Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate

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Background: Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, a common mutation of the gene encoding the enzyme that catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a carbon donor in the metabolism of folate, determines a striking reduction in the enzyme activity in carriers of mutation at homozygous status.

Patients and methods: We retrospectively analyzed the incidence of MTHFR C677T and the influence of genotype on methotrexate (MTX) toxicity in patients with acute leukemia undergoing maintenance chemotherapy. Seventy-eight patients were analyzed and 61 were evaluable for toxicity. MTX toxicity was assessed on bone marrow, liver and mucosae.

Results: The incidence of the C677T mutation was as expected in the general Italian population with 23.08% of patients being TT, 38.46% of patients CT and 38.46% of patients CC. The TT genotype was significantly associated with an increase of toxicity during MTX administration. No specific pattern of toxicity was detected, although in TT patients myelosuppression and liver toxicity were more pronounced.

Conclusions: TT genotype may indicate a need to reduce the dose of MTX during prolonged administration. Considering the high prevalence of homozygous individuals in the Italian population, pretreatment screening may be worthwhile.

Key words: methotrexate, MTHFR, polymorphism

Introduction
Methylenetetrahydrofolate reductase (MTHFR) has a pivotal role in the metabolism of folate and methionine, both important factors in DNA methylation and synthesis in humans. C677T is a common polymorphism; the incidence of this mutation in homozygous or heterozygous state is 18–20% and 40% in the Italian population, respectively, which is higher compared with other Northern European Caucasian ethnicity. In fact in the Northern European Caucasian population, the incidence of this polymorphism in the homozygous state is 10% [1, 2]. As a result of this mutation, homozygotes have ~30% of the normal MTHFR enzyme activity, while heterozygotes have 60% of normal MTHFR activity, causing impaired remethylation of homocysteine to methionine and subsequent hyperhomocysteinemia. This polymorphism has been associated in the last few years with cancerogenesis, neural tube defect or cardiovascular disease secondary to mild hyperhomocysteinemia in combination with low folate status [3, 4]. Treatment with antimetabolites such as methotrexate (MTX) can increase homocysteine, causing additional toxicity. In fact, more recently this polymorphism has been associated with MTX toxicity in transplanted patients receiving a short course of MTX as graft-versus-host disease (GvHD) prophylaxis [5].

Patients and methods
We retrospectively analyzed the MTHFR genotype of 78 patients with acute leukemia receiving maintenance chemotherapy including MTX (Figure 1). These patients were included in different Gruppo Italiano Malattie Ematologiche dell’Adulto (GIMEMA) protocols for acute lymphoblastic leukemia (ALL) or acute promyelocytic leukemia (APL) over a period of 10 years at two institutions [6, 7]. All patients included in this study were of European Caucasian ethnicity. Briefly, MTX was
**ALL maintenance protocols:**

0288  
MTX 30mg/m² 1-8-15 day
6-MP 60mg/m² 1 to 21 day
VCR 1.5mg/m² 22-29 day
PDN 40mg/m² 22 to 36 day

0496  
6-MP 45mg/m² during cranial irradiation
6-MP 90mg/m²/day
MTX 15mg/week

Course A. VCR 1.4mg/m² day 1  
DNR 30mg/m² day 1  
PDN 60mg/m² for 7 days

Course B. VCR 1.4mg/m² day 1  
CTX 600mg/m² day 1  
PDN 60mg/m² for 7 days

Months: 1 2 3 4 5 6 9 12 18 24 30 36  
A A B A B A B A B A B A B

**APL maintenance protocol:**

AIDA 2000  
ATRA 45mg/m² for 15 days every 3 months  
MTX 15mg/m²/week  
6-MP 50mg/m²/day  
for two years

**Figure 1.** Maintenance protocols. ALL, acute lymphoblastic leukemia; APL, acute promyelocytic leukemia.

Data collection

Clinical records of patients on maintenance were reviewed by a blinded single abstractor and maintenance intolerance was defined as the occurrence of hematopoietic toxicity (polymorphonuclear leucocytes <0.5 x 10⁹/l), hepato-toxicity (>2-fold increase in serum bilirubin and liver transaminases) and mucositis (assessed by the oral mucositis index, OMI) [8] or other toxicities (nausea, vomiting, diarrhea or fever) that resulted in dose reduction or delays in subsequent chemotherapy. Assessment of MTX toxicity was carried out at the time when patients were receiving full doses of the chemotherapeutic agent and throughout the maintenance period. Toxicity was assessed weekly by hemogram and biochemistry before administration of MTX. Concurrent use of other medications was also assessed. The progressive delay from the sixth month onward, the interval between reinductions being progressively longer, and the abrogation of daunorubicin from the eighteenth month onward allowed us to discriminate the role of MTX in hemopoietic toxicity.

**MTHFR genotyping**

Genomic DNA was used to determine MTHFR genotype using a PCR-based method that detects a single point mutation at nucleotide 677 [9]. Genotypes were in Hardy–Weinberg equilibrium.

**Statistical analysis**

Statistical analysis was performed using pair-wise comparison among the genotype groups and two-tailed Fisher’s exact test comparing CC and CT patients with TT patients with respect to residual enzyme activity in the heterozygous. Statistical significance was defined as $P \leq 0.05$.

**Results**

Seventy-eight patients were evaluated for MTHFR genotype. Patients’ characteristics are showed in Table 1. The wild-type genotype (CC) was present in 30 patients (38.46%), heterozygous genotype (CT) in 30 patients (38.46%) and homozygous genotype (TT) in 18 patients (23.08%). The allelic frequency of the T allele was comparable to the frequency of this allele in the Italian population [1]. Seventeen patients were not evaluable for toxicity during maintenance, because of high-risk leukemia sufferers being shifted to high-dose chemotherapy and stem-cell transplantation, or early relapse after induction chemotherapy. Sixty-one patients were considered evaluable for toxicity. CC was present in 21 patients (34.4%), CT was present in 25 patients (41%) and TT was present in 15 patients (24.6%). The pair-wise comparison test
among the different genotypes was as follows: TT versus CT, \( P = 0.04 \); TT versus CC, \( P = 0.17 \); and CC versus CT, \( P = 0.52 \).

For the statistical analysis we cumulated data from CC and CT patients and compared them with TT patients with respect to the residual enzyme activity in the heterozygotes.

Toxicities are reported in Table 2. No major toxicities on mucosae were reported in the three different groups. Among these, MTX intolerance was encountered in seven of 21 CC patients (33.3%), in six out of 25 CT patients (24%) and in nine of 15 TT patients (60%) (\( P = 0.03 \), Fisher’s exact test). Twenty-three of 78 patients were also genotyped for thiopurine S-methyltransferase (TPMT) polymorphism (TPMT2, TPMT3B and TPMT3C): we found three patients to be heterozygous for TPMT3C, but none of these developed toxicity during maintenance therapy (preliminary data).

### Discussion

Maintenance chemotherapy represents the mainstay of treatment of ALL. Currently, there is no explanation for the difference in compliance to prolonged administration of MTX and 6-MP (6-mercaptopurine). Study of pharmacodynamic and pharmacokinetics has failed to demonstrate a relationship between the plasma pharmacokinetics of MTX and 6-MP and disease outcome. A great deal of interpatient variability has been observed, although gender appears to have some impact on MTX bioavailability [10].

Recently, in a group of patients affected by breast cancer with a mutated MTHFR genotype, Toffoli et al. [11] described the occurrence of severe hematological toxicity after cyclophosphamide, MTX and fluorouracil treatment. More recently, reduced tolerance to maintenance chemotherapy with MTX and 6-MP has been attributed to TPMT deficiency [12]. Genetic polymorphism of TPMT causes a reduction of the enzyme activity and thus leads to accumulation of thioguanine in erythrocytes, which is proportional to the toxicity and efficacy of these drugs.

When MTHFR C677T was considered, further evidence of correlation between MTHFR genotype and MTX intolerance were demonstrated. Patients reported here were submitted to prolonged administration of MTX as part of their therapeutic program for at least 2 years. The vast majority of TT patients experienced MTX intolerance more frequently, requiring dose modification and temporary MTX withdrawal leading to overall poor compliance with maintenance. Time to completion of maintenance chemotherapy was also prolonged, although it was not significant (28.5 versus 26.5 months) in TT patients. Heterozygotes did not show any difference in terms of toxicity compared with wild-type patients. Combined toxicity on liver and bone marrow function was encountered more frequently in patients with the TT genotype (Table 2). After MTX dosage adjustments, patients who developed toxicity were subsequently treated with full doses of another chemotherapy.

Interpatient variability in drug response in terms of efficacy and toxicity is potentially regulated by several processes including drug transport and metabolism.

Currently, an empirical treatment approach is taken in most diseases, although at least nine enzymes metabolizing anticancer agents exhibit genetic polymorphisms [13]. It is probable that in the near future a panel of disease-specific genotypes will be utilized to identify subsets of patients who are genetically predisposed to develop toxicity from specific drugs. Recently, MTHFR polymorphism has been associated with higher risk of toxicity after very low doses of MTX given for GvHD prevention in patients submitted to allogeneic stem-cell transplantation [5]. The attribution of MTX toxicity to MTHFR genotype could be difficult in this setting [14], where toxicity is the result of conditioning regimen, GvHD occurrence and infections. In our study, maintenance chemotherapy that includes administration of small and continuous doses of chemotherapy allowed us to ascertain the role of MTHFR genotype in the development of toxicity during MTX administration. We conclude that the high prevalence of TT subjects in the Italian population suggests a benefit of genetic screening for this mutation in patients with acute leukemia undergoing prolonged administration of MTX.

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### References


