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AN ANALYSIS OF THE UTILITY OF BONE MARROW EXAMINATIONS CARRRIED OUT IN AN INFECTIOUS DISEASE WARD

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

Johannesburg, 2018

DECLARATION

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

Candidate Signature: _____

The _____ day of _____, 2018

ABSTRACT

Introduction:

Human immunodeficiency virus (HIV) infection in South Africa places a large burden on the public healthcare sector. This necessitates quick and effective diagnostic decision making skills in acutely ill hospitalized patients, in order to improve patient outcome and reduce the length of hospital stay. Bone marrow aspirate and trephine examinations have long been utilized to aid with diagnosis in those patients who present diagnostic dilemmas in advanced stages of HIV infection.

Aim:

The aim of the study was to review the bone marrow examinations carried out in the Infectious Disease ward at the Charlotte Maxeke Johannesburg Academic Hospital between January 2012 and December 2014.

Methods:

A retrospective record review of bone marrow examination results from the National Health Laboratory Service database was undertaken. Individual patient records were reviewed if information was omitted from the bone marrow examination request forms.

Results:

A total of 327 patients underwent bone marrow examinations during this time period. Diagnoses unique to the bone marrow examination were obtained in 77 cases (23.5%). A unique diagnosis in this study was defined as any diagnosis made solely on bone marrow examination and not by any other means, or a diagnosis which was made faster on bone marrow examination as compared to

other forms of investigation. In three of these cases there were two unique diagnoses obtained, resulting in a total of 80 unique diagnoses. The most common unique diagnoses obtained were mycobacterial infection (69 cases), malignant lesions (5 cases) and pure red cell aplasia (4 cases). A white cell count $< 4 \times 10^9$/L was a predictor of a unique outcome on bone marrow investigation ($p < 0.01$). A neutrophil count of $< 0.5 \times 10^9$/L was found in those with unique diagnoses; however, this was not significant. A lower platelet and white cell count as well as a lower CD4 cell count were found to be significant predictors of the diagnosis of mycobacterial infection on bone marrow examinations, either via positive bone marrow culture, observation of granulomata on histology, or Ziehl-Neelsen staining on trephine specimens.

Concluding statement:

Bone marrow examinations may assist in aiding diagnoses in patients in whom other less invasive investigations have not yielded positive outcomes. Those patients in whom the diagnosis of disseminated mycobacterial infection is of primary concern and who have advanced HIV infection with lower peripheral blood counts may benefit the most from this modality of investigation.

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ABBREVIATIONS

AIDS – Acquired immune deficiency syndrome

ART – Antiretroviral therapy

BME – Bone marrow examination

CCM – Cryptococcal meningitis

CD4 – Cluster of differentiation 4

Hb – Haemoglobin

HCT - Haematocrit

HIV – Human immunodeficiency virus

HL – Hodgkin's lymphoma

ITP – Idiopathic thrombocytopenic purpura

KS – Kaposi's sarcoma

MAC – *Mycobacterium avium* complex

MOTT – Mycobacteria other than tuberculosis

MTB – *Mycobacterium tuberculosis*

NHL – Non-Hodgkin's lymphoma

NHLS – National Health Laboratory Service

NP –Neutrophil

OI – Opportunistic infection

PAS – Periodic acid-Schiff stain

PCR – Polymerase chain reaction

PLT – Platelet

PRCA – Pure red cell aplasia

RPI – Reticulocyte production index

TB - Tuberculosis

VL – Viral load

WCC – White cell count

WHO – World Health Organization

ZN –Ziehl-Neelsen

CHAPTER 1. INTRODUCTION

1.1 General Introduction

South Africa has the largest number of people living with Human Immunodeficiency Virus (HIV) in the world.[1] These individuals present more frequently to health care establishments with various complications of HIV infection and often pose diagnostic challenges in advanced stages of the disease. The need exists to evaluate the investigative modalities available in the public sector, with the collective aim of achieving faster and accurate diagnoses, allowing directed treatment, while decreasing the number of days spent in hospital, and ultimately improving morbidity and mortality outcomes.

1.2 Literature Review

1.2.1 HIV infection in South Africa

Sub-Saharan Africa, and in particular South Africa, experiences an immense burden of HIV infection. The 2016 mid-year statistical release by Statistics South Africa estimated that 12.7% of the population was affected by HIV infection in the 2015-2016 period.[2] This translates into 7 million individuals, a fifth of whom are females of child-bearing age.[2] Since 2005 the easier and earlier access to antiretroviral therapy (ART) as well as increasing destigmatization of the disease has led to healthier individuals with a decline in deaths due to acquired immune deficiency syndrome (AIDS) from 47.4% in 2005 to 27.9% in 2016.[2] Despite this, up to 33% of this population still present with advanced disease, namely a CD4 cell count below 100 cells/mm^3 together with an array of opportunistic infections (OI).[3] These individuals frequently present a diagnostic challenge to modern day infectious disease physicians as their presentation is associated

with cytopenias, fever and non-specific symptomatology.[4,5] When investigating such individuals multiple tests are carried out in order to establish diagnoses. One such investigation is that of the bone marrow aspirate and trephine examination.

1.2.2 Bone marrow aspirate and trephine examination as a diagnostic modality

The use of bone marrow aspiration dates to as far back as 1903, with the first bone marrow trephine being carried out in 1943.[6] Although the technique of execution and the instruments utilized have been refined, the primary purpose of performing a bone marrow examination (BME) has remained unchanged over the years. The purpose of this investigation is to assist with patient diagnosis in specific clinical scenarios through detailed examination of the haematological system.[7]

The haematological system is affected by HIV in multiple ways. Infection, be it bacterial, mycobacterial or fungal, as well as haematological malignancies, may be seen on bone marrow examination.[5] In addition, immune system dysfunction secondary to HIV infection itself, together with the medications utilized in immune-compromised patients, may also affect cell line production and function.[5] It is thus important to understand exactly how useful performing an invasive and uncomfortable bone marrow aspiration and trephine is in order to assist with patient diagnoses and management.

The most common indications for performing a BME are clinical symptoms of weight loss, fever or night sweats, as well as laboratory findings in the form of peripheral blood cytopenias.[8–11] Other indications include for the investigation, or staging of, suspected malignant lesions or to investigate for specific disseminated infections caused by mycobacterial, parasitic or fungal organisms.[8–11]

1.2.3 Bone marrow investigations in the setting of HIV

Since 1995 various studies have been carried out in order to evaluate the indications for, and the diagnostic utility of, bone marrow examinations in different clinical scenarios. The majority of these studies have been conducted outside of South Africa, with a particular focus on the HIV-infected population. These studies have demonstrated that between 25-47% of bone marrow examinations have aided with diagnostic decision making.[4,5,10–15] A higher diagnostic yield has been obtained from bone marrow trephines and aspirates as opposed to aspirates alone.[7,11] Evaluation of bone marrow aspirates in isolation have been found to be diagnostic in 8.5% of the HIV-seropositive population.[11]

These studies on international cohorts have collectively found that the most common positive finding from BMEs is the presence of mycobacterial infections. [8–12,14,16,17] The strains predominantly cultured in these cohorts were mycobacteria other than tuberculosis (MOTT), namely *Mycobacterium avium* complex (MAC) and *Mycobacterium kansasii*. Riley et al[9], evaluated 433 bone marrow culture specimens from 1983 to 1992 and found that 82.3% of the positive mycobacterial cultures grew MAC. Of these, a third were diagnoses unique to BME, highlighting the importance of bone marrow investigations for the diagnosis of MAC. This finding was in contrast to the results of those patients in whom *Mycobacterium tuberculosis* (MTB) was cultured on bone marrow, as the diagnosis of MTB was made more frequently by modalities other than BME. Additional diagnosis which have been found on BME include the presence of disseminated fungal infections such as histoplasmosis and cryptococcosis, as well as the presence of haematological malignancies.[8,9,18]

1.2.4 What is known in the South African setting

There are two studies, in particular, that have focused on the South African population. Karstaedt et al[4], reviewed bone marrow examinations carried out at the Chris Hani Baragwanath Academic Hospital between 1996 and 1997. This study took place prior to the nationwide roll-out of antiretroviral therapy in 2004.[19] The investigators found that 38% of bone marrow examinations performed on HIV-seropositive individuals provided a diagnosis and of these 24% were diagnoses unique to the BME. The remaining 14% of positive BME had similar diagnoses to those made by other investigative means, this most commonly being MTB diagnosed on sputum examination. Unique diagnoses found in that study included MTB, MAC, disseminated cryptococcal infection and haematological malignancies.

The findings of Karstaedt et al[4], were then mirrored by work published by Van Schalkwyk et al[5], in which BMEs carried out at the Groote Schuur Hospital in Cape Town were analyzed from 2004 to 2007. Of the 147 patients in the HIV-seropositive study group, diagnoses where obtained through bone marrow examination in 47%.[5] Of these, 33% were found to be unique diagnoses and included findings of immune thrombocytopenic purpura (ITP), disseminated tuberculosis (TB) and haematological malignancies. Although the indications for the BMEs were noted, it was uncertain as to how many were done with the primary suspicion of disseminated TB. Thus it was impossible to correlate how many suspected cases were actually proven on bone marrow and hence difficult to assess the utility of a bone marrow specifically for diagnosing TB. In addition, only 23% of the study patients were receiving antiretrovirals at the time of investigation, meaning that the full impact of antiretroviral therapy on patient presentation is also uncertain as no studies on the results of BME have been carried out since expanded antiretroviral coverage in South Africa.[5,19]

Of note, both Van Schalkwyk et al[5] and Karstaedt et al[4] noted haematological malignancies as unique diagnoses. This highlights the usefulness of bone marrow investigation in individuals with suspected haematological malignancies. Bone marrow involvement has been found to be the most prevalent site of extra-nodal disease in HIV-seropositive patients with Hodgkin's lymphoma (HL), with 17-62% having bone marrow disease on presentation.[20] The major indication for bone marrow examination found by Van Schalkwyk et al[5] was for the investigation of anaemia and thrombocytopenia.

There have also been attempts to identify the specific factors which tend to be associated with a positive diagnosis on BME. Across various studies the following factors, namely, a previous diagnosis of TB, haemoglobin (Hb) level less than 6 g/dL, neutrophil (NP) count of less than 0,5 x 10^9/L, white cell count (WCC) less than 4 x 10^9/L, platelet (PLT) count less than 150 x 10^9/L and cluster of differentiation 4 (CD4) cell count less than 50 x 10^6/L, have been shown to be associated with a higher likelihood of obtaining a unique diagnosis on BME.[5,12,17] A haematocrit of less than 25% was found to be more predictive of a diagnostic bone marrow examination by Keiser et al[17] as opposed to a study by Luther et al[12] which found a hematocrit of less than 30% to be predictive.

There have also been clinical factors identified that can help to predict positive bone marrow findings, such as wasting, oral candidiasis and splenomegaly.[4,17] Bone marrow examinations performed purely for the investigation of a thrombocytopenia have shown increased megakaryopoiesis in keeping with the diagnosis of immune thrombocytopenic purpura in the majority of cases. Subsequently, it has been recommended that such patients should be treated empirically if the clinical suspicion of ITP exists, with a bone marrow investigation being indicated in patients with advanced immunosuppression who are at risk of other opportunistic infections, as

well as in those with poor response to steroid therapy.[10] Of interest, studies have also found that bone marrow examinations for the presence of cytopenias alone in patients who are afebrile do not assist with diagnosis as the most common finding is of HIV-associated marrow dysregulation.[4,10] Identification of those factors which are associated with diagnostic BMEs, enables one to differentiate more efficiently as to when a bone marrow examination should or should not be performed.

1.2.5 TB and the bone marrow

One of the most common indications for performing a bone marrow examination is the suspicion of disseminated TB.[14] With South Africa having one of the highest prevalence's of TB worldwide, the usefulness of diagnosing TB via bone marrow investigation needs to be explored.[21] Establishing quick diagnoses in such patients is often difficult when patients present with constitutional symptoms, and are sputum GeneXpert and auramine stain negative, and have no palpable lymph nodes to biopsy. These individuals, and in particular those with fever and cytopenias, are often subjected to a BME in the hope of establishing a TB diagnosis.[9,14] In comparison to blood or bone marrow TB cultures, there is a quicker time to diagnosis with the examination and special staining of a trephine specimen.[15] With a normal culture taking up to four to six weeks in order to yield a positive response, the shorter time needed to perform a histological examination thus accelerates the time to diagnosis and therefore facilitates patient throughput and management.[5,13,14] Brook et al[10] found that the diagnosis of MTB was made twenty five days earlier through bone marrow trephine examination as opposed to TB culture. A recent study published in the South African Medical Journal by Sedick et al[22] also confirmed the findings of bone marrow examination yielding a faster time to diagnosis of MTB, namely, one day for an aspirate and up to

four days for a trephine, as opposed to the four to six week wait for TB blood culture results. In contrast, however, Pacios et al[23] found that when investigating for disseminated MAC infection, as opposed to MTB, peripheral TB blood culture had a higher diagnostic yield than BME.

Granuloma formation is also a common finding noted on bone marrow trephine examination. These are seen more commonly in patients with clinically advanced retroviral disease, lower CD4 counts, as well as lower neutrophil counts.[4,5] Karstaedt et al[4] found that 93% of patients with TB had granulomas on bone marrow trephine examination. In HIV-seronegative individuals, however, bone marrow examination for the investigation of disseminated TB has not been found to be a useful investigation.[9]

1.2.6 The current situation in South Africa

The last study evaluating the utility of bone marrow examinations in the South African population analyzed data from 2004 to 2007; since then the access to ART in the health care setting has increased considerably, and the availability of, and access to, investigative means other than bone marrow examination for the diagnosis of TB and OIs, has expanded.[4,5,16,24] These developments prompts the question of whether such changes have impacted on the use and utility of BME for diagnostic purposes in our current setting.

Charlotte Maxeke Johannesburg Academic Hospital has an Internal Medicine department divided according to sub-specialties. This institution serves as a referral site for further investigation and management of those patients for whom primary and secondary level care does not suffice. The Infectious Disease ward admits on average seventy patients a month into a twenty-five bed ward. The large patient load and the obligation to ensure high patient turnover, without compromising

care, necessitates the need for quick and accurate diagnostic decision making. In the various studies described above, bone marrow investigation showed diagnostic utility in up to one third of patients.[11] Thus many HIV-infected patients who present with unexplained cytopenias or the suspicion of TB without supporting diagnostic evidence, are subjected to an invasive bone marrow examination on the basis of this being understood to be a useful diagnostic test.[7,13] The current study aimed to explore if the investigative modality of a BME still holds utility in the current South African context.

1.3 Aim of the Study

The aim of this study was to assess the utility of bone marrow investigations in aiding diagnoses in an adult Infectious Disease ward at a tertiary academic hospital.

1.4 Study Objectives

1.4.1 Primary Objectives

The primary objective was to review the results and assess the utility of bone marrow aspirates and trephines as a diagnostic test performed in an Infectious Disease ward over a three-year period.

1.4.2 Secondary Objectives

a. To review patient demographics, as well as common indications for performing bone marrow investigations
b. To determine the proportion of samples sent which were inadequate

c. To review any unique findings on bone marrow investigation as well as to determine if any haematological parameters were associated with an increased likelihood of obtaining a diagnostic bone marrow result
d. To review the results of those individuals in whom tuberculosis (TB) was diagnosed on bone marrow
e. To compare results found in HIV-seronegative versus HIV-seropositive patients

CHAPTER 2. METHODS

2.1 Study Population

A retrospective analysis of the results of bone marrow aspirates and trephines performed between January 2012 and December 2014 in the adult Infectious Disease ward at the Charlotte Maxeke Johannesburg Academic Hospital was undertaken. Ethics approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (clearance number M150847, Appendix 1). Approval was also obtained from the medical superintendent of the Charlotte Maxeke Johannesburg Academic Hospital for the use of patient records as well as the National Health Laboratory Service (NHLS) for utilization of their patient result database. Inclusion criteria were any patient admitted to the adult Infectious Disease ward during the study period (namely individuals ≥ 16 years of age) who had a bone marrow examination during the study period. No exclusion criteria existed.

2.2 Study Site

The Charlotte Maxeke Johannesburg Academic Hospital is a 1088 bed hospital servicing an estimated population of 4 million people in the Johannesburg Metropolitan District, as well as surrounding districts in Gauteng.[25] The Internal Medicine Department of the hospital is divided according to sub-specialties with patients being triaged on arrival. The presence of a dedicated adult Infectious Disease ward allowed information pertaining to this specific population group to be readily accessed.

2.3 Measurements

The results of bone marrow aspirate and trephine studies performed between January 2012 and December 2014 were obtained from the National Health Laboratory Services Database. Data was extracted from these reports onto a standardized data collection sheet (Appendix 2). This data collection sheet included the clinical information provided by the requesting physician, as well as the hematological and microbiological laboratory results.

Patient anonymity was maintained by allocating numbers to each bone marrow report, starting at 001. The information documented from the bone marrow trephine and aspirate reports were patient demographics (age, gender, HIV status, CD4, viral load (VL) and co-morbidities) as well as pre-BME blood results, namely the full blood count, reticulocyte production index (RPI), and vitamin B12, ferritin and folate levels. Any cytopenias noted were documented. The indications for performing the BME were also documented.

Bone marrow aspirate and trephine findings were tabulated according to cell types, marrow observations, unique findings, evidence of granulomata or other infections and positive special staining. The adequacy of the marrow aspirate and trephine for analysis were also documented. If relevant clinical information or data were not available on the electronic NHLS database, the individual patient's record was then obtained and reviewed at the Charlotte Maxeke Johannesburg Hospital records department. The term "unique diagnosis" referred to any diagnosis made on the bone marrow aspirate or trephine, which was not made by any other diagnostic means or alternatively a diagnosis made faster on bone marrow examination than by any other modality of investigation. The diagnostic utility behind identifying cases with a unique diagnosis on bone marrow investigation was that this information would subsequently be utilized in order to initiate and/or adjust patient treatment accordingly, thus likely affecting patient outcomes.

The results of TB blood cultures performed on the bone marrow, as well as TB culture results on blood or other submitted specimens were documented together with time to positivity and the specific microorganism(s) cultured. A positive finding of conclusive TB included a positive TB culture or a positive TB polymerase chain reaction (PCR) on bone marrow. The presence of a positive Ziehl-Neelsen (ZN) stain on trephine or granulomata observed on histology alluded to a possible diagnosis of TB. Patients' results were reviewed to document other means by which malignancies, TB or other opportunistic infections had been diagnosed apart from bone marrow investigations.

2.4 Data Analysis

The statistical programs Statistica, version 13 and Stata were used to analyze data. Raw data was tabulated in a Microsoft Excel spreadsheet (Appendix 2). Descriptive analysis was used for patient demographics, indications for bone marrow examination, as well as the quality of the samples obtained. The Student's t test was used for comparison between continuous data with a normal distribution, namely in comparing platelet count as a predictive variable against TB culture results, ZN stain results and granuloma findings on BME. The Mann-Whitney-U test was used in comparisons between data without a normal distribution, specifically in the comparison between possible predictive variables and unique diagnosis, and the presence of granulomata, ZN stain results and TB culture results. In the comparison between categorical data a Chi-Squared test was utilized, namely in comparison between gender and unique diagnosis. A statistically significant result was defined as a p value of <0.05. Odds ratios were calculated for predictive determinants of a unique diagnosis on BME.

CHAPTER 3. RESULTS

3.1 Patient Demographics, Clinical Findings and Haematological Results

A total of 327 bone marrow aspirate and trephines were carried out in the adult Infectious Disease ward at the Charlotte Maxeke Johannesburg Academic Hospital between January 2012 and December 2014. The study population consisted of 162 (49.5%) males and 165 (50.5%) females. The mean age of the study population was 36 years with a range of 17 – 65 years. The mean ages amongst males and females were similar, with males having a mean age of 37 years (range 18 – 65 years) and females having a mean age of 35 years (range 17 – 62 years).

The results of the haematological investigations at the time at which the bone marrow investigations were performed were evaluated. The mean haemoglobin level was found to be 7.7g/dL (range 2.1 g/dL – 15.8 g/dL). The mean platelet count was 154 x 10^9/L (range 1 – 966 x 10^9/L) with a mean reticulocyte production index of 0.9 (range 0 – 7.7). The mean white cell count and neutrophil count were 5.6 x 10^9/L (range 0.2 – 73.9 x 10^9/L) and 4.19 x 10^9/L (range 0.1 – 58.0 x 10^9/L), respectively. Haematinic studies, namely vitamin B12, folate and ferritin levels, at the time of the bone marrow are presented in Table 3.1.

Table 3.1: Summary of laboratory parameters measured at the time of the bone marrow investigation

Variables	Mean	Standard Deviation	Minimum	Maximum
Haemoglobin (g/dL)	7.7	2.3	2.1	15.8
Platelets (x 10^9/L)	154	169.7	6	966
White Cell Count (x 10^9/L)	5.4	6.2	0.2	73.0
Neutrophils (x 10^9/L)	4.2	5.3	0.1	58.0
Ferritin (ug/L)*	6276	14968.4	5	189600
Vitamin B12 (ng/L)*	1065	676.7	152	6144
Folate (ug/L)*	746	477.2	40	4438
Reticulocyte Production Index	0.9	1.21	0	7.7
Viral Load (copies/mL)	816400	167030	20	9483100
CD4 cell count (cells/mm^3)	94	132	1	1069

*Reference Ranges → Ferritin: 20 – 250 ug/L, vitamin B12: 130 – 700 ug/L, Folate: 280 – 790 ug/L

Amongst the 327 patients in the study, 314 patients (96%) were HIV-seropositive, with 12 testing HIV-seronegative (3.7%). One patient's HIV status was unknown (0.3%). Amongst those with HIV infection, the mean CD4 count was 94 cells/mm^3 with a range of 1 – 1069 cells/mm^3. Of the 314 HIV-seropositive patients, there were 271 patients (86.3%) with a CD4 cell count of ≤ 200 cells/mm^3 at the time of the BME. There were 186 patients (59.2%) who were not on ART at the time of the bone marrow investigation, while 128 patients were already on ART (40.8%). The mean CD4 cell count in those on ART was 111 cells/mm^3 and those not on ART had a mean CD4 cell count of 78 cells/mm^3. Of the 111 patients in whom viral load was measured, the mean value obtained was 816 400 copies/mL with a range of 20 – 9 483 100 copies/mL.

TB treatment had been commenced prior to the bone marrow examination in 130 patients (39.8%), with the remaining 197 patients not on TB treatment (60.2%). Of the patients on TB treatment, 93 (71.5%) were on empiric TB treatment guided by either clinical symptoms, a miliary picture on CXR or on the basis of abdominal ultrasound findings. The remaining 37 patients (28.5%) on

TB treatment, had TB proven by other means prior to the bone marrow examination being performed.

3.2 Indications for Bone Marrow Examination

The indications for which a BME was performed were grouped into three broad categories, which were to investigate the cause of a peripheral blood cytopenia, to investigate for disseminated TB or to investigate for suspected disseminated malignancy. Overlap existed between the indications for bone marrow examination, with some patients having more than one indication for the BME. A total of 541 indications were recorded (Table 3.2). Thirty-two percent of the BMEs (173/541) were performed due to a clinical suspicion of TB. Fifty-four percent (293/541) were performed due to the presence of a cytopenia on peripheral blood. Nine percent (48/541) of BME were carried out owing to the suspicion of a malignant lesion with bone marrow involvement. The two main malignancies queried on bone marrow examination were disseminated Kaposi's sarcoma and haematological malignancies, in particular lymphoma.

Other indications for the bone marrow examination accounted for 5% (27/541) of the marrow examinations performed. These indications included the suspicion of a pure red cell aplasia (PRCA) in 12 patients, the suspicion of an idiopathic thrombocytopenic purpura in 6 patients and for investigation of a thrombocytosis in 1 patient, a leukocytosis in 1 patient and a possible microangiopathic haemolytic anaemia in 2 patients. Other indications were to exclude marrow involvement as a consequence of disseminated cryptococcal infection (3 patients), to look for a drug-related cause of cytopenias (2 patients) and for the suspicion of disseminated sarcoidosis (1 patient).

Table 3.2: Indications for bone marrow examination

Indication	No. of patients	Percent (%)
Suspected Tuberculosis	173	32
Cytopenia	293	54
Suspected Malignant Lesion	48	9
Other	27	5

A total of 541 bone marrow examinations were performed in 327 patients. Indications were sometimes multiple

3.2.1 Peripheral blood cytopenias

The presence of a peripheral blood cytopenia prompting the bone marrow investigation was present in 89.6% of the total study population (293/327), representing 54% of the BME (Figure 3.1). For the purpose of the study anaemia was defined as a haemoglobin < 11 g/dL, leukopenia as a white cell count < 4 x 10^9/L and thrombocytopenia as a platelet count < 150 x 10^9/L. Of the patients for whom a cytopenia prompted the bone marrow investigation the most common peripheral blood abnormality found was the presence of a pancytopenia, occurring in 100 (34%) patients (Figure 3.1). The next most frequent cytopenias noted were anaemia and leukopenia (64 patients, 22%), anaemia with thrombocytopenia (57 patients, 20%) and anaemia alone (53 patients, 18%). Leukopenia only, thrombocytopenia only and leukopenia with thrombocytopenia each represented 2 % of the peripheral blood cytopenias prompting a bone marrow investigation. There were six patients (2%) in whom a thrombocytopenia alone prompted the bone marrow investigation, the diagnosis of idiopathic thrombocytopenic purpura being the primary concern. Of the 327 study patients anaemia was present in 302 patients (92.3%) and amongst those with HIV infection, 290 individuals had anaemia (92.4%).

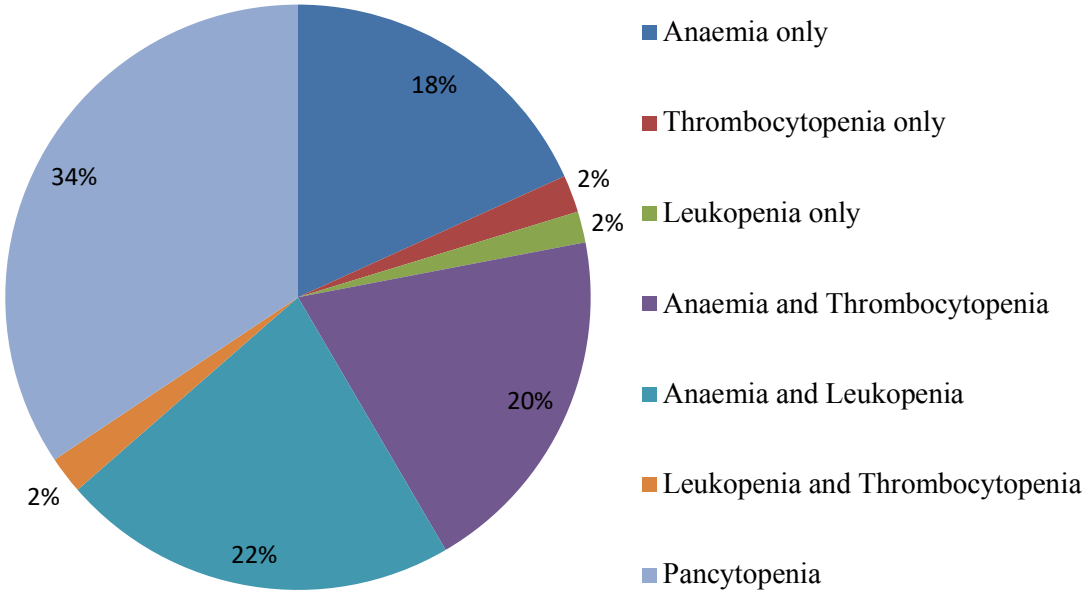


Figure 3.1: Classification of the cytopenias prompting bone marrow examination

3.3 Peripheral Blood TB Cultures

In the study population, 191 (58.4%) of patients had peripheral blood TB cultures performed (Table 3.3). Of this group the majority, namely 111 (58.1 %) patients, had negative culture results, with 65 (34.0%) having positive culture results and the remaining 15 (7.9%) of specimens were reported as either being contaminated or having a sample error.

Of the 65 positive TB blood cultures, MTB was isolated in 58 (89.2%) of cases and cultured in a mean of 27 days. MAC grew in a mean of 20 days in the five (7.7%) cases in whom it was isolated. One patient had rifampicin-resistant MTB cultured in 22 days and one patient was noted to have both MTB and MAC on peripheral blood TB culture which both flagged by 38 days.

Table 3.3: Organisms cultured on blood TB culture

Organism	No.	Percent of positive cultures (%)	Mean no. of days to a positive result	Standard Deviation
Mycobacterium tuberculosis	58	89.2	27	9
Mycobacterium avium complex	5	7.7	20	10.9
Mycobacterium tuberculosis and *Mycobacterium avium* complex	1	1.5	38	-
Rifampicin-resistant *Mycobacterium tuberculosis*	1	1.5	22	-

3.4 Bone Marrow Aspirate Results

Of the 327 cases reviewed, 12 cases had had no aspirate specimens submitted. The reason behind this was not documented, but may be assumed to be owing to technical difficulty. These patients for whom no aspirate specimens were found, however, all had trephine specimens submitted for analysis. Of the 315 aspirate specimens submitted, 40.6% (128/315) were found to be of an adequate quality (Figure 3.2). The remaining 59.4% of specimens (187/315) were documented as being inadequate owing to various reasons, including being aparticulate or haemodilute samples, clotted or thick specimens, particle fragments being noted and in one case the specimen was found to have been contaminated. Disordered erythropoesis was noted in 67.0% (211/315) of aspirates. Increased iron stores were noted on the aspirate in 46.7% (147/315) of cases. In 69.2% (218/315) of cases, granulopoiesis was noted in all stages of maturation, disordered maturation was documented in 8.9% (28/315) and toxic granulation was noted in 28.6% (90/315).

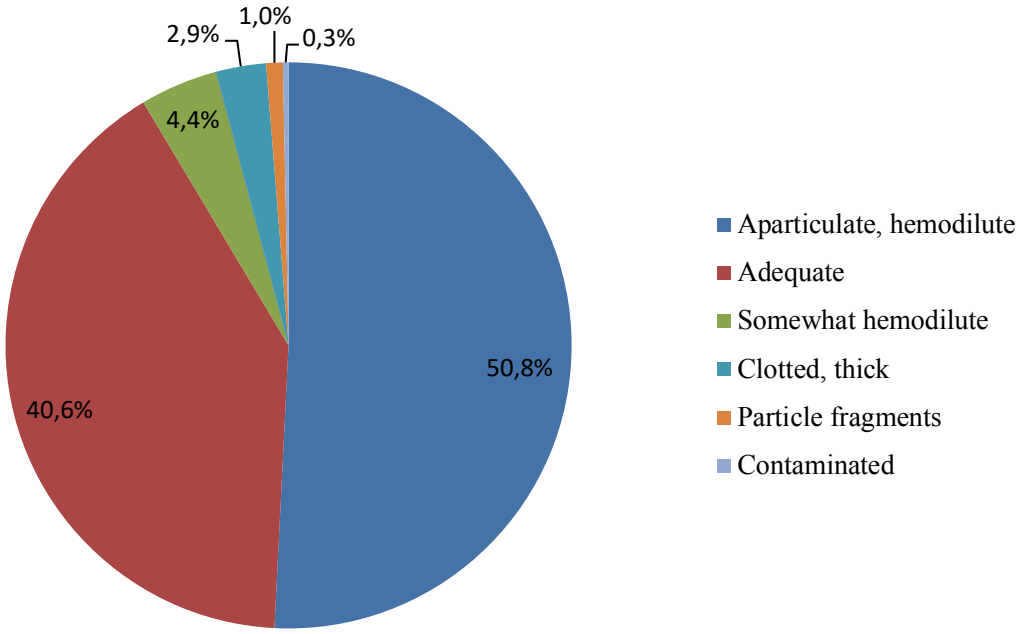


Figure 3.2: Bone marrow aspirate quality

3.5 Bone Marrow Trephine Results

Of the 327 bone marrow examinations reviewed, trephine specimens were not located on the NHLS database in 11 cases, all of whom had aspirate specimens submitted. The remaining 316 trephines analyzed were assessed for adequacy (Figure 3.3). The percentage of adequate specimens submitted were found to be higher than that of aspirate samples with 69.6% (220/316) being adequate. Overall, 13.6% (43/316) were inadequate and subsequently unable to be interpreted and 16.8% (53/316) were suboptimal and fragmented providing only partial information. Of the 220 adequate specimens, 37.3% (82/220) had ineffective haemopoiesis and 62.3% (137/220) had effective haemopoiesis. Granulomas were observed on trephine specimens in 41.1% (130/316) of cases.

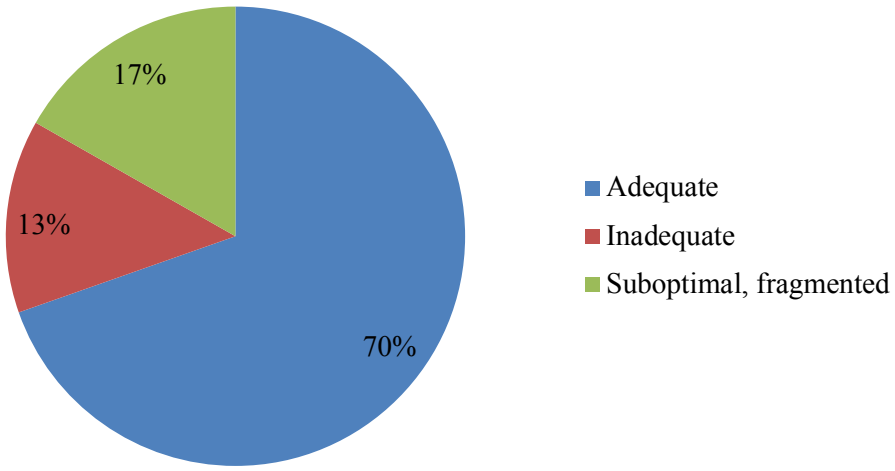


Figure 3.3: Bone marrow trephine quality

3.5.1 Special staining on bone marrow trephine

Of the 182 cases in which Ziehl-Neelsen staining of the trephine was performed, 28% (51/182) of the cases were positive. TB PCR testing was positive on trephine in six patients. Mycobacterial findings on BME are further elaborated on in section 3.8.

Periodic acid-Schiff (PAS) stain was positive in three patients. The first case was a patient in whom intra-cytoplasmic organisms were observed, together with a positive PAS stain on bone marrow trephine. The ZN staining in this case was also positive and these findings together with the morphological features on trephine examination were suggestive of MAC. This patient, in addition, had a positive sputum culture at 19 days confirming MAC infection, as well as a positive bone marrow culture at 47 days. Owing to laboratory processing the exact organism was unable to be clearly identified on the bone marrow culture.

The second patient with a positive PAS stain was already known to have cryptococcal meningitis, with a positive blood culture for *Cryptococcus neoformans.* In addition, this trephine specimen was mucicarmine stain positive confirming disseminated cryptococcal infection. The third patient who had a positive PAS stain on BME subsequently had a lumbar puncture performed which confirmed the diagnosis of *Cryptococcus neoformans* infection.

3.5.2 Parvovirus B19 staining on bone marrow trephine

Three patients had positive Parvovirus B19 PCR results noted. This final diagnosis, however, was not the primary concern when the BME were performed for these patients. Of the patients with a positive Parvovirus B19 PCR result, the first patient was a 28-year old female who had defaulted ART, with a CD4 count of 62 cells/mm^3 and a prior history of pulmonary TB. The indication for the BME in this case was to investigate a pancytopenia. The aspirate showed features concerning for Parvovirus B19 infection, namely an under representation of erythropoesis with early pronormoblasts together with features of anaemia of chronic disorders. Parvovirus B19 PCR was positive; however, the trephine specimen submitted was inadequate for interpretation.

The second patient was a 20-year old female on TB treatment and ART for 3 months with a CD4 count of 100 cells/mm^3 for whom the BME was performed to investigate a bicytopenia. She had a mildly hypocellular marrow with disordered prominent erythropoesis. The Parvovirus B19 PCR was positive and the suggestion was that this may be either recovery from acute infection or an evolving process. Further monitoring was recommended in this case.

The third patient with a positive Parvovirus B19 PCR was a 23-year old male patient, HIV-seropositive, on ART, with a prior history of TB three years before. He presented with a

normocytic anaemia for investigation. Bone marrow trephine revealed inclusions suspicious for Parvovirus infection and the subsequent Parvovirus B19 PCR was positive on the bone marrow.

Two additional patients were noted to have Parvovirus B19 inclusions on trephine; however, in both cases the PCR was negative. The first was a 41-year old male, HIV-seropositive on ART, with an isolated anaemia in the setting of a chronic empyema and renal dysfunction. Bone marrow examination showed features of a secondary PRCA; however, Parvovirus B19 PCR was negative. The second was a 40-year old female patient, HIV-seropositive on treatment, known with disseminated TB based on chest radiograph (CXR), who had presented with an anaemia for investigation. BME revealed the presence of granulomata, as well as marked erythroid hypoplasia, with no features of parvovirus infection and negative PCR, requiring the consideration of secondary causes, such as drug-related disease.

3.5.3 Other findings on bone marrow trephine

One patient had features suggestive of an aplastic anaemia. Additional findings unique to the trephine investigation included one patient in whom features of an anaplastic variant of a Non-Hodgkin's Lymphoma (NHL) were observed on trephine examination and another patient with generalized lymphadenopathy and hepatosplenomegaly with a large cell high grade NHL observed on trephine alone. A further case with a peripheral T cell lymphoma and an additional patient in whom the trephine showed Hodgkin's lymphoma, also confirmed on subsequent lymph node biopsy were additional unique diagnoses found on trephine examination.

3.6 Unique Diagnoses Made on Bone Marrow Examination

Of the 327 bone marrow examinations performed 158 (48.3%) yielded positive results. Although just under half of the bone marrow examinations provided a diagnosis, the primary objective of this study was to focus on those with diagnoses unique to the bone marrow examination. Of these 158 positive results, 77 diagnoses (23.5% of the study population) were unique to the bone marrow examination (Table 3.4). A unique diagnosis in this study was defined as any diagnosis made solely on bone marrow examination and not by any other means, or a diagnosis which was made faster on bone marrow examination as compared to other forms of investigation. Of the 77 patients in whom the BME provided a unique diagnosis, three patients had two simultaneous unique diagnoses made on BME.

Mycobacterial infection formed the majority of the unique diagnoses made. In nine patients MTB was cultured on bone marrow and provided a definitive diagnosis of the disease. Three of these individuals had already been on empiric TB treatment at the time of the BME. Of the 37 patients who had only granulomata observed on BME with no culture growth of MTB, 24 (64.9%) were already on empiric MTB treatment at the time, perhaps accounting for the lack of positive TB culture results on the BME.

Table 3.4: Unique findings on bone marrow investigation

Unique Diagnoses		
Mycobacterial Infection	***n*. of patients**	***n*. of patients on TB treatment already**
TB culture positive on BME only	9	3
Granulomata observed only	37	24
ZN positive only	1	0
Granulomata observed & ZN positive	11	5
BME TB culture positive & granulomata observed	6	1
BME TB culture positive & ZN positive & granulomata observed	2	1
MAC cultured on BME only	1	1
MAC cultured on BME faster than on peripheral blood	2	0
Total	**69**	**35**
Pure Red Cell Aplasia	***n.* of patients**	
Parvovirus B19 PCR positive	2	
Suspected Parvovirus B19 infection but PCR negative	1	
Secondary PRCA, Parvovirus PCR negative	2	
Total	**5**	
Malignancies	***n*. of patients**	
Non-Hodgkin's Lymphoma	2	
Hodgkin's Lymphoma	1	
Peripheral T cell lymphoma	1	
Total	**4**	
Others	**No. of patients**	
PAS stain positive, cryptococcus on BME	1	
Aplastic anaemia	1	
Total	**2**	

Abbreviations: TB – Tuberculosis, ZN – Ziehl-Neelsen, BME – bone marrow examination, MAC – *Mycobacterium avium* complex, PCR – polymerase chain reaction, PRCA – pure red cell aplasia, PAS – Periodic acid-schiff

One patient had a positive Ziehl-Neelsen stain on BME as the only means by which TB was diagnosed. Eleven patients had TB diagnosed with both granulomata and a positive Ziehl-Neelsen stain on BME, with five of these patients being on empiric TB treatment at the time of the BME.

TB bone marrow culture was positive, with granulomata being observed on the trephine examination, in six patients, one of whom was on empiric TB treatment. In two patients, TB bone marrow culture and ZN stains were positive with granulomata observed, one of whom was on empiric TB treatment. Three patients had MAC cultured on bone marrow culture, two of whom grew MAC faster on BME as compared to blood culture and in one for whom only the bone marrow culture was positive; however, the patient was already on empiric MTB treatment.

Five patients had the diagnosis of a pure red cell aplasia made on BME. In two of the cases the Parvovirus B19 PCR was positive, in one case the morphological and haematological findings were highly suspicious for Parvovirus infection, although the PCR was negative, and in the last two cases secondary causes of PRCA, potentially drug- or toxin-related aplasia, was suspected.

Four cases of haematological malignancies were identified on bone marrow alone. These findings comprised two cases of Non-Hodgkin's lymphoma, one case of Hodgkin's lymphoma and one case of peripheral T cell lymphoma.

There were two other unique findings on BME, one being the diagnosis of an aplastic anaemia and the second being a patient in whom the PAS and cryptococcus mucicarmine stains were positive, as discussed previously.

Three patients had two unique diagnoses obtained on bone marrow investigation. The patient with Hodgkin's lymphoma also had the finding of granulomata on BME and was on empiric TB treatment. One of the patients with a positive Parvovirus B19 PCR slide also had a positive TB

bone marrow culture. This patient was also on empiric TB treatment at the time of the BME. The third patient, who was not on TB treatment at the time of the BME, had both the diagnosis of NHL made on BME as well as a positive ZN stain together with granulomata observed on BME.

3.6.1 Predictive factors of a unique diagnosis on bone marrow examination

The Mann-Whitney-Wilcoxon test was used to determine which variables were more likely to predict positive results on BME were performed (Table 3.5). HIV status and gender did not impact the likelihood of obtaining a unique diagnosis on BME ($p = 0.57$ and $p = 0.65$ respectively). Of the haematological values, a haemoglobin < 7g/dL and platelet count < 150 x 10^9 /L were also not significant in predicting a unique diagnosis on BME (both with $p = 0.58$). CD4 cell count < 50 cells/mm^3 was also not significant as a predictor ($p = 0.62$). A white cell count < 4 x 10^9/L was, however, found to be a statistically significant predictor of a unique diagnosis on BME, with an odds ratio of 2.38 (confidence interval of 1.37 – 4.14) ($p = 0.002$). A neutrophil count < 0.5 x 10^9/L was found more commonly in those with a unique diagnosis; however, this was not found to be significant ($p = 0.05$).

Table 3.5: Predictors of unique diagnoses on bone marrow examination

Variable	Unique Diagnosis (n = 77)	Not a Unique Diagnosis (n = 250)	p value	Odds ratio
	n.	*n.*		
HIV positive	75 (97.4%)	239 (95.6%)	0.57	1.56 (0.33 - 7.32)
Male sex	40 (51.9%)	122 (48.8%)	0.65	1.12 (0.67 - 1.88)
Hb (< 7 g/dL)	32 (41.6%)	95 (38.0%)	0.58	1.16 (0.69 - 1.95)
Platelet count (<150 x 10^9/L)	52 (67.5%)	177 (70.8%)	0.58	0.86 (0.50 - 1.49)
WCC (< 4 x 10^9/L)	55 (71.4%)	128 (51.2%)	0.002	2.38 (1.37 - 4.14)
Neutrophil count (< 0.5 x 10^9/L)	5 (6.5%)	5 (2.0%)	0.05	3.55 (1.00 – 12.67)
CD4 (< 50 cells/mm^3)	41 (53.2%)	121 (48.4%)	0.62	1.13 (0.67 - 1.92)

Abbreviations: HIV – Human immunodeficiency virus, Hb – Haemoglobin, WCC – white cell count, CD4 – cluster of differentiation 4.

3.7 TB diagnosis on Bone Marrow Examinations

Of the 327 bone marrow investigations performed, MTB was proven in 94 (28.8%) cases, either from a positive bone marrow TB culture, presence of granulomata on BME, positive ZN stain or a positive TB PCR. The individual yields from each TB testing method are represented in Table 3.6. Six TB PCR stains were commented on as positive; however, it was not noted how many TB PCR tests were performed which yielded negative results, and thus comparisons could not be performed.

Table 3.6: Detection of MTB on bone marrow examination

Method	Number tested	Positive Result (%)
Tuberculosis bone marrow culture	226	55 (24.3%)
Ziehl-Neelsen Stain	182	51 (28.0%)
Granulomata	316	130 (41.1%)

A lower platelet count, WCC and CD4 count, as well as a higher VL, were found to be significantly associated with the likelihood of obtaining a positive finding of TB on BME, as expanded on below.

3.7.1 Mycobacterial culture results on bone marrow examination

Of the 225 bone marrow specimens submitted for TB culture, 146 (64.9%) were culture negative, 55 (24.4%) yielded positive results and 24 (10.7%) were contaminated. Of the 55 positive bone marrow cultures, 49 (89.1%) yielded MTB. Four cases (7.3%) cultured MAC, one case cultured rifampicin-resistant MTB and in one case the organism was not identified (Table 3.7).

Table 3.7: Organisms cultured on bone marrow TB culture

Organism	*n.*	Percent (%)	Mean Days to Positivity	Standard Deviation
Mycobacterium tuberculosis	49	89.0	27	10.7
Mycobacterium avium complex	4	7.3	13	8.4
Mycobacterium tuberculosis rifampicin-resistant	1	1.8	26	-
Unknown culture result	1	1.8	47	-

There was one particular patient in the cohort who cultured rifampicin-resistant TB on both bone marrow and blood culture. This was a 31-year old female, who presented with a severe anaemia of 2.7g/dL, was HIV-seropositive with a CD4 count of 16 cells/mm^3 and not on ART or TB treatment at the time of the bone marrow examination. The TB blood culture flagged positive after 22 days, whereas the bone marrow blood culture flagged positive after 36 days. Granulomata were observed on trephine and the Ziehl-Neelsen staining was positive.

One study patient had a bone marrow TB culture which flagged positive at the end of the 47 day culture period; however, no specific organism was able to be identified by the microbiology laboratory. This patient, however, subsequently cultured MAC on sputum and in addition had positive PAS and ZN stains on trephine examination.

3.7.2 Predictors of positive bone marrow TB culture results

In those patients with a positive TB bone marrow culture result, the mean white cell and platelet counts were 5.0 x 10^9/L and 111 x 10^9/L respectively. This was found to be significantly different ($p < 0.05$) from the mean white cell and platelet count of those who did not culture TB on bone marrow examination (6.3 x 10^9/L and 166 x 10^9/L, respectively). There was also a significant difference observed between the CD4 cell count and VLs (46 cells/mm^3 versus 110 cells/mm^3 and 2170699 copies/mL versus 603340 copies/mL, respectively) between those with a positive TB culture on BME and those without (Table 3.8).

Table 3.8: Predictors of positive TB culture on bone marrow examination

	TB Culture Negative		TB Culture Positive		p Value
	Mean	Standard Deviation	Mean	Standard Deviation	
WCC (x 10^9/L)	6.3	5.5	5.0	9.7	0.007
Hb (g/dL)	7.8	2.3	7.5	2.5	0.20
Platelets (x 10^9/L)	166	186	111	90	0.039
CD4 (cells/mm^3)	110	151	46	60	<0.001
VL (copies/mL)	603340	1272887	2170699	3157339	0.029

Abbreviations: WCC – white cell count, Hb – Haemoglobin, , CD4 – cluster of differentiation 4, VL – viral load

3.7.3 Granulomata on bone marrow examination

Granulomata were observed on trephine specimens in 41.1% (130/316) of cases, as noted previously. Significant predictors of the presence of granulomata on BME were WCC, platelet count, CD4 count and VL (Table 3.9). The group in whom granulomata were found had a WCC of 4.46 x 10^9/L, platelet count of 125 x 10^9/L, CD4 cell count of 60 cells/mm^3 and VL of 1318816 copies/mL as compared to those in the group without granulomata observed on bone marrow (WCC of 6.15 x 10^9/L, platelet count of 174 x 10^9/L, CD4 count of 119 cells/mm^3 and VL of 430584 copies/mL, respectively) (Table 3.9).

Table 3.9: Predictors of granulomata on bone marrow examination

	No Granulomata Observed		Granulomata Observed		p Value
	Mean	Standard Deviation	Mean	Standard Deviation	
WCC (x 10^9/L)	6.2	7.4	4.5	4.2	0.04
Hb (g/dL)	7.8	2.5	7.6	2.2	0.86
Platelets x 10^9/L)	174	195	125	121	0.01
CD4 (cells/mm^3)	119	145	60	101	<0.001
VL (copies/mL)	430584	965885	1318816	1284585	0.03

Abbreviations: WCC – white cell count, Hb – Haemoglobin, , CD4 – cluster of differentiation 4, VL – viral load

3.7.4 Ziehl-Neelsen stain on bone marrow examination

Those patients in whom the ZN stain was positive on the bone marrow trephine examination were found to have significant differences in their platelet count, CD4 cell count and viral load as compared to those without a positive ZN stain on bone marrow examination (Table 3.10).

Table 3.10: Predictors of positive ZN stain on bone marrow examination

	ZN Negative		ZN Positive		p Value
	Mean	Standard Deviation	Mean	Standard Deviation	
WCC (x 10^9/L)	5.5	6.5	4.5	3.5	0.83
Hb (g/dL)	7.3	2.3	7.7	2.3	0.43
Platelets (x 10^9/L)	152	152	104	82	0.03
CD4 (cells/mm^3)	86	121	41	43	0.004
VL (copies/mL)	829586	1880361	1866499	227274	0.012

Abbreviations : WCC – white cell count, Hb – Haemoglobin, , CD4 – cluster of differentiation 4, VL – viral load

3.7.5 Bone marrow versus peripheral blood mycobacterial cultures

Although a comparison between the yield of blood versus bone marrow cultures was not one of the objectives of this study, it was noted that 23 patients had both blood and bone marrow cultures positive for mycobacterial infection. Two of these patients were already on TB treatment at the time of the BME. Nineteen patients had MTB on both blood and bone marrow culture. Two patients had MAC on both cultures. One patient had MAC on blood culture and MTB on bone marrow culture and one patient had MAC and MTB on blood culture and MAC on bone marrow culture. In 11 cases (47.8%) the time to positivity was faster with blood culture and in 10 cases (43.5%) the time to positive result was faster with bone marrow culture. In two cases (8.7%) the time to culture positivity was the same.

3.7.6 MAC diagnoses on bone marrow examination

There were seven patients with the diagnosis of MAC in the study cohort (Table 3.11). Five of the seven were males with the age ranging from 18 – 60 years. All patients were HIV-seropositive. In all cases the CD4 cell counts were ≤ 35 cells/mm^3. Five of the seven patients were already on ART at the time of the BME, with four of the five having virological failure and one of the five presenting as a suspected immune reconstitution syndrome after having been on ART for three months.

Table 3.11: Summary of *Mycobacterium avium* complex diagnoses made on bone marrow examination

	Gender	Age	HIV status	CD4 Count	ON ART	On TB treatment	TB Blood Culture	Days to positivity	WCC	HB (g/Dl)	PLT	TB Bone Marrow Culture	Days to Positivity	Granuloma on Bone Marrow Trephine	Unique Diagnosis
Patient 1	Female	31	+	4	No	Yes, empiric	Contaminated	-	0.77	5.4	273	Positive	22 days	No	Yes
Patient 2	Female	33	+	35	Yes, VF	No	Positive- MAC	26 days	3.53	5.7	218	Positive	8 days	Yes	Yes
Patient 3	Male	51	+	16	Yes, VF	No	Positive- MAC	21 days	3.02	6.9	468	Positive	18 days	No	Yes
Patient 4	Male	18	+	12	Yes, VF	No	Positive- MAC/MTB	38 days	6.31	6.8	61	Positive	4 Days	No	Yes
Patient 5	Male	39	+	8	Yes, VF	Yes, LN biopsy & pleural fluid dx	Positive-MAC	8 days	2.38	6.7	317	None	-	No	No
Patient 6	Male	48	+	2	Yes, VL LDL ? IRIS (Rx 5/12)	No	Positive-MAC	21 days	0.19	5.7	72	Positive- MTB	31 days	No	Yes
Patient 7	Male	60	+	27	No	Yes, AUS emperic	Positive- MAC	20 days	3.83	6.6	136	Negative	-	-	No

* LN biopsy & pleural fluid diagnosis
** Suspected immune reconstitution syndrome, on ART for 3 months
Abbreviations: HIV – Human immunodeficiency virus, CD4 – cluster of differentiation 4, ART – antiretroviral therapy, WCC – white cell count, Hb – Haemoglobin,, PLT – platelet, VL – viral load, BME – bone marrow examination, TB – Tuberculosis, MTB – *Mycobacterium tuberculosis*, MAC – *Mycobacterium avium* complex, VF – virological failure, LDL – lower than detectable

Three of the seven patients were on MTB treatment prior to the BME, with two having been placed on empiric TB treatment and one starting TB treatment based on a positive lymph node biopsy result. Six of the seven patients had positive peripheral blood cultures for MAC with the remaining patient having a contaminated blood culture specimen.

Three of the seven patients had both bone marrow and peripheral blood culture flag positive for MAC. The time to diagnosis was faster on BM culture in all three cases. From this group, one patient flagged positive on peripheral blood culture for both MTB and MAC; however, was only culture positive for MAC on bone marrow culture. One patient had MAC cultured on bone marrow culture only, with the peripheral blood specimen having been contaminated and one patient who cultured MAC on peripheral blood, cultured MTB on the bone marrow culture. One patient had MAC cultured only on peripheral blood culture with no bone marrow culture being submitted and the BME did not provide a unique result. The last patient cultured MAC only on peripheral blood culture with a negative bone marrow culture and the BME in this case also did not provide a unique diagnosis.

Of the seven patients with MAC diagnosed in the study group all presented with a haemoglobin less than 7g/dL. The platelet count was > 100 x 10^9/L in those patients who cultured MAC alone. In the two patients who cultured both MAC and MTB the platelet count was < 100 x 10^9/L. These two patients had platelet counts of 61 x 10^9/L and 72 x 10^9/L, respectively.

3.8 Findings in HIV-seronegative Patients

Amongst the 327 records reviewed, 12 of the patients were HIV-seronegative. The median age in this group was 41 years (range 22 - 65 years). Two thirds (8 cases) had a platelet count < 150 x 10^9/L, with five cases (41.7%) having a WCC < 4 x 10^9/L and 3 cases (25.0%) having a haemoglobin < 7g/dL. The presence of a peripheral blood cytopenia was the indication for BME in all 12 patients. In half the group (n = 6) there was also the concern of TB and in two patients there was a concern of a NBL.

Four patients had a diagnosis made on BME. In all cases that diagnosis was of mycobacterial infection. Two of these cases were diagnoses that were unique to the BME. The first case with a unique BME diagnosis was a patient who was already on empiric TB treatment who had granulomata observed on BME and the second case was a patient with TB bone marrow culture positive for MTB as well as being ZN positive and having granulomata observed on BME. A summary of data from the HIV-seronegative cases is documented in Table 3.12

Table 3.13: Summary of HIV-seronegative patients

Variable	*n.* = 12	Percent (%)
Laboratory Parameters:		
White cell count < 4 x 10^9/L	5	41.7%
Haemoglobin < 7 g/dL	3	25.0%
Platelets < 150 x 10^9/L	8	66.7%
Indications:		
Suspected Tuberculosis	6	50.0%
Peripheral Blood Cytopenia	12	100.0%
Suspected Malignancy	2	16.7%
Diagnosis on bone marrow examination:		
Any diagnosis	4	33.3%
Unique diagnosis	2	16.7%
Tuberculosis Diagnosis:		
Blood culture	2	16.7%
Bone marrow culture	1	8.3%
Ziehl-Neelsen stain positive	2	16.7%
Granulomata present	4	33.3%
TB PCR positive	1	8.3%

Abbreviations: TB- Tuberculosis, PCR – Polymerase chain reaction

There was one patient with a positive TB PCR. This patient had a positive TB PCR on BME and was already on TB treatment based on a positive sputum GeneXpert result when the BME was performed. The indication for the BME in this case was for the investigation of a thrombocytopenia. Of the two patients who had positive blood culture results for MTB, one was already on empiric TB treatment at the time of the BME. This patient was, additionally, found to have granulomata on BME and a positive ZN stain. The second patient with a positive peripheral TB blood culture and a bicytopenia (Hb 7.5 g/dL and platelet 92 x 10^9/L), was not on empiric TB

treatment at the time of BME, but was, however, initiated on TB treatment after the BME due to a positive sputum GeneXpert result. This particular patient had a bone marrow TB culture specimen that was contaminated and an inadequate trephine specimen submitted; therefore, mycobacterial involvement in the bone marrow was not able to be accurately assessed.

CHAPTER 4. DISCUSSION

4.1 Demographics

In the current study, a total of 327 bone marrow investigations were performed during the three year study period in the Infectious Disease ward at the Charlotte Maxeke Johannesburg Academic Hospital. This sample size is, therefore, larger per time period as compared to international studies in which, on average, 200 – 400 patients have been reviewed over 8 to 9 year periods [9,10,12]. This difference may be accounted for by the higher burden of HIV infection and TB in the third world compared to first world nations.[1] Samples were also specifically from patients in an infectious disease ward at a tertiary level referral hospital covering a population group of over 4 million individuals from Central and Northern Johannesburg.[25] This may also account for the higher numbers of bone marrow investigations needing to be performed.

The mean age of the population group was 36 years (17 - 65 years), with 96% of these individuals being HIV-seropositive. This reflects the high burden of HIV infection in the South African public sector.[24] The mean age amongst males was 37 years (18 - 65 years) and amongst females was 35 years (17 - 62 years). These averages are similar to national figures published in the 2012 South African National HIV Prevalence, Behaviour and Incidence Survey which showed the highest HIV prevalence in the 30 – 34 year age group in females and 35 – 49 year age group in males.[24] The similarity in numbers of males and females in the current study, in terms of HIV status, namely 49.4% males and 50.6% females, does not reflect national trends in which the presence of HIV in females is 1.45 times higher than in males. However, this may be influenced by gender-specific health seeking behavior.[24] Four percent of individuals were HIV-seronegative. This small number of HIV-seronegative cases (12 patients) may be attributed to the fact that the samples emanated

from an infectious disease ward in which HIV infection and TB, particularly in the setting of HIV infection, are the most prevalent diagnoses. Unfortunately, the considerably smaller number of patients in the HIV-seronegative group did not permit meaningful comparisons between the HIV-seronegative and -seropositive cohorts (4% versus 96% of the study population).

4.2 Laboratory Parameters

The prevalence of anaemia in the current study, among those patients with HIV infection was 88.7%. This correlates with the high prevalence of anaemia in more advanced HIV disease owing to multifactorial factors such as infection, drugs and anaemia of chronic disorders.[26] In this study, however, the presence of anaemia, together with other cytopenias (leukopenia and thrombocytopenia), occurred in 76% of patients and this often prompted the bone marrow examination in these cases. Only 2% of the BMEs were performed for an isolated thrombocytopenia, despite the prevalence of ITP of up to 30% in advanced HIV infection.[27] This, however, is likely due to patients with isolated thrombocytopenia and suspected ITP being investigated in the haematology ward as opposed to the infectious disease ward.

In those patients with HIV infection, the mean CD4 cell count was 94 cells/mm^3. The lower CD4 counts obtained in this in-hospital population group possibly reflect the higher incidence of opportunistic infections in those with advanced stages of HIV infection and CD4 counts < 200 cells/mm.[3,28] At the time of the bone marrow examination, 41% of patients were already on ART. This is a higher percentage of patients on ART when compared to the findings by Van Schalkwyk et al[5] who documented that 23% of 147 patients were on ART in their study undertaken between 2004 and 2007. This difference may be accounted for by the expanded role out of ART

from 2004 onwards.[19] The need for hospitalization in individuals already on ART may be due to virological failure, immune reconstitution syndrome or the high community burden of TB; however, this distinction was not further explored in this study. As the study was a retrospective record review, individual patient's adherence to ART was unknown. Fifty-nine percent of study patients were not yet initiated on ART at the time of the BME. The mean CD4 count in this group was 77 cells/mm^3, again reflecting the advanced stage of the HIV disease and associated higher risk of OIs in this group.

4.3 Adequacy of the specimens

In this study, 60.4% of the aspirate and 13.6% of the trephine specimens were found to be of inadequate quality. An additional 16.8% of trephine specimens submitted were suboptimal, providing only partial information. Brook et al[10] in their 1997 review of 246 bone marrow samples also looked at the adequacy of samples submitted. Their study revealed that 7% of aspirate samples and 1% of trephine samples were inadequate. Similar to our study, however, there was no documentation as to what level of staff had performed the BME. According to Bain et al[7], in their 2001 paper on bone marrow trephine biopsies, the ideal trephine specimen length should be at a minimum of 1.6 cm. The length of the trephine specimens submitted in the current study was, however, not documented. It is also recommended that should obtaining an aspirate be technically difficult, the alternative would be to do a slide imprint utilizing a trephine specimen; however, it was not indicated in those specimens with inadequate results in the current study if this had been attempted.[7] The high proportion of inadequate samples in the current study may reflect the fact that in the public hospital sector in South Africa there are different levels of experience amongst

staff and more junior staff members often perform the investigations. The impact of the high rate of inadequate aspirate specimens on our study findings, as well as the implication of this on the TB bone marrow culture results, is difficult to assess.

4.4 Indications for bone marrow examination

The most common indication for BME in the current study was the presence of a peripheral blood cytopenia (54%). Sedick et al[22], similarly, found that in their cohort of 410 patients the investigation of peripheral blood cytopenias made up 45.6% of the indications for BME. The second most frequent indication for a BME in the current study was the concern of disseminated TB (32%). This was, however, noted to have been the indication for BME in only 15.8% of cases in the Sedick et al cohort.[22] Northfelt et al[8] and Luther et al[12] both noted the presence of peripheral blood cytopenia, together with an unrelenting fever, as the most common indication for a BME. The presence of pyrexia as an indicator for BME was not specifically noted on any of the requisition forms as a reason for a BME in our study.

4.5 Unique diagnosis on bone marrow examination

Positive results on BME were found in 48.3% of cases. This was higher than previous studies in which diagnoses made on BME accounted for 25 - 47% of cases.[4,5,8,10,13–18] In the current study 23.6% of diagnoses made were unique to the BME. This finding was similar to the 24.9% unique diagnoses on BME found by Karstaedt et al[4]; furthermore, this is also higher than international

studies in which the prevalence of unique diagnoses has ranged between 8 - 10%.[4,17] Van Schalkyk et al[5], however, had an even higher yield of 33% unique diagnoses in their cohort of 147 patients.

The predominant unique diagnosis made on BME in the current study was that of MTB, which encompassed 20.2% (66/327) of all the BMEs performed. This finding is in agreement with the findings of the study by Karstedt et al[4], which also revealed MTB as the predominant unique diagnosis on BME. Brooke et al[10] in 1997, reviewed 215 bone marrow samples over a 9-year period in London and similarly found MTB as the predominant diagnosis (20% of all cases). Van Schalkwyk et al,[5] found l4% of BMEs yielded MTB and this together with ITP was the most common diagnosis made on BME. The higher prevalence of ITP in their study compared to the current study in which ITP was not a diagnosis made on BME, was most likely owing to their cohort not being limited to an infectious disease ward.

Other unique diagnoses found in the current study, which were similarly found in other studies, were disseminated MAC, haematological malignancies and disseminated cryptococcosis.[8,9,18] Most international studies, however, found the prevalence of MAC to be higher than that of MTB, reflecting differing disease profiles of mycobacterial infection in third world countries like South Africa in which MTB is predominant versus the first world countries in which MAC appears to be a more common diagnosis on BME.[21]

Pure red cell aplasia and aplastic anaemia were two additional unique diagnoses made on BME in the current study. These diagnoses, however, were not common additional findings in other studies in the literature. It must be noted, however, that the majority of similar studies limited their inclusion criteria to patients who were undergoing bone marrow examination for pyrexia of unknown origin, which is most commonly absent in patients presenting with these two conditions.[9–11,13,14] All five patients in whom the unique diagnosis of a PRCA was made on BME

were HIV-seropositive. Two of these patients had positive Parvovirus B19 PCR. Patients with PRCA typically present with isolated anaemia.[29] These cases, in the absence of fever or other clinical or biochemical evidence to suggest sepsis, may also have been investigated in a haematology ward at the study hospital; thus the true incidence of PRCA on BME cannot be adequately evaluated based on the findings in the current study. This disease entity, be it secondary to drug-related causes, Parvovirus B19 infection, or as part of an immune reconstitution syndrome, has been recognized as a cause of isolated anaemia that is important to consider in HIV-seropositive patients.[29] Parvovirus B19 infection has also been noted to have a higher incidence in advanced HIV infection. This predilection is due to immunoparesis from the overwhelming HIV infection, which prevents the generation of appropriate IgG antibodies to target the Parvovirus. [30,31]

4.6 Predictors of a positive bone marrow examination result

This study found that a WCC < 4 x 10^9/L was a positive predictors of a unique diagnosis on BME (p value < 0.05). A neutrophil count of < 0.5 x 10^9/L, although found more commonly in those with a unique diagnosis, was not found to be significant. No other variables showed a significant difference when comparing those with or without a unique diagnosis on BME. This is in contrast to published studies in which more advanced HIV disease, with a lower CD4 count, was found to be a predictor of a positive BME.[4,17] Karstaedt et al[4], however, similar to our study, found that leukopenia, namely a WCC < 4 x 10^9/L, predicted a unique diagnosis on BME. Van Schalkwyk et al[5] found that a neutrophil count < 0.5 x 10^9L predicted a unique BME result; however, that study additionally found that a Hb < 6g/dL was also a significant predictor of a unique diagnosis. Keiser et al[17] and Luther et al[12] both found that a HCT < 25% and 30%, respectively, predicted positive results. This variable was not measured in the current study.

4.7 Mycobacterial infection on bone marrow examination

In the current study, MTB was confirmed on BME by either a positive TB bone marrow culture or a positive TB PCR or a positive ZN stain. The suggestion of TB was made from the presence of granulomata on BME. The diagnosis of MAC was made by the presence of a positive bone marrow culture for MAC. The majority of the unique diagnoses obtained on BME in this study were mycobacterial infections; namely 89.6% of all unique diagnoses (69 of 77 cases). These 69 cases formed 21.2% of the entire study population. This translates into 1 in 5 study cases yielding a unique diagnosis of mycobacterial infection on BME, thus highlighting the utility of the BME for the investigation of disseminated TB. Of those with mycobacterial infection found uniquely on BME, 35 patients (50.7%) were already on empiric treatment for MTB at the time of the investigation. The value of a BME in these cases would be to provide definitive diagnoses in those on empiric TB treatment, as well as to rule out the co-existence of an additional disseminated infection or a non-benign lesion.

4.8 Bone marrow TB culture

Twenty four percent of bone marrow TB cultures yielded positive results. The majority of positive cultures yielded *Mycobacterium tuberculosis* (89.1%) with an average time to positivity of 27 days. MAC was the second most common organism cultured in an average of 13 days. Similar to our study, Van Schalkwyk et al[5] found that the average time to MTB growth was 700 hours (namely 29 days). The average time for MAC growth of 582 hours (24 days) in that study was, however, almost double the 13 days found in the current study. The reason for this difference is

not clear, with the same MycoF/Lytic culture system medium being utilized for TB culture in both studies. The sample sizes of four patients in the study by Van Schalkwyk et al[5] and seven patients in this study are too small to conclusively comment on this finding.

A higher percentage of the peripheral mycobacterial blood cultures submitted yielded a positive diagnosis of mycobacterial infection as compared to those of the bone marrow culture (34% versus 24.4%, respectively). Northfelt et al[8], however, in their study carried out in San Francisco between 1988 and 1989 reviewing bone marrow examinations of 387 HIV-seropositive patients, found no significant difference between blood and bone marrow TB culture yields (sensitivity of 77% and 86% respectively).

In the twenty three patients who had both peripheral blood and bone marrow cultures sent in the current study, neither proved to be notably faster than the other, with 47.8% being culture positive faster on blood culture and 43.5% being bone marrow culture positive earlier. Pacios et al,[23] in 2004, reviewed a similar cohort of 23 patients with paired bone marrow and blood TB cultures with the primary aim of assessing their comparative yield. Their study revealed that blood culture was faster than bone marrow culture in the diagnosis on disseminated MAC. This finding, however, was not statistically significant. The time to diagnosis of MTB on blood versus bone marrow culture was however similar.[23] Brook et al[10], however, found that patients with TB on bone marrow culture had a diagnosis made 25 days earlier than blood culture taken at the same time. These results are in contrast to Lin et al[16], who in a similar size cohort of 24 patients, found that those with MTB had a lower likelihood of the bone marrow culture yielding a positive result. We thus support the final recommendation put forward by Pacios et al[23] and Lin et al[16] that blood and bone marrow culture performed in conjunction would ideally provide the highest positive yield.

4.9 Granulomata on bone marrow examination

Granulomata are also a common finding noted on bone marrow trephine examination. There were granulomata observed in 130 of the 316 cases tested (41.1 %) in the current study cohort. The prevalence of granulomata is similar to that found by Karstaedt et al (44% of cases).[4] In addition, Karstaedt et al[4] found that 93% of patients with proven TB infection had granulomata on bone marrow trephine examination (77 of 83 cases). Similarly Van Schalkwyk et al[5] also found that the majority of granulomata were observed in those subjects with proven mycobacterial infection, with 83% of positive TB culture patients having granulomata observed on BME (15 of 18 patients). An additional finding in our study was that 67 of 94 cases (71.3%) cases of mycobacterial infection confirmed either on blood culture or positive ZN stain, had granulomata observed on BME.

The prevalence of granulomata on BME of 41.1% in our study is higher than international studies, which have shown a 20-30% prevalence of BMEs being positive for granulomata.[9,16] This difference may be attributable to the higher prevalence of mycobacterial infection in South Africa.[12] Lin et al[16], in their study based in Taiwan, found that patients with MTB infection were more likely to have granulomata observed on BME than those with MOTT infections (82% compared to 30% of their 24 patients; $p = 0.03$). The higher prevalence of MTB versus MOTT in South African studies may also account for the higher prevalence of granulomata observed on BME.

Riley et al[9], found that there were no positive AFB stains on BME in the absence of granulomata leading to their suggestion that the ZN stain is of no use in the absence of granulomata. A similar finding was observed in our study with 48 patients having both a positive ZN stain and granulomata

observed on BME as compared to only three patients with a positive ZN stain who had no granulomata observed on BME.

4.10 Ziehl-Neelsen stain on bone marrow examination

Of the 182 tests for ZN performed on the bone marrow, 51 were positive (28%). This is lower than that found in the study by Sedick et al[22], in which 43.4% of ZN stains performed were positive. Of the patients with positive TB culture results in the current study, 16 of the 55 had ZN stains positive (29%). This is much less than that observed by Van Schalkwyk et al[5], who amongst a smaller sample size found that 44% of those with proven TB on BME had a positive ZN stain (8/18 patients) on BME. [5] This finding is also less than that found by Chosamata et al[32], who reported that 65% of those with a positive BM mycobacterial culture had a positive ZN stain. In the current study, 17 (43.6%) of the 39 patients with positive TB BM cultures with negative ZN tests were on TB treatment at the time of the BME. The presence of TB treatment in just under half of the BM TB culture positive patients may have accounted for the lower rates of a positive ZN yield in these patients.

4.11 Predictors of *Mycobacterium tuberculosis* on bone marrow examination

The presence of granulomata on trephine examination, positive ZN stain on BME and positive TB bone marrow culture results were seen to be significantly associated with lower mean CD4 cell counts, lower platelet counts and higher viral loads in the study population. Granuloma observation and positive bone marrow TB culture were also found to be significantly associated with a lower

WCC. Granulomata are documented to be more commonly seen in patients with more clinically advanced retroviral disease, lower CD4 cell values, as well as lower neutrophil counts.[4,5] A similar finding of a lower CD4 count being more prevalent in those with positive mycobacterial findings on BME was found in the 2015 study by Sedick et al,[22] in which the median CD4 cell count range amongst the different methods of diagnosing TB on BME was between 7 - 33 cells/mm^3. The statistical significance of this finding was, however, not evaluated.

4.12 *Mycobacterium avium* complex on bone marrow examination

Four individuals in this study had MAC cultured on BME, as compared to 49 who had MTB cultured. This finding is in contrast to studies conducted in the first world, which reflect a higher prevalence of MAC infection as compared to MTB. [9,10,12,14–17] Bishburg et al [14], in a study in New Jersey in 1986 also found in their examination of 47 patients that bone marrow examinations were a useful diagnostic tool to detect MAC infection, with 16 of their 17 patients known with MAC infection testing positive on BME. Quesada et al[15], in 2004, similarly found that amongst the 57 BMEs reviewed the primary organism identified was MAC (4 cases) as opposed to MTB (2 cases).[15] Keiser et al[17] in their cohort in Texas also found MAC to be the predominant organism amongst 30 patients. Brook et al[10] found that of the 34 patients in their cohort with MOTT infection, 22 were also found on BME and hence concluded that the BME had a sensitivity of 65% for diagnosing MOTT. In the London study by Riley et al[9], MAC was the organism most commonly found on bone marrow culture (42 of 51 cases). The investigators also found that of the patients in their study diagnosed with MAC, this was a unique diagnosis in one third of the cases, which for them highlighted the usefulness of BME in aiding with MAC diagnosis. The utility of BME in diagnosing MAC in the current study was difficult to comment on given the small sample

of patients who cultured MAC, namely 4 out of 55 positive bone marrow culture results. Given the available literature, however, BME appears to be a useful means to diagnose MAC in areas with higher disease prevalence.

What was noted in our study, however, was the lower haemoglobin level amongst those with MAC as compared to those with MTB diagnosis, who commonly had bicytopenias on peripheral blood. This is in keeping with MAC infection typically being associated with anaemia more commonly than other haematological abnormalities.

4.13 HIV-seronegative patients and the bone marrow examination

Twelve patients in this study were HIV-seronegative. The discrepancy between the numbers of those with HIV infection and those without (96% versus 4%, respectively) prevented valuable comparison between the two groups from being made. Four patients in the HIV-seronegative group obtained a positive diagnosis of mycobacterial infection on BME; however, in only two of these cases was this finding a unique diagnosis. There are very few reports on research exploring the value of BME in HIV-seronegative individuals. Riley et al,[9] in their study cohort, found that in those patients who were HIV-seronegative and not immune-compromised from other disease processes (a total of 199 bone marrow investigations over a period of 10 years), there was no benefit of a BME over alternative investigations for the identification of mycobacterial infection. In the current study, diagnosis of mycobacterial infection was less likely in the HIV-seronegative group as compared to the HIV-seropositive group (2% versus 20.1% respectively). In addition, the HIV-seronegative group were more likely to have TB proven elsewhere in the body and to have a negative bone marrow result as compared to the HIV-seropositive cohort. These finding suggests

a decreased utility of BME in those without HIV infection, specifically for the investigation of mycobacterial infection and additionally allude to the occurrence of less bone marrow involvement of mycobacterial infection in HIV-seronegative individuals. Repeat studies, however, need to be conducted to corroborate this result.

4.14 Potential limitations of the study

There were a few potential limitations of this study. This was a single centre study undertaken in a single unit and so the results may not be generalizable to other units or to other hospitals in South Africa. Bone marrow examination findings were obtained from the retrospective review of published reports and individual bone marrow aspirate and trephine specimens were not reanalyzed. The subjectivity associated with the interpretation of the BMEs by the different assessors has to be noted. Their findings have been accepted in good faith without independent confirmation. In addition, the study cohort was obtained specifically from an infectious diseases ward in which the yield of TB and the HIV seroprevalence is anticipated to be higher than that of a general medical ward having patients with both communicable and non-communicable diseases. This factor may influence how the study findings may be applied to primary or district hospital settings in South Africa. The high percentage of inadequate bone marrow aspirate specimens submitted may have also influenced the reliability of the interpretation of the bone marrow TB cultures namely, inoculation of peripheral blood as opposed to bone marrow aspirate blood may have affected the yield of positive TB bone marrow culture results.

CHAPTER 5. CONCLUSION

The findings in this study lead us to conclude that bone marrow aspirate and trephine examinations provide utility as a diagnostic tool in certain circumstances. This applies particularly to patients in an infectious diseases ward in whom non-invasive investigations for the presence of cytopenias on peripheral blood have been negative and the concern of disseminated mycobacterial infection remains high. Performance of a bone marrow aspirate and trephine in these circumstances, through aiding with diagnosis allows initiation and/or changes in patient treatment plans and by inference would most likely improve patient outcomes.

The poor adequacy of aspirate specimens submitted in this study potentially highlights the absence of formal teaching on bone marrow examination technique and slide preparation in the medical curriculum. There is a need for better dissemination of this knowledge in order to allow more meaningful evaluation of the bone marrow aspirate results as well as a focus on supervision from more experienced members of the medical team.

Our final recommendation would be to conduct the least invasive investigations first. If no success is obtained in making a diagnosis, a bone marrow examination is then recommended. TB culture on blood specimens and bone marrow TB culture should be performed together to allow for optimal yield. Bone marrow trephine results should ideally be made available to the clinician within two weeks of performance, although this is often difficult in the resource-limited public sector in South Africa. It is imperative in those patients who have been started on empiric TB therapy to follow up the results of these invasive investigations in order to confirm the diagnosis and to exclude other unexpected diagnoses.

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APPENDIX 1: Ethics Approval Certificate

R14/49 Dr Nirvana Bharuthram

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150847

NAME: (Principal Investigator)	Dr Nirvana Bharuthram
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	An Analisis of the Utility of Bone Marrow Examinations Carried out in an Infectious Disease Ward
DATE CONSIDERED:	28/08/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Charles Feldman
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/01/2016

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2: Data Collection Table

	PATIENT DEMOGRAPHICS																
PT NO.	GENDER	AGE	BLOOD RESULTS											INDICATION			
			HB	PLT	WCC	TB B/C	RVD	VL	CD4	Ferritin	B12	Folate	RPI	?TB	Cytopenia	NBL	Other
001																	
002																	
003																	
004																	
005																	
006																	
007																	
008																	
009																	
010																	
011																	
012																	
013																	
014																	
015																	
016																	
017																	
018																	
019																	
020																	

	ASPIRATE					
QUALITY	RESULT					BONE MARROW
	Erythropoesis	Iron Stores	Granulopoesis	Megakaryopoesis	Other	TB Culture

	TREPHINE							
PT NO.	QUALITY	RESULT						
		Ineffective haemopoesis	Effective haemopoesis	Multifactorial Anaemia	Granuloma	TB	Other	Positive Stains
001								
002								
003								
004								
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006								
007								
008								
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010								
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015								
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017								
018								
019								
020								

APPENDIX 3: National Health Laboratory Service Reference Ranges

National Health Laboratory Service Laboratory Parameter Reference Ranges	
Laboratory Parameter	**Reference range**
Platelet count	150 - 400 x 10^9/L
White Cell Count	4.00 - 10.00 x 10^9/L
Neutrophil Count	2.00 - 8.00 x 10^9/L
Ferritin	20 – 250 ug/L
Vitamin B12	130 – 700 ng/L
Folate	280 – 790 ug/L

APPENDIX 4: Turn It In Report



0600816k:MMED_-
_AN_ANALYSIS_OF_BONE_MARROW_EXAMINATIONS_CA...

ORIGINALITY REPORT

5%	1%	4%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Abstracts of papers presented at the Second Meeting of the European Haematology Association, Paris, France, 29 May-1 June 1996", British Journal of Haematology, 12/1996 Publication	1%
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