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The Effects of Rituximab on Lipids, Arterial Stiffness, and Carotid Intima-Media Thickness in Rheumatoid Arthritis

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The aim of the study was to examine lipid profiles, arterial stiffness (AS), and carotid intima-media thickness (cIMT), in 55 women with RA without overt cardiovascular disease (CVD) treated with rituximab (RTX).The following parameters were recorded before and 24 weeks after RTX therapy (2 infusions of 500 or 1,000 mg RTX intravenously, fortnightly): plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, DAS 28-ESR, serum C-reactive protein (CRP), RF IgM, AS (SI - stiffness index, RI – reflection index) by digital volume pulse contour analysis (Micro Medical, UK), and common cIMT by high-resolution B-mode carotid ultrasound. Based on the European League Against Rheumatism (EULAR) criteria, patients were divided into two groups: 1) moderate/good response to RTX therapy after 24 weeks (41 patients, 75%), 2) no response to RTX therapy (14 patients, 25%). Effective RTX therapy resulted in 9% increase in TC, 23% increase in HDL-C and 14% decrease in atherogenic index, 57% decrease in SI and 24% decrease in RI. We observed a 9% decrease of cIMTmax at 24 weeks. The improvement of cardiovascular parameters was accompanied by statistically significant decreases of CRP, ESR, RF IgM, and DAS 28 in group 1 ($P < 0.05$). There were no significant changes in lipid profile, AS parameters, and cIMT in group 2. Two infusions of RTX in case of moderate/good EULAR effect of therapy exerted favorable effects on lipid profile, AS and cIMT in women with RA without overt CVD.

Keywords: Rheumatoid Arthritis; Lipids; Carotid Atherosclerosis; Arterial Stiffness; Rituximab

INTRODUCTION

Rheumatoid arthritis (RA) confers a high risk of cardiovascular morbidity and mortality due to traditional cardiovascular risk factors, uncontrolled systemic inflammation, endothelial dysfunction, enhanced atherosclerosis, and prothrombotic state (1-4). Proinflammatory states in RA may also damage the elastic structures of arteries, increase arterial stiffness, and eventually contribute to peripheral overload and heart failure (5). Deteriorated elastic properties of the arterial wall and thickened carotid artery intima-media complex are independent predictors of cardiovascular events in the general population (6,7). Enhanced arterial stiffness and carotid intima-media thickness (cIMT) are both associated with systemic rheumatoid inflammation (5,8,9), all of which add to the cardiovascular phenomenon in RA.

Over the past decade, significant achievements in the treatment of RA have resulted in improved prognosis of the disease due to the prevention of irreversible changes in the musculoskeletal system (10). Drug-induced remission and low activity of RA are also associated with lowered cardiovascular risk (11).

Rituximab, the chimerical monoclonal antibodies to CD20 of B-lymphocytes, has been successfully used in the treatment of highly active RA, and few studies pointed to the beneficial effects of the biologic therapy on systemic inflammation, endothelial function and arterial stiffness of patients with RA (12-14). Pilot studies of rituximab in RA have also proved that the drug therapy ameliorates lipid profiles and variability of cardiac rhythm in RA (15-17).

The aim of the current study was to examine blood lipid profile, systolic and diastolic blood pressure (SBP and DBP), body mass index (BMI), blood glucose level, elastic properties of the arterial wall and cIMT in women with RA without clinical manifestations of cardiovascular disease during 6-month rituximab therapy.

MATERIALS AND METHODS

Subjected patients

A total of 55 women with definite diagnosis of RA (18) and high disease activity (DAS28-ESR ≥ 5.2) (18) were enrolled in the study. Mean age of the patients was 50.4 years, mean disease duration ≤ 98 months, and DAS28-ESR ≤ 6.2 (Table 1). Most patients were positive for rheumatoid factor (RF) and antibod-

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Table 1. Baseline characteristics of women with RA (n = 55)

Parameters	No. of patients
Age, yr	50.4 ± 1.7
Disease duration, mo	98 ± 9
DAS 28-ESR	6.2 ± 0.1
HAQ score, points	1.77 ± 0.1
Radiological stage (I/ /II/ III/ IV), %	2/33/38/27
RF+, %	82
Anti-CCP+, %	79
Extraarticular manifestations, %	47
NSAIDs, %	98
GC, %	80
GC dose at the moment of estimation, mg per day	8.0 ± 0.5
DMARDs use, %	86
Methotrexate use, %	55
Dose of methotrexate, mg/wk	12.1 ± 0.38
Leflunomide use, %	38
Dose of leflunomide, mg/day	20
Other DMARDs, %	7

Data are presented as M ± m, unless otherwise noted. RF+, rheumatoid factor positivity; anti-CCP+, positivity for anti-cyclic citrullinated peptide antibodies; NSAIDs, use of non-steroidal anti-inflammatory drugs; DAS28-ESR, disease activity score 28-joints based on erythrocyte sedimentation rate; DMARDs, disease-modifying anti-rheumatic drugs; RA, rheumatoid arthritis; GC, glucocorticoids.

ies to cyclic citrullinated peptide (anti-CCP), with Steinbrocker's radiological stage ≥ II. Mean score of functional insufficiency of joints HAQ (20) was 1.77. Extraarticular features of RA were present in 26 patients (47%), including 12 (22%) with rheumatoid nodules, 11 (20%) with neuropathy, 4 (7%) with dermatovasculitis (erosive-ulcerative vasculitis, nail-bed hemorrhage, digital arteritis), 2 (3.5%) with pleuritis/pericarditis, 3 (5.2%) with eye lesions.

Medication

Disease-modifying anti-rheumatic drugs (DMARDs) were administered in 47 patients (86%): methotrexate-in 26 (55%), leflunomide-in 17 (38%), and other DMARDs (hydroxychloroquine, sulfasalazine, chlorobutine) in 4 (7%). Doses of DMARDs were constant during the 6-month study period. Nonsteroidal anti-inflammatory drugs (NSAIDs) were used by 54 patients (98%) and glucocorticoids (GC < 10 mg/day) in 44 patients (80%). In all the patients previous DMARD therapies were not efficient for decreasing DAS28-ESR or they were intolerant to two and more DMARDs. Inhibitors of tumor necrosis factor alpha were inefficient in 38% of them before the start of rituximab therapy.

Rituximab was administered intravenously, fortnightly: 500 mg in 12 patients (22%), 1,000 mg in 43 (78%) on top of DMARDs, NSAIDs and GCs. All patients were tolerant to the biological therapy. The clinical efficiency of the therapy was assessed by the EULAR criteria at a 6-month point (21). Based on the criteria, patients were divided into two groups: group 1-41 (75%) with good and satisfactory response to the therapy and group 2-14 (25%) with no response.

Inclusion criteria

Patients fulfillingthe American College of Rheumatology (ACR) classification criteria for RA, 18-60 years ofage, and designated to receive rituximab, after evaluation by a clinicalrheumatologist independent of this study.

Exclusion criteria

Age above 60 years, established coronary artery disease (angina pectoris, previous myocardial infarction), clinical manifestations of chronic heart failure of New York Heart Association (NYHA) functional class II-IV, cerebral stroke, diabetes mellitus (DM), valvular heart defects, 3-4 degree of obesity, gastric and duodenal ulcers, oncological diseases, infectious diseases, thyroid disease and refusal to participate in the study. Patients on regular drug therapies for concomitant diseases (hypotensive, hypolipidemic, and anti-arrhythmic) were also excluded.

Clinical examination

Patients were examined before and after 6 months following rituximab administration. Descriptive information about traditional cardiovascular risk factors (hypertension, smoking, overweight, family anamnesis of CVD, menopause, dyslipidemia, and hypodynamia) and aggregate cardiovascular risk by the SCORE (22) were recorded.

Biomarkers

To assess elastic properties of the arterial wall, indicators of digital volumetric pulse were recorded by the Pulse Trace apparatus (Micro Medical, UK). The stiffness index (SI, m/s) and the pulse wave reflection index (RI, %) were analyzed. In cases of inability to estimate the value of arterial stiffness ("stiff arteries"), maximal values for SI (20 m/s) and RI (90%) were recorded.

Duplex scanning of carotid arteries was performed the Voluson 730 Expert device (Austria) using a transducer probe with a frequency of 7.5 MHz. The cIMT values in mm were recorded at three points from each side: at 10 mm to the carotid bulb on the common carotid artery; 5-10 mm cranially from the bulb; and at a 10 mm distance from the bifurcation on the internal carotid artery. The mean IMT was calculated based on three values from each side. Thickened IMT corresponded to values from 0.9 to 1.2 mm, and values of IMT ≥ 1.2 mm were interpreted as atherosclerotic plaques (23).

Total cholesterol (TC) values were recorded using the fermentative photometric test Chod-PAP, and triglycerides (TG) concentrations were identified using the fermentative colorimetric method (GPO-PAP) with glycerol-3-phosphatoxidase (variation coefficient < 5%). The values of high density lipoprotein cholesterol (HDL-C) were estimated by the fermentative method on the biochemical analyzer "Bayer" (Germany) using "DiaSys" reagents (Germany). The values of low density lipoprotein cholesterol (LDL-C) were calculated using the Fried-

wald formula with concentration of TG lower than 400 mg/dL (LDL-C = TC–[HDL-C +TG/2.19] in mmol/L). To determine the correlation between atherogenic and antiatherogenic lipoproteins, atherogenicity index (AI) was calculated: (TC–HDL-C)/HDL-C: the level > 4 reflects the atherogenicity of lipoproteins).

C-reactive protein (CRP) and IgM RF in blood serum were determined by highly sensitive immune nephelometric method using an automatic analyzer BN 100, BEHRING (Germany). Concentrations of anti-CCP were determined by immune enzyme analysis with the use of the commercial kit "Axis – Shield Diagnostic Limited" (UK).

Statistical analysis

Statistical analyses were performed by SPSS 15.0 software. Distribution of all parameters was checked by the Kolmogorov-Smirnov test. Normally distributed data were presented as M ± m. Statistical significance of the difference between not normally distributed variables was checked by the Mann-Whitney test. Matched samples were analyzed by the Wilcoxon Z-criterion. Significance of the differences between percentages was tested by the chi-squared test or the Fisher's exact test. Association between continuous variables was analyzed by the Spearman's rank correlation coefficient. Two-sided *P* values < 0.05 were set as significant.

Ethics statement

The local ethics committee of the V.A.Nasonova Research Institute of Rheumatology, Moscow, Russia approved the study and the patients gave a signed informed consent on inclusion (IRB No. 19-18-09-2009f).

RESULTS

At 6-month, values of DAS28-ESR and HAQ score, concentrations of IgM RF, CRP, and ESR were significantly lower in group 1 compared to group 2. There was only a tendency to a decrease of these indicators in group 2 (Table 2).

Group 1 demonstrated a significant increase in TC (9%) and HDL-C (23%) without any significant changes in LDL-C and TG, resulting in lowered atherogenicity index (by 14%) (Table 3). No statistically significant differences in fasting blood glucose, SBP, DBP, BMI, and the aggregate cardiovascular risk (SCORE) were noted in group 1. No significant changes in indicators of the lipid profile, glucose, SBP, DBP, BMI, and SCORE were recorded in group 2 (Table 3).

Rituximab therapy led to the improvement of elastic properties of the arterial walls in group 1 due to the decreased stiffness in major arteries (SI–decreased by 57%) and arterioles (RI–decreased by 24%) (Table 4). The rate of "very stiff" arteries lowered 3.5 times in group 1, whereas no significant changes in parameters of arterial stiffness were recorded in group 2 (Table 4).

Table 2. Dynamics of DAS28, HAQ score, RF, ACCP, CRP, and ESR in patients with RA on rituximab therapy

Parameters	Group 1 (n = 41)		Group 2 (n = 14)	
	Baseline	After 6 mo	Baseline	After 6 mo
DAS 28-ESR	6.3 ± 0.12	3.5 ± 0.1*	6.2 ± 0.3	5.6 ± 0.3
HAQ score, points	1.78 ± 0.09	0.80 ± 0.07*	1.80 ± 0.1	1.35 ± 0.16
IgM RF, IU/mL	488 ± 138	100 ± 34*	392 ± 179	243 ± 109
Anti-CCP, U/mL	70 ± 6	67 ± 7	64 ± 12	59 ± 12
CRP, mg/L	46 ± 6	10 ± 3*	28 ± 7	24 ± 10
ESR, mm/hr	53 ± 3	21 ± 3*	60 ± 7	47 ± 6

Data are presented as M ± m. *$P < 0.05$ before and after rituximab therapy.

Table 3. Dynamics of lipids profile, glucose level, systolic blood pressure, diastolic blood pressure, body mass index, and SCORE in patients with RA on rituximab therapy

Parameters	Group 1 (n = 41)		Group 2 (n = 14)	
	Baseline	After 6 mo	Baseline	After 6 mo
TC, mmol/L	5.64 ± 0.2	6.15 ± 0.2*	6.34 ± 0.5	6.73 ± 0.5
TC > 5 mmol/L, %	67	88	79	92
LDL-C, mmol/L	3.61 ± 0.2	3.73 ± 0.2	4.2 ± 0.5	4.2 ± 0.5
LDL-C > 3 mmol/L, %	74	85	74	85
TG, mmol/L	1.33 ± 0.1	1.36 ± 0.1	1.55 ± 0.2	1.59 ± 0.3
TG > 1.7 mmol/L, %	28	28	21	35
HDL-C, mmol/L	1.42 ± 0.1	1.73 ± 0.1*	1.44 ± 0.1	1.59 ± 0.1
HDL-C < 1.2 mmol/L, %	28	11*	28	14
Atherogenicity index	4.14 ± 0.2	3.78 ± 0.2*	4.52 ± 0.4	4.35 ± 0.4
Atherogenicity index > 4, %	55	31*	56	41
Fasting blood glucose, mmol/L	4.87 ± 0.1	4.96 ± 0.1	4.51 ± 0.1	5.1 ± 0.1
Fasting blood glucose > 6.0 mmol/L, %	5	2	0	0
SBP, mmHg	119 ± 2.8	119 ± 2.4	112 ± 2.8	125 ± 2.4
DBP, mmHg	74 ± 1.3	74 ± 1.3	71 ± 1.3	76 ± 1.3
BMI, kg/m^2	24.8 ± 0.6	24.5 ± 0.7	23.8 ± 1.3	22.6 ± 1.3
BMI < 20/20-25/ > 25 kg/m^2, %	52/31/17	50/33/17	43/36/21	30/40/40
SCORE, %	0.84 ± 0.2	0.83 ± 0.2	0.59 ± 0.2	0.61 ± 0.2

Data are presented as M ± m unless otherwise noted. *$P < 0.05$ before and after RTX treatment.

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Table 4. Dynamics of arterial stiffness and carotid intima-media thickness in patients with RA on rituximab therapy

Parameters	Group 1 (n = 41)		Group 2 (n = 14)	
	Baseline	After 6 mo	Baseline	After 6 mo
SI, m/s	14.7 ± 0.9	9.9 ± 0.9*	12 ± 3	12 ± 3
RI, %	76.5 ± 3	67 ± 3	68 ± 2	75 ± 2
Stiffarteries, %	52	15*	28	36
IMTmean, mm	0.77 ± 0.03	0.69 ± 0.02*	0.77 ± 0.02	0.73 ± 0.03
IMTmax, mm	0.97 ± 0.03	0.87 ± 0.02*	0.93 ± 0.04	0.92 ± 0.04

Data are presented as M ± m unless otherwise noted. *$P < 0.05$ before and after rituximab therapy. SI, stiffness index; RI, reflection index; IMT, intima-media thickness.

Finally, significant changes of cIMT were noted in group 1 at 6-month: mean cIMT decreased by 11% and maximal cIMT - by 9% (Table 4). There was a correlation between decreasing cIMT and IgM RF ($r = 0.49$, $P < 0.001$). No significant changes of cIMT were recorded in group 2.

DISCUSSION

Controlling systemic inflammation is currently viewed as an effective strategy for modifying cardiovascular risk factors and preventing vascular events in RA (24). Recent cohort data suggest that moderate doses of glucocorticoids (above 7.5 mg/day) increase HDL in patients with RA at high risk of cardiovascular events without increasing the atherogenicity index (25). However, long-term use of these powerful anti-inflammatory agents is associated with numerous side effects, limiting their cardiovascular benefits (26). Widely used anti-TNF agents seem to be safer for controlling risk of vascular events in RA (27), though some patients fail to respond due to intolerance and secondary inefficacy. In case of anti-TNF failure, switching to other biologic agents is advisable, and rituximab hold promise as a safe and effective alternative agent (28).

Rituximab therapy leads to the depletion of B-lymphocytes, which is facilitated by the system of complement, antibody-dependent cytotoxicity, and apoptosis (29). Recent experimental and clinical studies have demonstrated that anti-CD20 therapy modulates the course of atherosclerosis in RA (30). However, there are two different subtypes of B-lymphocytes, which have contrary effects on atherosclerosis: B1-cells are atheroprotective and B2-cells represent proatherogenic family of B-cells (30). Rituximab therapy deplets proatherogenic B2-cells and preserves atheroprotective B1-cells, resulting in inactivation of B-cells and macrophages as well as lowered production of proinflammatory cytokines and antibodies to oxidized LDL. The selective inhibition of B-cells may also indirectly affect endothelial function and prevent vascular events in RA (29).

The present study demonstrated that rituximab therapy increases HDL-C by 22% and ameliorates lipid profile in patients with RA, decreasing the atherogenicity index. Our results are in line with the data of the pilot study by Kerekes et al. (31), who reported an improved lipid profile (lowered TC and raised HDL-C in 5 female patients with RA on rituximab. Our results are also in agreement with the data by Raterman et al. (15), who reported a 9% decrease of the atherogenicity index in 49 patients after 6-month rituximab therapy. In contrast, another small study on the effects of one-year anti-TNF and rituximab therapies in patients with RA, showed anti-CD20 therapy (n = 53) decreased level of atheroprotective IgM antibodies against phosphorylcholine by 14% (32). Additionally, a one-year study of arterial stiffness, lipid profile and systemic inflammation in 33 patients with RA on rituximab, mostly nonrespondents to previous anti-TNF therapies, found no improvement of arterial function and even an increase of LDL-C and the atherogenicity index (33). To sum up, preliminary studies of rituximab in RA have provided contradictory data, which highlight multifaceted effects of anti-CD20 therapies on cardiovascular risk factors. The prognostic cardiovascular significance of the shifts in lipid and vascular parameters in response to rituximab remains uncertain.

The results of the current study point to an improvement of elastic properties of the arterial wall in patients with RA without cardiovascular disease responding to 6-month rituximab therapy. In nonrespondents, we found a tendency toward an aggravation of elastic properties of arteries. These findings are supported by the literature data, suggesting that the dampening of systemic inflammation by rituximab is not always accompanied by the amelioration of arterial stiffness in RA (33). Overall, the effects of rituximab therapy on the vascular wall should be interpreted in the context of the patients' age, preexisting cardiovascular disease, and duration and activity of RA. An important confounding factor, preventing the generalization of the results, is the difference in methodologies for assessing arterial stiffness in different studies.

Previous pilot studies have demonstrated that the depletion of B-cells in RA leads to the ameliorated endothelial function on the background of decreased disease activity (low DAS28) (12,14). A 24-month study of rituximab therapy in 38 patients with RA proved that the biologic therapy may not only improve endothelial function, but also decrease carotid IMT and reduce the progression of atherosclerosis (13). The results of our study prove that rituximab therapy may have positive effects on subclinical atherosclerosis as early as at 6-month, which is associated the reduction of IgM RF - an independent predictor of cardiovascular mortality (34).

Overall, rituximab therapy is well tolerated, and there is no evidence of a direct cardiotoxic effect of the biologic agent (35). One of the largest and longest studies of the safety of rituximab (3,194 patients with RA, who had received up to 17 courses of rituximab over 9.5 years) showed no increased risk of myocardial infarction and stroke over the time (33). The development of infusion reactions and vascular events in the course of rituximab therapy are rarely reported, and their association with the

biologic therapy is debatable (37).

The mechanisms of adverse cardiovascular effects in the course of rituximab therapy in RA remain uncertain. It is possible that platelet activation, overproduction of interleukine-6 and tumor necrosis factor alpha, subsequent coronary spasm, and rupture of atherosclerotic plaques underlie related vascular events. Perhaps the most important driving factor of the adverse vascular events is the preexisting overt or silent cardiovascular disease, necessitating comprehensive cardiovascular assessment prior to the initiation of the therapy. In our study, we enrolled patients without cardiovascular disease, and there were no major and minor side effects over the 6-month period.

In conclusion, rituximab therapy potently suppresses systemic inflammation, improves lipid profile and the atherogenicity index, decreases carotid IMT, and ameliorates elastic properties of the arterial wall in patients with RA without established cardiovascular disease. Further prospective studies are warranted to assess the effects of rituximab on cardiovascular risk factors and associated vascular events in RA.

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DISCLOSURE

There are no potential conflicts of interest related to the contents of the article. No any pharmaceutical agency was involved in the study design, collection, management, analysis, interpretation of the data, writing of the manuscript, and decision to submit the manuscript for publication.

AUTHOR CONTRIBUTION

Conception & design of the study: Novikova DS, Popkova TV, Nasonov EL. Data collection: Novikova DS, Popkova TV, Lukina GV, Luchikhina EL, Volkov AV, Novikov AA, Nasonov EL. Statistical analysis: Novikova DS, Popkova TV. Interpretation and analysis of the data: Novikova DS, Popkova TV. Writing the draft and paper: Novikova DS, Popkova TV. Critical review and revision: Novikova DS, Popkova TV, Lukina GV, Karateev DE, Volkov AV, Novikov AA, Aleksandrova EN, Nasonov EL. Approval of the final manuscript and submission: all authors.

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