0.18	NS
CIC vs. CIC (IU/ml)	7.43 ± 13.40	3.01 ± 2.92	$p < 0.05$
CIC SF vs. CIC SF (IU/mL)	13.47 ± 21.1	5.33 ± 7.53	$p < 0.001$

Table IV Comparison of C3c, C4 levels vs. CIC in serum and SF between control group and group with severe activity of RA.

RA activity groups Control vs. Severe (N = 24) (N = 15)	$\bar{x}$ ± SD (g/L)	$\bar{x}$ ± SD (g/L)	Statistical significance
C3c vs. C3c	1.33 ± 0.40	1.12 ± 0.26	NS
C3c SF vs. C3c SF	0.44 ± 0.30	0.28 ± 0.16	NS
C4 vs. C4	0.29 ± 0.10	0.25 ± 0.08	NS
C4 SF vs. C4 SF	0.10 ± 0.10	0.07 ± 0.06	NS
CIC vs. CIC (IU/mL)	7.43 ± 13.40	1.81 ± 0.76	$p < 0.001$
CIC SF vs. CIC SF (IU/mL)	13.47 ± 21.1	2.42 ± 0.66	$p < 0.001$

Abbreviations: CIC – circulating immune complexes; SF – synovial fluid; vs. – versus; N – number of patients; $\bar{x}$ – arithmetic mean; SD – standard deviation; p – statistical significance.

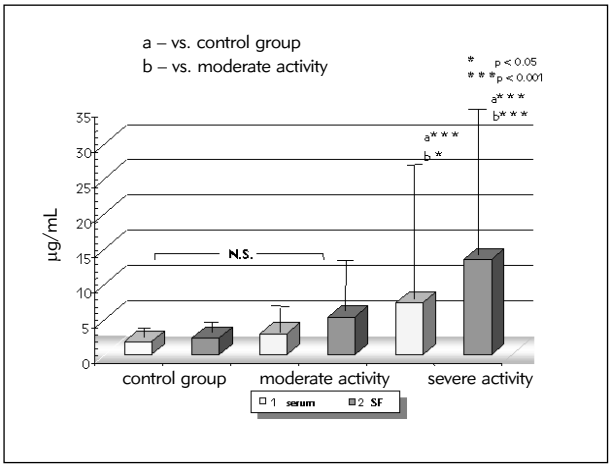

Figure 1 Comparison of CIC values in sera and SF in the control and groups with moderate and severe activity of RA.

Discussion

Under normal conditions, ICs are rapidly removed from the blood stream by macrophages in the spleen and Kupffer cells in the liver. Large ICs can be easily removed, while small ones do not provoke pathological states. Therefore, ICs of intermediate size usally have the largest potential for developing autoaggression. However, ICs continue to circulate in some circumstances. Eventually, they become trapped in the tissues of the kidneys, lung, skin, joints, or blood vessels. Certain properties influence their potential pathogenicity. Features of particular importance include nature, size, and concentration of the antigen, nature, size, and concentration of the antibody (22), rate of formation and clearance of the ICs (23). Just where they end up they set off reactions that lead to inflammation and tissue damage. ICs develop in autoimmune disease, resulting in the continuous production of autoantibodies which overload the ICs removal system. Up to date, unclear etiopathology of RA involves a large spectrum of biological molecules with several antigens or autoantigens and adequate antibodies, producing high levels of CIC, which are important for the severity of RA. Higher CIC level in SF than in serum implies its local production, that can be seen from the results obtained in this examination. However, to what extent CIC levels belong to the mentioned types of complexes and complement activation via the classical pathway remains an open question. Complement proteins are a major determinant for the size and solubility of an immune complex, which affects clearance. The evidence regarding intraarticular activation of the complement system in RA could elicit their involvement in pathogenesis and inflammation. The obtained results show no evidence of complement consumption in the sera or SF. Complement activation or consumption through C3 and C4 in the sera is hard to detect because all mentioned molecules belong to the family of acute phase proteins. Their production as a consequence of inflammation possibly masks the C3 and C4 consumption. Some authors for that reason suggest novel activation products (C1q-C4 complex, C3dg), as more relevant for complement activation in RA (24, 25). Our previous results revealed the connection between RA severity and the high level of different RF isotype combinations in both fluids (9), and confirmed the hypothesis about their involvement as a part of ICs in the pathological mechanisms.

More than 40 assay techniques used to detect or measure CIC have been described. Those tests include the Raji cell assay, C1q deviation test, conglutinin test, fluid and solid phase C1q binding procedures, RF assay, PEG precipitin test, etc. Since the size and physiochemical properties of CIC vary markedly, none of these assays has been accepted as a standard. A collaborative study (26) sponsored by the World Health Organization determined that no single method was appropriate in all suspected disease states and recommended that at least two different assay techniques should be performed to detect and measure CIC adequately. Everything mentioned above makes the interpretation of CIC results difficult for clinicians (27, 28). CIC concentration is relevant only in cases with clearly established clinical symptoms of the disease. Our results support the hypothesis (28,

29) that circulating as well as intraarticular ICs together with complement split products may play an important role in some RA pathogenetic aspects. The Clq binding test with anticomplementary activity may be the method of choice for evaluating CIC levels in RA.

The findings presented here imply a possible putative role of the CIC interaction in complement activation in RA. Quantification of CIC concentrations in sera and in SF showed that it could be a good laboratory parameter as a marker for the RA high activity, especially in combination with IgMRF (9) and anti-CCP Ab (30, 31). Measurement of total C3 and C4 in serum as a routine laboratory method is time-consuming without marked relevant data.

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