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# 677C>T 5,10-methylenetetrahydrofolate reductase reductase (MTHFR) polymorphism and early toxicity of high-dose methotrexate in children treated for acute lymphoblastic leukemia

## Polimorfizm 677C>T genu reduktazy 5,10-metylenotetrahydrofolianowej (MTHFR) a wczesne powikłania leczenia wysokimi dawkami metotreksatu u dzieci z ostrą białaczką limfoblastyczną

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Summary

**Introduction.** High doses of methotrexate (HD-Mtx) in some patients may cause severe adverse effects despite monitoring of Mtx elimination and administering of calcium folinate.

One of the most important enzymes of the folate metabolic pathway affected by Mtx is methylenetetrahydrofolate reductase (MTHFR) which activity depends on genetic polymorphisms.

**Aim.** Analysis of occurrence and intensity of Mtx therapy related early toxicities in children treated because of acute lymphoblastic leukemia (ALL) in dependency on 677C>T MTHFR gene polymorphism.

**Material and methods.** One hundred and sixty children (age: 1-17 years) treated in one pediatric oncology department between 1993 and 2007 according to ALL-BFM-90 and ALL-IC-2002 protocols were included into retrospective study. The presence of 677C>T MTHFR polymorphism was evaluated with the use of PCR-RFLP technique, and Mtx pharmacokinetics parameters were evaluated with immunoenzymatic method. Early toxicities of 617 chemotherapy cycles (Mtx dose 2 g/m$^2$ and 3 g/m$^2$) were analyzed according to NCI-CTC scale.

**Results.** In analyzed group the patients with wild-type genotype CC, heterozygotes CT and homozygotes TT were 42.5, 48.75 and 8.75%, accordingly. Nausea and vomiting were significantly more common in CT heterozygotes ($p = 0.03$). In TT patients delays of therapy ($p = 0.01$), thrombocytopenia ($p = 0.03$) and acute neurotoxicities ($p = 0.05$) were more common.

More severe liver toxicities, nausea, stomatitis, and infections were observed in TT homozygotes. Acute renal insufficiency of unknown etiology occurred in two patients (CT heterozygote and TT homozygote).

**Conclusions.** Obtained results indicate the possible correlation between the presence of T allel and high risk of acute toxicities of HD-Mtx therapy in children. It is necessary to continue studies including higher number of patients with evaluation of other important genetic polymorphisms.

Streszczenie

**Wstęp.** Wysokie dawki metotreksatu (HD-Mtx) pomimo monitorowania eliminacji leku i podaży leukoworyny u niektórych pacjentów wywołują nasilone objawy toksyczne. Jednym z kluczowych enzymów szlaku przemian folianów, występującym w zmiennej, zależnej od polimorfizmów genowych aktywności, którego działanie zaburza Mtx, jest reduktaza metylenotetrahydrofolianowa (MTHFR).

**Cel pracy.** Analiza występowania i nasilenia wczesnych toksyczności terapii HD-Mtx u dzieci leczonych z powodu ALL, w zależności od polimorfizmu genowego 677C>T MTHFR.

**Materiał i metody.** Badaniem retrospektywnym objęto 160 dzieci w wieku 1-17 lat, leczonych w jednym ośrodku onkologii dziecięcej w latach 1993-2007 zgodnie ze zmodyfikowanym

protokołem ALL-BFM-90 i ALL-IC-2002. Nosicielstwo polimorfizmu 677C>T MTHFR oceniono techniką PCR-RFLP, a parametry farmakokinetyki Mtx metodą immunoenzymatyczną. Bliskie toksyczności łącznie 617 cykli chemioterapii z zastosowaniem dawki 2 g/m$^2$ i 3 g/m$^2$ Mtx analizowano zgodnie ze skalą NCI-CTC.

**Wyniki.** W badanej grupie pacjenci o „dzikim" genotypie CC, heterozygoty CT i homozygoty TT stanowili odpowiednio: 42,5; 48,75 i 8,75%. Nudności i wymioty częściej występowały u heterozygot CT (p = 0,03). U pacjentów TT odnotowano częstsze: opóźnienia w kontynuacji leczenia (p = 0,01), małopłytkowość (p = 0,03) i ostre objawy neurotoksyczne (p = 0,05). Wyższe stopnie toksyczności wątrobowych, nudności, zapalenia śluzówek jamy ustnej i zakażeń obserwowano u homozygot TT. Ostra niewydolność nerek o nieznanej etiopatogenezie wystąpiła u dwóch pacjentów: heterozygoty CT i homozygoty TT.

**Wnioski.** Uzyskane wyniki wskazują na związek allela T z większym nasileniem ostrych toksyczności terapii HD-Mtx u dzieci. Celowa jest kontynuacja badań z udziałem dużych grup pacjentów i uwzględnieniem także innych istotnych polimorfizmów genowych.

## INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a most frequent neoplasm of childhood. With use of multimodal, multidrug therapy it can be cured in about 80% of cases. Standard chemotherapy is expected to result in toxicities that rarely are considered as life-threatening ones but in most of the patients these toxicities can be strongly pronounced and thus constitute a reason for therapy delay what increases a risk of leukemia relapse. Acute toxicities are particularly frequent in case of high doses of cytotoxic drugs used in repeated cycles as for example in case of high dose methotrexate (HD-Mtx) administration in remission consolidation phase in ALL (1-4). This cytostatic agent belongs to the antimetabolite group of cytostatics. It's action comprises blockage of several enzymes involved in folate metabolism pathway what results in block in purine synthesis, protein methylation inhibition and i.a. transformation of homocysteine to methionine (3, 5-9). Inhibition of cell divisions resulting from disturbed DNA synthesis and repair acts against the leukemic cell clone but also injuries healthy tissues, especially these that undergo rapid cell division as epithelial cells, mucosae, and bone marrow. Most frequently observed methotrexate toxicities are: myelosuppression, mucositis, skin changes, liver and kidney dysfunction, acute neurological toxicities and also infections. Life-threatening toxicities of high-dose methotrexate administration are however relatively rare, of which most important one is kidney failure. Prevalence and degree of observed toxic side effects of methotrexate results mainly from prolonged methotrexate exposition as well as individual patient's susceptibility to that drug. Gene polymorphisms resulting in modulation of enzyme activity that results in important metabolic pathways modification has been widely studied lately. Certain gene polymorphisms can influence not only the effectiveness of anti-cancer therapy and risk of relapse but also the occurrence and intensity of chemotherapy side effects (2-6, 8-14). In case of folate metabolic pathways that are influenced by methotrexate, methylenetetrahydrofolate reductase (MTHFR) activity is of special meaning (MTHFR). This enzyme is involved in the equilibrium of protein methylation processes (including homocysteine homeostasis) and urine and thymidine synthesis (DNA regeneration) as well. Among abundantly described MTHFR gene polymorphisms, the commonly observed 677C>T polymorphism is of special relevance as it results in significant decrease of MTHFR enzyme activity (10, 11). The prevalence of polymorphic T allel varies between different geographical regions that i.a. are known to differ in nutritional habits. This specific allel is most frequently observed in mediterranean and central-African population where diet is generally considered as folate-rich one. In Caucasian population MTHFR activity is observed in about half of population as the T allel is present in 40% of this population in its heterozygotic CT form and in about 10% of population in homozygotic TT form (5, 7, 10, 11, 15, 16). As a result of heterozygotic CT form occurrence and even more in case of 677 nucleotide TT homozygosity a thermolabile enzyme of decreased activity is produced (60 and 30% of activity, respectively). This can influence the individual patient's methotrexate sensitivity. Numerous published papers indicate a relationship between the presence of 677C>T MTHFR gene polymorphism and the observed intensity of acute methotrexate toxicities both in case of low as well as high methotrexate doses use (1, 9, 10, 17-20). Some papers indicate a relationship between this gene polymorphism and worse treatment results in children treated for ALL. These treatment failures may result not only from deaths of therapy toxicities (influenced i.a. by endothelium dysfunction secondary to hiperhomocysteinemia) but also an increased risk of relapse in T allel carriers in whom DNS synthesis suppression is decreased (1, 2, 21).

## AIM

Aim of present paper is analysis of high-dose methotrexate acute toxicities frequency and intensity in children treated for ALL depending on 677C>T MTHFR gene polymorphism presence.

## MATERIAL AND METHODS

**Retrospective analysis of 160 children with ALL (age: 1-17 years), treated from 1993 to 2007 at Pediatric Oncology and Hematology Department,**

**Polish-American Institute of Pediatrics, Jagiellonian University Medical College in Kraków with high-dose methotrexate as a consolidation ALL therapy (doses: 2 g/m² – modified ALL-BFM-90 protocol and 3 g/m² – ALL-IC-BFM-2002 protocol) was performed.** Therapy inclusion criteria comprised: good general status of the patient, no evidence for acute infections, normal liver and kidney function and elimination of drugs known to interfere with methotrexate metabolism. Therapy was administered four times, every two weeks. Patients were initially hydrated and received proper alkalization prior to 24-hour continuous methotrexate infusion administered with use of volumetric pump and central venous access. A lumbar puncture with intrathecal methotrexate or methotrexate/cytarabine/hydrocortisone administration (in case of 3 g/m² and 2 g/m² methotrexate dose respectively) was performed within two hours from the beginning of methotrexate infusion. Patients also received oral mercaptopurine (25 mg/m²) daily. A specific antidote – leucovorin (15 mg/m²) was administered in 42, 48 and 54 hour from the start of methotrexate infusion. Methotrexate serum concentration was routinely assessed in 24, 36, 42 and 48 hour form start of methotrexate infusion (EMIT® – Syva immunoenzymatic method, with use of VIVA-Vitalab analyzer, DADE-BEHRING, USA). Methotrexate serum concentrations obtained after completion of methotrexate infusion were considered as representative for steady state. Hour 48 serum methotrexate concentration exceeding critical values (0.40 $\mu$mol/l) resulted in leucovorin dose escalation, hydration intensification and in prolonged methotrexate serum concentration until safe values were reached (< 0.25 $\mu$mol/l) in each case. Methotrexate elimination pharmacokinetics were assessed with use of first degre elimination constants for both phases of methotrexate elimination estimated with Wagner's formula ($K_{el} = \ln c_1 - \ln c_2 / t_2 - t_1$).

Presence of 677C>T MTHFR gene polymorphism was identified in DNA isolated from peripheral blood leukocytes with use of PCR-RFLP technique. Starter sequences used in DNA amplification reaction were: CTG ACC TGA AGC ACT TGA AGG (forward) and AGT GAT GCC CAT GTC GGT (reverse). PCR reaction products were processed with restriction enzyme *Hinfl1*, which detects the locus of cytosine to thymidine substitution in 677th nucleotide. Obtained DNA fragments were separated with use of agarose gel electrophoresis (fig. 1).

National Cancer Institute Common-Toxicity-Criteria in GPOH modification (German Society of Pediatric Oncology/Hematology) (22) was used to assess organ and systemic toxicities observed during the high-dose methotrexate treatment.

An analysis of total of 617 chemotherapy cycles with use of 2 g/m² and 3 g/m² methotrexate doses was performed. Statistical analyses were performed with use of STATISTICA software. Chi-square test and Fisher exact test were used to identify relations between qualitative features. Consistency of allele separation within observed group of patients with expected allele distribution according to Hardy-Weinberg's rule was checked with use of the Chi-square test. One-sided fraction test was applied to check the influence of 677C>T MTHFR gene polymorphism on the incidence of acute HD-Mtx therapy toxicities. Multiple logistic regression analysis was performed to identify risk factors of increased HD-Mtx therapy toxicities. Statistical results were verified at the significance level of $p = 0.05$.

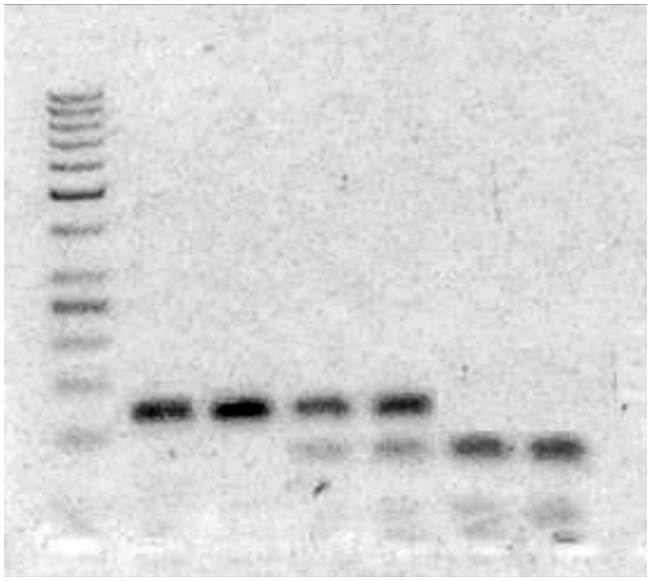

**Fig. 1.** Result of agarose gel electrophoresis of DNA samples treated with *Hinfl1* enzyme – 677C>T MTHFR gene polymorphism. From left to right: ladder, two samples – CC homozygotes (122bp DNA fragments), two samples – CT heterozygotes (two DNA fragments: 122bp – 677C allel and two sequences of 82bp – 677T allel), two samples – TT homozygotes (DNA fragments of 82bp corresponding to 677T allel with *Hinfl1* restriction site).

## RESULTS

Among 160 analyzed cases treated with HD-Mtx, 78 (48.75%) were CT heterozygotes, 68 (42.5%) were identifies as a wild-type CC gene carriers and 14 (8.75%) were TT homozygotes. The distribution of 677C>T MTHFR gene polymorphisms within analyze group was consistent with the Hardy-Weinberg equation conditions ($\chi^2 = 0.323$; $p = 0.57$).

Two of the most frequent treatment toxicities: liver dysfunction (elevated transaminase activity, increased serum bilirubin, decreased total protein and albumin concentrations) and mucositis were observed in equal proportions, independently from the MTHFR genotype of the subject. However in CT heterozygotes: nausea and vomiting ($p = 0.03$) and in TT homozygotes: acute CNS toxicities, thrombocytopenia and therapy delay ($p = 0.05$, 0.03 and 0.01 respectively) were more frequently observed than in carriers of the wild-type CC genotype. Summary of analyses of high-dose methotrexate acute toxicities and therapy side effects depending on 677C>T MTHFR gene polymorphism for 617 cycles analyzed is presented in table 1 and figure 2.

**Table 1.** Acute toxicity frequency in course of HD-Mtx (2 g/m$^2$ and 3 g/m$^2$) therapy depending on the carrier of T allel of 677C>T MTHFR gene polymorphism: analysis of 617 chemotherapy cycles.

| Treatment toxicity | Percentage of chemotherapy cycles depending on the genotype, p-value | | |
|---|---|---|---|
| | CT (n = 304 cycles) | CC (n = 257 cycles) | TT (n = 56 cycles) |
| Hepatic toxicity | 29.3% | 33.5% | 30.4% |
| | p = 0.143 | p = 0.328 | |
| Nausea | 23.4% | 16.7% | 19.6% |
| | p = 0.025 | p = 0.301 | |
| Infections | 14.5% | 18.3% | 17.9% |
| | p = 0.112 | p = 0.472 | |
| Stomatitis | 11.2% | 7.8% | 7.1% |
| | p = 0.087 | p = 0.429 | |
| Diarrhoea | 5.6% | 5.4% | 1.8% |
| | p = 0.459 | p = 0.126 | |
| Neurotoxicity | 2.3% | 0.8% | 3.6% |
| | p = 0.124 | p = 0.05 | |
| Skin toxicities | 3.0% | 2.7% | 0.0% |
| | p = 0.416 | p = 0.107 | |
| Anemia (1-4 grade) | 3.9% | 5.4% | 7.1% |
| | p = 0.199 | p = 0.306 | |
| Leukopenia (1-4 grade) | 7.6% | 6.2% | 7.1% |
| | p = 0.258 | p = 0.401 | |
| Thrombocytopenia (1-4 grade) | 5.6% | 3.1% | 8.9% |
| | p = 0.077 | p = 0.025 | |
| Kidney toxicities | 0.7% | 0.4% | 1.8% |
| | p = 0.343 | p = 0.128 | |
| Therapy delay > 7 days | 4.9% | 2.7% | 8.9% |
| | p = 0.090 | p = 0.014 | |

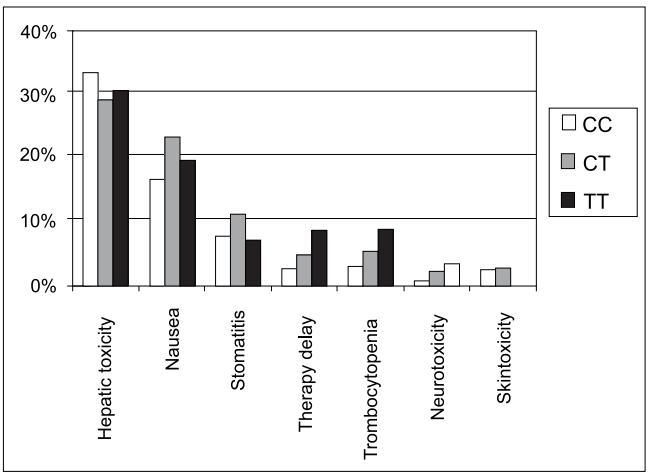


**Fig. 2.** Acute toxicity frequency in course of HD-Mtx (2 g/m$^2$ and 3 g/m$^2$) therapy depending on the 677C>T MTHFR gene polymorphism: analysis of 617 chemotherapy cycles.

Intensity of acute toxicities of HD-Mtx therapy for three patient 677C>T MTHFR polymorphism groups was also analyzed. Liver dysfunction was observed in about 30% of chemotherapy cycles irrespective of MTHFR genotype. In 92.5% of cycles only transient grade 1 toxicity was observed. It usually presented as a decrease of total protein and albumin serum concentration. Major hepatotoxicity (grade 3 and 4) were rare and occurred in TT homozygotes (1.8% chemotherapy cycles) more frequently than in CC homozygotes (0.4% of cycles). However differences were not statistically significant (p = 0.49). Mucositis and nausea (grade 2 and 3) were observed in allel T carriers (fig. 3) but no statistical differences were observed in comparison to CC homozygote group (p = 0.14 and 0.20 respectively). Neurotoxicity presenting as seizures and irritation with hyperesthesia (grade 2 and 3) were observed in 2 of 160 analyzed patients. These two patients were identified as CT heterozygotes. Other, less pronounced CNS toxicities (grade 1) were consistent with typical symptoms of arachnoid irritation and were significantly more common (p = 0.05) in children with 677TT genotype in comparison to patients with 677CC genotype. In case of infectious complications, presence of C allel was correlated to more frequent incidence of upper respiratory tract infection of a mild course (grade 1), T allel however was connected to more severe complications as pneumonia (grade 2) or generalized infections (grade 3) but no statistically significant differences were identified (p = 0.36). None of patients died of major toxicities resulting from HD-Mtx therapy. The most severe complication observed was acute kidney failure. It occurred in two girls (3.5 and 16 years old) during the first HD-Mtx chemotherapy cycle administered. In both cases coincidence of well-known risk factors (i.a. primary kidney dysfunction, administration of drugs interfering with methotrexate clearance, presence of transsudates, infections) was excluded. These two children were carriers of the CT and TT 677C>T MTHFR polymorphism.

To identify the factors influencing the intensity of acute therapy toxicities, a logistic regression model was created which included: methotrexate dose, age, BMI, presence of 677C>T MTHFR polymorphisms, hour 48 methotrexate serum concentration, late-phase

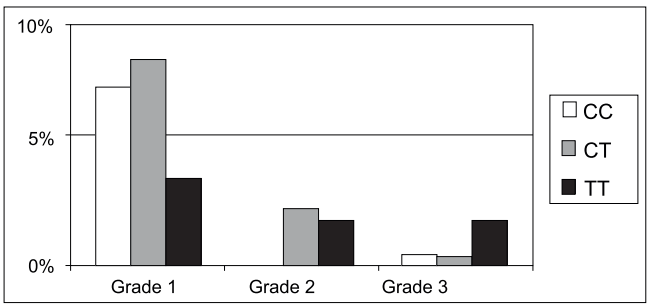


**Fig. 3.** Stomatitis intensity according to NCI-CTC scale depending on 677C>T MTHFR gene polymorphism: analysis of 617 chemotherapy cycles.

methotrexate elimination constant ($K_{el}$ 36.-48.). Logistic regression model revealed that hour 48. Serum methotrexate concentration is the only statistically significant parameter (p = 0.03) for the risk of occurrence of at least two different acute toxicities of HD-Mtx therapy (fig. 4). No influence of any of variants of 677C>T MTHFR gene polymorphism on disturbance of Mtx elimination was identified.

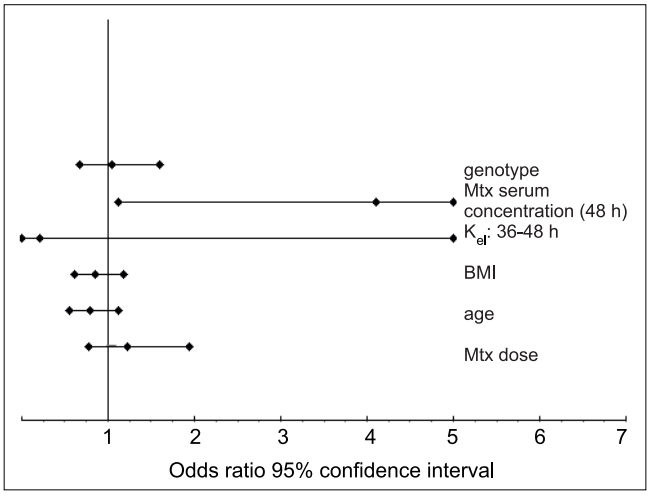


**Fig. 4.** Odds ratio and confidence interval for parameters influencing the occurrence of at least two different Mtx (2 g/m$^2$ and 3 g/m$^2$) chemotherapy toxicities.

## DISCUSSION

Methotrexate is one of fundamental antimetabolites which have been used in oncological therapy of hematopoietic system neoplasms and solid tumors since 1948. In ALL treatment it is used as a part of consolidation therapy – depending on the treatment protocol – in different high-doses as intravenous infusions of variable duration. It is also administered orally as a part of maintenance leukemia treatment. Polymorphism of genes encoding enzymes of the folate metabolism pathway which are essential i.a. for the DNA regeneration may be responsible for modification of the response to this drug or may influence the occurrence of acute treatment toxicities (2, 5, 8-11, 13, 15, 17, 19, 23, 24). One of well-known polymorphisms is the abundantly carried MTHFR C>T 677 polymorphism, which results in thermolability of the enzyme and it's substantially decreased activity (1, 10, 11, 15, 19, 20, 24). The key role of this enzyme in the folate metabolic pathway is the maintenance of equilibrium between nucleotide production and methylation reactions in the organism. Results published in numerous papers indicate that CT genotype and – especially TT genotype results in decreased MTHFR activity and it also results in increased serum and cerebrospinal fluid homocysteine concentration (7, 21). Hiperhomocysteinemia interferes with the endothelium structure and may induce serious toxic side effects in numerous organs and systems of the body. It was proved that hiperhomocysteinemia is related to grade 4 CNS toxicities i.e. thrombotic CNS events and seizures or coma occurrence (7). Available evidence for influence of 677C>T MTHFR gene polymorphism on methotrexate therapy tolerance is unequivocal. Some major experts in oncology (i.a. Pui) share the opinion that 677 TT homozygosity is connected to increased risk of mucosal inflammatory reactions of the gastrointestinal tract as well as to increased hepatotoxicity. It has been observed in low-dose methotrexate schedules however (9). Some pilot *in vitro* studies on ALL lymphoblast drug sensitivity in children had shown increased drug sensitivity in 677 TT homozygotes (18). Ulrich, as one of the first authors described the relationship between the presence of TT allele and increased mucosal toxicities and longer hematopoietic reconstitution (mainly due to prolonged thrombocytopenia) in a group of 220 patients treated for AML with use of methotrexate as a part of conditioning treatment before the allogeneic hematopoietic stem cell transplantation (19). In adult patients treated with methotrexate as a part of conditioning treatment before the allogeneic hematopoietic stem cell transplantation as a graft-versus-host reaction prophylaxis statistically significant correlation between the presence of 677 TT genotype and hepatotoxicity (p = 0.04) and slower platelet reconstitution (p = 0.01) was observed (25). It was shown that presence of T allel results in statistically significant increase in frequency of thrombocytopenia (p = 0.02) in a group of 181 children treated for ALL with HD-Mtx (17). El-Khodary has analyzed ALL therapy with use of 2 g/m$^2$ of methotrexate in 40 children and found that there had been statistically significant increase in myelotoxicity (grade 3 and 4) and hepatotoxicity (2 grade) in 677C>T MTHFR gene variant carriers (1). In a multicenter polish study, analyzing 7 different gene polymorphisms, 389 children were observed and a significant correlation between T allel 677C>T MTHFR presence and increased risk of death due to therapy toxicities (p = 0.03) was observed, moreover 1/3 of the relapses occurred in TT homozygotes. Most of deaths occurred in intermediate and high-risk group of patients, in T-ALL and in children with poor response to initial therapy, who were intensively treated. Among 20 of 30 (64.5%) deaths that occurred were deaths in remission and in most of the cases they resulted from infections and bleeding episodes in period of bone marrow aplasia. The authors had formulated a hypothesis that hiperhomocysteinemia resulting from decreased MTHFR activity may prone to heavily course of infections, vascular endothelium dysfunction and deaths resulting from multiorgan failure. They do also propose that chemotherapy intensity reduction should be considered in 677 TT homozygotes (21). In a study conducted by Aplenc et al. 520 children treated with low Mtx doses as a part of ALL maintenance treatment were observed. No influence of MTHFR 677C>T polymorphism on incidence of acute therapy toxicities (including infections) was identified (5). Presence of T allel was connected to statistically significant (p = 0.04)

increase of risk of relapse in a multiparameter Cox model (including age, initial WBC, initial response to therapy). Basing on this observation a hypothesis on influence of analyzed polymorphism on decrease of cytotoxic Mtx activity was proposed. Decrease MTHFR activity results in accumulation of 5,10-metylenetetrahydrofolates which are substrates for thymidilate synthase but it can also activate the parallel DNA production path and lead to increased DNA renewal. Similar observations were made by El-Khodary who analyzed 40 children treated due to ALL. Significantly lower EFS rates were observed in T allel carriers of 677C>T MTHFR gene polymorphism (1).

It should be mentioned that numerous papers showing relationship of T allel 677 C>T MTHFR gene polymorphism to increased acute Mtx therapy toxicities refer to prolonged use of low Mtx doses in patients treated due to ALL and rheumatoid diseases as well (16, 20). Toleration of low Mtx doses used typically in ALL maintenance treatment was assessed by Japanese investigators who found that there is a relationship between the presence of T allel and chemotherapy gaps due to its hematologic or hepatic toxicities (26). Also in one study conducted in adults significantly higher incidence of hepatic, intestinal and hematological toxicities of maintenance therapy was identified in 677 TT genotype carriers. Compromised liver function and leucopenia were almost five times more frequent in TT genotype carriers in comparison with CC genotype homozygotes (2). Another publication focusing on patients treated with low methotrexate doses due to ovarian cancer there was significantly higher incidence of hematological toxicities (grade 3 and 4) and mucositis described ($p = 0.0001$) in case of TT genotype in comparison with CT and CC variants (24).

However other papers have shown alternative opinions. Costea et al. in a study comprising 186 pediatric patients shown that in carriers of the T allel there was much less frequent incidence of major (grade 3 and 4) hematological and hepatic toxicities in course of low methotrexate dose treatment in consolidation and maintenance ALL therapy. Earlier study by Costea had indicated (as study of Aplenc) a relationship between the presence of T allel and lower EFS rates what made the author propose a controversial opinion on methotrexate dose escalation in TT homozygotes during maintenance therapy (10). De Jonge et al. in an *in vitro* study performed in 157 patients did not show an influence of the presence of T allel on methotrexate sensitivity of leukemic cells (6). Other papers have also not shown any relation between 677C>T MTHFR polymorphism with increased HD-Mtx therapy toxicities (7, 13, 23, 27-29). The lack of unequivocal opinions on the influence of 677C>T MTHFR gene polymorphism on acute Mtx therapy toxicities may be related to small number of study subjects in analyzed patient groups, differences between used therapy protocols, possible interactions with other drugs, diet, influence of ethnic and environmental factors on individual folate metabolism and other important gene polymorphisms with possible correlation that were not analyzed (1, 7, 23, 27, 29).

In the present study a series of 617 analyzed chemotherapy cycles more frequent or increased toxicities were observed for: nausea and vomiting, thrombocytopenia, therapy delay due to observed toxicities, or acute neurological toxicities in a T allel 677C>T MTHFR gene polymorphism carriers than in CC patients. Reports about the most serious HD-Mtx toxicity i.e. acute kidney failure are still incidental but information on the possible relation of this toxicity to the TT variant of 677C>T MTHFR polymorphism is available (30). Patient's sensitivity to HD-Mtx therapy is related not only to numerous well-known factors as patient's age, hydration, drug interactions etc. but is also related to genetic factors which are being intensively studied by numerous investigators. It can be assumed that polymorphisms of other genes involved in different compounds of folate pathway, not studied in present paper may influence the tolerance of HD-Mtx therapy or they may mutually correlate (4, 12, 13).

## CONCLUSIONS

Polymorphisms of genes involved in: transport of methotrexate to the cells, synthesis of more efficient polyglutamates and folate pathway can modify the antimetabolic effect of HD-Mtx therapy and result in increased therapy toxicity. To investigate the relevance of these genes more studies on large and homogeneous patient groups should be performed with special focus on polymorphisms of other genes involved in Mtx mechanisms of action i.a. transport proteins, family of multi-drug resistance proteins, and other enzymes of folate pathway. Pharmacogenomic studies are hoped to have additional impact on further progress in ALL treatment.

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