

Lymphopenia in Inflammatory Bowel Disease Patients on Immunosuppressive Medications

Bincy P Abraham, Joseph H Sellin

Bincy P Abraham, Houston Methodist Hospital, The United States
Joseph H Sellin, Baylor College of Medicine, Houston, TX, The United States

Correspondence to: Bincy P Abraham, Houston Methodist Hospital 6550 Fannin St. Suite 1001 Houston, TX 77030, The United States.

Email: bpabraham@houstonmethodist.org

Telephone: 713-441-0006

Fax: 713-797-0622

Received: June 28, 2015

Revised: July 24, 2015

Accepted: July 29, 2015

Published online: January 12, 2016

ABSTRACT

Azathioprine and 6-mercaptopurine (AZA/6MP) are effective immunosuppressives used for the treatment of inflammatory bowel disease. The optimal strategy for monitoring therapeutic efficacy and adequate immunosuppression remains to be defined. White blood cell count, mean corpuscle volume (MCV), and 6-mercaptopurine metabolites (6TGN, 6MMP) have all been suggested as potential parameters to monitor efficacy and safety. However, immunomodulators target lymphocytes specifically rather than leukocytes overall; little data is available on the relationship between lymphocytes and immunomodulators in IBD. This study evaluates the incidence of lymphopenia with immunosuppressive use, and correlates metabolite levels (6TGN and 6MMP) with leukocyte, lymphocyte, and MCV levels in IBD patients treated with AZA/6MP. Medical records of 125 adult IBD patients were analyzed based on the use of AZA/6MP, blood counts, and mercaptopurine metabolites 6TGN and 6MMP. Forty three percent of patients taking AZA/6MP developed lymphopenia. Of those with lymphopenia, only 11% had leukopenia. A low but statistically significant correlation of $r = -0.3$ ($p=0.04$) for 6TGN to leukocyte counts as well as to MCV levels was found. No correlation between 6TGN levels and lymphocyte counts was noted. This analysis shows that not only leukocyte counts

but lymphocyte counts should be monitored closely in patients on these immunosuppressives. Metabolite markers do not provide information regarding who will develop lymphopenia. Consequences of lymphopenia such as opportunistic infections need to be discussed with patients prior to starting this type of therapy.

© 2016 ACT. All rights reserved.

Key Words: Lymphopenia; Leukopenia; Immunosuppression; Mercaptopurine metabolites; Inflammatory Bowel Disease; Azathioprine; 6-mercaptopurine

Abraham BP, Sellin JH. Lymphopenia in Inflammatory Bowel Disease Patients on Immunosuppressive Medications. *Journal of Gastroenterology and Hepatology Research* 2016; 5(1): 1890-1894 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/1564>

INTRODUCTION

Approximately 1.1 million people in the United States suffer from IBD^[1]. The key to controlling symptoms and complications from IBD is to induce remission and maintain control of the inflammation. Azathioprine (AZA) and 6-mercaptopurine (6MP) are effective immunosuppressive medications used for the treatment of IBD, with about 70% of patients showing improvement of symptoms on these medications^[2]. The exact mechanism of action of these drugs has not been fully delineated; however, apoptosis of activated T lymphocytes may play an important role^[3,4]. Although it is known that leukopenia and lymphopenia can occur in patients taking AZA/6MP^[5], we have observed that lymphopenia defined by absolute lymphocyte count <1000 , can occur in these patients despite having normal leukocyte counts.

No standard of care exists on optimal monitoring for safety and efficacy of AZA/6MP use. Over the last several decades, multiple

laboratory parameters have been employed as surrogates to guide dosing of immunomodulators, including white blood cell (WBC) counts, mean corpuscle volume (MCV) levels and the mercaptopurine metabolites 6-thioguanine (6TGN) and 6-methylmercaptopurine ribonucleotides (6MMP).

WBC counts have traditionally been monitored. Since AZA/6MP specifically target lymphocytes, one might suspect that pharmacologic effect may be more evident on tracking lymphocytes. However there are no recommended guidelines on following lymphocytes.

Increased mean corpuscular volume (MCV) has also been found in patients taking AZA/6MP. Since MCV represents the volume of the RBC, and 6TGN is found inside RBC's, higher MCV may correlate to higher 6TGN levels. A Danish study did show a statistically significant correlation between MCV levels and 6TGN metabolite levels, but only with an *r*-value of 0.33^[6]. Since the routine CBC includes MCV, it would be clinically useful to determine if any higher correlation could be found to make this of any clinical significance.

In the era before drug metabolite measurement and the establishment of a therapeutic window for 6TGN levels, clinicians followed WBC counts to gauge the likelihood of response and toxicity. However, maintaining leukopenia (<3,000) does not necessarily correlate with clinical response^[7]. In a study by Dubinsky and others, only 8% of clinical responders were found to have leukopenia^[8]. Thus, maintained leukopenia is unnecessary and potentially dangerous. In addition to an increased risk of infections, data suggest an increased risk of malignancy, especially lymphoma, in patients maintained in a leukopenic state with AZA or 6MP for treatment of IBD^[7].

The AZA/6MP metabolites, 6TGN and 6MMP, have been suggested as surrogate markers for the safety and efficacy of immunosuppressive therapy. 6TGN levels may reflect therapeutic efficacy with an optimal range is 235-400 pmol/8 x 10⁸ RBC^[7]. Higher levels would indicate a greater risk of myelotoxicity. Whereas leukopenia may suggest that the 6TGN level is high (usually >450 pmol/8 x 10⁸ RBC), there is no evidence that such myelotoxic levels yield higher efficacy than 6TGN in the target range^[7]. A level below 235 pmol/8 x 10⁸ RBC suggests suboptimal dosing that may lead to inadequate response to treatment^[7]. On the other hand, 6MMP levels greater than 5700 pmol/8 x 10⁸ RBC indicate an increased risk of hepatotoxicity^[2].

If metabolite levels do correlate to these routine labs or even to clinical activity, monitoring these levels can better guide us in optimizing the safety of the drug as well as in assessing patients' disease activity. Others have suggested that clinical algorithms based on routine labs may provide equivalent information at less cost^[9].

The first aim this study is to determine the incidence of lymphopenia (defined by absolute lymphocyte count <1000) in patients with IBD with and without treatment using the immunosuppressives AZA/6MP. The second aim of this study is to determine how closely the metabolite markers 6TGN and 6MMP correlate with WBC, lymphocyte counts, MCV and, in severely lymphopenic patients, CD4⁺ & CD8⁺ counts.

METHODS

Patients

The study population includes 125 patients with inflammatory bowel disease from our Gastroenterology clinics. Patients were consecutively included into the study if they met the following criteria: adults (18 years of age or older) with biopsy proven IBD, and taking either azathioprine or 6-mercaptopurine for a minimum

of 6 months of therapy. Patients were started on this medication as per standard of care based on physician global assessment. If they had active disease, and failing mesalamine therapy or were steroid-dependent, the immunomodulator was started by the provider. All patients underwent TPMT testing and was started on weight based dosing accordingly. Normal TPMT activity patients was started on 2.5mg/kg for AZA and 1.5mg/kg for 6MP, and intermediate metabolizers had half the weight based dosing to optimize efficacy. Patients were excluded from the study if they were less than 18 years of age, pregnant, those with non-IBD colitis, those with either congenital or acquired immunodeficiency, or those who had a history of allergy or adverse reaction to AZA/6MP. This study was approved through our institutional IRB for retrospective chart review.

Data Extraction

The medical records of the patients that met the above inclusion criteria were reviewed and followed for 2 years. Variables measured include the use of all IBD therapies, specifically the use of the immunosuppressives AZA/6MP, 6TGN & 6MMP levels, leukocyte counts, MCV levels, and lymphocyte counts. CD4⁺ counts were measured in a select group of patients who were profoundly lymphopenic (absolute lymphocyte count <500). All other medications especially corticosteroid and anti-TNF use was noted.

Data Analysis

The number of IBD patients taking the immunosuppressives AZA/6MP and those who were not were identified. Based on this medication use, the incidence of lymphopenia was determined. The metabolite markers 6TGN and 6MMP levels were correlated with WBC counts, lymphocyte counts, MCV levels, and in the severely lymphopenic patients who had them drawn, CD4⁺ & CD8⁺ counts. Since 6MMP is a marker for hepatotoxicity and not for bone marrow toxicity, this metabolite served as a surrogate control. Clinical activity based on controlled or uncontrolled disease was analyzed in regards to metabolite levels and the routine blood levels as above.

Analysis was based on a correlation coefficient using Spearman's correlation. A two-tailed probability calculated with the appropriate degrees of freedom was used to obtain the statistical significance.

RESULTS

Incidence of Lymphopenia

A total of 125 IBD patients were found to have data regarding routine blood tests (CBC) and metabolite markers, and thus, were analyzed. A total of 73 males, 52 females were evaluated with 71 patients diagnosed with CD, 52 with UC, and 2 with IC.

Fifty-three of 125 patients (42%) were found to have mild lymphopenia (absolute lymphocyte count less than 1000). Forty-four (35% of 125 patients) had moderate lymphopenia (absolute lymphocyte count less than 850), and 19 patients (15%) had severe lymphopenia (absolute lymphocyte count less than 500). (See Table 1)

Of the 125 patients analyzed, 79 (63%) were taking the immunosuppressive AZA/6MP. Duration of treatment ranged from 6 months to 11.5 years. Of the 79 patients on AZA/6MP, 45 (57%) had developed mild lymphopenia, 39 (49%) had moderate lymphopenia and 17 (22%) had developed severe lymphopenia. Of the 46 patients not on AZA/6MP, 8 patients (10%) developed mild lymphopenia, 6 had moderate and 3 had severe lymphopenia. (See Table 2)

On evaluation of concurrent medications, 53 patients were on mesalamines, with 10/53 on them currently when lymphopenia occurred. However the majority were on mesalamines prior to starting immunomodulator therapy. Only four of 23 patients on

Table 1 Incidence of Lymphopenia in all IBD patients.

Level of lymphopenia	Patients
Mild Lymphopenia <1000	53/125 (42%)
Moderate lymphopenia <850	44/125 (35%)
Severe Lymphopenia <500	19/125 (15%)

Table 2 Incidence of Lymphopenia in IBD patients on AZA/6MP.

Level of lymphopenia on AZA/6MP (79)	Patient Number
Mild Lymphopenia <1000	45/79 (57%)
Moderate lymphopenia <850	39/79 (49%)
Severe lymphopenia <500	17/79 (22%)

infliximab, had lymphopenia. Three of the 4 patients were already lymphopenic prior to induction. Seven of 22 patients on prednisone were lymphopenic. Out of those 7, four had lymphopenia prior to start of corticosteroid therapy. A total of 2 patients were on budesonide, but both were lymphopenic prior to starting therapy.

Of the 44 patients with moderate lymphopenia (absolute lymphocyte count < 850), only 3 patients (7%) were leukopenic (WBC count < 3,000/mm³). Thus, leukopenia does not predict lymphopenia. In the patients found to be lymphopenic, AZA/6MP was used as treatment by 84% of them. However, 16% (7/44) of the lymphopenic were not using immunomodulators.

Seventy-nine (63%) of the patients evaluated were on chronic AZA/6MP therapy. Of those taking AZA/6MP, 39 (49%) patients developed lymphopenia, giving a relative risk of 3.39 with 95% CI (1.7 to 7.0). The use of AZA/6MP did not play a role in who developed leukopenia. Of those taking AZA/6MP and found to be lymphopenic, only 5% were leukopenic.

Serious Infections

In five patients, severe lymphopenia developed and CD4 counts, range (52 to 361), were monitored closely during this time. Two of these patients developed significant infections. Although the number of patients was small, multiple measurements of CD4 counts took place and these levels were analyzed with WBC and lymphocyte levels. Lymphocyte levels and CD4 counts were closely related $r=0.95$ ($p<0.000001$), however, WBC counts and CD4 counts were not ($r=-0.27$, $p=0.17$). In one patient, despite undetectable levels of 6TGN after discontinuation of azathioprine, lymphopenia was prolonged and took over 6 months to return to normal range. This patient had a CD4 count as low as 84 during treatment on azathioprine and developed herpes zoster infection. Interestingly, this patient maintained a normal leukocyte count (range 5700 to 11,200) prior to, during, and after the infection despite the low CD4 counts and lymphopenia. Therefore, we believe the lymphopenia could have contributed to his infection. At the time of initial diagnosis of lymphopenia, the patient's disease was under control. However, when the lymphocyte levels normalized, the ulcerative colitis worsened.

A second patient with pre-existing lymphopenia (lymphocyte count of 360 on 20 mg prednisone daily) worsened when placed on azathioprine. His absolute lymphocyte count dropped to 200, and his CD4 count was found to be 52. His leukocyte count also remained normal (range 4100 to 4800). Although his ulcerative colitis had improved, he developed night sweats, dyspnea on exertion, and was admitted and treated for pulmonary coccidiomycosis. The patient recovered successfully, albeit after a prolonged hospitalization. No opportunistic infections occurred in patients with normal lymphocyte counts.

The three other patients with severe lymphopenia did not develop significant infections and their lymphocyte levels improved to the normal range soon after the discontinuation of AZA/6MP.

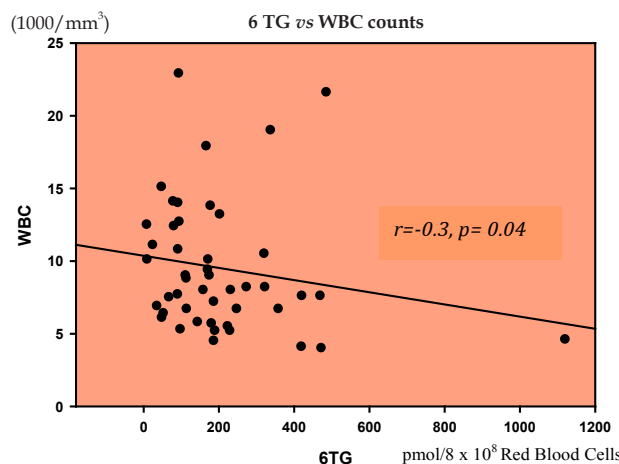


Figure 1 Correlation of 6-Thioguanine (6TG) levels to leukocyte (WBC) counts.

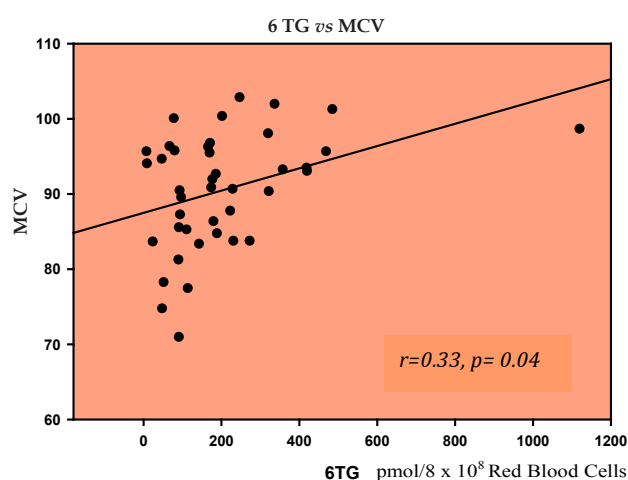


Figure 2 Correlation of 6-Thioguanine (6TG) levels to mean corpuscular volume (MCV).

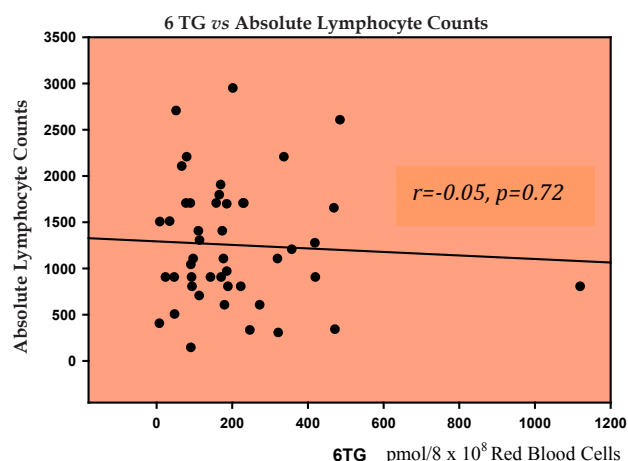


Figure 3 Correlation of 6-Thioguanine (6TG) levels to absolute lymphocyte counts.

Correlation of Metabolite Markers to Complete Blood Counts

The 6TGN levels were compared to WBC counts, absolute lymphocyte counts and MCV levels using Spearman's correlation coefficient. 6TGN levels compared to WBC counts showed a correlation of -0.3 ($p=0.04$) as depicted in Figure 1. As shown in Figure 2, 6TGN to MCV levels were found to be correlated by an r of 0.33 ($p=0.04$).

Since very few patients with lymphopenia had leukopenia, it was not unexpected that the 6TGN to lymphocyte correlation coefficient was very low ($r=-0.05$, $p=0.72$) as seen in Figure 3. 6MMP, as expected, showed no significant correlation to WBC counts ($r=-0.11$, $p=0.45$), to MCV levels (0.1 ($p=0.54$), or to lymphocyte counts ($r=-0.1$, $p=0.5$).

DISCUSSION

This study demonstrates that lymphopenia occurs in a significant number (42%) of patients with inflammatory bowel disease. The use of AZA/6MP increases this risk by almost 3 fold. However, a small group of patients develop lymphopenia without the use of these immunomodulators. Given the increasingly complex medical regimens used in treating IBD, including TNF-alpha antagonists, with possible effects on leukocytes and lymphocytes, it might be difficult to ascribe lymphopenia to a single class of medications. However, in this series of patients, no other medications increased the risk of lymphopenia.

In a similar study by Al Rifal and colleagues that assessed lymphopenia in IBD patients from 2 large tertiary care centers, 18/52 (35%) developed lymphopenia and 10/52 (19%) had severe lymphopenia which they defined as less than 600^[10]. In their patients, the lymphopenia lasted on average 85 days, resolved spontaneously and none developed any opportunistic infections. In contrast to our findings, they showed that steroid use was associated with a higher rate of lymphopenia than immunomodulators.

Nguyen and colleagues found higher rates of lymphopenia in pediatric patients on combination of aminosalicilate and thiopurine treatment which they linked to an aminosalicilate induced increase in both 6-TGN and 6-MMPN concentrations^[11].

Our study clearly demonstrates the association of lymphopenia with immunomodulators treatment in IBD. The clinical significance of this finding remains to be determined, but there are several potential implications that need to be clarified in future studies, including: 1) the association between lymphopenia and other markers of AZA/6MP dosing, 2) the correlation between lymphopenia and either biologic efficacy or complications of these medications in treating IBD patients.

In evaluating the metabolite markers 6TGN and 6MMP for any correlations to blood counts, only two were found to be statistically significant ($p < 0.05$) between 6TGN levels and WBC and MCV. However, correlation coefficients of 0.3 and 0.33 respectively, in the clinical setting cannot be translated as a sufficiently strong association to employ in clinical practice.

The two most significant complications of immunomodulators, infection and lymphoma, may be related to lymphopenia. Lymphocyte counts may be low enough to cause opportunistic infections, such as herpes zoster and pulmonary coccidiomycosis as found in our patients. CD4 counts measured in these patients were found to be in the AIDS defining range. Consideration of this should be taken into account when advising patients of side effects of this medication and the amount of immune suppression that could potentially place the patient at risk of infectious complications. A similar association between lymphopenia and infection was found by a study by Govani and Higgins that evaluated the combination of thiopurines and allopurinol use in IBD patients. They found that 5/27 subjects developed infectious complications which were related to lower absolute lymphocyte counts compared to those without an infection^[12].

Although the number of patients that developed infections were

small in their study and ours, the possibility that lymphopenia may identify a subset of patients at risk for significant drug induced complications will be need to be clarified in future studies.

Although conventional wisdom has implicated leukopenia (WBC <3000) as a risk factor for developing lymphoma, there is no direct evidence, to our knowledge to support this theory. Given recent data that suggests a several fold increase in lymphoma in azathioprine treated IBD patients, it is reasonable to consider lymphopenia rather than leukopenia as a risk factor^[13]. Thiopurines are cytotoxic for natural killer and cytotoxic T cells. A decrease in their inhibition effect on Epstein Barr virus-infected and immortalized B cells may be a trigger mechanism for development of lymphoma^[13]. Larger scale prospective studies would be required to adequately address this issue.

A study of 6MP metabolites in children with acute lymphoblastic leukemia showed correlation of WBC counts to 6TGN levels only if the medication caused myelotoxicity^[14]. When these patients were grouped into those who had an expected myelotoxicity to 6MP, the correlation coefficient was $r = -0.463$, $p < 0.0001$ ^[14]. In the group of patients that did not show myelotoxicity to the drug, 6TGN, no significant relationship was found^[14]. This raises the possibility that there may be subsets of populations (such as in children or in patients on 6MP for other treatments besides IBD) where there is a good correlation between increased 6TGN levels and myelotoxicity while in others not.

In our study, there was no correlation between 6TGN levels and lymphocyte counts. This is not a type II error; to have achieved any statistical significance, the study would have required over a thousand patients. Leukopenia does not correlate to lymphopenia. Therefore, one cannot rely on either CBC or 6TGN levels to determine who may develop lymphopenia. In our patients, lymphopenia was sometimes prolonged & persisted even with undetectable metabolite levels. Using MCV levels and WBC counts to monitor the efficacy of the medication would not be advised due to their poor correlation with 6TGN levels.

Lymphopenia may represent an integral component of the therapeutic effect as well as a significant risk factor of AZA/6MP therapy. Prior studies suggest that AZA/6MP exert the immunosuppressive effect by inhibiting Rac1 activation in T lymphocytes and inducing apoptosis^[3]. The retrospective nature of study did not permit for an accurate correlation of disease activity to the blood counts and metabolite levels. Therefore, we cannot determine a correlation between lymphopenia and clinical response in this study. As with other components of the immune response in IBD, the link between the intestinal environment and circulating lymphocytes is problematic.

Clinical significance of these findings still needs to be fully defined. A more detailed analysis of clinical activity in relation to blood markers can put the relationship between lymphopenia and disease activity into perspective. Although is possible that there is a dichotomy between therapeutic and toxic effects, we suspect that the two may be linked.

Identifying individuals with AIDS-like lymphopenia with simultaneously normal WBC and 6TGN levels provides a clear indication of the complexities of the pharmacometabolism of these drugs and the risks of opportunistic infections. The relatively modest effort of tracking lymphocyte counts may reduce rare, but serious, complications and improve outcome in IBD patients on immunomodulators.

More recently, a missense variant NUDT15 gene mutation has been found to confer increased susceptibility to thiopurine-induced

leukopenia independently of the 6-thioguanine mechanism in East Asians, specifically studied in Koreans, Japanese, and Taiwanese populations^[15,16,17,18]. We had a very low Asian population, and the fact that the NUDT15 mutation is rare in IBD patients of European descent, may explain the extremely low incidence of leukopenia in our study.

Until further information can be obtained with larger number of patients to determine the correlation between laboratory assessment and clinical activity, we should be cautious in relying solely on total WBC, leukocyte counts, or drug metabolite levels to predict disease response to treatment or drug toxicity. Laboratory values should be used as a guide in conjunction with assessment of clinical activity. This multifactorial approach is perhaps the most optimal management for these patients.

CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

REFERENCES

- Loftus CG, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Tremaine WJ, Melton LJ, Sandborn WJ. Update on the Incidence and Prevalence of Crohn's Disease and Ulcerative Colitis in Olmsted County, Minnesota, 1940-2000. *Inflamm Bowel Dis.* 2007; **13**: 254-261.
- Korelitz BI, Adler DJ, Mendelsohn RA, Sacknoff AL. Long-term experience with 6-mercaptopurine in the treatment of Crohn's disease. *Am J Gastroenterol.* 1993; **88**: 1198-1205.
- Tiede I, Fritz G, Strand S, Poppe D, Dvorsky R, Strand D, Lehr HA, Wirtz S, Becker C, Atreya R, Mudter J, Hildner K, Bartsch B, Holtmann M, Blumberg R, Walczak H, Iven H, Galle PR, Ahmadian MR, Neurath MF. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest.* 2003; **111**: 1133-1145.
- Thomas CW, Myhre GM, Tschumper R, Sreekumar R, Jelinek D, McKean DJ, Lipsky JJ, Sandborn WJ, Egan LJ. Selective inhibition of inflammatory gene expression in activated T lymphocytes: a mechanism of immune suppression by thiopurines. *J Pharmacol Exp Ther.* 2005; **312**: 534-545.
- Winkelstein A. The effects of azathioprine and 6 MP on immunity. *Journal of Immunopharmacology.* 1979; **1**: 429-454
- Thomas CW Jr, Lowry PW, Franklin CL, Weaver AL, Myhre GM, Mays DC, Tremaine WJ, Lipsky JJ, Sandborn WJ. Erythrocyte mean corpuscular volume as a surrogate marker for 6-thioguanine nucleotide concentration monitoring in patients with inflammatory bowel disease treated with azathioprine or 6-mercaptopurine. *Inflamm Bowel Dis.* 2003; **9**: 237-245.
- Siedman EG. Clinical Use and Practical Application of TPMT Enzyme and 6-Mercaptopurine Metabolite Monitoring in IBD. *Rev Gastroenterol Disord.* 2003; **3**: S30-S38.
- Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sennett D, Théorêt Y, Seidman EG. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology.* 2000; **118**: 705-713.
- Waljee AK, Joyce JC, Wang S, Saxena A, Hart M, Zhu J, Higgins PD. Algorithms outperform metabolite tests in predicting response of patients with inflammatory bowel disease to thiopurines. *Clin Gastroenterol Hepatol.* 2010 Feb; **8(2)**: 143-50.
- Al Rifal A, Prasad N, Shuttleworth E, McBurney H, Pushpakom S, Robinson A, Newman W, Campbell S. Natural history of azathioprine-associated lymphopenia in inflammatory bowel disease patients: a prospective observational study. *Eur J Gastroenterol Hepatol.* 2011 Feb; **23(2)**: 153-8.
- Nguyen TM, Le Gall C, Lachaux A, Bouliou R. High thiopurine metabolite concentrations associated with lymphopenia in inflammatory bowel disease (IBD) pediatric patients receiving aminosalicylates combined with azathioprine. *Int J Clin Pharmacol Ther.* 2010 Apr; **48(4)**: 275-81.
- Govani SM, Higgins PD. Combination of thiopurines and allopurinol: adverse events and clinical benefit in IBD. *J Crohns Colitis.* 2010 Oct; **4(4)**: 444-9
- Beaugerie L, Brousse N, Anne Marie Bouvier AM Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F; CESAME Study Group. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *The Lancet.* 2009; **374**: 1617 – 1625.
- Innocenti F, Danesi R, Favre C, Nardi M, Menconi MC, Di Paolo A, Bocci G, Fogli S, Barbara C, Barachini S, Casazza G, Macchia P, Del Tacca M. Variable Correlation Between 6-Mercaptopurine Metabolites in Erythrocytes and Hematologic Toxicity: Implications for Drug Monitoring in Children With Acute Lymphoblastic Leukemia. *Therapeutic Drug Monitoring.* 2000; **22**: 375-382.
- Asada A, Nishida A, Shioya M, Imaeda H, Inatomi O, Bamba S, Kito K, Sugimoto M, Andoh A. NUDT15 R139C-related thiopurine leukocytopenia is mediated by 6-thioguanine nucleotide-independent mechanism in Japanese patients with inflammatory bowel disease. *J Gastroenterol.* 2015 Nov 21. [Epub ahead of print]
- Liang DC, Yang CP, Liu HC, Jaing TH, Chen SH, Hung IJ, Yeh TC, Lin TH, Lai CL, Lai CY, Shih LY. NUDT15 gene polymorphism related to mercaptopurine intolerance in Taiwan Chinese children with acute lymphoblastic leukemia. *Pharmacogenomics J.* 2015 Oct 27. doi: 10.1038/tpj.2015.75. [Epub ahead of print]
- Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, Pei D, Chen Y, Crews KR, Komegay N, Wong FL2, Evans WE2, Pui CH2, Bhatia S2, Relling MV2. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *J Clin Oncol.* 2015 Apr 10; **33(11)**: 1235-42.
- Yang SK, Hong M, Baek J, Choi H, Zhao W, Jung Y, Haritunians T, Ye BD, Kim KJ, Park SH, Park SK, Yang DH, Dubinsky M, Lee I, McGovern DP, Liu J, Song K. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. *Nat Genet.* 2014 Sep; **46(9)**: 1017-20. doi: 10.1038/ng.3060. Epub 2014 Aug 10.

Peer reviewers: Emad H Aly MBBCh, MS, MD, FRCS, MEd, Consultant Colorectal & General Surgeon, Laparoscopic Colorectal Surgery & Training Unit - Aberdeen Royal Infirmary, Aberdeen, UK; Hideki Iijima, M.D. Ph.D., Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871 Japan.