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Editorial

Smoking and Methotrexate Inefficacy in Rheumatoid Arthritis: What About Underlying Molecular Mechanisms?

Gerrit Jansen[1], Maurits C.F.J. de Rotte[2], and Robert de Jonge[2]

The study by Safy-Khan, *et al* in the current issue of *The Journal of Rheumatology*[1] reports that in a methotrexate (MTX)-based treatment regimen for patients with early arthritis, current smoking was significantly associated with a smaller reduction of Disease Activity Score in 28 joints (DAS28) over time compared to noncurrent smoking. This negative effect of current smoking on DAS28 was dose-dependent: patients who smoked 10–19 cigarettes per day did worse than patients who smoked 1–9 cigarettes per day. Moreover, the effect was independent of concomitant prednisone use. These results support data from other clinical studies (reviewed in Ling, *et al*[2]) referring to smoking as a contributing factor to MTX nonresponse in patients with rheumatoid arthritis (RA). However, within these observations, questions related to underlying molecular mechanisms by which smoking adversely affects MTX efficacy have been largely underexposed. Here we will discuss some relevant factors emerging from MTX nonresponse prediction models from the perspective of the mechanism of action of MTX, drug resistance, and the role of smoking therein.

In brief, the mechanism of action of low-dose MTX treatment in RA involves MTX uptake in immune-competent cells, followed by its intracellular retention through conversion to polyanionic MTX-polyglutamate (MTX-PG) forms by the enzyme folylpolyglutamate synthetase. Polyglutamylation of MTX prevents MTX from being extruded from cells by selected members of the adenosine triphosphate (ATP)-binding cassette (ABC) drug efflux transporter family, including ABCC1–5 and ABCG2. Intracellularly, MTX-PGs inhibit several key enzymes in folate metabolism and purine biosynthesis *de novo*. These pharmacological inhibitions ultimately lead to the release of adenosine exerting antiinflammatory effects as well as inhibition of downstream signaling pathways driving inflammation.[3] Polyglutamylation and efflux by ABC transporters are also critical processes in cellular homeostasis of natural folates, which are being hijacked by MTX.

Over the past decades, clinically directed laboratory studies on blood cells and synovial tissue identified multiple predictors for their association with MTX (in)efficacy in RA. These markers were based on the following: (1) altered pharmacogenetic/genomic profiles of folate metabolism–related genes; (2) folate status–related altered epigenetics; (3) inflammation-related altered proteomic and metabolic profiles; and (4) altered serological and/or altered immunological marker profiles between MTX responders and nonresponders.[2] Combinations of clinical and lifestyle variables, and selected laboratory variables determined at baseline, were then used to construct and validate models to predict MTX nonresponse after 3–12 months.[4,5,6,7] Studies by Teitsma, *et al* for the U-Act-Early study utilized MTX response after 1-year treatment as an outcome measure and defined baseline DAS28 as the major contributor in the prediction model, along with current smoking and alcohol consumption. Inclusion of these variables revealed an area under the receiver-operating characteristic curve (AUC) of 0.75 for the U-Act-Early as development cohort and 0.68 for a validation cohort.[4] De Rotte, *et al* included data from 2 clinical studies, MTX-R and tREACH, to predict insufficient response to 3-month MTX treatment

[1]*G. Jansen, PhD, Biochemist, Amsterdam Rheumatology and Immunology Center, Amsterdam University Medical Center, location VUmc;*
[2]*M.C. de Rotte, PhD, Clinical Chemist, R. de Jonge, PhD, Professor of Clinical Chemistry, Department of Clinical Chemistry, Amsterdam University Medical Center, Amsterdam, the Netherlands.*

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Address correspondence to Dr. G. Jansen, Amsterdam Rheumatology and Immunology Center, Amsterdam University Medical Center, location VUmc, CCA - Rm 2.46, De Boelelaan 1117, 1081 HV Amsterdam, Amsterdam, the Netherlands. Email: g.jansen@amsterdamumc.nl.

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in patients with RA before disease-modifying antirheumatic drug (DMARD) initiation.[6] In their prediction model, baseline DAS28 (> 5.1) was again a contributor. Other variables such as the Health Assessment Questionnaire (HAQ; > 0.6), current smoking, BMI (> 25 kg/m^2), single-nucleotide polymorphisms for ABC transporters ABCB1 (rs1045642) and ABCC3 (rs4793665), and erythrocyte folate were additional variables contributing to the model that had an AUC of 0.80 for both the derivation and validation cohorts. Notably, within this model, current smoking had the highest relative contribution of the variables to the prediction scores. This model was recently externally validated for the U-Act-Early study, revealing an AUC of 0.75 and 0.71 for MTX nonresponse after 3 and 6 months, respectively.[7] Interestingly, Sergeant, *et al* constructed an MTX nonresponse prediction model for European Alliance of Associations for Rheumatology response after 6 months, with an AUC of 0.74 that included disease activity measures (HAQ, tender joint count [TJC], DAS), and laboratory (rheumatoid factor [RF]) and psychosocial (anxiety/depression) variables.[5] Interestingly, current smoking was positively associated with MTX nonresponse in the univariate analysis. Together, lifestyle variables (BMI, smoking, alcohol), demographic factors (age, sex), disease activity measures (DAS, HAQ, TJC), and to a lesser extent, laboratory variables (erythrocyte folate, C-reactive protein/erythrocyte sedimentation rate, RF), are the major contributors to MTX nonresponse. The future importance is dependent on the type of prediction model used, such as logistic regression or more complex machine learning models.[8] Current smoking, lifestyle variables, and demographic factors have the advantage over laboratory variables of being relatively easy to implement in clinical practice.

Tobacco and (e-)cigarette smoke contain thousands of individual constituents, including many potentially toxic and carcinogenic components.[9] When released in airway tissues and circulation, natural defense systems will be mobilized to minimize the harmful effects in the short and long run.[10] Nevertheless, smoking is associated with extraarticular manifestations contributing to cardiovascular and respiratory comorbidities in patients with RA by imposing oxidative stress to endothelial cells, airway epithelium, and residing immune cells (e.g., macrophages).[11,12] One prominent cellular defense system to toxic compounds is formed by the protein family of ABC drug efflux transporters.[10,13] This family of 49 members with functional redundancy covers a broad substrate specificity of expelling toxic compounds as well as therapeutic drugs varying in chemical structure and charge. The prototypical ABC transporter P-glycoprotein (P-gp; ABCB1) has hydrophobic compounds as preferred substrates, whereas ABCC1–5 and ABCG2 preferentially export amphiphilic/charged compounds/drugs. To this end, DMARDs are also among the substrates of ABC transporters, with ABCB1 capable of exporting glucocorticoids (prednisone) and ABCC1–5 and ABCG2 capable of extruding MTX, hydroxychloroquine, sulfasalazine, and leflunomide.[13,14] Thus, upregulation of ABC transporters can contribute to conferring diminished drug response.[14] An important aspect of ABC transporters is that beyond their pharmacological function, they also harbor a physiological function by exporting substrates related to immune response (e.g., prostaglandins, leukotrienes, and sphingolipids) to facilitate the migration of immune cells to lymph nodes.[13] In fact, in RA, expression of selected ABC transporters on peripheral blood lymphocytes and synovial macrophages has been associated with RA disease activity (role for P-gp) and diminished response to DMARDs, including MTX (through ABCC1–5 and ABCG2) and prednisone (through P-gp).[13,15,16,17]. Moreover, ABCC1–5 and ABCG2 play a role in folate homeostasis,[14] which indirectly affects MTX polyglutamylation and efficacy. For these reasons, selected ABC transporters (wildtype variant) and erythrocyte folate were incorporated as variables in MTX nonresponse prediction models.[6] Of note, a low baseline erythrocyte folate content has been associated with MTX nonresponsiveness after 3 months of therapy.[18] From this perspective, smokers were also identified by significantly lower erythrocyte folate levels than nonsmokers.[19] There is no particular evidence that concentrations of smoke components may inhibit intestinal and cellular folate transporters; rather, lower blood folate concentrations may be due to lifestyle-related lower folate intake of fruits and vegetables by smokers.[20] Smoking has also been associated with lower accumulation of long-chain MTX-PGs in erythrocytes of patients with RA during long-term MTX treatment.[21] In prospective clinical studies, erythrocyte accumulation of these long chain MTX-PGs over 3–9 months was shown to correlate with a better reduction of DAS28 scores in both patients with RA and those with juvenile idiopathic arthritis, although large interpatient variabilities in MTX-PG concentrations were observed.[22,23] Acknowledging that assessments of erythrocyte folate concentrations and MTX-PG levels serve as a *bona fide* marker for MTX response and MTX nonresponse prediction models, it has to be realized that erythrocytes are not the primary blood cells involved in RA disease pathogenesis. Also, mature erythrocytes have no regulated folate metabolism such as in peripheral blood mononuclear cells. As such, future directions for MTX therapeutic drug monitoring and MTX-PG analyses warrant extension to relevant immune cells, which are analytically feasible by combined liquid chromatography and mass spectrometry technology with labeled internal standards.[24] Also, inclusion of a laboratory marker to better define the extent and duration of smoking, such as cotinine,[25] may be helpful for correlations with clinical response and laboratory variables. These studies may guide further improvement of current MTX nonresponse prediction models by inclusion of novel variables.

The results in the study by Safy-Khan, *et al*[1] show that the adverse effect of current smoking on MTX treatment efficacy for patients with early arthritis was independent of concomitant prednisone use; therefore, it suggests no apparent drug interaction between MTX and prednisone. The distinct mechanism of action of both drugs, and the notion that prednisone has no involvement with ABC transporters being relevant for folate/MTX metabolism, is consistent with this clinical observation.

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