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THE PRACTICE AND USEFULNESS OF BONE MARROW EXAMINATIONS IN A COHORT OF HUMAN IMMUNODEFICIENCY VIRUS INFECTED CHILDREN IN SOUTH AFRICA: A DESCRIPTIVE STUDY

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Paediatrics

Johannesburg, 2011

DECLARATION

I, Biance Rowe, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed on this 2nd day of February, 2011.

PUBLICATIONS AND PRESENTATIONS

Accepted in part for a poster walk presentation entitled “The practice and usefulness of bone marrow examinations in a cohort of HIV+ children in South Africa: A descriptive study”, at the 26th International Pediatric Association Congress of Pediatrics held in Johannesburg, 4-9 August 2010.

ABSTRACT

INTRODUCTION: Bone marrow examination (BME) is performed in Human Immunodeficiency Virus-infected (HIV+) children with haematologic abnormalities to exclude specific disease (**SD**).

AIMS: *To describe the*:

(1) indications for BME , (2) utility of BME to diagnose **SD**, (3) patient characteristics associated with **SD** or **non-specific disease (NSD)**.

METHODS:

Design: Retrospective review.

Definitions:

SD: BME positive for opportunistic infection (OI) or HIV-related malignancy. **NSD**: HIV-related changes *only*.

RESULTS:

Eighty six BME's were done. Suspected SD in 56/86(65.1%) was the most common clinical indication. Bicytopaenia(n=32) and isolated cytopaenia(n=31) were the most common haematologic indications. NSD 48/86 (55.8%) was a more common finding than SD 32/86 (37.2%). Granulomas, pure red cell aplasia and malignancy were the SD identified. Pre- highly active antiretroviral therapy (HAART), advanced stage, and not being virally suppressed were significantly associated with NSD.

CONCLUSION:

The yield of SD (37.2%) on BME is comparable to adult studies. HAART should be instituted before BME as NSD will be the most likely finding.

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1. INTRODUCTION & BACKGROUND

1.1 Human Immunodeficiency Virus (HIV) – the face of the enemy

HIV is responsible for essentially all cases of the acquired immunodeficiency syndrome (AIDS) in the Western world. Humans were first infected via zoonotic transmission of the virus from a chimpanzee (1), and transmission was enhanced by worldwide travel and urbanization.

Based on mathematical modeling of HIV sequence variations, it is estimated that the first human infections with HIV occurred as early as the 1930's (2).

The majority of HIV-infections in southern Africa today have sequence variation C, also referred to as clade C (3).

HIV belongs to the family Retroviridae and the genus Lentiviridae. The virion measures 100 nanometer and contains two single RNA strands coated with the nucleocapsid protein. This viral RNA-protein complex core is enclosed in a capsid protein. The outer viral membrane (envelope) is acquired during budding from the host cell, while the glycoproteins (gp) embedded in this envelope, gp 41 and 120, are virally encoded. In addition to these structural proteins, the virion contains key enzymes

necessary for viral function, namely reverse transcriptase, integrase and protease (4).

The complete life cycle of HIV is beyond the scope of this document, but is readily available for reference (4).

The HI-virus infects CD4 T-lymphocytes having a cytolytic effect and depleting the CD4 T-lymphocyte pool over time. In addition to having direct cytotoxic effects on CD4 T-lymphocytes and cellular immunity, other mechanisms are also involved in causing the progressive destruction of the immune system (5). These mechanisms vary from inducing apoptosis through viral antigens to limitation of T-cell regeneration in the thymus and depleting the repertoire of antigen recognition. The T-cell proliferative response necessary for optimal B-cell function is adversely affected. Monocytes and macrophages are also infected by HIV, but instead of leading to cell death, these cells are used as reservoirs for latent infection.

A patient with HIV-infection manifests a range of clinical features as the immune system is progressively destroyed.

The clinical features of HIV-infection described in the untreated adult are those of an acute infection with flu-like symptoms (seroconversion) associated with a high-grade viraemia, followed by a variable asymptomatic period of immune containment of viral replication, and

finally progressive symptomatic immunocompromise with increasing viral loads, declining CD4 T-lymphocyte numbers and the occurrence of opportunistic infections (OI) and HIV-related malignancies.

Depletion of the CD4 T-lymphocyte pool may not be as readily apparent in infants and young children as they have higher absolute lymphocyte numbers. In the paediatric population, dysregulated B-cell function with hypergammaglobulinaemia and the production of inappropriate polyclonal antibodies is more common than in adults. HIV-infected infants and young children without prior antigen exposure respond poorly to new environmental antigens and this leads to a greater frequency and severity of invasive bacterial infections at a young age (6).

1.2 The scope of the HIV epidemic in South Africa and the paediatric context

An estimated 64% of people living with HIV-infection are resident in sub-Saharan Africa (7) and HIV-infection is a major public health problem in South Africa.

The South African National HIV survey in 2008 (8) estimated that 10.9% of all South Africans over 2 years old were living with HIV-infection at the

time. This figure translates into 5.4 million people, 280 000 of whom are children under the age of 15 years.

The prevalence in adults over the age of 25 years was 16.9%.

The South African Department of Health study in 2008 (9) estimated that 29.3% of pregnant women were living with HIV according to figures recorded based on data from antenatal clinic attendees.

More than 95% of HIV-infected children less than 15 years of age contract HIV-infection through vertical transmission which may occur intra-uterine, peripartum, or through breastfeeding postpartum. Based on data from the Actuarial Society of South Africa (ASSA) 2003 study (7) (10), an estimated 65 000 babies were infected during 2008, 39 000 babies were infected perinatally and 26 000 were infected through breastfeeding.

Without optimal management, most children that acquire HIV-infection in utero or during the intrapartum period will have a more progressive disease course and 35% of these children will die by the age of one year, whilst 50% of these children will die by the age of 2 years. Adolescents and older children who acquire HIV by horizontal transmission follow an adult disease course with more slowly progressive disease.

HIV-infection is a major cause of the high under-five mortality rate (U5MR) in South Africa, and it has contributed to more than 40% of the under-five deaths in South Africa (11).

The U5MR is a principal indicator used by the United Nations Children's Fund (UNICEF) to assess human and economic progress in a country. The United Nations has set a millennium development goal to reduce the incidence of HIV-infection in children by 20% in 2005 and by 50% in 2010, and in so doing, decrease the U5MR (11).

As noted by Saloojee (11), an important step towards reducing the incidence of HIV-infection in children is the prevention of mother to child transmission where the use of dual nevirapine and zidovudine (AZT) prophylaxis to mother and baby has reduced the absolute vertical transmission rate to approximately two percent.

The HIV-epidemic has had far-reaching consequences for health care in South Africa in both the urban and rural health care settings. Up to 60% of all paediatric beds at hospitals are occupied by HIV-infected children at some point (11), and many more patients are seen as outpatients in community clinic-based primary healthcare.

An integral part of the management of patients with HIV-infection is the introduction of highly active antiretroviral therapy (HAART), but unfortunately, according to figures from June 2008 from the ASSA 2003 model (10), an estimated 520 000 people in need of HAART (a combined figure for children and adults) would not have received it.

The HAART-treatment guidelines for children have recently been reviewed by the Department of Health and recommend initiating HAART in all children less than 1 year of age irrespective of CD4 count, and significantly lowering the CD4 count at which children aged 1-5 years and children over the age of 5 years would qualify for HAART (12).

Sadly, the picture of HIV-infection in children in the public health sector in South Africa is still bleak, with children presenting to public health care facilities with advanced HIV-infection, not on HAART, malnourished, with untreated OI's, in poor social circumstances, either orphaned or with HIV-infected, ill parents.

As HIV-infection is a multisystem disease, a certain level of knowledge and skill is needed from health care workers in order to identify and manage the disease manifestations. The haematologic system is frequently affected, the features of which are described below.

1.3 The clinical effects of HIV-infection with emphasis on the haematologic system

Haematologic abnormalities are among the most common manifestations of HIV-disease in the paediatric population and often produce multilineage blood defects (13). These abnormalities are initially detected on a peripheral full blood count and include various combinations of cytopaenias, including isolated cytopaenia (only one blood cell line abnormal), bicytopaenia (two blood cell lines abnormal) and pancytopaenia (three blood cell lines abnormal).

These haematologic abnormalities are often the initial indication for requesting a bone marrow examination (BME) in order to elucidate the aetiology of these abnormalities.

HIV-infection itself can be the cause of a central, bone marrow-related aetiology for the cytopaenias, as well as produce a peripheral mechanism of destruction to cause cytopaenias.

1.3.1 The effect of haematologic abnormalities on the staging of HIV infection

The interim revised World Health Organisation (WHO) clinical staging of HIV/AIDS (14) for infants and children defines four stages of HIV-infection. Stage 1 refers to mainly asymptomatic children, and stage 4 refers to children with advanced immunosuppression and having AIDS-defining conditions.

Haematologic abnormalities are defined in the WHO staging system as unexplained anaemia (<8g/dl), and/or neutropaenia (<500 cells/mm^3), and/or thrombocytopaenia (<50 000 cells/mm^3) for more than one month. If present, these haematologic abnormalities would place the patient in stage 3, defined as moderate immunosuppression.

1.3.2 The effect of HIV-infection on central bone marrow haematopoiesis and cytokine expression

Studies on the bone marrow cultures of HIV-positive patients have revealed that haematopoietic progenitor cell and colony growth were inhibited (15). The HI-virus was not isolated in these samples, suggesting that HIV has its effect on haematopoiesis through an indirect mechanism, rather than through direct infection of the progenitor cells.

Reduced colony growth was observed for colony-forming-unit multipotential haematopoietic progenitor cells (CFU-GEMM), colony-forming-unit-granulocyte-macrophage progenitor cells (CFU-GM), megakaryocytic colonies (CFU-Meg) and erythroid progenitor colonies (burst-forming-unit-erythrocyte) (16). Controversy exists as to whether direct infection of CD34+ progenitor cells in the bone marrow occurs as they carry both the CD4 receptor and the CXCR4 co-receptor necessary for viral entry into the cell, but numerous investigators have found that these cells are not susceptible to direct infection by HIV in vitro or in vivo (17) (18).

An alteration in the bone marrow micro-environment through infection of stromal cells, rather than direct infection of progenitor cells, is the most likely mechanism responsible for the abnormalities in haematopoiesis as described by Schwartz el al (19). Cytokine expression from stromal cells produces the signals to induce cellular differentiation in the progenitor cells and so regulate normal haematopoieisis.

A dysregulation of cytokine expression from stromal cells produces abnormalities in progenitor cell differentiation leading to various dysfunctions in differentiation, and deviation from the lineage that the progenitor cell was originally committed to. The dysregulation of cytokine expression by bone marrow stromal cells and disordered haematopoieisis

is at its maximum in HIV-infected patients with advanced stages of immunosuppression with high viral loads and low CD4 counts (20).

1.4 The effect of HIV-infection in producing a mechanism for peripheral destruction of blood cells

HIV-infection affects the number and function of B-cells, and causes as an early abnormality a non-specific hypergammaglobulinaemia accounting for the high globulin fraction seen in the serum of HIV-positive patients. The presence of autoantibodies directed against blood elements may be explained by abnormal B-cell regulation by HIV-infected T-cells, direct activation of B-cells by HIV-infection or a B-cell response to coincident infection with Ebstein-Barr virus or cytomegalovirus (21) (22). These autoantibodies are directed against erythrocytes, platelets, and less commonly, leucocytes (23). Auto-antibody coated blood elements are destroyed in the reticulo-endothelial system and provide a mechanism for peripheral destruction of these cells.

1.4.1 HIV-mediated autoantibodies directed against erythrocytes

Autoimmune haemolytic anaemia (AIHA) results from the destruction of erythrocytes by autoantibodies. The anti-erythrocyte antibodies are

caused by a general defect in the regulation of antibodies as occurs in the setting of HIV-infection. The presence of hypergammaglobulinaemia in patients with HIV-infection may result in the non-specific coating of the overabundant immunoglobulin G (IgG) to erythrocytes. Immune-complex-associated IgG may also bind to erythrocytes via the C3b receptors on their cell surface and lead to their destruction through the activation of the complement pathway (24) (25).

Various infectious agents that are frequently encountered in the setting of HIV-infection, namely Mycobacterium avium complex (MAC), Mycobacterium tuberculosis (MTB), Cytomegalovirus, Pneumocystis jiroveci, Parvovirus B19 and Histoplasma capsulatum may also be associated with the production of autoantibodies to erythrocytes (26).

Although 20-40% of HIV-positive patients have a positive direct antiglobulin (Coombs) test, overt haemolysis due to these antibodies is a rare occurrence.

Warm autoantibodies of the IgG type active at 37°C, and cold agglutinins of the immunoglobulin M (IgM) type active at 4°C have been described.

Reticulocytopaenia is a frequent finding in the setting of HIV-associated haemolysis and may be explained by the abnormal bone marrow micro

environment that prevents the release of reticulocytes from the bone marrow (27).

The current recommendation is that patients with a positive direct Coombs-result, minimal evidence of haemolysis, and a stable haematocrit level should be followed up clinically and not receive any specific treatment except HAART, as the autoantibodies present may be “clinically silent”.

Treatment options available in the setting of clinically significant haemolysis include blood transfusions, corticosteroids, immunoglobulin therapy, other immunosuppressives and splenectomy (28).

1.4.2 HIV-mediated autoantibodies directed against platelets

Immune thrombocytopaenic purpura (ITP) occurs in HIV-infected children with elevated levels of platelet-associated IgG and/or circulating immune complexes. Patients with HIV-ITP often have platelet counts above 20 x 10^9 cells/L and the overall incidence of haemorrage is low (29).

Immune complexes contain anti-HIV gp120 and anti-idiotypes directed against the anti-HIV antibodies. Cross-reactivity has been described between antibodies against HIV gp120/160 and platelet gpIIb/IIIa.

Platelets coated with these antibodies and/or immune complexes are destroyed in the reticulo-endothelial system (30).

The BME in patients with HIV-related ITP shows normal to increased numbers of megakaryocytes consistent with a peripheral destructive process (30).

HIV-associated ITP has been described as a cause for thrombocytopaenia in HIV-infected patients with early stage HIV-infection and mild immunosuppression (23)

The current recommendation for the management of patients with HIV-ITP is to institute HAART to reduce plasma HIV viraemia (31). Other treatment modalities should be reserved for patients with clinically significant, severe bleeding.

These treatment modalities have varying degrees of success in ameliorating the thrombocytopaenia and include corticosteroids, immunoglobulins, other immunosuppresives, attenuated androgens and splenectomy (32).These agents are expensive and their durable response is poor. This emphasizes that the treatment of choice for patients with HIV-related ITP is HAART. This recommendation is based on the results of several studies that showed a significant increase in platelet count in patients on HAART (33) (34) (35).

1.4.3 HIV-mediated autoantibodies against leucocytes

An autoimmune mechanism to account for granulocyte destruction has been postulated by some investigators, but has not been proven yet, especially in the paediatric setting (36). Secondary causes for leucopenia as discussed below should be given consideration first in HIV-positive patients with a low white cell count.

1.5 The indications for a BME in HIV-positive children

The aetiology of bone marrow suppression is often multifactorial with haematinic deficiencies, OI's, malignancies and drug side-effects contributing to the haematologic abnormalities in addition to HIV-infection.

In an attempt to differentiate between some of these contributors, a BME is often requested.

Indications for a BME quoted in adult literature include clinical indications such as pyrexia of unknown origin to exclude disseminated OI's or neoplastic infiltration of the bone marrow (37).

The most common indication for a BME though, is to explain haematological abnormalities, namely cytopaenias (38).

1.5.1 Cytopaenias as an indication for BME

Cytopaenias may be the initial finding in children with undiagnosed HIV-infection, and a high index of suspicion for HIV should be present when a child manifests with unexplained cytopaenias. Cytopaenias may involve one cell line (isolated cytopaenia), two cell lines (bicytopaenia) or three cell lines (pancytopaenia). Anaemia, thrombocytopaenia and leucopaenia may occur as an isolated finding or in various combinations.

Morphologic abnormalities can be detected in the peripheral blood of HIV-infected patients and include macrocytosis, reticulocytopaenia, circulating erythroblasts, granulocyte band-forms, immature myeloid cells, atypical lymphocytes and monocytes with cytoplasmic vacuoles and nuclear abnormalities (39).The presence of these haematologic abnormalities may prompt a BME to be done in an attempt to explain them, and to exclude a specific disease affecting the bone marrow.

If cytopaenias alone was the indication for BME, it was associated with a finding of specific disease in only 4.9% (4/82) as shown by Tanaka et al from Brazil (40). In the same study it was shown that if the indication for the BME was anaemia as a single lineage cytopaenia, a specific disease was diagnosed in only 3.7% (3/82).

This Brazilian study (40) concurs with previous findings by Brooke et al (41), where a low yield was found if BME was done in patients with isolated thrombocytopaenia, anaemia or leucopaenia, as HIV was found to be the underlying cause.

These studies highlight the fact that cytopaenias are common in patients with HIV-infection, but that a BME does not necessarily identify a specific disease besides HIV-infection as the aetiology of the cytopaenias.

A thorough knowledge of the possible aetiology of anaemia, thrombocytopaenia and leucopaenia as they pertain to HIV-infection is imperative in the care of paediatric HIV+ patients. These cytopaenias need to be investigated, explained, and where possible treated specifically in order to ameliorate their effects.

1.5.1.1 Anaemia in HIV-positive children

Anaemia is defined according to age-related values (42).

Anaemia is the most common haematologic abnormality described in HIV-infected patients and has been reported to occur in 60 – 80% percent of patients over the course of their disease.

The incidence of anaemia increases with the clinical stage of disease and is found in most HIV-positive patients with advanced stage disease (43).

The significance of anaemia in children with HIV-infection is highlighted by Ellaurie et al (44), who found that anaemia was present in 94% of 100 symptomatic, HIV-infected children, and that anaemia was a major predictor of disease progression. This emphasizes the importance of investigating anaemia in HIV-infected patients, and where possible, to correct the cause.

The anaemia in HIV-infection is mostly of the normocytic, normochromic type as described by Perkocha et al (26), and the aetiology is often multifactorial.

Two main pathophysiological processes produce anaemia either separately or in combination, namely through a central depression of erythropoiesis at the level of the bone marrow, and/or through the peripheral destruction of red blood cells by auto-immune mechanisms or sequestration of red cells.

Anaemia of chronic disease secondary to central bone marrow depression of erythropoeisis is the most frequent cause of anaemia in HIV-positive patients. HIV-infection of bone marrow stromal cells disrupts cytokine expression as described above in section 2.1.

Cytokines such as tumour necrosis factor alpha and interleukin 1 have potent suppressive effects on bone marrow erythropoiesis (45).

Erythropoietin (EPO), which is an important hormone regulating red cell production, failed to rise in response to the degree of anaemia in HIV-infected patients, and a blunted EPO-response has been cited as a contributing factor in producing anaemia in these patients (46).

Nutritional causes such as haematinic deficiencies of iron, folate and vitamin B12 suppress haematopoiesis and contribute to anaemia. A defect in iron utilization has also been suggested in patients with HIV-infection. Iron-staining of the bone marrow in these patients indicates normal storage iron in the presence of a decreased amount of iron in the normoblast red cell precursors (26). Vitamin B12 deficiency in HIV-infected patients may be secondary to altered serum transport of vitamin B12 due to altered cobalamin transport proteins in patients with advanced HIV-disease (47).

Certain drugs used in the treatment of HIV-infection and its complications may produce anaemia through bone marrow suppression. These drugs include AZT (48), trimethoprim-sulfamethoxazole, amphotericin B, gancyclovir and dapsone (49). A drug history is thus imperative in investigating the aetiology of anaemia in HIV-positive children.

Bone marrow infiltration by OI's may disrupt erythropoiesis and cause anaemia. Disseminated MAC infection in HIV-positive patients is a common cause of anaemia in patients with advanced HIV-infection (50). In these patients, anaemia tends to occur out of proportion to the other cytopaenias.

Other OI's may disseminate to the bone marrow and suppress erythropoiesis in a similar way, namely MTB infection and disseminated fungal infections such as Histoplasma capsulatum.

Human Parvovirus B19 can cause severe, recurrent anaemia in HIV-infected patients. This single-stranded DNA-virus invade erythroid progenitor cells in the bone marrow via the group P antigen and replicates extensively in erythroid progenitor cells in the bone marrow. The erythroid progenitor cell is ultimately lysed. The inefficient humoral immunity present in HIV-positive patients allows the viraemia to persist and lysis of erythroid progenitor cells in the bone marrow continues until a pure red cell aplasia (PRCA) develop with a block in erythoid maturation and the characteristic presence of giant pronormoblasts is noted in the bone marrow (51).

Bone marrow infiltration by a neoplastic process such as an HIV-associated malignancy namely a non-Hodgkin's lymphoma may depress erythropoiesis at the production level in the bone marrow resulting in anaemia. The myelosuppressive chemotherapy used in the treatment of

these malignancies also routinely causes anaemia. AIDS-related lymphoma occurs late in the disease process in the setting of severe immunosuppression (52).

1.5.1.2 Thrombocytopaenia in HIV-positive children

Thrombocytopaenia is defined as a platelet count of less than 150 x 10^9 cells/L across the age spectrum (42).

Thrombocytopaenia has been reported to occur in 10-20% of HIV-infected patients (53).

Isolated thrombocytopaenia may frequently be the presenting haematologic manifestation of HIV-infection (54). According to a study by Ellaurie et al (44), thrombocytopaenia is more likely to occur as an isolated haematologic abnormality than anaemia or leucopaenia and this may reflect the different mechanisms involved in producing cytopaenias.

Anaemia and leucopaenia are more likely to be secondary to a central bone marrow depression and ineffective haematopoiesis and they occur later in the disease process. Thrombocytopaenia, especially in early stage disease, is caused by peripheral destructive mechanisms involving immune-based mechanisms. HIV-related ITP is the most common cause

of thrombocytopaenia through peripheral destruction in HIV-positive patients and HAART is recommended as the treatment of choice for this condition.

Secondary causes of thrombocytopaenia in HIV-infected patients are drug-related bone marrow suppression, folate and vitamin B12 deficiency, infiltration of the bone marrow by OI's or malignancy, hypersplenism and microangiopathic processes such as thrombotic thrombocytopaenic purpura (55).

1.5.1.3 Leucopaenia in HIV-positive children

Leucopaenia in children is defined according to age-related values and may include various combinations of lymphopaenia and/or neutropaenia (42).

The peripheral blood smear may show characteristic abnormalities associated with leucopaenia in HIV-positive patients. These abnormalities may include an increased number of circulating neutrophil band forms, immature myeloid cells, atypical lymphocytes and monocytes with cytoplasmic vacuoles and nuclear aberrations (39).

Lymphopaenia in HIV-positive patients refers to a decrease in the number of circulating lymphocytes. The HI-virus is a lymphotropic virus and cause lymphopaenia through infecting the CD4 T-lymphocyte and causing cell lysis through viral replication.

HIV-uninfected CD4 T-lymphocytes may fuse with HIV-infected CD4 T-lymphocytes and cause syncitiae with multinucleated giant cells that lyse easily. HIV-uninfected CD4 T-lymphocytes may further be lysed through a mechanism involving lymphocytotoxic antibodies that react with OKT4 receptors on CD4-T-helper cells. Lymphopoiesis is often ineffective at a central bone marrow level in compensating for the above-mentioned mechanisms of loss of lymphocytes (56).

Neutropaenia is a common finding among HIV-infected patients with advanced stage disease, and is present in 50-75% of these patients (57). As mentioned in the study by Ellaurie et al (44), leucopaenia, and specifically neutropaenia, is most commonly due to ineffective granulopoiesis secondary to HIV-related bone marrow suppression. HIV-related anti-granulocyte antibodies have been described, but their correlation with neutropaenia is not 100%, and they are more common in adults (58).

Hypersplenism from a variety of causes may lead to the peripheral destruction of leucocytes.

Drug side-effects provide an important aetiology of neutropaenia in HIV-infected patients. These drugs are widely used and include dapsone, amphotericin B, antineoplastic chemotherapy, trimethoprim-sulfamethoxazole, acyclovir, gancyclovir as well as AZT. Indeed, nearly 50% of children taking AZT have been found to have absolute neutrophil counts of less than 750 cells/microlitre (59).

Infiltration of the bone marrow by opportunistic pathogens such as mycobacterial and fungal organisms, and neoplasms such as a non-Hodgkins lymphoma provide another mechanism for bone marrow suppression and resultant neutropaenia.

Neutropaenia is more common in HIV-positive patients with an OI (65%) compared to those without an OI (34%) (44). Other confounding factors such as disease stage, and medication status should be taken into account when attempting to explain neutropaenia in these patients.

Qualitative defects in neutrophil function have been described and these relate to abnormalities in chemotaxis, diapedesis, degranulation and intracellular killing via the respiratory burst system (60).

Infants and children seem to tolerate low absolute neutrophil counts better with relatively infrequent infectious complications for the degree of neutropaenia. If, however, the neutropaenia is severe and prolonged, the

risk of serious bacterial infections and hospitalization increases proportionally (61).

Several studies have suggested that quantitative and qualitative leucopaenia can be ameliorated by the use of HAART (62) (63). In the study by Huang et al (62), 66 HIV-infected patients were studied prospectively and a statistically significant increase was found in the total leucocyte and absolute neutrophil count after 6 months of initiating HAART.

The HAART-regimens in this study included a protease inhibitor such as ritonavir or indinavir. Protease inhibitors have been shown to directly stimulate haematopoiesis and reduce apoptosis (63). As most of the HAART-regimens studied included protease inhibitors, these results are relevant mainly in the setting of protease inhibitor-containing HAART-regimens.

It is clear that cytopaenias are used as an important indication for a BME, and their aetiology can rarely be explained by one factor only. The overall value of cytopaenias to predict a specific disease affecting the bone marrow is low. The direct effect of HIV-infection plays a major part in producing cytopaenias, and a BME often merely confirms this fact.

This has led to the clinical question as to what the usefulness of a BME really is in HIV-positive patients in terms of diagnosing specific disease.

If a BME is found to produce a high yield of specific disease, it may justify performing this invasive investigation on HIV-positive patients, and in particular, children.

1.6 The bone marrow in HIV-positive children

1.6.1 The morphology of the bone marrow in HIV-infection

Several studies, mainly in HIV-infected adults, have been published that describe the characteristics of HIV-associated myelopathy (64) (65).

Abnormalities have been reported in the cell lines and the matrix of the bone marrow.

1.6.1.1 The cytological features of HIV-associated myelopathy

The bone marrow in HIV-infected patients may be normocellular, hypocellular or hypercellular. Hypercellularity of the bone marrow has

been reported even in the presence of peripheral blood cytopaenias (66). Hypercellularity and peripheral blood cytopaenias often co-exist and reflect myeloid dysplasia and ineffective haematopoiesis.

In a study by Mueller et al (67), bone marrow cellularity was evaluated specifically in a paediatric cohort, and hypercellularity was the most common finding. A diffuse lymphocytosis was the most common factor contributing to the hypercellularity.

A lymphocytosis may be a normal finding in children, and cellularity is reported according to age-related norms. In this study (67), haematopoietic growth factors were also available to patients and contributed to the finding of hypercellularity. In this paediatric study (67), 76% of patients were on antiretroviral therapy, but this was restricted to either monotherapy or two-drug combinations. These were not as effective as HAART, and there was still a high incidence of bone marrow abnormalities.

Dysplasia has been noted in erythroid, myeloid and platelet precursors.

Erythroid dysplasia is generally present in over 50% of patients, and erythrocyte precursors in the bone marrow may show nuclear aberrations including fragmentation and lobulation (68). Megaloblastic change that is unrelated to serum folate and vitamin B12 levels and/or treatment with

AZT has been reported. Similar findings of erythroid dysplasia have also been documented in a paediatric cohort (67). Myeloid dysplasia is characterized by left shifted, hyposegmented myeloid precursors and a maturation arrest at the metamyelocyte stage. High nuclear:cytoplasmic ratios, cleaved nuclei, and vacuolization in granuloblasts have been reported (69).

Megaloblastosis and a left shift were the most common myeloid dyplastic features noted in paediatric patients (67).

Megakaryocytic dysplasia is more common in patients with advanced stage disease. Changes affecting megakaryocytes relate mainly to nuclear abnormalities and include fragmented or denuded nuclei (70). Small, uninuclear megakaryocytes were found in paediatric HIV-patients, similar to those seen in myelodysplastic syndromes, but more rarely in adults (67).

Abnormalities in plasma cell morphology are common in the bone marrow of HIV-infected patients, seen across the stage spectrum of the disease in 31-85% of cases. The plasmacytosis may be related directly to HIV through a dysregulated B-cell proliferation, or be present secondary to antigenic stimulation due to another infection involving the bone marrow. Plasma cells were found to occur in clusters and showed nuclear abnormalities (70).

Bone marrow eosinophilia, without a concomitant peripheral blood eosinophilia is seen in 9-61% of patients (71).

In adults, bone marrow iron stores were found to be adequate to increased and this indicates a defect in iron utilization, rather than an iron deficiency per se (72).

In a paediatric cohort (67) however, 44% of patients had decreased or absent iron stores in the bone marrow, while only 26% had increased iron stores. This is in keeping with the common finding of iron deficiency in children. Serum iron studies can identify patients with an iron deficiency that may benefit from iron supplementation, and a BME is not needed to establish an iron-deficiency state.

In a study by Harris et al in HIV-infected adults (71), well circumscribed and poorly defined lymphoid aggregates were described in up to 50% of patients with advanced stage HIV-infection. Some lymphoid aggregates may contain histiocytes and form noncaseating “loose” granulomas consisting of lymphocytes, plasma cells and histiocytes. In the majority of these patients, an infective cause for the granuloma formation was not found, and it was attributed to HIV-infection initiating histiocyte proliferation and promoting haemophagocytosis. The dysregulated cytokine profile in the bone marrow of HIV-infected patients plays a role in stimulating macrophage and histiocyte proliferation.

1.6.1.2 Abnormalities in the bone marrow matrix in HIV-associated myelopathy

The bone marrow matrix may show fibrosis, increased reticulin fibers and “gelatinous” transformation (73). These abnormalities in the bone marrow matrix may be focal or diffuse. As a result of these abnormalities, bone marrow aspiration may be difficult, not infrequently yielding an insufficient specimen for analysis.

1.6.2 The diagnostic utility of a BME in HIV-positive patients

A BME is often performed in the clinical settings of unexplained pyrexia, unexplained haematological abnormalities, in the staging of previously diagnosed AIDS-related lymphomas and to exclude bone marrow involvement by disseminated OI’s and malignancies.

The goal of a BME is to identify and differentiate between potentially treatable specific diseases in a timely manner. A potential advantage of a BME is that a specific diagnosis may be made rapidly in a critically ill patient based on histopathological examination of the core biopsy.

An important question that several earlier and more recent studies, as discussed below, aimed to answer is whether BME has a favourable risk to benefit profile to justify its use.

In the pre-HAART period, an early retrospective study by Northfelt et al (74), found that 16% of patients had a mycobacterial infection diagnosed on BME, and the conclusion was made that a BME is a useful investigation for detecting mycobacterial infection in these patients. Similar findings were found by Engels et al (75) and Nichols et al (76), where mycobacterial or fungal infection was diagnosed by BME in 32% and 20% respectively.

Benito et al (77) reported a yield of 37.9% of specific disease in HIV-infected patients with unexplained pyrexia. These and other studies (78), (79) are from industrialized countries and the yield of specific disease on BME is reported between 22-42%. The diagnosis was unique in only 8-10% of cases in industrialized countries with access to mycobacterial blood culture facilities.

Several series have shown that a peripheral blood mycobacterial culture is as effective as a bone marrow mycobacterial culture in diagnosing mycobacterial infection (80) (81).

Akpek et al (82) found that the diagnostic sensitivity of peripheral blood mycobacterial cultures is greater than that of bone marrow histopathology alone, further reducing the potential unique value of a BME.

In a retrospective study from South Africa by Karstaedt et al (83), the BME provided a yield of 38% of specific disease, and a unique diagnosis in 24% of HIV-positive adults in the pre-HAART era. The patients in this study (83), all had advanced disease, as reflected by their in-hospital mortality of 23%, and none had received HAART. The specific diseases identified on BME were MTB in 32.3% (83/257), MAC in 1.6% (4/257), Cryptococcus in 1.6% (4/257) and haematological malignancy in 3.1% (8/257).

This study (83) was done before lysis-centrifugation mycobacterial blood culture facilities were available in South Africa, and the authors concluded that the unique diagnostic yield of a BME would have been significantly less had these peripheral blood cultures been available as a first-line investigation in excluding disseminated mycobacterial infection.

In a study from Brazil (40), mycobacterial infections were isolated in 26.8% of cases. This study population were adults with advanced HIV infection and 85% of patients were prescribed HAART, but compliance was reported in only 65%.

In both the South African (83) and Brazilian (40) studies, the incidence of mycobacterial infections was much higher than the 10% reported elsewhere (84), (85). This can be explained by the fact that South Africa and Brazil are countries with a higher prevalence of mycobacterial infection compared to other more developed countries.

Mycobacterial infection may be associated with granuloma formation in different organs of the body. Granuloma formation was present in 44% (113/257) of patients in the study by Karstaedt et al from South Africa (83). In this study, the vast majority of patients with tuberculosis (92.8%) on bone marrow (BM) culture also had granulomata present on histopathological examination of the bone marrow.

This high incidence of granulomata (44%) contrasts with the 20-30% incidence reported elsewhere (76), (79).

A possible explanation for the higher incidence of granulomata in this study is that South Africa has a high overall incidence of mycobacterial infection, especially tuberculosis, compared with other more developed countries.

The presence of granulomata should alert the clinician to the high probability of an OI ranging from 80 to 100% in immunocompromised patients (41), especially in countries with a high incidence of mycobacterial infection.

The caveat in this statement is that not all granulomata on BME are due to mycobacterial infection.

The finding of a granuloma on a core biopsy is not pathognomonic for any one specific disease. Instead, its presence may be due to a variety of infectious and non-infectious causes.

Non-infectious causes of BM granuloma include malignant diseases, notably the lymphoma group, where it is more common to find granuloma in association with Hodgkin's disease than with non-Hodgkins lymphoma.

Malignant causes have been described as the cause of BM granuloma formation in 20 – 25% of cases in adults (86). Other non-infectious causes include drugs such as procainamide and sulfonamide.

Autoimmune diseases and sarcoidosis in adults were reported to cause BM granuloma less frequently (87).

In patients with advanced HIV infection, a 30% granuloma incidence was reported by Nichols et al (76). The vast majority of these patients (80%) had an infectious cause for the granuloma, notably mycobacterial or fungal infection. In 20% of cases the cause of granuloma formation was undetermined, and it was suggested that these loosely cohesive, poorly organized granulomata can be due to the HIV-infection itself.

Infectious causes of BM granuloma include viral, bacterial, mycobacterial, fungal and parasitic causes. Ebstein-Barr and cytomegalovirus are the most common viral causes, while Brucellosis and typhoid fever are bacterial causes of BM-granulomata in endemic areas. Disseminated fungal infection, in particular Histoplasmosis, is an important cause of BM-granuloma, especially in patients with HIV-infection (87).

Tuberculosis is one of the most common causes of BM-granuloma with reported incidence in the literature varying from 6 – 48%, and 33 – 100% in cases with disseminated tuberculosis (87) (88). These series do not exclusively describe immunocompromised patients, and the lower 20 – 30% incidence of granuloma formation (76) reported in HIV-positive individuals in some studies may be related to the inability of HIV-positive patients to mount a sufficient cellular immune response that is necessary for granuloma formation.

The pathological feature of caseation has historically been associated with tuberculosis, although in a study by Kinoshita et al (89), only 29% of patients had caseation necrosis.

In addition to the finding of a low rate of caseation in cases with tuberculosis, it was found that the sensitivity of Ziehl-Neelsen (ZN) staining to identify acid fast bacilli (AFB) on microscopy of the bone marrow is low (76) (89). The proposed advantage of achieving a rapid diagnosis of specific disease by microscopy or histopathological identification of granulomata on BME may be outweighed by the low sensitivity of microscopy to identify AFB's and the fact that the finding of granulomata in the bone marrow is non-specific. Nevertheless, as stated before (41), the presence of a BM granuloma in a country with a high tuberculosis incidence should prompt a careful search for tuberculosis by culture, and consideration should be given to institute empiric anti-tuberculosis therapy pending the result of mycobacterial culture.

Non-caseating BM granulomata have been described in small series of patients with MAC and disseminated Bacillus Calmette-Guerin (BCG) infection (90) (91).

In the pre-HAART as well as the HAART era, mycobacterial infections were found to be the most common specific disease identified on BME of adults with advanced stage HIV-infection (92).

Controversy exists in the adult literature as to the value of a BME in diagnosing these infections.

Akpek et al (82) concurred with previously documented findings (80) (81) that the diagnostic sensitivity of a blood and bone marrow mycobacterial culture is similar, but found that histopathological examination of bone marrow specimens resulted in the rapid identification of mycobacterial and/or fungal infections in a third of their patient cohort. Based on this, their recommendation is that the continued use of BME is justified.

On the other hand, Marques et al (93) found that the maximum sensitivity on histology with combined mycobacterial stains was only 50%, and that granulomata were not sensitive for the detection of mycobacterial and/or fungal infections when culture-proven cases were evaluated together. The authors recommend a peripheral blood culture as the initial step in investigating disseminated mycobacterial or fungal infection.

Few studies evaluate the value of a BME in diagnosing specific disease in the paediatric population exclusively.

In one of the largest studies from the Paediatric branch of the National Cancer Institute, Mueller et al (67) described normal or HIV-related findings in the majority of patients with advanced HIV-infection.

Although 76% of these patients were on anti-retroviral therapy, most were on monotherapy or two-drug combinations, now known to be inferior to multi-drug HAART. Dyspoietic changes in the bone marrow were non-specific and did not correlate with anti-retroviral therapy, age, stage of disease or the presence of OI's. Bone marrow cultures were not more helpful than peripheral blood cultures in diagnosing OI. Repeated peripheral blood cultures were in fact more sensitive than morphology or culture of the bone marrow in diagnosing disseminated cytomegalovirus-infection and MAC infection. The latter appears to be a unique finding in the paediatric population.

Due to the paucity of data on the value of BME in the HIV-positive paediatric population, the aim of my study was to describe the indications used for a BME in a tertiary health care setting, the diagnostic utility of this investigation, and the clinical and haematologic features that may be associated with the finding of non-specific or specific disease on BME.

Based on the literature review, the hypothesis is that non-specific HIV-related changes will be the major finding in this cohort of patients with advanced disease stage and prior to the initiation of HAART. In South Africa, with its high prevalence of tuberculosis, the most common specific disease expected to be identified is opportunistic mycobacterial infection.

As described previously (67), cytopaenias alone and fever alone have low value in identifying specific disease on BME. If this finding can be replicated in this paediatric cohort, it may serve as a recommendation to reconsider the indications for a BME in paediatric clinical practice.

2.0 METHODS

2.1 Study design

A retrospective, cross-sectional descriptive study of HIV positive children that underwent a BME during the study period.

2.2 Study site

Chris Hani Baragwanath Hospital was the base hospital for patient selection and three study sites were included namely:

1.) The General Paediatric wards at Chris Hani Baragwanath Hospital (Inpatients).

The four general paediatric wards admit newly diagnosed children with HIV-infection and acute illnesses as well as children with known HIV-infection requiring admission for an intercurrent illness. The incidence of HIV-infection among general paediatric admissions averages 30% (personal communication, Prof U Kala).

Haematologic abnormalities are commonly identified in this patient population when routine full blood counts are done. The identification of said haematologic abnormalities creates a common clinical dilemma - that is to decide what the relative importance of HIV-infection, and/or OI and/or HIV-related malignancy is in contributing to the abnormalities. This is an important question to answer as it has important treatment implications. The current practice is that the haematologists are consulted on these patients, and a BME is requested to assist in explaining the haematologic abnormalities.

2.) The Harriet Shezi Paediatric HIV Outpatient Clinic at Chris Hani Baragwanath Hospital (Outpatients).

Harriet Shezi Children's Clinic is the public sector paediatric HIV clinic at Chris Hani Baragwanath Hospital. The clinic has started more than 2000 children on HAART since the roll-out in April 2004. It is the largest single site paediatric HAART cohort known to date. The majority of children are referred here after hospital admission to continue management and follow-up on an outpatient basis.

3.) The Paediatric HIV Research Unit (PHRU) at Chris Hani Baragwanath Hospital (Outpatients).

PHRU was established as a prevention of mother-to-child transmission (PMTCT) site in 1996 and established as a paediatric clinic in 1998. It currently has 1280 children on HAART.

The current practice is that outpatients from (2) and (3) with haematologic abnormalities are routinely discussed with the haematologists and in most instances referred to the haematology service for a BME.

Permission was granted by the Chief Executive Officer (CEO) of Chris Hani Baragwanath hospital to review files for inpatients. Files for inpatients were obtained from the central records department at Chris Hani Baragwanath hospital. Files for outpatients were reviewed with permission from the heads of the Harriet Shezi and PHRU clinics respectively. The head of the Haematology Laboratory at the National Health Laboratory Service (NHLS) granted permission to access laboratory records.

2.3 Study period

The study was conducted by retrospective case-file review covering the three year period from 01 June 2006 to 01 June 2009.

2.4 Study sample

Eighty six patients were identified that met the inclusion criteria (see below), and further data was available for 69 out of the 86 patients to describe the characteristics of those patients with either specific or non-specific disease on BME.

2.5 Study method and data collection

A laboratory record listing all HIV-positive children that underwent a BME during the study period was obtained from the Haematology Laboratory at the NHLS.

A BME was defined as a bone marrow aspiration and trephine core biopsy. The procedure is done under conscious sedation with cardiovascular monitoring and sterile precautions are followed.

In the laboratory, bone marrow aspirates are stained with Giemsa stain. A standard method of reporting is followed to assess the overall cellularity of the specimen, the quality of haemopoiesis along the three cell lines, the relative proportion of lymphocytes, plasma cells and macrophages, the presence of viral inclusions and iron stores.

A ZN-stain is done to assess for the presence of AFB. No other routine mycobacterial stains are done.

Trephine core biopsies are decalcified in 5% formic acid and tissue architecture is assessed. The cellularity of the specimen, the degree of fibrosis present and the presence of reticulin fibers, granulomas and abnormal infiltrates are described. Trephine biopsies are stained with PAS para-amino salicylic acid (PAS) and Grocotts methenamine silver stain to assess for fungi and non-tuberculous mycobacteria.

Bone marrow aspirate material is incubated in a BACTEC 12B culture bottle containing 4ml of broth culture medium including antibiotics to suppress the growth of contaminants. The BACTEC 460 (BD Diagnostics) instrument is used where the culture bottle is placed in an incubator at 35° Celsius, and if growth is present, carbon monoxide is released into the headspace of the instrument and the culture is designated as being positive.

Once a bone marrow culture is found to be positive by the method described above, a ZN stain of the material is undertaken, and if positive, signifies the presence of mycobacterial growth. The identity of the organism (mycobacterial vs non-mycobacterial) can already be suspected on the morphology of the organisms on microscopy as tuberculous organisms show cording, but specifically designed probes are used to

distinguish between tuberculous (MTB-complex) and non-tuberculous mycobacteria (MAC).

Blood or bone marrow material with a positive mycobacterial growth on the BACTEC culture media can be transferred into a mycobacterial growth indicator tube (MGIT) and a polymerase chain reaction (PCR) can then be done to distinguish the different species that form the MTB-complex namely MTB or Mycobacterium bovis.

At present, a direct PCR is only validated for smear positive sputum on direct microscopy. All other specimens have to be incubated in the MGIT system first and subsequently undergo PCR-testing.

Inclusion criteria for entry into the study were the following:

(1) Age ≤ 16 years at the time of BME.

(2) HIV positive diagnosis by HIV ELISA blood test after 18 months of age or by HIV PCR before 18 months of age.

(3) Had a BME done during the study period.

The following patients were excluded from the study:

(1) Patients who are HIV+ and underwent a first or follow-up BME as staging purposes for a non HIV-related malignancy, defined as all malignancies *other than a* non-Hodgkins lymphoma.

Eighty six children met the inclusion criteria, and a retrospective case file review of these children was conducted.

The files were reviewed to obtain the following information:

2.5.1 Indication for BME

1.) Clinical and/or

2.) Haematological

AND

2.5.2 Major finding on BME

1.) Specific disease or

2.) Non-specific disease or

3.) Insufficient sample

2.5.1 Indication for BME:

Indications for a BME were divided into clinical and/or haematologic indications.

1.) Clinical

Clinical indications were defined as the suspicion of an OI or HIV-related malignancy, based on the presence of unexplained, new or

enlarging lymphadenopathy, and/or hepatosplenomegaly, and/or loss of weight, and/or persistent pyrexia.

Staging was chosen as a clinical indication if the BME was done as part of a staging work-up for an already diagnosed HIV-related malignancy (non-Hodgkins lymphoma) elsewhere (e.g. a fine needle aspirate or biopsy of a lymph node or mass lesion indicating an HIV-related malignancy.)

2.) <u>Haematological</u>

Haematologic indications were defined as the presence of an isolated cytopaenia (one cell line abnormal), bicytopaenia (two cell lines abnormal), or pancytopaenia (all three cell lines abnormal).

Anaemia and leucopaenia were defined according to age-related values, and thrombocytopaenia as a platelet count of less than 150×10^9/L (42).

2.5.2 <u>Major finding on BME</u>

The findings on the BME were grouped into findings on microscopy/histopathology and finding on bone marrow culture result in order to determine whether specific disease, non-specific disease, or an insufficient sample were present.

1.) <u>Specific disease (SD)</u>

Defined as:

Microscopic identification of AFB's, granulomata, fungal elements, malignant infiltrate or pathological features suggestive of a PRCA.

Any collection of epithelioid histiocytes was regarded as a granuloma. As mentioned in section 1.6.2, granulomata are a common manifestation of disseminated tuberculosis, especially in countries with a high tuberculosis prevalence such as South Africa. Therefore, the presence of granulomata in the bone marrow of our patient cohort was taken as indicative of disseminated tuberculosis.

The presence of fungal hyphae was defined as fungal elements.

A PRCA was present if giant pronormoblasts, severely depressed selective erythrocyte maturation and/or the direct visualization of viral inclusions in erythrocyte precursors were present.

A disseminated HIV-related malignancy was defined as bone marrow involvement by a non-Hodgkin's lymphoma

OR

Bone marrow cultures positive for mycobacterial organisms and/or positive viral studies.

A positive bone marrow culture of MTB-infection or non-tuberculous mycobacterial infection namely MAC infection, and Mycobacterium bovis infection. PCR to detect mycobacterial DNA in specimens was not widely available at the time of this study.

A positive PCR for Parvovirus B19, if done, confirmed the presence of Parvovirus infection. Fungal cultures of bone marrow are not routinely done at this institution.

2.) <u>Non-specific disease (NSD)</u>

Defined as:

Non-specific HIV-related changes, anaemia of chronic disorder and "peripheral cause for cytopaenia" without the concomitant presence of SD

The features of HIV-related myelopathy were described in section 1.6.1.1. In accordance with this, non-specific HIV-related changes were present if the BME yielded any of the following:

hypercellularity, myelodysplasia, disordered hematopoiesis, plasmacytosis and lymphocytic and histiocytic infiltrates without granulomas. Anaemia of chronic disorder was present if there were features of increased iron deposition in bone marrow macrophages, together with sideropaenia. A “peripheral cause for cytopaenia” was present if the BME was normal in the presence of cytopaenias, and would suggest the possibilities of immune destruction or hypersplenism.

If both specific disease as well as non-specific findings were present, the BME was classified under “SD”.

3.) <u>Insufficient sample (IS)</u>

Defined as:

An inability to comment on the bone marrow sample due to the sample being too haemodilute, or not containing enough marrow spaces to comment meaningfully on possible pathology, or samples not containing bone marrow at all.

(See complete Data Collection sheet 1 in Appendix A)

Further data was available for 69 out of the 86 patients to describe the characteristics of those patients with either SD or NSD identified on BME.

The following variables were recorded in order to describe the characteristics of HIV+ patients that had a BME done:

1.) Age and sex of the patient.

2.) Clinical stage of HIV infection as defined by the WHO (14)

- Stages 1-4

3.) Immunological category as reflected by the CD4 count (14)

- Category 1: CD4 ≥ 25%
- Category 2: CD4 15-24%
- Category 3: CD4 < 15%

4.) Virological stage as reflected by whether the patient is virally suppressed or not.

- Viral suppression: < 25 viral copies/ml of blood

5.) Haematologic profile of the patient – Whether an isolated cytopaenia, bicytopaenia or pancytopaenia is present.

6.) Treatment status namely whether the patient is taking HAART or not. On HAART was defined as patients taking HAART for ≥ 3 months duration and without an interruption in treatment in the 3 months preceding the BME (14).

7.) Potentially marrow suppressive drugs the patient was taking (excluding HAART). These drugs were defined as taking cotrimoxazole (Bactrim),

gancyclovir, dapsone, amphotericin B, and/or anti-neoplastic chemotherapy.

8.) The concomitant presence of a documented serious bacterial infection that may contribute to bone marrow suppression.

9.) The presence of a documented haematinic deficiency namely iron, vitamin B12 or folate deficiency. These deficiencies may contribute to haematological abnormalities.

10.) The presence of a known HIV-related malignancy or opportunistic infection at the time of analysis. An already diagnosed HIV-related malignancy or opportunistic infection elsewhere may disseminate and involve the bone marrow with resultant haematologic abnormalities.

(See complete data Collection sheet 2 in Appendix B)

2.6 Data analysis

Data was collected on 2 separate Microsoft Excel data collection sheets attached in Appendices A and B.

The data was analysed using the Stata Statistical software package version 10.1 in conjunction with a biostatistician.

Associations between patient characteristics and bone marrow findings were tested using the Fisher's exact and chi-square tests for categorical variable analysis.

Statistical significance was set at $p \leq 0.05$.

2.7 Ethics

Individual patient data was coded with a unique number and access to patient files was restricted to the investigator and study supervisor. It was in no way necessary to reveal a patient's identity in the final paper and adherence to strict levels of confidentiality was adhered to at all times.

Ethics approval was obtained from the Ethics Committee of the University of the Witwatersrand. (Reference number: M090913)

3.0 RESULTS

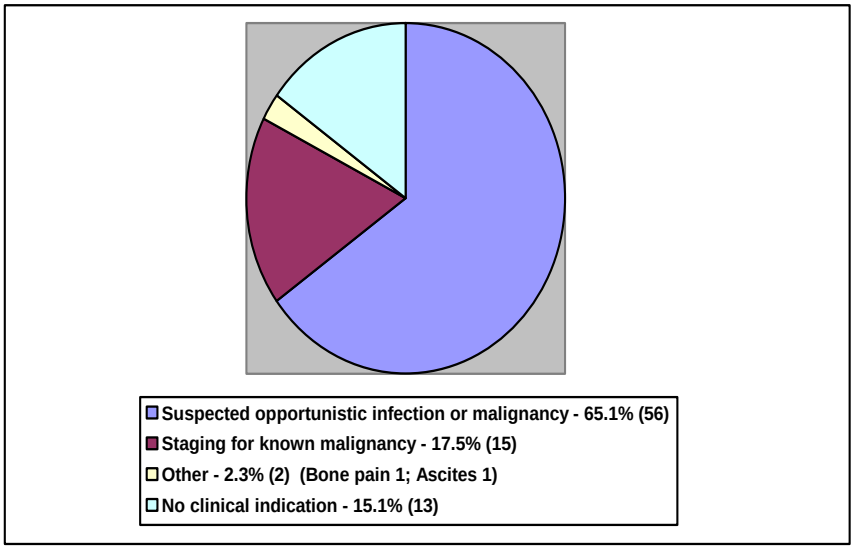
Eighty six patients had a BME done during the study period from the three study sites.

3.1 The clinical indications for performing a BME and the yield per clinical indication on BME

The most common clinical indication for performing a BME was the clinical suspicion of a disseminated OI or malignancy in 65.1% of (56/86) cases.

Other indications for a BME were bone pain (n=1) and ascites (n=1). In 15.1% (13/86) there were no clinical indications for the BME.

Figure 3.1 The clinical indications for performing a BME (n=86)



The association between different clinical indications and the findings on BME was tested with chi square and Fisher's exact tests and, as can be seen from table 3.1 below, no statistically significant associations were found between specific clinical indications and findings on BME.

Table 3.1 The relationship between clinical indications and findings on bone marrow examination

BMAT findings (n=86)	Suspected OI/malignancy % (n=56)	Staging for known malignancy % (n=15)	Other % (n=2)	No clinical indication % (n=13)
Specific disease	41.1% (23)	33.3% (5)	0 (0)	30.8% (4)
Non-specific disease	50.0% (28)	66.7% (10)	100% (2)	61.5% (8)
Insufficient sample	8.9% (5)	0 (0)	0 (0)	7.7% (1)

Pearson chi^2 p = 0.662

Fishers exact p = 0.748

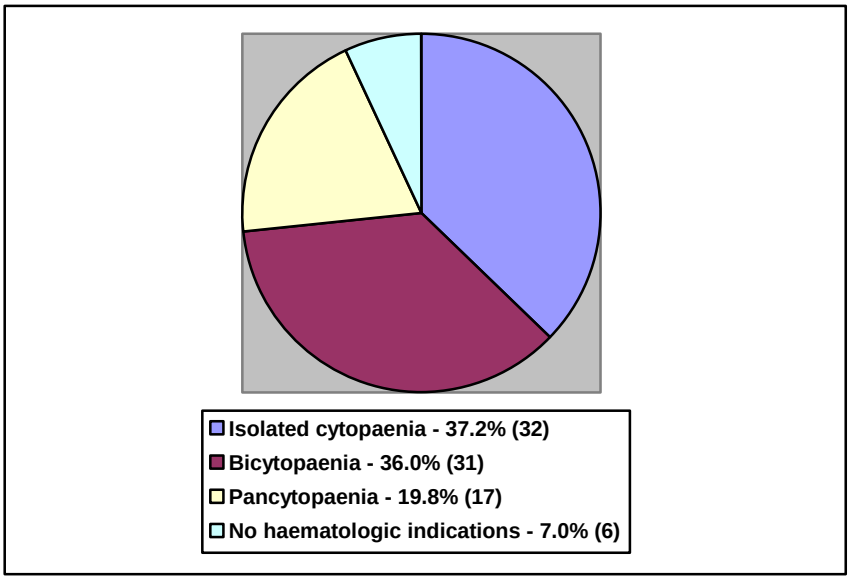
3.2 The haematologic indications for performing a BME and the yield per haematologic indication on BME

Isolated cytopaenia in 37.2% (32/86) and bicytopaenia in 36.0% (31/86) of patients were the most common haematologic indications for a BME.

Pancytopaenia was present in 19.8% (17/86) of patients.

No haematologic indication for the BME was present in 7.0% (6/86) of patients.

Figure 3.2 The haematologic indications for performing a BME (n=86)



Patients who had an isolated cytopaenia as haematologic indication for the BME had isolated anaemia in 78.1% (25/32) of cases, and an isolated thrombocytopaenia in 21.2% (7/32) of cases. No patients had an isolated leucopaenia as haematologic indication for BME.

Of those patients with an isolated anaemia as indication for BME, 52% (13/25) had specific disease on BME, and 48% (12/25) had non-specific disease as finding on BME.

Table 3.2.1 The specific disease identified on BME where isolated anaemia was the haematologic indication for performing the BME

Specific disease identified	(n=13)
Parvovirus infection	7
Non-Hodgkins lymphoma	3
MAC	2
MTB Complex	1

No patient who had an isolated thrombocytopaenia (7/32) as haematologic indication for BME had specific disease as finding on BME.

The association between different haematologic indications and the findings on BME was tested with chi square and Fisher's exact tests and no statistically significant associations were found between specific haematologic indications and findings on BME.

Table 3.2.2 The relationship between haematologic indications and findings on bone marrow examination

BMAT findings (n=86)	Isolated cytopaenia % (n=32)	Bicytopaenia % (n=31)	Pancytopaenia % (n=17)	No hematologic indication % (n=6)
Specific disease	40.6% (13)	38.7%(12)	41.2% (7)	0 (0)
Non-specific disease	56.3% (18)	51.6% (16)	47.1% (8)	100% (6)
Insufficient sample	3.1% (1)	9.7% (3)	11.8% (2)	0 (0)

Pearson chi^2 p = 0.336

Fishers exact p = 0.336

3.3 Patients with both clinical and haematologic indications for performing a BME

The majority of patients had both clinical and haematologic indications for a BME in 77.9% (67/86).

Table 3.3 Patients with both clinical and haematologic indications for performing a bone marrow examination

Indications	% (n=86)
Clinical **OR** hematologic	22.1% (19)
Clinical **AND** hematologic	77.9% (67)

3.4 The findings on BME and the utility of BME to diagnose specific disease

The table below lists the findings on BME:

Table 3.4.1 Individual findings on BME

Findings on BMAT	(n)*
Non-specific changes only	48
Granulomas	12
PRCA	11
Malignancy	7
Bone marrow culture positive	7
Insufficient sample	6

* Total adds up to >86 as some had more than one finding on BME

Non specific HIV-related changes indicative of HIV myelopathy namely hypercellularity, myelodysplasia, disordered hematopoiesis, plasmocytosis and lymphocytic and histiocytic infiltrates without granulomas, anaemia of chronic disorder and "peripheral cause for cytopaenia", were the major finding on BME in this cohort of patients in 55.8% (48/86).

SD, namely the presence of granulomas, PRCA, malignant changes, and positive bone marrow cultures comprised 37.2% (32/86) of the total.

No patients had fungal elements identified on microscopic examination of the BME. Only 16.7% (2/12) of patients with granulomas on BME had a positive ZN-stain.

In 7.0% (6/86) of cases, the samples were insufficient for analysis.

Table 3.4.2 Specific vs Non-specific disease on BME

Findings on BMAT	% (n=86)
NSD only	55.8% (48)
SD	37.2% (32)
IS	7.0% (6)

3.4.1 SD on BME

SD identified on BME were granulomas (n=12), PRCA (n=11), malignancy (n=7), and positive BM culture (n=7).

3.4.1.1 Granulomas (n=12)

All patients with granulomas on BME had a ZN-stain done, and of these, only 16.7% (2/12) were positive. Most patients with granulomas identified on bone marrow microscopy had a negative bone marrow culture for an opportunistic pathogen in 58.3% (7/12). The remaining five patients (41.2%) with granulomas on bone marrow also had a positive bone marrow culture namely MAC (n=3), and MTB Complex (n=2).

Two patients with positive bone marrow cultures had no granulomas on pathological examination of the bone marrow, one each with MAC and MTB Complex.

Further data was available for 10/12 patients who had granulomas on BME.

Patients with the finding of bone marrow granulomata were severely immunosuppressed as evidenced by having stage four clinical disease in all (n=10), the majority in immunological category three (n=7), and not virally suppressed (n=8).

Most patients with granulomata on bone marrow, were not on anti-retroviral therapy (n=7), with only 3/10 on HAART. The 3 patients on HAART with granulomata on BME had the following characteristics based on available data:

1.) One patient was on HAART for an unknown duration, but had an unsuppressed viral load. The patient was on treatment for MTB, but MAC was cultured on the bone marrow mycobacterial culture.

2.) One patient was on HAART for 4 years and was virally suppressed. Granulomatous inflammation as well as HIV-related changes was identified on the BME, but both the ZN-stain and bone marrow mycobacterial culture were negative.

3.) One patient was on HAART for 6 months. Data was not available with regard to viral load after the start of HAART. The patient was on treatment for disseminated MTB already, but had received a shortened intensive phase of treatment.

Patients with granulomata on bone marrow had pancytopaenia (n=5) as the most common haematologic abnormality, with bicytopaenia in 4/10 and isolated cytopaenia in 1/10 of patients.

3.4.1.2 PRCA (n=11)

Seven patients had giant pronormoblasts on morphological examination of the BME, which is pathognomonic of Parvovirus infection of the bone marrow. Four patients had microbiologically proven Parvovirus infection of the bone marrow with PCR positive for Parvovirus on the bone marrow.

3.4.1.3 Malignancy (n=7)

All patients with an HIV-related malignancy involving the bone marrow (n=7), had infiltration of the bone marrow by a non-Hodgkins lymphoma. In most cases, the diagnosis of malignancy was already made from another site, and BME was done for staging purposes (n=5). In two patients, the malignancy was primarily diagnosed on BME.

A total of 15/86 patients underwent a BME as a staging procedure, and of these, 33.3% (5/15) had bone marrow involvement by the malignancy.

3.4.1.4 Positive bone marrow cultures (n=7)

Positive bone marrow cultures were present in 8.1% (7/86) of patients. The responsible organisms were mycobacteria in all seven cases – three with MTB-complex and four with MAC.

Table 3.4.3 Bone marrow culture findings

Bone marrow culture	% (n=86)
Positive	8.1 % (7)
Negative	91.9% (79)

Table 3.4.4 Organisms grown from bone marrow culture

Organism	(n=7)
MTB Complex	3
MAC	4

3.5 Characteristics of patients that underwent a BME

Data was available for 69 out of the 86 patients who underwent a BME to determine the characteristics of patients undergoing a BME.

3.5.1 Gender and age

A BME was done in 55.1% (38/69) of males and 44.9% (31/69) of females. The majority of patients were >5 years of age in 56.5% (39/69), 1-5 year age group were 37.7% (26/69), and the minority were less than 1 year of age 5.8% (4/69).

3.5.2 Clinical, immunological and viral disease status

The clinical stage of patients who underwent a BME were stage four 71.0% (49/69) in the majority of cases, with stage three 18.8% (13/69), stage two 8.7% (6/69) and stage one 1.4% (1/69) comprising the remainder.

Most patients had immunologically advanced disease in immunological category three 71.0% (49/69), with 18.8% (13/69) in immunological category two, and 10.1% (7/69) in immunological category one.

Most patients were not virally suppressed at the time of BME with 88.4% (61/69) having a viral load of >25 copies/ml, and only 11.6% (8/69) being virally suppressed with a viral load of <25 copies/ml.

3.5.3 Antiretroviral and other medication status

The majority of patients were not on HAART in 73.9% (51/69), with 26.1% (18/69) patients on HAART. Only 31.9% (22/69) of patients were taking other medication defined as any of the following: cotrimoxazole (Bactrim), gancyclovir, dapsone, amphotericin B, and anti-neoplastic chemotherapy. Most patients were not taking any other medication 68.1% (47/69).

3.5.4 Haematologic abnormalities and presence of haematinic deficiency

Bicytopaenia in 37.7% (26/69) and isolated cytopaenia in 34.8% (24/69) were more common than pancytopaenia in 21.7% (15/69) in this cohort, while 5.8% (4/69) had no haematologic abnormalities. An isolated anaemia was the most common isolated cytopaenia identified.

Data was available in 85/86 patients with regard to the mean cellular volume (MCV) of the erythrocytes. The MCV-value is used to guide investigations into an underlying haematinic deficiency as a cause of

anaemia. MCV-values are age-specific (42), and are routinely available when a standard full blood count test is done.

In this cohort of patients, 64.7% (55/85) had normal MCV-values. Despite normal MCV-values, 29% (16/55) of patients had haematinic investigations done. These investigations included 15 iron studies, 10 vitamin B12-levels and 6 folate levels. The majority of patients with normal MCV-values that had haematinic investigations done, had no deficiency identified in 62.5% (10/16), while an iron-deficiency state was identified in 83.3% (5/6).

A high MCV was present in 23.5% (20/85) of patients. The majority of these patients had no haematinic investigations done in 65% (13/20). Of those that had haematinic investigations done, 5 iron studies, 5 vitamin B12-levels and 3 folate levels were done. No deficiency was identified in any of the patients with a high MCV. The majority of patients with a high MCV were taking HAART already in 70% (14/20), and one patient was on anti-epileptic medication.

A low MCV was present in 11.8% (10/85) of patients. Of these, 70% (7/10) had haematinic investigations done. These investigations included 7 iron studies, 3 vitamin B12-levels, and 4 folate levels. A deficiency was identified in all 7 patients that had haematinic investigations done, and all 7 had an iron-deficiency state.

3.5.5 Presence of concomitant septicaemia at the time of BME

Only one patient, 1.4%, had a positive bacterial blood culture at the time of BME, the organism was Salmonella paratyphi. This patient was not on antiretroviral therapy and had advanced immunosuppression (clinical stage four and immunological category three) with pancytopaenia. Four patients (5.8%) had no bacterial blood culture sent at the time of undergoing the BME, and 92.3% (64/69) had negative bacterial blood cultures at the time of BME.

3.5.6 Known presence of an OI or HIV-related malignancy at the time of BME

Most patients, 71.0% (49/69), had no known OI at the time of BME. Fewer patients, 29% (20/69), were already on treatment for an OI at the time of BME: 18 patients on treatment for MTB, 1 patient on MAC treatment and 1 patient with previously positive Parvovirus IgM and IgG serology. A previously diagnosed non-Hodgkins lymphoma was present in 14.5% (10/69) of patients, who underwent the BME as part of the staging workup.

The characteristics of patients who ultimately had SD and NSD as finding on BME are described in table 3.5.

Associations were tested between different patient characteristics and findings on BME.

As shown below, statistically significant associations were observed between clinical disease stage (p=0.05), viral load status (p=0.03), anti-retroviral treatment status (p=0.05) and the finding of NSD (HIV-related changes) on BME. Further to this, advanced clinical disease stage, not being virally suppressed, and not taking HAART were statistically significantly associated with the finding of NSD on BME as calculated by the chi square and Fisher's exact tests.

No statistically significant associations were observed between the other characteristics as listed in the table below and finding on BME.

Table 3.5 Clinical characteristics of patients who underwent BME and their association with the findings on BME

Clinical features	BM Specific disease (n=25)	BM Non specific disease (n=42)	BM insufficient (n=2)	Significance p ≤ 0.05
Sex				NS
Male	16	21	1	
Female	9	21	1	
Age				NS
< 1 year	1	3	0	
1-5 years	7	17	2	
> 5 years	17	22	0	
Clinical stage				**S (p=0.05)**
Stage 1	0	1	0	
Stage 2	0	6	0	
Stage 3	2	11	0	
Stage 4	23	24	2	
Immunological category				NS
Imm Cat 1	0	6	1	
Imm Cat 2	5	8	0	
Imm Cat 3	20	28	1	
Viral Load				**S (p=0.03)**
VL <25	5	2	1	
VL ≥25	20	40	1	
HAART				**S (p=0.05)**
Pre-Haart	15	35	1	
On Haart	10	7	1	
Other treatment				NS
Yes	10	11	1	
No	15	31	1	

Haematologic abnormalities				NS
Isolated cytopaenia	10	13	1	
Bicytopaenia	8	18	0	
Pancytopaenia	7	7	1	
No abnormality	0	4	0	
Haematinic deficiency				NS
Fe/VitB12/Folate deficiency	5	7	0	
Test not done	8	26	2	
No deficiency	12	9	0	
Bacterial Blood culture				NS
Positive	1	0	0	
Negative	24	38	2	
Not done	0	4	0	
Known OI				NS
Yes	7	12	1	
No	18	30	1	
Known HIV malignancy				NS
Yes	3	7	0	
No	22	35	2	

4.0 DISCUSSION

Health care practitioners that consult haematologists to request a BME on an HIV positive child are not uncommon in current day paediatric clinical practice.

A common clinical dilemma is that of an HIV positive child with haematologic abnormalities such as cytopaenias, identified on a peripheral blood count done as an in- or outpatient. This raises the important question of whether HIV infection is the major contributor to the abnormalities in blood parameters, or if, in addition, a SD, is involving the bone marrow with resultant cytopaenias.

This question is very relevant as it has major treatment implications.

In this study, I set out to determine what indications are used by health care workers in a tertiary hospital in both in- and outpatient settings when requesting a BME on an HIV positive child, as well as what the yield per indication for BME is.

I further aimed to determine what the utility of this invasive investigation is in order to diagnose SD.

Combining clinical, laboratory and therapeutic data, I aimed to describe patient characteristics that are associated with either **NSD** (HIV-related changes only on BME) or **SD** (disseminated OI or malignancy on BME).

4.1 The indications for performing a BME

A paucity of data exists on the indications for and utility of a BME in the paediatric population, with most studies reported in adults (94). Common indications (both clinical and haematological) for a BME used in adult studies are pyrexia of unknown origin, cytopaenias, a search for opportunistic pathogens and to exclude infiltration of the bone marrow by a neoplastic process (37) (75).

In the current paediatric study, the majority of patients had both clinical and haematologic indications for performing a BME in 77.9% (67/86). The indications for performing a BME were similar to those from above-mentioned adult studies, namely a suspicion of disseminated OI or malignancy accounting for the major clinical indications to perform a BME in 65.1% (56/86).

In this study, isolated cytopaenia in 37.2% (32/86), and bicytopaenia in 36.1% (31/86) of patients were the most common haematologic

indications for a BME. Pancytopaenia was the indication for BME in only 19.8% (17/86) of patients.

In this paediatric study, the most common isolated cytopaenia identified in patients who had a BME done was isolated anaemia, defined according to age-related values (42). Literature has reported anaemia as the most common haematological abnormality in HIV-infected patients, occurring in 10-20% of cases at the time of presentation and in 60-80% of patients over the course of the disease (43).

Anaemia in HIV-infected individuals often has a multifactorial aetiology as eluded to in section 1.5.1.1 In this study, anaemia was present in near equal numbers among those patients diagnosed with non-specific 48% (n=12) and specific 52% (n=13) disease on BME. NSD was present mostly in the form of anaemia of chronic disorder.

In patients with isolated anaemia as an indication for BME, the most common SD identified was opportunistic infections as a group. This included parvovirus infection (n=7), disseminated MAC infection (n=2) and disseminated MTB Complex infection (n=1). HIV-related malignancy namely non-Hodgkins lymphoma (n=3) formed the remainder of patients with specific disease on BME that had isolated anaemia as an indication for the procedure.

The literature recommends assessing iron stores and vitamin B12 and folate levels in the routine assessment of HIV-positive patients with anaemia to identify haematinic deficiencies in these patients (23). As is evident from section 3.5.4 above, the majority of patients in this cohort had a normal MCV-value. Despite this, haematinic investigations were done in nearly a third of patients (29%). This has important cost implications, as the majority of these with normal MCV-values did not have any haematinic deficiency. Similarly, in those patients with low MCV-values, vitamin B12 and folate levels were done in addition to iron studies, while low MCV-values are not commonly associated with a vitamin B12 or folate deficiency state. The recommendation is that MCV-values should be used as a guide to direct haematinic investigations appropriately and cost-effectively.

The effect of other possible contributors to anaemia namely AIHA and drug-induced anaemia were not specifically analysed in this study, but should be borne in mind as further contributors to anaemia in HIV-infected children.

With regards to yield per indication, no statistically significant association was found between any specific clinical or haematologic indication and finding on BME.

NSD however, was the major finding irrespective of whether the indication was clinical or haematologic.

A South African study among HIV positive adults (83) evaluated the indications for, and yield of, a BME as used by adult physicians and found that a BME should be reserved for when a patient is critically ill, or in geographic areas where opportunistic diseases such as histoplasmosis or leishmaniasis are prevalent or suspected.

In an earlier study by Brook et al (41), the highest yield of SD on BME in adult studies was found in patients with extremely advanced HIV disease, fever of unknown origin, pancytopaenia, and in the staging or investigation of lymphoma.

In the study by Tanaka et al (40) from Brazil, it was found that if fever of unknown origin alone was the indication for BME, it was only associated with a finding of SD in 3.7% (3/82). If cytopaenias alone (type of cytopaenias not defined), was the indication for BME, it was associated with a finding of SD in 4.9% (4/82). The highest diagnostic value was found if the indication for BME was both fever **and** cytopaenias in 25.6% (21/82). In this study, 85.3% (70/82) of patients were on HAART, but adherence was only reported in 65.8% (54/82).

The Brazilian study (40) accentuates that *after the introduction of HAART*, the value of a BME in HIV-positive patients with fever and cytopaenia without obvious localizing signs may prove useful in identifying disseminated OI's and/or malignancies. It further found that fever of unknown origin is mostly due to mycobacterial infections in this population.

In the current paediatric cohort where the majority of patients were not taking HAART, neither fever nor cytopaenia was significantly associated with SD on BME. A disadvantage in my study was that fever was grouped as part of the clinical indications for a BME and not clearly defined and evaluated separately, thus making any conclusions about the value of fever as an indication and possible predictor of a positive BME difficult in this cohort.

In my study, pancytopaenia was the least common haematologic indication for a BME, suggesting that, in this paediatric cohort, a BME is requested earlier by health care practitioners - even if only isolated cytopaenia or bicytopaenia is present. In the majority of these cases, NSD was found on BME. This compares favourably with the study by Brooke et al (41), showing that a low yield was found if BME was done in patients with isolated thrombocytopaenia, anaemia or leucopaenia, as HIV is usually the underlying cause in patients not yet on HAART.

An important recommendation following from the above mentioned studies is to discourage a BME if patients present with isolated cytopaenia or bicytopaenia, and to favour the initiation of HAART first with follow-up of the cytopaenias, as anti-retroviral therapy has been shown to be the definitive treatment of HIV-related haematologic abnormalities (13). This is of particular relevance in this paediatric cohort, as the majority of patients 73.9% (51/69) were not on HAART.

A further incentive for initiating HAART is that the diagnostic value of a BME is increased in patients with fever and cytopaenia who are already on HAART as shown by Tanaka et al (40).

4.2 Utility of BME to diagnose disease

In the current study, NSD, defined as HIV-related changes, anaemia of chronic disorder and “peripheral cause for cytopenia” without the presence of concomitant SD, accounted for the majority of bone marrow findings in 55.8% (48/86).

SD was identified in 37.2% (32/86) of patients.

An insufficient sample was reported in 7% (6/86), reflecting the technical difficulties often encountered in attempting to perform a BME on a young child.

NSD

NSD, defined as HIV-related changes only, was the most common finding on BME in this paediatric cohort in 55.8% (48/86) of cases. As referred to in section 1.6.1.1, the common pathological features attributed to HIV infection found in the bone marrow of HIV positive patients include hypercellularity, myelodysplasia, plasmacytosis and lymphocytic and histiocytic infiltrates. Reticular fibrosis, evidence of reticulo-endothelial blockade with iron deposition, vascular congestion and mucoid degeneration of fat were frequently observed (74).

In one of the few paediatric studies on the bone marrow features in children with HIV infection and peripheral blood cytopaenias by Meira et al (95), it was found that HIV-related changes in paediatric bone marrows were similar to those found in adults.

SD

The yield of SD on BME in my study was 37.2% (32/86), comparing favourably with both local and international data. The current study did not examine whether a specific diagnosis was made *exclusively* by BME, and thus can not comment on the value of BME in making a *unique* diagnosis of specific disease in children. This would require further study.

The South African study by Karstaedt al (83) documented a similar high yield of 38% of SD on BME in adults. However, in their study, the BME gave a unique diagnosis in 24% of cases. Several studies from developed countries have documented a yield of 25-42% of SD on BME (79) (81) (82), but with diagnoses unique to the bone marrow in only 8-10% of cases.

Blood culture facilities for mycobacterial culture were already available in developed countries at the time of study and this influenced the value of a BME in producing a unique diagnosis of specific disease in HIV+ patients.

The South African study (83), with its high rate of unique diagnosis on BME was conducted before lysis centrifugation blood culture for mycobacterial disease (TB BACTEC) was available, and the authors concluded that their yield of unique diagnosis would have been much lower had mycobacterial blood culture facilities been available.

Numerous studies have found that the diagnostic sensitivity of a blood mycobacterial culture is equal to a BM mycobacterial culture in identifying disseminated mycobacterial infection, and have recommended that a single lysis centrifugation blood culture should be the first step in the routine evaluation of HIV-infected adults when disseminated mycobacterial infection is suspected (74) (81).

One could thus speculate that the same would hold true as far as diagnosing disseminated mycobacterial infection in HIV-infected children is concerned. Should this same recommendation be applied in children, it would be particularly advantageous as a blood culture is certainly a much less invasive investigation compared to a BME.

Specific diseases identified in the current study were bone marrow granulomas (n=12), PRCA (n=11), malignancy (n=7) and a positive BM culture for opportunistic pathogens (n=7). No AFB's or fungal elements (hyphae) were identified on microscopy in this cohort of patients.

Granulomas and bone marrow cultures

Granulomas are the typical inflammatory response formed in response to a persistent antigen or as a hypersensitivity reaction. The presence of granulomas should alert the clinician to the high probability of an OI

ranging from 80 to 100% (41), especially in countries with a high mycobacterial, especially tuberculosis, incidence such as South Africa. As referred to in section 1.6.2, the etiologic spectrum of granulomata can encompass a variety of disorders besides mycobacterial and fungal infections.

In the current paediatric study, granulomas on BME were identified in 14% (12/86) of cases. The majority of patients (70%) (7/10), with granulomas on BME were not on HAART yet, had advanced immunosuppression, namely stage four clinical disease in 83% (10/12), immunological category three disease in 70% (7/10), and not virally suppressed in 80% (8/10). In the 3 patients with granulomas identified on HAART, there remains a possibility that the granulomatous inflammation could have been attributable to the HIV-infection and not true mycobacterial infection especially in view of the negative ZN-stain and mycobacterial culture, and that the unsuppressed viral load in the other patient contributed to the susceptibility to mycobacterial infection. The presence of granulomas in these patients on HAART does not change the conclusion that it is unlikely to detect granulomas in patients on HAART that are well and virally suppressed, and that a BME is not indicated in this population.

A South African study among HIV-infected adults (83) has reported a 44% incidence of granuloma formation on BME. This figure is higher than the 20-30% incidence of granuloma formation from internationally published

studies (76) (79). The higher incidence of granuloma formation in South African adults may reflect the high prevalence of mycobacterial infection in South Africa.

In the same study however (83), it is not stated what the distribution of disease stage among their patient cohort was. This is important to know, as a relatively intact immune system is required for granuloma formation, and in a cohort with more advanced immune suppression, granuloma formation would be expected to occur less frequently. This may be the explanation for the 14% incidence of granuloma formation from the current paediatric study, where a significant proportion of the cohort had advanced disease.

Only 5 of 12 patients (41.7%) with bone marrow granulomas had a positive bone marrow mycobacterial culture (MTB in 2, MAC in 3) as well. The remaining 58.3% (7/12), had granulomas on BME, but a negative BM mycobacterial culture.

A common clinical practice in South Africa, with its high incidence of mycobacterial infection, especially tuberculosis, is to start empiric anti-tuberculosis treatment based on the histopathological finding of a bone marrow granuloma, before waiting for culture confirmation. This would have been micriobiologically appropriate in only 5/12 patients (41.7%) with bone marrow granuloma and a subsequent positive mycobacterial BM

culture. Nevertheless, given the high incidence of mycobacterial infections among HIV-infected individuals in South Africa, the finding of a bone marrow granuloma should prompt the treating clinician to seriously consider empiric anti-tuberculosis treatment, and to follow-up the BM culture and clinical response to treatment.

Very few patients in this study had positive ZN staining of the bone marrow. Similar findings have been reported previously with low numbers of positive stains identified in other studies (81) (96) (97), and recommendations were made for the ZN stain to be replaced by, or used in combination, with two other stains namely the auramine-rhodamine stain, and the polyclonal antibody to Mycobacterium bovis. These stains are not in widespread use in the public health sector in South Africa at present.

PRCA

PRCA was the second most common SD identified on BME in this cohort (n=11), and accounted for a third, 34.3% (11/32), of cases of SD on BME. Human parvovirus B19 infection has been reported as one important cause of acquired PRCA in HIV-infected individuals (51).

The diagnosis of parvovirus B19 infection is made on the pathological basis of characteristic giant pronormoblasts in the bone marrow, and/or a positive PCR for parvovirus B19 DNA on a peripheral blood or BM specimen.

A BME though, is not essential to diagnose Parvovirus B19 as the cause of cytopaenias, as the diagnosis may also be made from a PCR-test for Parvovirus B19 DNA on a peripheral blood sample (98).

In this paediatric cohort, (7/11) patients had giant pronormoblasts morphologically characteristic of Parvovirus B19 infection of the bone marrow, and (4/11) patients had PCR-proven Parvovirus B19 infection of the bone marrow. Correlation of bone marrow Parvovirus B19 positivity with PCR-testing for Parvovirus B19 in the peripheral blood was not undertaken in this cohort and forms an opportunity for further study.

Identification of Parvovirus B19 has important therapeutic implications as treatment is available in the form of intravenous immunoglobulin therapy to ameliorate the haematologic abnormalities and decrease transfusion requirements. More importantly though, is that multiple reports suggested that the institution of HAART will result in the spontaneous clearance of parvovirus B19 infection, with subsequent improvement in the cytopaenias (99).

This forms an important recommendation to initiate HAART whenever Parvovirus B19 is identified during the evaluation of cytopaenias, and that, according to the above-mentioned studies reported in adults (51) (98), a PCR-test can identify this organism from a peripheral blood specimen, so that a BME is not essential when this diagnosis is considered.

Malignancy

The third most common specific disease identified on BME was a malignancy. In this cohort, BM involvement by a non-Hodgkin's lymphoma accounted for 21.9% (7/32) of SD identified on BME, and for 8.1% (7/86) of cases overall.

In all 7 cases of malignancy involving the bone marrow, the malignancy was a non-Hodgkins lymphoma. This corresponds to literature reporting that the most common malignancy in immunosuppressed children, whether secondary to HIV-infection or otherwise, is a non-Hodgkins lymphoma (100).

In this paediatric cohort, fifteen patients (17.4%) had a diagnosis of non-Hodgkins lymphoma established from another site, all of these patients underwent BME as a staging procedure, and 5/15 (33.3%) had confirmed disseminated non-Hodgkins lymphoma involving the bone marrow.

In 2/15 (13.3%) patients, the non-Hodgkins lymphoma was primarily diagnosed by BME. The indication for BME in these 2 patients was bone pain and ascites respectively, leading to a clinical suspicion of disseminated malignancy.

Most cases of lymphoma in children with AIDS present with extranodal manifestations such as organomegaly, jaundice or abdominal distention with or without ascites, bone marrow involvement or central nervous system symptoms. It is thus important to maintain a high index of suspicion for possible disseminated malignancy when these clinical features are present and a BME would be an appropriate investigation to rule out disseminated malignancy involving the bone marrow. A study in HIV positive adults has confirmed that a high yield was found if a BME was done in the investigation or staging of lymphoma (41).

4.3 Patient characteristics and bone marrow findings

Clinical data was available for 69/86 patients who underwent a BME in this cohort of patients. The aim was to test the association between different variables and findings on BME.

As can be seen from table 3.5, three variables were significantly associated with the finding of NSD on BME, namely advanced clinical

disease stage (p=0.05), not virally suppressed (p=0.03), and not on HAART at the time of BME (p=0.05).

The spectrum and pathogenesis of bone marrow abnormalities in HIV-infection has been described in section 1.0. Bone marrow abnormalities are frequently encountered in patients with HIV-infection with 40 – 60% of HIV-positive patients having dysplastic erythroid or myeloid bone marrow progenitor cells. The morphologic changes on BME in children were similar to those seen in adults (101). It is not surprising that NSD are the most common abnormality found on BME in HIV-positive patients (85), especially when not on HAART.

The plasma HIV RNA titer (viral load) is a strong predictor of the speed of progression of HIV-infection and hence the severity of the haematologic manifestations of HIV-infection. This is especially true in the first six months of life, and it was reported that an inverse correlation exists between viral load and the age of patients (102). Rapid disease progression and higher viral loads are generally seen in children compared to HIV-infected adults as reported in the study by Meira et al (95). In this paediatric cohort, although the numbers were small, all the infants less than 1 year of age (4/69) had unsuppressed viral loads, and all had NSD on BME.

It is thus possible that especially in younger patients, not on HAART and with a high viral load, the incidence of HIV-related changes on BME could be even higher – providing further strength to the recommendation to start HAART early to ameliorate the bone marrow changes and resultant haematologic abnormalities in these patients. Earlier initiation of HAART in infants less than 1 year of age forms part of the recently updated new treatment guidelines for children with HIV from the Department of Health stating that all children less than 1 year of age should be initiated on antiretroviral therapy regardless of CD4 count (12).

In this paediatric cohort, no single variable was significantly associated with the finding of SD on BME.

Literature from adult studies reports a varying spectrum of findings when a BME is done on HIV-infected patients. These findings ranged from HIV-related changes only (103), to identifying variables which may be associated with SD. A history of prolonged fever and an elevated direct bilirubin, was found to be associated with disseminated OI by logistic regression analysis in one study (82).

A recent study (85) from a major adult HIV/AIDS treatment centre in London, UK, found normal or HIV-related changes in the majority of BME's. In those that had SD on BME, the diagnostic yield was highest if cytopaenia **and** fever co-existed in the absence of localizing signs of

infection. If fever without cytopaenias and cytopaenias without fever were present, a BME had significantly less diagnostic value. All the patients in this study were *on HAART* already.

A common problem with most reported studies on all the aspects pertaining to BME's in HIV-positive patients, is that their patient population differ significantly from the current paediatric cohort studied. These studies are often from specialist clinics in developed countries, inner-city adult HIV clinics, HIV-infection acquired through homosexual transmission and intravenous drug abuse as opposed to mainly vertical transmission in children. In light of this, one should be cautious in applying the results from these studies to paediatric patients from sub-Saharan Africa as the context is vastly different.

Many reported studies are retrospective series with small patient numbers, in different settings (some outpatient and others inpatient-based), different exposure of patients to antiretroviral therapy, different or undisclosed disease stage of patients, and an uncertainty as to whether confounding variables that may influence variables were controlled for in all cases. Whether the BME provides a unique diagnosis or merely the same diagnosis from a different site, is also not always stated. This makes comparison between studies and recommendations based on data extrapolated from adult studies difficult.

5.0 CONCLUSION

In this paediatric cohort, the patient population can be defined as HIV positive children with advanced disease stage, the majority not on HAART, and mainly from an inpatient-setting in a tertiary hospital.

This retrospective, cross-sectional descriptive study aimed to identify the indications for BME in HIV+ children, the yield per indication for BME, the utility of BME to diagnose SD, and to describe the association (if any) between different patient variables and the finding of SD or NSD on BME.

The major findings were that BME's were requested by health care practitioners for a wide variety of clinical and haematologic indications, but that no statistically significant association was found between specific indications and finding on BME.

Isolated cytopaenia and bicytopaenia were the most frequent haematologic indications for this invasive procedure, despite it's low yield of SD in previously reported studies (40) (41).

As expected, given the advanced disease stage of these patients, non-specific HIV-related changes were found in the majority 55.8% (48/86).

The BME identified SD in 37.2% (32/86) patients, but the study did not examine whether this finding of SD on BME was unique to the bone marrow.

The most common SD identified were OI's as a group.

The following patient variables were significantly associated with the finding of NSD (HIV-related changes) on BME, namely advanced clinical disease stage (p=0.05), unsuppressed viral load (p=0.03), and pre - HAART (p=0.05).

Problems incumbent in any retrospective study were encountered namely the incompleteness of data and clinical, laboratory and therapeutic data was only available for 69/86 patients who underwent a BME during the study period. The study numbers are small and findings should be confirmed by larger, prospectively designed studies controlling for other variables which may influence the outcome and select patients that are representative of the paediatric HIV-positive community at large.

The majority of patients were referred from the general paediatric wards as inpatients, and as no standard referral protocol exists with regard to HIV-infected children with haematologic abnormalities, referral bias may have contributed to some patients being referred for a BME and others not, despite identical clinical presentations.

Most patients in this study were inpatients, symptomatic and newly diagnosed. They had advanced disease stage, unsuppressed viral loads and low CD4 counts. Thus, the sample is not representative of the general paediatric HIV-population at large and further study from a variety of settings in which patients across the stage-spectrum of HIV-infection is seen is necessary before general inferences about HIV-infection and bone marrow abnormalities can be made.

Despite the small numbers, retrospective nature and patient population that is slanted towards patients with advanced disease, the value that this study adds is that careful consideration should be given to instituting HAART first in the setting of non-critically ill patients with advanced immunosuppression, not on HAART, and with an unsuppressed viral load, before embarking on an invasive procedure such as a BME to explain haematologic abnormalities or search for OI's.

Less invasive procedures such as peripheral blood testing for mycobacterial infections (BACTEC) and Parvovirus B19 PCR-testing have been shown to be of equivalent value to bone marrow in diagnosing disseminated OI's (74) (81) (98), and these would be more appropriate initial investigations.

Prospects for future studies would be to evaluate the value of a BME in patients that are on HAART and present with fever without localizing signs *and* cytopaenias as this has been shown to produce the highest yield of SD in the HAART-era (40).

This paediatric cohort study highlights the fact that, as in adults, a BME is not an undisputed panacea for providing the answer in HIV-positive patients in whom disseminated OI's or malignancies are suspected, and sound clinical judgement and appropriate ancillary investigations should prevail before referring children for this invasive procedure.

Appendix A

DATA COLLECTION SHEET 1

No.																																		
	Indications for BME												**Confounders**							**Histopathology**												**Culture**			**BME+ for**
		Clinical						**Hematological**					**H/D**			**B/I**			**Drugs**	**Specific disease**						**Non-specific disease**		**Other**				Pos	Org	Neg	**any spec dis.**
	LOW	Pers fever	HSM	LAP	Susp OI / M	Staging	Other	Isol cyto	Bicyto	Pancyto	Other	Y	N	ND	Y	N	ND	Y	N	*AFB'S*	*Granuloma*	*Fungal*	*Malignancy*	*Parvo*		*HIV ONLY*	*ACD*	*Norm*	*PC*	*Other*	*Insuff*				

KEY

BME	Bone marrow examination
Y	Yes
N	No
ND	Not done
LOW	Loss of weight
Pers fever	Persistent fever
HSM	Hepatosplenomegaly
LAP	Lymphadenopathy
Susp OI/M	Suspected opportunistic infection or malignancy
Isol cyto	Isolated cytopaenia
Bicyto	Bicytopaenia
Pancyto	Pancytopaenia
H/D	Haematinic Deficiency
B/I	Documented Bacterial Infection
Drugs	Y Yes if on Bactrim, Ganciclovir, Dapsone or antineoplastic chemotherapy
	N No

AFB	Acid fast bacilli
Parvo	Parvovirus B19 infection
ACD	Anaemia of chronic disorder
PC	Peripheral cause for cytopaenia
Dx	Diagnosis
Insuff	Insufficient for analysis
HIV related	HIV Related changes on BME

Appendix B

DATA COLLECTION SHEET 2

NO.	BME Findings		SEX		AGE			HAART			Other Rx			STAGE										Nutr. Def				Blood Abnormalities				B/C			Known opp		Known HIV	
	1	2	M	F	< 1 YR	1-5 YRS	>5 YRS	PRE	ON	U	Y	N	U	Clinical					Immunological			Viral Load			Fe	B12 / Fol	ND	Nil	Isol. Cytop	Bicytop	Pancytop	No abn	Pos	Neg	ND	infx on Rx		malignancy	
	Specific	Non-specific												1	2	3	4	U	Cat I	Cat II	Cat III	VL < 25	VL > 25												Y	N	Y	N
	disease	disease																	CD4 >25 %	CD4 15-24	CD4 <15%																

KEY

BME Findings	Bone marrow examination findings				
1	Specific disease: Disseminated opportunistic infections or HIV-related malignancy				
2	Non-specific disease: HIV-related changes, anaemia of chronic disorder or peripheral cause for cytopaenia				
M	Male				
F	Female				
HAART	Highly active antiretroviral treatment				
	Pre	Not on HAART, or on HAART for less than 3 months at time of study or an interrupted period of HAART in preceding 3 months			
	On	On HAART for more than 3 months			
Other Rx	Other treatment: Y if on Bactrim, Ganciclovir, dapsone, amphotericin B or anti-neoplastic chemotherapy				
U	Unknown				
Nutr def	Nutritional deficiency				
Fe	Iron				
B12/Fol	Vitamin B12 / Folate				
BC	Blood culture				
Pos	Positive	**Neg**	Negative	**ND**	Not done
Opp Infx	Opportunistic infection				
HIV-related malignancy	Non Hodgkins lymphma				

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