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LABORATORY EXAMINATION OF SERONEGATIVE AND SEROPOSITIVE RHEUMATOID ARTHRITIS

LABORATORIJSKE PRETRAGE KOD SERONEGATIVNOG I SEROPOZITVNOG REUMATOIDNOG ARTRITISA

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Summary

In response to the continuing debate whether seronegative and seropositive rheumatoid arthritis (RA) are parts of the same disease spectrum or are distinct disorders, we performed a comparative analysis of some laboratory characteristics. The test group consisted of 125 seronegative RA (93 female, 32 male), with titers lower than 1/64 as defined by Rose-Waaler test. The control group consisted of 125 seropositive RA patients (93 female, 32 male), with titers of 1/64 or higher. Patients all belonged to the 2nd and 3rd functional classes (ARA), and were between 25-60 years of age. The duration of the disease was 1-27 years. Elevated average values of erythrosedimentation (ERS), C-reactive protein (PCR) and erythrocytes (Er) were found in seropositive patients, but they did not present statistically significant difference with regard to sero-status. Reduced values of hemoglobin (Hb) were found more frequently in seropositive patients ($t=2.26$, $p<0.05$), especially female seropositive patients ($t=4.38$, $p<0.01$), without correlation to disability and duration of the disease. Statistically significant difference was found in average values of fibrinogen in the seropositive subset ($t=2.10$, $p<0.05$), especially in female seropositive patients ($t=2.65$, $p<0.01$), and in average values of leukocytes ($t=1.37$, $p<0.05$) among male seropositive patients. Elevated immunoglobulin (IgM) values were more prominent in the seropositive subset ($\chi^2=47.6$, $p<0.01$), especially among seropositive females ($\chi^2=35.68$, $p<0.01$). Values of IgA and IgG did not present statistically significant difference with regard to sero-status. Levels of C3 and C4 components of the complement were reduced in seropositive tested subjects, without significant difference between sero-subsets. Increased values of gamma-globulin were confirmed with statistical significance ($\chi^2=3.39$, $p<0.05$) in seropositive subjects, while alpha-2 globulin values were nearly equally distributed in both subsets.

Keywords

rheumatoid arthritis, seropositive, seronegative, laboratory analysis

Sažetak

U cilju doprinosa kontinuiranoj raspravi o pitanju jesu li seronegativni i seropozitivni RA dio spektra iste, ili su dvije zasebne bolesti, ostvarili smo komparativnu analizu u odnosu na neke laboratorijke karakteristike. Ispitivana grupa je obuhvatila bolesnike sa seronegativnim RA sa titrom manjim od 1/64 određenim pomoću Waaler-Rose testa, dok je kontrolna grupa obuhvatila bolesnike sa seropozitivnim RA sa titrom 1/64 ili više. Svi ispitanici su pripadali II, III funkcionalnom razredu (ARA), životne dobi između 25-60 godina (Xb=49,96) sa trajanjem bolesti od 1-27 godina (Xb=6,41). Povišene vrijednosti eritrosedimentacije (ERS), C-reaktivnog proteina (PCR) i eritrocita (Er) su pronađene kod seropozitivnih bolesnika, ali nisu predstavljale statistički značajnu razliku u odnosu na sero-status. Srednje vrijednosti hemoglobina (Hb) bile su niže kod seropozitivnih bolesnika ($t=2,26$; $p<0,05$), posebno izraženije kod seropozitivnih ženskih bolesnika (Xb=10,77 g/l) ($t=4,38$,

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p<0,01), i nisu korelirale sa funkcionalnom nesposobnošću i trajanju bolesti. Statistički značajna razlika pronađena je za srednje vrijednosti fibrinogena u seropozitivnih bolesnika (t=2,10, p<0,05), posebno kod seropozitivnih ženskih bolesnika (t=2,65, p<0,01), i za srednje vrijednosti leukocita (t=1,37, p<0,05) kod seronegativnih muških bolesnika. Povišene srednje vrijednosti imunoglobulina (IgM) bile su izraženije kod seropozitivne skupine (χ^2=47,6, p<0,01), posebno kod seropozitivnih ženskih bolesnika (χ^2=35,68, p<0,01). (Vrijednosti IgA i IgG nisu pokazale statistički značajne razlike u odnosu na sero-status. Komponente C3 i C4 komplementa bile su niže kod seropozitivnih bolesnika, bez statističkog značaja u odnosu na sero-status. Povećane vrijednosti gama-globulina kod seropozitvnih bolesnika potvrđene su statistički značajnom razlikom (χ^2=3,93, p<0,05), dok su vrijednosti alfa 2-globulina bile približno jednako raspostranjene kod obje grupe.

Ključne riječi

reumatoidni artritis, seropozitivan, seronegativan, laboratorijske analize

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology. Because it can affect multiple organs of the body, rheumatoid arthritis is referred to as a systemic illness, primarily involving the peripheral joints, usually equally affecting both sides of the body (1,2,3,4,5,6).

Laboratory features are acute-phase reactants, rheumatoid factor and anemia (7,8). These parameters, which fluctuate during the course of the disease, represent large number of diverse reactions aiming to adjust the organism to the effects of stress/injury. Acute-phase reactants like CRP are primarily produced by hepatocytes, and their chief inductor is the proinflammatory cytokine IL-6. There is also a contribution by other inflammation-associated cytokines such as tumor necrosis factor-α or IL-1. Functionally, CRP provides downstream integration of overall cytokine activation (9). ERS provides a non-specific screening test for the presence of acute phase reaction (10). C-reactive protein in the serum most accurately reflects disease activity (11) and this test is performed in patients where ERS does not follow clinic activity of the disease (12,13,14). Results achieved from combination of ERS and PCR performed together are more reliable than when performing only one of them (15,16,17). Hemoglobin (Hb) is a good indicator of inflammatory activity and correlates with ERS levels and serum iron values. Typically, the anemia is normocytic, and either normochromic or hypochromic (18). Both blast forming units - erythroid and colony forming units inhibited by IL-1 and TNF-alpha - but their role "in vivo" in RA anemia is unknown. The reticuloendothelial system (RES) plays two major roles in iron metabolism: it recycles iron from senescent red blood cells and it serves as a large storage depot for excess iron (19). Leukocytosis is present in 25% of the patients, but it is not prominent for the diagnosis of RA (20,21,22). Levels of some proteins may be reduced during an acute phase response, increased levels of alpha-2 globulins, fibrinogen and gamma-globulins equivalent to the activity of the disease (23,24). At early stage, these levels are increased and are recommended to perform their investigation if equivalent activity of the disease is missing (25). The amount of complement in serum of the patients is normal or slightly increased, but in synovial fluid is reduced (26). Sometime, to prove the diagnosis, it is preferred to perform chemical and immunochemical test of the synovial fluid (27). New laboratory tests have appeared for the diagnosis and prognostic prediction of RA. Among them, anticyclic citrullinated peptide antibodies (anti-CCP), which bind epitopes containing citrulline, seem to be attracting the most attention (28).

Purpose

In response to the continuing debate whether seronegative and seropositive rheumatoid arthritis (RA) are parts of the same disease spectrum or are distinct disorders, we performed a comparative analysis of some laboratory characteristics.

Patients and Methods

In total 250 patients were selected from population-based study in the 1991-2008 period, and diagnosed as seronegative and seropositive RA at the Clinic for Sport Medicine in Prishtina and at internal medicine facilities in Kosovo. All patients had the classic form of RA, and fulfilled the ARA criteria (33). The test group consisted of 125 seronegative RA patients (93 female, 32 male), with titers lower then 1:64 as defined by Rose-Waaler test. The control group consisted of 125 seropositive RA patients (93 female, 32 male), with titers of 1:64 or higher. Patients all belonged to the 2nd and 3rd functional classes (ARA), and were between 25-60 years of age. The duration of the disease was 1-27 years.

The erythrocyte sedimentation rate (ESR) was performed by Westergreen's method expressed in mm/h. Upper limit for normal value was accepted as 20 mm/h for the first hour. C-reactive protein (CRP) was determined by agglutination method. Maximum value up to

6 mg/h was accepted as normal. Fibrinogen was determined by nephelometry technique, and maximum value regarded as normal was 4 g/l. Also tested were erythrocytes (minimum value was 3.9×10^{12}/l), hemoglobin (values below 120 g/l were accepted as minimum) and leukocytes (maximum value accepted was above 10.0×10^{9}/l). Correlations between the above mentioned laboratory and clinic parameters were investigated. Alpha-2 globulins and gamma-globulins were tested by agar method and values were expressed in g/l. Quantitative determination of immunoglobulins (IgG, IgM, IgA) in serum was done by Radial Immune Diffusion (RID) method (g/l). Fractions of complement component C3 and C4 (g/l) were determined by the same method.

Statistical parameters used for presentation of the results were: structure, prevalence, arithmetic average (Xb), standard deviation (SD), variation coefficient (CV %) and variation interval (Rmax-Rmin). T test and χ^2 test were used to determine differences between factors or features. Probability level was expressed by $p<0.01$ and $p<0.05$. Relationship between the variables was measured by Pearson linear correlation.

Results

Seropositive patients had higher average levels of erythrosedimentation (ERS) (table 1) (Xb=46.09mm/h, SD=22.17) compared to seronegative (Xb=45.10mm/h, SD=19.70), without statistically significant difference with regard to sero-status and sex. Elevated values of C-reactive protein (PCR) (table 2) had 39 (56.5%) seronegative and 40 (60.6%) seropositive patients. No statistically significant difference was found with regard to sero-status and sex.

Table 1. **Erythrocyte sedimentation** (ERS) **rate with regard to sero-status and sex**
Tablica 1. **Vrijednosti brzine eritrosedimentacije** (ERS) **u odnosu na sero-status i spol**

ERS (mm/h)		SNRA	SPRA	Total	T-test
Female	N	93	93	186	
	Xmax	128	125	128	
	Xmin	12	10	10	
	Xb	45.80	46.47	46.13	T=0.21
	SD	20.85	22.19	21.47	p>0.05
Male	N	32	32	64	
	Xmax	72	83	83	
	Xmin	18	17	17	
	Xb	43.06	44.97	44.02	T=0.39
	SD	16.01	22.41	19.34	p>0.05
Total	N	125	125	250	
	Xmax	128	125	128	
	Xmin	12	10	10	
	Xb	45.10	46.09	45.59	T=0.37
	SD	19.70	22.17	20.93	p>0.05
T-test M/F	T	T=0.77	T=0.33	T=0.73	
	p	p>0.05	p>0.05	p>0.05	

The average values of the number of erythrocytes (Er) of both sero-groups (table 3) were almost equal (Xb=3.53×10^{12}/l seronegative, Xb=3.5×10^{12}/l seropositive). Seropositive female patients had lower values compared to seronegative, but this difference was not significant. Male patients had almost equal values, no significance was found with regard to sero-status.

Seropositive patients had lower average Hb (hemoglobin) values (Xb=11.23 g/l,) compared to seronegative (Xb=11.70 g/l, SD=1.09), and these differences were statistically significant (t=2.26; $p<0.05$). Alike, seropositive female patients had lower Hb average values (Xb=10.77 g/l, SD=1.16) compared to seronegative (Xb=11.49 g/l, SD=1.07), with statistically significant difference (t=4.38, $p<0.01$). Male patients, in both groups, had nearly same Hb values (t=0.48, $p>0.05$).

Leukocyte (Le) average values did not show a significant difference with regard to sero-status (t=1.50, $p>0.05$), although seronegative patients had higher values (Xb=7.34×10^{9}/l, SD=2.04) compared to seropositive (Xb=6.98×10^{9}/l, SD=1.79×10^{9}/l). Seronegative male and female patients had higher Le values, but statistically significant difference was found only for seronegative male patients (t=1.37, $p<0.05$).

Seropositive patients had higher fibrinogen average values (Xb=4.76 g/l, SD=1.16) compared to seronegative (Xb=4.46 g/l, SD=1.06), and this difference was statistically significant (t=2.10, $p<0.05$). Statistically significant (t=2.65, $p<0.01$) was also the difference between seropositive (Xb=4.86 g/l, SD=1.19) and seronegative (Xb=4.43 g/l, SD=1.04) female patients, which was not the case in male patients (t=0.41, $p>0.05$).

Table 2. **C-reactive protein** (CRP) **with regard to sero-status and sex**
Tablica 2. **C-reaktivni protein** (CRP) **u odnosu na sero-status i spol**

PCR (mg/l)	Female				Male				Total			
	SNRA		SPRA		SNRA		SPRA		SNRA		SPRA	
	N	%	N	%	N	%	N	%	N	%	N	%
Examinees	47	50.5	47	50.5	22	68.8	19	59.4	69	55.2	66	52.8
Elevated	28	59.6	30	63.8	11	50.0	10	52.6	39	56.5	40	60.6
Test	χ^2=0.05		p>0.05		χ^2=0.02		p>0.05		χ^2=0.09		p>0.05	

Seropositive patients (table 4) had lower Hb average values (Xb=11.0, SD=1.3) compared to seronegative (Xb=11.6, SD=0.9) and this was expressed with statistically significant difference only for III[d] functional class (t=2.76, p<0.01). Correlation between functional classes and average Hb values was low and not significant: r=0.04, p>0.05 seronegative, r=0.10, p>0.05 seropositive.

Hb average values of seropositive patients were prominent only in case when the duration of the disease was in interval 1-10 year (t=2.09, p<0.05), and statistical difference was not significant in case when duration of the disease exceeded 10 years (t=1.00, p>0.05). Correlation coefficient between duration of the disease and Hb average values was low for both subsets, seronegative (r=-0.05, p>0.05) and seropositive (r=-0.02, p>0.05) (figures 1 and 2).

Elevated IgG (immunoglobulin) values (table 5) had 42 (68.9%) seronegative and 41 (68.3%) seropositive patients without statistically significant difference. Significantly elevated IgM values had 9 (15%) seronegative patients and 49 (79%) seropositive, (χ^2=47.6, p<0.01). Significance was also found for female seropositive patients (χ^2=35.68, p<0.01), which was not the case with male patients (χ^2=3.33, p>0.05). IgA values were elevated in 14 (24.1%) seronegative and 23 (37.7%) seropositive patients, without any statistically significant difference with regard to sero-status and sex.

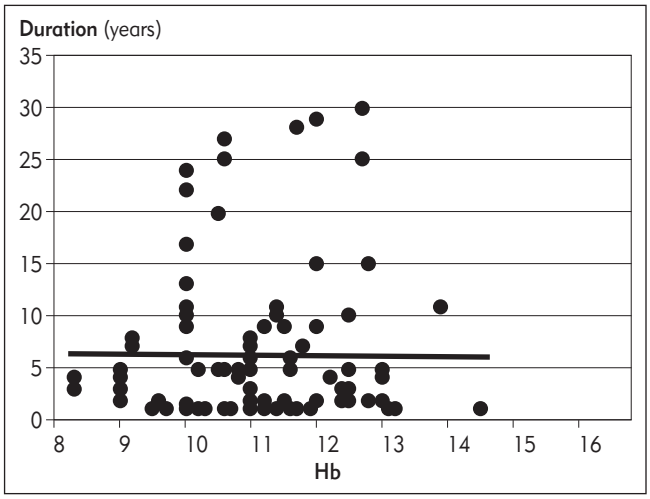
Table 3. **Laboratory parameters with regard to sero-status and sex**
Tablica 3. **Laboratorijski parametri u odnosu na sero-status i spol**

Examinations		SNRA	SPRA	Total	T-test
Examinees	N	125	125	250	
Er (10^{12}/l)	Xmax	4.9	4.9	4.9	
	Xmin	2.8	2.3	2.3	
	Xb	3.53	3.50	3.51	
	SD	0.46	0.54	0.50	T=0.36
	CV	13.17	15.49	14.35	p>0.05
Hb (g/l)	Xmax	14.8	23.8	23.8	
	Xmin	9.6	8.3	8.3	
	Xb	11.70	11.23	11.47	
	SD	1.09	2.02	1.64	T=2.26
	CV	9.33	18.01	14.29	p<0.05
Le (10^9/l)	Xmax	14.3	11	14.3	
	Xmin	4.3	3.9	3.9	
	Xb	7.34	6.98	7.16	
	SD	2.04	1.79	1.92	T=1.50
	CV	27.75	25.69	26.87	p>0.05
Fibrinogen (g/l)	Xmax	7.2	7.7	7.7	
	Xmin	2.3	3	2.3	
	Xb	4.46	4.76	4.61	
	SD	1.06	1.16	1.12	T=2.10
	CV	23.69	24.32	24.21	p<0.05

Table 4. **Correlation between average Hb values and RA functional status II-III** (ARA) **with regard to sero-status**
Tablica 4. **Korelacija izmedu srednjih vrijednosti Hb i funcionalnog statusa II-III** (ARA) **u odnosu na sero-status**

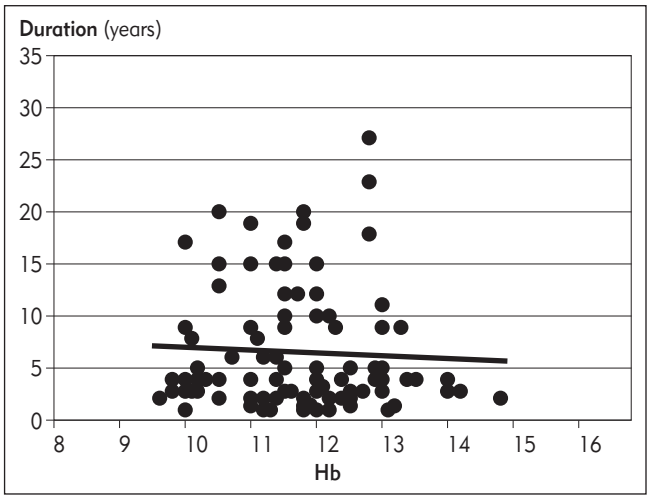
Hb (g/l)		SNRA	SPRA	Total	T-test
Functional class II	N	79	80	159	
	Xb	11.7	11.4	11.6	T=1.21
	SD	1.2	2.3	1.8	p>0.05
Functional class III	N	46	45	91	
	Xb	11.6	11.0	11.3	T=2.76
	SD	0.9	1.3	1.2	p<0.01
Total	N	125	125	250	
	Xb	11.7	11.2	11.5	T=2.26
	SD	1.1	2.0	1.6	p<0.05
II/III t-test	T	T=0.48	T=1.22	T=1.25	
	p	p>0.05	p>0.05	p>0.05	
Correlation	R	-0.04	-0.10	-0.07	
	T	>0.05	>0.05	>0.05	

Figure 1. **Correlation and regression between Hb values and duration of the disease in SPRA**
Slika 1. **Korelacija i regresija između vrijednosti Hb i trajanja bolesti u SPRA**



y=-0.06x+7.05 R=-0.05 T=-0.52 p>0.05

Figure 2. **Correlation and regression between Hb values and duration of the disease in SNRA**
Slika 2. **Korelacija i regresija između vrijednosti Hb i trajanja bolesti u SNRA**



y=-0.26x+9.45 R=-0.02 T=-0.20 p>0.05

Table 5. **Immunoglobulin values** (IgM,IgG,IgA) **with regard to sero-status and sex**
Tablica 5. **Vrijednosti imunoglobulina** (IgM,IgG,IgA) **u odnosu na sero-status i spol**

Immunoglobulin (g/l)		Female				Male				Total			
		SNRA		SPRA		SNRA		SPRA		SNRA		SPRA	
		N	%	N	%	N	%	N	%	N	%	N	%
Ig G	Examinees	47	50.5	48	51.6	14	43.8	12	37.5	61	48.8	60	48.0
	Elevated	31	66.0	33	68.8	11	78.6	8	66.7	42	68.9	41	68.3
	Test	χ^2=0.01		p>0.05		χ^2=0.06		p>0.05		χ^2=0.02		p>0.05	
Ig M	Examinees	45	48.4	49	52.7	15	46.9	13	40.6	60	48.0	62	49.6
	Elevated	9	20.0	41	83.7			8	61.5	9	15.0	49	79.0
	Test	χ^2=35.68		p<0.01		χ^2=3.33		p>0.05		χ^2=47.60		p<0.01	
Ig A	Examinees	46	49.5	48	51.6	12	37.5	13	40.6	58	46.4	61	48.8
	Elevated	12	26.1	18	37.5	2	16.7	5	38.5	14	24.1	23	37.7
	Test	χ^2=0.93		p>0.05		χ^2=0.59		p>0.05		χ^2=0.63		p>0.05	

Table 6. **Complement component C3 and C4 in serum with regard to sero-status and sex**
Tablica 6. **Komponente komplementa C3 i C4 u odnosu na sero-status i spol**

Complement component (g/l)		Female				Male				Total			
		SNRA		SPRA		SNRA		SPRA		SNRA		SPRA	
		N	%	N	%	N	%	N	%	N	%	N	%
C3	Examinees	45	48.4	38	40.9	14	43.8	14	43.8	59	47.2	52	41.6
	Elevated	6	13.3	2	5.3			3	21.4	6	10.2	5	9.6
	Reduced	16	35.6	18	47.4	1	7.1	2	14.3	17	28.8	20	38.5
	Test	p>0.05				p>0.05				p>0.05			
C4	Examinees	45	48.4	40	43.0	13	40.6	14	43.8	58	46.4	54	43.2
	Elevated	3	6.7							3	5.2		
	Reduced	6	13.3	9	22.5			2	14.3	6	10.3	11	20.4
	Test	p>0.05				p>0.05				p>0.05			

Table 7. **Plasma proteins with regard to sero-status and sex**
Tablica 7. **Proteini plazme u odnosu na sero-status i spol**

Protein electrophoresis (g/l)		Female				Male				Total			
		SNRA		SPRA		SNRA		SPRA		SNRA		SPRA	
		N	%	N	%	N	%	N	%	N	%	N	%
Alpha 2-globulins	Examinees	53	57.0	46	49.5	17	53.1	22	68.8	70	56.0	68	54.4
	Elevated	24	45.3	29	63.0	8	47.1	12	54.5	32	45.7	41	60.3
	Test	χ^2=2.15		p>0.05		χ^2=0.02		p>0.05		χ^2=2.39		p>0.05	
Gamma-globulins	Examinees	55	59.1	53	57.0	20	62.5	16	50.0	75	60.0	69	55.2
	Elevated	34	61.8	40	75.5	11	55.0	13	81.3	45	60.0	53	76.8
	Test	χ^2=1.74		p>0.05		χ^2=1.70		p>0.05		χ^2=3.93		p<0.05	

Elevated values of complement component C3 were found in 6 (10.2%) seronegative and 5 (9.6%) seropositive patients (table 6). Reduced values were found in 20 (38.5%) seropositive and 17 (28.8%) seronegative patients. Elevated values of complement component C4 were found in only 3 (5.2%) seronegative, and reduced values in 11 (20.4%) seropositive patients. No statistically significant difference was found with regard to sero-status and sex.

Alpha 2-globulins (table 7), were elevated in 41 (60.3%) seropositive patients and 32 (45.7%) seronegative without any statistically significant difference. Gamma-globulin levels were higher in seropositive patients: 53 (76.8%) seropositive, and 45 (60%) seronegative, and this difference was statistically significant (χ^2=3.93, p<0.05). Seropositive male and female patients had higher levels of alpha 2-globulins and gamma-globulins, but statistically not significant.

Discussion

There were found biochemical changes in patient with RA, but non one was specific for this disease (1,29). We found within acute-phase reactants elevated average values for erythrosedimentation rate (t=0.37,

p>0.05) and fibrinogen (t=2.10, p<0.05) in seropositive patients, whereas PCR values were almost equally positive in both subsets. Alike, was confirmed by Delpuech P. et al. (30), Shadick NA. et al. (31) that CRP measurement in blood did not separate seropositive versus seronegative rheumatoid arthritis. Average values of the leukocytes (t=1.37, p<0.05) in seronegative male patients, and average values of fibrinogen (t=2.65, p<0.01) in female patients show a statistically significant difference with regard to sex. Some authors have found also elevated levels for ERS and CRP in seropositive patients during the course of the disease (32,33). Ernst E. et al. (34) have found correlation between the titer of RF and the activity of the disease. At the same time, Listing J. et al. (35) have found elevated CRP in values in erosive RA and in patients with present DRB1*04 or DRB1*01. Van Leeuwen MA. et al. (36) have confirmed that FRIgM levels represent better activity of the disease compared to FRIgA and FRIgG levels, however their clinical significances are limited compared with CRP. We found that average value of the number of erythrocyte did not change with regard to sero-status and sex, whereas average values of hemoglobin were reduced in seropositive patients (t=2.26, p<0.05), especially in seropositive female (t=4.38, p<0.01) and did not correlate with functional disability and the duration of the disease. We did not find considerable alterations in average values of leukocytes with regard to sero-status, although there were detected frequently elevated values in seronegative patients. Poulter LW. et al. (37) didn't find significant difference in proportions of lymphocyte or macrophage subsets between the groups with seropositive and seronegative inflammatory arthritis. Kohler T. et al. (38) have compared seropositive and seronegative patients, where IgM was detected more in seropositive RA which matches with our findings that elevated IgM values were frequently detected in seropositive patients (χ^2=47.6, p<0.01) undistinguished regarded to sex. According to our data, average values of IgA and IgG did not show differences with regard to sero-status. Similar results we can find in the study of Kim NH. et al. (39), who find elevated levels of immunoglobulins in both sero-groups, in serum and synovial fluid, slightly more in seropositive patients. Similar opinion has Kholmogorova GT. et al. (40) that IgD was more often in the seropositive patients to rheumatoid factor (60%) than in seronegative ones (33%) but the difference was not statistically significant.

At the same time, Pai S. et al. (41) did not find significant diference in IgA levels in serum within patients with RF IgA positive and negative, in our data we can find that complement component C3 and C4 were reduced in seropositive patients, but there was not any significance with regard to sero-status and sex (39,42).

Conclusion

There was insufficient data to conclude that the differences between seronegative and seropositive are evident.

Acute-phase reactants, IgA, IgG levels and alpha-2 globulins levels, with some irrelevant differences, are almost equal with regard to sero-status. IgM, gammaglobulins and reduced complement component C3 and C4 in serum are prominent in seropositive patients. With regard to sero-status differences within sex, with some exceptions, are not relevant.

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