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THE IMMUNE STATUS AND VASCULAR BED STIFFNESS INDICES AT THE RHEUMATOID ARTHRITIS PATIENTS DURING THE INFLIXIMAB THERAPY

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The indices study results of the cytokine status in the blood serum, and the functional state parameters of the vascular wall and its dynamics during the infliximab medical treatment at the 38 patients with the advanced stages ACCP – negative variant of the rheumatoid arthritis have been presented in the given paper. So, the significant increase in the serum concentration of the proinflammatory cytokines (e.g. TNF-α, IL-1β, IL-6, IL-8, and IL-17) at the patients with the RA advanced stage, compared with the control group, has been found. The significant decrease at all the examined RA-pulse wave propagation time, the stiffness and the augmentation indices increase, having reflected the vascular wall elasticity decrease patients, have been revealed. The pulse wave velocity (PWV) determination has been shown its further increasing up to 14,2 ± 1,2 % (e.g. $p < 0,05$), that it is indicated in the arterial bed stiffness increase at the examined RA patients. The multivariate correlation analysis carrying out has been shown, that there is the significant inverse correlation between the pulse wave propagation time, the IL-1β, IL-6, IL-8, IL-17, TNF-α serum concentration, and the direct one – between the proinflammatory cytokinesis, the pulse wave velocity (PWV), and the augmentation index at the deployed RA patients. So, the infliximab medical therapy is associated with the content significant reduction of the IL- lβ, IL-6, TNF- α, IL-8, IL-17 blood serum, the stiffness decrease and the vascular wall elasticity increase (e.g. by the lower pulse wave velocity, by the augmentation and the stiffness indices) at the RA patients.

The rheumatoid arthritis (RA) is one of the most common and wide – spread chronic inflammatory diseases in the humans; it is characterized by the symmetrical chronic erosive arthritis (e.g. the synovitis) of the peripheral joints and the systemic immuno-inflammatory visceral injuries [4]. As the important and the significant challenge, in this pathology condition, the premature mortality rate is considered (e.g. up to 50 % of the cases) from the cardio–vascular system diseases, having caused by the atherosclerotic vascular disease (e.g. the myocardial infarction, the congestive heart failure, the sudden death, the acute ischemic stroke), having developed for 10 years earlier, than in the population [8]. So, the cardio – vascular events (CVE) risk at the RA is quite significantly higher, than in the general population, and at the patients, having suffered from the diseases with the proven high – level cardio – vascular risk (e.g. the diabetes mellitus and the arterial hypertension) [8,9,11]. The CVE development risk increase at the RA is associated with the immuno – inflammatory mechanisms, having underlied in the basis of the RA pathogenesis and the atherosclerosis [2]. So, it is believed, that the inflammation, having contributed to the lipids deposition in the vascular wall, is the significant pathogenetic factor «destabilization» of the atherosclerotic plaque and the atherothrombotic complications [11]. It has been shown, in the recent years that the high frequency of the «subclinical» atherosclerotic vascular disease is characterized for the RA. The endothelium dysfunction, the small and the large vessels of the elasticity decrease, the systemic vascular resistance increase are reflected the earliest stage of the atherosclerotic vascular disease, and they have already been detected the RA onset, though these changes degree manifestation is practically increased with the disease duration further increasing.

The atherosclerosis development and the increased risk of the premature death at the RA are associated with the extra – articular manifestations, the disease progressive course, the inflammatory process activity, and the sulpur–positivity for the RF. However, in the overwhelming majority of the cases, the cardio – vascular complications are further developed at the RA patients, with the low or the moderate risk, according to the traditional factors risk, that is why the pressing challenge of the new predictors finding out of their occurence is the most actual one [2, 11].

The effective anti – inflammatory therapy carrying out is played the important role in these complications prevention, having taken into consideration the chronic inflammation and the autoimmune disorders place, in the atherosclerosis development and the CVE related with it at the RA [6].

Recently, the genetically engineered biological agents (GEBA) are successfully used, that are permitted to be controlled the immuno-pathological processes further development for the patients' medical treatment with the RA high level activity [7]. However, up to the present time, there is no clear understanding and its presentations on the genetically

engineered biological agents (GEBA) operation on the cardio – vascular system, since the data inconsistencies and also the insufficient knowledge of the development mechanisms, and the cardio – vascular diseases progressiveness at the RA [7, 9].

Therefore, the absolute practical – scientifically interest is presented the further study, as the immune status and the arterial stiffness indices relationship, well as the GEBA cardio – vascular effects, including infliximab, which may be of the great practical significance not only to be highlighted the high risk groups of the cardio – vascular pathology disease at the RA patients, but it is enable to be personalized the whole therapy, to be reduced the cardio – vascular complications occurrence frequency, and also to be improved the life quality, and the prognosis of this category patients.

This present study **main purpose** has been to be examined the relationship between the pro-inflammatory cytokines level (e.g. TNF-α, IL-1β, IL-6, IL-8, and IL-17) and the vascular wall stiffness at the RA patients, and their dynamics during the infliximab medical therapy.

The Materials and Methods. The 38 patients have been under their observation with the advanced stages of the rheumatoid arthritis, from the moderate up to the high level disease activity; the RA duration has not been exceeded 2 years (e.g. 24 months). So, the patients' average age has been made up 32,6 ± 8,5 years (e.g. 390 ± 102 months). The patients' inclusion criteria into this study have been the following: the RA presence 2–3 degrees of their activity, the ACCP – negative variant of the disease, the evidence availability, and the contraindications absence for the infliximab to be used, the patient's informed consent presence for the further inclusion into the study. The patients' exclusion criteria from this study have been the following: the Ra 1 activity degree, the ACCP – positive variant of the disease, the infectious processes of any localization, the diabetes mellitus, the liver and the kidney dysfunction diseases, and the congestive heart failure. So, the rheumatoid arthritis diagnosis has been prescribed, in accordance with the «ACR/EULAR» criteria, 1987/2010. The RA general activity and the functional class (FC) have been determined, according to the RA classification, having adopted at the Plenary Session of the Russian Rheumatologists Association in 2007. So, the RA activity quantitative assessment has been carried out with the DAS 28 (Disease Activity Score) index use, having recommended by «EULAR» [4]. The «EULAR» criteria have been used, having based on the «DAS 28» index dynamics, to assess the therapy effectiveness.

So, the content in the TNF-α, IL-1β, IL-6, IL-8 blood serum has been measured by the immune – enzyme analysis with the test systems use (e.g. the «Protein Contour» LLC, the Saint – Petersburg city), the IL-17 – by the solid – phase immune – enzyme analysis sandwich – option method with the «R&D Diagnostics Inc.» (USA) firm reagents use, the TGF-β1 has been determined by the immune – enzyme method (e.g. («Amersharm Pharmacia Biotech»).

The investigation of the vascular wall state parameters has been evaluated by means of the daily arterial blood pressure (ABP) monitor of the «Peter Telegin» company of Nizhny Novgorod city and the «BPLab®» software. So, the following parameters have been determined: TPWP – the time pulse wave propagation (ms); ASI – the arterial wall stiffness index; AIx – the augmentation index (%); SAI – the systolic area index (%); PWV (cm/sec) – the pulse wave velocity (e.g. it has been calculated by the formula:

$$PWP = \frac{l_{Ao} + l_{av}(ASc + AA + 1/3AB)}{TPWP},$$

where l_{Ao} – the distance between the aorta mouth and the subclavian artery, having measured sonographically; l_{av}(ASc + AA + 1/3 AB) is the total length of the subclavian axillary and the one third shoulder.

The biochemichal and the functional studies methods had been carried out by the conventional methods and the generally accepted methodologies, that was allowed to be evaluated the various organs function, to be identified the comorbidities and the concomitant diseases.

Thus, the 20 practically healthy persons at the age of 42,5 ± 6,4 years (e.g. 509 ± 76 months), having made up the control group members, have been included in the study, for the parameters' comparaive evaluation of the cytokine status and the vascular wall stiffness.

The cytokine status evaluation and the vascular wall stiffness parameters have been determined just before the medical treatment after the 12 months of the infliximab therapy.

So, the basic anti – inflammatory therapy of the patients has been included the methotrexate 11,8 ± 8,2 mg/week, prior the infliximab medical treatment. The infliximab has been administrated into/venously infusion driply by 200 mg/day, according to the recommended scheme: 0, 2, 4, 6 weeks, then every 8 weeks during the 12 months.

So, the digital data statistical processing has been produced with the «Microsoft Excel» applied programs standard package and the «STATISTICA 6.0» for Windows. The quantitative variables statistical analysis has been based on the difference of the average arithmetical populations. In the case of the two independent samples, the t – Student test criterion with the variance separate evaluation for the independent groups has been used. The Pearson's linear correlation coefficients have been calculated to be evaluated the relationship between the quantitative parameters. So, the Student and Wilcoxon tests criteria have already been used to be assessed the indices changes validity during the medical treatment.

The Results and Discusion. It is quite well – known, that the cytokines are invoved, and they are taken part in the inflammation, the hematopoiesis,

the immunocompetent cells' differentiation and its further growth, the immunoregulation, the lymphocytes' chemotaxis processes regulation [6].

The synovial tissue is the main synthesis site, as the main pro-inflammatory, well as some anti – inflammatory cytokines at the RA, while their concentration density is increased, as in the synovial fluid, well as in the blood serum [3, 10].

It should be noted, that these immunological markers of the atherosclerosis, as the TNF-α, IL-1β, IL-6, and the others, on the one hand, are the atherothrombotic complictions «predictors», and they are associated with the traditional risk factors of the cardiovascular diseases, on the other hand, – they are reflected the chronic inflammatory process course at the RA [2]. Therefore, it has been interesting to be determined the TNF-α, IL-1β, IL-6, IL-8, and IL-17 content in the blood serum of the RA patients.

Thus, the study results have already been shown the significant increase in the TNF-α, IL-1β, IL-6, IL-8, and IL-17 concentration at the patients with the advanced RA stages, in comparison with the control group members (Table 1). The content in the blood serum at the RA patients in the TNF-α $5,1 \pm 0,6$ times (e.g. $p < 0,05$); in the IL-1β – $4,3 \pm 0,7$ times (e.g. $p < 0,05$); in the IL-6 in $9,6 \pm 0,9$ times (e.g. $p < 0,05$); in the IL-8 in $8,1 \pm 0,6$ times (e.g. $p < 0,05$); in the IL-17 in $4,6 \pm 1,1$ times (e.g. $p < 0,05$) has been higher than the control level.

Table 1

The Pro-Inflammatory Cynokines Content Dynamics in the Blood Serum of the Advanced RA Patients During the Infliximab Therapy

Indices (pkg/ml)	Control ($n = 20$)	RA Patients	
		Before treatment ($n = 38$)	After the infliximab therapy ($n = 38$)
	1	2	3
TNF-α	32,4 ± 3,6	216,7 ± 18,2*1	94,2 ± 10,4*1-2
IL-1β	35,4 ± 4,1	148,2 ± 12,9*1	98,2 ± 9,6*1-2
IL-6	15,8 ± 3,9	128,5 ± 11,2*1	76,4 ± 8,8*1-2
IL-8	8,8 ± 2,4	37,6 ± 3,8*1	18,9 ± 2,4*1-2
IL-17	7,8 ± 1,2	31,4 ± 1,8*1	24,1 ± 1,6*1-2

N o t e . The significant differences of the arithmetic averages (e.g. $p < 0,05$) have been indicated by the asterisk (e.g. *), the numbers next to the asterisk – in relation to that group indices some of these differences are the significant ones.

The elastic – flexibly properties study of the vascular wall has been shown the significant decrease at all the examined RA patients, in comparison with the control pulse wave propagation time (PTT) –for $28,1 \pm 1,6\%$; the increase stiffness index (ASI) – for $24,3 \pm 2,8\%$ (e.g. $p < 0,05$), the augmentation index (AIx) – $64,8 \pm 5,8\%$ ($p < 0,05$), SAI – the systolic area index – for $21,4 \pm 1,1\%$ ($p < 0,05$), which is characterized the vascular wall elasticity degrease (Table 2).

Table 2

The Vascular Wall Stiffness Indices Dynamics at the RA Patients During the Infliximab Treatment

Parameters	Control ($n = 20$)	RA Patients	
		Before treatment ($n = 38$)	After the infliximab therapy ($n = 35$)
	1	2	3
PWPV (sm/sec)	117,8 ± 14,5	148,4 ± 6,8*1	132,3 ± 6,4*1,2
PTT (ms)	164,5 ± 2,4	122,4 ± 11,4*1	136,1 ± 9,1*1,2
ASI	37,3 ± 5,1	49,6 ± 3,1*1	42,5 ± 4,8*1,2
AIx (%)	−30,9 ± 4,2	−10,6 ± 1,1*1	−16,8 ± 1,6*1,2
SAI (%)	54,8 ± 2,3	66,3 ± 2,4*1	58,4 ± 1,6*1,2

N o t e . The significant differences of the arithmetic averages (e.g. $p < 0,05$) have been indicated by the asterisk (e.g. *), the numbers next to the asterisk – in relation to that group indices some of these differences are the significant ones.

The pulse wave propagation velocity (PWPV) determination, which is charaterized the vascular bed stiffness, and it is considered, as the integral indicator of the cardiovascular risk, had been shown its further increase for 14,2 ± 1,2 % (e.g. $p < 0,05$), that it is indicated on the arterial channel stiffness further increase at the examined RA patients.

So the multivariate and the multi-factor correlation analysis carrying out has been established the significant direct correlations and the correlative relationships presence between the Il-1β and the AIx (e.g. $r = 0,46$, $p < 0,05$); the IL-6 and the AIx (e.g. $r = 0,48$, $p < 0,05$); the TNF-α and the AIx (e.g. $r = 0,51$, $p < 0,05$); the IL-8 and the AIx (e.g. $r = 0,41$, $p < 0,05$); IL-17 and the AIx (e.g. $r = 0,39$, $p < 0,05$) correlations, the inverse correlation dependences have already been determined between the level in the blood serum IL-8 and the RTT (e.g. $r = –0,57$, $p < 0,05$), the IL-17 and the PWPV (e.g. $r = –0,54$, $p < 0,05$), the IL-1β и the PTT (e.g. $r = –0,43$, $p < 0,05$), IL-6 and the PWPV (e.g. $r = –0,61$, $p < 0,05$), the TNF-α и PTT ($r = –0,57$, $p < 0,05$), which is allowed to us to be considered the pro-inflammatory cytokines hyper-expression, as the one from the mechanisms of the stiffness progression and the elasticity decrease of the arterial bed at the RA. So, it should to be mentioned, that such cytokines, as the IL-17, IL-1β, IL-6, TNF-α, and the IL-8 are the chronic inflammation markers of the vascular wall, their assessment is allowed to be identified the individuals with the high risk of the cardiovascular complocations development [2].

It is well – known, that the TNF-α is played the significant role in the inflammatory response and the cytokine caskade coordination. This cytokine is practically activated the blood coagulation system, it is enhanced the trombogenic and the vasoconstrictor endothelium activity, it is reduced the NO synthesis, it is activated the RAAS, and it is potentiated the ET-1 formation by the endothelial cells. The TNF-α is the strongest incentive for the IL-1β production, which is the major development mediator, as the local inflammatory response in the vascular wall, as well as the acute – phase response at the organism level [1].

The IL-1β is synthesized by the macrophages and by the monocytes, and also by the vascular endothelial cells. The IL-1β is practically exhibited the wide range of the local and the systemic effects, which are included the following: the T- and B-lymphocytes activation, the synthesis induction of the adhesion and the IL-8 molecules [3]. Therefore, the IL-1β increase is the essential mechanism of the endothelial dysfunction progression, which is the leading cause of the elastic – flexibly properties of the vascular bed.

Among the IL-6 multitude effects, it should to be mentioned its role in the systemic inflammation, as the major mediator of the acute phase, having stimulated the production in the inflammation secondary participants' hepatocytes – the acute phase proteins: the C-reactive protein (CRP), the A amyloid, the apolipoprotein-α, the fibrinogen, and the compliment components. So, the IL-6 significant property – is the blood procoagulant activity further increase. The IL-6 serum level is practically reflected the inflammatory process activity in the systemic blood flow circulation, and it is the progostic criterion of the cardiovascular complications [3].

In its turn, the IL-17, having produced by the T-cells (e.g. CD4 +), is exhibited the highly expressed inflammatory activity, it is able to be induced the various mediators inflammation synthesis, including the TNF-α, IL-1, the IL-6, and the IL-8, thereby, having contributed to the autoimmune pathological reactions development, including the inflammation induction at the rheumatoid arthritis (RA). Then, it has been revealed the IL-17 capability to be activated the IL-1 and the IL-6 expression, having possessed the destructive potential in the inflammatory process, as well as the metalproteinaz (e.g. MMP-9) expression, having resulted in the tissue modeling, and the degradation production release of the II type collagen. And, moreover, it has been found out the IL-17 role in the chondrocytes and the synoviocytes regulation, the granulopoiesis stimulation [3, 10].

So, these above-indicated mechanisms are potentiated the immune inflammation activity of the arteries walls, the endothelial dysfunction progression, which is increased the vascular bed stiffness increase at the rheumatoid arthritis (RA) patients.

The medical treatment results assessment showed, that at all the examined patients during the inflixmab use the positive clinical dynamics had been achieved, having characterized by the «good response» presence to the medical treatment (e.g. DAS28 < 3,2) at 78,9 % (e.g. 30 persons); the remission (e.g. DAS28 < 2,6) has been identified at the 5 patients (e.g. 13,15 %), the moderate disease activity (e.g. 3,2 > DAS28 < 5,1) had been saved, and it had been lasted, totally, at the 3 (e.g. 7,95 %) patients.

Thus, the laboratory indices study and its parameters reserch just after the carried out infliximab therapy the significant level reduction of the pro-inflammatory cytokines had been established: the TNF-α concentration decreased in 2,6 ± 1,1 times, the IL-1β – for 66,4 ± 3,2 % (e.g. $p < 0,05$), the IL-6 – in 2,1 ± 0,6 times, the IL-8 – for 19,9 ± 0,4 % (e.g. $p < 0,05$), and the IL-17 – in 1,8 ± 0,6 time.

The vascular wall stiffness parameters study, after the infliximab therapy, has been revealed the significant increase in the time pulse wave propagation (TPWP) for 14,5 ± 2,1 % (e.g. $p < 0,05$). It has also been achieved the significant increase in the augmentation index (AIx) for 1,2 ± 0,4 time, and the area stiffness index (ASI) – for 9,7 ± 1,4 % (e.g. $p < 0,05$), having determined the vascular wall elasticity.

It has been found out the significant decrease the PWPV for 17,4 ± 1,4 % (e.g. $p < 0,05$) at the RA patients after the infliximab therapy. It should to be

mentioned, that the PWPV is the strongest predictor of the cardio-vascular mortality at the patients not only with the cardiac pathology, but at the RA [9]. The PWPV is directly depended from the arterial elasticity and its stiffness: the greater vascular wall stiffness, and lower its elasticity, the higher the PWPV, and, correspondingly, less the PTT. In our study, at the patients with the advanced stages ACCP-negative RA option, under the infliximab therapy influence, along with the high level clinical efficiency, the level reduction in the blood serum of the pro-inflammatory cytokines had been achieved, that it was accompanied by the arterial bed stiffness decrease, and the elasticity increase, and it was potentially associated with the risk reduction of the heart vascular complications development.

Thus, all these recieved data are supported the assumption, that the TNF-α biological effects neutralization has the beneficial impact upon the arterial vessel wall condition, and, consequently, it may be reduced the occurrence risk of the various cardio-vascular pathology disease at the ACCP-negative form of the RA.

The Conclusions

1. At the advanced stage of the ACCP-negative RA version violations of the structural and the functional properties of the arterial bed are usually characterized by the elasticity decrease (e.g. the augmentation and the stiffness indices increase), and by the stiffness increase (e.g. the pulse wave propagation velocity increase).

2. At the patients with the advanced stages of the ACCP-negative RA version is taken its place the significant inverse correlation between the time pulse wave propagation, the increase in the serum IL-1β, IL-6, IL-8, IL-17, and the TNF-α concentration, and the direct correlation – between the inflammatory cytokinesis, the rise pulse wave velocity, and the augmentation index.

3. The infliximab therapy at the patients with the ACCP-negative RA version, along with the clinical efficiency, is accompanied with the content significant reduction in the blood serum IL-lβ, IL-6, TNF-α, IL-8, IL-17, the stiffness decrease, and the vascular wall elasticity increase (e.g. the lower pulse wave velocity, the augmentation index, and the stiffness).

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