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LETTER TO THE EDITOR

The impact of methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism on transplant-related variables after allogeneic hematopoietic cell transplantation in patients receiving MTX as GVHD prophylaxis

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Genomic screening before allogeneic hematopoietic cell transplantation has emerged as an investigational tool used to predict events that elicit subtle effects following transplantation. Single-nucleotide polymorphisms are by far the most prevalent type of variants in the human genome, with an average occurrence every several 100 bases. Most single-nucleotide polymorphisms have no known effect on gene function, but a proportion of them may alter the expression and/or biological activity of encoded proteins, contributing to variation in disease susceptibility and treatment toxicity.[1]

MTX is an antifolate chemotherapeutic drug used to prevent GVHD in allogeneic hematopoietic cell transplantation. MTX functions by inhibiting dihydrofolate reductase and limiting the function of other enzymes, such as methylenetetrahydrofolate reductase (*MTHFR*), that play roles in folate metabolism. As it has been shown that *MTHFR C677T* polymorphism (*CT*/*TT*) results in reduced activity of the enzyme (30% in *TT* and 60% in *CT* genotype),[2,3] use of MTX in patients with *CT*/*TT* variations may influence side effects of MTX such as mucositis and medullary toxicity acquired from low folate levels. Some studies show that the presence of the *C677T* (*TT*) genotype leads to variations in the severity of medullary and extra medullary toxicities of MTX whereas others show no effect.[4–6] We assessed our data to determine if the *MTHFR C677T* genotype has any effect on transplant-related toxicity and engraftment kinetics in patients undergoing allogeneic hematopoietic cell transplantation who received MTX for GVHD prophylaxis.

We analyzed data collected from 82 patients who received transplants from their HLA-identical siblings in our Transplantation Unit between November 1999 and July 2004. The median age was 31 (range, 14–50) years and F/M was 32/50. The majority of the patients had standard risk acute or chronic leukemia. The conditioning regimen consisted of BU–CY ($n=70$), CY–TBI ($n=6$) and CY–antithymocyte globulin ($n=2$). All patients received CsA 6 mg/kg/day and short-term MTX 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6 for GVHD prophylaxis. Stem cell source was BM in 23 and PB in 59 of the patients. Recipients of BM were given G-CSF starting on day +1 until neutrophil engraftment. Patients did not receive folinic acid rescue, and no MTX dose modification was necessary for any patient because of underlying or developing renal or hepatic dysfunction. The first three consecutive days showed neutrophil count that was over 0.5×10^9/l (NEU500) and 1×10^9/l (NEU1000), which was considered as neutrophil engraftment, and sustained platelet count over 20×10^9/l (PLT20) and 50×10^9/l (PLT50) for 7 days without platelet transfusion was considered as platelet engraftment.

Thirty-two (39%) out of 82 patients were found to have *CT*/*TT* variation; two homozygotes (*TT* genotype) and 30 heterozygotes (*CT* genotype). The mononuclear cell and CD34$^+$ cell counts were similar between *MTHFR* variation (*CT*/*TT*)-positive and -negative patients in BM (median 0.48 vs 0.62×10^8 per kg and 1.96 vs 1.55×10^6/kg, respectively) and PB (5.37 vs 4.69×10^8/kg and 5.65 vs 5.51×10^6/kg, respectively) groups.

In the whole cohort of transplanted patients, there was no statistically significant difference for neutrophil engraftment, with a slight tendency for delayed engraftment of neutrophil 1×10^9/l in the *MTHFR CC*/*CT* genotype group (median 17 vs 15 days and 19.5 vs 17 days for neutrophil 0.5×10^9 and 1×10^9/l, respectively). Platelet engraftment was similar in both groups (median 13 vs 14 days and 16 vs 18 days for platelet 20×10^9 and 50×10^9/l, respectively). When comparing the engraftment kinetics of *CT*/*TT* patients with negative ones according to stem cell source separately, there was a remarkable delay in neutrophil engraftment and a difference at time to PLT20 in BM recipients with the exception of time to PLT50. We observed no significant difference in neutrophil and platelet recovery in the PB recipients (Table 1).

When evaluating the impact of *CT*/*TT* variation on regimen-related toxicities—especially for mucositis—we were not able to find any significant difference in the incidence, grade and duration both in the whole cohort and also according to stem cell source. Number of patients requiring total parenteral nutrition was similar in *CT*/*TT* variation-positive and -negative groups (79 vs 69%, $P=0.3$), and none of the remaining patients received multivitamins during the transplant procedure. We did not observe any difference for other extramedullary toxicities, which may be related to MTX (for example, nausea, vomiting and diarrhea).

MTHFR C677T (*CT*/*TT*) polymorphism had no effect on other early transplant-related complications. The incidences of acute GVHD (grades 2–4) were similar in all patients (59 vs 46%, *CT*/*TT* and *CC* groups respectively; $P=0.13$) and in BM (50 vs 50%, *CT*/*TT* and *CC* groups, respectively) and PB (66 vs 48%, *CT*/*TT* and *CC* groups, respectively; $P=0.19$) groups according to *CT*/*TT* variation.

Table 1 Engraftment kinetics according to stem cell source and *MTHFR C677T* variation

	BM (n = *23*)			*PB* (n = *59*)		
MTHFR C677T variation	*CT/TT* (*n* = 7)		*CC* (*n* = 16)	*CT/TT* (*n* = 25)		*CC* (*n* = 34)
NEU500 (range)	21 (16–25)	*P* = 0.05	15 (14–20)	16 (12–20)	*P* = 0.8	15 (11–22)
NEU1000 (range) days	22.5 (21–37)	*P* = 0.0001	17 (15–21)	19 (12–24)	*P* = 0.3	17 (11–23)
PLT20 (range) days	27 (21–36)	*P* = 0.03	21 (11–30)	13 (9–19)	*P* = 0.5	13 (9–23)
PLT50 (range) days	28 (27–38)	*P* = 0.3	27 (16–45)	16 (12–29)	*P* = 0.8	17 (11–25)

Abbreviation: MTHFR = methylenetetrahydrofolate reductase.
CT/TT: variation positive (heterozygous and homozygous); CC: no variation.

Our data revealed that the *C677T* variation of *MTHFR* has some effect on prolongation of engraftment, especially in BM recipients who receive MTX for GVHD prophylaxis, but we could not show any increase in extramedullary toxicity or GVHD incidence in our patient cohort.

The major limitation of this study was the small number of patients in our cohort. In addition, it was also difficult to determine engraftment kinetics within our study due to the low cumulative MTX dose administered to these patients.

Epigenetic (pharmacogenetic) variables related to both the donor and the recipient are important determinants of tailored transplant approaches and are especially relevant when analyzing the genetics associated with the underlying disease. Taking into account the importance of those variables, we designed the above single-center analysis. We were able to show a difference in allogeneic BM recipients with *MTHFR C677T* gene polymorphism in terms of neutrophil engraftment. Multigenic analysis with established transplant-related variables is necessary to further support our conclusions.

E Soydan, P Topcuoglu, K Dalva and M Arat
Stem Cell Transplantation Unit, Hematology Department, Ankara University School of Medicine, Cebeci, Ankara, Turkey
E-mail: arat@medicine.ankara.edu.tr

References

1 Kallianpur AR. Genomic screening and complications of hematopoietic stem cell transplantation: has the time come? *Bone Marrow Transplant* 2005; **35**: 1–16.
2 Ulrich CM, Yasui Y, Storb R, Schubert MM, Wagner JL, Bigler J *et al*. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. *Blood* 2001; **98**: 231–234.
3 Ulrich CM, Robien K, McLoad HL. Cancer pharmacogenetics: polymorphisms, pathways and beyond. *Nat Rev Cancer* 2003; **3**: 912–920.
4 Chiusolo P, Reddiconto G, Casorelli I, Laurenti L, Sora F, Mele L *et al*. Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. *Ann Oncol* 2002; **13**: 1915–1918.
5 Robien K, Schubert MM, Bruemmer B, Lloid ME, Potter JD, Ulrich CM. Predictors of oral mucositis in patients receiving hematopoietic cell transplants for chronic myelogenous leukemia. *J Clin Oncol* 2004; **22**: 1269–1275.
6 Besisik-Kalayoglu S, Caliskan Y, Sargin D. Methylenetetrahydrofolate reductase C677T polymorphism and toxicity in allogeneic hematopoietic cell transplantation. *Transplantation* 2003; **76**: 1775–1777.