CASE REPORT

Azathioprine-associated myelodysplastic syndrome in two patients with ulcerative colitis

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ABSTRACT

Azathioprine is a commonly used immunosuppressive agent in post-transplantation regimens and autoimmune diseases. An increased risk of lymphoma with thiopurine therapy in patients with inflammatory bowel disease has been described previously; however, there are few reported cases of azathioprine therapy-related myelodysplastic syndrome and acute myeloid leukaemia. We report two patients with ulcerative colitis who subsequently developed azathioprine-related myelodysplastic syndrome. It is imperative that gastroenterologists remain vigilant for this rare complication as this subset of patients has a particularly poor prognosis. These cases are also important in considering the risk of open-ended thiopurine therapy.

CASE 1

A 68-year-old female patient with a suspected neutropenic fever was advised to attend the accident and emergency department by her general practitioner (GP). Her GP reported a neutrophil count of $0.5 \times 10^9/l$. She described a 3 week history of night sweats, low-grade fever, increasing fatigue and shortness of breath on exertion. She had a medical history of ulcerative pancolitis diagnosed 6 years prior to this presentation and type 2 diabetes mellitus. Current medication included azathioprine 125 mg once daily, Asacol 3.2 g daily, ferrous fumarate 210 mg once daily and Calcichew D3 tablets taken twice daily. Thiopurine methyltransferase (TPMT) enzyme assay levels were within the normal range. She had not been exposed to any other immunomodulatory drugs, and her colitis had been relatively quiescent. Prior to this, she had been undergoing routine follow-ups, and her blood counts did not reveal any

abnormalities. There was no family history of cancer in her first-degree relatives. Physical examination was unremarkable. Observation revealed a temperature of 37.5°C. Pulse 80 was beats/min, blood pressure 128/35 mm Hg and oxygen saturation by pulse oximetry 99% on room air.

INVESTIGATIONS

Blood tests showed a haemoglobin of 8.9 g/dl, mean cell volume (MCV) 107.5 fl, platelets $118 \times 10^9/l$, white cell count $9.14 \times 10^9/l$, neutrophils $2.84 \times 10^9/l$, lymphocytes $3.8 \times 10^9/l$ and monocytes $2.48 \times 10^9/l$.

Blood biochemistry showed the following; urea 2 mmol/l, creatinine 52 µmol/l, sodium 139 mmol/l, potassium 3.6 mmol/l, C reactive protein 22.9 mg/l, albumin 38 g/l, bilirubin 5 µmol/l, alkaline phosphatase 66 IU/l and alanine transaminase 17 IU/l.

Iron, vitamin B₁₂ and folate were normal.

Chest radiograph was unremarkable.

A peripheral blood film showed a leucoerythroblastic picture with 10% blasts on film. Bone marrow aspirate and trephine revealed 15% blasts, which were confirmed as being of myeloid origin by flow cytometry. Morphologically, there was significant dyserythropoiesis in the marrow. Cytogenetic testing by fluorescence *in situ* hybridization (FISH) analysis revealed monosomy 7 detected in 70% of the interphase nuclei. The findings were consistent with the diagnosis of myelodysplasia of the subtype refractory anaemia with excess blasts-2.

Outcome and follow-up

The patient was started on 5-azacitidine therapy. She was re-staged 6-months post

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diagnosis with a bone marrow aspirate and trephine, showing approximately 20%–30% myeloid blasts. Cytogenetic analysis still showed monosomy 7. She had a progressive increase in her peripheral blast count despite continuing 5-azacitidine. Eight months post diagnosis, she died of pneumonia.

CASE 2

A 22-year-old man was referred to our department of haematology following routine blood counts at a local hospital showing an increasing monocytosis and neutrophilia. He had a medical history of ulcerative pancolitis, which was diagnosed 4 years prior to this. His regular medication consisted of azathioprine 125 mg once daily. TPMT enzyme assay levels were within the normal range. His colitis had been relatively quiescent and he had not been exposed to any other immunomodulatory drugs. There was no family history of cancer in his first-degree relatives. Initial blood tests revealed a haemoglobin of 8.8 g/dl, white cell count $12.8 \times 10^9/l$ and monocytes $2.2 \times 10^9/l$. He was asymptomatic at this point. Prior to this, he had been undergoing a routine follow-up, and his blood counts did not reveal any abnormalities. Three months later, he was admitted to his local hospital following a flare of ulcerative colitis, which required hospital admission and treatment with intravenous steroids. A blood count showed a haemoglobin of 10.4 g/dl, white cell count $32 \times 10^9/l$ and monocytes $7.4 \times 10^9/l$. His bone marrow biopsy revealed a hypercellular dysplastic marrow with monosomy 7. Physical examination was unremarkable. Azathioprine was stopped and he was commenced on Asacol 3.8 g daily along with a reducing regimen of prednisolone. He was referred to our centre as there was a concern that he may have developed therapy-related myelodysplasia.

Investigations

Blood tests revealed a haemoglobin of 8.9 g/dl, MCV 92.9, platelets $43 \times 10^9/l$, white cell count $48.9 \times 10^9/l$, neutrophils $5.38 \times 10^9/l$, lymphocytes of $4.4 \times 10^9/l$ and monocytes $28.39 \times 10^9/l$.

Bone marrow aspirate and trephine showed trilineage dysplasia and 12% blasts. Cytogenetic analysis demonstrated monosomy 7 detected in 95% of interphase nuclei by FISH analysis. The findings were consistent with the diagnosis of myelodysplasia of the subtype chronic myelomonocytic leukaemia.

Outcome and follow-up

The patient was admitted electively for induction chemotherapy with the fludarabine, cytarabine and idarubicin regimen, with a view to proceeding to allogeneic stem cell transplantation as definitive therapy. Unfortunately, during chemotherapy, he developed neutropenic sepsis requiring a prolonged intensive treatment unit admission and treatment with broad spectrum antibiotics. Following this, cytomegalovirus

(CMV) colitis was diagnosed after biopsies revealed inclusion bodies with a high CMV PCR DNA titre. The patient was treated with valganciclovir. Subsequent bone marrow aspirate and trephine showed morphological and cytogenetic remission. An unrelated donor was identified as a match allowing the prospect of performing a bone marrow transplant. The patient decided to consider the option of a pan-proctocolectomy prior to proceeding to a bone marrow transplant, in view of the increased risk of transplant-related mortality, should he develop an episode of severe colitis. While considering this option over a period of 5 months, the patient suffered from disease progression with increasing bone marrow blast count, skin and central nervous system infiltration with leukaemia cells and died 8 months post diagnosis.

DISCUSSION

Azathioprine has a number of well-described adverse effects. However, an association between azathioprine treatment and therapy-related myelodysplastic syndrome and acute myeloid leukaemia (t-MDS–AML) is a rare complication reported in the literature.

Kwong¹ reviewed 56 cases of t-MDS–AML secondary to azathioprine. Azathioprine had been administered for a median of 65 months (range 6–192) to a median cumulative dose of 146 g (range 19–750) before t-MDS–AML was diagnosed. Cytogenetic analysis was available in 33 cases, with aberrations of chromosome 7 being the predominant abnormality occurring in 22 cases as either monosomy 7 or deletion of the long arm of chromosome 7. Outcome was poor with the majority of patients having died of the disease at the time of reporting. A median survival of 9 months post diagnosis has been reported in another case series of 14 patients with t-MDS–AML following treatment with azathioprine.²

We report two cases of therapy-related myelodysplasia following azathioprine in patients with ulcerative colitis. There were no other clear predisposing factors for development of MDS. Both patients had previously received short courses of steroids for flares of their ulcerative colitis but had not used any previous medications known to be oncogenic. Karyotype analysis revealed monosomy 7 as the sole aberration in both cases (figure 1). Cytogenetic aberrations involving partial or complete deletions of chromosomes 5 and/or 7 are characteristic findings in t-MDS–AML secondary to alkylating agents or radiotherapy.³ The most common single abnormality is monosomy 7, followed by deletion of the long arm of chromosome 5 and then monosomy 5.⁴

It has been suggested that the risk of developing t-MDS–AML secondary to azathioprine may be dose- and duration related.^{1 5} Case 1 had taken an approximate cumulative dose of 176.5 g over a period of 63 months. Case 2 had taken an approximate

Figure 1 Interphase fluorescence *in situ* hybridization using two probes binding to centromere (green) and long arm of chromosome 7 at position 7q31 (red). Only one is present in each cell indicating monosomy 7 for Case 1 (A) and Case 2 (C). Karyotype with arrow indicating monosomy 7 for Case 1 (B) and Case 2 (D).

cumulative dose of 126 g over a 48-month period. This is similar to the median duration and cumulative dose reported by Kwong.¹ This suggests that leukaemogenesis occurs in most cases after azathioprine has been taken for a prolonged duration.

Another hypothesis is that azathioprine metabolism and individual susceptibility may contribute to the development of t-MDS-AML. TPMT is a key enzyme involved in the metabolism of thiopurine drugs such as azathioprine and 6-mercaptopurine (6-MP).⁶ TPMT exhibits genetic polymorphism. It has been estimated that 1 in 300 individuals has a very low TPMT activity, 11% have intermediate activity and 89% have normal or high activity.^{7, 8} Individuals with a low TPMT activity may be at an increased risk of thiopurine toxicity and an association with the development of t-MDS-AML has been suggested.⁹ However, in our two cases, TPMT enzyme assay levels were within the normal range. Genotyping or measurement of 6-thioguanine levels was not performed.

It is unclear whether there is an increased risk with multiple immunomodulatory therapies. In the case review of 56 patients with t-AML-MDS secondary to

azathioprine (AZA), the majority of patients were taking AZA with concomitant or previous exposure to prednisolone only.¹ Four of the cases had been exposed to cyclophosphamide, which may have contributed to leukaemogenesis. Of the four reported cases of t-AML/MDS secondary to AZA in inflammatory bowel disease (summarised in table 1), not one case had been exposed to immunosuppressive therapy aside from prednisolone.^{10–13} Although in three cases of 6-MP-related AML/MDS, there had been prior use of anti-tumour necrosis factor therapy.^{14, 15}

Early recognition of this complication is challenging. Kwong¹ found that of the 27 cases where previous haematological results were available, 11 cases had cytopenias (a range including isolated anaemia, leucopenia, thrombocytopenia or combinations) preceding the diagnosis of t-MDS-AML.¹ The cytopenias recovered after cessation of azathioprine which was subsequently reintroduced. It is important to note that cytopenias are not uncommon during azathioprine treatment particularly when compared with other immunosuppressants used in the treatment of ulcerative colitis.¹⁶ In a cohort of 739 patients with

Table 1 Characteristics of patients with t-MDS–AML and Crohn’s disease

Age	Gender	Cumulative dose (g)	Duration of treatment (months)	TPMT genotype/ assay levels	Other immunosuppressants	Haematological diagnosis	Cytogenetics	Outcome (months)	Reference
42	M	35	NA	Not performed	Prednisolone	MDS	–Y, +8, +9	Alive, 12	¹¹
43	M	7.5	1	Not performed	Prednisolone	AML (FAB M1)	–21	Alive, 12	¹³
21	F	109	36	Heterozygous *3A variant	Prednisolone	AML with multilineage dysplasia	–7, Complex abnormalities	Dead, 3	¹²
71	M	NA	84	No mutation/ normal level	Nil	AML with multilineage dysplasia	–7, Complex abnormalities	Dead, 9	¹⁰

NA, not available; t-MDS–AML, myelodysplastic syndrome and acute myeloid leukaemia; TPMT, Thiopurine methyltransferase.

inflammatory bowel disease (IBD) treated for a median of 12.5 months, 5% developed bone marrow toxicity.¹⁷ Leucopenia appeared to be the most common abnormality occurring in 3.8% and 4.6% of the patients in two large IBD cohorts.^{17–18} A more recent retrospective cohort study of 1997 patients reported incidence of severe leucopenia, neutropenia and thrombocytopenia to be 0.16, 0.51 and 0.08 per 100 person-months, respectively.¹⁹ Our two cases underwent regular monitoring and did not display any preceding haematological abnormalities on blood counts. When presented with cytopenias in practice, it would be prudent to review peripheral blood films and consider t-MDS–AML as a differential.

Patients with IBD have an increased incidence of haematological malignancies, including MDS and AML, even prior to a widespread use of immunomodulatory therapy. In a prospective population-based cohort study of 47 679 Swedish patients with IBD, a significantly higher than expected risk of myeloid leukaemia was found in patients with ulcerative colitis.²⁰ The study period covered four decades (1969–2001) allowing assessment of variations in risk over time, there was a non-significant tendency towards higher risks for myeloid leukaemia in ulcerative colitis (UC) during the 1990s; however, information about pharmacotherapy was not available. In another US-based cohort of 15 000 patients with IBD, 25 patients were diagnosed with MDS of which 11 had UC. None of these patients had been treated with thiopurines.²¹

An increased risk of lymphoma with thiopurine therapy in patients with IBD has been described in a number of studies.^{22–23} However, the less well-recognised complication of t-MDS–AML has been reported in a small number of IBD patients treated with azathioprine^{10–13} or 6-MP.^{14–15–24} These cases display characteristic cytogenetic hallmarks indicative of t-MDS–AML. To the best of our knowledge, these are the first-reported cases of azathioprine-associated MDS–AML in patients with ulcerative colitis. Further large observational studies are required to evaluate the possible mechanism of azathioprine-related

leukaemogenesis and associated risk factors. Given the small number of reported cases, it is likely that the benefit of thiopurine therapy in IBD still outweighs the risk of developing t-MDS–AML. However, the subset of patients who develop t-MDS–AML have a particularly poor prognosis; it is therefore imperative that clinicians remain vigilant for this rare complication particularly in patients on long-term azathioprine therapy. Patients who develop unexpected cytopenias should undergo prompt haematological assessment.

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