CASE REPORT

Azathioprine-induced pancytopenia with normal TPMT activity presenting with HSV oral ulcers

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SUMMARY

A 65-year-old man with treatment-resistant psoriatic arthritis, hypertension, dyslipidaemia and benign prostatic hyperplasia (BPH) presented with herpes simplex virus (HSV) oral ulcers and a recent 15 lb weight loss due to reduced consumption. Five weeks previously, his methotrexate was tapered and he had begun taking azathioprine. The patient's thiopurine S-methyltransferase (TPMT) activity level was normal prior to starting azathioprine. He was found to have pancytopenia with normal folate levels and azathioprine was discontinued. His pancytopenia worsened, with a nadir 8 days after stopping azathioprine, before returning to normal levels. His oral ulcers improved and he was able to tolerate solid food. This case illustrates that decreased TPMT activity is not the only risk factor for pancytopenia as an adverse reaction to azathioprine. Furthermore, HSV stomatitis may be the presenting symptom of pancytopenia. The timeline of improvement in cell counts illustrated in this patient has implications for the management of suspected azathioprine-induced pancytopenia.

BACKGROUND

Azathioprine is often used to treat autoimmune disorders, and it has a known risk of myelosuppression, most often leucopenia. 6-Mercaptopurine (6-MP) is a metabolite of azathioprine that is further metabolised by the rate-limiting enzyme thiopurine S-methyltransferase (TPMT); some polymorphisms of TPMT lead to decreased TPMT activity. Pancytopenia is a rare complication that has been associated with reduced TPMT activity. Research shows that homozygous and heterozygous variant TPMT alleles (TPMT*2, TPMT*3A and TPMT*3C) predispose individuals to greater azathioprine toxicity.^{1 2} With reduced TPMT activity, more 6-thioguanine nucleotides accumulate in the serum, increasing the potential for toxicity.³ The US Food and Drug Administration (FDA) recommends routine testing of TPMT activity or TPMT variant alleles prior to azathioprine administration to reduce the risk of serious adverse outcomes.⁴ The Clinical Pharmacogenetics Implementation Consortium recommends dose reductions if the patient has intermediate or low TPMT activity, as patients with low TPMT activity are more susceptible to severe myelosuppression and 30%–60% of patients with intermediate TPMT activity have moderate rates of myelosuppression.⁵ The connection between pancytopenia and low and intermediate TPMT activity levels is documented, yet there are few

reported cases of azathioprine-induced pancytopenia in patients with normal TPMT activity.^{1 6–8} This has implications for the clinical monitoring of a patient with normal TPMT levels after administration of azathioprine.

CASE PRESENTATION

A 65-year-old man with a history of treatment-resistant psoriatic arthritis had a history of minimal benefit and adverse reactions to multiple immunosuppressive drugs. He had used etanercept 50 mg weekly for several years until it lost its efficacy. He had taken adalimumab 40 mg for 1 year with marginal control of his symptoms, but he then developed clinical and laboratory evidence of recurrent disease activity and alopecia. He took apremilast 30 mg for 12 days before developing swelling and pain on his left hand, which caused him to discontinue the medication. He tried ustekinumab 45 mg every 12 weeks for 6 months without benefit. The patient was then prescribed methotrexate 15 mg weekly, prednisone 20 mg daily, folic acid 3 mg daily and leucovorin calcium 10 mg weekly, but he had worsening disease activity, including hair loss and symmetrical joint arthritis. Prior to starting azathioprine, his C-reactive protein level was normal at 0.4 mg/L, his TPMT activity was within normal range at 21.0 µmL red blood cells (RBC) (15.1–26.4 µmL), and serological workup was negative for infection with tuberculosis, hepatitis B virus (HBV) and hepatitis C virus (HCV).

The patient's rheumatologist decreased his methotrexate dose from 15 mg to 7.5 mg weekly and ordered azathioprine 50 mg daily with plans to up-titrate the dose to 75 mg daily after 1 week, while continuing his prednisone 20 mg daily (figure 1). Repeat complete blood count 2 weeks after initiating azathioprine was normal. One month after starting azathioprine, the patient noted worsening alopecia, which could have been a late adverse effect of methotrexate. He was advised to stop methotrexate entirely and to increase azathioprine to 125 mg daily. Shortly after increasing his azathioprine dose, the patient developed severe herpetic stomatitis (figure 2). He was unable to consume solid food due to pain, and he lost 15 lbs between 8/14 and 8/29. The patient subsequently presented to his rheumatologist and was found to have pancytopenia. Relative to his pre-azathioprine cell counts, his white cell count (WCC) decreased from 8.3 cells x 10⁹/L to 1.0 cells x 10⁹/L, his platelets decreased from 215 x 10⁹/L to 61 x 10⁹/L and his haemoglobin decreased from 13.8 g/dL to 10.3 g/dL.

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Figure 1 Timeline of symptoms and blood counts relative to medication administration. AZA, azathioprine; CBC, complete blood count; Hgb, haemoglobin; HSV, herpes simplex virus; MTX, methotrexate; plts, platelets; WCC, white cell count.

dL. The patient was instructed to immediately stop azathioprine. Approximately 1 week after discontinuation of azathioprine, repeat labs showed worsening pancytopenia with WCC of 0.74 cells x 10⁹/L, platelets of 27 x 10⁹/L and haemoglobin of 9.3 g/dL (table 1). He was admitted to the hospital for evaluation and

management of his pancytopenia and weight loss of 10–15 lbs over a 2-week period due to his inability to eat solid foods.

INVESTIGATIONS

Other causes of pancytopenia were effectively ruled out as below.

Infectious: an infectious aetiology of the patient's pancytopenia was unlikely due to recent negative testing for HCV, HBV and tuberculosis (Quantiferon TB Gold test) prior to initiation of azathioprine and negative testing for HIV (antibody screen) and cytomegalovirus (DNA Quantitative PCR) during the patient's hospital stay.

Prior to hospitalisation, the patient had a positive HSV 1 rapid culture of oral lesions with typing confirmed by monoclonal antibody microscopic immunofluorescence and a positive serum HSV 1 immunoglobulin G. The patient's immunosuppression was the likely trigger for oral HSV reactivation. Serum HSV 1/2 PCR result was negative early in the patient's hospitalisation (8/31), effectively ruling out the possibility of HSV viraemia as the cause of pancytopenia (table 2).

Metabolic: given his recent use of methotrexate, folate levels were checked to rule out folate deficiency as the cause of pancytopenia, notwithstanding the patient's concurrent regimen of folic acid and leucovorin. The patient's folate level was normal. The patient's B12 levels were also normal.

Haematologic: a bone marrow biopsy was not performed during his hospitalisation due to the high likelihood of azathioprine-induced pancytopenia and the low likelihood that results of the biopsy would impact management.

Figure 2 Photo of herpes simplex virus 1 oral ulcer (black arrow) taken by patient prior to hospitalisation.

DIFFERENTIAL DIAGNOSIS

Please see table 2 for the differential diagnosis of pancytopenia.⁹

Table 1 Patient's serum lab values

Blood parameter	Before azathioprine (6/29)	Day 1* (8/24)	Day 7† (8/30)	Day 8 (8/31)	Day 10 (9/2)	Day 12 (9/4)	Day 14 (9/6)
Haemoglobin (g/dL)	13.8	10.3	9.3	8.6	7.7	6.8	7.1
White cell count (10 ⁹ /L)	8.3	1.0	0.74	0.3	1.29	3.07	7.69
Platelets (10 ⁹ /L)	215	61	27	29	46	72	110

*Patient discontinues azathioprine.

†First day of patient's hospitalisation.

TREATMENT

During his hospital course, the patient's oral ulcers were treated with intravenous acyclovir on the first day of his hospital admission, and then he was transitioned to oral valacyclovir after his serum HSV PCR was negative. Although the time course of his history did not suggest it, he was given three doses of intravenous leucovorin for possible methotrexate toxicity. His cell counts slowly began to improve on hospital day 3—approximately 9 days after his last dose of azathioprine. The patient experienced febrile neutropenia during his hospitalisation, for which he was given piperacillin/tazobactam, metronidazole and three doses of intravenous filgrastim. The patient had one flare of arthritis during his hospital stay when we attempted to decrease his prednisone dose from 20 mg daily to 15 mg daily.

OUTCOME AND FOLLOW-UP

The patient's blood counts reached their nadir 8 days after stopping azathioprine and returned to normal levels approximately 14 days after stopping azathioprine (table 1). His oral ulcers improved during his hospitalisation and he was able to tolerate solid food. He was placed on prednisone 15 mg daily for treatment of his psoriatic arthritis.

One month after discharge from hospital, the patient had a WCC of 5.8 cells x 10⁹/L, haemoglobin of 11.6 g/dL and platelets of 30 x 10⁹/L. With persistent thrombocytopenia, the medical team proceeded with a bone marrow biopsy. The results of the bone marrow biopsy showed hypoplasia of haematopoietic elements with normal maturation and no evidence of dysplasia. The patient's blood counts improved on prednisone 60 mg daily,

which was subsequently tapered gradually to 7.5 mg. The patient plans to continue routine blood checks on an outpatient basis.

DISCUSSION

Using the Naranjo Adverse Drug Reaction (ADR) Probability Scale, the likelihood of this patient's pancytopenia being caused by an ADR is 'probable' (score of 5).¹⁰ While there are other possible aetiologies of the patient's pancytopenia, the temporal relationship with azathioprine and course of recovery make an ADR the most likely cause in this patient's case.

Severe cytopenias and bone marrow suppression are known adverse effects of azathioprine. Case reports show myelosuppression rates in patients after azathioprine ranging between 8% and 25%.^{11–13} Although leucopenia is most common, pancytopenia is also possible. Reports of pancytopenia due to azathioprine have been documented for patients undergoing treatment for a range of rheumatological illnesses, including lupus nephritis, myasthenia gravis, inflammatory bowel disease, organ transplant, dyshidrotic eczema, psoriatic arthritis and rheumatoid arthritis.^{1 2 6 14–18} Patients with lupus may have increased risk of azathioprine toxicity, given the reduced stimulation of granulocytes in patients with lupus.¹⁹

Higher azathioprine doses and low TPMT activity levels confer increased likelihood of azathioprine-induced pancytopenia. TPMT converts the azathioprine derivative 6-MP to inactive metabolites. Lower TPMT activity causes shunting of 6-MP down an alternate pathway, leading to a buildup of 6-thioguanine nucleotides. The incorporation of these nucleotides into DNA and RNA leads to cytotoxicity and myelosuppression.³ The FDA recommends that physicians consider TPMT genotype or phenotype testing in all patients before initiating treatment with azathioprine.⁴ FDA labelling of azathioprine (Imuran) clearly states 'TPMT testing cannot substitute for complete blood count (CBC) monitoring'.⁴ There is at least one documented case of azathioprine-induced aplastic anaemia and fatal myelosuppression in a patient with lupus with normal TPMT activity.⁸ All patients on azathioprine should have complete blood counts checked weekly during the first month of therapy, twice monthly for the second and third months and then monthly or more often if dosing changes are made.⁴

Patients typically develop pancytopenia within the first 3 weeks of exposure, although the exact time course of azathioprine-induced pancytopenia may be variable and is often complicated by coadministration of other drug therapies. Most reported cases show a similar timeline of recovery once hospitalised, patients' cell counts tend to recover after 1–3 weeks with discontinuation of azathioprine and treatment with folic acid, filgrastim and supportive care. In one reported case of azathioprine-induced pancytopenia in a patient with lupus, the patient had been taking azathioprine for over 2 months before developing pancytopenia after a decrease in the administered dose of corticosteroids.¹⁸ In this case, the stimulatory effects of corticosteroids may have temporarily protected the patient from the marrow toxicity of azathioprine.^{18 20}

Table 2 Causes of new-onset adult pancytopenia⁹

Aplastic anaemia	Idiopathic Drugs Chemicals (benzene, dichlorodiphenyltrichloroethane, lindane, hydrocarbon-based glue vapours) Radiation Anorexia
Autoimmune disease	Systemic lupus erythematosus Rheumatoid arthritis Sarcoidosis
Infection	Viral (HIV, hepatitis, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, adenovirus, parvovirus B19) Fungal Tuberculosis
Splenomegaly	Portal hypertension
Nutritional deficiency	Vitamin B12 Folate Zinc toxicity Copper deficiency
Bone marrow infiltration	Myelodysplastic syndrome Multiple myeloma Acute leukaemias Chronic leukaemia Non-Hodgkin's lymphoma Metastatic cancer

After discontinuation of azathioprine, cell counts typically decrease for several days before returning to baseline levels, with one case documenting pancytopenia as long as 3 weeks after the discontinuation of azathioprine.¹⁴ This delayed improvement has been attributed to the persistence of serum and intracellular levels of 6-thioguanine nucleotides after azathioprine has been discontinued.^{14,21} Physicians should take caution not to misinterpret this continued decrease in cell counts after medication discontinuation as a sign of an alternative explanation for cytopenias.

This case adds to the few reported cases of azathioprine-induced pancytopenia in patients with normal TPMT levels in the literature

Patient's perspective

I was surprised when I received a call from my rheumatologist telling me that there were abnormalities with my lab work and shocked when she told me to go to the emergency room for admission into the hospital. I had recently developed very painful mouth sores and I was struggling through the pain and discomfort, but I had no out-of-the-ordinary symptoms that would lead me to believe I needed to be hospitalised. At this point I thought that if I were in the hospital, they could treat my painful mouth sores.

Once I was admitted to the hospital and was seen by various teams of doctors, is when I realised the gravity of my situation. That was the eye-opener, and that's when it 'hit home'. I was in danger of getting an infection that I might not be able to survive because of my extremely low white blood cell count. I hadn't focused on that at all. Instead, all I could think about was the pain from the mouth sores—my number one priority.

I felt comfort in knowing that each of the medical teams were constantly monitoring me and knowing that their number one priority was to find out what caused the drastic change in my blood cells. Their main objective was getting my blood counts up to normal and avoiding an infection. As it turns out, I did develop a fever, and the teams of doctors got together to come up with a plan. Their planned approach worked and I started to see improvement. At one point, they discussed the possibility of a bone marrow biopsy but, fortunately, their approach to raising my blood counts worked without the need for it.

Having gone through this experience, I no longer just take medication without reviewing the potential side effects and the importance of ongoing lab work.

Learning points

- This case demonstrates that having a normal thiopurine S-methyltransferase (TPMT) activity level does not preclude the development of pancytopenia due to azathioprine therapy.
- After initiating azathioprine, physicians should closely monitor patients' blood counts, regardless of TPMT activity or variant allele status.
- Manifestations of pancytopenia may be clinically variable and may be mistaken for direct adverse reactions to drug therapy. Signs of immunosuppression, such as herpes simplex virus 1 oral ulcers, should trigger physicians to consider pancytopenia.
- Understanding the time course of azathioprine-induced pancytopenia may prevent misdiagnosis and unnecessary bone marrow biopsies.

and highlights the need for continuous monitoring in the weeks and months following initiation of azathioprine therapy.

Contributors CHJ conceived the project and drafted the initial manuscript. She obtained the patient's consent for the case report and was responsible for incorporating the other authors' revisions and submitting the final work. JT was involved in the planning and conception of the project, acquisition of patient consent and data and revising the manuscript. JSC was involved in the planning, background research, analysis of data and editing of the manuscript. All authors were involved in the care of the patient. All authors approved the submitted manuscript and agree to be accountable for all aspects of the work.

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