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Research Article

Malignancy Risk and Safety Issues of Azathioprine in Patients with Inflammatory Bowel Disease: Clinical Experience from a Referral Center

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Abstract

Background: Despite the development of new agents for the treatment of inflammatory bowel disease (IBD), azathioprine (AZA) is widely used as monotherapy or combination therapy for the induction and maintenance of remission in both Crohn's disease (CD) and ulcerative colitis (UC). The aim of this study was to investigate both the prevalence of malignancy development in IBD patients receiving AZA as well as long-term safety of AZA and the management of adverse events.

Methods: Data were retrospectively extracted from the patient records of the Gastroenterology Department of University hospital of Ioannina from 2003 to 2019.

Results: 222 IBD patients treated with AZA, under regular follow-up intervals were included in the study. 65.6% of IBD patients (146/222) had CD and 34.4% had UC (76/222). The mean duration of AZA administration was 51 months (range: 0-135 months). The most common dose of AZA was 100mg/day (range: 50-200mg). The most common adverse events were gastrointestinal symptoms (8.6%, 18/222), leucopenia (2.7%, 6/222), hepatotoxicity (5.4%, 12/222) and pancreatitis (1.8%, 4/222). Two patients were diagnosed with basal cell cancer, while no hematological malignancy was detected. Infections were recorded in 19 patients (8.6%) of whom 6 needed hospitalization.

Conclusion: According to this retrospective observational study, approximately one-third of IBD patients receiving AZA presented clinical significant adverse event during follow-up. Azathioprine withdrawal or dose reduction was needed in 47 (21%) patients.

Keywords: *Azathioprine; Safety; Thiopurine; Inflammatory Bowel Disease; Ulcerative Colitis; Crohn's Disease*

Introduction

In recent decade, the development of anti-TNF agents, vedolizumab, ustekinumab and tofacitinib have revolutionized the treatment of inflammatory bowel disease (IBD). However, the use of azathioprine (AZA), both as monotherapy or combination therapy, remains a significant part of IBD treatment, offering induction and maintenance of remission both Crohn's disease (CD) and ulcerative colitis (U) and is widely used in IBD treatment [1,2].

Nevertheless, concerns regarding the safety of AZA use, mainly as combination therapy and the optimizing of azathioprine therapy present a usual clinical challenge. It is estimated that up to 15% of patients receiving AZA may present adverse reactions, leading to dose reduction or drug withdrawal. These adverse events may be divided into dose-dependent, such as nausea and myelotoxicity and dose-independent, such as pancreatitis [3,4] (Table 1). The activity of thiopurine methyltransferase (TMPT) seems to play a significant role to the myelotoxicity development in patients with AZA. TMPT metabolizes azathioprine to inactive metabolites and its activity varies, presenting a trimodal distribution; high, reduced and very low activity. There is an inverse association between TMPT activity and myelotoxicity development. Patients with reduced or very low activity have a greater risk of AZA-induced myelotoxicity [5].

Dose independent adverse reactions
Rash
Arthralgia
Hepatitotoxicity
Flu-like symptoms
Pancreatitis
Nausea
Dose dependent adverse reactions
Myelotoxicity
Nodular regenerative hyperplasia
Veno-oclusive disease
Hepatic peliosis

Table 1: *Adverse events of azathioprine.*

Furthermore, AZA use have been associated with a higher risk of malignancies and infections [6]. Many studies have conducted that IBD patients receiving thiopurines have an increased risk of lymphoid tissue cancer and skin cancer via damage to DNA, alteration of immune control of chronic viral infection and decreased immunosurveillance of cancer cells [7]. In addition, AZA has been associated with a higher risk of opportunistic infection, serious infection [8] and tuberculosis reactivation [9].

Aim of this study is to investigate the long-term safety of AZA, to document the prevalence of adverse events of AZA in patients with IBD, to identify possible predictive factors and describe the management of these adverse events. Furthermore, a great emphasis has been placed on documentation of malignancies development in these patients.

Methods

In this retrospective study, we included 222 patients with IBD, who were monitored at University hospital of Ioannina from 2003 to 2019. The diagnosis of IBD was made based on endoscopic and histologic findings after ileocolonoscopy with biopsies. Inclusion criteria of study were diagnosis of IBD and regular follow-up intervals with laboratory tests at least every six month after start of AZA administration. A registry of duration and location of IBD, extra-intestinal manifestations, AZA-related adverse events and their management was made.

Definitions of adverse events

AZA-induced pancreatitis: several abdominal pain, elevation of serum amylase three times above the upper limit and exclusion other causes, e.g. cholelithiasis. In this study, re-challenge test was not performed.

Rash: new onset rash after AZA administration that resolved after AZA withdrawal

Leucopenia: white blood cell count <3.5 x 10^9/L

Neutropenia: neutrophil count < 1.5 x 10^9/L

Thrombocytopenia: platelet count <150 x 10^3/μL

Bone marrow suppression: anemia, leucopenia and thrombocytopenia.

Gastrointestinal manifestations: nausea, vomiting.

AZA-induced hepatotoxicity: abnormal liver enzymes after AZA administration, when other causes of liver injury have been excluded, such as primary sclerosing cholangitis and viral hepatitis. Furthermore, we used the Roussel Uclaf Causality Assessment Method (RUCAM) for the definition of drug-induced liver injury (DILI)

Infection

Infection and malignancies development after AZA administration.

Statistical analysis

Data were analyzed using SPSS software version 23. All clinical data were categorized as either categorical or continuous variables. Continuous variables were categorized as means and standard deviation.

Results

The mean age of patients starting AZA was 46.9 years (Std. Dev. 17,9) at start of AZA administration. 65.6% of IBD patients (146/222) had CD and 34.4% had UC (76/222). 62.1% of patients were males and 37.8% were females. The mean duration of AZA administration was 51 months (range: 0 - 135 months), while the most common indication of AZA use was the induction of remission both in UC and in CD. Additionally, most patients started AZA treatment within five years after IBD diagnosis (Table 2).

	Patients with ulcerative colitis	Patients with Crohn's disease
Localization of IBD	6.6% (5/76) Proctitis 54.0% (41/76) Left sided colitis 39.4% (30/76) Pancolitis	35.6% (52/146) Ileitis 26.0% (38/146) Colitis 38.4% (56/146) Ileitis and colitis
Sex (Male/Female)	48 (63%) /28 (37%)	90 (61%) /56 (39%)
Indication of AZA use Induction of remission Maintenance of remission Prophylaxis of postsurgical recurrence	 63% (48/76) 37% (28/76) -	 55% (81/146) 42% (60/146) 3% (5/146)
Start of AZA treatment after IBD diagnosis <5 years 6-10 years > 10 years	 72% (55/76) 21% (16/76) 7% (5/76)	 77% (112/146) 16% (23/146) 7% (11/146)

Table 2: *Characteristics of IBD patients receiving azathioprine.*

The most common dose of AZA was 100mg/day (1.74mg/kg, range: 50 - 200 mg). Approximately a quarter of patients presented an adverse effect related to AZA treatment. The most common adverse events were gastrointestinal symptoms (8.6%, 18/222), leucopenia (2.7%, 6/222), hepatotoxicity (5.4%, 12/222) and pancreatitis (1.8%, 4/222) (Table 3). Gastrointestinal symptoms, such as nausea and mild abdominal pain, were the most frequent adverse event, leading to 83% (15/18) of patients to AZA discontinuation. 8 of these patients suffered from UC and ten from CD. The symptoms resolved a few days to weeks after AZA withdrawal. The mean duration of symptoms to drug discontinuation was 3 weeks (range: 0-13 weeks). In two patients, symptoms improved after dose reduction, while one patient continued to receive AZA with symptoms relief after two months.

Adverse event	Number of patients, Incidence (%)
Gastrointestinal symptoms	18 (8.1)
Leucopenia	6 (2.7)
Hepatotoxicity	12 (5.4)
Bone marrow failure	3 (1.4)
Pancreatitis	4 (1.8)
Thrombocytopenia	2 (0.9)
Arthralgia	2 (0.9)
Alopecia	2 (0.9)
Infection	19 (8.6)
Malignancy	2 (0.9)

Table 3: *Adverse events related to azathioprine use.*

According to revised Atlanta classification, all cases of AZA-induced pancreatitis were mild and all patients were treated with AZA withdrawal and adequate intravenous fluid resuscitation. Re-challenge test was not performed and the median time of acute pancreatitis onset after AZA administration was 18 days.

Furthermore, AZA caused hepatotoxicity in 12 patients, while none of whom had preexisting liver disease. DILI occurred in the first weeks (range: 3 - 9 weeks). After AZA discontinuation, the liver function tests were returned to normal. According to R factor (serum alanine aminotransferase (ALT) / upper limit of normal (ULN) divided by serum alkaline phosphatase/ ULN), the pattern of DILI was hepatotoxicity (R > 5) in 10 out of 12 cases, in two cases the pattern of liver injury was mixed (R: 2 - 5), while no cholestatic pattern was observed.

Hematological adverse events were documented in 8% (11/222) of patients. Mild leucopenia (white blood cell count from 2 to $3x10^9$/L) occurred in six patients. No infection was detected in these patients with a median follow-up period of 23 months (range: 10 - 38 months). In addition, mild thrombocytopenia (platelet count from 100 to 150 x 10^3/μL) was detected in two patients. In addition, three cases of bone marrow failure occurred.

During AZA treatment, 19 cases of infection were documented of whom six patients needed hospitalization. Two out of six patients suffered from pyelonephritis and were females; one patient had acute prostatitis, while the other three suffered from respiratory infection. Four out of six patients received azathioprine with anti-TNF agent.

Two cases of basal cell cancer were documented, which were treated with local surgery. These patients suffered from UC and treated with AZA and mesalazine, while the duration of AZA administration was seven and eight years and the dose of AZA was 2mg/kg AZA in both.

Overall, adverse events development leaded to drug withdrawal or doe reduction in 21% (47/222) of patients. The most common cause was nausea. The table 4 presents the frequency rates of discontinuance or dose reduction in relation to adverse events (Table 4).

Adverse Events	Patients N (%)	Azathioprine Discontinuance N (%)	Dose Reduction N (%)
Gastrointestinal symptoms	18 (8.1)	15 (83)	2 (11)
Hepatotoxicity	12 (5.4)	12 (100)	-
Bone marrow failure	3 (1.4)	3 (100)	-
Pancreatitis	4 (1.8)	4 (100)	-
Arthralgia	2 (0.9)	1 (50)	1 (50)
Alopecia	2 (0.9)	-	2 (100)
Infection	19 (8.6)	2 (11)	3(16)
Malignancy	2 (0.9)	2 (100)	-

Table 4: *Frequency rate of discontinuation or dose reduction due to adverse events.*

Discussion

In this study, the frequency rate of adverse events of azathioprine in IBD patients was 22% (49/222). Gastrointestinal symptoms, such as nausea and abdominal pain, were the most frequent AZA-related adverse event, leading to the AZA discontinuation in 6.7% of patients. It seems that gastrointestinal symptoms frequently cause AZA intolerance and their frequency rate ranges from 5% to 8% [10,11], while their pathogenesis appears to be associated with AZA-imidazole ring and is independent of TMPT activity [12].

Myelotoxicity of AZA appears to be a common and dose dependent adverse event, causing bone marrow failure, thrombocytopenia and leucopenia. According to a meta-analysis, the cumulative incidence of AZA-induced azathioprine is 7% (CI 95%; 6%-8%) [13]. In our study, the frequency rate was 5% and the leucopenia was the most frequent manifestation of myelotoxicity. In a long-term follow-up

cohort study of 3931 patients, leucopenia was the most common hematological adverse event of thiopurine use with a prevalence of 4% (162/3931); however, only 6 patients discontinued thiopurine use [11]. In our study, AZA therapy modification due to leucopenia was not performed and none of patients with leucopenia suffered from infection. As mentioned above, the low activity of TMPT is associated with myelotoxicity development; however, we did not perform measurement of TMPT activity due to no availability. It is worth noting that myelotoxicity may be developed at any time after AZA administration with a range from a few days to 27 years; however, the most cases have been described during the early period of AZA treatment [13]. In cases of bone marrow failure, blood counts returned to normal within two months after drug withdrawal. In the cases of thrombocytopenia, splenomegaly should be excluded due to potential nodular regenerative hyperplasia and non-cirrhotic portal hypertension development [14].

AZA-induced hepatotoxicity may include mild elevation of liver enzymes, nodular regenerative hyperplasia, peliosis and veno-occlusive disease. It seems that three possible mechanisms may contribute to liver injury: idiosyncratic reaction, hypersensitivity and endothelial cell injury [15]. The prevalence of AZA-induced liver damage ranges from 3% to 10% and the most cases of hepatotoxicity are mild and asymptomatic rises of transaminases in serum [16]. In this study, 5.4% of patients developed AZA-induced DILI.

Regarding the pancreas, acute pancreatitis was observed in three patients with CD and one patient with UC. Acute pancreatitis is the most common pancreatic manifestation of IBD with an incidence rate from 1.4% to 4.3% depending on follow-up period of study and IBD group [17]. In our study the incidence rate of AZA-induced acute pancreatitis was lower compared to the literature. In a Spanish retrospective multicenter study the incidence rate of thiopurine-induced pancreatitis was 3.1% [18], while a prospective multicenter study from Germany detected an incidence rate of 7.3% [19].

Many studies have been suggested that AZA may increase the risk of serious and opportunistic infections both as monotherapy but in particular as combination therapy with anti-TNF agents [20]. Previous studies have reported a prevalence of infections in patients receiving thiopurines between 0.3% and 7.4% and the most frequent are viral infections, such as CMV, Epstein-Barr virus and varicella zoster virus [21]. In our study, the prevalence of infection during AZA treatment was 8.6% and the most patients, who needed hospitalization, received combination therapy. In the era of COVID-19 pandemic, thiopurine use has been associated with a higher risk of severe COVID-19 [22]. This study collected data until 2019 and there are no data about AZA and COVID-19.

Furthermore, many studies have conducted that patients receiving AZA may have a higher risk of lymphoid tissue cancer and skin cancer [7], via damage to DNA, alteration of immune control of chronic viral infection and decreased immunosurveillance of cancer cells [23]. Risk factors of lymphoma development seem to be combination therapy with anti-TNF agent, Epstein Bar virus infection during thiopurine therapy and male gender [24]. Due to increased risk of thiopurines-associated lymphoma increases in uninfected patients from Epstein-Barr virus (EBV) when they are infected during thiopurine therapy, it has been recommended that to test for EBV antibodies prior to thiopurine exposure and to avoid using thiopurines in uninfected patients [24]. In this study, no cases of hematological malignancy were detected.

Regarding skin cancer development, a recent meta-analysis described that IBD patients have 1.88-fold higher risk of overall skin cancer after thiopurine exposure (RR = 1.88, 95% CI: 1.48-2.38, $P < 0.001$), while no increased risk for melanoma skin cancer was observed (RR = 1.22, 95% CI: 0.90-1.65, P = 0.206) [25]. Current evidence has suggested a duration-dependent effect of AZA on skin cancer development. A Canadian case-control study found that the use of thiopurines for more than 5 years is associated with increased risk of skin cancer development (odds ratio: 1.78; 95% CI: 1.25-2.54) [26]. However, this increased risk of nonmelanoma skin cancer seems to decrease or return to baseline after withdrawal of thiopurines [27]. In our study, two cases of basal cell cancer were documented, while the both patients received AZA over five years. Therefore, IBD patients receiving azathioprine should be informed for the higher risk of skin cancer in order to use daily sun protective measures, while regular skin cancer screening may be benefit in this IBD group [28].

Furthermore, two patients complained for hair loss after AZA initiation (2mg/kg/day). They suffered from UC and received also mesalazine for five months before AZA treatment. Other causes of hair loss, such as low folic acid or serum iron, were excluded. Hair loss was improved after reduction of dose (1mg/kg/day). AZA-induced alopecia has been associated with NUDT15 polymorphisms [29].

The lack of control group and the retrospective design of study should be reported as a limitation as well as the inclusion of patients from a single center.

Conclusion

In this retrospective single center study, the prevalence of adverse events was 31% and the most common adverse reactions were gastrointestinal symptoms followed by hepatotoxicity, leucopenia and pancreatitis. Discontinuation or dose reduction of AZA treatment was required in 21% of patients, while 2.7% of patients needed hospitalization due to infection development. Our findings are in agreement with previous studies. Therefore, close clinical and laboratory monitoring are required in patients receiving AZA and personalized risk-benefit assessment based on disease severity and extend, comorbidities and history of EBV infection is essential in order to optimizing IBD treatment.

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Data Availability

Data was generated at the University hospital Ioannina, Greece. The data that support the fndings of this study are available on request from the corresponding author.

Declarations Conflict of Interest

The authors have no conflict of interest to declare that are relevant to the content of this article.

Bibliography

1. Torres J., *et al*. "ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment". *Journal of Crohn's and Colitis* 14.1 (2020): 4-22.
2. Raine T., *et al*. "ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment". *Journal of Crohn's and Colitis* (2021).
3. Jong DJd., *et al*. "Safety of thiopurines in the treatment of inflammatory bowel disease". *Scandinavian Journal of Gastroenterology* 38 (2003): 69-72.
4. De Boer NKH., *et al*. "Drug Insight: pharmacology and toxicity of thiopurine therapy in patients with IBD". *Nature Clinical Practice Gastroenterology and Hepatology* 4.12 (2007): 686-694.
5. Gisbert JP., *et al*. "Thiopurine Methyltransferase (TPMT) Activity and Adverse Effects of Azathioprine in Inflammatory Bowel Disease: Long-Term Follow-Up Study of 394 Patients". *The American journal of Gastroenterology* 101.12 (2006): 2769-2776.
6. Goel RM, Blaker P., *et al*. "Optimizing the use of thiopurines in inflammatory bowel disease". *Therapeutic Advances in Chronic Disease* 6.3 (2015): 138-146.

7. Pasternak B., *et al*. "Use of Azathioprine and the Risk of Cancer in Inflammatory Bowel Disease". *American Journal of Epidemiology* 177.11 (2013): 1296-1305.

8. Wisniewski A., *et al*. "Increased incidence of systemic serious viral infections in patients with inflammatory bowel disease associates with active disease and use of thiopurines". *United European Gastroenterology Journal* 8.3 (2020): 303-313.

9. Lorenzetti R., *et al*. "Higher risk of tuberculosis reactivation when anti-TNF is combined with immunosuppressive agents: a systematic review of randomized controlled trials". *Annals of Medicine* 46.7 (2014): 547-554.

10. Moran GW. "Clinical predictors of thiopurine-related adverse events in Crohn's disease". *World Journal of Gastroenterology* 21.25 (2015): 7795.

11. Chaparro M., *et al*. "Safety of Thiopurine Therapy in Inflammatory Bowel Disease". *Inflammatory Bowel Diseases* 19.7 (2013): 1404-1410.

12. McGovern DPB., *et al*. "Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity". *Gastroenterology* 122.3 (2002): 838-839.

13. Gisbert JP and Gomolln F. "Thiopurine-Induced Myelotoxicity in Patients With Inflammatory Bowel Disease: A Review". *The American Journal of Gastroenterology* 103.7 (2008): 1783-1800.

14. De Boer NKH., *et al*. "Thiopurines in Inflammatory Bowel Disease: New Findings and Perspectives". *Journal of Crohn's and Colitis* 12.5 (2018): 610-620.

15. Calabrese E and Hanauer SB. "Assessment of non-cirrhotic portal hypertension associated with thiopurine therapy in inflammatory bowel disease". *Journal of Crohn's and Colitis* 5.1 (2011): 48-53.

16. Fousekis FS., *et al*. "Hepatobiliary Manifestations and Complications in Inflammatory Bowel Disease: A Review". *Gastroenterology Research* 11.2 (2018): 83-94.

17. Fousekis FS., *et al*. "Pancreatic Involvement in Inflammatory Bowel Disease: A Review". *Journal of Clinical Medicine Research* 10.10 (2018): 743-751.

18. Bermejo F., *et al*. "Acute pancreatitis in inflammatory bowel disease, with special reference to azathioprine-induced pancreatitis". *Alimentary Pharmacology and Therapeutics* 28.5 (2008): 623-628.

19. Teich N., *et al*. "Azathioprine-induced Acute Pancreatitis in Patients with Inflammatory Bowel Diseases-A Prospective Study on Incidence and Severity". *Journal of Crohn's and Colitis* 10.1 (2016): 61-68.

20. Kirchgesner J., *et al*. "Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases". *Gastroenterology* 155.2 (2018): 337-346.

21. Ardizzone S., *et al*. "Review: Immunomodulators for all patients with inflammatory bowel disease?" *Therapeutic Advances in Gastroenterology* 3.1 (2010): 31-42.

22. Ungaro RC., *et al*. "Effect of IBD medications on COVID-19 outcomes: results from an international registry". *Gut* 70.4 (2021): 725-732.

23. Annese V., *et al*. "Impact of New Treatments on Hospitalisation, Surgery, Infection, and Mortality in IBD: a Focus Paper by the Epidemiology Committee of ECCO". *Journal of Crohn's and Colitis* 10.2 (2016): 216-225.

24. Nakase H., *et al*. "Evidence-based clinical practice guidelines for inflammatory bowel disease 2020". *Journal of Gastroenterology* 56.6 (2021): 489-526.

25. Huang S-Z., *et al*. "Risk of skin cancers in thiopurines-treated and thiopurines-untreated patients with inflammatory bowel disease: A systematic review and meta-analysis". *Journal of Gastroenterology and Hepatology* 34.3 (2019): 507-516.

26. Kopylov U., *et al*. "Risk of Lymphoma, Colorectal and Skin Cancer in Patients with IBD Treated with Immunomodulators and Biologics". *Inflammatory Bowel Diseases* 21.8 (2015): 1847-1853.

27. Hagen JW and Pugliano-Mauro MA. "Nonmelanoma Skin Cancer Risk in Patients With Inflammatory Bowel Disease Undergoing Thiopurine Therapy: A Systematic Review of the Literature". *Dermatologic Surgery* 44.4 (2018): 469-480.

28. Giagkou E., *et al*. "Dermal Lesions and Skin Cancer in Patients with Inflammatory Bowel Disease Receiving Immunosuppressive Therapy". *Asian Pacific Journal of Cancer Prevention: APJCP* 19.10 (2018): 2845-2851.

29. Nomura H., *et al*. "Azathioprine-induced alopecia and leukopenia associated with NUDT15 polymorphisms". *Journal of the European Academy of Dermatology and Venereology* 32.10 (2018): e386-e389.

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