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Effect of methotrexate on serum levels of anti-CCP antibodies and different classes of rheumatoid factors in rheumatoid arthritis patients

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Abstract

Background: *Anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific to rheumatoid arthritis and are predictive of rapid progression and erosive disease. There are contradictory reports on the impact of therapy on the titers of these autoantibodies and their correlation with treatment response.*

Objective: *To investigate the effect of methotrexate treatment on anti-CCP antibodies and rheumatoid factors (RFs) in patients with rheumatoid arthritis (RA). We also analyzed whether treatment response was associated with specific changes in anti-CCP antibodies.*

Material and methods: *The study group consisted of 44 patients with RA. All patients were treated with MTX once weekly (dose range: 10-25 mg). Disease activity was determined by the DAS 28 index and treatment response was assessed according to EULAR response criteria. Anti-CCP antibodies and IgA, IgM, and IgG RFs were tested in serum samples in methotrexate-naïve patients and after six months of treatment using a commercial second-generation ELISA kit.*

Results: *The serum titers of anti-CCP antibody and IgA, and IgM RFs (but not IgG RF) decreased significantly after six months of treatment with MTX. The differences in IgA and IgM RF were most pronounced in patients with good treatment response according to EULAR criteria. Anti CCP antibodies differed significantly before and after treatment in patients with good and moderate response ($p = 0.02$). In patients with no response there were no significant differences.*

Conclusions: *Methotrexate treatment of RA patients results in a decrease in the serum titers of anti-CCP antibodies and IgM and IgA RFs in patients showing clinical improvement. These parameters may be used as complementary indicators of treatment efficacy.*

Key words: *methotrexate, anti-cyclic citrullinated peptide antibodies, rheumatoid factor, rheumatoid arthritis.*

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Methotrexate (MTX) remains a gold standard in the therapy of rheumatoid arthritis (RA) and is the most commonly administered disease-modifying antirheumatic drug (DMARD) [1, 2]. Improvement defined as at least moderate according to the EULAR criteria or as 20% improvement (ACR20) according to the American College of Rheumatology (ACR) is achieved in approximately 45-65% of patients. According to various authors, therapy with MTX is continued by 55-81.8% of patients after 2 years, by 46-62% after 5 years, and by approximately 30% after 10 years. The mechanism of action of MTX in RA therapy is not fully understood. Studies are still underway to discover whether the main mechanism of action is of immunosuppressory, immunomodulatory, cytostatic, or anti-inflammatory character.

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific to RA and have a diagnostic and prognostic role. Various meta-analyses showed that the sensi-

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tivity of rheumatoid factor (RF) in RA patients is 68-69% and specificity 77-85%, and the corresponding values for anti-CCP antibody are 67-76 and 92-95% [3-5]. The detection of anti-CCP antibodies is even more significant in early RA. In their meta-analysis, Nishimura *et al.* did not find any diagnostically significant differences between IgG RF, IgM RF, and IgA RF, but anti-CCP antibodies were a better diagnostic marker than all three RF subclasses [5]. Anti-CCP antibodies may appear in serum several years before the onset of the first clinical signs of the disease and are present in 35-40% of patients seronegative for classic rheumatoid factors [6]. Anti-CCP antibodies also seem to be a better marker indicating the erosive form of the disease. There are contradictory data on the effect of therapy on autoantibody levels and the correlation between their initial concentration and treatment response.

The purpose of this study was to determine whether therapy with MTX affects rheumatoid factor and anti-CCP antibody levels and if their initial concentrations play a role in treatment efficacy.

Material and methods

Fifty-five RA patients meeting the ACR criteria for RA of 1987 were recruited to the prospective study. The participants were patients of the Rheumatology Clinic and the Rheumatology and Internal Diseases Department in Wroclaw. Forty-four patients treated with MTX for at least 6 months and regularly reporting to control examinations were qualified for the final analysis. The patients' characteristics are presented in Table 1. The following inclusion criteria were accepted:

- informed consent to participation in the study,
- confirmed RA diagnosis based on ACR criteria,
- active form of the disease defined as ESR > 30 mm/h and/or CRP > 1.5 mg/dl, minimum 4 painful and 3 swollen joints, DAS 28 > 3.2,
- age over 18 years,
- females and males with reproductive potential had to use reliable contraception,
- stable doses of NSAIDs and glucocorticosteroids (max. 10 mg of prednisone a day) for the last 4 weeks were admissible.

Patients fulfilling the following criteria were excluded from the study:

- pregnancy or breastfeeding,
- coexistence of other systemic connective tissue diseases besides RA,
- clinically significant hepatic and renal dysfunction (AspAt or AlAT > 1.5-times over the normal range, serum creatinine > 1.5 mg%),
- leucopenia (< 3500 WBC/μl), thrombocytopenia (< 100.000 PLT/μl), anemia (hemoglobin < 8.5 g/l),
- alcohol abuse,
- infection with hepatotropic viruses type B hepatitis (HBV) or type C hepatitis (HCV),
- infections refractory to therapy,
- cancer currently diagnosed or in medical history, if no recovery was achieved,
- uncontrolled diabetes,
- patient unwilling or unable to cooperate.

The study was approved by the Ethics Committee of the Wroclaw Medical University.

Drug dosage principles

MTX was administered orally in an initial dose of 10-15 mg once a week. Then, in weeks 12-16 the patients were given MTX in a dose of 15 mg once a week. If at least moderate improvement was not achieved and there were no significant adverse effects, the dose was increased to the maximum level of 25 mg.

Table 1. Comparison of patients with no response (non-responders) and those who had a moderate or good response (responders) during treatment with MTX

	Responders	Non-responders	*p*
number of patients	32	12	
age (average ± SD)	52 ±9	50 ±11	NS
sex, women (%)	88	75	NS
DAS 28	5.9 ±0.6	5.6 ±0.8	NS
MTX dose (min.-max.)	15 (12.5-25)	15 (12.5-25)	NS
folic acid supplementation (%)	100	100	NS
glucocorticosteroids (%)	54	58	NS
NSAID (%)	76	75	NS
smoking (%)	30	33	NS

NS – not statistically significant
** statistically significant p < 0.01*

Clinical and laboratory evaluation

Clinical and laboratory activity of the disease was evaluated during the observation period. Clinical evaluation was based on medical history, physical examination with tender and swollen joint count, pain intensity evaluation using a 100-millimeter visual analogue scale, evaluation of the patient's general physical fitness (based on the standard Health Assessment Questionnaire, HAQ), and laboratory test results (erythrocyte sedimentation rate – ESR, C-reactive protein level – CRP). The parameters allowed determination of improvement according to the criteria recommended by the European League Against Rheumatism (EULAR) based on the change in the DAS 28 disease activity index.

Based on the effectiveness of the therapy, the patients were divided into three groups according to the EULAR response criteria:

- group A – no response (DAS28 reduction < 0.6),
- group B – moderate effectiveness of the therapy: DAS28 reduction > 0.6 and < 1.2,
- group C – good response to the therapy: DAS28 reduction > 1.2.

Moreover, in some analyses the non-responders were compared with the responders (patients in whom a moderate or good response was observed) because of the limited number of participants in each group.

The evaluation of treatment safety was performed based on medical history, physical examination, and selected additional tests. Before the start of the therapy, X-ray of the chest and ECG were performed and HBV and HCV infections were excluded. Additionally, before inclusion and during all study visits the following laboratory parameters were measured: circular blood cell count, hepatic and renal function (serum activity of alanine aminotransferase AlAT, aspartate aminotransferase AspAT, creatinine level, urinalysis). Rheumatoid factor and anti-CCP antibody titers were determined at the beginning of the therapy and after 6 months. Clinical and laboratory tests were performed before the start of the therapy and in months 2, 4, and 6 of the observation period.

Joint damage was assessed on plain radiographs and classified according to the Steinbrocker criteria before the start of the therapy. All patients were given 5-15 mg of folic acid 24 to 48 h following the administration of MTX.

Methodology of selected laboratory tests

Commercially available ELISA tests (Euroimmun, Germany) were used to determine IgG, IgM, and IgA rheumatoid factor concentrations and anti-CCP antibodies (EUROIMMUN, Germany). Cut offs of the test used for RF (IgG, IgA, IgM) were ≥ 20 RU/ml and for anti-CCP ≥ 5 RU/ml.

Statistical methods

The statistical significance of differences between mean values of the tested parameters before the therapy and during MTX use was determined with Student's *t*-test (U Mann-Whitney test – for variables that were not normally distributed or had not homogenous variance) for dependent samples. *P* values of less than 0.05 were considered significant. Correlation of rheumatoid factor and anti-CCP antibody concentrations with disease activity was tested with Pearson's linear correlation and Spearman's correlation coefficient.

Results

A total of 55 patients with RA were included in the study. A final analysis was performed on 44 patients (37 females and 7 males). Eleven patients were withdrawn from the study because of adverse effects (9 patients) and irregular control visits (2 patients). All the patients were Caucasians treated with MTX for at least 6 months. Mean MTX dose was 15 mg/week (range: 12.5-25 mg).

Analysis of treatment effectiveness

Treatment response was determined according to EULAR criteria in the 44 patients who were followed during the whole study period. In the majority of patients, statistically significant improvement in disease activity was achieved already after 8 weeks of the therapy. Good response to the therapy was found after 2 months in 48% moderate improvement in 25% and no improvement or deterioration in 27% of the patients. This study confirmed that such parameters as sex, rheumatoid factor seropositivity, the use of glucocorticosteroids, or disease stage do not affect MTX treatment response (Table 1).

In this study we evaluated the effect of MTX on anti-CCP antibody and rheumatoid factor (in three classes: IgM, IgG, and IgA) concentrations. Serum concentrations of anti-CCP antibodies decreased significantly after 6 months of the therapy ($p < 0.01$). The therapy with MTX caused statistically significant reductions in the serum levels IgM RF, and IgA RF antibodies only in patients who showed good response to the therapy (Table 2, 3). Anti-CCP antibody titer changes in different patients groups divided according to treatment response (good and moderate vs. no response) differed significantly ($p = 0.02$) (Table 3). This suggests that these values may be taken into account when evaluating the effectiveness of the therapy. Moreover, the initial anti-CCP antibody levels in responders were lower than in non-responders, which was not statistically significant. Mean titers of anti CCP antibodies before treatment was 124 ±218 IU/ml in responders ($n = 32$) and 164 ±130 IU/ml ($n = 12$) in no responders.

Discussion

Methotrexate is used all over the world in more than 0.5 million patients with RA [7]. However the efficacy of MTX therapy is hard to predict. No single reliable bio-

Table 2. Serum titers of IgA RF, IgM RF, and IgG RF before and after six months of MTX treatment

DAS28 changes	RF	Patients number`	Mean IU/ml ± SD IU/ml	Statistical significance
DAS28 changes > 1.2	IgA RF before	21	132.7 ±89.7	$p < 0.01$
	RF IgA after 6 months	21	93.5* ±86.7	
	RF IgM before	21	465.2 ±346.4	$p < 0.01$
	RF IgM after 6 months	21	308.5* ±206.9	
	RF IgG before	21	21.1 ±15.8	NS
	RF IgG after 6 months	21	16.7 ±17.8	
DAS28 changes > 0.6 to < 1.2	RF IgA before	11	144.4 ±90.5	NS
	RF IgA after 6 months	11	136.7 ±96.0	
	RF IgM before	11	357.9 ±212.0	NS
	RF IgM after 6 months	11	347.4 ±221.7	
	RF IgG before	11	14.2 ±3.7	NS
	RF IgG after 6 months	11	15.2 ±12.9	
DAS28 changes < 0.6	RF IgA before	12	188.0 ±91.3	NS
	RF IgA after 6 months	12	197.4 ±104.7	
	RF IgM before	12	490.5 ±273.8	NS
	RF IgM after 6 months	12	466.2 ±219.6	
	RF IgG before	12	26.9 ±26.7	
	RF IgG after 6 months	12	16.9 ±12.1	NS

NS – not statistically significant
** statistically significant p < 0.01*

chemical marker has been identified and routinely determined clinical and laboratory parameters are poor predictors of treatment response. One of the first studies investigating the effect of MTX on IgM RF titer was published by Olsen *et al*. They demonstrated a reduction in serum IgM RF concentration and a reduction in IgM synthesis by peripheral blood mononuclear cells in patients treated with MTX [8]. Results concerning MTX's effect on RF are contradictory. Alarcon *et al*. demonstrated that IgM RF and IgA RF concentrations were reduced during MTX therapy, but correlation with clinical improvement was demonstrated only for IgM RF [9]. Spadaro *et al*. also found a reduction in RF concentration in classes IgG, IgM, and IgA, but it did not correlate with improvement in clinical parameters [10]. In another study, Spadaro *et al*. evaluated the effect of MTX therapy on RF and anti-CCP antibody levels. They found no correlation of initial anti-CCP antibody levels with the effectiveness of the therapy. They showed that MTX treatment resulted in a significant reduction of IgM RF, but anti-CCP antibodies were not affected. However, their study group was small ($n = 20$) and the MTX doses were relatively low (7.5-15 mg/weekly) [11]. Other studies showed both a reduction in serum IgM RF level and reduced production of IgM RF by mononuclear cells only in patients who responded well to MTX therapy [12, 13]. However, no correlation between reduced IgA RF level and clinical improvement of patients was demonstrated [12]. De Rycke *et al*. did not find a correlation between initial anti-CCP antibody concentration and the efficacy of combination therapy of infliximab and MTX, but patients with lower baseline IgM RF concentrations showed better response. After 30 weeks of therapy, a statistically significant decrease was seen only for IgM RF, but not for anti-CCP antibodies [14]. Both anti-CCP antibody and RF concentrations decreased significantly after 6 and 12 months of therapy with adalimumab and MTX as reported by Atzeni *et al*. Those changes correlated with a decrease in disease activity. No such changes were reported in patients treated with MTX alone. However, they had been given MTX previously and their disease was stable. Therefore the authors did not rule out the possibility that if the patients had been evaluated before and during the MTX therapy, the results could have been different [15]. In our study, anti-CCP antibody, IgA RF, and IgM RF serum concentrations were significantly reduced after 6 months of MTX therapy. This concerned primarily patients who responded well to the therapy. These results suggest that these parameters may be considered when evaluating the effectiveness of the therapy. The other finding of this study is that patients with lower baseline concentrations of anti-CCP antibodies responded better to the therapy. Similar results were obtained in the PROMPT study and the BeSt study [16].

Table 3. Serum titers of anti-CCP before and after six months of MTX treatment

DAS28 changes	anti-CCP	Patients number	Mean IU/ml ± SD IU/ml	Statistical significance
Δ DAS28 > 0.6	anti-CCP before	32	123.8 ±218,9	$p < 0.02$
	anti-CCP after 6 months	32	80.2* ±155.7	
Δ DAS28 changes < 0.6	anti-CCP before	12	164.6 ±130.5	NS
	anti-CCP after 6 months	12	151.9 ±137.7	
DAS28 changes in all patients	anti-CCP before	44	133.3 ±197.0	$p < 0.01$
	anti-CCP after 6 months	44	95.0* ±149.8	

NS – not statistically significant
** statistically significant p < 0.01*

The mechanism by which MTX could affect anti-CCP antibody level is not completely understood. It is possible that the anti-inflammatory and pro-apoptotic effects of the drug may reduce citrullination and the synthesis of antibodies [17].

Conclusions

Methotrexate therapy is associated with significant decreases in anti-CCP and IgM RF, IgA RF antibodies in good responders to therapy.

Disclosures – none.
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