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Review Article

Therapeutic Drug Monitoring of Thiopurine Therapy in Patients with Inflammatory Bowel Disease

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1. Abstract

The number of patients with inflammatory bowel disease (IBD) is increasing in the worldwide. Thiopurine S-methyltransferase (TPMT) plays a significant role in the metabolism of thiopurine drugs. Low TPMT activity in body is associated with pathological thiopurine drug metabolisms, overproduction of cytotoxic metabolites and myelosuppression.

The aim of this study and review was to make a comparative TPMT enzyme activity analysis using TPMT enzyme expression determination method in IBD patients who are already taking azathioprine drug therapy, with patients who have not yet begun this therapy. The long-term aim is to decrease overall expenses using azathioprine, that could be done if patients would be tested for TPMT expression level before starting therapy with azathioprine, thereby excluding this therapy for patients with higher risk of adverse side effects, reducing medical expenses treating these side effects.

20 IBD patients (55% female, n=11; 45% male, n=9) data was obtained and analysed. 70 % of patients (n=14) was diagnosed with ulcerative colitis (UC), 30 (n=6) with Crohn's disease (CD). 75% (n=15) of patients had not previously received azathioprine (Imuran 50 mg). 15% (n=3) had received azathioprine therapy, but stopped using it because of negative side effects like dyspepsia, acute pancreatitis, symptom exacerbation. 10% (n=2) was still receiving azathioprine therapy. Activity of TPMT was low (<5.5 U/mL) in 10% of patients (n=2), average (5.6-15.5 U/mL) in 5% (n=1), normal(15.6-44.0 U/mL) in 70% (n=14) and high (>44.0 U/mL) in 15% (n=3)

The results of this study and review suggests that the TPMT enzyme activity should be determined before administering azathioprine drug therapy for patients diagnosed with inflammatory bowel disease to prevent adverse reactions and evaluating treatment risks.

2. Key Words: Inflammatory bowel disease;Azathioprine;*Myelosuppression*;Thiopurine S-methyl transferase

3. Introduction

The spread of IBD patients in the world tends to increase. The treatment of these patients is an important healthcare problem nowadays, and it has been shown that effective personalized treatment reduces the risk of disability, complications and side effects. Analysing and evaluating drug metabolism plays a crucial role in predicting the effectiveness of pharmacotherapy and in preventing adverse reactions [1]. The Thiopurine drugs (azathioprine, mercaptopurine and thioguanine) are mainly used in a treatment of autoimmune diseases [2]. TPMT is one of the enzymes essential for the metabolism of thiopurines. Low TPMT enzyme activity is associated with abnormal metabolism of thiopurine drug substances, overproduction of cytotoxic metabolites, and reason of myelosuppression [3,4]. TPMT enzyme activity is regulated by genetic polymorphism. It has been found that about 0,3% of individuals are homozygous for *TPMT* mutation, while 11% have a heterozygous allele variant indicating low enzyme activity [1]. Therefore, the British National Formulary strongly recommends that the TPMT enzyme should be identified prior to starting thiopurine therapy [3].

4. Materials and Methods

All patient's blood tests were collected in the 5-7 mL vacutainer tubes. The samples were centrifuged for 30 minutes after collection for 15 minutes at 1000 rpm at 4 °C. Samples were stored frozen at -80° C, avoiding re-freezing. After collecting blood samples, a survey was completed. It includes patient of IBD diagnosis – ulcerative colitis (UC) or Cronh's disease (CD), demographic data on age, gender; as well as the duration of the disease, the history of the disease, the use of medication, intolerance and allergies, routine blood laboratory tests to assess what could affect TPMT expression. TPMT expression was determined by the ELISA using the *MyBioSurce* reagent kit Human TPMT ELISA Kit (catalogue number MBS938845).

5. Results

All 20 respondents included in the study had histological diagnosis of IBD (UC in 70%, n=14; CD in 30%, n=6).Patients had moderate to severe disease activity according Mayo score in UC patients and Crohn's Disease Activity Index (CDAI) in Crohn's disease patients. 50% of respondents (n=10) were diagnosed with particular IBD for more than 10 years ago. UC was diagnosed in 8 men and 6 women; CD was more diagnosed in 5 women and only 1 men.

Summarizing information on the usage of medications, 45% of respondents (n=9) used per oral form of mesalazine; 40% (n=8) a combination of mesalazine per oral and suppositories. 75% of respondents (n=15) have never used azathioprine before, 15% (n=3) have used it, but have stopped taking due to side effects, while 10% (n=2) used azathioprine during the study. Patients who discontinued due to adverse reactions reported side effects such as gastrointestinal symptoms and acute pancreatitis. Patient's TPMT expression ranged from 1.4 to 50 U/mL. All respondents were divided into TPMT enzyme activity: 10% (n=2) patients had low (<5.5 U/mL) TPMT activity, 5% (n=1) patient had intermediate (5.6-15.5 U/mL) activity, 70% (n=14) patients normal (15.6-44.0 U/mL) and 15% (n=3) patients high (>44.0 U/mL) TPMT activity.

6. Discussion

IBD continues to spread rapidly; it is a global health care and society problem. Patients with IBD should have early diagnostics methods and personalised treatment from the early steps of disease. As well is important therapeutic drug monitoring drug treatment, as it can decrease risks of complications and side effects and improve quality of life. All respondents in our study had an age range from 22 to 79 years, with an average age of 42 years. Both Northern Europe and USA, Canada have the highest prevalence of IBD compared to other countries. In these countries, the disease is most commonly diagnosed in patients aged 15 to 35 and the average age is 31 years [5]. In contrast, in other countries (both in Europe and Asia), the disease is most commonly diagnosed between the ages of 15 and 45 and the highest prevalence is found in young people around 20 years of age, but only 10-15% of all patients are aged 60 or over [6].

According to the respondent's data on the usage of azathioprine, most or 75% of patients have not used it, so it would be useful to find out the TPMT expression of each individual. This would make it possible to find out if the chosen therapy with one of the thiopurines will be effective and there will not be side effects. In countries such as the United States and the United Kingdom, the level of this enzyme is already established prior to initiation of therapy [2, 7].

One of the most commonly used methods is the enzymatic assay, or phenotyping, of TPMT enzyme to measure the activity of the enzyme in the blood [8]. The results of the TPMT enzyme activity test may be influenced by several factors. One of them is a recent blood transfusion that can produce false results. Medications used before may also reduce the level of this enzyme in the blood, for example if the patient has taken sulfasalazine, mesalazine, thiazide, allopurinol, salicylic acid 48 hours before the test. This is why this test is recommended to be repeated during azathioprine treatment [9].

The second approach to determining the amount of TPMT in a subject is genotyping, which determines polymorphisms in DNA. The TPMT genotypes are usually determined using the polymerase chain reaction (PCR) method. Continuing our research in the future, it would be interesting to carry out TPMT genotyping in patients with reduced TPMT enzyme activity.

Unlike phenotyping, the genotype test is not affected by external factors responsible for TPMT coding and does not need to be repeated during therapy. The sensitivity of the genotype test depends on the number of polymorphisms required to be detected [10]. Several mutation variants associated with thiopurine toxicity have been identified. The most commonly found non-functional alleles are *TPMT * 3A, TPMT * 3C* and *TPMT * 2* [4]. It has been shown that a patient carrying any of these TPMT alleles can accumulate large amounts of 6-TGN in the body, which may exacerbate the side effects [6].

Most patients with IBD have normal TPMT activity with two functional alleles, however, all patients receiving azathioprine therapy should be monitored and identified for TPMT enzyme activity [11]. Following the Clinical Pharmacogenetics Implementation Consortium (CPIC) for genotype and thiopurine dosing ~ 1 in 178 to 1 in 3,736 patients has a homozygous genotype with two non-functional *TPMT* alleles, which means that these patients have low / inadequate TPMT enzyme activity and have severe risk of myelosuppression during therapy. ~ 3-14% of the populations are heterozygous, with moderate risk of toxicity at 30-60% of therapy, therefore, caution and lower doses of medication are needed during therapy. In turn, 86-97% are wild-type with two functional *TPMT* alleles and high levels of enzyme activity [12].

According the increased risk of toxicity and high treatment costs, Food and Drug Administration recommends *TPMT* genotyping or phenotyping prior to initiation of thiopurine therapy. This allows patients to identify an effective starting dose of thiopurine and, if necessary, to choose other alternative medications [3,13]. CPIC has published recommendations for *TPMT* genotyping results based on the usage of azathioprine, underlining the need to consider medication substitution or a reduction in the dosage of azathioprine in patients with low or inadequate TPMT activity [2,14].

Determination of TPMT enzyme activity in IBD patients would be necessary prior to thiopurine therapy in order to prevent adverse reactions and to evaluate the risk of therapy.

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