CASE STUDY

Azathioprine and diffuse alveolar haemorrhage: the pharmacogenetics of thiopurine methyltransferase

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ABSTRACT: Current guidelines support the use of corticosteroids and azathioprine as one possible treatment strategy for idiopathic pulmonary fibrosis (IPF). However, some patients with genetic polymorphisms of thiopurine methyltransferase (TPMT) are at risk of severe azathioprine myelotoxicity.

The current authors present the case of an 85-yr-old Caucasian male with IPF who developed diffuse alveolar haemorrhage as a complication of azathioprine-induced myelosuppression.

Leukocyte genetic TPMT testing revealed that the patient had homozygous polymorphisms associated with the absence of TPMT activity and severe azathioprine-induced myelotoxicity.

Thiopurine methyltransferase deficiency should be considered in patients who develop leukopenia early in treatment with azathioprine, or who present with severe marrow suppression at usual doses. For centres with equipped laboratories, a dosing suggestion is provided based on thiopurine methyltransferase testing. Even with screening strategies, frequent monitoring of complete blood count and liver biochemistry should remain the mainstay of surveillance for azathioprine toxicity.

KEYWORDS: Azathioprine, diffuse alveolar haemorrhage, myelotoxicity, pharmacogenetics, polymorphism, thiopurine methyltransferase

Despite the lack of data to support the use of immunosuppressive drugs for idiopathic pulmonary fibrosis (IPF), clinicians should not necessarily dismiss them as ineffective [1, 2]. In fact, the American Thoracic Society/European Respiratory Society consensus statement suggests moderate doses of steroids combined with either azathioprine or cyclophosphamide [3]. Azathioprine is a pro-drug of 6-mercaptopurine (6-MP). It is used as an immunosuppressant for solid organ and haematological transplants, as well as a steroid-sparing agent for a variety of immune-mediated diseases. Its use is limited by both its slow onset of action (3–4 months) and its toxicity (sometimes occurring before any anti-inflammatory effect), which includes hepatitis, bone marrow suppression, infection and malignancy.

CASE REPORT

An 85-yr-old Caucasian male was admitted to hospital with fatigue and increasing dyspnoea of

2 weeks' duration. He had been diagnosed with IPF 4 yrs previously. His other past medical history included transurethral resection of bladder cancer, hypertension and chronic kidney disease (creatinine $150 \mu\text{mol}\cdot\text{L}^{-1}$, normal levels $44\text{--}106 \mu\text{mol}\cdot\text{L}^{-1}$). His dyspnoea had progressed over the months prior to hospitalisation, limiting his mobility and necessitating supplemental home oxygen therapy. Due to the patient's worsening clinical status and deteriorating pulmonary function tests, he was prescribed azathioprine 100 mg daily and prednisone 40 mg daily starting 2 months prior to hospital admission. His other medications included beclomethasone inhalations, nadolol and hydrochlorothiazide. Shortly after the patient was admitted to hospital, he developed acute respiratory failure. Significant findings on physical examination included paradoxical breathing, tachypnoea ($36 \text{ breaths}\cdot\text{min}^{-1}$), a high oxygen requirement (oxygen saturation 92% on oxygen $15 \text{ L}\cdot\text{min}^{-1}$ by a nonrebreather mask) and bilateral anterolateral crackles on chest auscultation. A chest

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radiograph revealed diffuse airspace disease with basal honeycombing. The patient was intubated and admitted to the intensive care unit (ICU) for mechanical ventilation. His complete blood count was as follows: haemoglobin $65 \text{ g}\cdot\text{L}^{-1}$ (normal level $130\text{--}180 \text{ g}\cdot\text{L}^{-1}$), with a high normal mean corpuscular volume; reticulocyte count $1 \times 10^9\cdot\text{L}^{-1}$ (normal level $10\text{--}75 \times 10^9\cdot\text{L}^{-1}$); platelet count $17 \times 10^9\cdot\text{L}^{-1}$ (normal level $150\text{--}400 \times 10^9\cdot\text{L}^{-1}$); and white blood cell count $0.4 \times 10^6\cdot\text{L}^{-1}$ (normal level $4.5\text{--}11 \times 10^6\cdot\text{L}^{-1}$), with $0.1 \times 10^6\cdot\text{L}^{-1}$ neutrophils and $0.2 \times 10^6\cdot\text{L}^{-1}$ lymphocytes. Electrolytes, liver function tests and creatinine kinase were unremarkable; serum creatinine was elevated but remained near baseline level. Fibreoptic bronchoscopy showed diffuse bleeding, and sequential bronchoalveolar lavages (BALs) were bloody. The diagnoses upon admission were azathioprine-induced pancytopenia and diffuse alveolar haemorrhage.

The patient developed a fever shortly after ICU admission and was treated empirically for febrile neutropenia with broad-spectrum antibiotics and filgrastim. Cultures of BAL fluid and blood grew *Staphylococcus aureus*; BAL was negative for atypical cells, viruses, fungal elements and *Pneumocystis jirovecii*. Transoesophageal echocardiography showed no evidence of endocarditis. A sample for thiopurine methyltransferase (TPMT) genetic testing was taken, and the patient received platelets and packed red blood cells. Due to nonresolving respiratory failure, and in accordance with the patient's previously expressed wishes, life support was withdrawn on the seventh hospital day. The family declined permission for a post-mortem examination. TPMT activity was not measured by the current authors' laboratory, but molecular genetic testing reported 2 weeks later revealed the patient to be homozygous for the TPMT*3A (460G>A and 719A>G) mutation and, thus likely to have low or no TPMT activity. The trigger for the patient's diffuse alveolar haemorrhage was not pathologically established, but the available results suggested the combination of infection and azathioprine-induced thrombocytopenia.

DISCUSSION

As shown in figure 1, azathioprine is converted to 6-MP *in vivo*, where it is subsequently metabolised to either 6-thioguanine (6-TG) nucleotides by the enzyme inosine-5'-monophosphate dehydrogenase (IMPDH), or to 6-methyl-mercaptopurine ribonucleotides by the enzyme TPMT [4, 5]. 6-TG is a cytotoxic metabolite associated with marrow suppression. TPMT also converts 6-MP to 6-methyl-mercaptopurine, which is associated with hepatotoxicity.

The activity of the enzyme TPMT has been shown to be trimodal, with 90% of people having high activity, 10% intermediate, and 0.3% low or no activity [6, 7]. In those with reduced activity, the metabolism of 6-MP is shunted to the IMPDH pathway, leading to accumulation of myelotoxic 6-TG nucleotides. The current authors are unaware of direct experimental evidence that explains why the xanthine oxidase pathway does not accommodate the excess 6-MP. TPMT activity is related to ~20 different single nucleotide polymorphisms [8]. Three key mutations, however, account for >90% of all clinically significant TPMT mutations and 80–95% of low or intermediate metabolisers found in Caucasian, African-American and Asian sub-populations. The common

mutant haplotypes are TPMT*2 (238G>C predicting Ala80Pro), the double heterozygote TPMT*3A (460G>A and 719A>G predicting Ala154Tyr and Tyr240Cys) and TPMT*3C (719A>G alone) [6, 9]. TPMT*3A is the most prevalent deleterious allele among Caucasians, while TPMT*3C is the most prevalent in African and South-East Asian populations. Patients with intermediate activity are usually heterozygous (*i.e.* have one mutated and one "wild-type" chromosome), while those with low or absent TPMT activity are almost always homozygous for TPMT mutations (*i.e.* both chromosomes are mutated).

TPMT phenotype correlates with 6-TG metabolite levels, thought to result in bone marrow toxicity [10, 11], and TPMT status may predict the duration from initiation of therapy to myelosuppression. In a series of rheumatoid arthritis patients taking azathioprine, five out of six subjects heterozygous for a mutant allele had therapy discontinued within 1 month due to low leukocyte counts [12]. In a study of Crohn's disease patients receiving azathioprine, cytopenia manifested within 2 months for homozygotes, within 16 months for heterozygotes, and as late as 87 months for those with no mutated TPMT alleles [13]. Similar studies in patients taking azathioprine for lung disease have yet to be carried out.

The use of other myelosuppressive drugs, or the presence of concurrent infections, could explain the lack of specificity in TPMT testing studies. Furthermore, concurrent use of allopurinol, a xanthine oxidase inhibitor, may cause a toxic shift in the metabolism of thiopurines. Preliminary studies looking at another enzyme involved in azathioprine metabolism, inosine triphosphate pyrophosphatase (ITPA), show that myelosuppression may also be related to certain relatively rare ITPA gene polymorphisms [14, 15]. In summary, while factors other than TPMT status are likely to be involved in azathioprine toxicity, the majority of evidence suggests that TPMT deficiency is a

FIGURE 1. A simplified schematic diagram showing the major pathways of mercaptopurine metabolism. Azathioprine (AZA) is converted to 6-mercaptopurine (6-MP) in the liver via a glutathione-dependent process accelerated by glutathione-S-transferase. 6-MP undergoes further metabolism by xanthine oxidase (XO) and thiopurine methyltransferase (TPMT). 6-MP is also metabolised by hypoxanthine guanine phosphoribosyltransferase (HPRT) within the red blood cells and is subsequently converted by TPMT to 6-methyl-mercaptopurine ribonucleotides or by inosine-5'-monophosphate dehydrogenase (IMPDH) to 6-thioguanine nucleotides. Deficiency of TPMT activity leads to accumulation of 6-thioguanine nucleotides, which may cause bone marrow toxicity.

TABLE 1 Genetic determinants of thiopurine methyltransferase (TPMT) activity and suggested azathioprine dosing

Frequency %	TPMT activity [#]	Genotype	Clinical correlate/azathioprine dosing
89–90	High	TPMT ^H /TPMT ^H	Myelotoxicity occurs late, likely multifactorial Initiate standard dose (1.5–2.5 mg·kg ⁻¹ ·day ⁻¹) Some patients may require higher doses.
10–11	Intermediate	TPMT ^H /TPMT ^L	Increased risk of myelotoxicity Reduce dose by 25–50% and monitor complete blood count closely
0.2–0.3	Low or absent	TPMT ^L /TPMT ^L	High risk of early myelotoxicity Avoid i.v. use Drop dose by 75–90%; avoid altogether if no TPMT activity Follow complete blood count weekly for at least 8 weeks.

TPMT^H: wild-type TPMT allele associated with high enzymatic activity; TPMT^L: mutated TPMT allele associated with low enzymatic activity. Intermediate metabolisers of thiopurines are heterozygous with TPMT^H and TPMT^L. Homozygotes with two deficient alleles are at risk of severe myelotoxicity early in therapy, generally within 1–2 months. [#]: erythrocyte TPMT activity may be measured in a variety of ways, including mass spectrometry and high-performance liquid chromatography. Clinicians should consult with a knowledgeable laboratory professional for specific interpretation of genetic and enzymatic measures of TPMT activity.

significant independent risk factor for myelotoxicity, especially early in the course of treatment.

A recent study in renal transplant patients initiated on 1.5 mg·kg⁻¹·day⁻¹ of azathioprine found that twice the proportion of patients with a heterozygous TPMT mutation required dose reductions due to leukopenia than patients with homozygous wild-type TPMT [16]. Azathioprine dosing based on erythrocyte TPMT activity has been offered as a means to reduce the risk of cytopenia [10, 17]. Although >70% of myelosuppression related to azathioprine use is not associated with TPMT polymorphism [13, 18, 19], screening as few as 20 patients for TPMT deficiency can prevent one adverse event over 6 months of therapy [20]. Furthermore, pharmacoeconomic models and prospective studies have demonstrated that genotype or phenotype screening for TPMT polymorphisms is cost-effective in patients with rheumatological disorders [20], inflammatory bowel disease [21, 22], paediatric leukaemia [23] and autoimmune skin disease [24]. Other small prospective screening studies of TPMT activity have not adequately predicted toxicity in patients with inflammatory bowel disease [25, 26]. Economic models are limited, as they are based only on literature estimates of the incidence of azathioprine toxicity. However, large-scale prospective screening studies are unavailable, and with mixed results in the small studies there is ongoing debate regarding the benefit of routine TPMT screening. The British Society of Gastroenterology guidelines state that TPMT measurement cannot yet be recommended as a prerequisite to therapy with azathioprine [27], a view shared by the American College of Gastroenterology [28]. The British Association of Dermatologists, however, suggests TPMT activity testing prior to initiating therapy with azathioprine [17]. The current authors are not aware of any pharmacoeconomic analyses or prospective screening studies of TPMT in patients with pulmonary diseases.

Conclusion

The patient was homozygous for the TPMT*3A mutation, thus putting him at greater risk for azathioprine myelotoxicity. The ensuing severe thrombocytopenia is likely to have contributed to the diffuse alveolar haemorrhage. In this case,

if the patient's TPMT status had been known in advance, it is possible that an alternative therapy may have been selected, especially given the lack of data supporting any particular pharmacological treatment strategy for IPF [29].

Since the utility and cost-effectiveness of thiopurine methyltransferase phenotype or genotype screening remains somewhat controversial, there are currently no national or international guidelines that recommend routine thiopurine methyltransferase testing prior to initiation of azathioprine for chest diseases. For those clinicians with access to thiopurine methyltransferase activity or genetic testing, table 1 summarises one possible clinical approach to the initiation of azathioprine. Where testing is unavailable, clinicians should be aware that one in 10 patients has a thiopurine methyltransferase polymorphism that may predispose them to azathioprine myelotoxicity. During the first 8 weeks of therapy, a minimum of weekly monitoring of the complete blood count and liver function tests should be considered, as this is the period of highest risk. If red blood cell thiopurine methyltransferase activity measurement is considered for patients on azathioprine presenting with pancytopenia, blood samples should be drawn before the administration of red cell transfusions and should be interpreted based on the guidelines provided by the testing laboratory. Molecular genetic testing of leukocytes can be done irrespective of the administration of blood products.

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