Personalized medicine of methotrexate therapy


The ‘one-carbon metabolism’ research group of the department of clinical chemistry focuses on one-carbon, folate and vitamin B12 metabolism. One-carbon metabolism is crucial for human life because it generates a) purines and pyrimidines, the building blocks of RNA and DNA, and b) methyl-groups necessary for methylation reactions, which are essential in cellular regulation. We have linked derangements in one-carbon and folate metabolism to many diseases such as cardiovascular disease (1), neurodegenerative diseases, midline defects/congenital heart defects (2-12), osteoporosis (13-15), infectious diseases (16), adult and pediatric arthritis (17-19), and cancer (20-23). In oncology, anti-folate Tomudex chemotherapeutic drugs such as methotrexate (MTX) and pemetrexed (41) block crucial steps in one-carbon-metabolism and thereby inhibit DNA replication and growth of rapidly growing tissues. Anti-folates are also used in the treatment of malaria, arthritis and dermatological diseases like psoriasis. The aim of the one-carbon metabolism research group is to perform genetic and metabolic profiling of one-carbon-metabolism in order to investigate a) the regulation of its metabolism in health and disease, b) how derangements in its metabolism affect human diseases such as cancer, and c) how the folate status affects anti-folate therapy (personalized medicine) in patients in order to be able to individualize anti-folate therapy to obtain maximal efficacy with minimal toxicity (medicijn op maat). In this paper, we will focus on the MTX study.

High-dose MTX (HD-MTX) originally was developed in the 1940s as a chemotherapeutic drug in the treatment of neoplastic diseases such as (pediatric) Acute Lymphoblastic Leukemia (ALL) and other proliferative diseases (24, 42). In the 1970s and 1980s, it appeared that low-dose MTX (LD-MTX) also was effective for the treatment of Rheumatoid arthritis (RA) and Juvenile Idiopathic Arthritis (JIA) and in today’s practise it is the cornerstone disease-modifying anti-rheumatic drug. Although MTX is an effective drug, there is large inter-individual variation in the efficacy and toxicity of MTX limiting its use (25, 26, 43). In RA and JIA, efficacy varies between 30-70% depending on the treatment regime and outcome measure. We showed that 33% of JIA patients are non-responders according to ACR30 criteria (17) and that adverse events such as grade-3 or grade-4 mucositis occurred in 25% of children treated with MTX for ALL (22). In children with JIA, hepatotoxicity and gastrointestinal toxicity are major problems and are the main reasons for MTX withdrawl (27). In RA, 10-30% of patients discontinue MTX because of toxicity (28). In current practice, MTX is administered based on historical precedent rather than on scientific knowledge and it is seldom individually tailored, implicating a wide range in MTX levels and variability in side effects (26).

Hypothesis MTX study

We hypothesize that derangements in the patient’s cellular one-carbon and folate status modifies the response to MTX treatment. Identifying predictors of MTX efficacy and toxicity may lead to the development of individualized treatment strategies with improved efficacy and lower toxicity. To this end, we initiated three multicentre retrospective and prospective MTX trials in the areas of RA (partly embedded in the ‘treatment in Rotterdam Early Arthritis CoHort’ [REACH] study in the South-West of the Netherlands), JIA (UMCU-WKZ and Erasmus MC-Sophia), and ALL (UMCG-Beatrix, VUMC, and Erasmus MC-Sophia).

Metabolism and mechanism of action of MTX

In HD-MTX treatment such as in ALL, ≥ 500 mg/m² MTX is infused followed by folinic acid (leucovorin) rescue. In RA and JIA, LD-MTX (15-25 mg/week in RA and 10-15 mg/m² in JIA) is given orally in a fixed dose that may be increased when response is insufficient; folic acid is used to prevent adverse events. Sub-
Determinants of MTX efficacy and adverse events

We associated the RFC1 80G>A variant to an increased susceptibility to ALL (21) indicating that this polymorphism may be related to reduced folate uptake. Furthermore, ALL patients carrying the MTHFR 1298 A>C and MTRR 66A>G variants showed decreased in-vitro MTX sensitivity (20). Thus, patients carrying these polymorphisms in folate-related genes might need more MTX. In 81 pediatric ALL patients, we further explored the relation between folate-related genes and MTX toxicity (22). In line with literature data, we observed side-effects such as mucositis in 25% of children. MTHFR 1298 C-allele carriers needed less blood transfusions (p=0.042) and showed a trend to more rapid recovery of leukocyte count in high-risk ALL patients (p=0.053). MTRR 66 G-allele carriers showed a higher incidence of oral mucositis (p=0.018) and the MTRR 66AG genotype was associated with a slower recovery of platelet count in high-risk patients (p=0.004). Elevation of transaminase levels occurred less frequently in patients with SHMT1 1420CT genotype (p=0.041). The MTHFR 677C>T SNP and the MTRR 66A>G SNPs were identified as determinants of impaired Bone Mineral Density (BMD) in 83 childhood ALL patients.

In LD-MTX treatment such as in RA and JIA, we investigated how a deranged folate metabolism influences MTX response. In 205 RA patients from the BeSt trial (18), purine pathway polymorphism (AMPD1 34C>T, ATIC 347C>G, ITPA 94A>C) were associated with good clinical response. The explained variance (R²) for being a responder of each single genotype was approximately 20%, which compared favourably to classical risk factors such as the disease activity score (DAS) at baseline (R²=12%). For the association with toxicity, only ATIC G-allelic carriers experienced more adverse drug events (OR=2.0, 95%CI 1.1-3.7) (18). A cohort of 287 JIA patients treated with MTX was studied longitudinally over the first year of treatment. The adenosine triphosphate-binding cassette transporter B1 (ABCB1) gene polymorphism rs1045642, OR: 3.66 (95%CI: 1.62-8.33; p=0.002) and the ABCC3 rs4793665, OR: 2.70 (95%CI: 1.30-5.59; p=0.008) showed higher odds to achieve MTX response (submitted).

Prediction models of MTX efficacy and adverse events

The first prediction model for MTX efficacy was successfully constructed in 205 RA patients (19). The model for MTX efficacy consisted of sex, rheumatoid factor and smoking status, the DAS, and 4 polymorphisms in the AMPD1, ATIC, ITPA, and MTHFD1 genes. This prediction model was transformed into a scoring system ranging from 0 to 11.5. Scores of ≥3.5 had a true positive response rate of 95%. Scores of ≥6 had a true negative response rate of 86%.

Similar to RA, MTX is the anchor drug in JIA. If JIA patients are unresponsive to MTX, early and effective combination treatment with biologicals (TNFα inhibitors, IL-1 receptor blockers or IL-6 blockers) is required to prevent joint damage. To ensure that only patients unresponsive to MTX receive early addition-
al treatment with biologicals and those responsive to MTX are spared costly drugs with potentially serious adverse effects, it is crucial to predict those patients who will be unresponsive to MTX monotherapy. In a discovery cohort of 183 patients, we developed a prediction model to identify JIA patients not responding to MTX. The prediction model included: erythrocyte sedimentation rate and SNPs in genes coding for methionine synthase reductase (MTRR), multidrug resistance 1 (MDR-1/ABCB1), multidrug resistance protein 1 (MRP-1/ABCC1), and proton-coupled folate transporter (PCFT). The area under the receiver operating characteristics curve (AUROC) was 0.72 (95% CI: 0.63-0.81). The prediction model was transformed into a total risk score (range 0 to 11). At a cut-off score of ≥3, sensitivity was 78%, specificity 49%, positive predictive value was 83% and negative predictive value 41%. In the validation cohort (n=104), the AUROC was 0.65 (95% CI: 0.54-0.77) (submitted). A prediction model for MTX-related gastrointestinal adverse events (MTX intolerance) is being developed. These prediction models should be improved by adding metabolic parameters such as indicators of a disturbed one-carbon metabolism and their diagnostic accuracy should be tested in randomized control trials.

**Intracellular MTX-PG measurement and treatment response**

Plasma MTX levels can be easily measured but LD-MTX is rapidly cleared from plasma and hence, plasma MTX levels do not correlate with MTX response and are therefore not routinely measured. In RA, measurement of MTX in erythrocytes (RBC-MTX-PG) or white blood cells (WBC) may be a strong predictor of response (33-38); in childhood ALL, high accumulation of MTX-PGs was related to efficacy to MTX trials (33-38); in childhood ALL, high accumulation of MTX-PGs was related to efficacy to MTX (42). However, intracellular MTX-PGs are generally not measured by clinical laboratories. This is mainly because there is no rapid and specific method to measure RBC-MTX-PG in routine laboratories. Therefore, we developed fast and high-throughput MALDI-MS/MS methods to measure MTX in erythrocytes and plasma (39, 40). Although this method is very fast, the machinery is not standard for routine laboratories. Using stable isotope dilution LC-ESI-MS/MS, we are now able to measure RBC-MTX-PGs (also plasma) in a fast and precise way (paper in preparation; figure 2). Sample pre-treatment is simple and consists of a lysing and a deproteinization step. The first results indicate that there is a large inter-individual variation in RBC-MTX-PGs accumulation in patients treated with the same dose of MTX at 3 months of treatment (figure 2). Whether RBC-MTX-PG accumulation can predict MTX response and adverse events (and also non-compliance) in an early phase of treatment and can be used for more early and aggressive dose escalation in patients is now subject of study.

**Conclusion**

Determinants of MTX efficacy and adverse events have been identified. In RA and JIA, the first prediction models were constructed to predict MTX response at the start of treatment in order to achieve early dose escalation or additional combination therapy with biologicals. Future research should aim at improving the diagnostic accuracy of the prediction models by adding metabolic predictors to the clinical and genetic predictors in the models. Also, randomized controlled trials should establish whether the prediction models can realize individualized MTX therapy to obtain maximal efficacy with minimal toxicity (medicijn op maat) in order to improve disease outcome.

**References**


