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Immune thrombocytopaenia at a central hospital in Johannesburg

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IMMUNE THROMBOCYTOPAENIA AT A CENTRAL HOSPITAL IN JOHANNESBURG

Dr Melvin Mbao

Johannesburg 2016

A Research Report submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the degree of Master of Medicine in the branch of Internal Medicine.

Declaration

I, Melvin Mbao, declare this research report to be my own unaided work. It is being submitted for the degree of Master of Medicine in Internal Medicine in the University of Witwatersrand, Johannesburg. It has not been previously submitted for any degree or examination at this or any other university.

_MELVIN MBAO _____

(Signature)

___15_____ Day of _August_2016

Dedications

This work is dedicated to my parents, Prof M l Mbao and Mrs Gertrude Mbao, who have taught me the values of perseverance and duty. A special thanks to my brothers and friends for their support.

Acknowledgements

I would like to acknowledge the following people for their invaluable support and inspiration:

- My supervisor, Prof. Johnny N Mahlangu, Head of the School of Pathology and Head of the Haematology ward and the Main Haematology Laboratory; Charlotte Maxeke Johannesburg Academic Hospital; and Co- Supervisor Dr Georgia Demetriou from the Department of Medical Oncology, Charlotte Maxeke Johannesburg Academic Hospital.

- The accommodating staff of the Charlotte Maxeke Johannesburg Hospital Haematology Department and the patients for without which this work would not be possible.

Abstract

Background. Primary immune thrombocytopenia (ITP) is a rare disease causing significant morbidity. South Africa has a high prevalence of HIV infection which may be associated with immune thrombocytopenia. There is a paucity of clinical, management and outcome data on immune thrombocytopenia in the local South African setting.

Objectives. To compare the demographics, clinical presentation, management and treatment outcomes of immune thrombocytopenia in HIV positive and HIV negative patients and to compare the treatment outcomes with established international guidelines.

Methods. This was a retrospective comparative study conducted at Charlotte Maxeke Academic Hospital, Johannesburg, from January 2003 to December 2014. Adults (≥ 18 years) with confirmed diagnosis of ITP were included. Hospital charts of eligible patients were reviewed to extract data on their clinical presentation, diagnosis, HIV status, treatment and outcomes. A comparison was made between HIV positive and negative patients. Descriptive analysis was performed on the data and results were presented graphically. The P-value of <0.05 was regarded as significant.

Results. A total of 250 patients were screened, of which 154 patients met eligibility criteria for the study. 91% of the patients were female, 58% were HIV negative and 42% were HIV positive**.** The 25-35 year age-group comprised the highest percentage of HIV positive patients (42%). There was no difference in the presentation of symptoms between HIV positive and HIV negative patients. Response to first line therapy was not significantly different between the HIV positive and HIV negative patients (p=0.1370). The patients who went on second line therapy, showed excellent response with approximately 80% reaching complete response. There was no difference in HIV positive and HIV negative groups.

Conclusion. In a large central hospital in a high HIV prevalence setting, there is no significant difference between HIV positive and HIV negative patients in terms of clinical presentation, treatment and outcomes in confirmed patients with immune thrombocytopenia. The management of ITP at the CMJAH is comparable to that of published guidelines.

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List of Abbreviations

1) AIDS: Acquired ImmuneDeficiency Syndrome

2) ELISA: Enzyme Linked Immunosorbent Assay

3) HIV: Human Immunodeficiency Virus

4) ITP: Immune Thrombocytopaenic Purpura

5) SLE: Systemic Lupus Erythematosus

Chapter 1: Introduction

1.1 Primary Immune Thrombocytopenia (ITP)

Primary immune thrombocytopenia (ITP) which was previously known as idiopathic thrombocytopenic purpura, is an important clinical condition associated with significant morbidity and often challenging management. The International Working Group consensus has defined Immune Thrombocytopenia as an autoimmune disorder which is characterized by isolated thrombocytopenia(1). In practical terms this refers to an isolated peripheral blood platelet count of less than 100×10^9/L. There should be no obvious underlying or initiating secondary cause of the thrombocytopenia(1, 2). ITP is therefore a diagnosis of exclusion. The thrombocytopenia is caused by the destruction of autoantibody platelets coated by the reticuloendothelial system; in the majority of cases the spleen is the site of destruction(3, 4). ITP is a heterogeneous disorder in terms of clinical presentation and can manifest with or without mucocutaneous bleeding as well as other sites of bleeding but is rarely life-threatening(1, 2).

1.2 Secondary Immune Thrombocytopenia

Secondary immune thrombocytopenia refers to thrombocytopenia arising from an identifiable underlying disease states or drug exposure (1, 5). A number of conditions can give rise to secondary ITP, such as systemic lupus erythematosus (SLE). Infections such as Human immunodeficiency virus (HIV) and hepatitis C infection are recognized causes of secondary ITP (6-8). In the hospital setting, the largest secondary cause of thrombocytopenia is drugs such as heparin, alcohol and quinine (8). In drug induced immune thrombocytopaenia, the most common mechanism is secondary to accelerated platelet destruction as a result of autoreactive, drug dependent antibodies(9). Drugs can also cause decreased platelet destruction by direct bone marrow toxicity(9).

1.3 Prevalence

Globally, ITP is a rare disease with an incidence ranging from 1.6 to 3.9 per 100 000 persons per year (10). The prevalence in Europe and the United States of America has been estimated to be approximately 9.5 per 100 000 persons per year(10). The true prevalence in the developing countries and South Africa is currently unknown. ITP has a definite female prevalence; all ages are affected by the disease (5, 11).

1.4 History of ITP

ITP has a long history with the clinical features of the disease being described early as 1735 by German poet Paul Gotlieb Werlhof (12). This preceded the discovery of platelets in the mid 1800s(12). In 1951, the seminal work of Harrington *et al* entitled "*Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura*" proved that a circulating humeral factor was responsible for rapid platelet destruction(13). Since then our knowledge of the pathogenesis of Primary ITP, although not fully elucidated, has broadened extensively.

1.5 Pathogenesis

The underlying aetiology of Primary ITP remains unknown, but the resultant effect is immune dysregulation leading to immune-mediated destruction of platelets and/or suppression of platelet production (3, 14).The "humeral factor" identified by Harrington *et al* was subsequently identified to be a platelet antibody of the immunoglobulin G type (IgG)(12, 13, 15). These antibodies have a specific antigenic target in the form of platelet membrane glycoproteins GP1b/IX and GPIIIa(14, 16). These autoantibodies coat the platelets and facilitate the process of binding or

opsonization by macrophages bearing the FcyR receptor in the reticuloendothelial system, particularly the spleen, thereby targeting them for elimination(14, 16).

Autoreactive T lymphocytes have been implicated in the production of autoantibodies by the process of epitope spread(17). The glycoproteins are internalized and broken down by macrophages and other antigen producing cells. The new peptides or “epitopes” are presented to T cells and, in coordination with co – stimulatory cells, induce autoreactivitiy in the T cells. The autoreactive T cells subsequently stimulate B lymphocytes, which produce the antibodies that opsonize platelets(18). Autoantibodies not only target platelets but also bind megakaryocyte progenitors, thereby interfering with megakaryopoiesis, with subsequent decrease in platelet production(19, 20). There is a generalized loss of T cell tolerance in the form of autoreactive T cells as well as a decrease in regulatory T cells (TREGs) which help maintain immune tolerance(14, 16).

The pathogenesis in secondary ITP is slightly different, particularly in HIV related ITP. An association between HIV and ITP was first noticed in 1982 when a group of 11 homosexual men with HIV were noted to have thrombocytopenia (21). The pathogenesis is multifactorial with increased destruction coupled to impaired production contributing to thrombocytopaenia. By the process of molecular mimicry, anti HIV antibodies cross react with platelet glycoproteins and form immune complexes that target the platelets for peripheral destruction(6, 22). Production of platelets is also impaired with direct infection of megakaryocytes by HIV as well as by the induction of premature apoptosis and inhibition of maturation of megakaryocytes (6, 22). In the pre-HAART era, the incidence of HIV associated ITP was between 10 -30% of HIV positive cases; this has subsequently improved in the era of HAART(7).

1.6 Clinical Features

The diagnosis of primary ITP is one of exclusion of secondary causes and is approached by taking a relevant history and a thorough focused physical examination followed by appropriate investigations. The complete blood count and smear need to be reviewed to exclude pseudothrombocytopenia and secondary causes(8). ITP often presents insidiously with no symptoms and is discovered accidentally on analysis of a complete blood count. The most common initial manifestation of ITP are the bleeding manifestations into the skin and mucous membranes (4). Mucocutaneous bleeding takes the form of petechiae, purpura, epistaxis and gum bleeding, which are reflective of the low platelet count (4, 10). There may be accompanying symptoms of anaemia due to the ensuing blood loss from genito-urinary bleeding or the gastrointestinal tract (4,10). In the context of HIV, ITP is often an early manifestation of the disease which predates AIDs and AIDs related conditions(7).

1.7 Diagnostic evaluation of ITP

The clinical hallmark of ITP is the presence of petechiae and purpura; however, the clinical examination may be normal. The IWG laid the threshold for diagnosis at a platelet count of less than 100×10^9 although symptomatic bleeding usually occurs with a platelet count of less than 30×10^9(1, 5). The thrombocytopenia is often isolated with a normal haemoglobin and white cell count, but associated microcytic anaemia may occur. It is critical to evaluate the complete blood count and the peripheral smear to exclude other secondary causes of the thrombocytopenia.

International guidelines do not suggest routine bone marrow aspirate investigations in patients with typical features of ITP(1, 23). In South Africa, however, there is a high prevalence of

intramedullary pathology such as tuberculosis, especially in our HIV positive population(24). Bone marrow aspirates are often done to exclude secondary pathologies. When done, the bone marrow aspirates often reveals the presence of normal to increased numbers of megakaryocytes (25).

1.6 Management

Management goals are different in Primary ITP as compared to Secondary ITP. In Primary ITP the two major indications for treatment are the platelet count level and extent of bleeding. The threshold for treatment of platelets is less than 30×10^9/l and the aim is to return the platelet count to a safe level of above 30×10^9/l that prevents further bleeding with minimal treatment related morbidity (1). Bleeding patients are treated irrespective of platelet count below 100 x 10^9/l (1). The response to therapy is further defined according to platelet counts into complete, partial and no response (1). A complete response is defined as a platelet count of 100 x 10^9/l and the absence of bleeding (1). A partial response is a platelet count of between 30 – 99 x 10^9/l as well as 2 fold increase in platelet counts (1). No response is defined as a platelet count of less than 30 x 10^9/l or a less than 2 fold increase in the baseline platelet counts (1). In secondary ITP, management is aimed at treating the underlying cause (1,2).

The first line therapy in Primary ITP is corticosteroids which, although they do not alter the natural history of the disease, result in a rapid rise in platelet counts. The mechanism of action involves prevention of the clearance of the opsonized platelets as well as increasing platelet production by inhibiting the destruction of platelets within the bone marrow (4). There is a high response rate of 66% to the use of corticosteroids; however, there are also high relapse rates once the patient is weaned from corticosteroids (4, 26). The most popular regime is oral prednisone at 1mg /kg bodyweight; however, other investigators such as Cheng have used shorter courses of high dose dexamethasone (i.e. 40mg per day for 3 days) with good effect

(26, 27). Common side effects of long term corticosteroids which limit prolonged usage are hypertension, diabetes and osteoporosis (27).

Other agents within the first line therapies include the usage of high dose intravenous immunoglobulins and anti D immunoglobulins, particularly in patients who are bleeding and for whom steroids are contraindicated or ineffective (1, 8). These agents raise the platelet count precipitously within 24 – 48 hours but do not have a durable response (28, 29). In terms of second line therapies for ITP, splenectomy remains the treatment of choice with highest durable response(1, 30). Approximately 60-80% achieve partial to complete response post splenectomy(30). Splenectomy is not without complications, such as sepsis secondary to encapsulated organisms and a risk of thrombosis(30). It is, however, the recommended therapy for patients with refractory ITP or cases which are refractory to corticosteroid therapy (11, 30). There is currently no difference in the outcome of laparoscopic splenectomy when compared to laparotomy (11,30).

1.8 Novel therapies in ITP

In the last 20 years novel “targeted” therapies have evolved to counter the immune dysregulation involved in ITP. Rituximab is an anti CD 20 chimeric monoclonal antibody which acts by depleting B- lymphocytes that produce the offending antibodies (31, 32). The approximate number of patients who achieve a complete response (platelets > 100×10^9/l and absence of bleeding) to Rituximab amounted to 40% at one year and 20% at 5 years (31, 33). The use of Rituximab is, however, associated with toxicities ranging from common ones such as first infusion reactions and serum sickness to rare complications such as progressive multifocal leukoencephalopathy (31, 33).

Another group of novel therapies are the thrombopoietin-receptor agonists aimed at both platelet production and platelet restoration. Thrombopoietin (TPO) is a glycoprotein hormone

which is synthesized in the liver and kidneys (34). By binding to the c-MPL receptor on the platelet surface, a cascade of chemical reactions is triggered resulting in increased megakaryocytopoiesis and ultimately enhanced platelet production (11, 31). Two agents, Romiplostim® and Eltrombopag®, have been developed which mimic the action of endogenous thrombopoietin without initiating antibody production, which was a complication of native thrombopoietin(11, 31). They are effective agents in both splenectomised and post-splenectomy patients and increase platelet counts in 80%-88% (34, 35). These agents are, however ,associated with bone marrow fibrosis and thrombosis(34). Thrombopoietic agonists are currently registered as rescue therapy for those patients with refractory ITP who are also refractory to corticosteroids, immunoglobulins and splenectomy(1).

In those asymptomatic patients with HIV associated ITP, the first line of therapy is Highly Active Anti Retroviral therapy (HAART)(6, 7). However in those patients with significant bleeding and low platelets under 30×10^9/l, the mode of therapy remains the same as primary ITP, with the same first and second line therapies (1, 8).

1.9 Aims and Objectives of the Study

We hypothesize that in South Africa, the clinical presentation, diagnosis, therapeutics and treatment outcomes in HIV positive patients may be different to those without HIV infection. There is a paucity of local data on the difference between HIV+ and HIV- patients, and published international data in this regard is also lacking. South Africa has a high burden of HIV infection and is therefore ideal in addressing this research question and producing data which would be critical in guiding appropriate management of these patients. The objectives of this study were therefore:

- To describe the demographics of patients presenting with ITP (Both HIV positive and negative).

- To compare clinical presentation, diagnosis, treatment and treatment response between HIV positive and negative patients with ITP.
- To compare the treatment response of ITP at CMJAH to those of published ITP guidelines.

Chapter 2: Materials and Methods

2.1 Ethics Clearance

Approval to conduct the study was obtained from the Human Research Ethics Committee of the University of Witwatersrand, Johannesburg prior to start of the study. Approval was granted with clearance certificate number

M131026.

This study was a retrospective study conducted by reviewing the hospital charts of patients with a diagnosis of ITP; no informed consent was required from study participants.

2.2 Study Design and Setting

This was a single centre retrospective comparative analysis of ITP in HIV+ compared to HIV- patients. The study was undertaken at Charlotte Maxeke Johannesburg Academic Hospital- (CMJAH), a quaternary care public facility located in Parktown, Johannesburg, from 1 January 2003 until 31 December 2014. The hospital has both inpatient and outpatient facilities and sees internally and externally referred patients with haematological conditions.

2.3 Study Population

Patients who were included in the study were diagnosed and managed at the CMJAH Haematology Department. All patients had been tested for HIV with an enzyme linked immunosorbent assay (ELISA) as part of their routine management.

Only patients who met the following criteria were included in the study:

Inclusion Criteria

- Males and females with confirmed ITP
- Had an HIV test done
- Had presented and were managed at the CMJAH
- Age 18 and above years of age, and
- Had complete clinical record of ITP management at CMJAH.

Patients with incomplete records as well as those with other secondary causes of thrombocytopenia other than HIV were excluded. The secondary causes which were excluded were the following.

Exclusion Criteria

- Incomplete clinical records
- Infections – herpes infection, hepatitis, tuberculosis-- excluded by appropriate testing where indicated
- Malignancies – chronic lymphocytic anaemia and non-Hodgkin's Lymphoma excluded on the basis of peripheral blood and bone marrow findings
- Drug-induced immune thrombocytopenic purpura (e.g. heparin, quinine/quinidine, sulphonamides)- excluded on history

- Connective tissue disorders - Systemic lupus erythematosus- excluded on connective screen where indicated.

2.4 Data Collection

Clinical data was obtained from the patients hospital files, which were drawn from the Haematology Department records system as well as the hospital archive system using the patient's hospital registration numbers. Only patient meeting the above criteria were included in the study. Files with incomplete clinical data were excluded.

The data taken from the files was then captured manually onto a standardized data collection sheet (see Appendix A). In order to preserve privacy and confidentiality, patients were assigned a unique study number with no names or personal details recorded in the data sheet. The data was captured onto the data collection sheet and then entered onto a Microsoft@ Excel database. Each case was identified with a study number during statistical analyses performed.

2.5 Statistical Analysis

The data was analysed using SAS (Statistical Analysis System, version 9.3, SAS Institute, USA). The demographic information of the patients was split according to HIV status and plotted as bar charts to visualise the distribution of variables (age groups and gender).

The assessment of symptoms at clinical presentation followed. Bar graphs were again utilised to illustrate the main presenting symptoms, namely the petechiae, purpura, genitourinary or gastrointestinal bleeding. Further statistical tests done included the following:

i. The chi-square test for association between variables
ii. The kappa test of agreement, and

iii. The McNemar test of independence of rows and columns.

In the diagnostic category, comparison between the HIV positive and negative groups was based on bone marrow aspirates performed and also the subsequent aspirate findings; these were displayed in bar graphs.

The management (treatment) of all patients was assessed and analysis was performed to look for differences between first line and second line therapies used. Lastly, the responses to treatment of the HIV positive and negative groups were checked on a three-scale metric namely, complete, response, partial response and no response; the results were extrapolated. The response rate was defined as follows:

- Complete response - Platelet counts > 100 x 10^9/l
- Partial response – Platelets between 30 – 99 x 10^9/l
- No response – Platelet count < 30x 10^9/l

Chapter 3: Results

3.1 Demographics

A total of 250 patients were screened in the period 1 January 2003 to 31 December 2014. Of these, 157 patients met the inclusion criteria. Of these eligible patients 59%(93) were HIV negative whereas 41%(64) were HIV positive. The median age of the 157 patients was 37 years, patients ranging from 18 to 75 years.

All 157 patients had HIV tests performed. In terms of gender distribution 92% (86) of HIV negative patients were female and 8% (10) male, where as in the HIV positive group, 91%(58) were female and 9% (6) male. Patients were also evaluated according to their age groups. In both HIV positive and negative patients, the age-group of 25-36 were most numerous, comprising 25% (23) of HIV negative patients and 42% (27) of the HIV positive group.

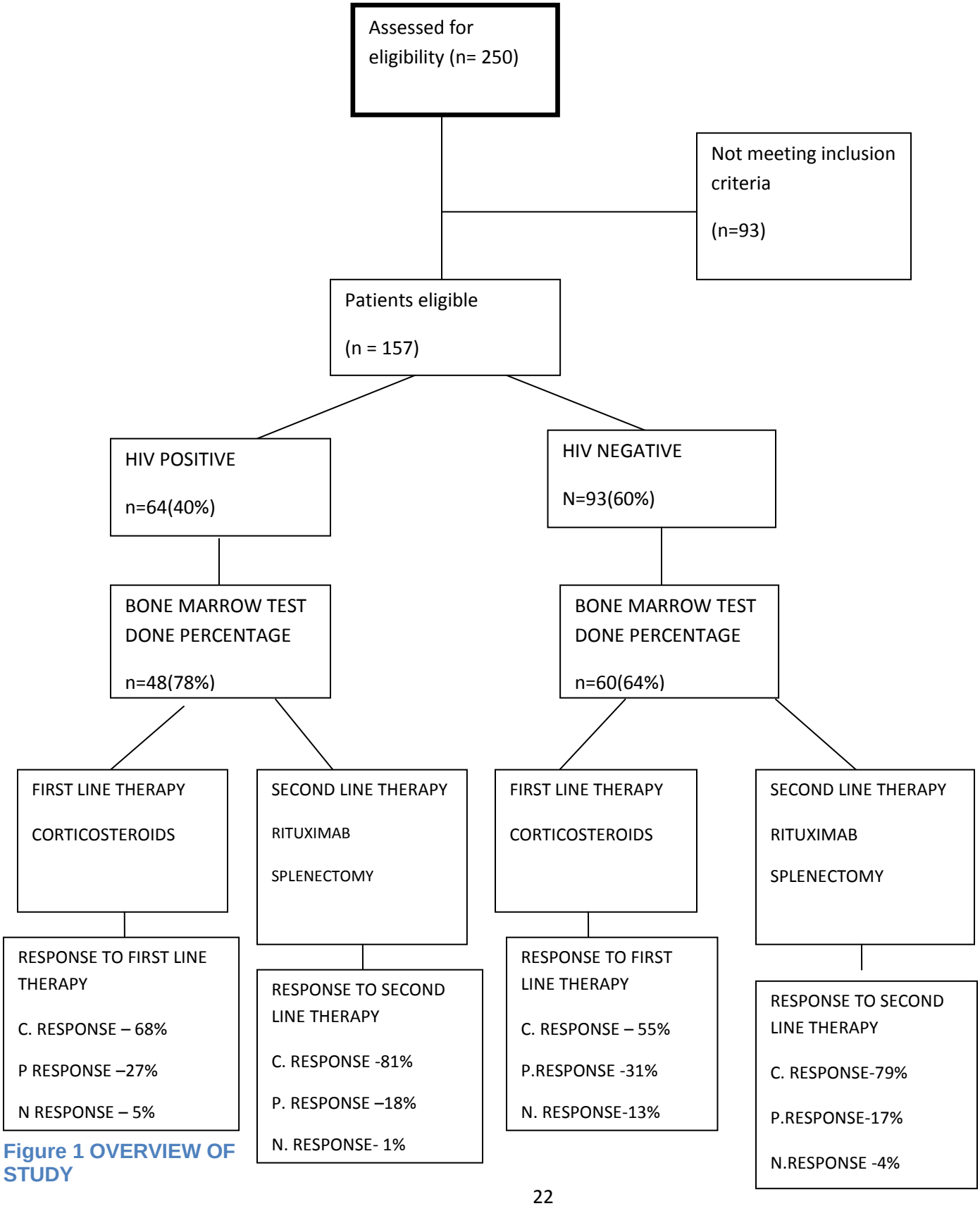


Figure 1 OVERVIEW OF STUDY

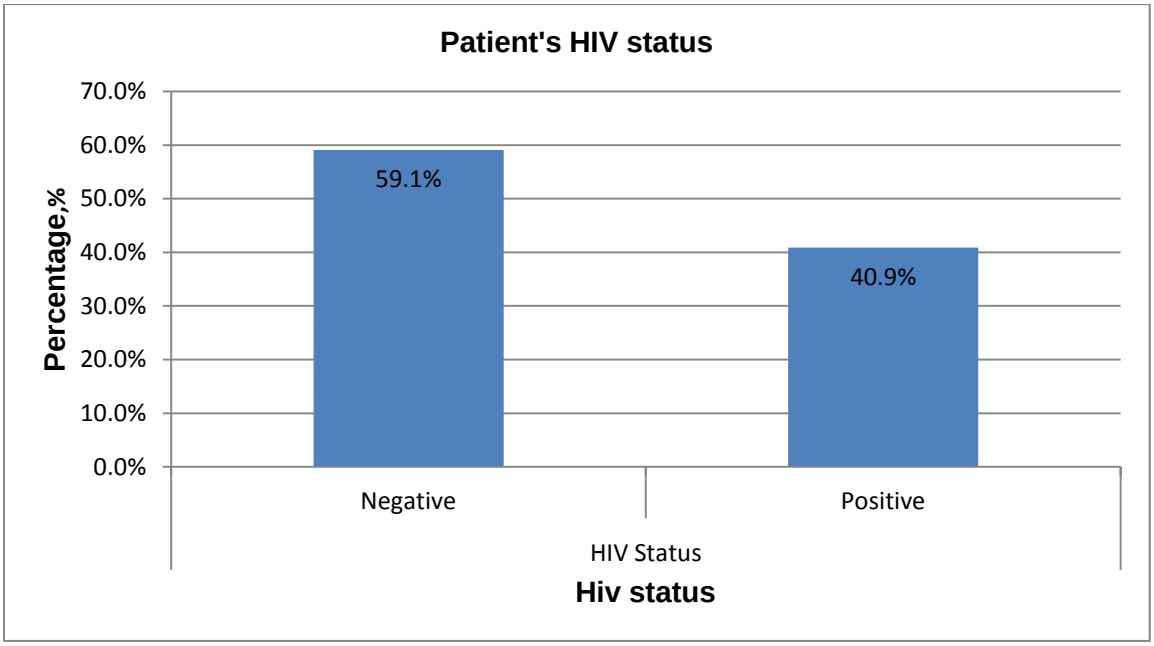


Figure 2 HIV status

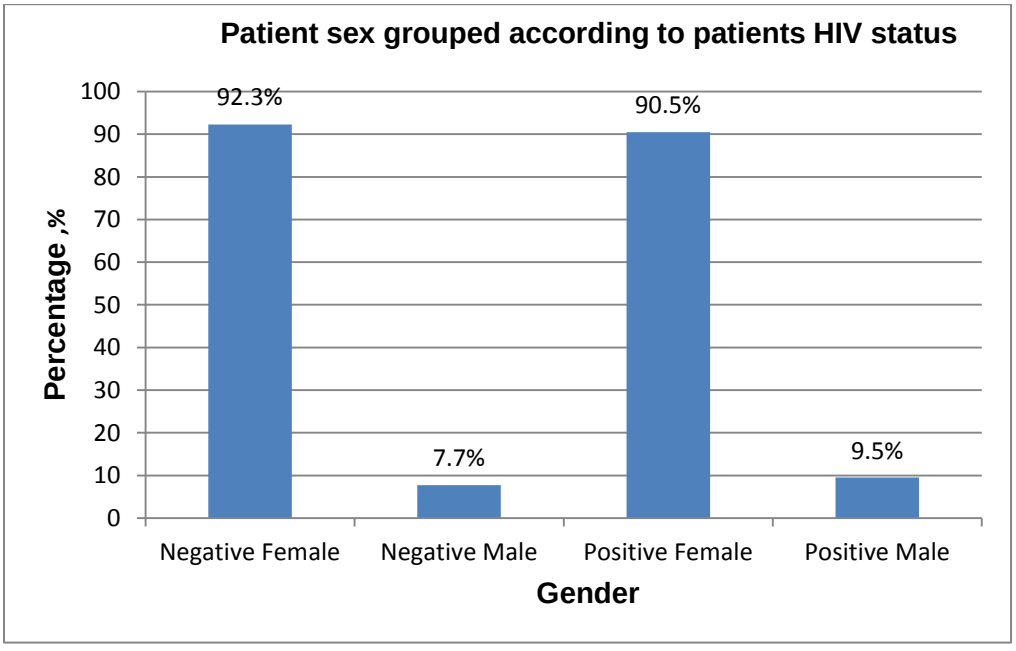


Figure 3 Gender by HIV status

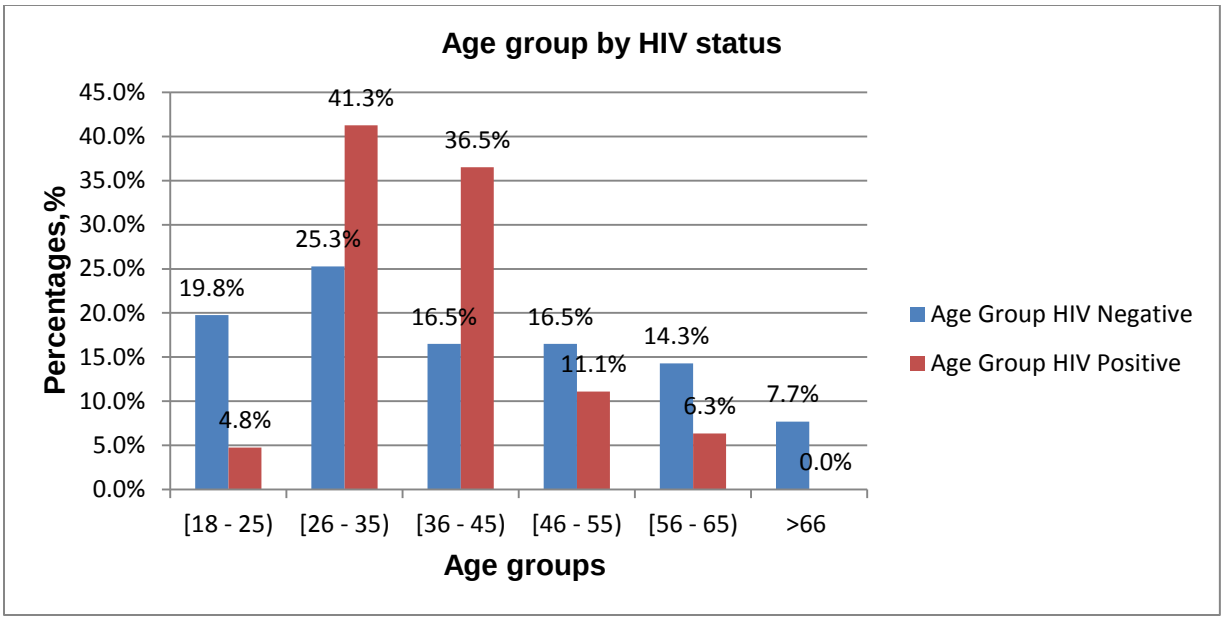


Figure 4 Demographics - Age group according to patients HIV status

3.2 Assessment of symptoms at clinical presentation

In terms of clinical symptoms, 96% (151) of patients were symptomatic and 4 %(6) of patients were not. The HIV negative group had more symptomatic patients with 98 %(154), whilst the HIV positive group had 95%(61). Petechiae and purpura were common presenting symptoms, with 84 %(131) in total presenting with petechiae and 85 %(133) presenting with purpura. Oropharyngeal and genitourinary bleedings were less common, with 70 %(109) of the total group presenting with oropharyngeal bleeds whilst 33%(52) presented with genitourinary bleeds.

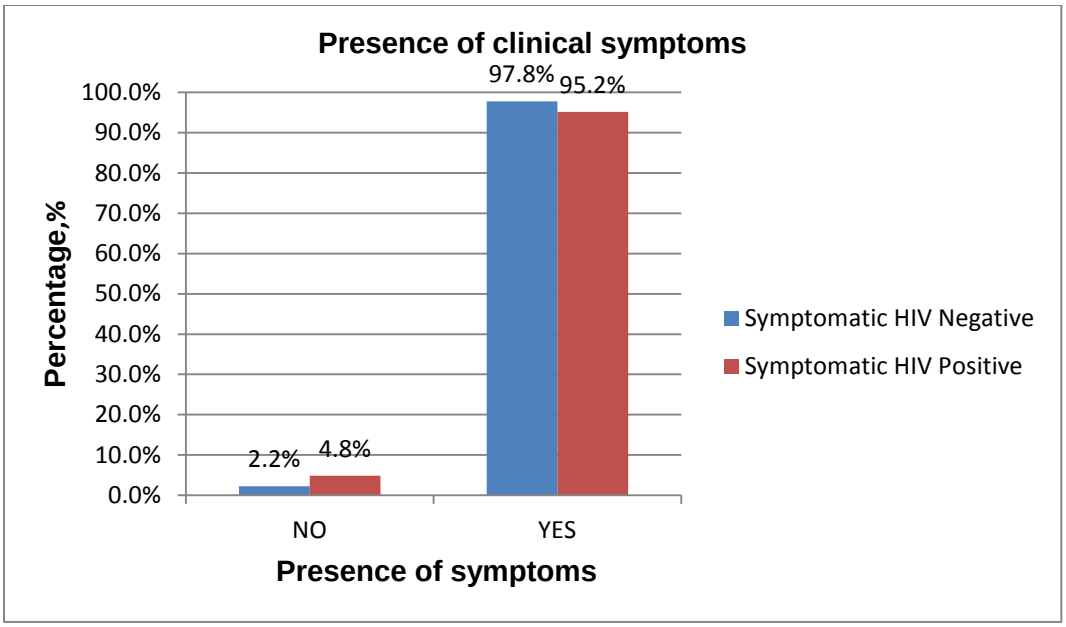


Figure 5 Clinical symptoms - symptomatic patients

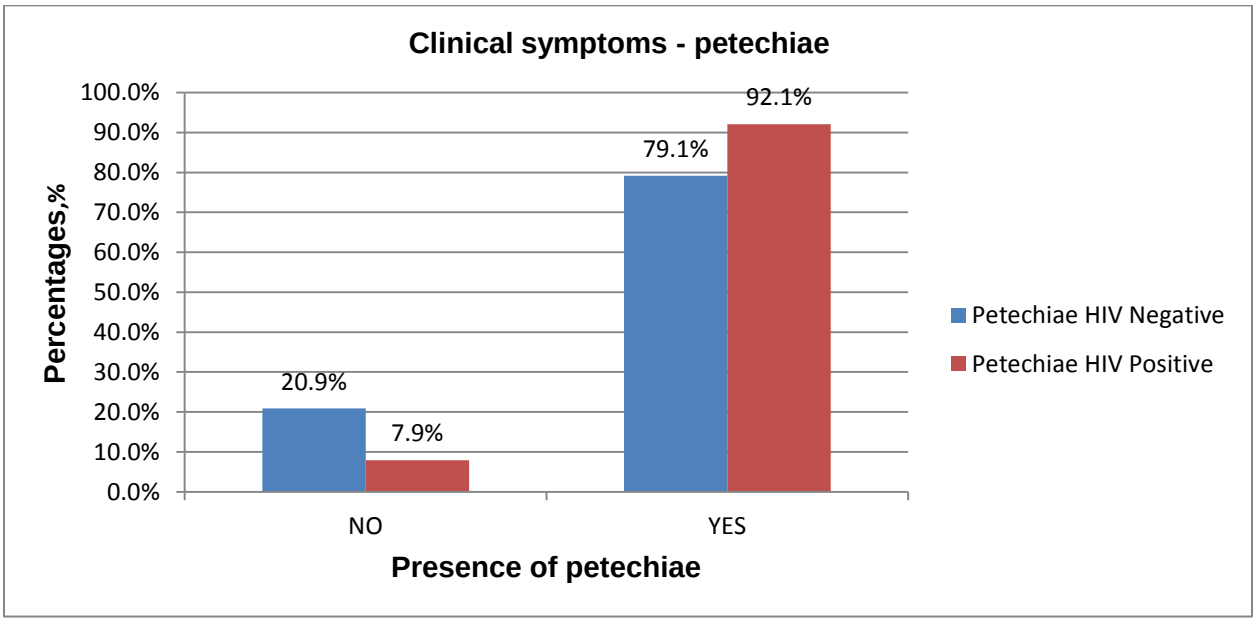


Figure 6 Clinical Symptoms - patients with petechiae

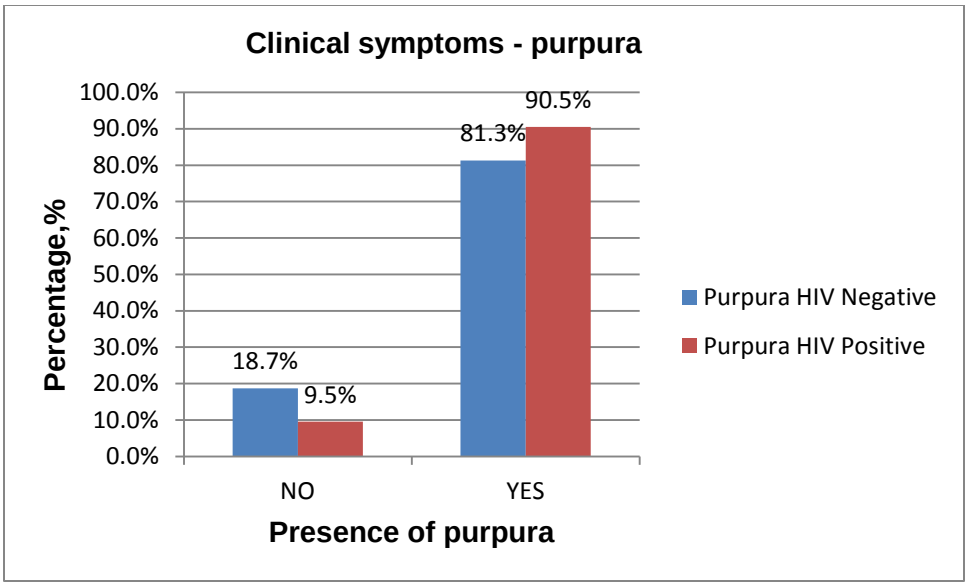


Figure 7 Clinical symptoms - patients with purpura grouped according to HIV status

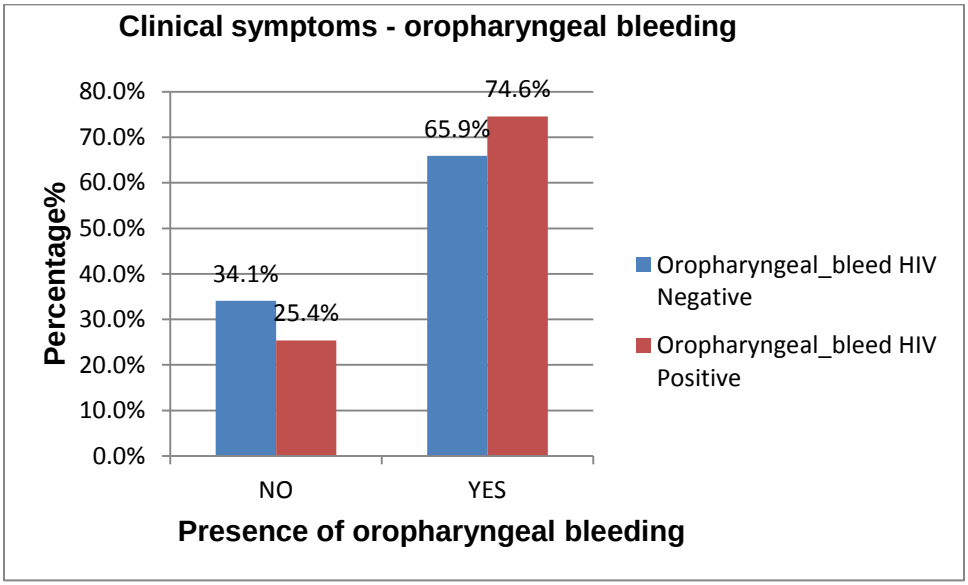


Figure 8 Patients with oropharyngeal bleeds grouped according to Patients HIV status

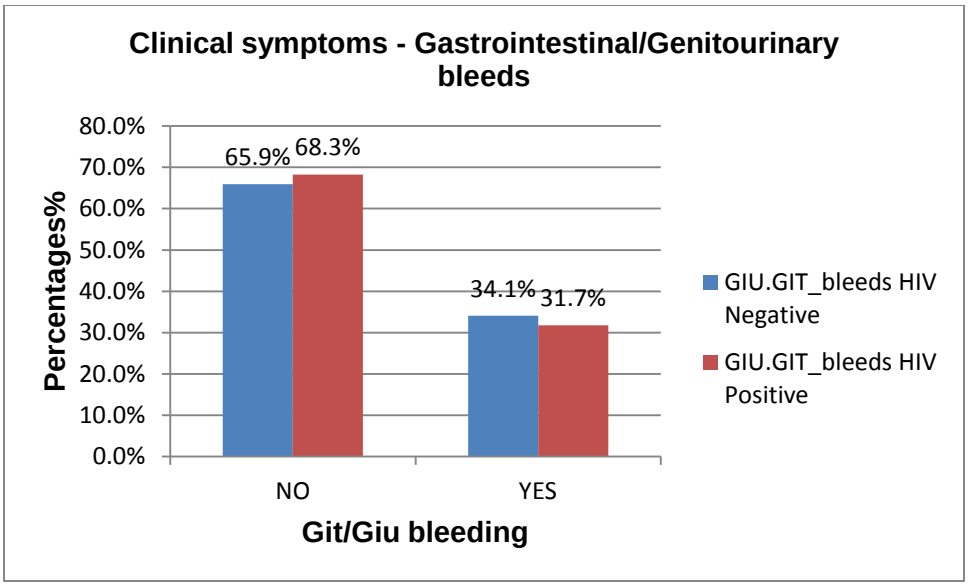


Figure 9 Genitourinary/Gastrointestinal Bleeds grouped by HIV status

3.3 Diagnosis

A significant proportion of the patients had bone marrow aspirations done 69% (106). Bone marrow aspirations were done on 75% (48) of the HIV positive group and 64%(60) of the HIV negative group. In terms of bone marrow findings the most common finding for both HIV negative and positive groups was the presence of well represented megakaryocytes; a tiny proportion of patients showed decreased megakaryocytes.

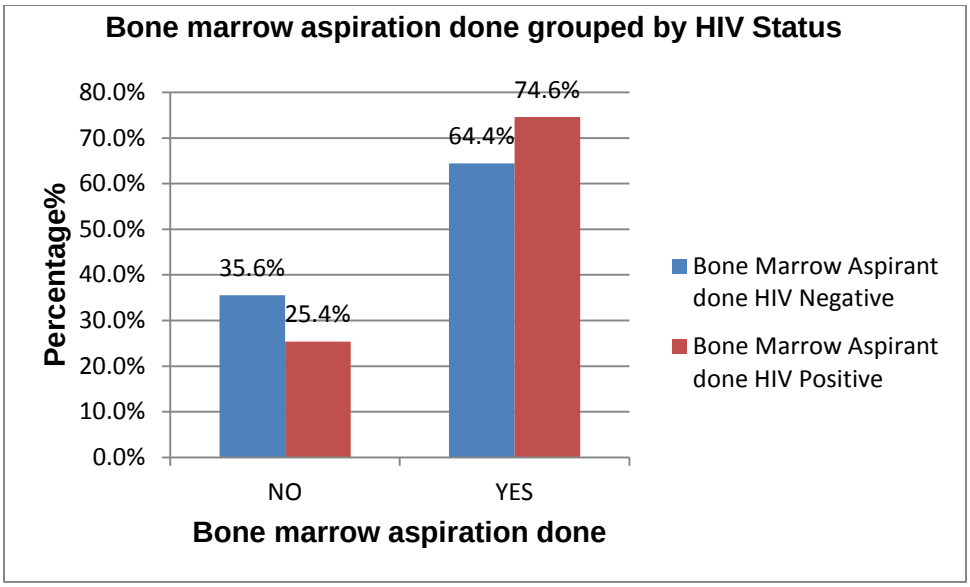


Figure 10 Bone marrow aspirate done grouped by HIV status

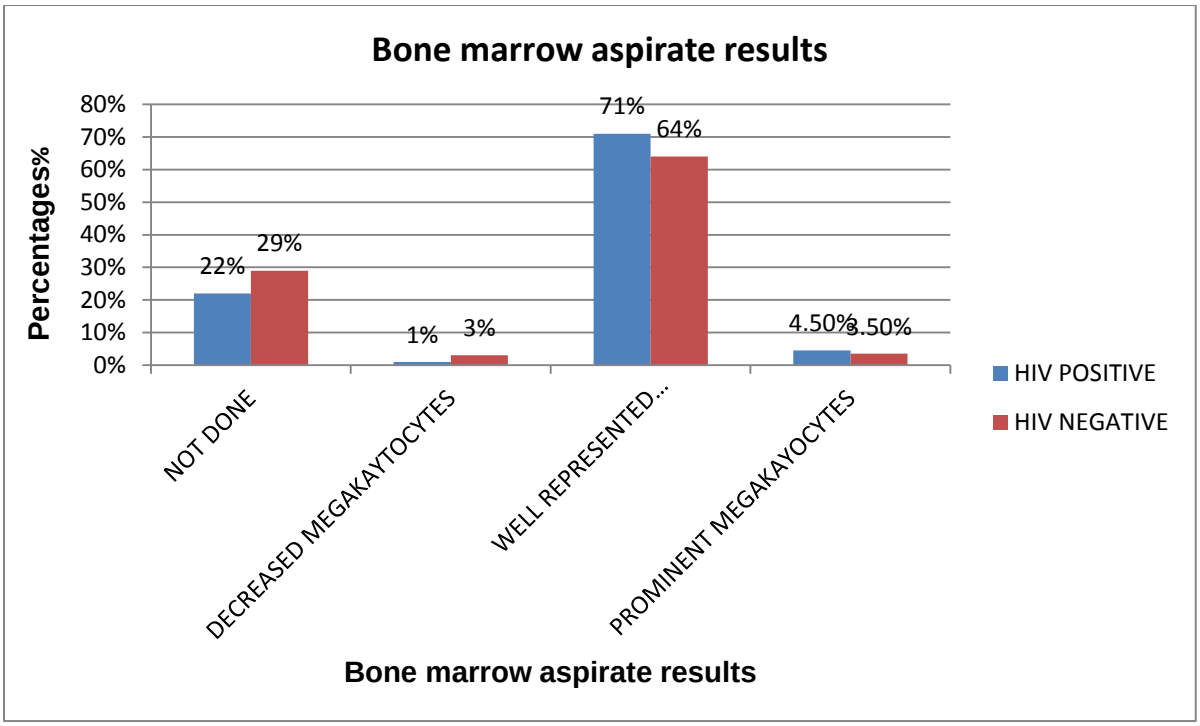


Figure 11 Megakaryocytes in bone marrow grouped according to HIV status

3.4 Management (Treatment)

In terms of therapy, both first and second line therapies were employed. Corticosteroid therapy was universally used with a total of 97%(152) of patients being on corticosteroids. Immunoglobulins used on a mere 7%(6) of HIV negative patients. A significant proportion of patients were on second line therapy in the form of immunomodulatory therapies such as Azathioprine. In the HIV positive group, 59% (38) received Azathioprine whilst 41%(26) did not receive that therapy. Newer therapies such as Rituximab were used only in the minority of cases. Rituximab was used by 14% (13) in the HIV negative group, 9%(6) in the HIV positive group. Splenectomy was also not a common second line therapy amongst our population with only 16%(24) of HIV negative patients and 10%(6) of HIV positive patients having had a splenectomy.

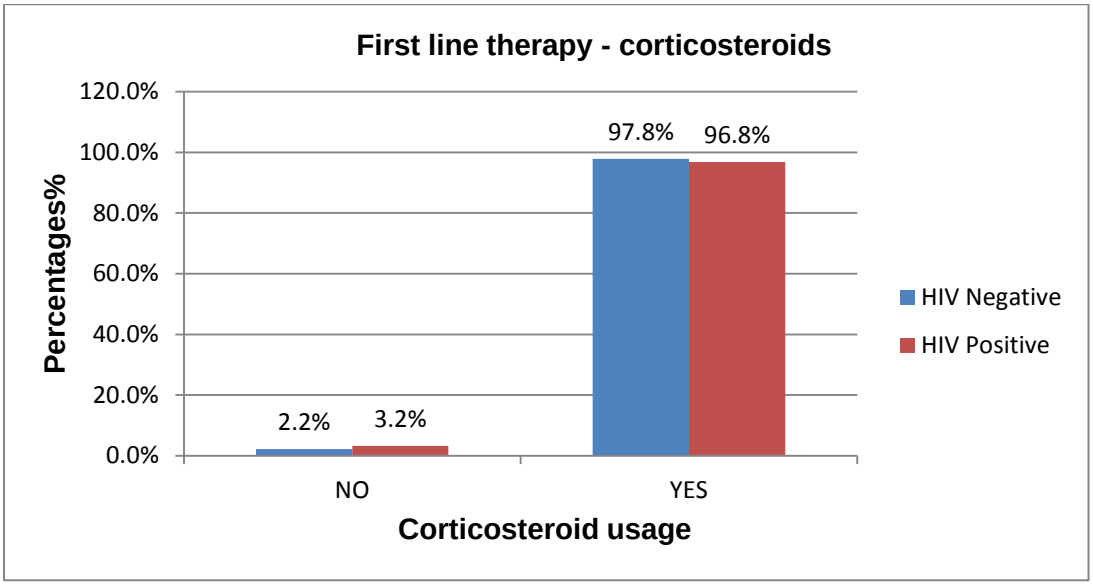


Figure 12 Corticosteroid usage by (first line therapy)

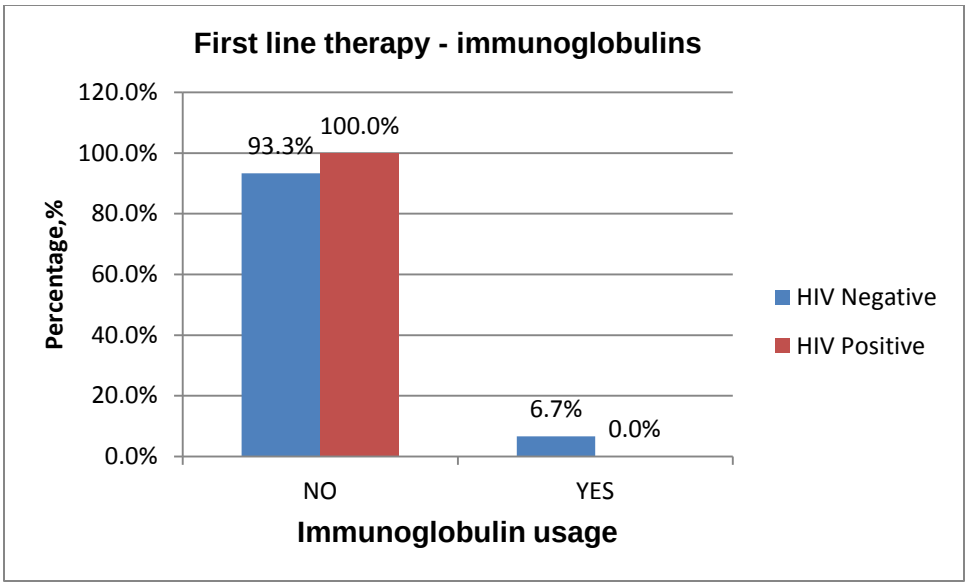


Figure 13 Immunoglobulin usage according to HIV status (First line therapy)

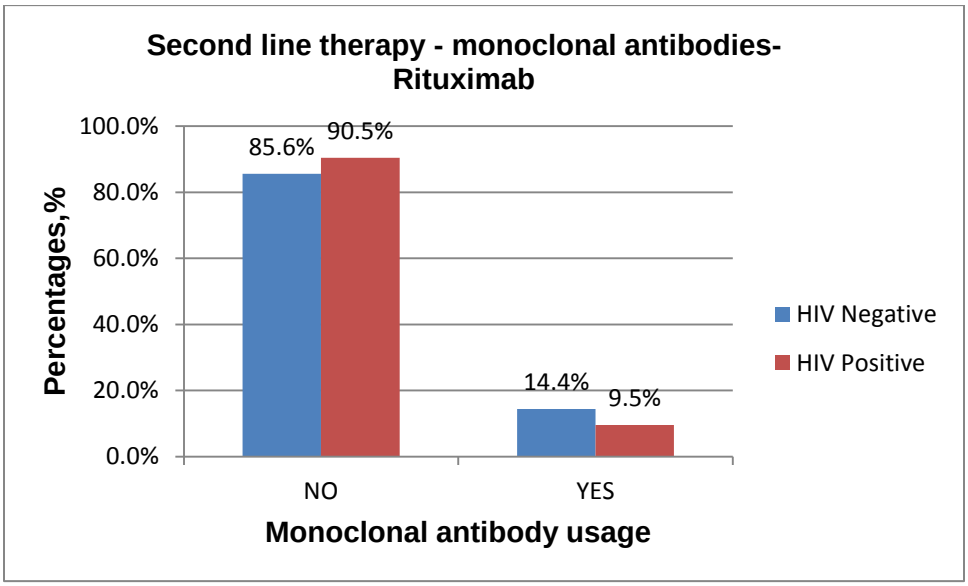


Figure 14 Monoclonal Antibodies (Rituximab) according to HIV status (First line therapy)

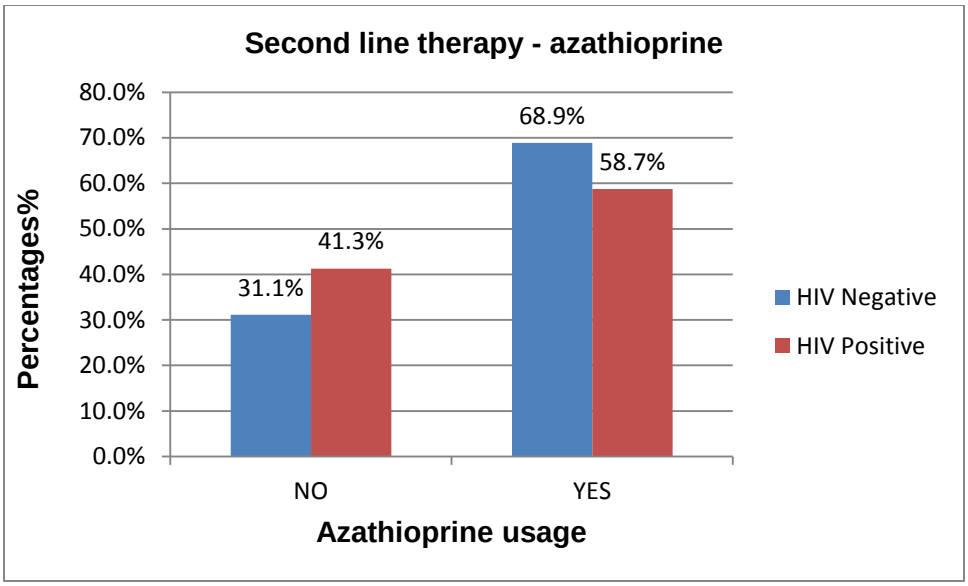


Figure 15 Immunomodulatory therapy (Azathioprine) according to patients HIV status (second line therapy)

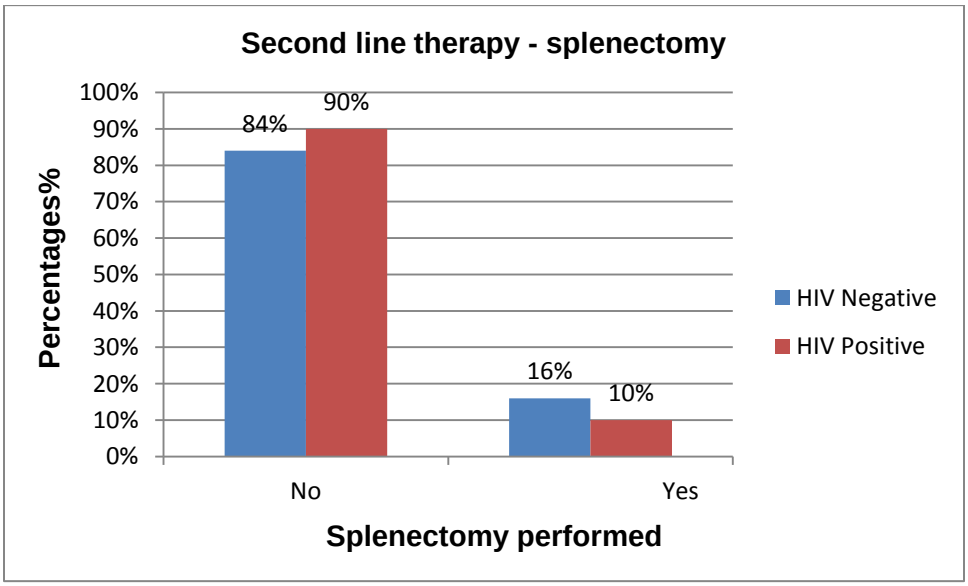


Figure 16 Splenectomy grouped to HIV status (second line therapy)

3.5 Treatment Response

The treatment response was analysed according to platelet counts which included the following:

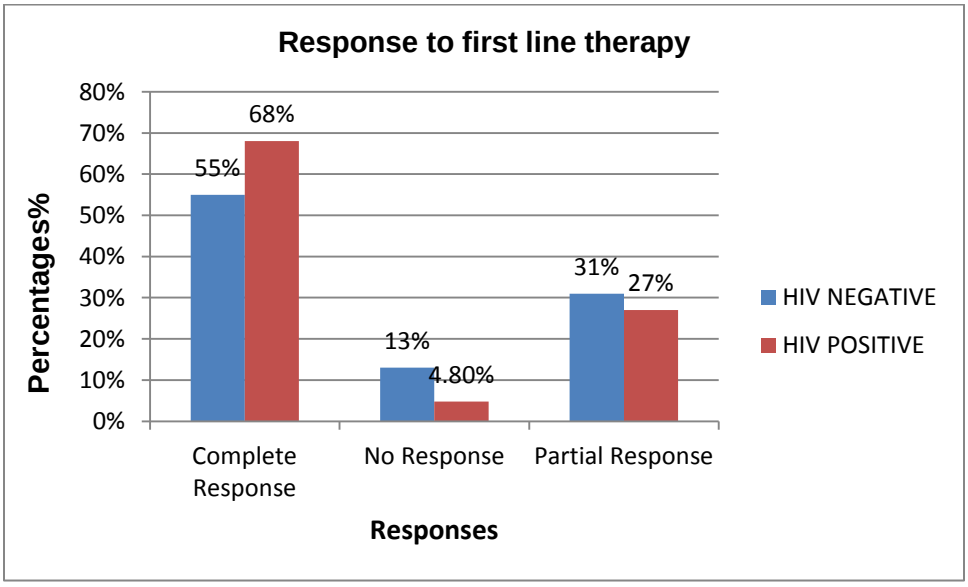
Complete response - platelets> 100 x 10^9/l

Partial response – platelets between 30 – 99 x 10^9/l

No response – platelets < $30x10^9$/l

The treatment response was also divided according to those who responded to first line therapy (i.e. corticosteroids, immunoglobulins, anti D anti immunoglobulins) and second line therapy (monoclonal antibodies e.g. Rituximab, splenectomy and immunomodulatory therapies such as azathioprine). In terms of first line therapies, the HIV positive group had a complete response rate of 68%(44) as compared to 56%(52) in the HIV negative patients. A small minority of patients both HIV positive (4.8%), and HIV negative (13%), did not respond to first line therapy at all. Comparison between the two groups (HIV positive and Negative) did not reveal a statistical difference in terms of first line therapy as suggested by a p-value of 0.137. 88 patients went on to second line therapy and there was no statistical difference between HIV negative and positive groups with a p-value of 0.655. Of these patients, 79%(73) of the HIV negative group had a complete response compared to 81%(52) of the HIV positive group. Some 17%(16)of the HIV negative group responded partially compared to 18%(12) of the HIV positive group. A mere 4%(4) of the HIV negative group did not respond at all to therapy.

Figure 17First line therapy according to HIV status



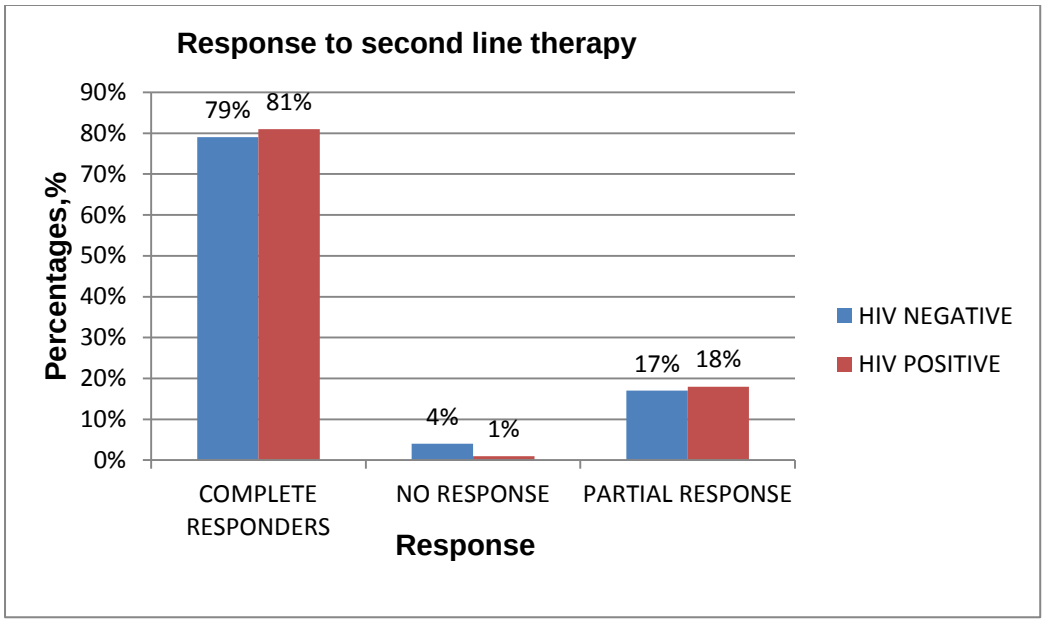


Figure 18 Second line therapy according to HIV status

Chapter 4: Discussion

This study revealed a number of interesting factors regarding ITP in our setting. There were a total of 157 patients with ITP (HIV positive and HIV negative) who met the criteria for eligibility. The majority of our patients were HIV negative (58%), whereas 42% were HIV positive. This finding is significant in an area with a high HIV burden, where most patients would be expected to be HIV positive. The exact reasons for the poor association between ITP and HIV infection are not clear. The fact that most patients were HIV negative may suggest that ITP requires an intact immune system to develop.

The overwhelming majority of the total population studied were female patients (91%). In both the HIV positive and HIV negative groups, females generally tend to have higher incidence of ITP than their male counterparts by a factor of 17 in the HIV negative and 11 in the HIV positive groups. The female gender predilection is consistent with the finding in literature (5, 11, 26). The exact reason for the female predominance still remains unknown. This female predilection in HIV patients mirrors the prevalence of HIV in our general population which is generally higher in females than in males.

When the patients were divided according their age groups, the 25-35 year age-group comprised the highest percentage of HIV positive patients (42%), followed by the 35-46 year age-group (36%); the remainder was spread across the other age-groups. Sexual activity also peaks in these age bands, which could explain their high rates of HIV associated ITP. It is also interesting to note that within the HIV negative patient base, the 25-35 age-group still constitutes the highest percentage of patients, though much lower (25%) than their HIV positive counterparts (42%). It appears as if the 25-35 age band is the modal class for ITP patients. Beyond the age of 25, the percentage of patients drops in both the HIV positive and HIV

negative groups. This correlates with international literature, which suggests that ITP is a disease of the young (4, 26)

In terms of clinical presentation, the vast majority of patients were symptomatic (97%). There was no obvious association between HIV status and symptoms at presentation. The most common presenting symptoms were petechiae and purpura. A Dutch study of 152 patients with ITP by Porteltjie et al, showed that ITP showed that the most common presenting complaints were severe thrombocytopenia (platelets< 30 x 10^9/l) and purpura or petechiae (36). Oropharyngeal and genitourinary symptoms were less common in our study population.Whilst there was evidence for association between HIV status and the presence of petechiae (p-value of 0.030) at 5% level of significance, there was no evidence for association between HIV status and purpura (p=0.117), oropharyngeal bleed (p=0.251) and genitourinary bleeds (p = 0.764). These results are consistent with those of the published literature (1, 8, 11).

Bone marrow aspirations were done in the majority of cases; however, a significant number of patients were diagnosed without a bone marrow aspirate being done as per published guidelines (1, 8, 24). In the HIV negative cases, 29% of patients did not have bone marrows aspirations done, compared to 22% of HIV positive patients. The reason for this is unclear but may reflect that it was considered unnecessary for patients who presented with typical symptoms of ITP to have bone marrow aspiration, as per published guidelines(1, 8, 24). In our study, the most common finding in the bone marrow was well represented megakaryocytes in both HIV positive and HIV negative patients. The minority of patients with decreased megakaryocytes especially in the HIV positive population are likely in keeping with direct infection of the megakaryocyte causing apoptosis (6, 25)

There was no association between HIV status and bone marrow aspiration (p=0.290). According to international literature, the most common bone marrow finding is normal to

increased levels of megakaryocytes in the absence of other abnormal findings. Consequently the findings in our study were in keeping with international literature (20, 25).

In terms of treatment, corticosteroids were the cornerstone of therapy, with 98% of HIV negative patients undergoing this therapy and 97% of HIV positive patients also receiving the treatment. Immunoglobulin therapy had minimal usage with 7% receiving therapy. Azathioprine was the most common of the second line therapies used with 69% of HIV negative patients receiving it. Splenectomy and monoclonal antibodies therapy were infrequently used with 16% of HIV negative patients having splenectomies and 14% of HIV negative patients subjected to monoclonal antibodies. This could reflect the ease of availability and affordability of Azathioprine compared to the other second line therapies

There was no statistical difference between HIV positive and HIV negative patients in terms of response to first line therapy($p = 0.137$). In terms of complete responders to first line therapy, the HIV positive group had a higher response rate ($p=0.097$). In a study by Ambler et al (2012) about 80% of patients with HIV – ITP achieved a partial or complete response (7). The study by Porteltjie et al (2001) showed comparable complete response rates to first-line therapy of 93% after 2 years (36). In our series of patients who went on second linetherapy (88), a majority showed excellent response with approximately 80% reaching complete response in both HIV and HIV negative groups. There was no statistical difference between the two groups. The Ambler study(2012) which had fewer patients had 50% (11) reach a partial response and 36% (8) reach complete response amongst patients who went on to second line therapy(7). The use of immunoglobulins was the predominant mode of therapy amongst these patients whereas in our series, azathioprine and splenectomies dominated. This may be the reason for the higher response rates. The study by Porteltjie et al (2001) also had few patients going onto second line therapy (14) and had 36% achieving complete or partial response (36).

Study Limitations

In our study, there were a number of possible limitations to be considered. This was a retrospective study so long term follow up of patients to gauge any divergence in outcomes in the groups studied was not possible. As a result of retrospective nature of the study, the number of patients enrolled was also limited.

An additional limitation was that this was a single centre study, so findings could be affected by specific local factors and may not be generalizable to other centres and different levels of care. Moreover, the population studies had access to all levels of health care in close proximity. It is possible that similar results may not be achieved in rural and remote settings.

Another limitation was that patients may have been excluded due to incomplete clinical methods and also poor follow up at our Haematology Department due to patient non-compliance.

Conclusion

This study has shed light on the demographics, clinical presentation and management outcomes of patients with Immune Thrombocytopenic Purpura in a Central Teaching Hospital in Johannesburg. The data from this study is comparable to that of international literature, showing a predominance of female ITP patients. In this study done in a high HIV burden setting, two thirds of patients were HIV negative. There were no significant differences between HIV negative and positive patients in terms of clinical presentation or management and response to therapy for immune thrombocytopaenia. This study is an important addition to ITP knowledge in the context of HIV knowledge which is currently lacking.

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Appendix A

Data Collection Sheet

Patient Number

Demographic Data

AGE (Years)	
SEX (M/F)	

Inclusion Data present

The following Criteria has to be present (Mark with an X)

	YES	NO
HIV Test Done		
Age >18		

Patient HIV Test Result

	HIV positive	HIV negative

If HIV POSITIVE CD4 COUNT_____

Exclusion Criteria absent

The following Criteria must be absent(mark with an X)

	YES	NO
Secondary Causes of thrombocytopaenia present		
Incomplete records		

E.g. of Secondary Causes

Infections – Herpes infection, Hepatitis, Tuberculosis

Malignancies – Chronic Lymphocytic Anaemia and Non Hodgkin's Lymphoma

Drug-induced immune thrombocytopenic purpura (e.g. heparin, quinine/quinidine, sulphonamides)

Autoimmune- Systemic Lupus Erythematosus

Clinical Presentation

Age at Presentation of Disease

Presenting Symptoms

	Yes	NO
Asymptomatic		
Petechiae		
Purpura		
Oropharyngeal Bleed		
Genitourinary bleeds		

Diagnosis

Platelet Count at presentation

Abnormalities of Haemoglobin or White Cell Count

	Yes	NO
Presence of Anaemia		
Abnormal White Cell Count		

Bone Marrow Aspirate Done

	Yes	NO

Result of Bone Marrow Aspirate

Megakaryocytes in Bone Marrow Aspirate

Normal	Increased	Decreased

Bone Marrow Trephine Result

Management

First Line therapy Offered

	YES	NO
Corticosteroids		
Immunoglobulins		
Other e.g. Anti D immunoglobulin Thrombopoietin Receptor Agonist e.g. Romiplostim		

Second Line Therapies

	YES	**NO**
Monoclonal Antibodies e.g.Rituximab		
Splenectomy		
Immunomodulatory therapy e.g. , azathioprine		

I

If HIV Positive is the Patient on HAART

	Yes	NO

HAART Regimens – Regimen 1 Tenofovir, Lamivudine, Efavirenz)

Treatment response- First Line Therapy

Definitions

Complete Response – A platelet count of at least 100 x 10^9/L

Partial Response – Platelets between 30 – 100 x 10^9/L

No Response – Platelet Count less than 30 x 10^9/L or doubling of baseline count

Response is defined as a rise in platelet count plus concurrent resolution of bleeding symptoms

	Complete response	Partial Response	No response

Treatment Response – Second Line Therapy

	Complete response	Partial response	No Response

Appendix B

Ethics Clearance Certificate

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131026

NAME: (Principal Investigator)	Dr Melvin Mbao
DEPARTMENT:	Molecular Med & Haematology/Internal Med CM Johannesburg Academic Hospital
PROJECT TITLE:	Immune Thrombocytopenic Purpura at a Central Hospital in Johannesburg
DATE CONSIDERED:	25/10/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Johnny Mahlangu
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/10/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature M131026 Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES