Acute lymphoblastic leukemia is the most common pediatric malignant disease. Contemporary paediatric co-operative group acute lymphoblastic leukemia (ALL) protocols cure approximately 80% of patients. High cure rate can be achieved with the risk-stratified therapies that employ multiple chemotherapy agents. Currently the risk-stratification is based on host and disease characteristics including age, gender, white blood cell count, karyotype, immunophenotype and early response to therapy, but unfortunately this model fails to identify a significant fraction of non-responding patients or an individual patient who will experience severe toxicity at drug doses tolerated by the majority. One of the challenges for the clinical decisions in future is the determination of factors which would provide better risk stratification and thus enable us to choose the most appropriate therapy for the individual patient. Beside already employed clinical and biologic factors, individual genetic differences in metabolic pathways which can affect individual responses to drugs, both in terms of therapeutic effect as well as adverse effects, may play an important role determining the outcome of the treatment of acute lymphoblastic leukemia. Pharmacogenetics, the study of genetic variations in drug-processing genes and individual responses to drugs, may enable the improved identification of patients at higher risk for either disease relapse or chemotherapy-associated side effects. In the following presentation we would like to present a brief overview of our results in pharmacogenetic studies in childhood acute leukemia. During the past years we have focused our work in the field, to explore pharmacogenetic factors that may play a role in individual susceptibility for development acute leukemia, the role of polymorphisms and gene-gene interactions in acute toxicity and the occurrence of certain late effects such as late cardiotoxicity and occurrence of secondary neoplasms.

**Folate pathway and susceptibility to leukemia**

Although the events leading to ALL are not clear yet, it seems that environmental and dietary factors as well as the genetic susceptibility play a role. One of the important dietary factors is the intake of folate, which is needed for normal cell growth. Intracellular folate metabolism is complex and involves several enzymes that channel the methyl group of tetrahydrofolate (THF) into the direction of the DNA synthesis or methylation. The key enzymes involved in the folate metabolic pathway are 5,10-methylenetetrahydrofolate reductase (MTHFR), thymidylate synthase (TS), methionine synthase (MS) and methionine synthase reductase (MTRR) (fig. 1). Two common polymorphisms C677T and A1298C are known in the MTHFR gene resulting in a lower enzyme activity and have been associated with a reduced risk for childhood and adult ALL. Besides MTHFR, other polymorphic enzymes are important for the utilization of the methyl group. Studies have demonstrated that TS polymorphism is a protective factor for adult ALL and malignant lymphoma and it also influences the treatment outcome of childhood ALL and colorectal cancer. However, there is no information on the influence of MS and MTRR polymorphisms on the risk for childhood ALL. Folate metabolic pathway is complex and the polymorphic enzymes competing for the folate supplies determine intracellular folate availability. It is to be expected that the combination of polymorphisms in these genes rather than a single polymorphic gene influence the...
The results of our study in which we analysed common genetic polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR), thymidylate synthase (TS), methionine synthase (MS) and methionine synthase reductase (MTRR) in 68 children with ALL and 258 healthy controls showed, that the combination of MTHFR 677T polymorphism and TS 3R/3R genotype did not influence the risk for childhood ALL, suggesting that the altered rate of dTMP synthesis was not associated with the risk for ALL. On the other hand, the combination of MTHFR 677T, MS 2756G and MTRR 66G polymorphisms, which may lower the availability of C1 group for methylation reactions, showed a tendency to reduce the risk for childhood ALL 3.2-fold (1).

Toxicity and outcome

In the study of the role of polymorphisms on toxicity we have focused on two drugs which are the key elements of childhood ALL treatment: methotrexate and 6 mercaptopurine. The prediction of high-dose methotrexate (HD-MTX) toxicity is a key issue in the individualization of treatment in childhood acute lymphoblastic leukemia (ALL). In our first study the aim was to evaluate the influence of MTX pathway polymorphisms on HD-MTX treatment outcome in children with ALL. In total, 167 children with ALL were genotyped for methylenetetrahydrofolate reductase; TS: thymidylate synthase; MS: methionine synthase; MTRR: methionine synthase reductase.
in the second exon of the RFC1 gene results in amino acid substitution of arginine for histidine (H27R) in the first transmembrane domain, a region implicated in substrate binding and/or translocation [2–4]. This substitution results in altered RFC transport properties and decreased RFC expression, and is correlated with altered MTX transport. In the study in 60 children treated for ALL we have studied whether RFC1 G80A polymorphism could be used as a predictor of MTX toxicity in children and adolescents with ALL treated with high dose MTX (HD-MTX). We have found no association between different RFC1 genotypes and MTX exposure after any of the applications. No association was also found between different RFC1 genotypes and investigated MTX toxicities (leukopenia, thrombocytopenia, mucositis, neurotoxicity). In addition, there was no significant difference in ALL outcomes between RFC1 genotypes (4).

In order to investigate the influence of common polymorphisms in SLC19A1, MTHFR and ABCB1 on plasma levels of MTX, we developed a population pharmacokinetic model of high-dose methotrexate (HD-MTX) in children with acute lymphoblastic leukaemia (ALL) and malignant lymphoma (ML). The study population comprised 64 children with ALL/ML (age 1.6–16.8 years) who had received a total of 252 MTX courses (2–4 per patient). Common putative functional polymorphisms in the SLC19A1, MTHFR, MS, MTRR, TS and ABCB1 genes were analysed by PCR-based genotyping. Nonlinear mixed effects modelling was used for the pharmacokinetic analysis. We found MTX clearance to be decreased to 73.8% in patients with the MTHFR 677TT genotype. Patients homozygous for the variant MTHFR 1298A>C and SLC19A1 80A>G were at decreased risk for leucopenia. The TS 2R>3R polymorphism was associated with a lower incidence of and mucositis. In contrast, the MTHFR 677TT polymorphism was associated with an increased incidence of mucositis. A population pharmacokinetic model developed in this study implies only a limited influence of genetic factors on the systemic disposition of MTX. Clearance is moderately reduced in patients with the MTHFR 677TT genotype. Genetic polymorphisms in the folate metabolic pathway and SLC19A1 were associated with HD-MTX toxicity (3).

6-MP is an antimetabolite widely used in the treatment of acute lymphoblastic leukemia. The clinical relevance of 6-mercaptopurine (6-MP) dose reduction in prevention of thiopurine-induced toxicity is well established in patients homozygous for low activity alleles of thiopurine S-methyltransferase (TPMT) but uncertain in heterozygous individuals or undetectable enzyme activity (mutated homozygous). Decreased TPMT activity is caused by mutations in the TPMT gene, the most prevalent being 460G4A and 719A4G, which are usually inherited together in cis as the 4*A allele. These polymorphisms destabilize the native structure of the TPMT enzyme, resulting in the formation of misfolded states, which subsequently undergo intracellular degradation. ALL patients with TPMT deficiency tend to respond better to 6-MP therapy because they accumulate TGN in cancer cells, but are at higher risk of developing toxic effects. To investigate the effect of TPMT haplo-insufficiency in heterozygous pediatric ALL patients on thiopurine treatment outcome, we have analyzed the association of 6-MP dose reduction and the incidence of 6-MP-induced toxic effects with TPMT and MTHFR genotypes. 6-MP dose reduction was recorded in 19% of the ALL patients, and was more likely to occur in patients with mutated PMT than in wild-type patients. 6-MP-related toxic effects, such as hematologic toxicity, stomatitis, infections and secondary tumors were present in 14, 5, 21 and 4% of the patients, respectively. Hematologic toxicity, stomatitis and infections were more likely to occur in patients with low activity TPMT alleles than in wild-type patients (5).

Secondary neoplasms and late toxicities

Patients after treatment of childhood cancer are at special risk for subsequent neoplasms. The risk of secondary neoplasm was studied in a cohort of 1,577 patients treated for childhood cancer registered in the Cancer Registry of Slovenia (CRS) between 1961 and 2000. Three groups of primary malignancies, namely: leukemia, CNS tumors, and lymphomas made for 70% of cases of secondary neoplasms. In leukemia patients, there was a 1.17% risk for second neoplasm at 5 years after diagnosis, 7.75% at 10 years, 11.14% at 15 years, 13.03% (95% CI 6–19.6%) at 20 years, and 16.14 at 25 years (Fig 2) (6).

In the study in which we tried to identify DNA repair polymorphisms contributing to the risk of second neoplasm in 359 long term survivors of childhood ALL, we observed a significant association of NBN 1197G allele with increased risk of developing second neoplasm, while the risk was decreased in carriers of XRCC3-316G allele compared with patients with wild-type genotype (7).
We hypothesized that deactivating variants of superoxide dismutase II (SOD2) [rs4880 (-9Val > Ala)], catalase (CAT) [rs1001179 (-262C > T) and rs10836235 (c.66 + 78C > T)], GSTT1, and GSTM1 may increase the risk of developing cardiac toxicity, in patients exposed to anthracyclines. The hypothesis was tested in a cohort of 76 long-term survivors of acute lymphoblastic leukemia in childhood. Cardiac damage was evaluated as an attributable variable and compared to gene polymorphisms. In our study group, we show statistically significant correlation between CC homozygosity for CAT (rs10836235 (c.66 + 78C > T)) and cardiac damage after anthracycline exposure ($p = 0.020$) (10).

Despite statistical significant results of some of our studies of association of genetic polymorphisms on toxicity, survival and development of second neoplasms in childhood leukemia, the major problem that we are facing is that, due to limited number of studied subjects, many of the studies lack statistical power. To overcome the obstacle of insufficient numbers of studied subjects, joint efforts of research groups are needed. The established IALLGC consortium is international initiative that may enable us to better understand genetic susceptibility to acute leukemia by making the framework for high quality studies.

In similar studies we were not able to show correlation between polymorphisms in MTHFR Glutathione S transferases and the occurrence of secondary neoplasms after the treatment of leukemia in childhood (8,9).

Anthracyclines have contributed significantly to the increased cure rate in pediatric oncology. Cardiac toxicity is an important late effect after anthracycline treatment and is thought to occur by reactive oxygen species mediated cardiac damage. We hypothesized that deactivating variants of superoxide dismutase II (SOD2) [rs4880 (-9Val > Ala)], catalase (CAT) [rs1001179 (-262C > T) and rs10836235 (c.66 + 78C > T)], GSTT1, and GSTM1 may increase the risk of developing cardiac toxicity, in patients exposed to anthracyclines. The hypothesis was tested in a cohort of 76 long-term survivors of acute lymphoblastic leukemia in childhood. Cardiac damage was evaluated as an attributable variable and compared to gene polymorphisms. In our study group, we show statistically significant correlation between CC homozygosity for CAT (rs10836235 (c.66 + 78C > T)) and cardiac damage after anthracycline exposure ($p = 0.020$) (10).

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References


