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Personalized medicine of methotrexate therapy

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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 chemoteherapeutic 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.

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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 antirheumatic 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' [tREACH] 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/m^2 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/m^2 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-

cutaneous (or intramuscular) injections are given when response is insufficient, when patients do not tolerate oral tablets or when compliance is low. Oral MTX is actively absorbed in a capacity-limited process by the proximal jejunum. Because of the relatively short half-live (6-15 hours), intermittent LD-MTX administration once a week does not lead to accumulation of MTX in plasma and hence, therapeutic drug monitoring is not possible in LD-MTX treatment. Plasma MTX is mainly eliminated by the kidneys; 65-80% is eliminated within 12 hours after administration. Circulating MTX is taken up into cells via the solute carrier 19A1/reduced folate carrier (SLC19A1/RFC) and is additionally transported into the cell via the solute carrier 46A1/proton coupled folate transporter (SLC46A1/PCFT) and folate receptors (FOLR) 1 and 2 (figure 1) (29). Members of the adenosine triphosphate (ATP) binding cassette (ABC) transporters including ABCB1/P-glycoprotein (P-gp), multidrug resistance proteins (MRP/ABCC) as well as breast cancer resistance protein (BCRP/ABCG2) function as ATP-dependent MTX efflux transporters (29) albeit with different affinity. Intracellularly, MTX is polyglutamylated (MTX-PG) by folyl-polyglutamate synthetase (FPGS) to a variability of chain-lengths (PG2-6) competing with γ-glutamyl hydrolase (GGH) that deconjugates glutamate residues (figure 1) (30). Polyglutamylation retains MTX intracellularly because it is no substrate for the MTX efflux proteins and a higher degree of MTX polyglutamylation leads to stronger inhibition of the target enzymes in one-carbon metabolism and purine the novo synthesis. In LD-MTX treatment, the pentaglutamate (PG5) is the highest order of glutamylation detected while the triglutamate form (PG3) of MTX predominates (31, 32).

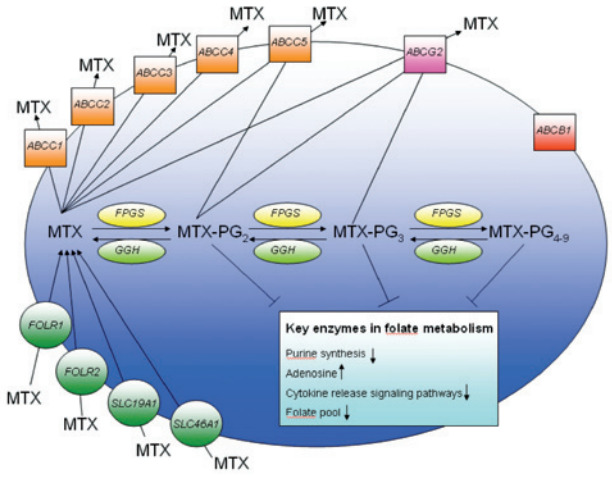

Figure 1. Cellular MTX transport routes for MTX influx and efflux in relation to polyglutamylation and mechanisms for arthritis suppression. MTX = methotrexate, MTX-PG = methotrexate polyglutamates, *ABCB1/ABCC1/ABCC2/ABCC3/ABCC4/ABCC5/ABCG2* = adenosine triphosphate-binding cassette transporter subfamily B/C/G member 1/2/3/4/5, *FPGS* = folylpolyglutamate synthetase, *FOLR1/2* = folate receptor 1/2, *GGH* = gamma-glutamyl hydrolase, *SLC46A1/19A1* = solute carrier 46A1/19A1.

Determinants of MTX efficacy and adverse events

We associated the *RFC*1 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 *SHMT*1 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 (R^2) 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 (R^2=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 *AMPD*1, *ATIC*, *ITPA*, and *MTHFD*1 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/*ABCB*1), multidrug resistance protein 1 (*MRP*-1/*ABCC*1), 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 respons (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.

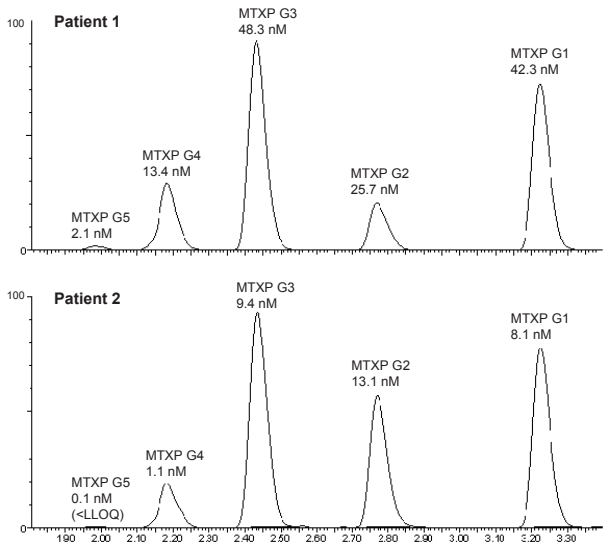

Figure 2. Chromatographs showing the MTX concentrations of RBC pellets in two different patients at 3 months of LD-MTX monotherapy. Both patients received the same amount of MTX. Concentrations are given in nmol/L RBC pellet.

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

1. Dunkelgrun M, Hoeks SE, Schouten O, et al. Methionine loading does not enhance the predictive value of homocysteine serum testing for all-cause mortality or major adverse cardiac events. Intern Med J. 2009; 39(1):13-18.
2. Verkleij-Hagoort A, Bliek J, Sayed-Tabatabaei F, Ursem N, Steegers E, Steegers-Theunissen R. Hyperhomocysteinemia and MTHFR polymorphisms in association with orofacial clefts and congenital heart defects: a meta-analysis. Am J Med Genet A. 2007; 143(9): 952-960.
3. Verkleij-Hagoort AC, de Vries JH, Ursem NT, de Jonge R, Hop WC, Steegers-Theunissen RP. Dietary intake of B-vitamins in mothers born a child with a congenital heart defect. Eur J Nutr. 2006; 45(8): 478-486.
4. Verkleij-Hagoort AC, van Driel LM, Lindemans J, et al. Genetic and lifestyle factors related to the periconception vitamin B12 status and congenital heart defects: a Dutch case-control study. Mol Genet Metab 2008; 94(1): 112-119.
5. Verkleij-Hagoort AC, Verlinde M, Ursem NT, et al. Maternal hyperhomocysteinaemia is a risk factor for congenital heart disease. BJOG. 2006; 113 (12): 1412-1418.
6. van Driel LM, de Jonge R, Helbing WA, et al. Maternal global methylation status and risk of congenital heart diseases. Obstetr Gynecol 2008; 112 (2 Pt 1): 277-283.
7. van Driel LM, Eijkemans MJ, de Jonge R, et al. Body mass index is an important determinant of methylation biomarkers in women of reproductive ages. J Nutr. 2009; 139 (12): 2315-2321.
8. van Driel LM, Smedts HP, Helbing WA, et al. Eight-fold increased risk for congenital heart defects in children carrying the nicotinamide N-methyltransferase polymorphism and exposed to medicines and low nicotinamide. Eur Heart J. 2008; 29 (11): 1424-1431.

9. van Driel LM, Verkleij-Hagoort AC, de Jonge R, et al. Two MTHFR polymorphisms, maternal B-vitamin intake, and CHDs. Birth Defects Res. 2008; 82 (6): 474-481.
10. Obermann-Borst SA, Isaacs A, Younes Z, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstetr Gynecol. 2011; 204 (3): 236 e1-8.
11. Obermann-Borst SA, van Driel LM, Helbing WA, et al. Congenital heart defects and biomarkers of methylation in children: a case-control study. Eur J Clin Invest. 2011; 41 (2): 143-150.
12. Obermann-Borst SA, Vujkovic M, de Vries JH, et al. A maternal dietary pattern characterised by fish and seafood in association with the risk of congenital heart defects in the offspring. BJOG 2011;118(10):1205-1215.
13. van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, et al. Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med. 2004; 350(20): 2033-2041.
14. Yazdanpanah N, Uitterlinden AG, Zillikens MC, et al. Low dietary riboflavin but not folate predicts increased fracture risk in postmenopausal women homozygous for the MTHFR 677 T allele. J Bone Miner Res. 2008; 23 (1): 86-94.
15. Yazdanpanah N, Zillikens MC, Rivadeneira F, et al. Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: the Rotterdam study. Bone. 2007; 41 (6): 987-994.
16. de Boer MG, Gelinck LB, van Zelst BD, et al. beta-D-glucan and S-adenosylmethionine serum levels for the diagnosis of Pneumocystis pneumonia in HIV-negative patients: a prospective study. J Infect. 2011 62 (1): 93-100.
17. de Rotte MC, Luime JJ, Bulatovic M, Hazes JM, Wulffraat NM, de Jonge R. Do snapshot statistics fool us in MTX pharmacogenetic studies in arthritis research? Rheumatol (Oxford, England). 2011; 49 (6): 1200-1201.
18. Wessels JA, Kooloos WM, De Jonge R, et al. Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis. Arthritis Rheum. 2006; 54 (9): 2830-2839.
19. Wessels JA, van der Kooij SM, le Cessie S, et al. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum. 2007; 56 (6): 1765-1775.
20. de Jonge R, Hooijberg JH, van Zelst BD, et al. Effect of polymorphisms in folate-related genes on in vitro methotrexate sensitivity in pediatric acute lymphoblastic leukemia. Blood. 2005; 106(2): 717-720.
21. de Jonge R, Tissing WJ, Hooijberg JH, et al. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. Blood. 2009; 113(10): 2284-2289.
22. Huang L, Tissing WJ, de Jonge R, van Zelst BD, Pieters R. Polymorphisms in folate-related genes: association with side effects of high-dose methotrexate in childhood acute lymphoblastic leukemia. Leukemia. 2008; 22 (9): 1798-1800.
23. Winkel te ML, Muinck de Keizer-Schrama SM, Jonge de R, et al. Germline variation in the MTHFR and MTRR genes determines the nadir of bone density in pediatric acute lymphoblastic leukemia: a prospective study. Bone. bbbb; 48 (3): 571-577.
24. Krajinovic M, Moghrabi A. Pharmacogenetics of methotrexate. Pharmacogenomics. 2004; 5 (7): 819-834.
25. Ranganathan P, Eisen S, Yokoyama WM, McLeod HL. Will pharmacogenetics allow better prediction of methotrexate toxicity and efficacy in patients with rheumatoid arthritis? Ann Rheum Dis. 2003; 62 (1): 4-9.
26. Relling MV, Fairclough D, Ayers D, et al. Patient characteristics associated with high-risk methotrexate concentrations and toxicity. J Clin Oncol. 1994; 12 (8): 1667-1672.
27. Hoekstra M, van Ede AE, Haagsma CJ, et al. Factors associated with toxicity, final dose, and efficacy of methotrexate in patients with rheumatoid arthritis. Ann Rheum Dis. 2003; 62 (5): 423-426.
28. Alarcon GS, Tracy IC, Blackburn WD, Jr. Methotrexate in rheumatoid arthritis. Toxic effects as the major factor in limiting long-term treatment. Arthritis Rheum. 1989; 32 (6): 671-676.
29. Assaraf YG. The role of multidrug resistance efflux transporters in antifolate resistance and folate homeostasis. Drug Resist Updat. 2006; 9(4-5): 227-246.
30. van der Heijden JW, Dijkmans BA, Scheper RJ, Jansen G. Drug Insight: resistance to methotrexate and other disease-modifying antirheumatic drugs--from bench to bedside. Nature Clin Pract 2007; 3(1): 26-34.
31. Dalrymple JM, Stamp LK, O'Donnell JL, Chapman PT, Zhang M, Barclay ML. Pharmacokinetics of oral methotrexate in patients with rheumatoid arthritis. Arthritis Rheum. 2008; 58 (11): 3299-3308.
32. Dervieux T, Orentas Lein D, Marcelletti J, et al. HPLC determination of erythrocyte methotrexate polyglutamates after low-dose methotrexate therapy in patients with rheumatoid arthritis. Clin Chem. 2003; 49(10): 1632-1641.
33. Dervieux T, Furst D, Lein DO, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. Ann Rheum Dis. 2005; 64 (8): 1180-1185.
34. Dervieux T, Furst D, Lein DO, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum. 2004; 50 (9): 2766-2774.
35. Dervieux T, Greenstein N, Kremer J. Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. Arthritis Rheum. 2006; 54 (10): 3095-3103.
36. Dervieux T, Kremer J, Lein DO, et al. Contribution of common polymorphisms in reduced folate carrier and gamma-glutamylhydrolase to methotrexate polyglutamate levels in patients with rheumatoid arthritis. Pharmacogenetics. 2004; 14(11):733-739.
37. Angelis-Stoforidis P, Vajda FJ, Christophidis N. Methotrexate polyglutamate levels in circulating erythrocytes and polymorphs correlate with clinical efficacy in rheumatoid arthritis. Clin Exp Rheumatol. 1999; 17(3): 313-320.
38. Kremer JM, Lee JK. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. Arthritis Rheum. 1986; 29(7): 822-831.
39. Meesters RJ, den Boer E, de Jonge R, Lindemans J, Luider TM. Assessment of intracellular methotrexate and methotrexate-polyglutamate metabolite concentrations in erythrocytes by ultrafast matrix-assisted laser desorption/ionization triple quadrupole tandem mass spectrometry. Rapid Commun Mass Spectrom. 2011; 25 (20): 3063-3070.
40. Meesters RJ, den Boer E, Mathot RA, et al. Ultrafast selective quantification of methotrexate in human plasma by high-throughput MALDI-isotope dilution mass spectrometry. Bioanalysis. 2011; 3(12): 1369-1378.
41. Walling J. From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. Investigational new drugs 2006; 24(1): 37-77.
42. Rots MG, Pieters R, Peters GJ, et al. Role of folylpolyglutamate synthetase and folylpolyglutamate hydrolase in methotrexate accumulation and polyglutamylation in childhood leukemia. Blood 1999; 93(5): 1677-83.
42. Bulatovic M, Heijstek MW, Verkaaik M, et al. High prevalence of methotrexate intolerance in juvenile idiopathic arthritis: development and validation of a methotrexate intolerance severity score. Arthritis Rheum 2011; 63(7): 2007-13.