Lymphopenia in Inflammatory Bowel Disease Patients on Immunosuppressive Medications

Bincy P Abraham, Joseph H Sellin

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.

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 mercaptourine 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 pharmacological 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. 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 to toxicity. However, maintaining leukopenia (<3,000) does not necessarily correlate with clinical response to toxicity. This metabolite served as a surrogate control. Clinical activity based on controlled or uncontrolled disease was analyzed in regards to metabolite markers 6TGN and 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.

**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...
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.

### 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).

<table>
<thead>
<tr>
<th>Level of lymphopenia</th>
<th>Patients</th>
<th>6 TG vs WBC counts</th>
<th>6 TG vs MCV</th>
<th>6 TG vs Absolute Lymphocyte Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Lymphopenia &lt;1000</td>
<td>53/125 (42%)</td>
<td>r=0.3, p=0.04</td>
<td>120/100</td>
<td>r=0.05, p=0.72</td>
</tr>
<tr>
<td>Moderate lymphopenia &lt;850</td>
<td>44/125 (35%)</td>
<td>110/100</td>
<td>70/100</td>
<td></td>
</tr>
<tr>
<td>Severe Lymphopenia &lt;500</td>
<td>19/125 (15%)</td>
<td>60/100</td>
<td>50/100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1 Incidence of Lymphopenia in all IBD patients.**

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

© 2016 ACT. All rights reserved.
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 affects 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 Rifai 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 aminosalicylate and thiopurine treatment which they linked to an aminosaliclylate 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[13].

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 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[14]. 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[15]. 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[16]. 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[15]. 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. 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 numbers of patients to determine the correlation between laboratory assessment and clinical activity, we should be cautious in relying solely on total WBC, leucocyte 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**


**Peer reviewers:** Emad H Aty MB BCH, 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.