ORIGINAL INVESTIGATIONS

Therapeutic drug monitoring of methotrexate and its metabolites in the red blood cells and mononuclear cells of patients with rheumatoid arthritis

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Objective: *to assess the time course of changes in the concentration of methotrexate (MTX) and its main metabolites in the red blood cells (RBC) and mononuclear cells (MNC) of patients with rheumatoid arthritis (RA), by taking into account individual characteristics (age, statin therapy, and smoking).*
Patients and methods. *The investigation enrolled 33 MTX-treated patients (mean age 53.2±11.7 years) with RA, who underwent therapeutic drug monitoring to measure the RBC and MNC concentrations of free MTX and MTX polyglutamates (MTXPGs) with 2, 3, and 4 glutamate residues (MTXPG 2–4) in using tandem chromatomass spectrometry after 4, 12, and 24 weeks of therapy.*
Results and discussion. *Following 12 weeks, the concentration of MTXPG4 in the MNC was higher in patients taking statins, while that of MTX and MTXPG2 in the RBC were significantly lower than in smokers. At 24 weeks, older patients were observed to have a higher MTX level and a lower MTXPG4 concentration in the RBC.*
Conclusion. *After 24 weeks of therapy, the RBC concentration of MTPG4 was lower and that of MTX was higher in older patients than in others, which confirms data on a slower MTX metabolism in the elderly. The use of statins is likely to have a positive impact on the accumulation of MTXPG. There is a statistically significantly lower RBC concentration of MTXPG in at 12 weeks of therapy.*

Keywords: *therapeutic drug monitoring; methotrexate; rheumatoid arthritis.*
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Background

It is well known that methotrexate (MTX) is a prodrug that is activated in cells by transformation into polyglutamates. After administration, MTX rapidly passes from the plasma into various cells, where it undergoes glutamination with the active participation of folyl polyglutamate synthetase. This enzyme attaches up to four additional glutamate residues to the parent molecule. The reverse process begins almost immediately: glutamate residues are cleaved by γ-glutamyl hydrolase with formation of original monoglutamate form, which is rapidly cleared from the cell using transmembrane proteins [1]. Practical relevance of the patterns of MTX active metabolites distribution cannot be overestimated. Since MTX is known to have a relatively narrow therapeutic window, dose adjustment based solely on clinical observation may be unreasonably long, and in addition, there is a possibility of treatment regimen violation (non-compliance): missed doses, self-change in dosage etc. in each individual case, which may affect the results of therapy. Therapeutic drug monitoring is a set of measures to determine the drug levels in the physiological fluids, in order to select the optimal dosing regimen for a particular patient, and to minimize the undesirable effects of a drug [2]. The main goal of drug monitoring is the pharmacokinetic control of drug therapy. Currently, tandem chromatography-mass spectrometry is used for this purpose [3]. Most of the major scientific works, including prospective studies using tandem chromatography-mass spectrometry, are carried out in Japan [4, 5], USA [6], as well as by international research collaborations [7]. The results obtained on the Japanese population cannot be extrapolated to the European population due to the peculiarities of MTX metabolism in patients of this ethnic origin. Thierry Dervieux's studies are relevant, but they do not cover the entire range of possible tests during therapeutic drug monitoring, specifically, the assessment of polyglutamates levels in mononuclear cells. In addition, there have been no prospective studies of MTXPG levels in RA patientsX blood over the past 3 years, and in earlier studies [8–10] an insufficiently sensitive research method was used. Amit Sandhu et al. [11] observed 117 subjects (the so-called Indian Asian population of RA patients) for 24 weeks and did not reveal a significant relationship between the MTXPG levels and the response to MTX. At the same time, MTXPG3 levels were significantly higher ($p = 0.001$) in the group of patients with adverse reactions (AR) (nausea, vomiting, dizziness, fever), and, using the method of logistic regression, the authors demonstrated that the level of this metabolite was a predictor of similar AR development. However, in patients with ALT/AST increase, no differences were found in MTXPG3 levels. Moreover, ethnic characteristics do not allow unambiguous extrapolation of these results to the population of Russian patients. Other studies are sporadic, not prospective, and are not applicable for the development of methotrexate therapeutic drug monitoring for patients in the Russian population. In recent years, there has been a keen interest in the possibility of treatment adherence assessment of patients using methotrexate. So, Kelley Brady in collaboration with Thierry Dervieux found that MTXPG3 level less than 5 nmol/L in erythrocytes indicates major therapy regimen violations or patient's immunity to MTX [12]. In the same work threshold values for subtherapeutic, intermediate and therapeutic levels for this metabolite were established. The technique was

Table 1. Characteristics of patients included in the study

Variable	Value	%
Women	26	78
Men	7	22
Age, years	53.2 ± 11.7	–
Body Mass Index, kg/m^2	26.5± 4.6	–
Disease duration, Median [25-й; 75-й percentiles], месяцы	8.0 [5.0; 36.0]	–
Rheumatoid Factor positive	26	78
anti-Cyclic Citrullinated Peptide antibody positive	25	76
DAS 28, points	5.4 ±1	–
Statins intake	6	18
Smoking	4	12

Note. ACCP antibodies – anti-cyclic citrullinated peptide antibodies.

Table 2. Average concentration values (nmol / L) (MTXPG 2-4) in RBC and MO

Visit	Cells type	Median	[25;75] quartiles	Min	Max
Week 4	red blood cells	42.8	19.0;155.0	3.0	987.7
	mononuclear cells	6.2	5.3;11.9	1.6	147.2
Week 12	red blood cells	48.1	17.1;89.0	0.1	519.9
	mononuclear cells	10.9	3.9;31.0	0.9	147.6
Week 24	red blood cells	39.4	17.2;70.6	2.7	191.8
	mononuclear cells	8.3	2.7;14.0	0.4	72.4

Table 3. Concentration of MTX metabolites (nmol/L) in groups of smokers and nonsmokers, Me [0.25; 0.75]

MTX metabolite	nonsmokers, n=23	smokers, n=4	p
MTX monoglutamate	46.5[25.3;97.5]	11.2 [2.6;21.9]	0.021
7-OH-MTX	28.2[7.1;64.7]	2.1[0.5;10.4]	0.008
MTXPG2	8.2[4.1;32.9]	0.5[0.1;1.3]	0.001
MTXPG 3	32[0.9;10.4]	2.8 [0.9;10.4]	0.069
MTXPG 4	3.1 [1.5; 7.3]	3.9[0.95-11.9]	0.864
MTXPG 2-4 (Sum)	54.8[2.2;23.4]	13.2[2.2;23.4]	0.038

proposed for determining the patientsX compliance and introduced into clinical practice. It has been suggested that, since the kinetics of MTX in the RBC differs from its kinetics in other types of cells, such as leukocytes, there may be no clear relationship between MTXPG levels in erythrocytes and RA control [9]. That is why the analysis of MTXPG levels in immunocompetent cells seems to be relevant.

Objective: we studied the concentration of methotrexate and its active metabolites in dynamics after 4, 12 and 24 weeks of MTX therapy to summarize the preliminary results obtained during therapeutic drug monitoring of the concentration of MTX in red blood cells (RBC) and mononuclear cells (MO) of RA patients.

Materials and Methods: the prospective study included 33 patients (26 women, 7 men) aged 53.2 ± 11.7 years with the diagnosis of RA, according to the ACR / EULAR 2010 criteria, who were MTX therapy naive. All patients had normal renal excretory function (GFR more than 60 ml / min / 1.73 m^2). MTX was administered subcutaneously at an initial dose of 10–15 mg per week with a gradual increase in the dose to the maximum of 25 mg per week or until adverse reactions (ARs) developed. The characteristics of the patients are shown in Table 1.

The patients were examined at 4, 12 and 24 weeks from the start of MTX therapy. During the visits, the following parameters were assessed: disease activity (DAS2), ARs, concomitant medications, BMI, and the cumulative MTX dose. The number and reasons for missing MTX injections were recorded. Blood sampling was carried out for a general clinical blood test; AST ALT, C-reactive protein levels were determined; venous blood samples were collected separately to determine the concentrations of MTX monoglutamate, MTX polyglutamates with 2, 3 and 4 glutamate residues (MTXG 2–4), 7-hydroxymethotrexate (7-OH-MTX) by tandem chromatography-mass spectrometry. The mononuclear cells (MO) fraction was isolated by layering the peripheral venous blood on verografin-ficoll. To assess the effect of age on the MTXPGs levels, the patients were divided into 2 groups. Group 1 (n=14) included elderly patients (men over 65 and women over 60). Group 2 (n=18) consisted of the rest of the patients. Statistical analysis using the methods of parametric and nonparametric statistics was carried out: Statistica 10 for Windows (StatSoft Inc., USA).

Results. Table 2 shows the dynamics of the mean values of the concentration of the main active metabolites of MTX in RBC and MO.

Pairwise comparison of the concentrations of MTXPGs 2–4, using the nonparametric Wilcoxon method, did not reveal statistically significant differences at Weeks 4,12, and 24 (Fig. 1.2).

There was a direct correlation between the level of total MTXPG, MTX monoglutamate, 7-OH-MTX in RBC and MO at all visits. The average total number of MTXPGs, both in RBC and in MO, did not differ at 4, 12 and 24 weeks of therapy, however, as can be seen from the figure, at earlier visits there was a greater scatter of values. The concentration of the studied MTX metabolites did not correlate with the body mass index, glucocorticoid intake, single and cumulative MTX doses, the incidence of ARs, total volume of RBC (MCV), and leukocyte count. Based on the available material, no correlations were found between ЖKDAS28 (0–4, 0–12 and 0–24) and the concentration of MTX metabolites.

Smoking. Samples of 27 patients were available for analysis at 12 weeks of therapy. Four (15%) of them were smokers. It was revealed that the concentration of MTX monoglutamate, MTXPG2, 7-OH MTX in the RBC was statistically significantly lower in smokers (Table 3).

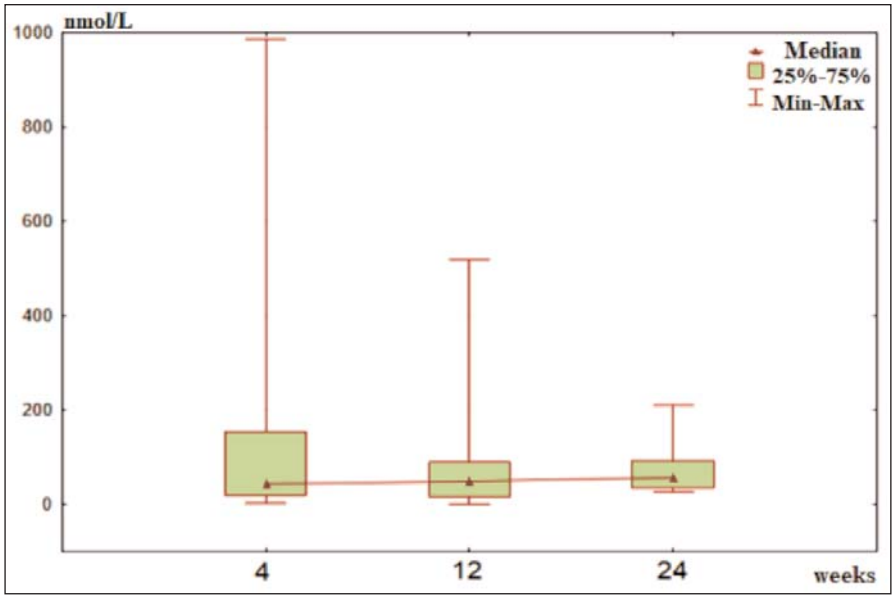

Figure 1. *The total number of MTXPG (2-4) in red blood cells*

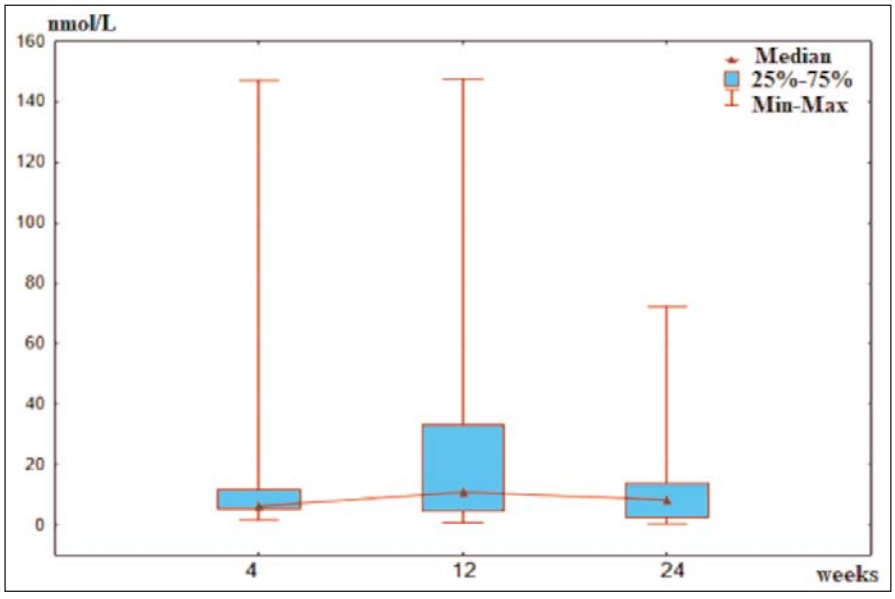

Figure 2. *The total amount of MTXPGs (2–4) in mononuclear cells*

Age. At Weeks 4 and 12, the concentrations of MTX metabolites, both in RBC and in MO, did not differ in the groups of patients of different ages. At week 24, there was a statistically more significant level of MTX monoglutamate in the RBC in group 1 compared with group 2 (5.6 [0.3; 26.5] versus 1.8 [0.7; 13.5]; p=0.03). At the same time, the concentration of MTXPG4 in the RBC, a high level of which indicates a good response to MTX therapy, in group 1 was statistically significantly lower than in group 2 (2.0 [1.3; 3.1] versus 4.5 [2.1; 6.0], respectively; p = 0.01).

Concomitant therapy. At week 12, the concentration of MTXPG4 in the MO was higher in the group of patients receiving statins (10.5 [7.1; 17.1] versus 3.5 [1.1; 7.8] nmol / L, p=0.04). The concentration of MTX metabolites did not depend on the glucocorticoid intake (neither with daily oral administration, nor as pulse therapy or intraarticular injections).

Short breaks (1–3 weeks) in treatment. A comparison was made of the concentrations of MTXPG 2,3 and 4 in the groups of patients who missed MTX injection (regardless of the reason and the number of misses) and who did not miss the injection. By week 4, only 1 patient had a deviation from the therapy regimen. By week 12, 11 (33%) patients showed a deviation from the therapy regimen; however, no differences in the concentrations of MTX metabolites in both RBC and MO were found (p> 0.05 in all cases). Twenty-nine patients completed 24 weeks of observation therapy. In 19 (57.6%) patients, a violation of the therapy regimen was revealed, however, there were no differences in the concentrations of MTX metabolites both in RBC and in MO (p> 0.05 in all cases).

Discussion.

The presented results are preliminary because of a small size of the study group. However, this study has already made it possible to answer the following questions regarding the tactics of MTX therapy.

According to Russian and foreign clinical guidelines [13–16], the recommended starting MTX dose is 10–15 mg/week with a rapid increase (2.5–5 mg every 2–4 weeks) to 25–30 mg/week depending on the effectiveness and tolerability. After 24 weeks of therapy, patients of the older age group had a lower MTXPG4 level and a higher level of original monoglutamate form of MTX, which confirms the data on a slower MTX metabolism and justifies a longer waiting period for the therapeutic effect of MTX.

Statins use is likely to enhance the MTXPG accumulation. These data should be used with caution, since there was no positive correlation between statin use and MTX metabolites levels at 24 weeks. In further research, special attention should be paid to drug interactions. Thus, it is known that omeprazole slows down the excretion of 7-OH-MTX [17]. Considering the fact that omeprazole is one of the most frequently prescribed Tnon-antirheumaticУ drug for patients with rheumatic diseases, it seems important to consider these possible interactions.

Smokers were found to have a significantly lower MTXPG RBC levels at 12 weeks; therefore, smoking cessation at the early stages of MTX therapy will increase the effectiveness of treatment.

Based on the data obtained, it can be assumed that short breaks (1–3 weeks) in treatment do not have a significant impact on the MTX metabolites levels; this impact, however, cannot be confirmed with the method used.

Conclusions. The applied method for determining the methotrexate metabolites levels seems to have important practical role, since it makes possible the therapeutic drug monitoring of MTX. This approach provides wide opportunities for everyday clinical practice, since it allows personalized management of RA patients by predicting the therapeutic effect, determining adherence to therapy, adjusting the dose and, if required, switching to another therapy. With further monitoring of this cohort of patients, the dependence of the therapeutic effect on the of MTX metabolites levels in RBC and mononuclear cells will be assessed. Treatment interruptions of insignificant duration (1–2 weeks) do not affect MTXPG 2,3 and 4 levels and distribution.

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Conflict of Interest Statement

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