


**Case Report**

## A Gastrointestinal Toxicity during Low-Dose Methotrexate Treatment in Two Pediatric Patients with Acute Lymphoblastic Leukemia

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**Abstract**

Low-dose Methotrexate (LD-MTX) and 6-mercaptopurine (6-MP) are used in the maintenance phase of treatment of childhood acute lymphoblastic leukaemia. It was reported that individuals with reduced activity of two of the enzymes involved in the pathway of purine metabolism, thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15), will be exposed to higher levels of active metabolites and will be at higher risk of side effects, such as myelosuppression. Therefore, dosing recommendations for 6-MP based on TPMT and NUDT15 genotype have been approved by FDA and have been published by the Clinical Pharmacogenetics Implementation Consortium and the Ditch Working Group. Though several studies were conducted on the toxicity of LD and HD-MTX in childhood ALL patients, none of the genetic markers so far have been used in MTX therapy protocols due to the lack of a clear association with the response and/or toxicity. We describe two pediatric patients who suffered from gastrointestinal toxicity following peroral administration of LD-MTX during maintenance therapy of ALL that diminished after switching to parenteral administration of the drug.

**Keywords:** Gastrointestinal Toxicity; Low-Dose Methotrexate; Acute Lymphoblastic Leukemia; Children; Polymorphism

**Introduction**

Methotrexate (MTX) is a competitive inhibitor of enzymes involved in folate metabolism, causing inhibition of DNA and RNA synthesis, methylation and cell death [1]. In childhood, acute lymphoblastic leukaemia (ALL) treatment MTX is used in high and low dosages [2]. According to the current ALL IC BFM 2009 protocol, patients who are in remission after intensive chemotherapy continue their treatment with maintenance therapy, consisting of two oral chemotherapeutic agents, 6-mercaptopurine (6-MP) in 50 mg/m<sup>2</sup> dose every day and low dose MTX (LD-MTX) in 20 mg/m<sup>2</sup> dose once per week for 104 weeks [3]. In certain children, 4 or 6 doses of intrathecal methotrexate are also given during the early phase of maintenance therapy [3]. The doses of 6-MP and LD-MTX are modified according to leukocyte count and differential, with targeted leukocyte count between 2x10<sup>9</sup>/L and 3x10<sup>9</sup>/L [3]. Common side effects of 6-MP and LD-MTX treatment include bone marrow suppression with pronounced leukopenia, neutropenia, lymphopenia, thrombocytopenia and significantly elevated liver enzymes and bilirubin that are usually manageable with reduction of drug dose or temporary discontinuation of treatment. In cases of serious side effects, such as long-standing diarrhoea or MTX pneumonitis, discontinuation of maintenance therapy is mandatory [3]. 6-MP, a purine analogue, is metabolically transformed into active thioguanine nucleotides (TGN) that are incorporated into DNA, which leads to cell death. Two of the enzymes involved in the complex pathway of these metabolites are thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15). It was reported that individuals with reduced activity of either enzyme will be exposed to higher levels of active metabolites, and will be at a higher risk of side effects, such as severe myelosuppression [4]. Therefore, dosing recommendations for 6-MP based on TPMT and NUDT15 genotype have been approved by FDA and have also been

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published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group [3].

MTX enters cells primarily via the solute carrier family 19 member 1 (SLC19A1) transporter. After completing its anticancer effect through a complex antifolate mechanism, MTX is removed from the blood into the hepatocytes by a liver-specific membrane transporter solute carrier organic anion transporter family member 1B1 (SLCO1B1). Several polymorphisms in the gene encoding SLC19A1 and SLCO1B1 are associated with impaired transporter function [5]. Though several studies were conducted on childhood ALL patients [6-10], including two meta-analyses that reached opposite conclusions [11, 12] none of the genetic markers so far have been used in MTX therapy protocols due to the lack of a clear association with the response and/or toxicity. Consequently, the FDA has not yet made recommendations concerning pharmacogenetic testing before MTX therapy. In our case report, we discuss pharmacogenomics as an important tool in preventing drug toxicity. We describe two pediatric patients who suffered from gastrointestinal toxicity following peroral administration of LD-MTX during maintenance therapy of ALL that diminished after switching to parenteral administration of the drug.

## Patients and Methods

Polymorphisms in SLC19A1 and SLCO1B1 were analyzed with allele-specific polymerase chain reaction in real-time (KASPar), employing appropriate positive (synthetic and biological) and negative controls. Beforehand, written informed consents of patients' parents were obtained for targeted molecular genetic analysis.

## Case Report

### Patient 1

A 4-year-old boy was diagnosed with B-cell ALL with initial central nervous system (CNS) involvement. Treatment with high dose (HD) intravenous MTX, as part of intensive treatment, underwent without significant toxicity. During maintenance therapy, he was given 6-MP every day and LD-MTX once per week and underwent radiotherapy due to the initial involvement of CNS. Later his mother reported an inappetence, failure to

thrive and mucositis in the child. The first signs of mucositis developed 2 weeks after the beginning of maintenance therapy. Mucositis with a duration of three days developed every single week, starting on the second day after oral LD-MTX ingestion. Significant failure to thrive followed and was an indication for total parenteral nutrition and feeding by nasogastric tube. Despite the reduction of doses of 6-MP and LD-MTX, severe cytopenia developed and maintenance therapy was stopped temporarily. Bone marrow aspiration showed hypocellularity with no signs of residual disease. MTX-induced gastrointestinal toxicity, related to oral ingestion of LD-MTX, was raised.

As polymorphisms in SLC19A1 and SLCO1B1 were associated with gastrointestinal toxicity in the literature, we decided on pharmacogenetic analysis of the most common polymorphisms in genes coding for these two transporters. The result confirmed that the patient was a heterozygous carrier of polymorphisms in both genes with a genotype SLC19A1 rs1051266 GA and had a heterozygous state for polymorphic allele \*1B with haplotype SLCO1B1 \*1/\*1B (Table 1). Although there are no guidelines for MTX-SLCO1B1 and MTX-SLC19A1 gene-drug pairs, our previous studies in our population showed that SLC19A1 and SLCO1B1 polymorphisms may be associated with an increased risk for gastrointestinal toxicity. Since our patient developed severe gastrointestinal toxicity and cytopenia and had had no major gastrointestinal toxicity during previous HD-MTX therapy, a decision, based on the results of molecular genetic analysis, was made to switch the patient from oral to the parenteral form of MTX. Afterwards, parenteral MTX was given on weekly bases until the end of maintenance therapy without major difficulties and the boy remains in remission for 3 years.

### Patient 2

A 15-year-old boy on maintenance therapy with 6-MP every day and LD-MTX once per week for T-ALL reported a loss of appetite and a loss of 10% of his body weight in the last few months. His daily food intake was estimated by a dietician, who reported highly suboptimal calorie intake. The patient himself reported a loss of appetite preventing him from eating more. Despite normal blood count and smear, suspicion of relapse as well as the fungal infection was raised. At the same time, polymorphisms associated with increased risk of gastrointestinal toxicity following LD-MTX treatment were analyzed. The results confirmed heterozygosity

Table 1: Results of molecular genetic analysis.

Results of molecular genetic analysis						
Patient	Gene	Polymorphism	Nucleotide	Amino acid	Genotype	Result interpretation
Number 1	SLC19A1	rs1051266	80 G>A	His27Arg	GA	Heterozygous for polymorphism
	SLCO1B1	rs2306283	388A>G	Asn130Asp	AG	Heterozygous for polymorphism
	SLCO1B1	rs4149056	521T>C	Val174Ala	TT	Homozygous for normal allele
Number 2	SLC19A1	rs1051266	80 G>A	His27Arg	GA	Heterozygous for polymorphism
	SLCO1B1	rs2306283	388A>G	Asn130Asp	AA	Homozygous for normal allele
	SLCO1B1	rs4149056	521T>C	Val174Ala	CT	Heterozygous for polymorphism

for polymorphism SLC19A1 with genotype *SLC19A1* rs1051266 GA and concomitant heterozygosity for polymorphic allele \*5 with haplotype *SLCO1B1* \*1/\*5\*1/\*5. No further diagnosing was needed and the patient was switched to parenteral maintenance LD-MTX therapy which was finished successfully. He is now in remission for 2 years.

## Discussion

Pharmacogenomics and individualized drug therapy are increasingly influencing the course of clinical medicine. Identification and characterization of genetic polymorphisms, involved in drug metabolism, transport and mechanism of action, provide important information on individual inherited differences in drug metabolism and treatment response. By preventing therapeutic failures and adverse drug events (ADEs), they enable treatment optimization and outcome [13]. Many genes are involved in the metabolism of MTX and polymorphism of some of them (gamma-glutamyl hydrolase (GGH), serine hydroxymethyl transferase 1 (SHMT1), thymidylate synthase (TS)) were linked to adverse events and others (*SLC19A1* and methylene tetrahydrofolate reductase (MTHFR)) to treatment efficacy [14].

### SLC19A1

The solute carrier 19A1 (*SLC19A1*), trivially referred to as reduced folate carrier (RFC), is coding for solute folate carrier (RFC1), a major transporter of folates and antifolates into the cell. It is involved in the transport of MTX from the intestinal lumen to enterocytes as well as in the transport of MTX from the blood into cells. The most frequently studied *SLC19A1* polymorphism is rs1051266 in the second exon of the *SLC19A1*, resulting in the amino acid substitution of arginine for histidine (H27R) [15 in transmembrane domain 1 (TMD1), a region implicated in substrate binding and/or translocation [16, 17], which is expected to alter SLC transport properties. Although to date, other polymorphisms have also been described, the functional consequences of other single nucleotide polymorphisms (SNPs) are not known at the moment [18]. The association between polymorphisms of *SLC19A1* and the occurrence of HD- [1, 19-28] and LD MTX treatment-related toxicity was studied in ALL patients and patients with rheumatoid arthritis (RA) respectively [29]. The results of different studies evaluating the role of *SLC19A1* c.80 AA and GA genotypes in oncogenesis and response to chemotherapy are contradictory [30-32]. In ALL patients some studies of HD-MTX related toxicity reported a protective role of rs1051266 AA genotype [24, 25] while others reported either increased risk of MTX-induced toxicities and fatal outcomes in rs1051266 A carriers [1, 19-20] or even absence of association between *SLC19A1* genotype and HD-MTX-induced toxicities [21, 23, 26-28].

Additionally, *SLC1A1* polymorphism can influence MTX metabolism and treatment efficacy. A study performed by Kotur et al. showed that the A allele of the *SLC19A1* c.80 variant

contributes to slow MTX elimination [1] and in Leyva-Vázquez's study GG and GA genotypes were linked with ALL relapse and poor survival [32]. Many studies on the possible association between polymorphisms of *SLC19A1* and the occurrence of LD-MTX-induced ADEs in patients with RA were published. Lima et al. reported a protective effect of wild type alleles of rs1051266 and rs2838956 regarding MTX induced gastrointestinal toxicity [33]. On the other hand association between GGAG haplotype for *SLC19A1* rs7499, rs1051266, rs2838956 and rs3788200 with MTX gastrointestinal toxicity<sup>33</sup> as well as polymorphism *SLC19A1* A were associated with increased risk for temporary discontinuation of treatment due to MTX-related toxicities [29].

### SLCO1B1

Apart from *SLC19A1*, the influence of *SLCO1B1* polymorphisms, hepatic organic anion transporter that mediates the transport of MTX in hepatocytes and elimination of MTX via bile [34-38] on MTX treatment-related toxicities was also reported. The majority of studies were again conducted in children with ALL receiving HD-MTX therapy and suggested an association between certain polymorphisms of the *SLCO1B1* gene and increased risk for MTX induced toxicities, particularly increased risk of gastrointestinal toxicity [39]. To date, studies on association between certain polymorphisms of *SLCO1B1* gene and risk for LD-MTX induced toxicities were also reported in patients with RA [33, 40], where *SLCO1B1* rs4149056 was associated with gastrointestinal toxicity. Three large GWAS studies showed that *SLCO1B1* variants rs11045879 and rs4149081 tied to functional rs4149056 are associated with MTX clearance and GI toxicity and this result was replicated in an independent cohort [39, 41]. An even larger replication GWAS study, enrolling around 1300 childhood ALL patients also reported an association of rs4149081, rs11045879, rs11045821, and functional rs4149056 with MTX clearance variability of around 10% [42]. A study published by Roszkiewicz et al. included 100 children with RA, treated with a low dose of MTX. The drug was given in peroral form or subcutaneously and adverse events were closely followed. In patients with *SLCO1B1* rs4149056, CT/CC variants resulted in lower MTX clearance, a higher plasma concentration of MTX and consequently a higher probability of gastrointestinal toxicity [40]. Patients with TT variant had a higher probability of hepatotoxicity and importantly none of the SNPs was related to MTX efficacy [40]. Contrary, Kotur et al. did not show a connection between the *SLCO1B1* rs4149056 T/C variant and MTX elimination in pediatric ALL patients [1].

Recently, the association between a combination of polymorphisms of *SLC19A1* and *SLCO1B1* rs4149056 T allele and gastrointestinal toxicity of LD-MTX was also observed in Portuguese patients with RA [33] and variant alleles in *SLCO1B1* rs4149056 and rs11045879 were found to be associated with lower 6-MP/MTX tolerance in ALL patients [43]. In our patient 1, no major difficulties with gastrointestinal toxicity except mild mucositis were noticed during HD-MTX, yet soon after starting

peroral LD-MTX, mucositis with aphthous stomatitis and severe failure to thrive developed, indicating the association of peroral intake of the drug with the SLCO1B1 polymorphism [1, 40]. In reported patient 2 loss of appetite lead to significant weight loss during the maintenance chemotherapy that improved after switching from peroral to parenteral LD-MTX. The results of our report on the relationship between SLCO1B1 and SLC19A1 polymorphisms and gastrointestinal toxicity are in accordance with the above-mentioned reports. The absence of gastrointestinal toxicity in case of intravenous application opposite to peroral treatment in our patients is in accordance with the published data that SLCO1B1 polymorphism is a risk factor for gastrointestinal toxicity in RA patients, who mostly receive MTX in a peroral form in opposite to absence of influence of SLCO1B1 rs4149056 T/C variant on the elimination of intravenous HD-MTX in pediatric ALL patients [1, 40]. SLCO1B1 and SLC19A1 genotyping before initiation of maintenance treatment could be one of the factors enabling safer and more effective LD-MTX treatment in ALL patients. Consequently, we could be able to identify the group of patients with a significant risk for gastrointestinal toxicity during treatment with peroral LD-MTX. In these patients, parenteral LD-MTX maintenance therapy could be introduced at the very beginning of treatment, thus successfully preventing treatment-related gastrointestinal toxicity.

## Conclusion

Our report could serve as an example of personalized therapy with LD-MTX in ALL patients, aimed at achieving better treatment outcomes.

## Statement of Ethics

Written informed consent was obtained from the patients' parents for publication of this case reports.

## Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

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The authors declare that no funding was obtained.

## Author Contributions

B.F.K., T.P., M.K., S.L.A., L.K., J.J. and V.D. have participated in writing the paper.

## Data Availability Statement

All data generated or analyzed in the report are included in the article. Further inquiries can be directed to the corresponding author.

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