



Severe Neutropenia Related with Azathioprine Treatment in Autoimmune Hepatitis: The Impact of Homozygous Inactive Allele Mutation of Thiopurine S-Methyltransferase (TPMT) Gene

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Abstract

Introduction: Azathioprine is considered part of the standard-of-care treatment strategy in autoimmune hepatitis. Despite the fact that several practice guidelines recommend Thiopurine Methyltransferase (TPMT) genotyping, phenotyping and/or measurement of metabolites activity, hepatology guidelines do not consider TPMT testing mandatory, due to scarce available information referring to its controversial utility. We report the case of a 56 year-old woman with recent autoimmune hepatitis diagnosis, who received two months of combined immunosuppressive treatment (decreasing doses of prednisone and azathioprine 50 mg during one month, with later increase to 100 mg per day). She was admitted in the Emergency Room due to severe febrile neutropenia. Azathioprine treatment was discontinued and TPMT genotyping was performed. A genetic homozygous variant (rs1800462) corresponding to homozygous TPMT*2 polymorphism was detected. The patient's treatment was modified to prednisone combined with mycophenolate with normalization of liver enzymes and resolution of the adverse event.

Discussion: TPMT is a polymorphic enzyme involved in the metabolism and inactivation of thiopurine substances (such as azathioprine) administered as immune suppressants in the treatment of malignancies and autoimmune diseases. Mutations in the gene that encodes this enzyme may augment the risk of adverse events. Three genetic variants in the TPMT gene (rs12201199, rs1142345, and rs1800460) account for more than 90% of inactivating alleles. There are four nonfunctional alleles responsible of 80% to 95% of low enzyme activity: *2, *3A,*3B, *3C. Performing genetic testing to determine the TPMT polymorphism prior to treatment initiation with azathioprine has proven to be a valuable tool to prevent severe adverse events.

Conclusion: Routine screening of TPMT polymorphisms in autoimmune hepatitis may prevent severe adverse events in patient with null enzyme activity.

Introduction

Treatment with thiopurine analogs is the cornerstone of several immunosuppressive regimens for malignancies and autoimmune diseases. Azathioprine is a purine analog that interferes with nucleic acid metabolism; it is widely used as part of the standard-of-care in several illnesses such as inflammatory bowel disease, acute lymphatic leukemia and autoimmune hepatitis [1]. The occurrence of serious adverse events related to its use is not infrequent: 10% of the patients manifest with bone marrow toxicity, pancreatitis, hepatotoxicity or a combination of these events [2,3]. Thus, it is important to assess which patients are at higher risk of suffering severe and potentially life-threatening adverse events, since proper dose adjustment may prevent its occurrence/diminish its severity [4,5].

Several clinical pharmacogenetics consensus and clinical practice guidelines endorse the determination of Thiopurine Methyltransferase (TPMT) polymorphisms prior to initiation of azathioprine treatment [5-7]. Different types of polymorphisms affect this enzyme performance, varying from having negligible to intermediate or even high levels of enzyme activity [8]. Thus,

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the presence of different types of TPMT polymorphisms has been linked to the occurrence of severe side effects, or to the lack of proper immunosuppressive activity in patients receiving standard azathioprine doses [4].

Despite the fact that TPMT testing is currently a standard practice for internal medicine and gastroenterology guidelines [7], it has not been proposed as a mandatory test in the treatment of autoimmune hepatitis, due to scarce data regarding its clinical utility [9]; thus it is seldomly solicited prior to treatment initiation in daily practice. We will describe the case of a patient with autoimmune hepatitis treated with a combination of steroids and azathioprine at standard doses that develops severe febrile neutropenia associated with a homozygous TPMT polymorphism with null activity.

Case Presentation

A previously healthy fifty-six year-old woman attended to the HPR Liver Unit with jaundice and elevated liver enzymes (up to forty-times the baseline levels) with preserved liver function. In etiological studies performed, hypergammaglobulinemia and high titles of both antinuclear and anti smooth muscle antibodies were detected. Viral hepatitis and other metabolic illnesses were discarded. The diagnosis of autoimmune hepatitis was based on clinical and biochemical findings, and treatment with prednisone 40 mg was initiated with excellent response. Azathioprine was added after two weeks of steroid treatment, initially at a 50 mg dose, and increased after one month to 100 mg. After two months of immunosuppressive therapy, the patient attended the hospital Emergency Room due to acute diarrhea non respondent to antibiotic therapy and paronychia in her left hand middle finger. At admission she was conscious, subfebrile (37.5°C) and hyperdynamic (heart rate 100 beats/minute). When examined, oral leukoplakia and small oral ulcers were observed, as well as a small abscess in her left hand middle finger and faint coarse crackles in the base of her right lung without pathological findings on her chest X-ray. In laboratory analysis, bicytopenia (white cell count 589 cells/mm³ and platelet count 29,000 cells/mm³) with severe neutropenia (65 cells/mm³), acute kidney injury (creatinine 1.40 mg/dl with metabolic acidosis) and elevated protein C reactive levels (344.9 mg/L) were observed. She was treated as febrile neutropenia with broad-spectrum antibiotics, antifungal and antiviral therapy, as well as with granulocyte colony stimulating factor. Since azathioprine was suspected as a cause of bicytopenia, it was suspended at admission. The patient's general condition as well as her platelet and white blood cell count improved during the course of the following week. She was discharged with a normal complete blood cell count after 8 days of hospitalization. To confirm the causality of azathioprine in the development of febrile neutropenia in this patient, the thiopurine methyltransferase genotype and activity was analyzed. The sample was processed following the protocol for extraction and purification of the genomic DNA that could be present in it by magnetic particle technology (Magna Pure Compact-Roche). The amplification of the regions of the TPMT gene is carried out by the Polymerase Chain Reaction (QIAGEN). The amplification products were subjected to restriction fragment length polymorphism (RFLP) analysis using restriction enzymes. (New England BioLabs Inc.). An homozygous variant p.Ala80Pro (rs1800462) corresponding to the genotype TPMT*2 was detected, which associates with low or nule enzymatic activity. Thus, azathioprine was not re-initiated and currently the patient is under treatment with low dose prednisone mycophenolate mofetil with normal liver enzymes.

Discussion

Thiopurine S-Methyltransferase (TPMT) is an S-Adenosylmethionine (SAM)-dependent cytosolic enzyme implicated in the metabolism of thiopurines such as azathioprine. This prodrug requires metabolic activation to generate its active metabolites thioguanine nucleotides (6-TGNs), which ultimately cause its cytotoxic effects. The majority of these antimetabolites are detoxified by TPMT and to a minor extent by xanthine oxidase. Among enzyme-deficient patients, the alternative pathway *via* hypoxanthine phosphoribosyltransferase is accelerated, leading to higher amounts of 6-TGN which in turn may cause bone marrow toxicity and even pancytopenia [1,10].

TPMT has more than 40 naturally occurring variants, most of which exhibit low or intermediate enzyme activity towards thiopurine substrates. TPMT has a trimodal distribution with 89.5% normal to high methylators, 9.9% intermediates and 0.6% deficient methylators. The TPMT activity can be determined by phenotyping procedures; just three genetic variants in the TPMT gene (rs12201199, rs1142345, and rs1800460) account for more than 90% of inactivating alleles. The majority of patients have two normal alleles, 3% to 14% of patients are heterozygous and 0.3% carries two non-functional alleles. There are four nonfunctional alleles responsible of 80% to 95% of low enzyme activity: *2, *3A, *3B, *3C. They are distributed with ethnic differences: *3A prevails in caucasians where as *3C predominates in Africans, Asians, and afro Americans [11,12].

In Argentina, a mixed ethnicity country, there is heterogeneous information regarding the most prevalent allelic mutation. Some studies have found higher prevalence of *3A, followed by *2 and *4, whereas other recent study refer to *2 allele as the most prevalent inactive phenotype (as described in the present case) [11,13].

Currently, the determination of TPMT genotype by protein chain reaction obtained from leukocytes's DNA is relatively inexpensive, reproducible and does not have methodological limitations. Since its correlation with the enzyme's phenotype has proven to be adequate, especially for low active alleles, it is the initial study of choice to determine TPMT activity in clinical practice [2,7,14]. The detection of the mutant allele would allow classifying patients in three groups: normal homozygotes with high enzymatic activity, heterozygous with intermediate activity and homozygotes with the low allele activity. This classification has implications in azathioprine dosing, since patients with high enzymatic activity could receive azathioprine at high doses with less apparent risk of toxicity, whereas in patients with intermediate and low activity alleles it would be advisable to perform the measurement of TPMT in erythrocytes in order to correlate genotype and phenotype. If confirmed, heterozygous patients should receive doses adjusted to the value of enzymes and their body weight, whereas patients with low activity would be candidates to alternative drugs with a different metabolic route [3,12,15].

TPMT polymorphisms are not the sole responsible of adverse events due to azathioprine: there are studies reporting the presence of gastrointestinal and bone marrow toxicity despite the presence of normal or near normal TPMT enzymatic activity [8,9]. Due to this evidence, in cost-effectiveness analysis, the benefit of TPMT testing is still controversial [1,2].

In the few existing publications discussing TPMT testing in autoimmune hepatitis patients, TPMT or metabolite dosing were controversial regarding its efficacy in predicting azathioprine

toxicity [8,16,17]. Currently, experts and hepatology clinical practice recommendations state that close monitoring of azathioprine toxicity should be performed in all patients, and TPMT testing is only recommended if available (although not mandatory) [9,18].

Due to the potential life threatening severity of myelosuppression secondary to azathioprine treatment, as portrayed in the presented case, the benefit of TPMT testing appears to be clear in all autoimmune hepatitis patients. Further studies, specially including patients of different ethnicities, may provide valuable information to reassess the benefit of TPMT testing prior to azathioprine treatment in patients with autoimmune hepatitis.

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