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Case Report

NUDT15 Genotype Guided 6-Mercaptopurine Dosing in a Filipino Child with Acute Lymphoblastic Leukemia Who Suffered from Life Threatening Infections Due to Severe Myelosuppression: A Case Report

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Abstract:

Rationale and Objectives: Patients harboring the *NUDT15* polymorphism tend to be more susceptible to 6-mercaptopurine (6-MP) toxicity. Interruption of treatment due to this is frequently associated with increased risk of relapse. In patients with severe myelosuppression during 6-MP treatment, it is strongly recommended that *NUDT15* testing should be performed. Unfortunately, in developing countries like the Philippines, different strategies have been employed to address this challenge with most attempts resulting to varied outcome. A case of a child with acute lymphoblastic leukemia (ALL) with *NUDT15* polymorphism is presented. The importance of appropriate 6-MP dose adjustment using *NUDT15* genotype guided dosing in this patient is emphasized.

Case Report: A 3-year-old Filipino-Chinese girl with standard risk ALL presented with prolonged myelosuppression during consolidation chemotherapy. This was associated with life-threatening episodes of bacterial and fungal infection. To determine role of 6-MP toxicity, *TPMT* and *NUDT15* genetic studies were performed. Results demonstrated normal *TPMT* variant. *NUDT15* testing revealed that the patient carried two mutations, a heterozygous polymorphism for *NUDT15* ins36_37 and homozygous polymorphism for *NUDT15* C415T.

Discussion and Summary: *NUDT15* gene encodes an enzyme that belongs to the Nudix hydrolase superfamily. Like *TPMT*, it is a negative regulator of thiopurine activation. However, studies have identified that there is higher prevalence of *NUDT15* gene mutations among Asian ancestry. Mutations result in poor metabolism of thiopurines and severe myelosuppression. These patients with two polymorphisms may require only 5mg/m^2/day dosing or 10 percent of the recommended daily dose. *NUDT15* genetic testing is strongly recommended for ALL patients who develop 6-MP toxicity, in order to determine the appropriate dose that will provide optimum disease control while preventing treatment interruptions that may negatively affect outcome.

Keywords: NUDT Polymorphism; 6-mercaptopurine toxicity; severe myelosuppression; Filipino child

Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, adolescents and young adults younger than 20 years, accounting for 18.8 percent of all cancer cases in this age group (1). According to the

Leukemia & Lymphoma Society, the 5-year survival rate of ALL is 91.9 percent for children and adolescents younger than 15 years, and 94.1 percent for children younger than 5 years (2). The disease-free survival has greatly increased with the improved treatment regimens. Despite this, treatment interruptions due to myelotoxicity is a common adverse event and results in a higher relapse rate, making ALL the second leading cause of cancer death among children (3).

6-Mercaptopurine (6-MP) is an important anticancer agent and remains a backbone of most ALL regimens (4). It is commonly used as a part of the consolidation, intensification and maintenance phases of the treatment. However, it is not uncommon to encounter severe myelosuppression and agranulocytosis while on 6-MP therapy (5). This is partly due to the large interindividual and intraindividual variations in 6-MP bioavailability and cellular pharmacokinetics affecting patient dosing. Patients receiving identical doses per body surface area may experience varied systemic and intracellular drug toxicity (1). Unfortunately, in developing countries like the Philippines, as a result different strategies have been employed to address this challenge with most attempts resulting to varied outcome. For instance, physicians are more accustomed to decrease 6-MP drug doses in case of toxicity or escalated doses in patients insufficiently myelosuppressed. Thus, the average 6-MP dose prescribed vary widely. To date, the utilization of genetic polymorphisms and pharmacogenomics that determine 6-MP efficacy and toxicity have been employed to improve ALL treatment (6).

Recently, polymorphisms involving the *NUDT15* gene have been identified as a major genetic cause for 6-MP related bone marrow suppression (7). This gene encodes an enzyme that belongs to the Nudix hydrolase superfamily which is a negative regulator of thiopurine activation and toxicity. There is now an increasing interest in the clinical implementation of *NUDT15* genotype-guided 6-MP dose individualization (8).

More importantly, studies have identified that there is high prevalence of *NUDT15* polymorphisms in Asian and Hispanic populations (9). Filipino children with ALL, who experience extreme sensitivity to 6-MP, might carry one or more of the *NUDT15* variants.

This case report is the first to present the case of a child of Filipino-Chinese ethnicity who experienced life threatening 6-MP induced severe myelosuppression during chemotherapy due to heterozygous polymorphism for *NUDT15* ins36_37 and homozygous polymorphism for *NUDT15* C415T.

Case Report

A 3-year-old girl, initially presenting with bone pain, was eventually hospitalized and admitted under the Department of Pediatrics Section of Hematology and Oncology. The patient had a normal medical history with no known drug allergies, no travel to epidemic areas and no know exposure to toxins. There was no family history of malignancy. Initial physical examination was unremarkable except for multiple bruises and petechiae on upper and lower extremities. There was no lymphadenopathy nor hepatosplenomegaly. There were no associated bone or joint abnormalities. Initial blood count showed a hemoglobin count of 91g/L (normal range, 110-150g/L), white blood cell (WBC) count of 5730 (normal range, 4800-10000mm^3), and platelet count of 21,000 (normal range, 150000-400000/mm^3). Bone marrow aspiration revealed bone marrow showed 87.39 percent lymphoblasts with immunophenotypic findings consistent with CD10+ ALL. Cerebrospinal fluid examination was negative for leukemic involvement. Karyotyping and fluorescence in situ hybridization (FISH) did not identify any mutations associated with high-risk disease. These findings were consistent with the diagnosis of standard risk acute precursor B-cell lymphoblastic leukemia.

The patient received induction therapy and achieved morphological remission four weeks post therapy. However, during the consolidation phase with cyclophosphamide (1g/m^2 day 1 and 15), cytarabine (75mg/m^2 days 2-5, 9-12, 16-19 and 23-26) and 6-MP (60mg/m^2 for 28 days), the patient was admitted due to severe sepsis due to myelosuppression. Culture studies were positive for *Eschirichia coli* and *Klebsiella pneumoniae,* in urine and blood, respectively. Subsequently, the patient developed *Candida* sp. central line associated infection and infectious endocarditis which resulted in central line removal and prolonged antifungal therapy. Moreover, consolidation phase could not be completed according to the scheduled outline in the protocol due to febrile neutropenia. Due to the severe myelosuppression during this phase of chemotherapy, 6-MP toxicity was considered. *TPMT* and *NUDT15* genetic analysis were performed. Although a normal *TPMT* genetic variant was identified, the patient was identified as carrying heterozygous polymorphism for *NUDT15* ins36_37 and homozygous polymorphism for *NUDT15* C415T. During the succeeding consolidation treatment, the dose of 6-MP was adjusted to 10 percent of the total recommended dose. Twenty-four months after the patient's initial presentation, the patient is having regular follow ups and is continuing maintenance therapy with 5mg/m^2/day or 10 percent of the recommended daily dose of 6-MP.

Discussion

6-mercaptopurine (6-MP) is an effective immunosuppressant and anti-cancer drug. Its benefits of several years of myelosuppressive maintenance therapy for ALL are well proven. It is a key component in the treatment of ALL with clear evidence showing that interrupted therapy results in an unfavorable outcome (4). Currently, there is no international consensus on drug dosing, and protocols reflect tradition rather than empirical evidence. In most, the starting dose of oral 6MP is 50 mg/m2/day then individually adjusted (10). Thus, the average 6-MP dose prescribed during maintenance therapy vary widely. This is mainly due to excessive myelosuppressive property of 6-MP. Interruption of scheduled treatment due to this is frequently associated with increased risk of relapse. Pharmacogenetic studies have indicated that cases of leukopenia can be largely explained by single nucleotide polymorphisms (SNPs) among these two specific genes: *TPMT* and *NUDT15* (11).

The first discovery of a clinically relevant genetic variant resulting to susceptibility to 6-MP toxicity identified mutations involving

the *TPMT* gene. 6-MP is a substrate of S-methylation by thiopurine methyltransferase (*TPMT*) and the activity of this enzyme. Patients who carry specific single nucleotide polymorphisms in the *TPMT* gene have increased sensitivity to both the cytotoxic and myelotoxic effects (12). However, studies have previously identified the high frequency of this polymorphism in both populations of European and African American descents but considerably extremely rare in the East Asian population, *so TPMT* polymorphisms alone cannot explain the observed in tolerance among Asians (13). Coulthard et al cited that the prevalence of variant *TPMT* genotypes to be as high as 80 percent in certain European regions (14). In comparison with the local study done by Lagaya-Arañas et al where among sixty-two children with ALL, only two (3.2%) had heterozygous variant phenotype (15). Recently, there are several genetic traits which are associated with 6-MP intolerance had been identified in East Asian cohorts.

Compared to *TPMT* polymorphisms, *NUDT15* genetic variants are more common in Hispanic and East Asians descents (15). This gene encodes an enzyme that belongs to the Nudix hydrolase superfamily. Like TPMT, the encoded enzyme is a negative regulator of thiopurine activation and toxicity. Members of this superfamily catalyze the hydrolysis of nucleoside diphosphates, including substrates like 8-oxo-dGTP, which are a result of oxidative damage, and can induce base mispairing during DNA replication, causing transversions. The gene is located in the long arm of chromosome 13 at position 14.2 and mutations in this gene result in poor metabolism of thiopurines, and are associated with 6-MP leukopenia. Previous studies have identified that 40 percent of Asian patients using thiopurine treatment discontinued therapy owing to life-threatening toxicity (17). This percentage can be attributed possibly due to the presence genetic variants of the *NUDT15* polymorphism.

It was first reported by Yang et al in 2014 the *NUDT15* R139C variant conferring susceptibility to thiopurine-induced leukopenia (18). Since then, there have been numerous publications, mostly in patients of Asian origin, that further supported Yang et al's findings. Furthermore, this variant is most common in East Asians, rare in Europeans, and not observed in Africans, thereby contributing to ancestry-associated differences. Studies reported that *NUDT15* may be a better predictor for thiopurine-reduced leukopenia than *TPMT* polymorphism in the Asian population (19). A genome wide association study (GWAS) in pediatric ALL population in 2015 revealed a significant association between a missense variant in the *NUDT15* gene and 6-MP dose intensity (16). Thus, *NUDT15* genotyping should currently be prioritized for the prediction of leukopenia among the Asian population, and its application in precision medicine should be considered in the future.

In a study conducted among 404 Taiwanese patients with ALL, five patients were homozygous for *NUDT15* variant and the maximal tolerable dose of 6-MP of 9.4±3.7mg/m^2/day was administered (20). In a similar study conducted among Korean pediatric patients with ALL, five out of 182 were homozygous for the *NUDT15* variant and received 6-MP dose of 7.5mg/m^2/day (21). These studies suggest that incorporating pharmacogenetics testing of *NUDT15* for personalized 6-MP dosing is highly recommended especially dose with East Asian descent.

Table 1 shows the guideline released by the Clinical Pharmacogenetics Implementation Consortium (CPIC) last 2018 for the *NUDT15* genotype guided 6-MP dosing (22). From the tests performed, this patient has two variants affecting both of her *NUDT15* gene. Her polymorphisms involving 2 alleles resulting to two no function alleles. This makes her very sensitive to 6-MP and classified as a poor metabolizer. Patients with two no function alleles require much reduced tolerated dose as low as 10mg/m^2/day dosing. The patient received much lower dose of 6-MP at 5mg/m^2/day. This was continued until maintenance chemotherapy with no observed severe myelotoxicity.

Table 1: *NUDT15* genotype guided 6-MP dosing based on CPIC 2018 Guidelines.

Genotype	Phenotype	6-MP Dosing Recommendation
An individual carrying normal *NUDT15* function alleles - normal activity of NUDT15	Normal metabolizer - normal risk of 6-MP related leukopenia, neutropenia, myelosuppression	Start with normal starting dose (75mg/m2/day) Adjust dose of 6-MP without any special emphasis on 6-MP compared to other agents.
An individual carrying one *NUDT15* no function allele - intermediate activity of NUDT15	Intermediate metabolizer - increased risk of 6-MP related leukopenia, neutropenia, myelosuppression	Start with reduced starting doses at 30% to 80% of normal dose.
An individual carrying two *NUDT15* no function alleles - low or absent activity of NUDT15	Poor metabolizer - greatly increased risk of 6-MP related leukopenia, neutropenia, myelosuppression	Initiate dose at 10mg/m2/day Adjust dose further based on myelosuppression. Allow 4 to 6 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing 6-MP over other agents.

Poor metabolizers, like our patient, is rare with frequencies of 0.91 percent and 0.46 percent among East Asians and South Asians, respectively (23). Though studies have been conducted on other Asian countries, there is still lack of data regarding *NUDT15*

polymorphisms in the Filipino population. Factors, especially our multiracial ethnicity, may contribute to ancestry associated differences. However, upcoming studies may shed light on the polymorphisms in Filipino patients.

Furthermore, the importance of uninterrupted treatment with 6-MP is continually emphasized in this report. Maintenance phase of therapy is deemed be important for most ALL subsets. Observational studies support that 6-MP/MTX maintenance therapy is superior to other drug combinations, and that poor patient adherence significantly increase the risk of relapse (24). Studies identified that if chemotherapy is truncated, the 5-year event free survival may be as low as 60 percent, even for non–high-risk ALL patients (25). In addition, studies show that recurrent unwarranted treatment interruptions as an adverse factor for increased risk of second malignancies (4).

Summary

This is the first report of a child with ALL of Filipino-Chinese ancestry presenting with life threatening 6-MP myelotoxicity and identified to carry heterozygous polymorphism for *NUDT15* ins36_37 and homozygous polymorphism for *NUDT15* C415T. This report emphasizes the impact of *NUDT15* polymorphisms on the management of ALL, maintaining the balance between the need for leukemia control and avoidance of excessive toxicity. This case emphasizes the need to be recognize potential 6-MP associated toxicity, the need to pursue pharmacogenetic analysis to identify polymorphisms that may affect 6-MP administration and the need to avoid unnecessary interruptions that may negatively affect overall survival. This report strongly recommends to perform pharmacogenetic analysis involving the *NUDT15* gene among individuals who present with susceptibility to 6-MP toxicity to avoid live threatening myelosuppression. Its application should be considered in the future especially due to our racial vulnerability.

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