Original Article:

Correlation of pharmacokinetic dispositionof methotrexate and serum cytokine levels in rheumatoid arthritis patients

Raisa Aringazina[1], Leysan Myasoutova[2], Liudmila Babaskina[3], Olga Pashanova[4]*

Abstract

Objective*:** Methotrexate is the principal basic anti-inflammatory medication used in the rheumatoid arthritis treatment.Therefore, research into its effect on cytokine concentrations may help improve the effectiveness of disease treatment. ***Study aim*:** was to investigate the neffect of methotrexate therapy on the blood content of proinflammatory cytokines in RA patients, as well as define the relation of proinflammatory cytokine blood concentration to clinical and laboratory disease features and methotrexate pharmacokinetics. ***Material and methods: Sixty-five patients with rheumatoid arthritis (46 women and 19 men; average age 50.83± 8.91 years) underwent an examination.Thirty-two patients (26 women and 6 men) participated in studyingmethotrexate pharmacokinetics. ***Results and Discussion*:** The course of rheumatoid arthritis is accompanied by a significant increase of TNF-α (40.36±4.01 pg/ml), IL-1β (17.68±1.74 pg/ml), andIL-6(8.39±0.46pg/ml) in the blood.Oral administration of methotrexate (MT) for patients with rheumatoid arthritis promotes persistent elimination of clinical disease symptoms and reduced production of proinflammatory cytokines:TNF-α drops by 2.08 times ($p<0.05$) compared to baseline, that of IL-1β and IL-6 – by 2.86 ($p<0.05$) and2.84times($p<0.05$), correspondingly.Blood levels for TNF-α and IL-6 are consistent with clinical markers for rheumatoid arthritis, such as joint pain, joint swelling, and morning stiffness in extremities.Blood cytokine concentrations were not correlated with AUC, C_{max}, and T_{max}. There was an inverse correlation between the blood rates of TNF-α and the weekly dose of MT ($r = -0.34$, $p<0.05$) and II-6 and the weekly dose of MT ($r = -0.32$, $p<0.05$). ***Conclusion:*** Rheumatoid arthritis is closely associated with increaseing of proinflammatory cytokine blood levels, which affects the clinical course of the disease.The dosage of methotrexate applied affects the blood concentrations of IL-6 and TNF-α.

Keywords: clinical markers of inflammation; cytokine profile; methotrexate in rheumatoid arthritis; pharmacokinetics of methotrexate; rheumatoid arthritis.

Bangladesh Journal of Medical Science Vol. 21 No. 02 April'22 Page : 335-343
DOI: http://doi.org/10.3329/bjms.v21i2.58066

Introduction

Nowadays, the problem of rheumatic disorders (RD) has become especially important in modern medical science.[1] This is because RDs are among the most common non-infectious diseases worldwide(third place after cardiovascular and digestive system deseases), which lead to patients disability.[1,2] Rheumatoid arthritis (RA) is the most common type of arthritis for people with RDs.The RA is common to all countries of the world, irrespective of climatic conditions and geographical features.[3] As reported by the 2017 Global Burden of Disease study, there were nearly 20 million RA patients in 195 countries, with the highest prevalencein the United Kingdom

1. Raisa Aringazina, Department of Internal Diseases № 1, Non-Commercial joint-stock Society "West Kazakhstan Marat Ospanov Medical University", Aktobe, Kazakhstan
2. Leysan Myasoutova, Department of Hospital Therapy, Kazan State Medical University, Kazan, Russian Federation.
3. Liudmila Babaskina, Department of Pharmacy, Sechenov First Moscow State Medical University, Moscow, Russian Federation.
4. Olga Pashanova, Department of Economics and Organization of Pharmacy, Sechenov First Moscow State Medical University, Moscow, Russian Federation.

Correspondence: Raisa Aringazina, Department of Internal Diseases № 1, Non-Commercial joint-stock Society "West Kazakhstan Marat Ospanov Medical University", Aktobe, Kazakhstan. Email: raringazina@rambler.ru

(471.8 cases per 100.000 population), Trinidad and Tobago(404.4 cases per 100.000 population), Barbados (40.6 cases per 100,000 population). The lowest rates were reported in Indonesia (91.1 cases per 100.000 population), Timor-Leste (91.4 per 100.000 population), and Sri Lanka (97.2 per 100.000 population).[4]The maximum incidence of this nosology occurs between the ages of 60 and 65, and females are 2 to 4 times more vulnerable than males.[5,6]In the US, the prevalence of RA among females is 0.78%, while among males –only 0.31%. The disease results in a significant deterioration in the quality of life, and lack of proper and timely treatment leads to disabilityin almost half of patients within the first five to seven years of illness.[7,8]Among patients with RA the mortality rate is 54 % higher than in the general population. At that,it is higher for the cardiovascular system, the musculoskeletal system, the respiratory and gastrointestinal organs.[9]The five-year survival rate for serious diseases with visceral symptoms is less than 50%.[10]A recent systematic review of 72 studies evaluatingthe costs of treating patients with RA reported that the annual direct and indirect costs associated with handling this disease ranged from USD 2,408 to USD 8,845.[11] InItially, the total cost of RA treatment is about EUR 2billionperyear,ofwhich45%representindirectcosts,45% are direct medical expenses, and 1 % accounts for direct non-medical costs.[12]

By its nature, rheumatoid arthritis is a chronically evolving autoimmune systemic disease that affects peripheral joints with symmetrical erosive-destructive lesions.[13] The etiology of the disease is still unknown, and various infectious agents (Epstein-Barr virus, retroviruses, parvovirus B19, bacterial stress proteins, superantigens), endogenous factors (citrullinated peptides and proteins), genetic factors, and harmful habits (smoking) are considered as possible triggers. The pathogenesis of RA is based on autoimmune inflammation, which is systemic, but primarily affecting the joints, i.e., the synovial membrane.[14,15] The uncontrolled progressive inflammatory process in the synovial membrane of the joints is a key characteristic of RA, distinguishing it from other rheumatic and non-rheumatic inflammatory diseases.Thus, the RA is characterized by the formation of an ectopic focus of hyperplasia (with active proliferative fibroblasts, macrophages, and lymphocytes) in the synovial tissue, i.e., pannus, which is a pathognomonic sign of the disease.Pannus is distinguished by invasive growth that causes to the joint cartilage destruction with its subsequent disappearance, the proliferation of granulation tissues, and, therefore, the development of ankyloses.[16,17]

Over the past few years, scientists have increasingly focused on the pathogenesis of RA in various cytokines.[18-20]During RA,proinflammatory cytokines of macrophage origin are being excessively produced in synovial tissue.These are interleukin-1 (IL-1) and IL-6, tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophagecolony-stimulatingfactor.[19,20]TheCKsproducedwithin the pannus induce the enzymes that destroy cartilage. That is, major clinical symptoms of RA (chronic synovitis, joint destruction) are associated with proinflammatory CKs, and TNF-α plays a key role in this process. This cytokine demonstrates its biological activity by interacting with specific receptors expressed on fibroblasts, neutrophil leukocytes, keratinocytes, endotheliosytes, and others.The binding of TNFα to these receptors activates such transcription factors as NF-kB, JNK, and AP-1, directly regulating the activity of the genes responsible for the biosynthesis of inflammatory mediators, primarily cytokines, and inducing cell apoptosis, i.e., their programmed death.[18,21,22]

Over the past few decades, the concept of RA therapy has changed slightly.Induction of persistent remissions is the primary goal of treatment, and the basic anti-inflammatory drug used to achieve this goal is methotrexate (MT).[23]Moreover,an intense MT therapy should begin as soon as possible after the disease onset (best in the first 3 months) witha rapid escalation of the drug dose (up to 20-25 mg/week).[24]By the chemical structure, TM is similar to folic acid and belongs to antimetabolic medications. The mechanism of action of the medicine is based on its antifolates propertiesIn particular, MT inactivates dihydrofolate reductase, an enzyme that splits folic acid in the human body, deactivating thus the formation of its active metabolites, namely tetrahydrofolate and dihydrofolic acids. They are necessary for converting homocysteine into methionine and for the synthesis of thymidylate and purines, essential substrates for DNA synthesis.[25-27] It is worth noting that this effect of MT (dihydrofolate reductase inhibition) results in a decrease of DNA biosynthesis and is manifested during therapy at drug doses from 100 to 1,000 mg/m2, which are used in the treatment of cancerous diseases. At lower doses, the mechanism of MT action is somewhat different: MT

metabolites formed due to its polyglutamation in the cell directly inhibit such folate-dependent enzymes as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transaminase and thymidylate synthetase. Therefore, polyglutamine metabolites of MT induce adenosine synthesis, which is the most important endogenous anti-inflammatory mediator.[27,28]

Even though MT is the reference standard for AR treatment with an undisputed basis of proof of its efficacy,[24] the present problem is that there are differences in its bioavailability when taken orally, which limits the efficacy of the treatment.Also, there is no clear relationship between the clinical response to MT therapy and its pharmacokinetics. The effectiveness of MT is assumed to be dependent upon its intracellular concentration.[27]Moreover, the influence of MT pharmacokinetic endpoints on proinflammatory cytokine levels in the blood of RA patients has not been sufficiently studied.Given that cytokinesare important in disease pathogenesis, the development of such knowledge can help improve the effectiveness of RA patients'treatment.

The aim of the study − to investigate the effect of methotrexate therapyon the blood content of proinflammatory cytokines in RA patients, as well as definethe relation of proinflammatory cytokineblood concentration to clinical and laboratory disease features and methotrexate pharmacokinetics.

Material and methods

Sixty-fivepatients with AR were examined: 45 (69.2 %) females and 19 (30.8%) males. The age of included patients was from 18 to 75 (the average age was 50.83 ± 8.91).20 apparently healthy humans (AHH) formed a control group.

Verification of the diagnosis RA was carried out according to recommendations of theAmericanCollege of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) 2010,[29] and EULAR recommendations for the management of early arthritis 2016.[30]

Study inclusion criteria: verified diagnosis of RA; duration of RA at least 6 months; moderate to a high degree of RAseverity; age over 18 years; no history of MT intake; intake of a stable dose of the nonsteroidalanti-inflammatory drug (NSAID) or prednisolone<10mg/day for at least 1monthbeforethe study; body mass index of20.00-29.99 kg/m2; signed informed consent for participating in the study.

Patients with at least one of the following exclusioncriteria werenot included in the study: age less than 18 years; history of MT administration; intra-articular injections of anti-inflammatory medicines less than a month prior to the study; treatment history with biologics; ACR functional class of IV RA; the presence of concomitant chronic diseases in the acute or sub/decompensation stage; infectious pathology; body mass index of 30.00 kg/m^2 or above or less than 20.00 kg/m^2; oncopathology of any localization; mental illness; pregnancy, lactation; alcohol and drug dependence. Patients were treated according to EULAR (2016),[31] and ACR 2015 recommendations.[24]During the study period, patients were taking either previously prescribed oral NSAIDs or prednisolone (at a dose of less than 10 mg/day).Additionally, a steady dose of prednisolone was maintained for four weeks before being included in the study.The initial dose of MTamounted to 7.5 mg/week and was gradually increased thereafter for maximum clinical efficacy.In case of anypronounced toxicity or adverse reactions, the dose of MT was reduced.

Each patient was monitored over 24months.The assessment of complaints, clinical status, laboratory and instrumental methods was completed before inclusion in the study and thereafter 14 days, 1 month, and every 6 months for the entire duration of the study.

The patient examination complex included detailed study of complaints and collection of medical history, physical examination, laboratory and instrumental assessment methods.

In accordance with the 2010 ACR/EULAR criteria, a detailed examination of the patients' joints was performed, indicating the number of painful and swollen joints, the presence of morning joint stiffness, and its duration(inminutes).'Small' and 'large' joints have been assessed as well.The intensity of joint pain was estimated using a 100 mm visual analog scale (VAS).[32]

Severity of RA was measured using the DAS28 index, including 28 joints: 2 shoulders, 2 elbows, 2 wrists, 2 metacarpophalangeal of 1st-5th fingers, 2 interphalangeal of the 1st finger, 2 proximal interphalangeal of 2nd-5th fingers, and 2 knee joints;[33] for the DAS28 index measurement, the calculator http://www.das-score.nl/das28/en was used. A DAS28 index below 2.6 points indicated an RA remission, 2.6–3.2 points – low RA activity, 3.2–5.1 – moderate severity, and over 5.1 – high RA severity.

The range of laboratory tests included general clinical blood and urine tests, thymol test, the measurement of blood glucose, urea, creatinine, and total bilirubin content, aspartate, and alanine aminotransferase activity, gamma-glutamyltransferase, alkaline phosphatase, ion-, protein-, lipid- and coagulogram.

The range of rheumatic tests involved the determination of C-reactive protein (CRP), rheumatoid factor, antistreptolysin O, which was performed by an immunoturbidimetric method using standard reagent kits from VitalDiagnostic (Russia).

Blood levels of IL-1β,IL-6,andTNF-α were determined by immunoassayusing IL-1 beta Human ELISA Kit (USA), Human IL-6 ELISA Kit (USA), and TNF alpha Human ELISA Kit (USA) on a StatFax 2100 immunoassay analyzer (Awareness Technology Inc.,USA).

The instrumental examination consisted of electrocardiography (ECG) and X-ray of the joints on the hands and feet.

PharmacokineticsofMTwas studied during patient visits: before treatment, 14 days after MT initiation, and after 1, 6, 12, 18, and 24 months. The pharmacokinetic disposition was examined in 32 patients, including 26 (81.3%) females and 6 (17.7%) males. Blood samples were taken before MT injection (after an 8-hour night fast), 30 min after MT injection, and 1, 2, 4, 6, 8, 12, and 24 h after MT administration.At similar intervals, blood samples were collected from these patients to assess the blood content of the cytokines under study.Urine was also taken within 24 hours to determine the quantity of MT.Additionally, blood and urine samples were taken to determine creatinine levels.Creatinine clearance was computed by Cockcroft-Gault formula using an online calculator (available at:https://www.mdcalc.com/creatinine-clearance-cockcroft-gault-equation). Blood and urine were analyzed for TM content by polarising the fluorescence immunoassay using Beacon 2000 (Panvera, USA) polarising fluorimeter. Given that the TM dose adjustment was carried out throughout the study (to ensure optimal control of disease symptoms and stable remission),the pharmacokinetics was studiedat different doses of the drug. The following indicators were determined for each volunteer: C_{max} – the patient's maximum plasma MT concentration; TC_{max} – the time at which C_{max} was reached; AUC_{0-t} –the area under the pharmacokinetic curve from the time of drug administration(timezero) to the collection of a specific blood sample, when the MT content in blood was equal to/above the minimum limit at which the drug could be quantified; $AUC_{0-\infty}$– area under the pharmacokinetic curve from time zero to infinity; $C_{max/AUC}$–relative drug absorption rate; K_{el}– drug elimination constant; $T_{1/2}$– elimination half-life; MRT –mean resident time (time of drug retention in the blood).

The findings were statistically processed using non-parametric Wilcoxon and Mann-Whitney tests, as well as the Spearman's rank correlation coefficient. The values were presented as mean and standard deviation (M±SD). Differences were considered statistically significant at $p < 0.05$. For comparation of the frequency of symptoms we used the Fisher's exact test. The AUC value was calculatedusing the trapezium method; AUC from 8 to 24 hours was estimated using the logarithmic trapezoidrule, $AUC_{0-\infty}$was defined as the ratio offinal concentration ok $_{el}$;the k_{el} was determined by non linear regression analysis. Systemic clearance was estimated by the ratio of the MTdose to AUC_0.

Ethical Standards

The study was conducted according to theprovisions of ICH Good Clinical Practice (1996), International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organization of Medical Sciences, Rules of ethical principles for scientific medical research with human participation approved by the Helsinki.Declaration (1964-2013).

The study design and form of informed consent for patients participating were approved during a meeting of the University's bioethics committee. Before being included in the study, patients had the opportunity and sufficient time to read all elements of the Informed Consent to Voluntary Participation form with a detailed description of the patient's rights, goals, objectives, and research methods, expected results, etc.Patients who had questions were provided with comprehensive responses.

Results

The design of the study is presented in Figure 1.

A total of 65 patients took part in the study, with female predominance. The mean disease duration among the patients studied was 9.86± 2.04 years. At that, 9 (13.9%) patients showed X-ray stage II according to Steinbrocker, 51 (78.5%) patients had stage III, and stage IV was observed in 5 (7.6%) patients.Among the patients included in the research,

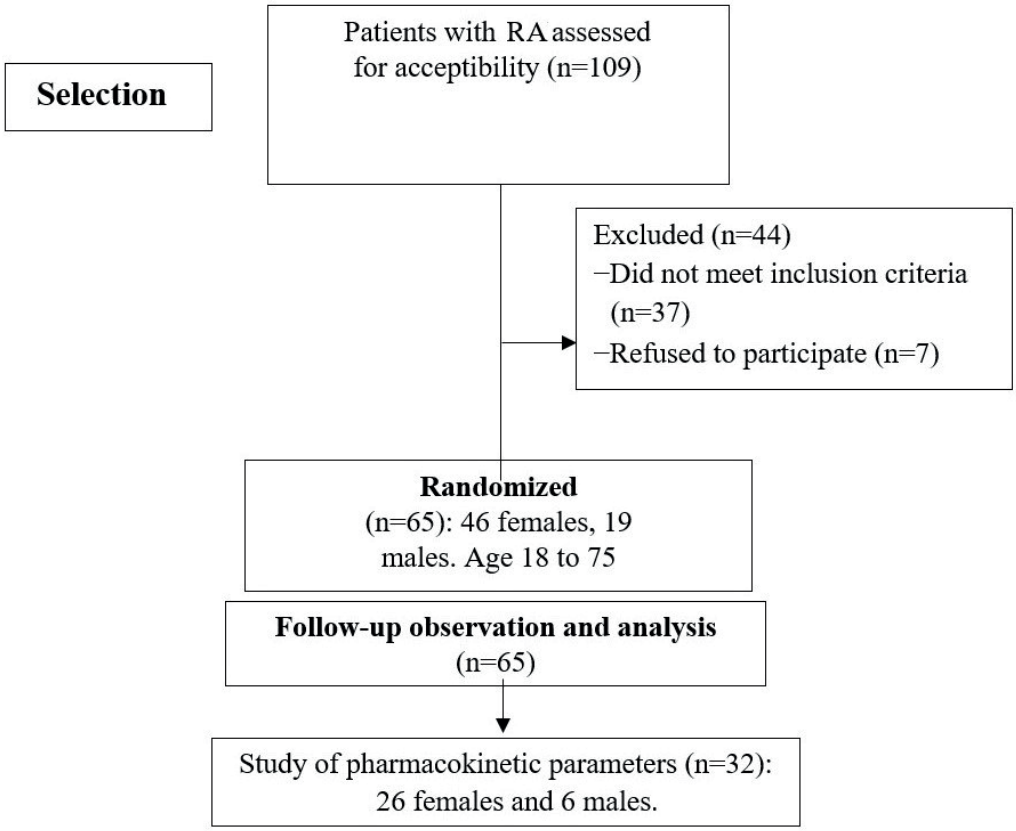

Figure 1. Study design.

12(18.5%) had the I functional class of the disease, 49(75.4%)–the II functional class, and another 4 (6.1%) – the I functional class. The average degree of RAseverity during the calculation of the DAS28 index was high, representing 5.18±0.73.Rheumatoid factor (RF) was detected in the blood of 46 patients (70.8%), meaning they were seropositive; no RF was found inother 19 (29.2%) patients, meaning they were seronegative.

The main clinical symptoms observed in all RA patients under study were joint swelling and pain with the average number of swollen joints being 13 (3.0 to 28.0), painful joints being 19 (4 to 28), and the duration of morning joint stiffness being 96.40±11.25 minutes. All patients (100.0%) had overall weakness, leucocytosis, and increased erythrocyte sedimentation rate (ESR). The average white blood cell count in patients with RA was 13.2 ± 1.85 $\times 10^9$/l and ESR was 61.20 × 10.37mm/h.

In addition to joint lesions, the patients under study had systemic manifestations ofthe disease: lymphadenopathy – in 32 (49.3%) patients, amyotrophy – in 27 (41.5%) patients, anemia of chronicdisease–in20(30.8%)patients, weightloss–in20(30.8%)patients, rheumatoid nodules – in 14 (21.5%) patients, fever – in 13 (20.0%) patients, diffuse interstitial fibrosis – in 6 (9.2%) patients, and rheumatoid vasculitis – in 2 (3.1%) patients.

Therapy with oral MT administration reduced the intensity of the main clinical and laboratory RAsymptoms already on the 30th day (the end of 1st month) of the treatment. Thus, on the 30th day of the treatment, only 19(29.2%) patients complained of joint pain versus 65(100.0%)before treatment($p<0.001$); joints welling was noted in16(24.6%) versus65 (100.0%) patients before treatment($p<0.001$); 22 patients (33.8%) reported morning stiffness compared to 65 (100.0%) before therapy ($p<0.001$). The DAS28 index decreased from (5.18 ± 0.73) to (3.12 ± 0.16) scores ($p<0.05$). In the general blood analysis, the white blood cell count decreased from (13.2 ± 1.85) $\times 10^9$/L to(5.93±0.72)$\times 10^9$/L($p<0.05$), and the ESR dropped from(61.20×10.37) mm/h to (22.47 ± 3.26) mm/h ($p<0.05$). The 6th month of treatment ended in obtaining a stable regression of the disease symptoms (clinical and laboratory).

Investigation of cytokine profile showed that patients with RA had a significant increasing of pro-inflammatory cytokines compared to AHHs (Table 1).The blood content ofTNF-αin RApatients beforetreatmentwas 2.03 times higher compared to AHHs ($p < 0.05$), IL-1β was 2.86 times higher ($p < 0.05$), and the levelof IL-6 exceeded reference values by 3.08 times ($p < 0.05$).

Table 1. Changes in cytokine content in the blood of RApatients after methotrexate therapy

Indicator	AHH (n=20)	Patients with rheumatoid arthritis (n=65)						
		Before treatmen t	After 14 days	After 1 month	After 6 months.	After 12 months	After 18 months	After 24 months
TNF-α, pg/ml	19.85± 1.31	40.36±4 .01*	31.57±3 .10*	25.59± 2.43**	20.17±1 .93**	19.71± 2.06**	19.80± 1.47**	19.44± 1.56**
IL-1β, pg/ml	6.19± 0.38	17.68±1 .74*	11.93±0 .95*/**	9.04±0 .90*/**	6.80±0 .62**	6.32±0 .56**	6.26±0 .33**	6.18±0 .21**
IL-6, pg/ml	2.73± 0.13	8.39±0.46*	6.29±0.33*/**	4.07±0.20*/**	3.15±0.16**	3.03±0.10**	2.90±0.11**	2.84±0.08**
Weekly MT dose, mg	-	-	7.5	8.0	12.5	14.0	15.5	17.0

Note. * - the difference is statistically significant compared to AHH ($p < 0.05$); ** - the difference is statistically significant compared to the index before treatment ($p < 0.05$).

During MT treatment, a positive dynamic was observed with respect to decreasing levels of all proinflammatory cytokines studied.Alreadyin14days after MT administration, there was a significant decrease of IL-1β content in the blood by 1.48 times ($p < 0.05$) and that of IL-6 – by 1.34 times ($p < 0.05$); as forTNF-α, only attend encytoa decrease of its contentinthe blood was noted onthe14th day; one month later, its level significantly decreasedby 2.58 times ($p < 0.05$) compared to the pre-treatment levels. At the sixth month of the MT therapy, the blood content of TNF-α, IL-1β, and IL-6 was not statistically different from that of AHH ($p > 0.05$).

Correlations between proinflammatory cytokine levels in the blood and clinical and laboratory parameters were establishedas well (Table 2).

Table 2. Correlations between proinflammatory cytokine blood levels, clinical disease manifestation and pharmacokinetic endpoints in RApatients.

Marker	**TNF-α**	**IL-1β**	**IL-6**
Joint pain	r=0.42 p<0.05	-	r=0.21 p<0.05
Swelling in thejoints	r=0.54 p<0.05	-	r=0.51 p<0.05
Morning stiffness	r=0.48 p<0.05	-	r=0.26 p<0.05
Fatigue	r=0.33 p<0.05	-	r=0.24 p<0.05
ESR*	r=0.28 p<0.05	-	r=0.30 p<0.05
Leukocytes	r=0.35 p<0.05	-	r=0.39 p<0.05
RF	r=0.39 p<0.05	-	r=0.25 p<0.05
Side effects of MT	-	r=0.36 p<0.05	-
AUC	-	-	-
C_{max}	-	-	-
T_{max}	-	-	-
Weekly MT dosemethotrexate	r=-0.34 p<0.05	-	r=-0.32 p<0.05

Note: ESR – erythrocyte sedimentation rate; RF – rheumatoid factor; MT – methotrexate; AUC – area under the curve.*

The analysis of MT pharmacokinetics did no reveal any correlation between the pharmacokinetic parameters of the drug and blood levels of TNF-α, IL-1β, and IL-6. Only a weak inverse correlation between the weekly dose of MT and blood levels of

IL-6(r=-0.32 p<0.05) and TNF-α (r=-0.34 p<0.05), as well asa direct correlation between the frequency of adverse MT events and IL-1β blood levels,was reported.

Discussion of the Results

It has been established that the course of RA was accompanied by a significant increase ofproinflammatory cytokines TNF-α(40.36±4.01 pg/ml in the blood (2.03 times (p<0.05) higher compared to AHH), IL-1β(17.68±1.74pg/ml,or 2.86times(p<0.05)highercomparedtoAHH),and IL-6(8.39±0.46 pg/ml, or 3.08 times (p<0.05) higher in comparison with AHH). In the authors' opinion,these data confirm the considerable role of TNF-α, IL-1β and IL-6 in pathogenesis of RA.This is also confirmed by the presence of correlations between blood levels of cytokines under study and some symptoms and laboratory indexes: for TNF-α and joint pain (r=0.42 p<0.05), joint swelling (r=0.54 p<0.05), morning stiffness (r=0.48 p<0.05),fatigue(r=0.33p<0.05),ESR(r=0.28p<0.05),white blood cell count(r=0.35 p<0.05), and RF (r=0.39 p<0.05); for IL-6 and joint pain (r=0.21 p<0.05), joint swelling (r=0.51 p<0.05), morning stiffness (r=0.26 p<0.05), fatigue (r=0.24 p<0.05), white blood cell count (r=0.39 p<0.05), ESR (r=0.30 p<0.05), and RF (r=0.25p<0.05).

The use of MT in RA patients is effective since it permanently eliminates the major clinical symptoms of the disease,standardizing thus laboratory parameters.Oral MT treatment contributes to a decrease in pro-inflammatory cytokine productionas demonstrated by the reduction dynamics of TNF-α, IL-1β, and IL-6 in the blood of patients studied from the 14th day after the start of MT therapy(Table 1). Such positive effects of MT on the RAcourse and pro-inflammatory cytokine levels persisted throughout the study.Moreover, from the 6th month of MT treatment, the levels of TNF-α, IL-1β, and IL-6 in RA patients did not differ statistically from AHH (p>0.05).This effect is of high importance, given the systemic effects of IL-6, such as stimulation of B-lymphocytes differentiation, biosynthesis of osteophase proteins in the liver, activation of osteoclast genesis, degradation of cartilage. The detected correlations between the IL-6 blood levels and clinical and laboratory RAsymptoms once again confirmthat this cytokine plays one of the leading roles in the RA pathogenesis. Similar correlations were found for TNF-α as a key pro-inflammatory cytokine the main clinical symptoms of the disease are associated with.The obtained findings are well consistent with the literature and studies on the involvement of proinflammatory cytokines inthe pathogenesis of RA,[18-21]the MT effectiveness for the treatment of RA patients, as well as its effect on the cytokine profile.[25-28]

In contrast, the blood levels of IL-1β did not correlate with the clinical markers of RA, indicating a minor role of this cytokine in the development and progression of the disease. Interestingly, no correlation between the level of IL-1β and the frequency of adverse events in RA patients was observed. The same applies for IL-6 and TNF-α. The significance of such correlation is not clear and no data was found in the literature that could explain it, which necessitates further investigation of thisissue.

An important part of this research is the study of the relation between MT pharmacokinetics and proinflammatory cytokine blood levels.It is preferable that the pharmacokinetic disposition of MT should be observed over a fairly long timeframe (24 months).We didn't found correlation between the blood levels of the cytokines studied and AUC, C_{max}, and T_{max}.However, a reverse correlation between the blood levels of TNF-α (r=-0.34 p<0.05) and IL-6 (r=-0.32 p<0.05) and the weekly dose of MT was established.This illustrates the importance of using an appropriate dose of MT in RA patients,and determining TNF-α and IL-6 in the blood may help in selecting the correct MT dose. Only one such study, carried out in the United States by Kremer et al.with 17 AR participants has been found in the literature.[34]The authors investigated the relationship between antiinflammatory and proinflammatory cytokines, markers of AR activity, and pharmacokinetic endpoints. Each patient was followed up for 36 months. This study also found correlations between IL-6 levels in the blood of patients with RA and clinical markers of the disease. The most significant changes were noted for IL-6; a correlation was also observed between AUC and IL-2 (r=0.23, p=0.045).[34]

Conclusions

The course of rheumatoid arthritis was established to be accompanied by a significant increase in blood concentrations of TNF-α (40.36±4.01 pg/ml in the blood(2.03 times higher (p<0.05) compared to AHH), IL-1β (17.68±1.74 pg/ml, 2.86 times (p<0.05) higher compared toAHH), and IL-6 (8.39±0.46 pg/ml, 3.08 times (p<0.05) higher compared toAHH).

Therapy with oral methotrexate administration in rheumatoid arthritis patients promotes a stable elimination of clinical disease symptoms and reduced proinflammatory cytokine production. Thus, the level of TNF-α decreases by 2.08 times ($p<0.05$) compared to baseline, that of IL-1β and IL-6 – by 2.82 ($p<0.05$) and 2.84 times ($p<0.05$), respectively. Blood levels of TNF-α and IL-6 correlated with clinical markers of rheumatoid arthritis: TNF-α and joint pain ($r=0.42$ $p<0.05$), joint swelling ($r=0.54$ $p<0.05$), morning stiffness ($r=0.48$ $p<0.05$), fatigue ($r=0.33$ $p<0.05$), ESR ($r=0.28$ $p<0.05$), white blood cell count ($r=0.35$ $p<0.05$), and RF ($r=0.39$ $p<0.05$); IL-6 and joint pain ($r=0.21$ $p<0.05$), joint swelling ($r=0.51$ $p<0.05$), morning stiffness ($r=0.26$ $p<0.05$), fatigue ($r=0.24$ $p<0.05$), white blood cell count ($r=0.39$ $p<0.05$), ESR ($r=0.30$ $p<0.05$), and RF ($r=0.25$ $p<0.05$). These values correspond to the incidence of methotrexate side effects ($r=0.36$ $p<0.05$).No correlation between the blood levels of the cytokines studied and AUC, C_{max}, and T_{max} were revealed.However, an inverse correlation between TNF-α ($r=-0.34$ $p<0.05$) and IL-6 ($r=-0.32$ $p<0.05$) blood levels and MT weekly dose were established.

Prospects for further research. Comparison of the effectiveness of oral and subcutaneous methotrexate forms in rheumatoid arthritis treatment.

Funding. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests. Authors declare that they have no conflict of interests.

Data Availability. Data will be available on request.

Authors's contribution.

Data gathering and idea owner of this study: Raisa Aringazina and Olga Pashanova

Study design: Leysan Myasoutova and Liudmila Babaskina

Data gathering: Liudmila Babaskina

Writing and submitting manuscript: Raisa Aringazina

Editing and approval of final draft: Leysan Myasoutova

References

1. McDougall C, Hurd K and Barnabe C. Systematic review of rheumatic disease epidemiology in the indigenous populations of Canada, the United States, Australia, and New Zealand. *SeminArthritis Rheum* . 2017; **46**(5): 675-686. https://doi.org/10.1016/j.semarthrit.2016.10.010 https://doi.org/10.1016/j.semarthrit.2016.10.010
2. Sarzi-Puttini P, Ceribelli A, Marotto D, Batticciotto A and Atzeni F. Systemic rheumatic diseases: From biological agents to small molecules. *Autoimmun Rev*. 2019; **18**(6): 583-592. https://doi.org/10.1016/j.autrev.2018.12.009 https://doi.org/10.1016/j.autrev.2018.12.009
3. Otón T and Carmona L. The epidemiology of established rheumatoid arthritis. Best practice & research. *ClinRheumatol*. 2019; **33**(5): 101477. https://doi.org/10.1016/j.berh.2019.101477 https://doi.org/10.1016/j.berh.2019.101477
4. Safiri S, Kolahi AA, Hoy D, Smith E, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of rheumatoid arthritis 1990-2017: *a systematic analysis of the Global Burden of Disease study* 2017. AnnRheum Dis2019; **78**(11): 1463-1471. https://doi.org/10.1136/annrheumdis-2019- 215920 https://doi.org/10.1136/annrheumdis-2019-215920
5. Davis JM. Rheumatoid arthritis: A severe disease that preventive approaches would greatly benefit. *Clin Ther*. 2019; **41**(7): 1240-1245. https://doi.org/10.1016/j.clinthera.2019.04.026 https://doi.org/10.1016/j.clinthera.2019.04.026
6. Favalli EG, Biggioggero M, Crotti C, Becciolini A, Raimondo MG and Meroni PL. Sex and management of rheumatoid arthritis. *Clin Rev Allergy Immunol*. 2019; **56**(3): 333-345. https://doi.org/10.1007/s12016-018-8672-5 https://doi.org/10.1007/s12016-018-8672-5
7. Hunter TM, Boytsov NN, Zhang X, Schroeder K, Michaud K and AraujoAB. Prevalence of rheumatoid arthritis in the United States adult population in healthcare claims databases, 2004-2014. *RheumatolInt*. 2017; **37**(9): 1551-1557. https://doi.org/10.1007/s00296-017-3726-1 https://doi.org/10.1007/s00296-017-3726-1
8. Markusse IM, Akdemir G, Dirven L, Goekoop-Ruiterman YP, van Groenendael JH, Han KH, et al. Long-term outcomes of patients with recent-onset rheumatoid arthritis after 10 years of tight controlled treatment: A randomized trial. *Ann Intern Med*. 2016; **164**(8): 523-531. https://doi.org/10.7326/M15-0919 https://doi.org/10.7326/M15-0919
9. van den Hoek J, Boshuizen HC, Roorda LD, Tijhuis GJ, Nurmohamed MT, van den Bos GA, et al. Mortality in patients with rheumatoid arthritis: a 15-year prospective cohort study. *Rheumatol Int*. 2017; **37**(4): 487-493. https://doi.org/10.1007/s00296-016-3638-5

https://doi.org/10.1007/s00296-016-3638-5

10. Lacaille D, Avina-Zubieta JA, Sayre EC andAbrahamowicz M. Improvement in 5-year mortality in incident rheumatoid arthritis compared with the general population-closing the mortality gap. *Ann Rheum Dis*. 2017; **76**(6): 1057-1063. https://doi.org/10.1136/annrheumdis-2016-209562 https://doi.org/10.1136/annrheumdis-2016-209562
11. Hsieh PH, Wu O, Geue C, McIntosh E, McInnes IB and Siebert S. Economic burden of rheumatoid arthritis: a systematic review of literature in biologic era. *Ann Rheum Dis*. 2020; **79**(6): 771-777.https://doi.org/10.1136/annrheumdis-2019-216243 https://doi.org/10.1136/annrheumdis-2019-216243
12. Mennini FS, Marcellusi A, Gitto L and Iannone F. Economic burden of rheumatoid arthritis in Italy: Possible consequences on anti-citrullinated protein antibody-positive patients. *ClinDrug Investig*. 2017; **37**(4): 375-386.https://doi.org/10.1007/s40261-016-0491-y https://doi.org/10.1007/s40261-016-0491-y
13. 13. Myasoedova E, Davis J, Matteson EL and Crowson CS. Is the epidemiology of rheumatoid arthritis changing? Results from a population-based incidence study, 1985-2014. *Ann Rheum Dis*. 2020; **79**(4): 440-444. https://doi.org/10.1136/annrheumdis-2019-216694 https://doi.org/10.1136/annrheumdis-2019-216694
14. 14. Scherer HU, Häupl T and Burmester GR. The etiology of rheumatoid arthritis.*J Autoimmun* 2020; 110: 102400.https://doi.org/10.1016/j.jaut.2019.102400 https://doi.org/10.1016/j.jaut.2019.102400
15. Roszyk E and Puszczewicz M. Role of human microbiome and selected bacterial infections in the pathogenesis of rheumatoid arthritis. *Reumatologia*.2017; **55**(5): 242-250. https://doi.org/10.5114/reum.2017.71641 https://doi.org/10.5114/reum.2017.71641
16. Zamanpoor M. The genetic pathogenesis, diagnosis and therapeutic insight of rheumatoid arthritis. *Clin Genet*. 2018; **95**(5): 547-557.https://doi.org/10.1111/cge.13498 https://doi.org/10.1111/cge.13498
17. Croia C, Bursi R, Sutera D, Petrelli F, Alunno A and Puxeddu I. One year in review 2019: pathogenesis of rheumatoid arthritis.*Clin Exp Rheumatol*. 2019; **37**(3): 347-357.
18. Alam J, Jantan I and Bukhari S. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomed Pharmacother*. 2017; 92: 615-633. https://doi.org/10.1016/j.biopha.2017.05.055 https://doi.org/10.1016/j.biopha.2017.05.055
19. Ridgley LA, Anderson AE and Pratt AG. What are the dominant cytokines in early rheumatoid arthritis? *CurrOpin Rheumatol* 2018; **30**(2): 207-214. https://doi.org/10.1097/BOR.0000000000000470 https://doi.org/10.1097/BOR.0000000000000470
20. Alunno A, Carubbi F, Giacomelli R and Gerli R. Cytokines in the pathogenesis of rheumatoid arthritis: new players and therapeutic targets. *BMC Rheumatol*. 2017;1: 3. https://doi.org/10.1186/s41927-017-0001-8 https://doi.org/10.1186/s41927-017-0001-8
21. Noack M and Miossec P. Selected cytokine pathways in rheumatoid arthritis. *SeminImmunopathol* 2017;**39**(4): 365-383. https://doi.org/10.1007/s00281-017-0619-z https://doi.org/10.1007/s00281-017-0619-z
22. Soomro M, Magsi M, Soomro MA, Akram M and Lahmar O. Patients' knowledge on rheumatoid arthritis presenting with arthralgia in a Tertiary Care Teaching Hospital, Pakistan. *Bangladesh J Med Sci.* 2019; **18**(4): 808-813. https://doi.org/10.3329/bjms.v18i4.42909 https://doi.org/10.3329/bjms.v18i4.42909
23. Drosos AA, Pelechas E and Voulgari PV. Rheumatoid arthritis treatment. A back to the drawing board project or high expectations for low unmet needs? *J Clin Med*. 2019; **8**(8): 1237. https://doi.org/10.3390/jcm8081237 https://doi.org/10.3390/jcm8081237
24. Singh JA, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, et al. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol*. 2016; **68**(1): 1-26. https://doi.org/10.1002/art.39480 https://doi.org/10.1002/art.39480
25. Pashanova OV, Lopatina NB, Krivosheev SA andBaranova NY. Comparative analysis of approaches and treatment results of patients with early and nonearly rheumatoid arthritis. *Open Access Mace J Med Sci*. 2019; **7**(17): 2802-2806. https://doi.org/10.3889/oamjms.2019.693 https://doi.org/10.3889/oamjms.2019.693
26. Brown PM, Pratt AG and Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. Nature reviews. *Rheumatology*. 2016; **12**(12): 731-742. https://doi.org/10.1038/nrrheum.2016.175 https://doi.org/10.1038/nrrheum.2016.175
27. Friedman B andCronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. *Joint Bone Spine*. 2019**;** **86**(3): 301-307.https://doi.org/10.1016/j.jbspin.2018.07.004 https://doi.org/10.1016/j.jbspin.2018.07.004
28. Maksimovic V, Pavlovic-Popovic Z, Vukmirovic S, Cvejic J, Mooranian A, Al-Salami H, et al. Molecular mechanism of action and pharmacokinetic properties of methotrexate. *MolBiolRep* 2020;**47**(6): 4699-4708.https://doi.org/10.1007/s11033-020-05481-9 https://doi.org/10.1007/s11033-020-05481-9
29. Gunawan P and Saharso D. A case of juvenile dermatomyositis responding to methotrexate and steroid. Bangladesh . i2018; **17**(4): 675-677. https://doi.org/10.3329/bjms.v17i4.38336 https://doi.org/10.3329/bjms.v17i4.38336
30. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2010; **69**(9): 1580-1588. https://doi.org/10.1136/ard.2010.138461 https://doi.org/10.1136/ard.2010.138461
31. Combe B, Landewe R, Daien CI, Hua C, Aletaha D, Álvaro-Gracia JM, et al. 2016 update of the EULAR recommendations for the management of early arthritis. *Ann Rheum Dis*.2017; **76**(6), 948-959.https://doi.org/10.1136/annrheumdis-2016-210602 https://doi.org/10.1136/annrheumdis-2016-210602
32. Smolen JS, Landewé R, Bijlsma J, Burmester G, Chatzidionysiou K, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis*. 2017; **76**(6): 960-977.https://doi.org/10.1136/annrheumdis-2016-210715 https://doi.org/10.1136/annrheumdis-2016-210715
33. Hawker GA, Mian S, Kendzerska Tand French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF- MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res* 2011;**63**(S11): 240-252. https://doi.org/10.1002/acr.20543 https://doi.org/10.1002/acr.20543
34. Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de PutteLB and van Riel PL. Modified disease activity scores that include twenty- eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*1995; **38**(1): 44-48. https://doi.org/10.1002/art.1780380107 https://doi.org/10.1002/art.1780380107
35. Kremer JM, Lawrence DA, Hamilton R and McInnes IB. Long-term study of the impact of methotrexate on serum cytokines and lymphocyte subsets in patients with active rheumatoid arthritis: correlation with pharmacokinetic measures. *RMD Open*. 2016; **2**(1): e000287. https://doi.org/10.1136/rmdopen-2016-000287 https://doi.org/10.1136/rmdopen-2016-000287