Lymphocytopenia In Patients with Inflammatory Bowel Disease on Immunosuppressive Medications

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Lymphocytopenia In Patients with Inflammatory Bowel Disease on Immunosuppressive Medications

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Lymphocytopenia In Patients with Inflammatory Bowel Disease on Immunosuppressive Medications

by

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Dedication

This work is dedicated to my loving husband, Philip, my son, Aidan, my soon to be born son, my parents, and my sister.

Acknowledgements

I would like to thank my mentor Dr. Joseph Sellin for providing me the opportunity to work with him and learn the art and practice of inflammatory bowel disease. I would also like to thank my committee members, Dr. Don Powell and Dr. Karen Szauter for providing me with valuable advice on my research. Dr. Dan Freeman has provided insight into the significance of statistics in relationship to my clinical research. I appreciate the help and guidance Marie Carr and Tonya Groh has done in all the administrative work to get me through the coursework.

Lymphocytopenia In Patients with Inflammatory Bowel Disease on Immunosuppressive Medications

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Azathioprine and 6-mercaptopurine (AZA/6MP) are effective immunosuppressives commonly used for the treatment of inflammatory bowel disease (IBD). The mercaptopurine metabolites, 6-thioguanine (6TG) and 6-methylmercaptopurine ribonucleotides (6MMP), have been suggested as surrogate markers of the safety and efficacy of immunosuppressive therapy. Elevated levels of 6TG have been found to be associated with bone marrow toxicity manifest as leukopenia. Elevated 6-MMP levels have been associated with hepatotoxicity. This study aims to evaluate the incidence of lymphocytopenia with immunosuppressive use, to correlate metabolite levels (6TG and 6MMP) with leukocyte (WBC), lymphocyte, mean corpuscle volume (MCV) levels in IBD patients treated with AZA/6MP, and secondarily to evaluate clinical status with these blood markers.

Medical records of adult IBD patients taking either azathioprine or 6-mercaptopurine were analyzed based on blood counts, mercaptopurine metabolites 6TG and 6MMP, and clinical status. Eighty three percent of the patients taking AZA/6MP developed lymphocytopenia. Of those with lymphocytopenia, only 12% had low WBC counts. A low correlation of $r = -0.3$ ($p=0.04$) for 6TG to WBC counts as well as to MCV levels was found. No correlation between 6TG levels and lymphocyte counts were noted. Clinical activity to any blood markers showed no significant correlation.

This analysis shows that lymphocyte counts and not leukocyte counts should be monitored closely in patients on these immunosuppressives, and that metabolite markers do not provide information regarding who will develop lymphocytopenia. Blood markers cannot be used as a marker for disease activity although further detailed studies will need to be done. Consequences of lymphocytopenia such as opportunistic infections need to be discussed with patients prior to starting this type of therapy.

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CHAPTER 1

Specific Aims

Inflammatory Bowel Disease (IBD), an autoimmune disorder in the form of either Crohn's disease or Ulcerative Colitis, can cause a spectrum of symptoms from diarrhea and abdominal pain to gastrointestinal bleeding. With long-standing disease, there is an increased risk of cancer at the site of severe disease as well as other non-intestinal cancers such as lymphomas. The key to controlling symptoms and complications from this disease 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. The mechanism of action of these drugs has not been fully delineated, although apoptosis of T lymphocytes may play an important role.[1] It is well known that leukopenia can occur in patients taking AZA/6MP. Recently, we have observed that lymphocytopenia can also occur in patient taking AZA/6MP.

In order to determine if patients on these medications are prone to developing leukopenia, Prometheus Laboratories, Inc. has commercialized a blood test that determines the levels of the metabolites of AZA/6MP. These metabolites, 6-thioguanine (6TG) and 6-methylmercaptopurine ribonucleotides (6MMP), have been suggested as surrogate markers of the safety and efficacy of immunosuppressive therapy. 6TG levels reflect therapeutic efficacy. The optimal range is 235-400 pmol/8 x 10^8 RBC.[2] Greater than 450 pmol/8 x 10^8 RBC would indicate a higher risk of myelotoxicity.[2] These patients are prone to developing leukopenia. If the level is below 235 pmol/8 x 10^8

RBC, this indicates a suboptimal dosing, that may lead to inadequate response to treatment.[2] On the other hand, 6MMP levels greater than 5700 pmol/8 x 10^8 RBC indicate an increased risk of hepatotoxicity.[2]

Despite this knowledge about the relationship between metabolite and total WBC levels, lymphocytopenia may or may not correlate to metabolite levels. Unfortunately, there is no test to date that can provide this information. Furthermore, no standard of care exists on optimal monitoring for safety and efficacy of AZA/6MP use. Some propose to use WBC counts looking for leukopenia as a sign of toxicity without checking for a WBC differential. Some may use MCV as a surrogate marker for optimal medication dosing and others use metabolite levels.[3] If metabolite levels do correlate to these routine labs or even to clinical activity, knowing these levels can better guide gastroenterologists in optimizing the safety of the drug as well as in monitoring the patient's disease activity. Thus, our specific aims are:

Primary Aim 1: To determine the incidence of lymphocytopenia in patients with IBD with and without treatment using the immunosuppressives AZA/6MP.

Primary Aim 2: To determine how closely the metabolite markers 6TG and 6MMP correlate with routine blood counts of WBC, lymphocyte counts, MCV and, in severely lymphopenic patients, $CD4^+$ & $CD8^+$ counts.

Secondary Aim: To determine how clinical activity relates to both metabolite markers and routine complete blood counts.

CHAPTER 2

Background and Significance

2.1 Inflammatory Bowel Disease:

Approximately 1.1 million people in the United States suffer from IBD.[4] Patients with ulcerative colitis most commonly complain of bloody diarrhea. Some patients may experience bloody bowel movements of upwards of 10 to 15 times a day which can contribute to severe anemia and fatigue. Patients with Crohn's disease may have diarrhea, but those with fibrotic disease can develop strictures which increase their risk of bowel obstruction, or fistulas and abscesses which can require multiple antibiotics and surgical treatment. With ulcerative colitis, surgery can essentially "cure" the disease if the entire colon is removed. However, having only small intestine comes with its own set of problems. Those undergoing colectomy also are subject to increased risk of infertility in both males and females due to disruption of pelvic organs and nerves. Patients may develop pouchitis, or inflammation of the surgically placed pouch that acts as a reservoir similar to the rectum. With Crohn's disease, surgery is not curative and has more limited goals. Since Crohn's disease can affect any segment of the GI tract, and frequently recurs after surgical excision, surgical resection of the diseased portions can eventually leave a patient with a limited bowel length, so malabsorption and short bowel syndrome can occur. Thus, the first line of treatment for both ulcerative colitis and Crohn's disease is medical therapy.

2.2 Medications:

Patients with IBD usually require multiple medications to control their disease. Almost all patients are placed on mesalamine (5-aminosalicylic acid) which has anti-inflammatory properties, and a very low side-effect profile. But when flare-ups occur, the most often used rescue medications are corticosteroids such as prednisone. This medication, although it works quickly at alleviating inflammation, is not without adverse side effects, especially when used long-term. These include and are not limited to diabetes mellitus type II, Cushingoid features, hypertension, weight gain, osteoporosis, and osteonecrosis. Due to either of these side effects, or due to non-response to corticosteroids, patients are placed on longer acting immunosuppressants, "steroid-sparing" agents. These agents include azathioprine (AZA) or 6-mercaptopurine (6MP). Some studies suggest that about 70% of patients have improvement of symptoms on these medications.[5]

2.3 AZA/6MP Side Effects:

Azathioprine is the pro-drug which is broken down to 6-mercaptopurine in the body.[6] The actions of both medications are considered essentially synonymous. These medications are not without their immediate side effects such as pancreatitis, bone marrow toxicity, and hepatitis. In most cases, drug induced hepatitis or pancreatitis dictates an immediate withdrawal from treatment to avoid any further complications. However, those with leukopenia are usually monitored and evaluated for its severity. These patients may remain on the medication assuming that the leukopenia contributes to the efficacy of the drugs. Thus, it is important to understand the type of leukocytes affected by these medications. Since lymphocytes are involved in cell-mediated and

humoral immunity, lymphocyte levels may provide more detailed information about the patient's disease and their response to treatment. We have recently observed that significant lymphocytopenia (low levels of lymphocytes defined by absolute lymphocyte count <1000) can occur in patients on AZA/6MP, despite having normal total WBC levels.

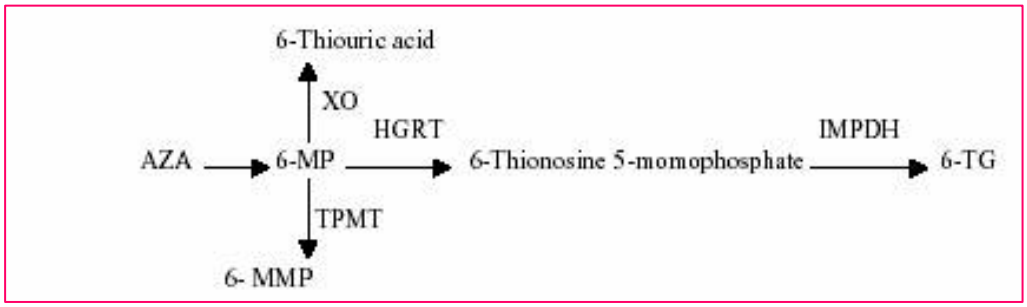
2.4 Lymphocytopenia:

Developing lymphocytopenia is not without its consequences. Lymphocytes are important in containing or eradicating infectious agents through cell- and humoral immunity. B cells produce immunoglobulin and antibodies and contribute to humoral immunity. T cells are comprised of two main subtypes, $CD4^+$ and $CD8^+$ T lymphocytes. Loss of cell-mediated immunity though low levels of these cell types contribute to severe infections that the immunocompetent patients either never develop or resolve quickly without major sequelae. These low levels are commonly seen in patients with severe, untreated HIV/AIDS. Thus, any patient that develops leukopenia or, specifically, lymphocytopenia, must be monitored closely. Considerations should be made about possible dose reduction or complete withdrawal of these medications. No set guidelines exist currently to determine which patients must be withdrawn from therapy and which patients can be continued on a lower dose. The metabolic pathway of these drugs may provide this information.

2.5 Medication Metabolism:

These medications are metabolized by three competing enzyme systems.[6] Xanthine oxidase (XO), found in the intestinal mucosa and liver, converts a small amount of 6MP to an inactive metabolite, 6-thiouric acid.[7] Once 6MP reaches the intracellular compartment, it is metabolized by two other enzymes. Thiopurine methyltransferase (TPMT) catalyses the methylation of 6MP, converting it to 6-methylmercaptopurine (6MMP).[7] Hypoxanthine phosphoribosyl transferase (HGRT) initiates a series of enzymatic reactions that convert 6MP to 6-thioguanine nucleotides (6TG).[7] 6TG is the active metabolite with cytotoxic and immunosuppressive properties.[8]

Figure 1: The thiopurine methyltransferase (TPMT) pathway.[6]



Approximately 11% of IBD patients have mutations in TPMT and cannot metabolize the medication normally. These patients are at higher risk for myelosuppression and leukopenia.[9] Thus, patients are usually started at very low doses, or if known to be homozygous for the mutation, they are usually not started on the medication. Due to this, our study will focus on the majority of patients with normal enzyme activity.

2.6 Metabolite Testing:

Thiopurine metabolite testing in IBD patients treated with AZA/6MP is indicated after initiating therapy for following dose adjustments to reach target 6TG levels, at the time of a clinical relapse, serially (twice yearly) to monitor adherence and /or TPMT induction, at the time of an adverse event (hepatotoxicity or myelotoxicity), and to monitor effect of change in weight or concomitant drug use (5-ASA, sulfasalazine, furosemide, etc).[2]

Some gastroenterologists have proposed to simply follow WBC counts and maintain leukopenia instead of measuring metabolites[2] In the era before metabolite measurement and the establishment of a therapeutic window for 6TG levels, this was practiced by some clinicians to increase the likelihood of a clinical response.[2] Whereas leukopenia may suggest that the 6TG level is high (usually >450 pmol/8x 10^8 RBC), there is no evidence that such myelotoxic levels yield higher efficacy than 6TG in the 300 pmol/8 x 10^8 RBC range.[2] 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.[2]

Increased mean corpuscular volume (MCV) has been found in patients taking AZA/6MP. Since MCV represents the volume of the RBC, and since 6TG is found inside RBC's, higher MCV may correlate to higher 6TG levels. A Danish study did show a statistically significant correlation between MCV levels and 6TG metabolite levels, but only with an r value of 0.33.[3] Since the routine CBC includes MCV, we will

determine if any higher correlation could be found to make this of any clinical significance.

2.7 Significance:

No study to date has sought to correlate leukopenia to lymphocytopenia in these patients, and no study to date has evaluated the relationship between 6TG levels and lymphocytopenia. Since lymphocytes rather than granulocytes are the target of these drugs, and since perhaps lymphocytopenia in addition to profound leukopenia predisposes patients to serious to life-threatening infections, this study aims to evaluate the importance of correlating the mercaptopurine metabolite levels to leukocyte subsets. This may help determine a standard of care for monitoring for safety and efficacy of this drug.

If the study shows no significant correlation of mercaptopurine metabolite levels to routine labs and especially to lymphocyte counts, patients would most likely need to be monitored with the use of metabolite levels until a more appropriate and less expensive marker for therapeutic efficacy and potential side effects is found.

Besides the importance of correlating metabolite levels to lymphocytopenia, this study can provide other important information. This may offer some insight into the mechanism of action of these drugs, in that AZA/6MP may act to reduce specific lymphocyte sub-sets ($CD4^+$ cells for example). If lymphocytopenia occurs, the mechanism of anti-inflammatory action may be due to actual suppression of proliferation or apoptosis of lymphocytes. However, if lymphocyte counts remain normal despite improvement in the symptoms of the patient, suppression in the activity of intestinal

lymphocytes rather than circulating lymphocytes, or even suppression in lymphocyte function, may be the determining factor.

Since there have been no set guidelines on maintenance doses, this study may provide information of appropriate maintenance doses for patients that will minimize complications from infection. For 6-mercaptopurine, patients are generally placed at a starting dose of 1.5 mg/kg daily. Since azathioprine is the pro-drug, a higher starting dose of 2.5 mg/kg daily has been used as a standard. The patient is usually monitored until side effects occur, and then only is the dose adjusted. Gastroenterologists may be able to follow set guidelines for dosing based on metabolite levels. The benefits include time and charges avoided in the hospital, reduction of number of labs drawn, and most importantly the reduction in morbidity and mortality from life-threatening infections.

This study may provide information on duration of effect of these medications. Usually AZA/6MP can take several weeks to reach steady-state levels and provide efficacy. Based on follow-up of patients who may require the discontinuation of AZA/6MP, the therapeutic effect in controlling symptoms as well as the duration of lymphocytopenia that continues despite medication withdrawal, can provide clues to the duration of residual effects of the drug, and perhaps laboratory markers of that effect.

Despite the use of surrogate laboratory markers, the ultimate marker of efficacy of a treatment is based on each patient's clinical activity. A German study of 84 patients with IBD showed that those who went into remission during treatment with azathioprine displayed significantly lower leukocyte counts compared to patients not in remission ($p = 0.004$ in Crohn's disease and 0.003 in ulcerative colitis).[10] This same study failed to show that the mean corpuscular volume correlated with the response to purine analogues.

Other studies however failed to show that leukopenia correlated with treatment response. In a French study, an increase in MCV levels were predictive of a favorable clinical response only when 6TG levels were above 250 pmol/8 x 10(8) red blood cells.[11] Our study will compare clinical activity based on physician's global assessment (PGA) to metabolite levels and routine blood work. How closely the patient's clinical activity matches these blood markers will provide perhaps the most significant data.

CHAPTER 3

Research Design and Methods

3.1 Research Design

The aim of our study is to determine if lymphocytopenia is correlated with metabolite levels of AZA/6MP. The study population includes approximately 125 patients with IBD. The medical records of these patients were reviewed and followed for approximately 1 year. Variables measured include the use of all IBD therapies, especially the use of the immunosuppressives AZA/6TG, 6TG & 6MMP levels, leukocyte counts, MCV levels, lymphocyte counts, and $CD4^+$ and $CD8^+$ cells. The results of the blood cell levels especially lymphocyte counts are compared to the metabolite levels to evaluate for any correlation.

3.2 Study Population

Patients who met the following inclusion criteria were analyzed for this study.

3.2.1 Inclusion criteria:

- Age 18 years of age or older
- Diagnosis of biopsy proven Inflammatory Bowel Disease (Crohn's disease, Ulcerative Colitis, or Indeterminate type)
- Follow up for at least 1 year of visits or hospitalizations.

3.2.2 Exclusion criteria:

- Children: younger than 18 years old.
- Colitis of unknown origin

- Infection colitis
- Suspected IBD, non biopsy proven
- Pregnancy
- Concomitant infection with HIV/AIDS
- Any other use of AZA/6MP for other autoimmune diseases.
- History of allergy or adverse reaction to AZA/6MP.
- Any evidence of prior immunodeficiency, acquired or congenital.
- History of pancreatitis, or hepatitis
- Any additional criteria that may preclude patient from taking AZA/6MP

3.3 Evaluation of Data:

Medical records of all IBD patients that met above criteria were evaluated. The type of IBD (Crohn's disease versus ulcerative colitis), as well as any other co-existing conditions will be noted.

CBC with differential was evaluated and leukocyte and lymphocyte counts, MCV levels, were placed in a database for each patient. The results of these labs as well as the timing of AZA/6MP therapy were analyzed. Liver Function Tests (LFTs) were usually drawn every 3 months to ensure the patient has had no underlying elevation of liver enzyme levels or other liver abnormality. Information on genetic testing for mutations of the TPMT enzyme was also noted. The type and dose of medications were also taken into account.

Prometheus Laboratories, Inc. measures quantitative metabolite levels using High Pressure Liquid Chromatography (HPLC) for 6TG in peripheral RBC, separate stationary

and mobile phase, as well as quantitative levels using HPLC for 6-methyl-mercaptopurine (6MMP) in peripheral RBC, separate stationary and mobile phase. Results sent to Prometheus were recorded for evaluation, especially with correlating leukocyte and lymphocyte counts.

During the study period, the patients were followed clinically, and treated based on symptoms, physician evaluation, and lab results, adjusting doses as needed. Since this is a retrospective chart review, no changes to patient management occurred because of the study itself. If the patient's physician made any changes to medications, or any relevant infections were found, these were noted.

For patients that become profoundly lymphopenic (absolute lymphocyte count < 850), $CD4^+$ and $CD8^+$ cell counts were drawn and were recorded. If $CD4^+$ cell count drops below 250, or the patient appears to have developed an opportunistic infection, AZA/6MP may be discontinued or have the dosage decreased depending on clinical situation. These patients are closely monitored, and any labs associated with these changes were also recorded.

Table 1: Database Per Patient

AZA/ 6MP dose	Other Meds	6TG level	6MMP level	WBC	MCV level	Absolute lymphocyte count	$CD4^+$ cell count	$CD8^+$ cell count	Adverse reactions

3.4 Clinical Activity Evaluation

Although the initial database for the study did not include clinical status, a retrospective chart review determined clinical activity for the patient as defined by physician global assessment. In general, in order to have controlled disease, the patient must not be using prednisone, have quiescent disease per endoscopy, or be on stable doses of medications. Flare-ups, worsening disease activity, increasing dose of medications placed the patient in the uncontrolled disease status.

CHAPTER 4

Data Analysis

4.1 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 lymphocytopenia was determined.

The metabolite markers 6TG and 6MMP levels were graphed and analyzed against WBC counts, lymphocyte counts, MCV levels, and in the severely lymphocytopenic 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.

Although clinical activity was not the initial focus of this research, a limited retrospective analysis was made based on the physician's global assessment. This was determined from hospital and clinic notes. Each patient was determined to have their disease either controlled or uncontrolled based on criteria as described in the methods section. This status was analyzed in regards to the metabolite levels and the routine blood levels.

Correlation between the metabolite levels and WBC subsets was based on a correlation coefficient. With much research involving humans, the potential for outliers exist. Spearman's correlation was used to minimize any possible effect on the general population. A two-tailed probability calculated with the appropriate degrees of freedom was used to obtain the statistical significance.

CHAPTER 5

Results

5.1 Incidence of Lymphocytopenia

From chart review of about 180 patients with IBD, a total of 124 patients were found to have data regarding routing blood tests (CBC) and metabolite markers, and thus, were analyzed.

Fifty-two of 124 patients (42%) were found to have lymphocytopenia (absolute lymphocyte count less than 1000). Forty-three of them (35% of 124 patients) actually had severe lymphocytopenia (absolute lymphocyte count less than 850).

Table 2: Total patients with Lymphocytopenia

Total patients (124)	Patient Number	Percentage
Lymphocytopenia <1000	52/124	42%
Severe lymphocytopenia <850	43/124	35%

In a select group of patients with severe lymphocytopenia, CD4 counts were measured and found to be significantly depressed: range (84 to 361). One patient developed herpes zoster infection, was taken off azathioprine, and had leukocyte and lymphocyte levels monitored closely. In the absence of azathioprine, it took over 6 months for the lymphocyte and CD4 counts return back to normal. At the time of initial diagnosis of lymphocytopenia, the patient's disease was under control. However, when the lymphocyte levels normalized, the patient's disease worsened. One patient with severe lymphocytopenia required hospitalization for pulmonary coccidiomycosis. The patient was taken off all immunosuppressives, including azathioprine.

Of the 124 patients analyzed, 78 (63%) were taking the immunosuppressive AZA/6MP and 46 (37%) were treated with other medications. Of the 78 patients on AZA/6MP, 43 (55%) had developed lymphocytopenia, 36 (46%) had severe lymphocytopenia (absolute lymphocyte count <850). See Table 3. Of the 46 patients that were not on AZA/6MP, 9 patients (20%) developed lymphopenia.

Table 3: Lymphocytopenia on AZA/6MP.

Patients On AZA/6MP (78)	Patient Number	Percentage
Lymphocytopenia <1000	43/78	55%
Severe lymphocytopenia <850	36/78	46%

To clarify this, Figure 2a and 2b gives a breakdown of lymphopenic patients in general, and those on AZA/6MP:

Figure 2: Breakdown of Lymphocytopenic patients:

Figure 2a:

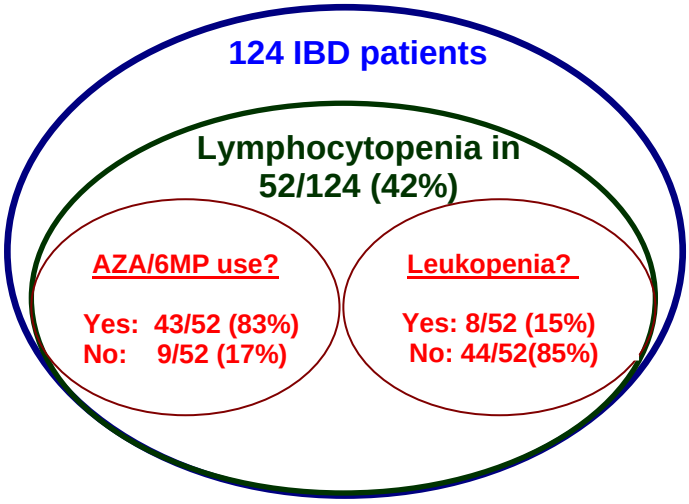
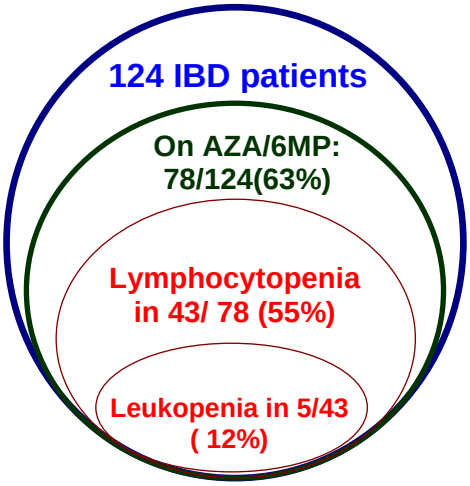


Figure 2b:



It is interesting to note (Figure 2a) that of all patients with lymphocytopenia (52/126), only 15% were leukopenic (white blood cell count of less then 4,000/mm^3). Thus, leukopenia does not predict lymphocytopenia. In the patients found to be lymphopenic, AZA/6MP was used as treatment by 83% of them. However, 17% (9/52) of the lymphopenic were not using the drug. Other immunosuppressive medications may have contributed, but on review of these 9 patients, only one patient was on infliximab, one was on budesonide, and one was on prednisone. The remainder (6/9) was not on any immunosuppressive medications. This may suggest other etiologies in the development of lymphocytopenia.

Figure 2b gives an overview of the role of AZA/6MP in the development of lymphocytopenia. Seventy-eight (63%) of the patients evaluated in the study were on chronic AZA/6MP therapy. Of those taking AZA/6MP, 43 patients (55%) developed lympocytopenia. 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 12% were leukopenic.

Table 4: Relative risk of lymphocytopenia with immunosuppressive use.

Total 124 patients	Lymphocytopenic	Not Lymphocytopenic
AZA/6MP use	43	35
No AZA/6MP use	9	37

Relative Risk = 2.82, with 95% CI (1.5 to 5.2)

5.2 Correlation of Metabolite Markers to Routine Blood Tests

The 6TG levels were compared to WBC counts, absolute lymphocyte counts and MCV levels using Spearman's correlation coefficient. 6TG levels compared to WBC counts showed a correlation of -0.3 (p=0.04) as depicted in Figure 3. That is, for higher 6TG, a lower WBC count was found with a correlation of 0.3. As shown in Figure 4, 6TG to MCV levels were found to be correlated only by an r of 0.33 (p=0.04).

Since the general data showed that very few patients with lymphocytopenia had leukopenia, it appears logical that the 6TG to lymphocyte correlation coefficient was very low (r=-0.05, p=0.72) as seen in Figure 5.

Figure 3:

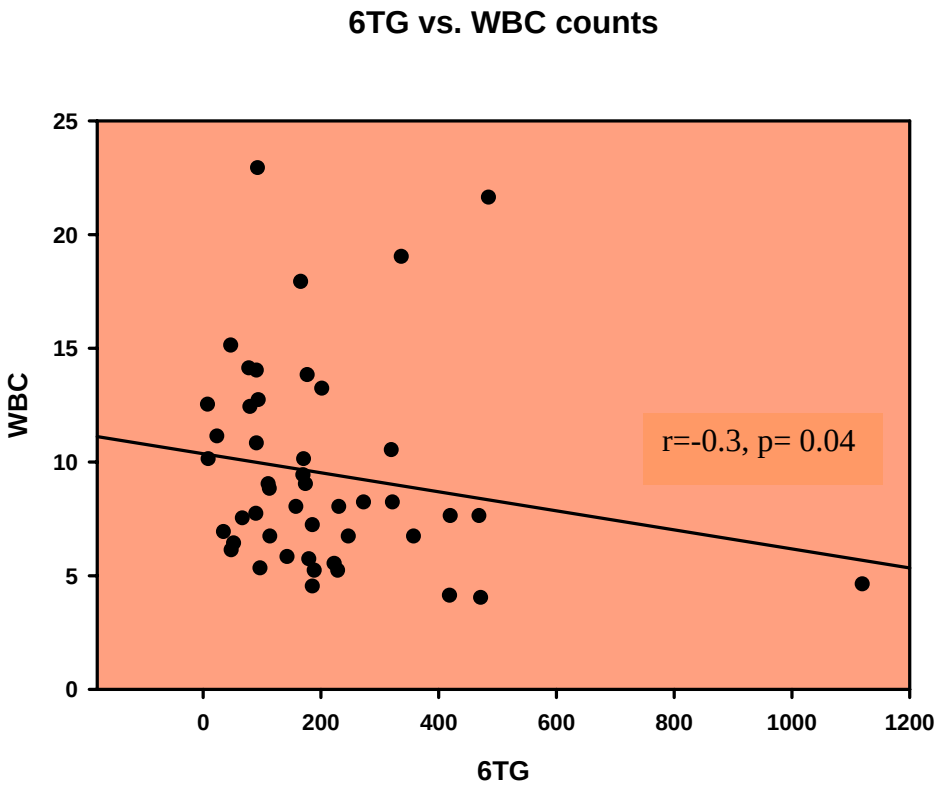


Figure 4:

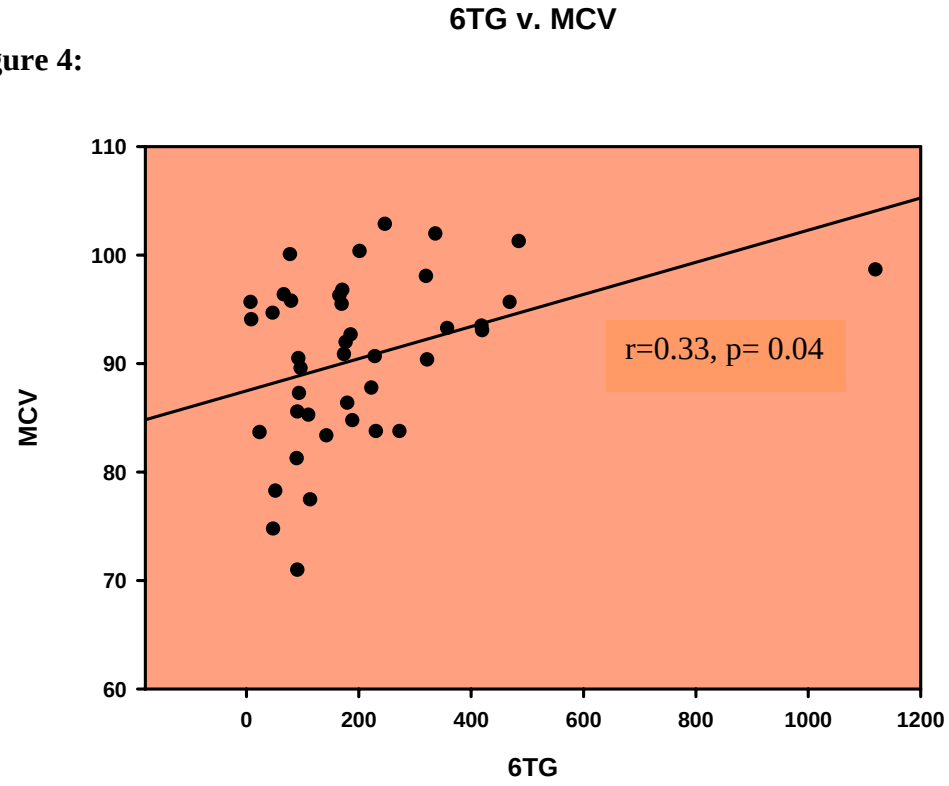
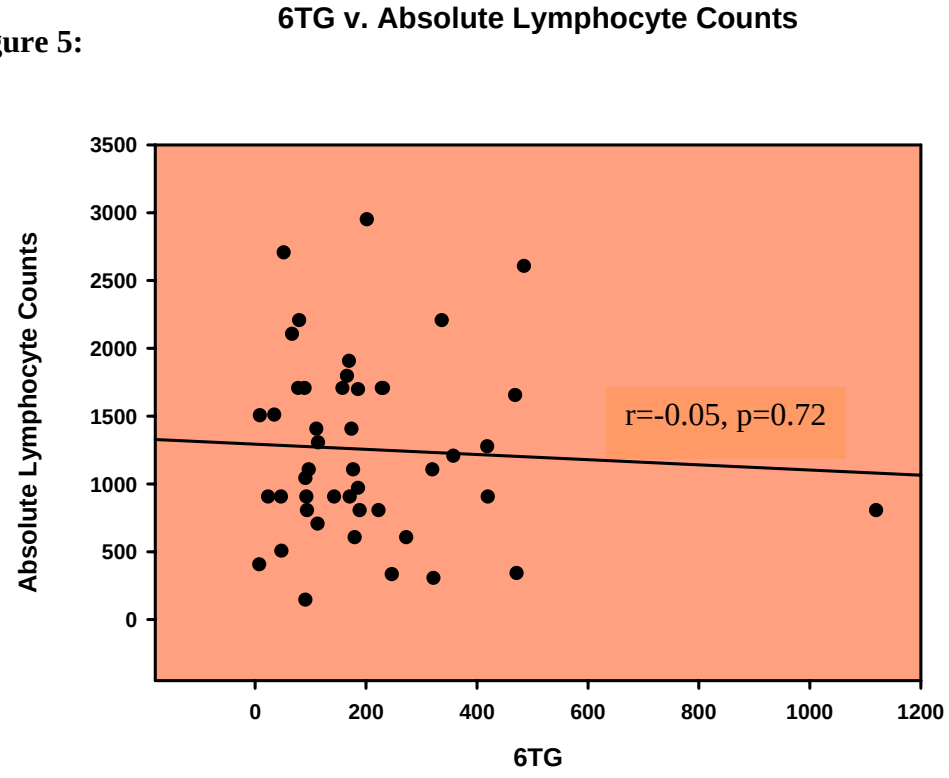


Figure 5:



The other metabolite, 6MMP, was used as a control to correlate with WBC, MCV, and lymphocyte counts. All levels were found to have no significant correlation: r values of -0.11 (p=0.45) (Figure 6), 0.1 (p=0.54) (Figure 7), and -0.1 (p=0.5) (Figure 8), respectively. Since 6MMP is an indicator of hepatotoxicity, the lack of correlation is not surprising.

Figure 6:

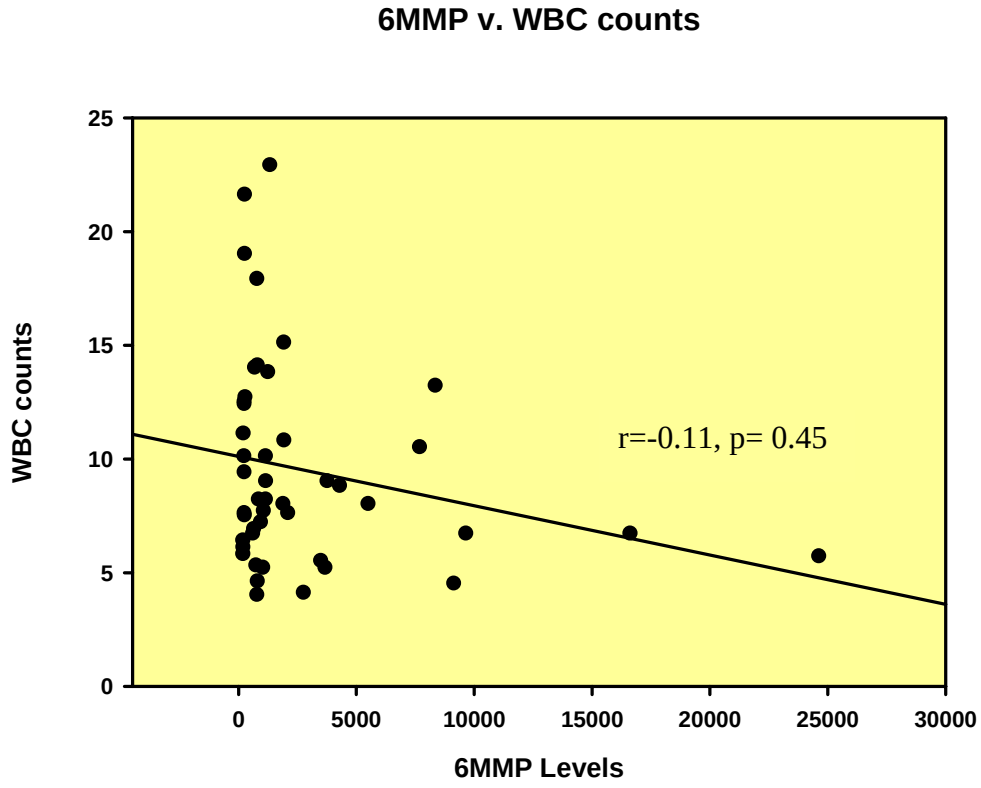


Figure 7:

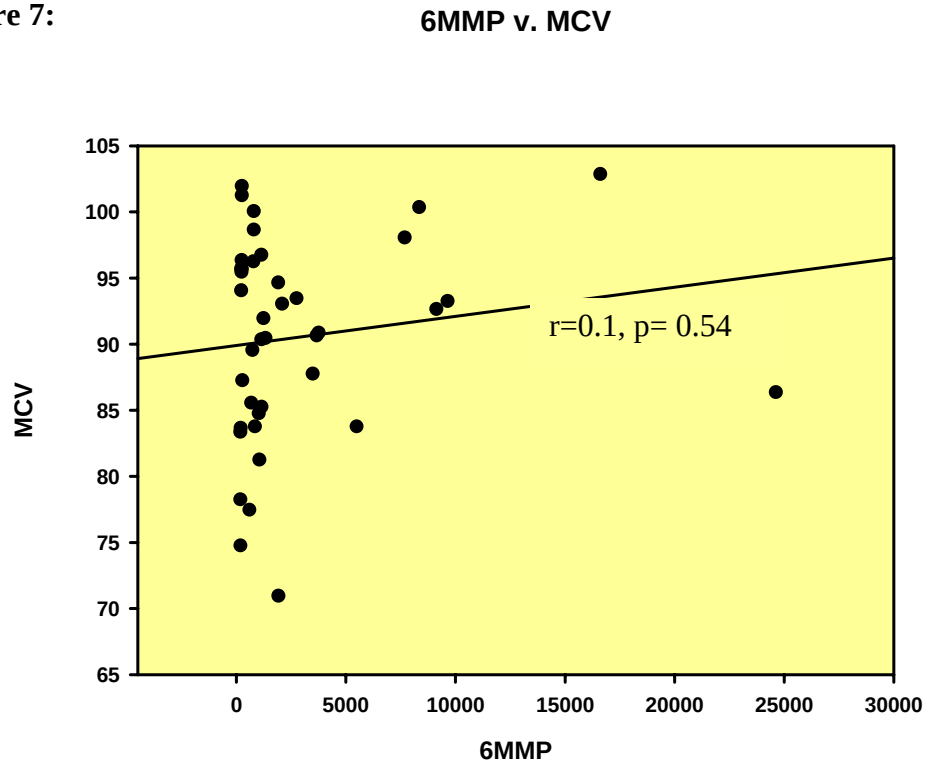
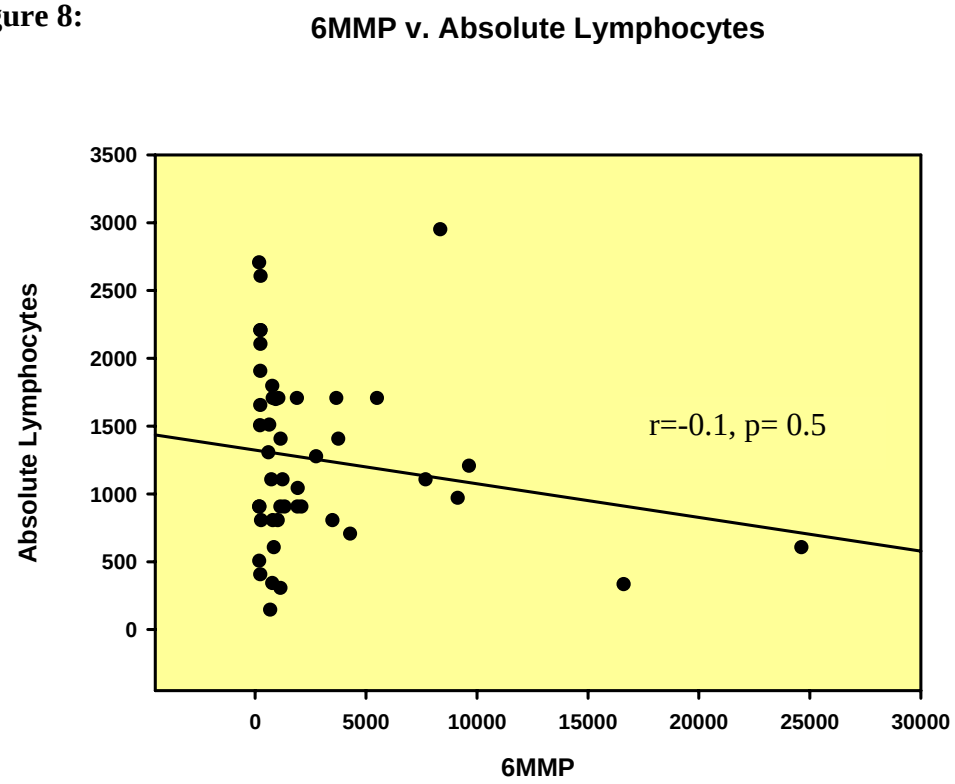


Figure 8:



5.3 Correlation of Clinical Activity to Metabolite Markers and Routine Blood Tests

No significant correlation was found when comparing those with controlled disease status to their WBC counts, MCV levels, absolute lymphocyte counts, or to the metabolite levels 6TG or 6MMP as shown in Table 5. Since the primary intent of the study did not include clinical activity evaluation, the number of patients on retrospective review that could be given a "controlled" versus "uncontrolled" clinical activity was significantly lower. Of the 124 patients, clinical activity was able to be analyzed for 60 patients in those who had routine blood markers (WBC, MCV, and lymphocyte counts) measured, while those with metabolites 6TG and 6MMP measurements, only 14 of those could a clinical activity be analyzed. The number of patients evaluated was small and thus, no correlations reached statistical significance. Of the 60 patients, 29 had controlled disease activity, and of the 14, only 5 had controlled disease activity.

Table 5: Correlation of Controlled-disease Clinical Status with Markers:

WBC (n=60)	MCV (n=60)	Lymphocytes (n=60)	6TG (n=14)	6MMP (n=14)
r=0.09 (p=0.47)	r=0.12 (p = 0.41)	r=0.08 (p=0.52)	r=-0.17 (p=0.57)	r=-0.24 (p=0.41)

5.4 Severe Lymphocytopenia

In four patients, severe lymphocytopenia developed and CD4 counts were monitored closely during this time. Although the number of patients was small, multiple measurements of CD4 counts took place and these levels were analyzed with WBC (Figure 9) and lymphocyte levels (Figure 10). The only significant correlation found was between lymphocyte levels and CD4 counts which took on a one-to-one ratio of $r=0.95$ ($p<0.000001$). In one patient, despite undetectable levels of 6TG after

discontinuation of azathioprine, lymphocytopenia 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.

Figure 9:

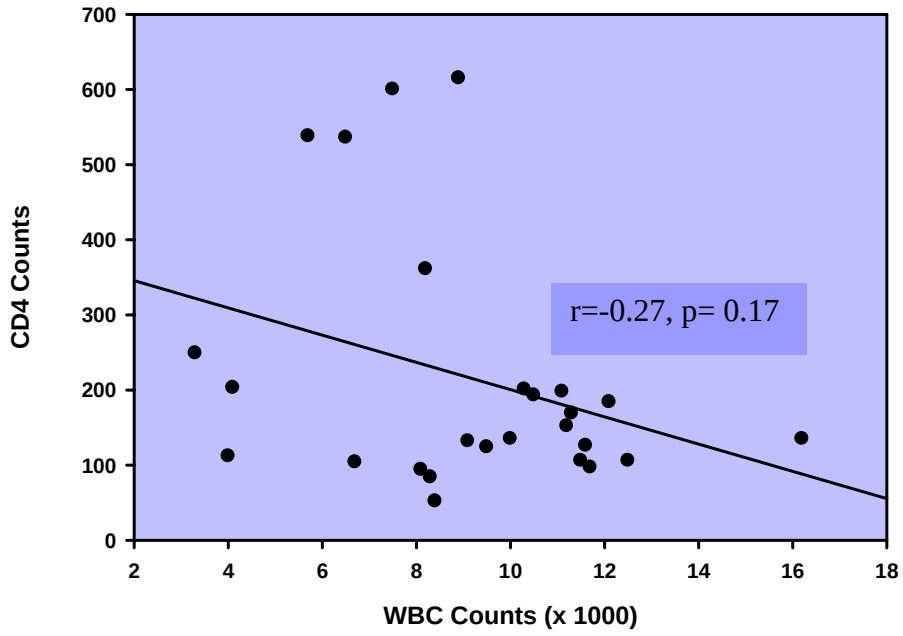
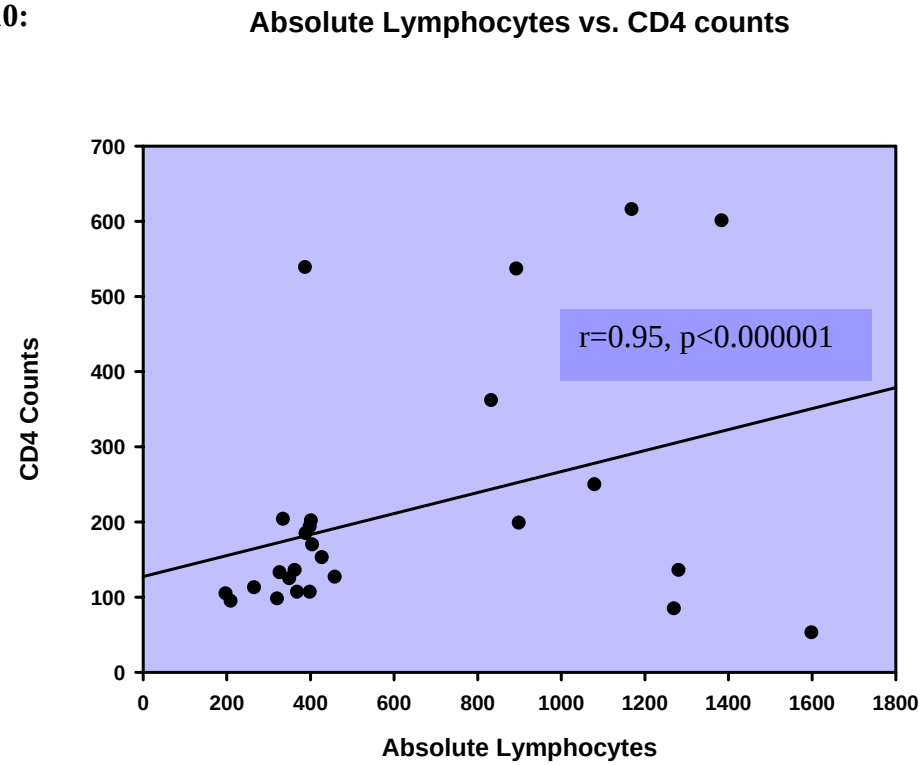


Figure 10:


Absolute Lymphocytes vs. CD4 counts
CD4 Counts
Absolute Lymphocytes
r=0.95, p<0.000001
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
1200
1400
1600
1800

CHAPTER 6

Discussion and Conclusion

6.1 **Incidence of Lymphocytopenia**

Lymphocytopenia 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 lymphocytopenia without the use of these medications. With the increasing use of biologics, TNF-alpha antagonists such as infliximab and adalimumab, their role in affecting leukocytes and lymphocytes may somewhat complicate this picture. However, in this study, the use of these agents, nor the use of corticosteroids did not appear to cause increase risk of lymphocytopenia.

Levels of the lymphocyte count may be low enough to cause opportunistic infections, such as herpes zoster and pulmonary coccidiomycosis as found in our patients. In the patients in this study with these infectious diseases and other select patients with very low lymphocyte counts, CD4 counts 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. Because the number of patients that developed infections were small, more information will be needed to clarify why these patients developed complications while others did not.

6.2 Correlation of Metabolite Markers to Routine Blood Tests

The metabolite markers 6TG and 6MMP did not show any clinically significant correlation to any of the routine blood tests. The only two correlations that were found

to be statistically significant were between 6TG levels and WBC and MCV, but both had a marginal correlation. A previous Danish study showed that 6TG was related to MCV by an r value of 0.33, the same as found in our study.[10]

6TG levels and lymphocyte counts had an extremely poor correlation. This is not a type II error, and to have achieved any statistical significance, the study would have required over a thousand patients. However, since leukopenia did not always correlate to lymphocytopenia, it appears reasonable to believe that even with larger n, a clinical significant correlation would not have been reached. As expected, 6MMP did not show correlations to any of the routine blood markers.

In general, carefully monitoring of lymphocyte levels must be done to prevent potential infections. We cannot rely on 6TG levels to determine who may develop lymphocytopenia. Using MCV levels and WBC counts to monitor the efficacy of the medication would not be advised due to their poor correlation with 6TG levels.

6.3 Correlation of Clinical Status to Metabolite Markers & Routine Blood Tests

Although the initial intent of the study was not to evaluate clinical activity to lab work, the findings instigated an interest to retrospectively evaluate these patients. From our findings, there is limited correlation of disease status to any of the blood markers. Thus, we cannot rely on biomarkers in monitoring the efficacy of the drug. In a time where we have placed emphasis on obtaining labs and using them to guide management of these patients, this study has affirmed that we must use caution in relying solely on blood 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, and this multifactored approach is perhaps the most optimal management for these patients. Until further information can be obtained with larger number of patients and perhaps a better clinical activity marker than a physician's global assessment, we may be able to determine more accurate information.

Lymphocytopenia, although a worrisome component of AZA/6MP therapy, may play a role in its efficacy of treatment. In some patients, we have noticed an improvement in symptoms while the lymphocyte counts were low, however, when the levels increased, symptoms recurred. However, analysis of all patients showed that there is no correlation between either WBC, lymphocyte, MCV, or 6TG levels to clinical status. Perhaps using detailed clinical trial markers of disease activity may provide more accurate evidence of this then what this study provided.

6.4 Future Directions

The underlying mechanism of lymphocytopenia of these patients remains unknown. Since theories exist that apoptosis of T lymphocytes plays a role in the mechanism of action of AZA/6MP, it would be of interest to further study the T lymphocyte subsets in these patients. The limited data in this study of CD4 counts indicated that CD4 counts as low as seen in HIV/AIDS patients can occur and is of concern especially in the realm of opportunistic infections. The close correlation between CD4 counts and lymphocyte levels suggests that CD4 counts may be the most important subtype, however more patients will need to be evaluated. Further analysis of T cell subsets may confirm the role of CD4 counts as well as determine other subsets such as regulatory T cells that could be contributing to lymphocytopenia. Further studies may be required to determine true clinical status or clinical significance behind the

lymphocytopenia. Ongoing data collection of patients with lymphocytopenia and the clinical background of the patients that go on to develop infections may be of value. Analyzing intestinal lymphocytes and its specific subsets may also provide additional clues on mechanism of action of these medications.

6.5 Conclusion

This study shows that lymphocytopenia does not always correlate with leukopenia. Significant lymphocytopenia and depressed CD4 counts can occur in the presence of normal WBC counts and metabolite (6TG/6MMP) levels. Lymphocyte as well as WBC counts should be monitored in patients receiving immunosuppressive therapy with AZA/6MP. Lymphocytopenia was sometimes prolonged & persisted even with undetectable metabolite levels. Clinical significance of these findings still needs to be fully defined. Perhaps a more detailed analysis of clinical activity in relation to blood markers can put disease activity into perspective. Lymphocytopenia may or may not be a part of the therapeutic effect. It can, however, predispose patients to opportunistic infections. These risks must be discussed with patients and taken into account when considering the use of these medications for control of their disease.

Glossary

6MP 6-mercaptopurine

6TG 6-thioguanine

6MMP 6-methylmercaptopurine

AIDS Acquired Immune Deficiency Syndrome

AZA Azathioprine

CBC Complete Blood Count

HGRT Hypoxanthine Phosphoribosyl Transferase

HIV Human Immunodeficiency Virus

HPLC High Pressure Liquid Chromatography

IBD Inflammatory Bowel Disease

LFTs Liver Function Tests

MCV Mean Corpuscular Volume

TNF Tissue Necrosis Factor

TPMT Thiopurine Methyltransferase

WBC White Blood Cell

XO Xanthine oxidase

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Vita

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